Biology, Ecology and Diversity

Aspects of the reproductive behaviour and development of two forensically relevant species, Blaesoxipha (Gigantotheca) stallensi (Lahille, 1907) and Sarcophaga (Liopygia) ruficornis (Fabricius, 1794) (Diptera: Sarcophagidae)

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A B S T R A C T

We studied aspects of the reproductive behaviour and development of two species of Sarcophagidae (Diptera) of potential forensic importance, Blaesoxipha stallensi (Lahille, 1907) and Sarcophaga ruficornis (Fabricius, 1794), which are dominant in assemblages in dry forests in Northeast Brazil. We described the behavioural acts associated with courtship and mating and estimated the development time (from egg/larva until adult) – of both species. Description of the reproductive behaviour was based on 50 couples of each species whereas 250 larvae were used for the estimation of the developmental time. A total of 55 successful copula were observed for B. stallensi and 142 for S. ruficornis. Pre-copulatory behaviour differed between the species, as S. ruficornis presented a high rate of competition among male specimens. Blaesoxipha stallensi copulated more frequently in the morning and the mean duration of copulation was similar for both species. The species showed different reproductive strategies: S. ruficornis follows the typical strategy in Sarcophagidae and are viviparous (larviparity), but we report here the first documented evidence of ovoviviparity of B. stallensi. Sex ratio of the emerged adults did not differ (p > 0.05) markedly for either species. Total development time in days was similar with 22.9 for B. stallensi and 21.3 for S. ruficornis. The pronounced similarities in the morphology of both species – combined with their similar time of development – may act as confounding factors for forensic entomologists and stress out the need for an accurate taxonomical identification.

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Introduction

Reproductive strategies among species of Diptera are remarkably diverse and include oviparity, ovoviviparity, larviparity and pupiparity, among others (Meier et al., 1999). Complex behavioural traits also vary among species within a family, which is the case in Sarcophagidae. Flesh flies typically deposit first instar larvae (larviparity) on the substrate, as displayed by species of the genus Sarcophaga Meigen, 1826 whose substrates include faeces, decomposing fruit, carcasses and cadavers (Pape, 1996; Mulieri et al., 2010). On the other hand, oviparity can also occur, especially in the case of parasitoids such as those of the speciose genus Blaesox-

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quantitative data on the duration of each developmental stage. In addition, information on reproductive behaviour is crucial in order to understand the differential competitive abilities of species in the colonization of ephemeral resources – which will determine the composition and temporal structure of necrophagous assemblages. Furthermore, biominical data are needed to elucidate processes of biological invasions and on the assessment of environmental conservation status – as demonstrated by the use of sarcophagids as biological indicators (Barbosa et al., 2017).

Recent studies performed in fragments of the seasonally dry forest (Caatinga) in Brazil have revealed the dominance of B. stalleni (Lahille, 1907) and Sarcophaga ruficornis (Fabricius, 1794) in rat carcasses (Oliveira and Vasconcelos, 2018), which poses interesting questions regarding their biotic potential and adaptation to xeric environments. Detailed description of the behaviour and life cycle of both species is scant, particularly for B. stalleni. Thus, the objective of this study was to investigate aspects of the reproductive behaviour and development of B. stalleni and S. ruficornis under laboratory conditions. Specifically, we aimed to: a) describe the behavioural acts associated with courtship and mating and b) estimate the development time from egg/larva laying until adult.

Materials and methods

Insect identification and establishment of