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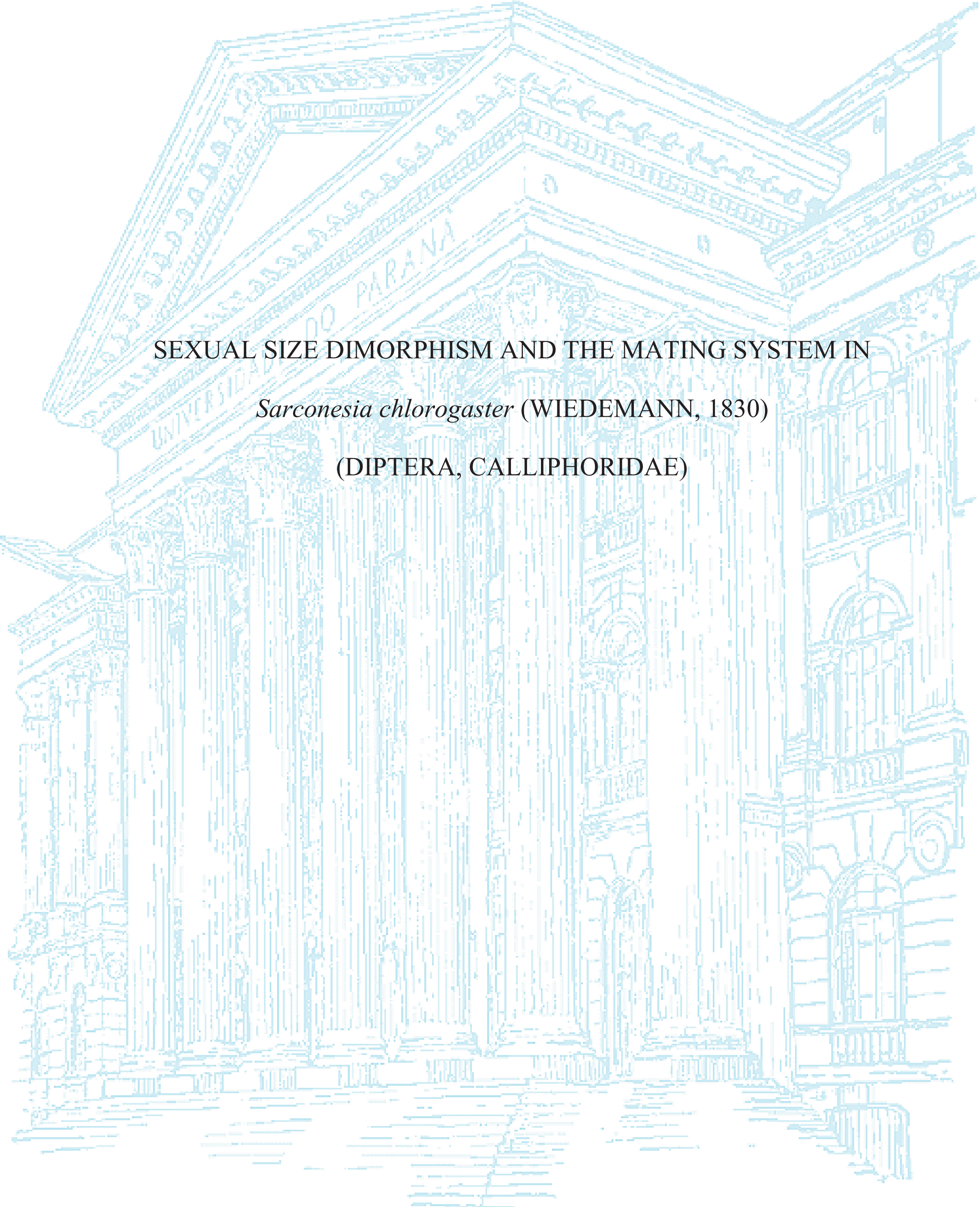

SEXUAL SIZE DIMORPHISM AND THE MATING SYSTEM IN *Sarconesia chlorogaster* (WIEDEMANN, 1830) (DIPTERA, CALLIPHORIDAE)

CURITIBA

2024

SABRINA MACHADO DA SILVA

SEXUAL SIZE DIMORPHISM AND THE MATING SYSTEM IN *Sarconesia chlorogaster* (WIEDEMANN, 1830) (DIPTERA, CALLIPHORIDAE),

Tese apresentada à Coordenação do Programa de Pós-Graduação em Ciências Biológicas, Setor de Ciências Biológicas, Área de Concentração em Entomologia, do Departamento de Zoologia da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Mauricio Osvaldo Moura

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“Tudo o que temos de decidir é o que fazer com o tempo que nos é dado”

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PREFÁCIO

O dimorfismo sexual de tamanho em insetos é uma das diferenças morfológicas mais comuns de ser observada entre os sexos, e pode ser resultado de seleção natural ou sexual. Em geral, em insetos, as fêmeas são maiores que os machos, devido a seleção natural resultando em uma maior fecundidade relacionada ao tamanho do corpo. No entanto, táxons em que o maior tamanho de corpo tem viés para os machos são comumente relatados entre os insetos, resultado de seleção sexual. Nestes casos os machos usam esse tamanho maior do corpo para ter vantagem na aquisição de cópulas e, consequentemente, essa característica influencia sua aptidão (fitness) e faz com que essa característica seja selecionada ao longo das gerações. O maior tamanho de corpo pode conceder vantagem em lutas de machos contra machos (competição intraespecífica), na aquisição de recursos (território e alimento) ou ainda na escolha pela fêmea. Além disso, essa diferença no tamanho pode ser importante em situações de conflito sexual entre os membros da mesma espécie (intersexual). Nesta dinâmica o macho submete a fêmea à cópula forçada (coerção sexual), impondo os custos (injúrias mecânicas, aumento no risco de predação, gasto energético) deste tipo de interação e impedindo que a fêmea selecione outro parceiro antes da cópula. Esse tipo de sistema de acasalamento está alinhado com a teoria de conflito sexual, que sugere uma assimetria no investimento de recursos na prole: enquanto fêmeas tendem a investir mais na qualidade da sua prole, machos investem mais na quantidade de cópulas, resultando em um conflito quase que unanime entre os sexos, devido ao trade-off de custos e benefícios da cópula para cada sexo.

Em *Sarconesia chlorogaster*, um Califorídeo endêmico da região sul da América do Sul, ocorre o padrão inverso (já citado) de machos maiores que fêmeas. Essa diferença no tamanho do corpo foi percebida inicialmente durante a descrição básica da morfologia externa de imaturos e adultos. Esse dimorfismo sexual de tamanho foi posteriormente identificado como resultado de um maior tempo de permanência dos machos no estágio imaturo, permitindo um aumento de tamanho corporal para a fase adulta. No entanto, nos interessa saber o porquê dessa diferença em termos evolutivos, como resulta em uma vantagem reprodutiva, e consequentemente como ela tem relação com a seleção sexual. Para entender melhor esse cenário se faz necessário o estudo detalhado do sistema de acasalamento espécie específico, um tópico chave para a compreensão de como essa característica evoluiu e permaneceu na morfologia da espécie. O objetivo desta pesquisa foi analisar detalhadamente como esse viés no tamanho do corpo dos machos afeta o sistema de acasalamento em *S. chlorogaster* a partir de dados da morfologia externa, comportamento sexual e medição dos parâmetros associados ao acasalamento.

Ao realizar os ensaios comportamentais em arenas, os experimentos-piloto nos mostraram um comportamento de coerção física do macho para obtenção da cópula. Uma luta física intensa entre machos e fêmeas para o início ou não da cópula. Posteriormente, ao estudar grupos de indivíduos com relação ao comportamento sexual, concluímos também que o sistema de acasalamento nessa espécie é do tipo promíscuo, o que significa que tanto machos quanto fêmeas possuem mais de um parceiro por período reprodutivo. Em táxons que possuem órgãos de armazenamento de esperma esse tipo de sistema abre precedente também para a competição pós-cópula, a competição espermática e possível escolha críptica da fêmea.

Com os dados relacionados a frequência de cópulas nos grupos de observação, tivemos múltiplos resultados que ajudaram a compreender melhor o sistema de acasalamento da espécie. Entre eles estabelecemos que a duração da cópula em *S. chlorogaster* é de, em média, 86.69 minutos. Além disso, o início da cópula e o comportamento de persistência do macho é crucial para a obtenção da cópula, comportamento esse agressivo quando comparados aos demais táxons relacionados e descritos em literatura. Também estabelecemos que os machos copulam em média (N:32) mais vezes durante o período fértil (aproximadamente 20 dias observados) do que as fêmeas (N:21), e que nesta espécie as fêmeas podem acasalar até 6 vezes no mesmo dia, o que aumenta a intensidade de competição espermática. Por fim, descrevemos comportamentos de resistência e persistência para fêmeas e machos, que condizem com o esperado em sistemas de acasalamento com conflito de interesses entre os sexos e os dados aliados ao comportamento sexual, demonstraram que o maior tamanho de corpo do macho é resultado da seleção sexual através da coerção sexual.

Little fly,
Thy summer's play
My thoughtless hand
Has brushed away.

Am not I
A fly like thee?
Or art not thou
A man like me?

For I dance
And drink and sing,
Till some blind hand
Shall brush my wing.

If thought is life
And strength and breath,
And the want
Of thought is death,

Then am I
A happy fly,
If I live,
Or i

William Blake (1757-1827)   P. 1793

RESUMO

O dimorfismo sexual de tamanho (SSD) é um padrão morfológico intraespecífico comum encontrado entre machos e fêmeas. Essas diferenças de tamanho sexual podem ser adaptativas ou não adaptativas e são mediadas por variações ambientais. Em insetos, o padrão geral de tamanho corporal tende a favorecer as fêmeas, como resultado da seleção natural para aumentar a fecundidade. O oposto, no entanto, quando o maior tamanho de corpo tem viés para os machos da espécie, é comumente encontrado na natureza e pode ser resultado da seleção sexual para aquisição de parceiros. Em *Sarconesia chlorogaster*, uma mosca-varejeira endêmica da América do Sul, foi observado um viés em favor dos machos e investigou-se sua origem. Descobriu-se que o tamanho corporal maior era produzido por meio de diferenças no tempo de desenvolvimento larval entre machos e fêmeas. Independentemente da causa imediata do dimorfismo sexual de tamanho, o sistema de acasalamento de uma espécie desempenha um papel fundamental para entender a evolução e a manutenção do dimorfismo sexual de tamanho. Para examinar as possíveis características e comportamentos associados a essa diferença de tamanho, investigamos grupos de indivíduos de *S. chlorogaster* e descrevemos o sistema de acasalamento em detalhes. Para a descrição da morfologia interna, congelamos tanto machos quanto fêmeas maduros (7 dias de idade), dissecamos sob estereomicroscópio, removemos os tratos reprodutivos, montamos em lâminas e os fotografamos. O comportamento sexual e as medidas relacionadas à cópula foram registrados com uma câmera GoPro e posteriormente analisados no R Studio por meio de progressão linear. O sistema reprodutivo feminino contém dois ovários pareados, um oviduto comum único e uma câmara genital que recebe duas glândulas acessórias e três espermatecas. O trato reprodutivo masculino de *S. chlorogaster* inclui um par de testículos, ductos deferentes, uma vesícula seminal triangular, um par de glândulas acessórias e uma bomba de esperma esclerotinizada. O tempo médio de cópula é de 86,69 minutos, os machos copulam em média (N=32) mais vezes durante o período fértil do que as fêmeas (N=21), e ambos os sexos podem copular até 6 vezes no mesmo dia, o que aumenta a intensidade da competição pós-cópula. Assim, concluímos que *S. chlorogaster* possui um sistema de acasalamento promíscuo e um intenso conflito sexual pré-cópula. Ambos os sexos realizaram comportamentos de resistência e persistência, consistentes com o conflito de interesses. Além disso, a descrição do comportamento sexual demonstrou que o tamanho corporal maior dos machos é resultado da seleção sexual, favorecendo a coerção sexual.

Keywords: SSD, comportamento sexual, coerção, tamanho do macho.

ABSTRACT

Sexual size dimorphism (SSD) is a common intraspecific morphological pattern found between males and females. Such sexual size differences may be adaptive or nonadaptive, and are mediated through environmental variation. In insects, the general pattern for body size is biased towards females, as a result of natural selection to increased fecundity. The opposite, however, when body size is biased towards males, is often found in nature and can be a result of sexual selection for mate acquisition. In *Sarconesia chlorogaster*, a South America's endemic blowfly, a male bias was noted and investigated for its origin. The larger body size was found to be produced through differences in larval development time between males and females. Independent of the proximate cause of sexual size dimorphism, the mating system of a species has a key role to understand the evolution and maintenance of sexual size dimorphism. To examine the possible characteristics and behaviors associated with this difference in size, we investigated groups of *S.chlorogaster* and described the mating system in detail. For the description of the internal morphology, we freeze both mature males and females (7 days of age), dissect them under stereomicroscope, remove the reproductive tracts, mount them on slides, and photograph them. The sexual behavior and copulation-related measures were taken with a GoPro recording camera, and posteriorly analyzed on *R studio* through linear progression. The female reproductive system contains two paired ovaries, a unique common oviduct and a genital chamber that receives two accessory glands and three spermatheca capsules. The male reproductive tract of *S. chlorogaster* includes a pair of testes, vas deferens, a triangular seminal vesicle, a pair of accessory glands and a sclerotized sperm pump. The average copulation time is 86.69 minutes, males copulate on average (N= 32) more times during the fertile period than females (N= 21), and both sexes can mate up to 6 times in the same day, which increases the intensity of post-mating competition. Thus, we conclude that *S. chlorogaster* has a promiscuous mating system and an intense pre-copulation sexual conflict. Both sexes performed resistance and persistence behaviors, which are consistent with conflict of interests. In addition, the description of the sexual behavior demonstrated that male's larger body size is a result of sexual selection by selecting for sexual coercion.

Keywords: SSD, sexual behavior, coercion, male size.

LIST OF FIGURES

Figure 1. Chronological scheme of main steps of methodology 21

Figure 2. Plate containing 4 steps of the methodology: a) female in ventral position; b) sexed samples; c) dorsal marking and d) modified insect rearing cage 24

Figure 3. Female reproductive system 26

Figure 4. Male reproductive system 27

Figure 5. Lateral view of the female ovipositor and spermatheca capsule 28

Figure 6. Mating behavior 29

Figure 7. Ethogram of mating behavior 31

Figure 8. Copulation duration 32

Figure 9. Frequency of copulations during the mating experiments 33

Figure 10. Number of copulations during the mating behavior experiment for females 34

Figure 11. Number of copulations during the mating behavior experiment for males 34

Figure 12. Frequency of mating with different males and females 35

Figure 13. Relationship between copulation time and male body size 56

Figure 14. Comparison of copulation time and the copulation order 57

Figure 15. Probability of mating between category body sizes of males and females 58

Figure 16. Number of matings for males and females correlated with body size 59

SUMÁRIO

1. GENERAL INTRODUCTION 12

1.2 REFERENCES 15

2. **CHAPTER I – MORPHOLOGY AND MATING BEHAVIOR OF** *Sarconesia chlorogaster* **(Wiedemann, 1830)** 17

2.1 INTRODUCTION 18

2.2 METHODOLOGY 21

- 2.2.1 TIMELINE OF THE PERFORMED EXPERIMENTS 21
- 2.2.2 COLONY ESTABLISHMENT 22
- 2.2.3 INTERNAL MORPHOLOGY 22
- 2.2.4 MATING EXPERIMENTS 23
- 2.2.5 VIDEO RECORDING 24

2.3 RESULTS 25

- 2.3.1 INTERNAL MORPHOLOGY 25
- 2.3.2 MATING BEHAVIOR 28

2.4 DISCUSSION 36

2.5 CONCLUSION 42

REFERENCES 43

3. CHAPTER II - DOMINANCE AND SIZE 49

3.1 INTRODUCTION 50

3.2 OBJECTIVES 53

- 3.2.1 SPECIFIC OBJECTIVES 53

3.3 METHODOLOGY 54

- 3.3.1 SAMPLING AND STATISTICAL ANALYSIS 54

3.4 RESULTS 56

3.5 DISCUSSION 59

REFERENCES` 63

1.GENERAL INTRODUCTION

Imagine that one common scene: a couple, of whichever species, copulating. We both amateurs and researchers know they will provide a new generation of offspring and then perpetuate the species. However, another aspect observed in many of those interactions is that there is a conflict between the male and the female involved in mating. Think in these events, how many times did you realize that one of the partners was literally firmly holding the other, which in turn is trying to escape? That happens because there is a conflict of interests in most matings and to understand it we also need to understand the natural history of sexual selection and its unfolding.

Firstly, the theory of sexual selection was proposed by Darwin in addition to the theory of natural selection (Darwin, 1871), this last one the better known of Darwin's contribution explains the biodiversity through differential reproduction of the organisms based on the ability to survive (Zuk and Simmons, 2018). In sexual selection theory, the success of an organism is based on the ability to successfully obtain mates and fertilize the gametes of their partners. In both theories the variability between organisms is a necessary condition for the operation of selection forces (natural or sexual). This occurs not only between species and the members of a population, but also between males and females. These sexual differences can be associated and reflect, physiologically and morphologically, the production and the contact of egg and sperm (primary sexual characteristics), whereas a second type of characteristics are strictly associated with the acquisition of partners (secondary sexual characteristics). When a variability in a heritable characteristic is associated with the success in mating it could, consequently, be influenced by sexual selection. This can lead to the evolution of a sexual dimorphism (Wilkinson and Johns, 2005).

In insects we observe a variety of morphological patterns associated with sexual dimorphism resulting from two main processes mediated by sexual selection: a) the ability to obtain resources, win battles or defend resources and b) female choice. The first process is in general associated with traits that confer advantages in a dispute such as larger bodies, horns, head and leg modifications, sperm length and ejaculate volume. Female choice, however, is associated with gifts, pheromones, courtship behaviors, morphological features, control of insemination time, sperm activation and neutralization, all these traits confer an advantage to a male because they are preferred and consequently chosen by the female (Wilkinson and Johns, 2005).

Sexual size dimorphism (SSD) is a common trait in nature and can evolve through: 1) natural selection to increase fecundity—where females are usually larger than males, as seen in most insects; and 2) sexual selection, which may confer an advantage in mate acquisition through competition, female mate choice (Darwin, 1871; Honek, 1993; Fairbairn, 1997; Rohner et al., 2018), or sexual coercion, where copulation is forced, limiting the ability to accept or reject a mate (Clutton-Brock and Parker, 1995).

The female-biased SSD was described for several flies (*Sepsis punctum* Fabricius, 1794; *Sepsis fulgens* Meigen, 1826; *Sepsis neocynipsea* Melander & Spuler, 1917; *Musca domestica* Linnaeus, 1758; *Sepsis cynipsea* Linnaeus, 1758; *Drosophila rhopaloa* Bock & Wheeler, 1972; *Drosophila melanogaster* (Meigen, 1830) (Rohner et al. 2018) and, despite not being the most common, the opposite, male-biased SSD were also described for several taxa in the literature (Puniamoorthy et al, 2012; Rohner et al. 2016), inclusive for flies (*Drosophila prolongata* Singh & Gupta, 1977; *Scathophaga stercoraria* (Linnaeus, 1758); *Sepsis punctum* Fabricius, 1794; *Sepsis neocynipsea* Melander & Spuler, 1917*; Sepsis lateralis* (Wiedemann, 1830) (Rohner et al. 2018).

The differences between the sexes are also responsible for the conflict of interests and this is an assumption of another theory: whenever there is an interaction between two organisms they will have different evolutionary interests, which is linked with the optimal individual fitness of each sex. This theory includes a discussion of the interaction between the sexes with an evolutionary perspective and the role each sex has in parental care (Trivers 1972). In general, one of the sexes will invest more energy than the other in his offspring, an asymmetry in reproductive investment (Trivers 1972). In nature, in most cases the female is the sex that allocates more energy per gamete and so, will prioritize the quality of each gamete while males invest in quantity so, many fertilizations mean more genes in the next generation (Bateman 1948). The general rule is that the sex who invests more energy in offspring would be the chooser sex, while the one who invests less will be the courter and, consequently, they will need to have the arms or the attractive characteristics to be selected or acquire its mates. Based on this, the theory of sexual conflict was built with the premise of an existing conflict of interests between the sexes, even in a genetically scale, and so opposite directions of the sexual selection forces can act leading to the evolution of sexually antagonistic coevolutionary traits (SAC) (Parker 1979). The evolutionary conflict of interests results in behavioral, morphological and physiological traits of resistance and persistence observed during mating (Parker 1979; Arnqvist and Rowe, 2005).

The Flies (Diptera), a megadiverse order of insects, are incredible in many aspects. It occurs through the most diverse habitats and are thus also morphologically and behaviorally diverse, which makes them a fit model system to the study of mating systems and sexual selection (Choe and Crespi, 1997; Wilkinson and Johns, 2005; Shuker and Simmons, 2014). The blowfly *Sarconesia chlorogaster* Wiedmann 1830 (Diptera: Calliphoridae) are endemic to South America (Brazil, Argentina, Uruguay, Bolivia, Peru, and Chile), has a necrophagous feeding habit and usually occurs in urban cities (Lopes 1973). During the past years this blowfly was investigated, especially focused in the possible applications on forensic issues, however the difference in size between males and females are noted through the years. Initially, this difference was on average between 8 and 12 millimeters for males and 6-12 millimeters for the females (Mello, 1972), so the female's minimum size was smaller. More recently this difference in size was noted during investigations on immature stages of development (larvae and pupae); life-history traits (Moura & Bonatto 1999, Lecheta & Moura, 2015; Flissak and Moura 2018), and finally by personal observations during years of rearing under laboratory conditions (Bonatto and Carvalho, 1996; Lecheta et al. 2015; Flissak and Moura 2018). It was found to be produced through differences in larval development time in which the males spend more time in immature stages and reach a bigger size and late emergence (Flissak & Moura unp. manuscript). The main objective of this thesis was investigating the evolutionary causes of the male biased size in *S. chlorogaster* through the detailed description of the mating system and measures associated with mating, mainly mating duration and frequency for males and females.

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CHAPTER I – MORPHOLOGY AND MATING BEHAVIOR OF *Sarconesia chlorogaster* (Wiedemann, 1830)

2.1 INTRODUCTION

Darwin (1871) began to characterize sexual selection by describing sexual dimorphism in animals. In several animal groups, the dimorphism between males and females is related to body size. The process that leads to such sexual size differences may be adaptative or nonadaptive, both mediated through environmental variation (Trivers, 1972, Fairbairn et al, 2007). For example, in insects' females are generally larger than males because of a size-fecundity relationship (Honek, 1993, Teder and Tammaru**,** 2005**)** which is considered to be produced by natural selection (Fairbairn, 1997). However, larger body size may also be attained by genetic architecture, such as in insects with larger eggs in one sex (Blanckenhorn et al., 2007). Or produced by growing in a favorable environment (Esperk, et al. 2007).

Independently of the proximate cause of sexual size dimorphism (SSD), the mating system of a species plays a key role in understanding the evolution and maintenance of sexual size dimorphism. It occurs because mating systems are the result of sexual selection (Emlen and Oring, 1977) and thus it is expected that SSD varies among mating systems. So, understanding why one sex is larger than the other also implies in describing its mating system. This includes all the activities that involves the male and the female sexual behaviors and set the arena where selection acts (Parker, 1970; Eberhard, 1996; Blanckenhorn, 2005; Arnqvist and Rowe, 2005). A mating system is classified accordingly to the number of partners males and females can copulate with during a determined period in a monogamous system characterized by a stable pair-bond between male and female. Other mating systems types are classified as polygamous, which is characterized by multiples partners and can be divided into: polygyny, when one male copulate with multiple females; polyandry, when the female copulates with multiple males; and polygynandry (or promiscuity), where males and females have multiple pairs (Emlen and Oring 1977; Shuster and Wade 2003; Shuker and Simmons, 2014).

When describing a mating system, in addition to the number of partners during the reproductive period, it is important to identify the behaviors that occur before, during, and after the copulation (Parker 1971; Danielsson, 2001; Arnqvist and Rowe 2002). The pre-copulatory behaviors include: the courtship, the actively search for mates, the establishment of territories or resources (such as gifts), competing with members of one's own sex (usually male-male intrasexual competition) for the access to the chooser sex, and the struggle for the copulation per se (sexual coercion in intersexual selection) (Clutton-Brock and Parker, 1995; Arnqvist and

Rowe, 2005). During mating, the copulation duration affects the number of sperm or seminal fluid volume transferred or even the size of the spermatophore and thus it influences the probability of paternity (Parker, 1993). Also, courtship movements, such as the amount of abdomen tapping or the size of gift-prey, can also bias the fertilization success (Spieth, 1952; Wolfner, 1997; Parker and Simmons, 2000; Klein et al., 2013). During copulation we can also study how the internal and external organs fit each other, which contributes to the understanding of the mating system (Mattei et al. 2015). This occurs because seminal fluids can modify and influence the reproductive tract of females, as well as the duration of the refractory period, which reflects in the parameters associated with copulation patterns (such as frequency of mating). The end of the copulation (or the post copulation period) occurs with the dislodgement of the external genitalia. In polygamous systems this links to the success in fertilization, another important aspect that configures a very important part of the individual fitness (Parker 1970; Birkhead and Moller 1998; Simmons 2019; García González 2008).

Some species can copulate with more than one partner (Parker, 1970). This implies that selection can also operate after copulation (Parker, 1970). The evolution of this promiscuos mating system is linked to a direct gain in fitness related to an increased lifetime offspring production (Arnqvist & Nilsson, 2000). However, multiple mating also has negative effects on female longevity (Chapman et al 1995) which implies there should be an optimal female mating rate (Arnqvist & Nilsson, 2000). Called as refractory period, the interval between copulations is almost species-specific considering the existing time variation (Arnqvist and Nilsson, 2000). Female remating can be associated with a direct gain from sperm nutrition and male resources, or with indirect gain by increasing the genetic variation of the offspring (Ridley 1988). Another post copulatory behavior strategy is the mating guarding, which is when the male remains attached to the female for a period of time that exceeds the necessary time to sperm transfer. This behavior limits the access of other males to the female and enhances the chances for the fertilization of the eggs since no other male can copulate with the guarded female (Parker, 1970; Alcock, 1994). Thus, the frequency and interval between copulations in the mating system of a given species depends on the limits of the trade-off that occurs between the number of matings and their effect on longevity.

In an evolutionary perspective of sexual selection, males invest less energy on gametes production and invest more in the number of matings. Consequently, the reproductive success for males is more variable but associated with an increasing number of matings (Trivers 1972). To obtain matings and thus rise the fertilization success, males can adopt some strategies like male-male fight; resource defense of food or territory (intrasexual selection), and sexual coercion (intersexual selection). All of these behaviors can select for larger bodies through sexual selection because larger males gain advantage in contests for access to females and, thus, achieve a higher reproductive success (Arnqvist and Nilsson 2000).

Sexual coercion is a behavior that allows males to bypass female mate choice (Clutton-Brock and Parker 1995). It is based on physical constraints and can occur through forced copulation, harassment, or intimidation and is expected to lead to high costs to the females (Clutton-Brock and Parker, 1995) which is translated in reduced female fitness through reduced lifetime fecundity and longevity (Chapman et al. 1995). In coercive mating, males use the physical strength of a larger body size to force the female to copulate, the female, in turn, resists these attempts, and these opposite forces was expected by the sexual conflict theory (Parker 1979; Arnqvist and Rowe, 2005). Female resistance behavior has been considered a response to male harm and thus behavior that reduces the mating rate (Blanckenhorn et al., 2002). However, resistance behavior can function as a mate choice if it selects males that can overcome female resistance or even a signal of lack of receptivity if it is not associated with reduced fitness costs imposed by male harm (Briceño and Eberhard, 2017).

Sarconesia chlorogaster Wiedmann 1830 (Diptera: Calliphoridae) is an endemic South American blowfly (Brazil, Argentina, Uruguay, Bolivia, Peru, and Chile) that has a research history linked to its forensic importance (Carvalho and Mello-Patiu 2008, Vairo et al. 2015, Lecheta et al. 2015). *Sarconesia chlorogaster* is sexually dimorphic either in life-history traits (Moura & Bonatto 1999, Lecheta & Moura, 2015) or in size-related traits (Flissak & Moura unp. manuscript). The sexual size dimorphism of *S. chlorogaster* is male biased (Mello 1972, Flissak & Moura unp. manuscript) and produced through differences in development time between males and females (Flissak & Moura unp. manuscript). As larger males are not the rule in insect sexual dimorphism (Darwin 1871; Fairbairn, 1997) and are linked to intrasexual selection (Anderson and Iwasa, 1996) this chapter studied the sexual size dimorphism from a sexual selection perspective.

In this chapter, our main objective is to fully describe the mating system of *S. chlorogaster* through morphological, behavioral and statistical data analysis. The internal morphology description allows us to access general characteristics of the reproductive organs and to do this we employ didactic draws and photographs. For describing the mating behavior, we outline experiments focusing on pre- during and post-copulation movements made by males and females and simultaneously on the acquisition of parameters that were linked to copulation.

The unity of all these pieces of observation helps us to understand and characterize for the first time the mating system of *S. chlorogaster* and answer our main question: **How is the male-biased SSD in *S. chlorogaster* linked to mating behavior and the opportunity for sexual selection?**

2.2 METHODOLOGY

The methodology described here was defined based on a series of tested pilot experiments with several male/female operational sex ratios, time of the observational period and populations.

2.2.1 Timeline of performed experiments:

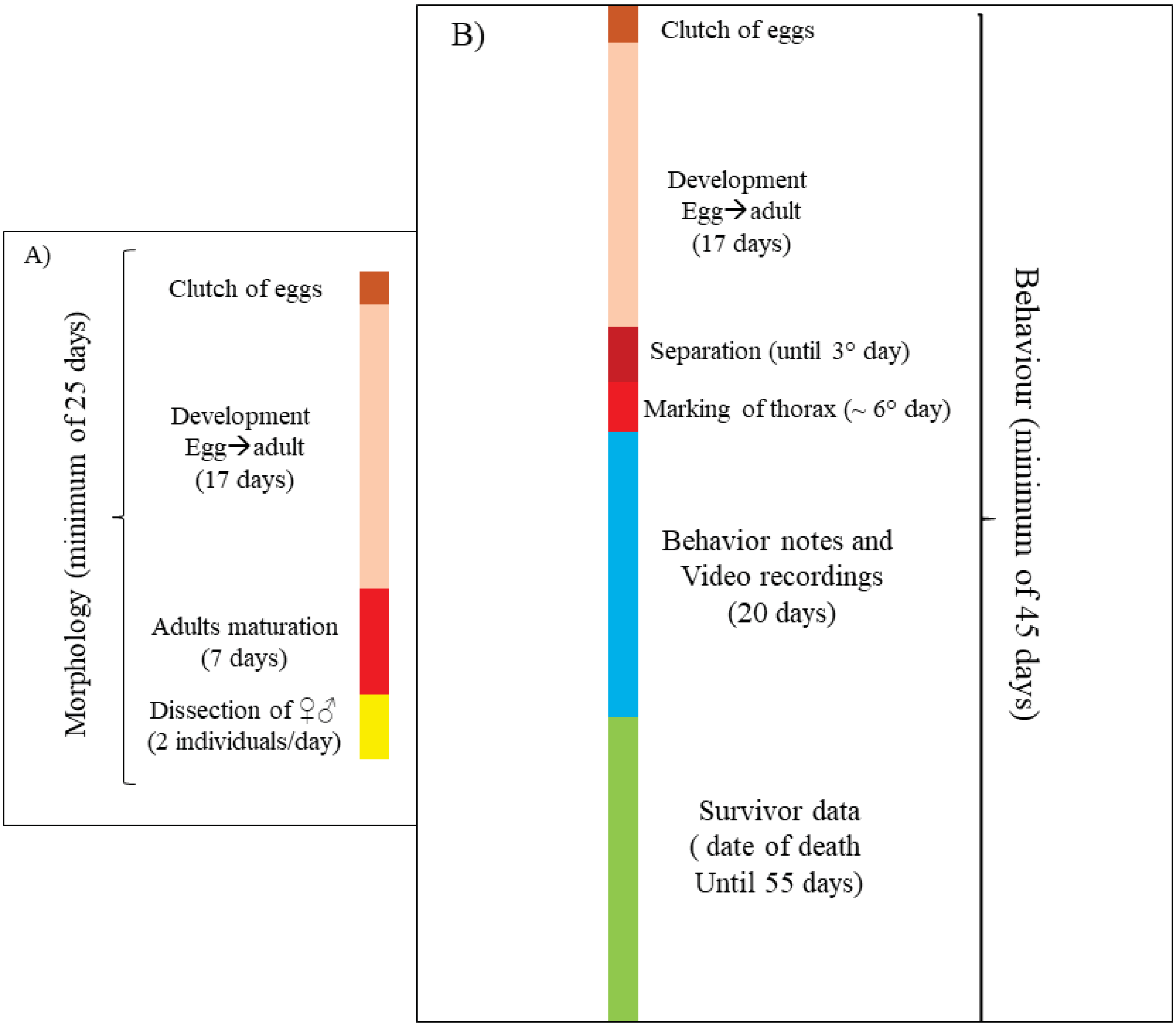

Figure 1. A chronological scheme showing the methodology employed. A) A scheme representing the main experimental steps for obtaining the individuals that were dissected. B) A scheme that shows the main experimental steps to access the mating behavior of *S. chlorogaster*.

2.2.2 Colony Establishment

The main colony of *S. chlorogaster* was established from wild adults collected in the city of Curitiba, state of Paraná, Southern Brazil (25° 25’ S and 49° 14’ W) on January of 2019, 2020 and 2021 (Summer). Also, comparative colonies were established from wild adults collected in the city of Chapecó, state of Santa Catarina, Brazil (27° 5′ 48″ S and 52° 37′ 7″ W), União da Vitória, state of Paraná, Brazil (26° 13' 48" S and 51° 05' 11" W) and Guarapuava, state of Paraná, Brazil (25° 23′ 37″ S and 51° 27′ 22″ W) on January of 2019 and 2020.All locations in Southern Brazil. However, the experiments and statistical analyzes contained in this document refer only to the data collected from adults in the population of Curitiba, no results were obtained from the other three populations.

The adults were actively collected with the aid of a Falcon tube, attracted by a mixture of powdered milk and sugar placed in a modified Van Somerem-Ryder trap. The collected adults were placed in a plastic cage and fed a diet composed of sucrose, powdered milk, and water *ad libitum*. A small portion of ground beef was placed on a culture dish, into the insect’s rearing cage, for the maturation of the ovaries and obtain of the oviposition. The eggs laid for the females were transferred to a 500-ml plastic container with a semisynthetic diet based on bovine stomach (Estrada 2009) provided *ad libitum* for larval development. The containers with the immatures were placed within a larger (1000 ml) container with vermiculite as a substrate for pupariation and placed in incubators adjusted to 25°C (±1) and relative humidity + −70% under constant conditions, and 12:12 h photoperiod (L: D cycle). The next generations from this couple formed the colony that was maintained under constant temperature (25°C), humidity (60%), and 12:12 h photoperiod (L: D cycle), and provided all the individuals used in the behavior and morphological experiments.

2.2.3 Internal Morphology

We dissected flies from the clutch of eggs obtained from the main colony (Curitiba). They were obtained on several occasions along the four years of experiment (2018-2022). All individuals aged between 7 to 10 days after emergence (FIG 1A). At this age, the reproductive tracts are mature and the organs are fully developed (Spradbery and Sands, 1976; Avancini and Prado, 1986). All the individuals were first euthanized in freezer at -4 °C for 60 minutes. After this they were individually dissected under a stereomicroscope. This procedure initiates with the pin of the individual in ventral position. The pin was positioned in the thoracic region to avoid damage to the abdominal organs (FIG 2A) and the ventral abdominal sclerites were removed with the aid of pointed and hard forceps and hypodermic needles. This method was utilized in both sexes. After complete removal, the reproductive tracts were placed in an excavated microscope slide with a drop of glycerin and immediately photographed with the aid of a Zeiss Microscope equipped with an AxioCam. The images were processed with Zen 2011 software and posteriorly edited in the Adobe Photoshop software (FIG 3 and 4).

2.2.4 Preparing Adult flies – Mating Experiments

To describe the mating behavior, eggs from the main colony were separated in different months throughout 2021 (that is, different generations) and reared in the same conditions as described in section 2.1. Three days after the adult's emergence, the individuals were identified by sex (FIG 2B) and placed in separate cages to ensure that all individuals used in the experiment remained virgins. After that, the females received a protein source (bovine meat) from the fourth day after emergence for ovary maturation (Thomas 1993). To enable individual discrimination and to identify which individual is performing the observed behavior we marked males and females with colored points in the thorax (FIG 2C). Both sexes were marked from five days after the emergence to at least one day before the experiment. For marking, we used water-based paint (gouache) and syringes without the needle (because of the rounded tip). All flies were frozen for at least five minutes to allow manipulation. This period of time is adequate for *Sarconesia chlorogaster* as it depletes individual's activity without providing any harm (Lecheta & Moura, unp. Data). There was no noticeable effect of individual marks on survivorship or individual behavior during the experiments.

This experiment had the aim to gather information about the mating system of *Sarconesia chlorogaster* including 1. the behavioral repertoire of pre, during and post copulation of both sexes; 2. Strategies of resistance (females) and persistence (males) employed during matings; 3. The mean time of mating (duration of copulation in minutes); 4. Number of copulations (♀ ♂) in each day and during all the experiment for each individual; 5. Time until the first copulation in the cage and 6. The effect of size on mating behaviors.

Experiment per se 5:5 operational sex ratio**:** After six days of emergence, five males (red, blue, white, yellow and green) and five females (light blue, light green, pink, orange and light pink), were randomly chosen and introduced into a modified insect rearing cage (Mating arena, L 30cm x W 30cm x H 30cm) with one transparent side and observed for the next 20 days, between 08:00 am - 18:00 pm (FIG 2D). During observations the following variables were scored: Start/End of copulation; presence of behavioral resistance (♀) and persistence (♂) for each mating (when possible); extra male interference; Size of each couple (posteriorly). Observations were made with groups formed by ten adults: five males (red, blue, white, yellow and green) (FIG 2C) and five females (light blue, light green, pink, orange and light pink).

We observe a total of 450 matings and 550 individuals through the experiments.

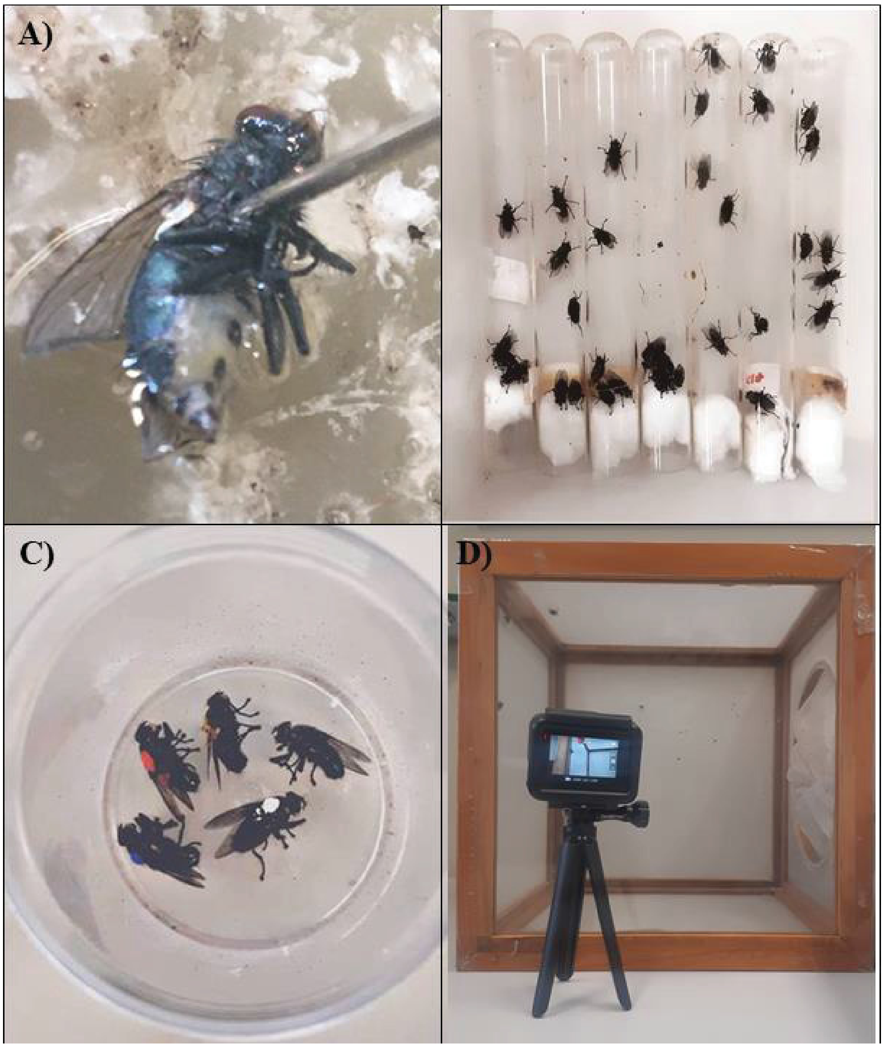

Figure 2. A) Sample of *Sarconesia chlorogaster* (♀) with central pin and the abdominal plates facing upwards, the position which facilitates its removal. B) Individualized males that will be marked. C) Five marked males. D) Arena (modified insect rearing cage, see methodology) settings to record the mating behavior of *Sarconesia chlorogaster*. Also, in D we have a positioned GoPro camera to recording the all behaviors in the cage.

2.2.5 Video Recording

For an accurate description of the reproductive behavior and construction of the ethogram (FIG 6 and 7) 10 groups were recorded using a GoPro Hero 5 Black camera. The recordings began upon the introduction of all males and females into the mating arena and ended after successful copulation or after 45 minutes of recording. The Ethogram (FIG 6 and 7) was constructed using more than 500 minutes of video recording analyzed in slow motion.

2.3 RESULTS

2.3.1 Internal Morphology

Female Reproductive System: The female reproductive tract of *S. chlorogaster* follows the general pattern of Calliphoridade and includes two paired *ovaries* (o*v*), that contains polytrophic *ovarioles* (*ovl*) of tapering shape. Each group of ovarioles converge to the corresponding lateral oviducts (lo), that, in turn, fuses in a unique c*ommon oviduct* (co). That unique oviduct opens posteriorly in a *genital chamber* (g*c*) that receives also one pair of long tubular *accessory glands* (ag) and three spiral *spermathecal ducts* (d) with sinuous sclerotized *spermathecae capsule* (*spt*) in his distal end (Figure 3A, 4A). In *S. chlorogaster* the accessory glands are a long tubular structure expanding from the proximal part and increasing in diameter until a distal part ending with a terminal swelling. The accessory glands are positioned in the anterior dorsal region of the genital chamber. The spermathecal ducts are slender, long, spiral and are also positioned in the anterior-dorsal region of the genital chamber. The distal portion of the ducts end up in the spermatheca, which has an unusual capsule shape for Calliphoridae (Figure 4B). The base has the tube form but changes from a transparent to a brown chitinized aspect, expanding into a sack like shape (which resembles a stomach), shortly after this chitinized structure changes to a spiral shape with two laps and again expand to a non-symmetrical inverted triangle, or briefly a sinuous shape.

Male Reproductive System**:** The male reproductive tract of *S. chlorogaster* includes a pair of orange *testes* (*t*) of “stomach” shape, all surrounded by tracheoles, with each testes connected in its posterior end to the paired *vas deferens* (*vd*). The vas deferens is very thin, short in length, transparent and difficult to observe and differentiate from the tracheoles, the paired vas fuse in a *medial ejaculatory duct (ed)* by a triangular *seminal vesicle* (*sv*). The seminal vesicle is connected laterally with the pair of *accessory glands* (*ag*) (Figure 3B). The accessory glands are tubular and enlarged with the same width until and its lumen is filled with an opaque substance. The ejaculatory duct is formed by a very long duct, twice the size of the testes, and has at its posterior end a sperm pump (sp). This organ consists of a sclerotized disc, an ejaculatory apodeme and an unsclerotized diverticulum with a valve system.

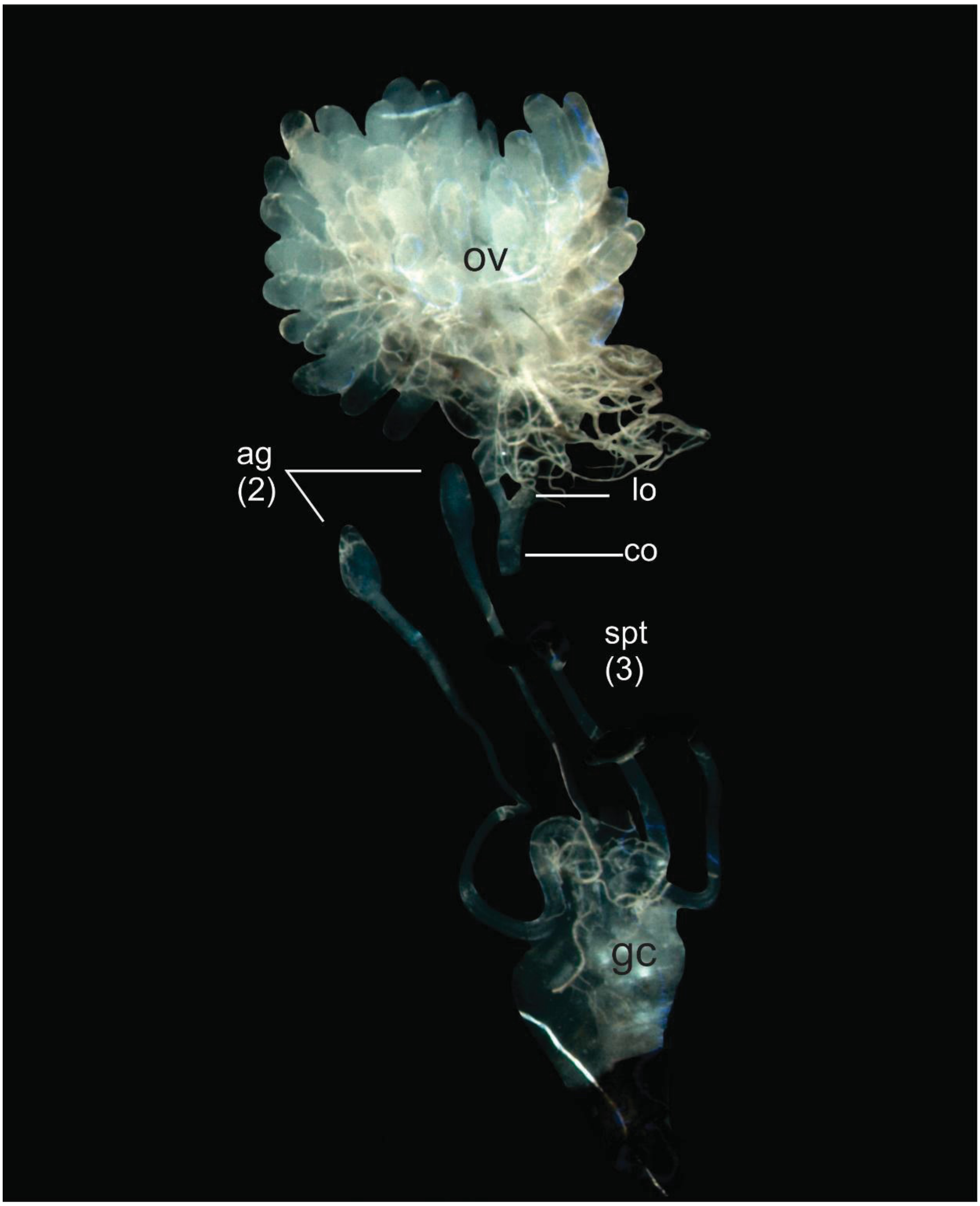

Figure 3. Female reproductive system of *Sarconesia chlorogaster*. ag: accessory glands; co: common oviduct; gc: genital chamber; lo: lateral oviduct; spt: espermathecae.

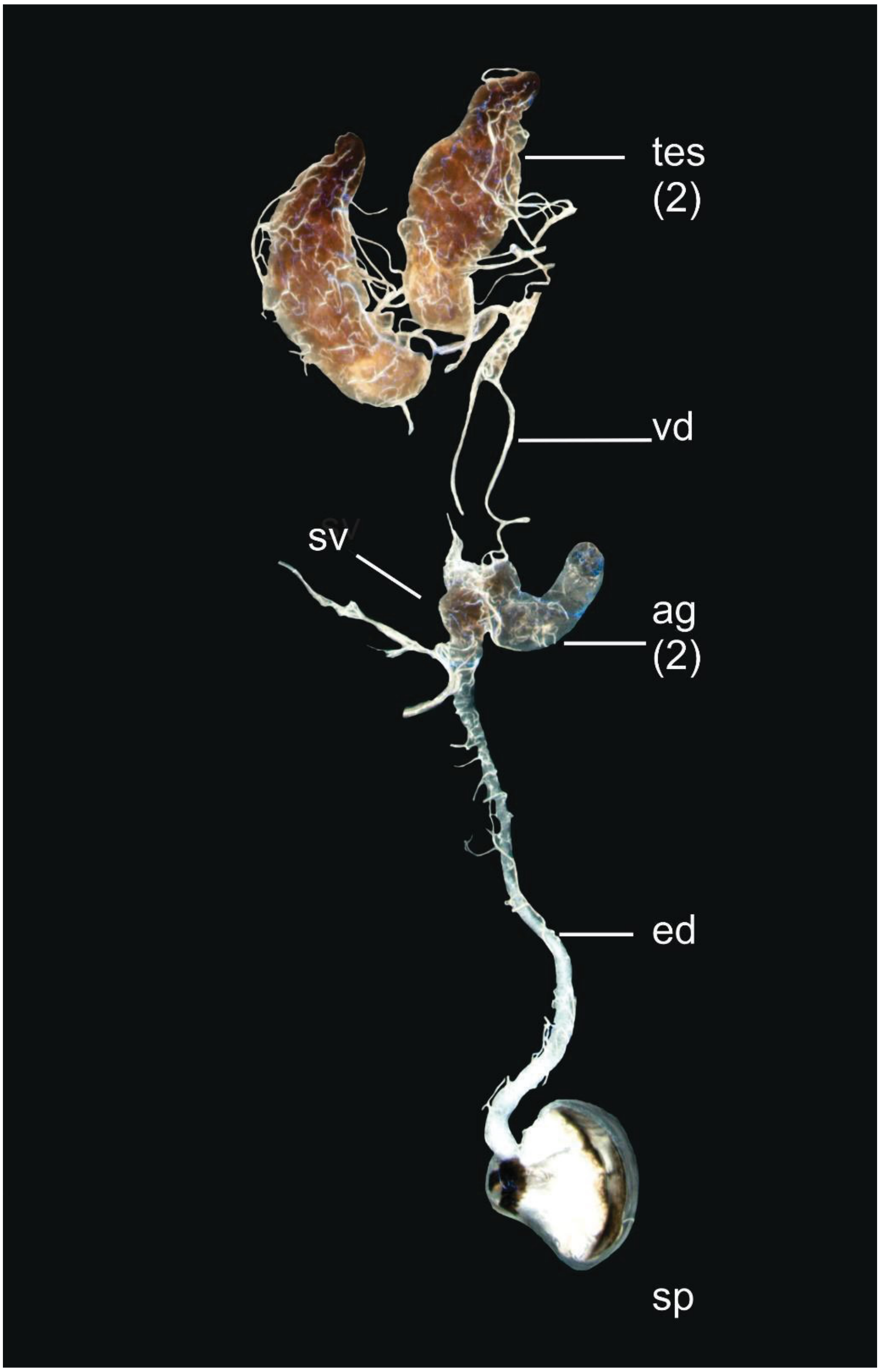

Figure 4. Male reproductive system of *Sarconesia chlorogaster*. ag: accessory glands; ed: ejaculatory ducts; sp: sperm pump; sv: seminal vesicle; tes: testicles; Vd: vas deferens.

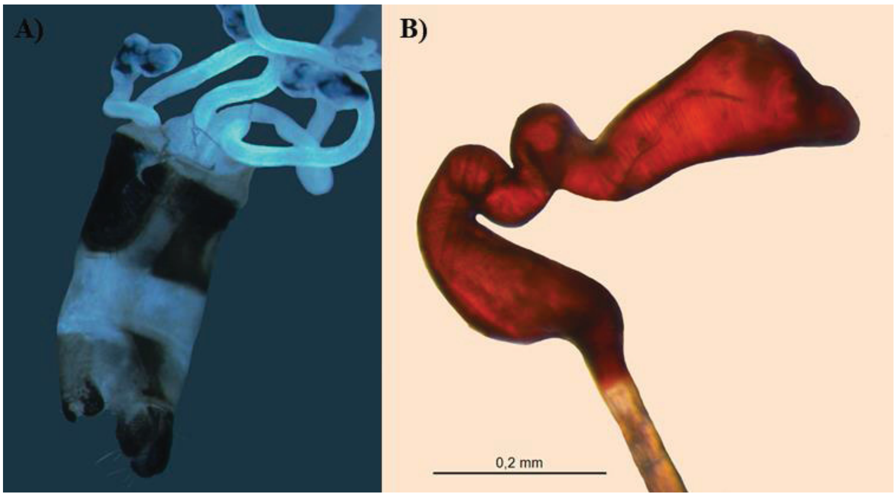

Figure 5. A) A lateral view of the female ovipositor with the ducts of spermathecae on the top. B) A close up of the sinuous spermatheca capsule.

2.3.2 Mating Behavior

List of Pre-copulatory behaviors: Walking; Standing; Grooming itself; Preening the appendages, head and the distal part of the abdomen; Kicking; Touching; Wing extension, Wing clapping; Wing flicking; Decamping and Immobile, performed by male and female and the last on performed only by male the Attempted Copulation.

Pre-copulation events

In general, there are similarities in behaviors displayed by sexes before mating, both usually preening the wings **(pw)**, legs **(pl)** and abdomen **(pa)**. Commonly, soon after the flies enter the mating arena, they start preening their hind legs **(phl)**, followed by the preening of their wings. Besides the preening of wings, we also observed that in both sexes adults clap their wings **(cw)** without using the hind legs. To perform the behavior, both wings move from the resting position horizontally, to the opposite sides and return. This movement was also observed with only one wing, in a unilateral clapping movement **(ucw)**. Both sexes also perform the preening of the abdomen using their hind legs **(pa)**. The movement for males and females is apparently the same: they use the hind legs to wrap the dorsal part of the abdomen and then slide, from the anterior to the posterior part of the body until reaching the genitalia. The difference between sexes is that males always have a bigger part of the genitalia exposed than the female because that the telescoped ovipositor will expose only its tip. The time individuals take before the first copulation is on average 50.67 minutes (± 47.53; n = 70). However, there is a huge variation in time to the first copulation, as the dispersion around the mean (SD) showed, meaning that the first copulation in the arena can occur as soon as the individuals are placed in the arena or even hours later.

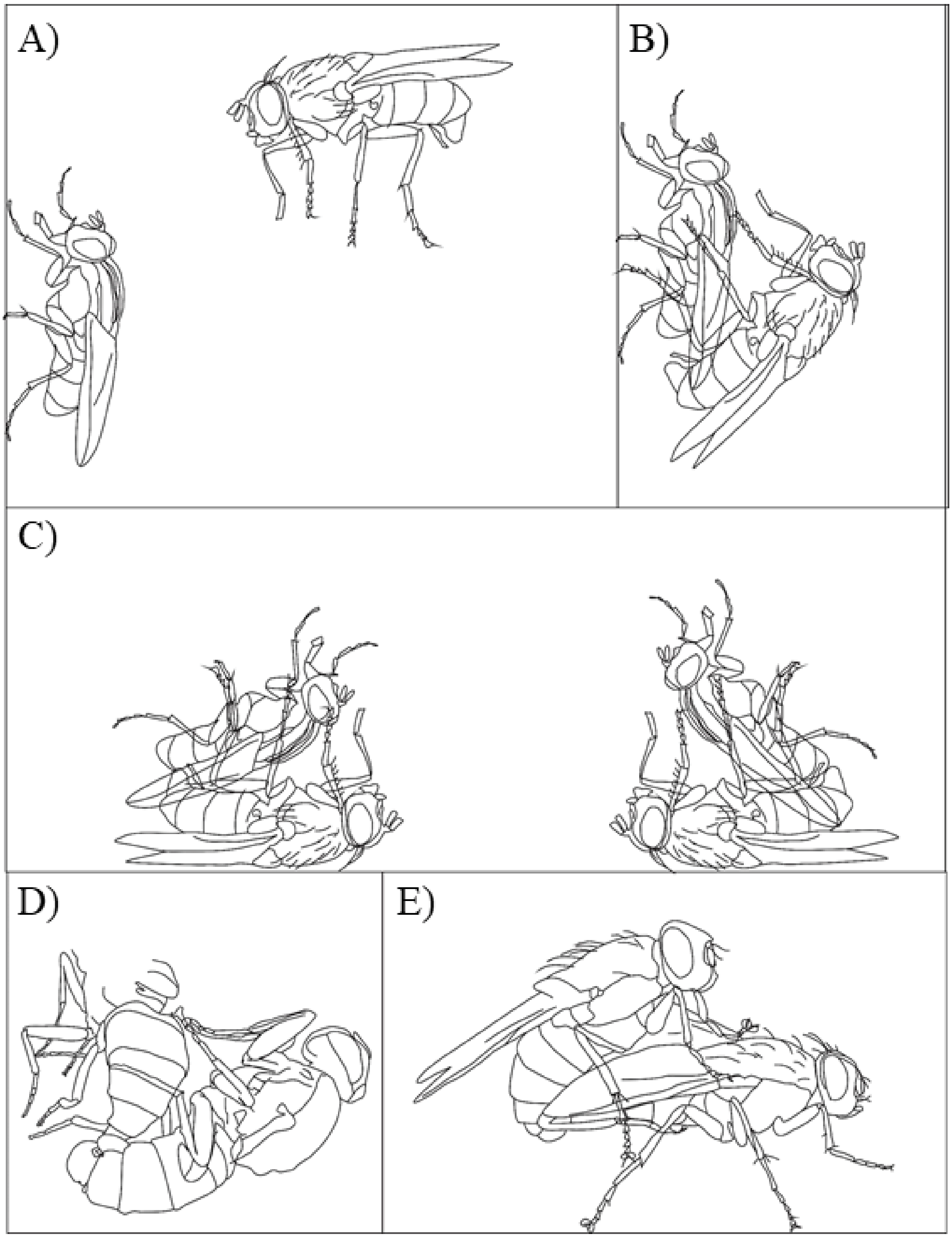

Figure 6. *Sarconesia chlorogaster* mating behavior. A) The male finds a female, B) The male makes an onslaught by mounting the female's back and tries to insert the sexual apparatus, C) Both (♀♂) fall to the floor of the arena and while the male continues attempting to insert his apparatus into the female ovipositor, she kicks the air trying to free herself, which can make both of them spin. D) focus of the external genitalia in contact and E) if the male has success, the couple ends up in the mounting position

Copula Attempted

This event occurs before copulation per se and is a crucial step to an unsuccessful or a successful mating. In *S. chlorogaster* the copulation is forced and the attempt to mating began with a sudden and rapid movement of lunge performed by males against the females, which in turn try to resist the attempt (FIG 6A). There are two types of lunges performed by the male. The first one is a slight struggle, where the male makes an onslaught by mounting in the female's back, trying to insert the sexual apparatus (FIG 6 B and C). The male holds the female's body with his legs, the front legs stay between the female head and thorax region, the middle legs wrap the female wings or the anterior part of the abdomen and the hind legs wrap the final part of the female abdomen. While holding the female the male introduces the sexual apparatus and the female resists attempting to dislodge the male by shaking the body laterally, walking and kicking (one at a time or all simultaneously) (FIG 6D). If a male can hold and introduce the sexual apparatus, the copulation initiates. In this type of behavior both are stand up (FIG 6D).

The second type of behavior involves an aggressive struggle. In this type of sexual behavior, the male initiates with an onslaught on the female which in turn resists somersaulting and rolling in the ground. The female’s movement results in twirls that can be intense or mild (which vary with the number and speed of the twirls). After that, the male attempts continue as in the slight struggle behavior, with the male trying to introduce the aedeagus. However, as the female resist vigorously to the attempt, the difficulty to start copulation increases because of the twirls. This type of resistance made by the female is more effective to dislodge the male than shaking, walking or kicking but did not prevent copulation if the male can persist in holding the female.

During Copulation

Once the genital organs are connected, the mating would occur and last an average time of 86.69 minutes (+ 44.95 min; n = 324, FIG 8). The mating in *S. chlorogaster* lasts a minimum of 25 minutes and the maximum observed time of 355 min. After the struggle in the attempt of copulation, the couple stands mounted with fewer movements than before the copulation. The following behaviors were observed (mainly for the females): Walking; standing immobile; grooming itself; flying; rubbing; kicking; jumping; somersaulting and twirling.

Throughout the copulation it is also common to observe a interference behavior performed by other males present in the arena, after the couple was stand up, a second, a third, and even a fourth male can climb on the couple and vigorously attempt to displace the copulating male.

This behavior was observed with 4 males at the same time (mounted in tandem) but is more commonly performed by one male at a time. In this situation the extra male or a take-over male can only mount the couple's back and try to dislodge the copulating male, mount and try to insert his genital apparatus on the female. It occurs while fighting with both male and female using the fore legs to kick both of them. This interference occurs sporadically during the copulation and rarely interrupted the copulation, which occurred only one time. The resistance behaviors performed by the female include: 1) kicking: the female kicks the external sexual apparatus of the male with his hind legs; 2) Jumping: the female walks, with the male mounted, for the sides or ceiling of the cage and jumping to the floor; 3) a somersaulting with twirls (See the section Attempt to Copulation). These resistance movements were observed during interactions performed for females during the copulation attempt, after the beginning, and also as a strategy to try to end copulation but none of them was capable of displacing the male or ending the copulation after it has started.

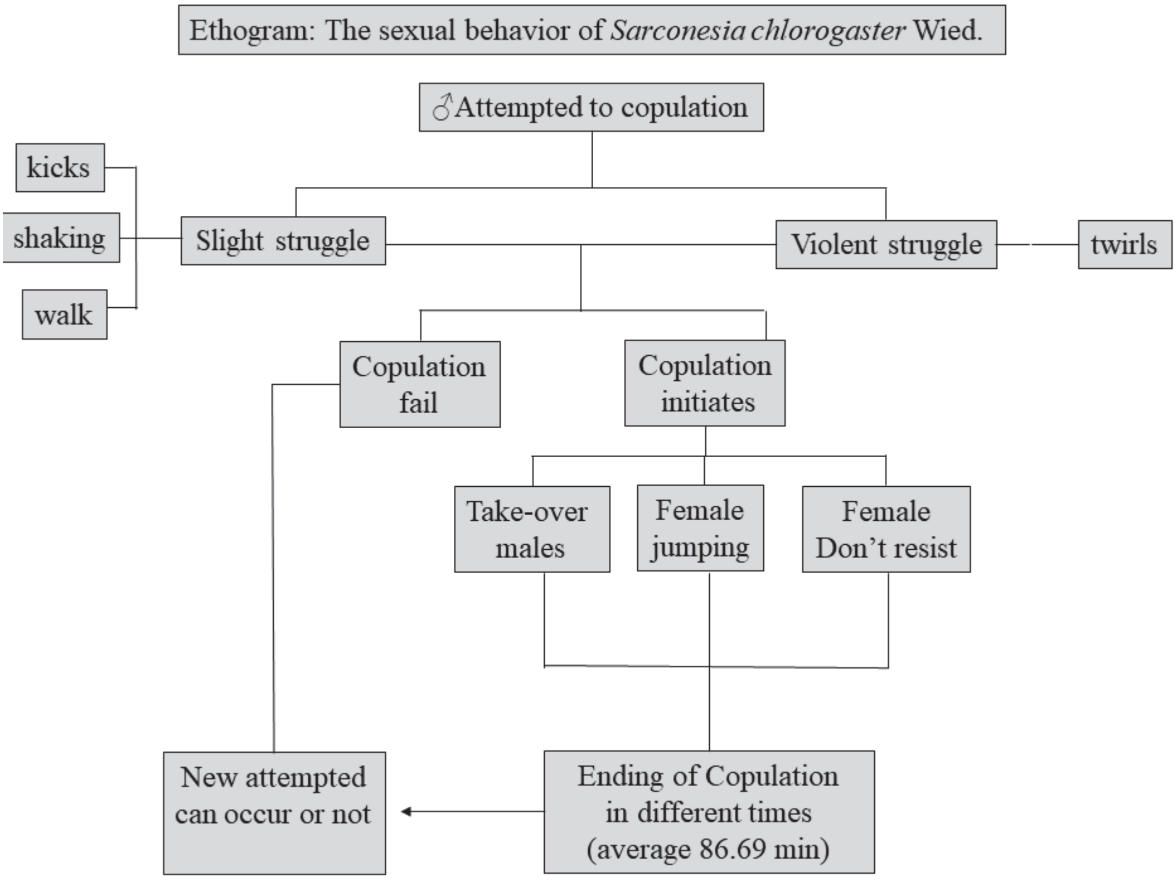

Figure 7. Ethogram of *Sarconesia chlorogaster*'s mating behavior, this graphic illustration demonstrates the main behaviors associated with the mating behavior of the species and the 2 main mating pathways according to the female's resistance intensity.

The movements involved in the end of mating are subtle, sometimes accompanied by a sound that results from the shaking of the female's body. The female also pushes back the male genitalia out with her hind legs and/or kicking.

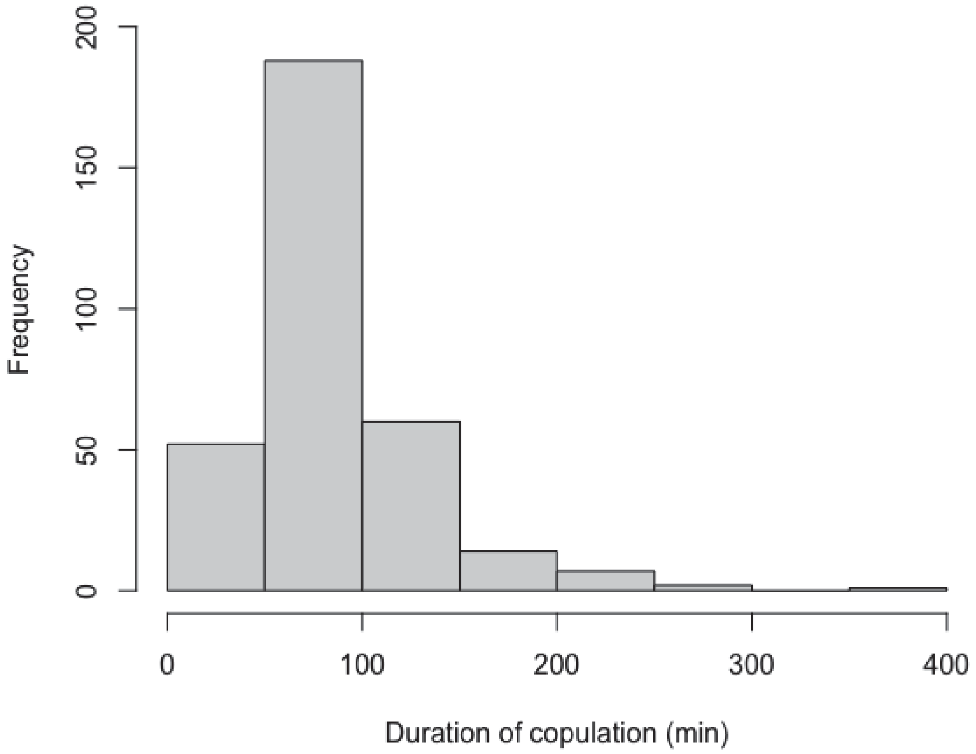

Figure 8. Frequency of the duration of copulation in minutes of *Sarconesia chlorogaster*. The histogram indicates that the highest frequency of copulations that occurred during this experiment last between 50 and 100 minutes.

Post-copulatory behavior

Immediately after mating, the mating pair performs the grooming of their genitals vigorously and both, male and female, can remate. The time between copulation varies. Generally, the males remate more often and on the same day than females. The maximum number of observed matings for males during the experiment was 32 times and the average number of copulations was 9.25 matings (± 7.78) while for females the maximum number of matings was 21 times (FIG 9) and the average mating number was 7.53 (± 5.14). On the same day males and females can copulate a maximum of six times (FIG 10, FIG 11), although it seems more common to males than for females (FIG 10 and 11). In *S. chlorogaster* the female has sperm storage organs, three spermathecas (See Morphology of the reproductive tract) and the mating is not followed by oviposition. The oviposition can occur days after the copulation and after multiple mating. The female produces around 200 eggs or more in each posture. This number varies, and we obtained a minimum of 15 and a maximum of 250 pupae from the females in the experiment.

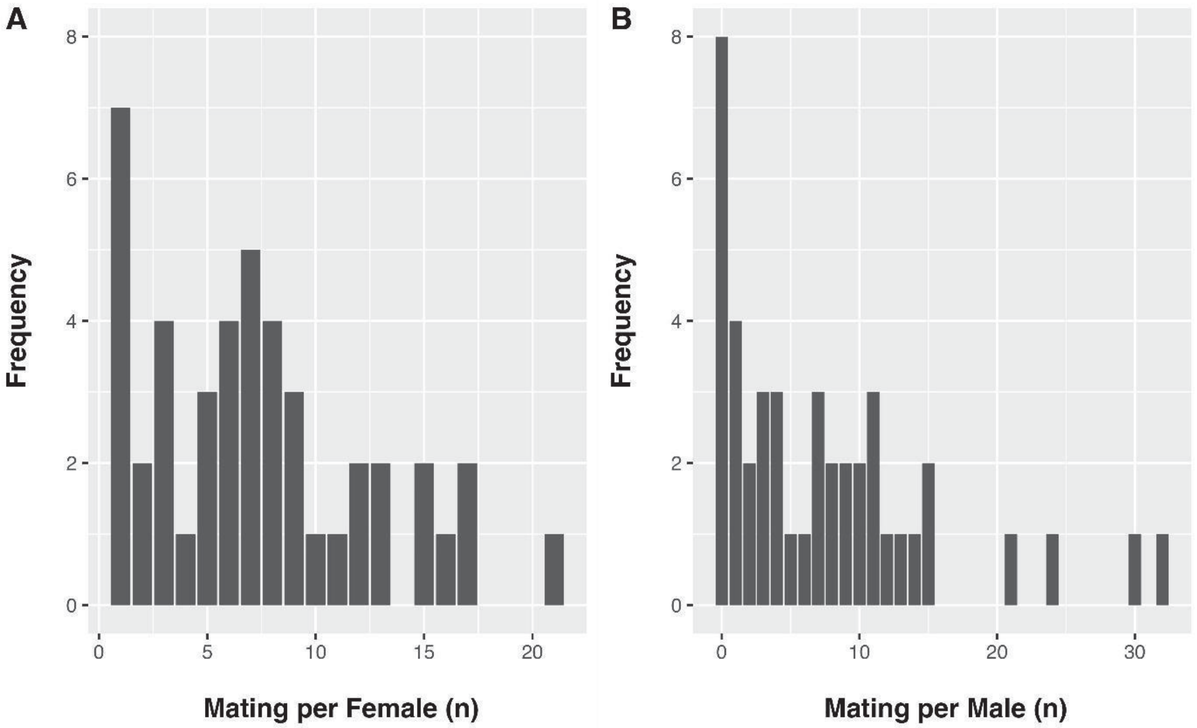

Figure 9. Frequency of copulations during the mating experiments (n = 331) for females (left) and males (right). The histogram shows that males are able to copulate more times than females during a reproductive season. Both sexes copulate with more frequency one-two times

Overall, each female mates with a maximum of four different males (Fig 12), although more frequently with two. On the other hand, males mated with a maximum of five females (Fig 12), more frequently with four females. Considering that the arena had 4 females and 4 males, and that the experiment lasted 20 days, males could copulate with all the available females more than once and females could avoid copulation from some males or were preferred by the same males in the same or subsequent days (fig 10 and 11).

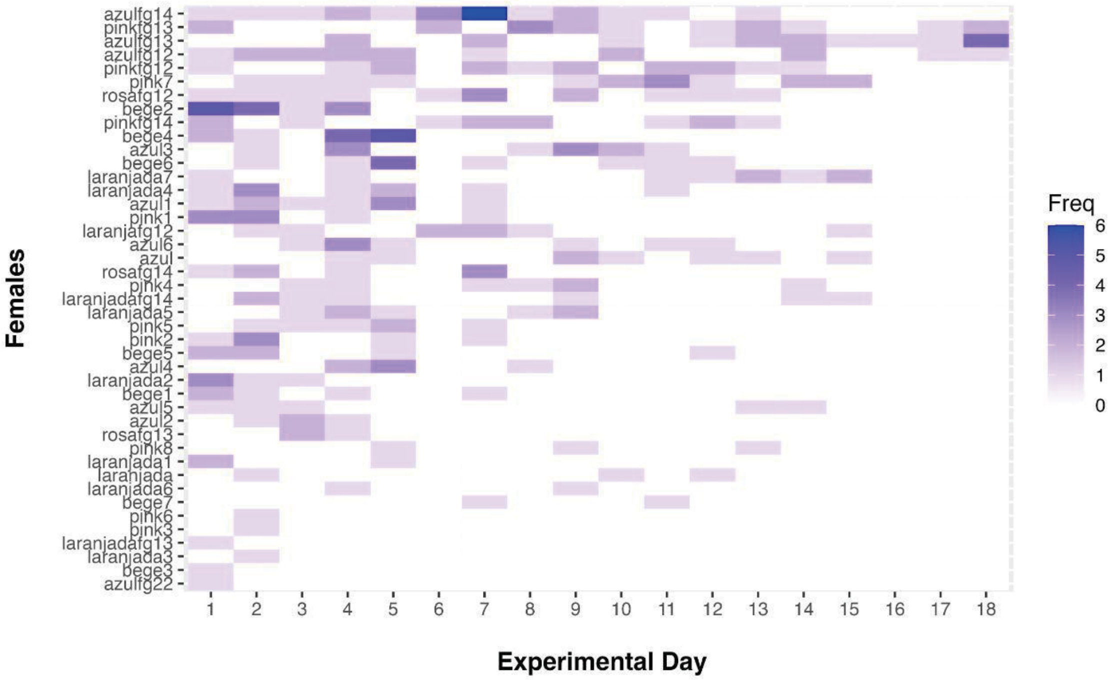

Figure 10. The number of copulations during the mating behavior experiment of *Sarconesia chlorogaster* performed during 18 days. The greater the intensity of the purple color, the greater the number of copulations in the same day. Focal females are displayed on x axis, while experimental days are shown on the y axis.

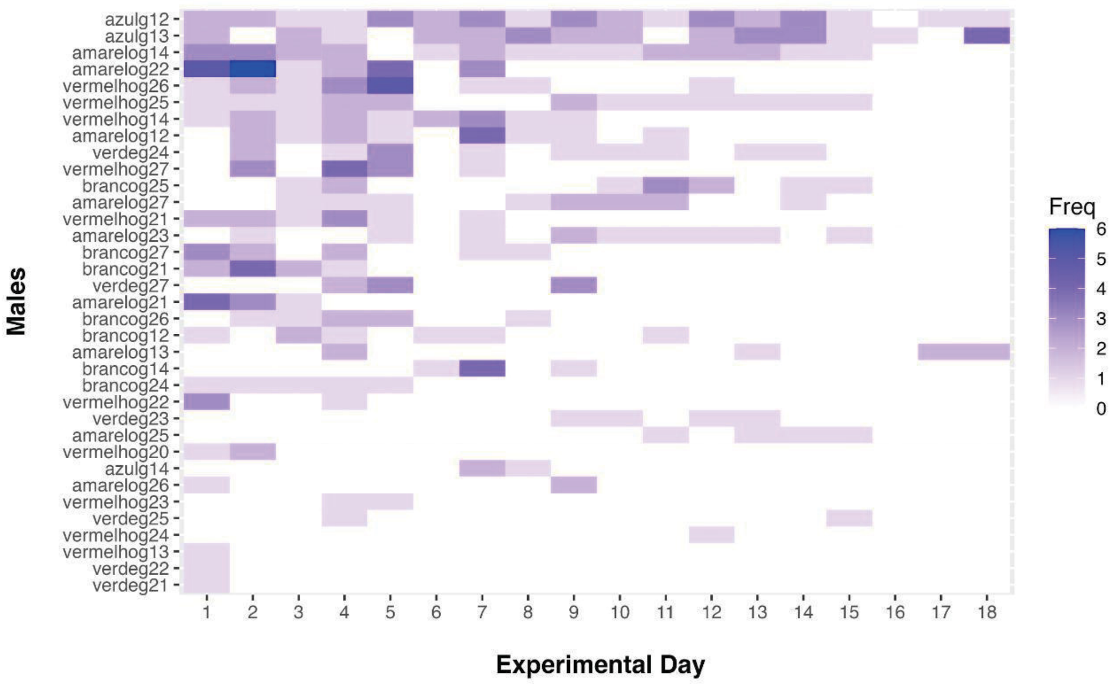

Figure 11. The number of copulations during the mating behavior experiment of *Sarconesia chlorogaster* performed during 18 days in June of 2022. The greater the intensity of the purple color, the greater the number of copulations in the same day. Focal males are displayed on x axis, while experimental days are shown on the y axis.

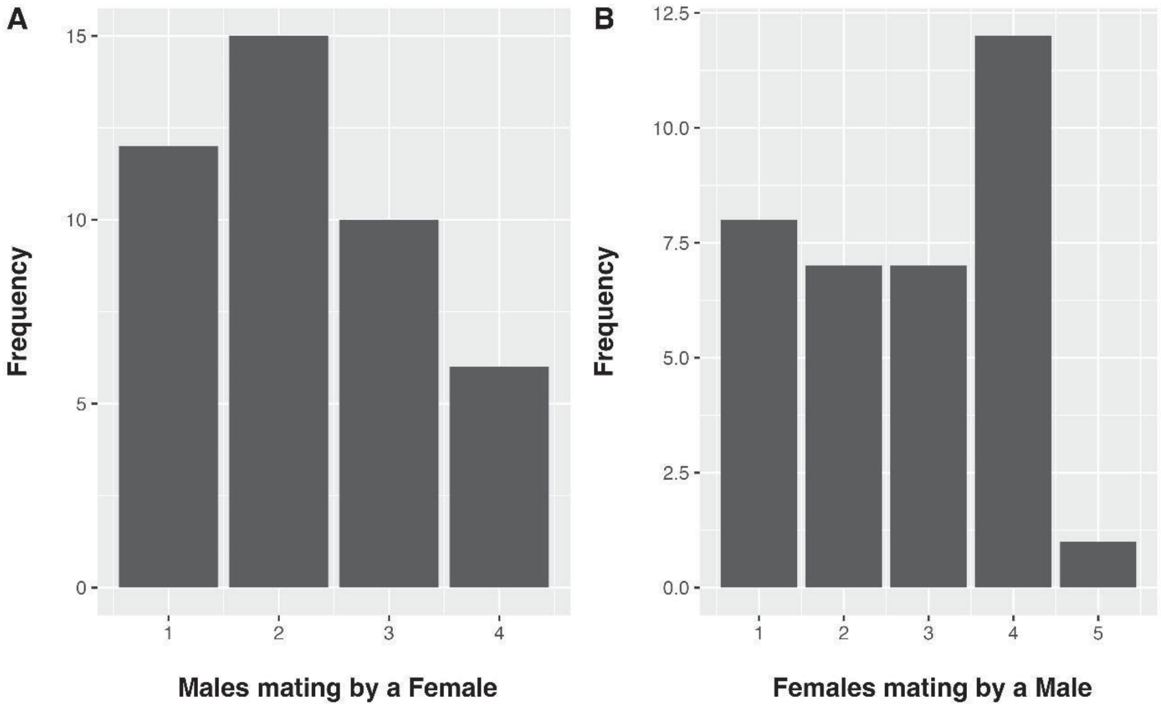

Figure 12. Frequency of mating with different males (A) and females (B) of *Sarconesia chlorogaster*.

2.4 DISCUSSION

Our analysis found that the the female reproductive tract in Calliphoridae comprises a pair of ovaries, lateral and common oviducts, 3 sinuous spermathecae with his own spiral ducts and a pair of accessory glands all that converges to the genital chamber and to the telescoped ovipositor. For males, the reproductive tract comprises a pair of testes, 2 thin vas deferens, tubular accessory glands, a triangular shape seminal vesicle and a slender and long ejaculatory duct with and ending sperm pump linked with the external genitalia. This is a basic morphological pattern previously described for other Calliphoridae (Snodgrass, 1993, Spradbery and Sands, 1976; Avancini and Prado 1986; Sukontason et al. 2009) and more specifically to *Chrysomya bezziana* Villeneuve 1914 (Calliphoridae) (Spradbery and Sands 1976); *Phormia regina* (Meigen, 1826) (Calliphoridae) (Merritt et al. 1994); *Chrysomya megacephala* (Calliphoridae) (Fabricius, 1794) (Sukontason et al. 2011); *Chrysomya megacephala* (Calliphoridae) (Name et al., 2010) *Lucilia cuprina* (Wiedemann, 1830)*, Lucilia eximia* Wiedemann, 1819*,* and *Lucilia peruviana* Robineau-Desvoidy, 1830 (Calliphoridae) (Name et al., 2012); *Chrysomya albiceps* (Wiedemann, 1819), *Chrysomya megacephala* (Fabricius, 1794) (Calliphoridae), *Cochliomyia macellaria* Fabricius, 1775 and *Cochliomyia hominivorax* Coquerel, 1858 (Calliphoridae) (Harterreiten-Souza and Pujol-Luz 2012) and now described also for *S. chlorogaster*. *Sarconesia chlorogaster* (Wied. 1830) had part of the genitalia described for both sexes, the female spermathecae and the male sperm pump (Mello, 1972). There were no morphological descriptions for the other reproductive tract organs or sexual behavior, however. Here, we describe for both sexes and in details the internal reproductive tracts of *S. chlorogaster*, mainly the non-chitinous. Some characteristics (spermatheca capsula) of the female reproductive tract are considered relevant in promiscuous system of mating and are associated with post copulatory selection such as the size, shape, number and disposition of the spermathecal capsules and the length of the spermathecal ducts (Otronen et al., 1997; Simmons et al., 1999; Hosken and Ward, 2000; Pitnick and Miller 2003).

The spermatheca capsule are frequently described on taxonomical studies of Diptera (Couri, 1987; Artigas, 1991; Couri, 2004; Vairo et al.2015; Harterreiten-Souza and Pujol-Luz, 2012) because of its taxonomic value (Theodor, 1976; McAlpine 1981). The most usual shape of spermatheca capsule among Diptera is rounded or subcylindrical (Harterreiten-Souza and Pujol-Luz, 2012). The unusual sinuous shape found in *S. chlorogaster* may be related with post copulatory strategies to control the fertilization process (Walker, 1980; Parker 1979). One of these strategies was the male sperm precedence, when the last or first male to transfer the sperm has the advantage in sire the eggs (Parker 1970). With this sinuous format the first male will fill the distal part of the capsule, and the constriction in the middle part can difficult the sperm displacement by any other male's sperm, decreasing the likelihood of fertilization. The shape of the capsule favors mixing sperms, with slender capsule shape favoring the last male sperm precedence (Schlager, 1960; Brower, 1975; Walker, 1980). As the sinuous shape with constrictions seems to hinder the movement of sperm contents, it favors the hypothesis of a first male precedence over the last male precedence. Also, in *S. chlorogaster* the capsule spermathecal and the ducts were fully filled with sperm since the first mating (personal observation), a behavior that reduces the chances of sperm displacement by subsequent males (Dybas and Dybas, 1981). The female of *S. chlorogaster* does not have a refractory period for the same day which increases the sperm competition due to the multiple matings which could select for these strategies and morphology.

The spermathecal ducts are less often described for Calliphoridae. Two of them are on the same side of the body and can either be two individual ducts ending in two separated capsules, or fused from the base with the two capsules involved for the same glandular tissue. The third duct always emerges from the other side of the body. The first arrangement occurs in the following Calliphoridae species, *C. macellaria* (Fabricius, 1775), *C. megacephala* (Fabricius, 1794), *C. hominivorax* (Coquerel, 1858) *C. albiceps* (Wiedemann, 1819) (Harterreiten-Souza and Pujol-Luz, 2012). The arrangement with three separated ducts is described for *L. cuprina*, *Phormia regina* (Clift and Mc Donald, 1973; Merrit et al., 1994) and *S. chlorogaster,* and can represent an increase in the control of the female in the allocation of sperm, allowing a posterior fertilization bias on the paternity (female choice control) (Eberhard, 1996; Miller and Pitnick 2003). In *S. chlorogaster* the spermathecal ducts are strongly spiral (convoluted) and it remains with this morphology even after being extracted from the female's body, which indicates a strong muscular tissue. This spiral arrangement makes the spermathecal duct lengthier which could increase the difficulty for males to fertilize a female, again enabling more female control over the fertilization process (Keller & Reeve, 1995; Eberhard, 1996; Miller and Pitnick, 2002; 2003). For example, in *D. melanogaster* an experiment selected for two different mean lengths of the seminal receptacle (primary storage organ) and sperm length found that the interaction between the male sperm and the female tract length was the most significant parameter that affect the fertilization success (Miller and Pitnick, 2002). The above considerations show the importance of studying the functional morphology of the reproductive tracts to understand the effects of sexual conflict on the behavior and morphology of males and females, especially in the game for copulation control performed by both sexes.

In males the pair of accessory glands in *S. chlorogaster* are thick, tubular and end in a rounded shape, which is very similar in morphology to *C. megacephala* (Sukontason et al., 2009); *Chrysomya bezziana* Villeneuve (Spradbery and Sands, 1976) but differs from *Lucilia eximia* and *L. cuprina,* that have slender and lengthier accessory glands (Clift and Mc Donald, 1973; Name et al., 2011). The accessory glands are involved in the production of seminal fluids and could contain proteins (SFPs), lipids, carbohydrates, salts, hormones, nucleic acids, and vitamins (Chapman et al., 1995). In general, the accessory glands and their content affect the reproductive behavior or/and the physiology of the female and are related to sexual conflict, increasing the chances of males to fertilizing the eggs or obtaining an advantage against other males in sperm competition (Leopold, 1976; Sharma et al, 2017). In *D. melanogaster* the seminal fluids affect various aspects of the reproduction of the female, the proteins present in the fluid can alter the refractory period, the egg production, reduce the female life span, gene expression and increase feed levels (Hollis et al. 2019). This range of possibilities makes the study of seminal gland proteins an important factor for understanding the mating system of species. As this is the first description of the mating system of *S. chlorogaster*, and despite not addressing the identification of the proteins contained in these glands, we can eliminate some of these functions by observing the behavior, mainly in the post-mating period. Observing the mating behavior of *S. chlorogaster* we found no refractory period for females, which suggests that the substances contained in the male accessory gland do not influence remating and consequently sperm competition is not reduced by this strategy. Furthermore, females do not oviposit immediately after mating, so the substance does not influence egg production or laying. However, we cannot exclude other functions of the gland's contents, like storage/utilization of sperm, altered gene expression and increased feeding (Frank et al., 2011; Sharma et al. 2017; Hollis et al., 2019).

The description of the sexual behavior of *S. chlorogaster* confirms that the mating system is of the promiscuous type (Figs 9, 10, 11 and 12), in which males and females copulate with different partners during the reproductive period (Emlen & Oring 1977; Shuker and Simmons, 2014). This mating type is very common in insects and among Diptera in particular (Shuster and Wade 2003; Yeates and Weigmann, 2005). The number of matings in *S. chlorogater* for males or females had (FIG 10 and 11), are bigger than other calliphorids such as *C. hominivorax*, which mate between 5 and 17 times (Crystal 1967; Baumhover 1966;

Bushland and Hopkins, 1951). However, the average number of copulations per day and the highest frequencies during the experiment is one or two matings (FIGS 9, 10, 11) which demonstrates a clear asymmetry in mating, a necessary condition for sexual selection to occur (Williams 1966; Kirkwood and Rose 1991).

The average duration of mating is a key component of the mating system, and it is under sexual selection because it affects reproductive success (Andersson, 1994). In species that remate, as in *Sarconesia chlorogaster*, the intensity of sperm competition is one of the factors that can affect the duration of copulation due to the variation in successful fertilization (Simmons, 2001). For *S. chlorogaster* this duration reaches the average of 86.69 minutes (+- 44.95 min; N: 324), a maximum of 355 and a minimum of 25 min (FIG 8). In Diptera, the average duration of copulation varies between taxa from seconds until several hours (Hieber and Cohen 1983). For example, *C. hominivorax* (Calliphoridae) has an average time of 1.6 to 3.8 (2.6 +- 0.1 min) minutes (Crystal et al.,1967), *Haematobia irritans* (L.) (Muscidae), between 0.5 to 5min (Bruce 1964), *Lucilia cuprina* (Wiedemann, 1830) (Calliphoridae) between 10 to 14.5 min (Brown, 1958), *Musca domestica* Linnaeus, 1758 (Muscidae), between 56 to 84 min (Chang, 1965); *Glossina palpalis* (Robineau-Desvoidy, 1830) (Muscidae) between 60 and 120 min (Jordan 1958). The advantages of the duration of copulation depend on the mating system. Coercive mating enables the larger sex to control the time expend in copula. In *S. chlorogaster* the male controls the copulation time, which remains longer than in other Calliphoridae species. This could be a strategy selected to cope with sperm competition. Overall, the longer the copulation time, the greater the number of transferred sperm, which would increase the male competitive ability (Parker et al. 1990; Parker 1993). Also, when the males continue copulating even after filling the storage organs of the female this prolonged copulation works as a mate-guarding strategy, an extreme behavior to prevent the number of competitors (Mazzi et al., 2009). *S. chlorogaster* seems to employ both strategies since the time to complete sperm transference is not as high as the copula time (personal observation). The next steps to understand the behaviors associated with the mating system in *S. chlorogaster* include quantifying how much each of these strategies influence the average copulation time.

The sexual behavior of *S. chlorogaster* in a general perspective is agressive. It includes mainly behaviors of female resistance and male persistence, which drives to a conflict interaction between the sexes. This violent struggle includes high-speed twirls, kicks and shaking while the male tries to achieve and maintain the copulation and consequently have the opportunity of fertilization. The rejection or resistance behavior performed by the female are species specific. In *Blaesoxipha stallengi* for example, all attempts performed by males result in a successful copulation, so there is no resistance. In *S. ruficornis* the females adopt two different strategies of rejection: fly away (to escape), or flap her wings while kicking with its forelegs. This movements are also observed for *S. chlorogaster* but does not seem to affect the attempts for copulation made by males. The males of *S. chlorogaster* adopt two attempted strategies, the violent one being the most common. In this strategy, males usually try to copulate through a strike against the female, which usually knocks her to the ground with the thorax on the floor (FIG 6). Then, the male grabs and holds the female while trying to insert the aedeagus, while the female resist with whirling movements that vary in intensity and speed. When the fight is established, the male tries to introduce the aedeagus while the female fights against the copula, kicking the male and their aedeagus. After a successful insertion of aedeagus, the couple stay at an angle of 45°, the same position observed for *B. stallengi* (Lahille) and *Sarcophaga ruficornis* (Fabricius) (Barbosa et al 2019). The resistance behavior of *S. chlorogaster* is similar to the described behavior for species of water strider (Rowe et al. 1994, Arnqvist 1997a, Watson et al. 1998). Males of *S. chlorogaster* does not have any structure capable of forcing the genitalia intromission, but male size limits the female's movement, which seems to fit in the indirect coercion by imposition (See Eberhard, 2002 for details). In addition, in Chapter 2, we present data demonstrating that copulation depends on the size of the male. Also, we show that female size influences copulation, and males chose females by its size.

The common repertoire of pre-copulation courtship behaviors (e.g., rapid flights and wing vibration) occurs in other species such as *Blaesoxipha stallengi* (Lahille, 1907) and *Sarcophaga ruficornis* (Fabricius, 1794) but was absent for *S. chlorogaster* (Barbosa et al. 2019). In *Drosophila melanogaster* (Meigen, 1830), the most studied specie among insects, the general behaviors of courtship comprise wing vibration, wing flicking, wing fluttering, tapping, wing waving, wing scissors movement, licking and circling the female (Spieth, 1952). Although some of these wing movements were observed in *S. chlorogaster*, none of them are directly related to the acquisition of mating. During copulation, the male was observed performing a stereotyped, rhythmic rubbing movement with his surstylus against the female's ovipositor ventrally. This movement can be a stimulant behavior, considered a pre-copulatory courtship (Briceño and Eberhard 2017), but in that case this function was not confirmed.

As the mating system of *S. chlorogaster* is indirect coercion by imposition, males are not expected to perform pre-copulatory courtship because the females will be forced to mate. In *Scatophaga stercoaria* (Linnaeus, 1758) a historical model system in sexual selection studies, the male grabs the female immediately after she arrives in the dropping of dung and does not perform a pre-copulatory courtship (Parker, 1970). The males fight each other for the females and after copulation, which lasts between 25 and 35 min (20ºC). They assume a passive behavior without genital contact while the female oviposits. In this phase, another male can take over the female and engage in a next copulation (Parker, 1970). It contrasts with the males of *S. chlorogaster* that do not initiate mating immediately in the presence of a female. On the other hand, they spend an average of 50.67 minutes (± 47.53; n = 70) before the first attempt to mate and have preferences for a female of a certain size (Chapter II). So, the male attempts and if successful the copulation takes on average 86.69 min. After the sperm transfer, the male continues with the genital contact and decides when the copulation finishes. During this phase, other males frequently try to separate the mating pair, which was rarely observed, i.e., take-over behavior performed by males in this species seems not effective. Although there are differences in the mating strategies employed by *S. chlorogaster* and *S. stercoraria*, it can be noticed that both species have no pre-copulatory courtship and that both of them coerce the female to mate.

2.5 CONCLUSION

- The blowfly *Sarconesia chlorogaster* mating system is of the promiscuous/polygynandry type (i.e., males and females could have several partners during the reproductive cycle);

- The mating is obtained by the male through indirect coercion by physical imposition;

- Males mate more times (n=32) during life than females (n=22), creating an opportunity for sexual selection to operate;

- Both males and females develop behavioral strategies of persistence and resistance which seem linked to minimizing the costs generated by the intrinsic sexual conflict;

- Sexual selection in this species may occur through sexual coercion, and this is probably the reason why males are larger than females;

- This type of qualitative (behavior and morphological) and quantitative (statistical) experiments and analyses allows not only access new information about behavior for *Sarconesia chlorogaster* but also support previous morphological descriptions from different taxa in insects and Diptera.

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CHAPTER II – DOMINANCE AND SIZE: COERCIVE MATING DYNAMICS IN *SARCONESIA CHLOROGASTER* INTERACTIONS

3.1 INTRODUCTION

In the field of biological sciences, a recurrent question is why are males and females different from each other (Darwin 1871). This is a complex question due to its involvement with major theories such as sexual selection, sexual conflict theory, the diversity of mating systems and the variations within taxa. These sexual differences can be associated and reflect, physiologically, behaviorally and morphologically, the production and meeting of egg and sperm (primary sexual characteristics), or with characteristics that are strictly associated with the acquisition of partners (secondary sexual characteristics). When a variation in a characteristic is linked to mating success, it is influenced by sexual selection, which may ultimately drive the evolution of sexual dimorphism (Wilkinson and Johns, 2005).

Sexual size dimorphism (SSD) (in body size) is one of the most common type of dimorphisms found in nature (Anderson 1994), and can be observed from extreme to moderate levels (Fairbairn 2007). From an evolutionary perspective, there are a few ways by which a larger body size can be selected. When females are larger than males, the larger body size could evolve through natural selection, because of a greater possibility of investment of resources in its progeny by a size–fecundity relationship. In parallel, it can be via sexual selection, where male or female size optima may evolve in response to: an advantage in acquiring mates via dispute, male-male competition; through female mate choice, when a certain male characteristic is preferred and selected by the female (Darwin, 1871; Honek, 1993; Fairbairn, 1997; Rohner et al. 2018, Jaenicke & Fromomntail, 2021), or by sexual coercion. In this last scenario, the copulation is forced and limits or even prevents the ability of the female to accept nor reject a copulation (Clutton-Brock and Parker, 1995). This occurs through modified characteristics, like grasping structures or a larger body (Arnqvist, 1989; Arnqvist Nilsson, 2000). Sexual coercion occurs in nature through three main forms: forced copulation, that includes physical restrains; harassment, when males make too many attempts of copulation, which has a greater cost to the female than to submit to copulation; and by intimidation, a strategy in which the male punishes the female, a behavioral strategy that increases the chances of a future mating with the same female (Clutton-Brock and Parker, 1995).

There is a general pattern among insects regarding body size. The female would tend to have the largest size compared to males due to the higher investment in the progeny, and also a greater fertility (Shine, 1989; Fairbairn, 1997). Female-biased SSD was indeed described for many flies, some examples are *Sepsis punctum* Fabricius, 1794; *Sepsis fulgens* Meigen, 1826;

Sepsis neocynipsea Melander & Spuler, 1917; *Musca domestica* Linnaeus, 1758; *Sepsis cynipsea* Linnaeus, 1758; *Drosophila rhopaloa* Bock & Wheeler, 1972; and *Drosophila melanogaster* (Meigen, 1830) (Rohner et al. 2018). Despite being less most common in insects, male-biased SSD occurs in several species (Puniamoorthy et al, 2012; Rohner et al. 2016), such as the flies *Drosophila prolongata* Singh & Gupta, 1977; *Scathophaga stercoraria* (Linnaeus, 1758); *Sepsis punctum* Fabricius, 1794; *Sepsis neocynipsea* Melander & Spuler, 1917; and *Sepsis lateralis* Wiedemann, 1830) (Rohner et al. 2018).

Previous studies have shown that larger male body size is a key factor in sexual selection, contributing to greater mating success and higher fitness (Borgia 1980; Thornhill 1980; Johnson 1982). This supports the hypothesis that sexual selection favors larger body size in males of ectothermic species (Rohner et al, 2018). In a male-biased operational sex ratio, larger body size is expected to be an adaptive trait for males in intrasexual competition for mates (Anderson, 1994; Parker, 1979; Fairbairn, 2007). Sexual selection operates both before and after copulation, with each stage selecting for different competitive traits. In pre-copulatory sexual selection, larger body size may be directly advantageous, enhancing a male's ability to compete in physical confrontations or to coerce females into mating (Parker, 1979; Anderson, 1994; Clutton-Brock and Parker, 1995, Gay et al., 2009). Post-copulatory selection may indirectly favor larger body size if it provides an advantage in sperm competition, possibly due to a correlation between body size and testes size (Puniamoorthy and Schafer, 2012). In all scenarios, higher mating rates of males could reduce female fitness (Costa et al, 2010).

The blowfly *Sarconesia chlorogaster* Wiedmann 1830 (Diptera: Calliphoridae) are endemic in South America (Brazil, Argentina, Uruguay, Bolivia, Peru, and Chile), they have forensic importance (Carvalho and Mello-Patiu 2008, Vairo et al. 2015, Lecheta et al. 2015), and were rearing for years under laboratory conditions (Bonatto 1996, Lecheta et al. 2015; Flissak and Moura 2018). Also, have a sexual size dimorphism biased for males (Flissak, 2023).

In this chapter, we aim to examine the impact of male body size on mating success within a competitive environment characterized by male-biased sex ratios. Our objective is to assess how variability in male body size can influence mating outcomes when compared to other males. Also, our goal is to evaluate the resulting fitness implications for females and the mating system as a whole. Specifically, we seek to determine whether an increased male body size confers advantages in mating success, as evidenced by mating frequency, copulation duration, and the number of mating partners. In the preceding chapter, we showed that mating duration is extended and that male size significantly influenced male-female interactions, often resulting in the imposition of mating (mating coercion). Furthermore, we ask whether larger male body size provides additional benefits, such as a selective advantage in choosing females of a particular body size. To address this hypothesis, we conducted behavioral experiments that quantified copulation duration, the frequency of mating events for both males and females, and the temporal changes in these parameters throughout the experimental period.

3.2 OBJECTIVES

To examine the effects of male-biased body size in *Sarconesia chlorogaster* and explore its potential links to a coercive mating system.

3.2.1 Specific Objectives

1. Does male body size significantly affect the copulation duration in *Sarconesia chlorogaster*?
2. Does the order of copulation affect copulation duration?
3. Is male size directly related to the size of the female that he copulates with?
4. Does the number of matings of males and females depend on body-size?
5. Does sexual conflict (female resistance) affect copulation duration?

3.3 MATERIAL AND METHODS

The flies were reared as described at Chapter 1- Section 2.1, Colony establishment and the experiments follow the described in Section 2.2- Preparing Adult flies – Mating Experiments and Experiment per-se 5:5.

3.3.1 Sampling and Statistical analysis

In all experiments the following parameters were recorded: the duration of each pairing success, and the thorax length of all individuals in the arena. Also, in some cases we can record the resistance event (female); the clutch size (if they occurred); if the males try to take-over a female and ending a copulation; the age of the individuals, the age they were submitted to the experiment, and the survivor. In the last and most embracing experiment, used for the analyzes described here, a total of 330 copulations were timed, the result of the interaction of 88 individuals distributed in 11 cages over the course of 2021, all experiments were carried out under the same laboratory conditions (average temperature ~25°C, same light position and cage type) . After the end of the experiment, all individuals, including those who did not copulate, were mounted and photographed with the aid of a digital microscope (Dino-Lite), the photos focused on the thorax region. Subsequently, each individual was measured using a straight line, starting at the prothorax and ending at the apex of the scutellum, the measured was made on pixels (using ImageJ software) and transformed in millimeters based on the photograph of a model scale. The thorax measurement is considered a good estimate of overall body size (Fairbairn 2007).

The data was curated on Excel and posteriorly transformed into a txt file to facilitate its processing in the R Core Team (2023). After confirming the existence of a male-biased SSD and observing that mating is forced (sexual coercion), we used the mating-pair size data to test for the role of male size and the copulation parameters (duration, number of copulations and resistance behavior). To better understand this scenario, we elaborated the following questions:

Question 1. Does male body size significantly affect copulation duration? And 1.2 Does the order of copulation affect its duration?

To test the relationship between body size and copulation duration, and whether the order of two or three consecutive matings affects the copulation duration we employed a linear mixed model (LMMs). For a better visualization of the model, body size and copulation duration data were log-transformed, and the random variable for the first question was the day of the experiment.

Question 2. Is male size related to the size of the female that he copulates with?

To analyze we applied a logistic regression with ordered variables. First, we ordered the body size measurements from both sexes and separated into 3 quartiles. For males we obtained these three categories of size and the respectively intervals; small (S; 0-3,865mm), medium (M; 3,865mm-4,11mm) and large (L; > 4,11mm), and for females small (S; 0-3,301mm), medium (M; 3,301mm-3, 931mm) and large (L; > 3,931mm). From this regression we obtained a table with the number of copulations between each of the categories (S, M and L), and later a graphic that demonstrates the relationships between each category of body size of the male and the category of females with which they copulated.

Question 3. Does the number of matings of males and females depend on body-size?

This analysis was made from the data set of body size and mating number fitted to a Generalized Linear Model (GLM), with error distribution of Poisson and weighted by the number of days that the male remained in copulation.

Question 4. Does female resistance affect mating duration?

For this question we employed LMMs and the random variable was the individual.

3.4 RESULTS

In *Sarconesia chlorogaster*, males had an average size of 3.90 mm (± 0.26 mm), while females averaged 3.62 mm (± 0.34 mm), confirming a moderate sexual size dimorphism (<10%) with a male-biased pattern (males > females).

The distribution of copulation duration exhibited a log-normal pattern, with primary peaks between 50 and 100 minutes, extending to 250 minutes and reaching a maximum of 350 minutes in one instance. The mean copulation duration was approximately 87 minutes (± 45 min), reflecting a pronounced skew in copulation times among males. Males that had copulated averaged a body size of 3.90 mm (± 0.26 mm), while copulating females averaged 3.62 mm (± 0.34 mm) with the frequency distribution of male body sizes concentrated between 3.6 mm and 4.3 mm. Male body size had a significant positive effect on copulation duration (LMM, $F_{1,\ 311} = 5.29$, $P = 0.01$), with a slope of 0.9 (log-transformed; 95% CI: 0.17–1.62) which means that the larger the male's body size, the longer the duration of copulation (Fig 13).

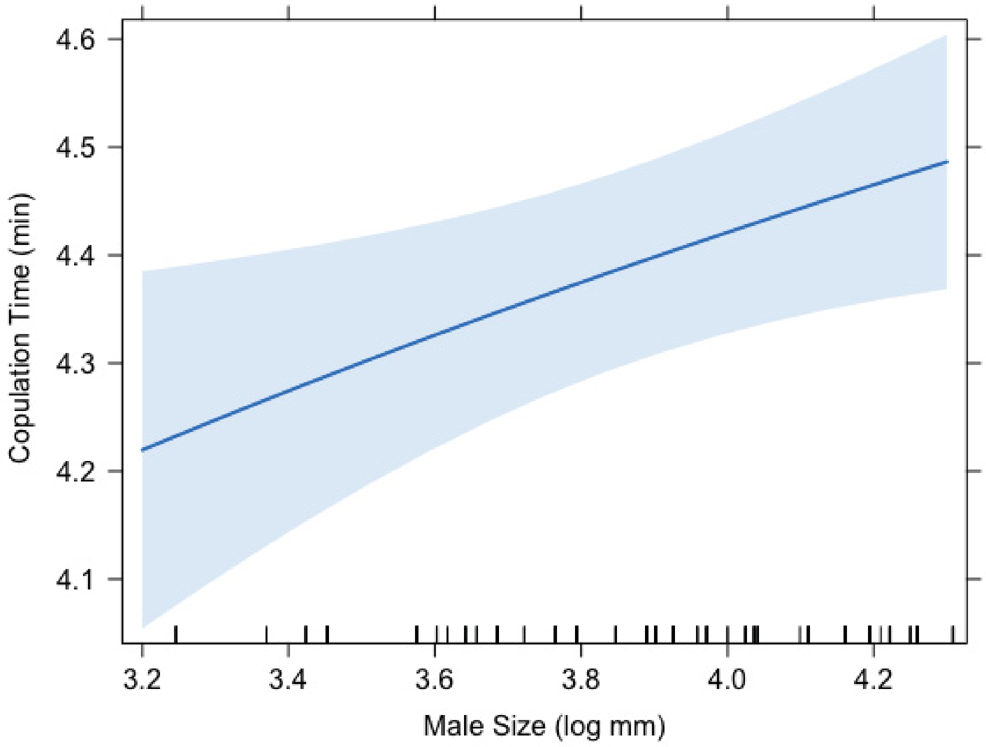

Figure 13. Relationship between copulation time (in minutes) and male body size of *Sarconesia chlorogaster*. Grey areas are the confidence bands.

The second question we explore focuses on the copulation duration and its relation to the order in which mating occurs. The duration of copulation may either increase or decrease when a male engages in multiple copulations within the same day. For three consecutive matings, we found that copulation duration significantly varies depending on the order of copulations ($F_{2,223}$ = 6.26, P = 0.002, Figure 14). On average, a male's first copulation tends to last longer (83.6 minutes, CI = 77.7 to 89.9) compared to the second (68.61 minutes, CI = 61.4 to 76.7, linear hypothesis test, difference = 0.19 ± 0.06, t = 2.96. P = 0.009) and third copulations (66 minutes, CI = 56 to 77.9, linear hypothesis test, difference = 0.23 ± 0.09, t = 2.58. P = 0.02). The duration of the second and third copulations does not differ significantly (linear hypothesis test, difference = 0.03 ± 0.10, t = 0.38 P = 0.92).

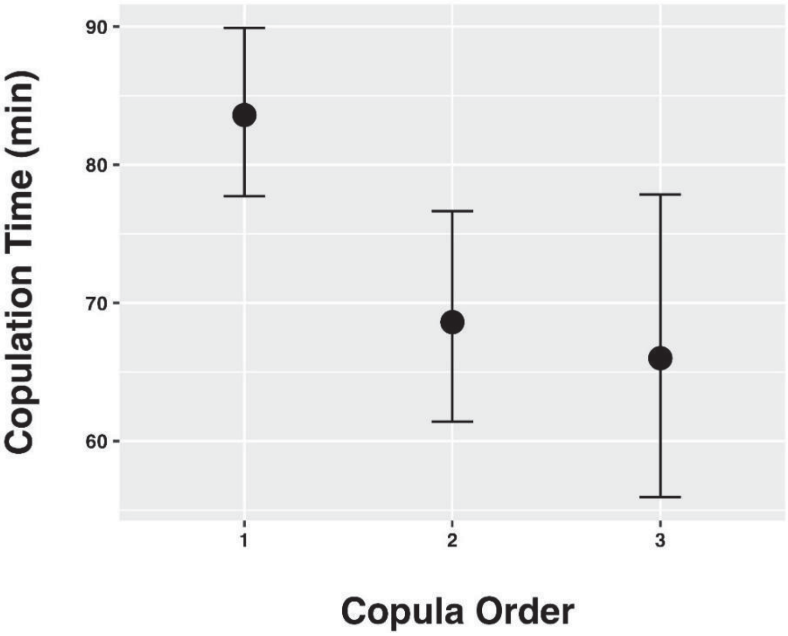

Figure 14. Comparison of copulation time and the order in which it occurred for males that copulated more than once in the same day.

Classifying males and females into size groups (see Methods) reveals a trend of size class matching between males and females during copulation (Table I, LRT - chi-square = 106.83, df = 2, P < 0.001). Overall, large males have a preference for copulating with large females when compared with small females (Table I, Fig 15). Medium males copulate with all the categories and the small males copulate only with small females, with some occurrence of mating with medium females (Table I, Fig 15).

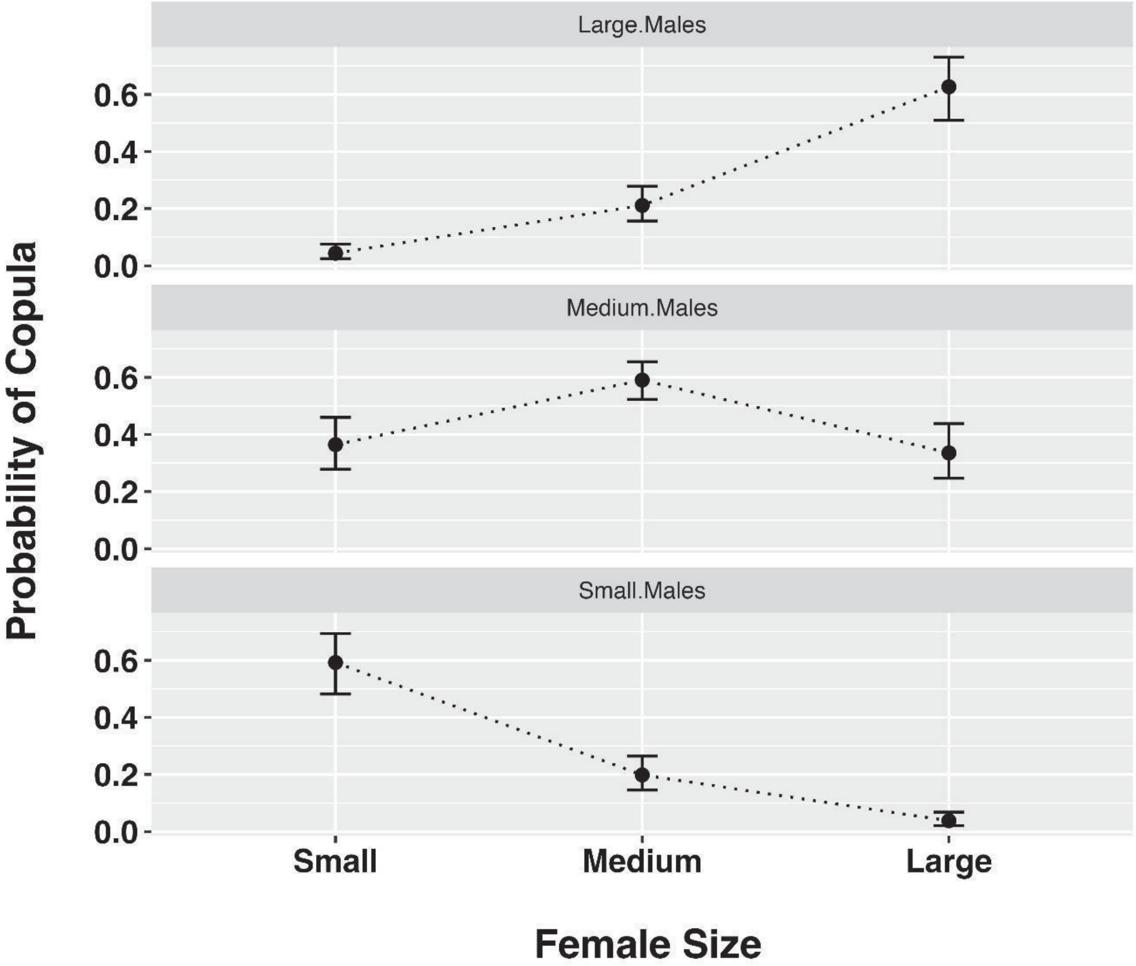

Figure 15. Predict probability (Mean ± SD) of males of different body size categories (Small, Medium, or Large) copulate with females of corresponding size categories (Small, Medium, or Large).

Considering the odds ratio of the ordinal logistic regression model, the probability of a large male to copulate with a medium female is 195% higher than the probability to copulate with a small female. Also, the probability of a large male to copulate with a large female is 748% higher than the probability to copulate with a small female.

Table I – The effect of the difference between the average male (Small: 0 - 3,865mm; Medium: 3,865mm - 4,11mm and Large :> 4,11mm) and female size (Small :0 - 3,301mm, Medium: 3, 301mm-3, 931mm; Large: > 3,931mm) on the total number of copulations observed during the experiments.

	Female Size Categories			
	S	M	L	Total
Male Size categories				
S	43	33	4	80
M	32	90	22	144
L	0	35	45	80
Total	75	158	71	304

Source: Author (2024)

The number of times males copulate are positively related to its body size ($\beta = 2.342 \pm 0.523$, $z = 4.479$, $P = 0.0001$) and negatively related to the female body size ($\beta = -2.5044 \pm 0.4260$, $z = -5.879$, $P = 0.0001$) and so the number of copulations increase with body size for males but, in contrast, decreases with female size (FIG. 16)

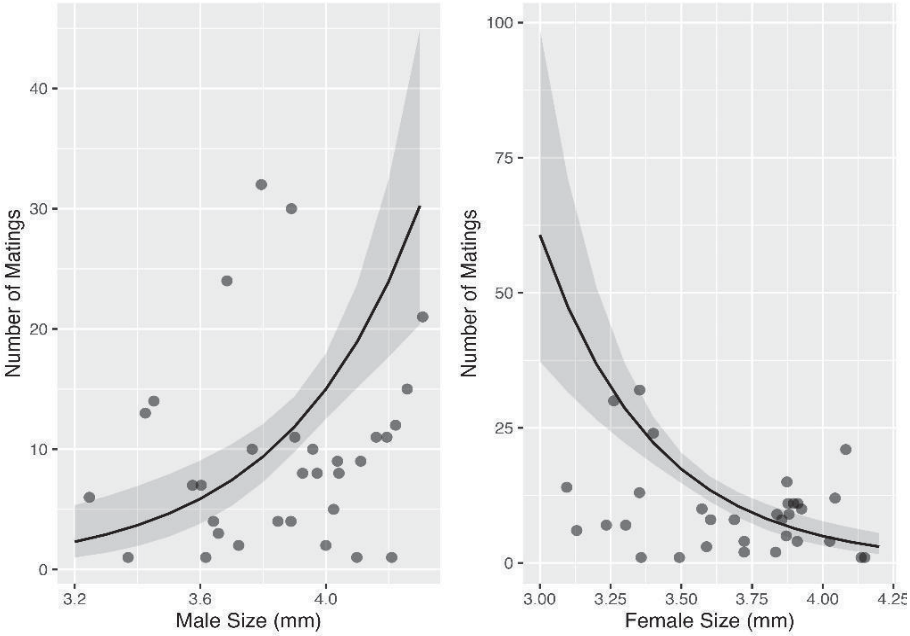

Figure 16. Number of matings and body size for male and females. In the left we can observe that for males the number of matings increases according to body size, the opposite happens with the curve that describes this relationship for females (on the right), the larger the female's body size, the smaller the number of copulations.

The duration of copulation also depends on female resistance ($F_{1,103}=10.48$, $P = 0.001$, $\beta = -19.87 \pm 6.14$). Notably, females who exhibited no resistance or displayed minimal resistance had longer copulation times (94.8 minutes, 95% CI = 83.3 to 106.3 minutes) compared to females who displayed a strong response (74.9 minutes, 95% CI = 66.5 to 83.4 minutes).

DISCUSSION

A variation in a given characteristic is subject to sexual selection pressures when it also reflects a variation in reproductive success (Darwin, 1871; Andersson, 1994; Clutton-Brock, 1988). In *S. chlorogaster*, a target characteristic for sexual selection is the male body-size. This occurs because larger male body-size affects copula duration, the frequency of copulations, and even provides to the larger male the ability to choose a certain category of female body-size. Additionally, in *S. chlorogaster*, the male-body size is, on average, 8.8% larger than that of the female. Such male-biased sexual size dimorphism, though moderate, is sufficient to give males control over various aspects of mating according to our results. This male size advantage provides opportunities for sexual selection both before mating (pre-mating) through coercive control of females, and during mating, by influencing the frequency and duration of copulation (Chapman, 2006). According to the theory of sexually antagonistic coevolution, females are expected to be under direct selection for traits that help them to avoid copulation (Chapman et al., 2003; Chapman, 2006; Arnqvist and Rowe, 2005). In *S. chlorogaster*, female size appears to be a trait that could regulate copulation and may be under selective pressure, reinforcing the hypothesis of sexual conflict. This also suggests that sexually antagonistic coevolution could be the underlying mechanism driving this sexual conflict (Chapman et al., 2003; Chapman, 2006; Arnqvist and Rowe, 2005), and further experiments are needed to test this hypothesis.

According to sexual conflict theory, males and females are in a continuous struggle for control over copulation. This is because their fitness optima often differ, which results in each sex adopting distinct strategies to maximize their reproductive success (Arnqvist and Rowe, 2005; Parker, 1979). As in *S. chlorogaster* the male size affects the control over mating (both pre-copulatory and copulatory stages), allows males to copulate more times and extend copulation time beyond what is necessary for sperm transfer this is expected to be a target trait for sexual selection. Female size, however, also plays a role because larger females copulated less than small and medium sized females and can resist males of different sizes. So, it seems that sexual selection, targeting male size, and natural selection, fecundity selection, could be occurring. Overall, results showed that in male biased sexual dimorphism, sexual selection is higher in males relative to females, suggesting and advantage toward bigger males (Janicke and Fromonteil, 2021). For instance, in *D. melanogaster*, smaller males were less successful in mating compared to larger males (De Nardo et al., 2021), similar to the pattern observed in *S.*

chlorogaster males. However, female size did not interact with male size in *D. melanogaster* (De Nardo et al., 2021), contrasting with our findings. Additionally, *D. melanogaster* females can bias sperm precedence by ejecting sperm from the first male in favor of a second, preferred male. Despite the similarity, the advantage due to the male larger size, the mating system differs, in *D. melanogaster* there is a female selection before copulation. The observed for *S. chlorogaster* is that the females did not seem to control mating by direct choice of larger males through assessment, but seems to be an indirect choice through a female resistance-male persistence game, as occurred in one of the possible mating strategies in *Sepsis cynipsea* (Blanckenhorn et al, 2001).

In the first chapter, we mentioned the strategies potentially associated with prolonged time of copulation. In *S. chlorogaster*, it is extended. This is not only in comparison to other Calliphoridae species, but also relative to the minimum time required to fill completely the spermatheca (empirical observation). If we compare copula durations among other well-studied Diptera species we found that for the sheep blowfly *Lucilia cuprina* (Wiedmann), the average copulation time remains around 11 minutes (at 27°C) (Smith et al., 1988, 1990; Cook, 1992; Cook, 1994). This is eight times shorter than the average time in *S. chlorogaster*. As mentioned earlier (Chapter I), this difference may confer advantages both in terms of competition with other males (sperm competition) and in potential female choice for males that engage in longer copulations (cryptic female choice) (Eberhard, 1996; Birkhead and Møller, 1998). In *Scathophaga stercoraria* (L.) for instance, a species that has historically importance in sexual conflict studies, males are also larger than females. This is similar to the pattern found for *S. chlorogaster.* However, in *S. stercoraria*, larger males copulate for shorter durations, and the number of copulations depends on testes size rather than overall body size (Parker, 1970; Simmons & Ward, 1991). In *Sarconesia chlorogaster*, this relationship is directly proportional—larger males have longer copulation time (Fig. 13). Usually, copula duration is largely explained by male sperm competition, in which males control the duration of copulation to optimize sperm transfer or mate guarding (Parker, 1970). However, as female *S. chlorogaster* also exercise control of copulas through resistance mechanisms it seems more probable that the copula duration is a product of male-female interaction, as occurs in *Calossobruchus maculatus* (Coleoptera, Eady and Brown, 2017, Pérez-Staples et al, 2010). Although female *S. chlorogaster* exerts some control of copula duration, the fact that sequential copulas of males have shorter duration could indicate that, at least to males, copula time is related to sperm transfer and mate guarding, common strategies in polyandrous species (Kelly and Jennions, 2011). The increase in the number of copulations plays a crucial role in intrasexual competition, as it directly impacts male fitness by enhancing the chances of fertilizing a greater number of females eggs (Bateman, 1948). In *S chlorogaster* we found that larger body-size is positively related with the number of copulations the male attains (FIG 16). For this species this number reached 35 copulations throughout the experiment period. The effect of male body size in reproductive success is well documented (Otronen, 1993; Sigurjónsdóttir and Snorrason, 1995; Harano et al. 2012; Miller & Svensson, 2014,). The same pattern was observed for *Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae)*, where there is a positively correlation between larger body size and mating success (Rodriguero et al. 2002), which implies this trait as a target for sexual selection. Although males in this species do not engage in direct combat for access to females, our data demonstrate that larger males have an advantage over smaller males to obtain mating (FIG 16), resulting in higher reproductive success.

In *S. chlorogaster*, larger males preferentially copulate with the largest available females (FIG 15). Body size affects various aspects of life history (Peters 1983), one of which is fecundity. The larger the female's body size, the greater the number of eggs available for fertilization, and the offspring tend to be of better quality compared to those of smaller females (Honek et al. 1993). So, the male choice for larger female body-size is expected since this trait affects the fecundity (Anderson, 1994) especially in a promiscuous scenery (Bonduriansky 2001). Despite this preference, in *S. chlorogaster* males obtain copulations by coercion, and smaller males cannot force mating with larger females (Table I). This is because this type of mating system depends on the physical strength of the males, and smaller males cannot overcome the resistance behavior of larger females. In this species, with our dataset, we find that the larger the female's body size, the fewer copulations occur (FIG 16). This is because there is a higher number of medium and small males in our samples, and medium or small males are not physically capable of holding and forcing copulation with larger females.

Collectively, our findings indicate that *Sarconesia chlorogaster* males exert control over mating dynamics by coercing females into copulation. Furthermore, our results suggest that the duration of copulation is likely associated with both sperm transfer efficiency and mate guarding behaviors. We observed that larger males copulate more frequently with larger females, despite the latter exhibiting resistance behaviors. Additionally, larger females exhibit a lower frequency of copulations compared to small or medium-sized females. Taken together, these observations lead us to hypothesize that male size is subject to sexual selection pressures, while female size may be influenced by both natural and sexual selection through an evolutionary arms race with males, as well as through fecundity selection.

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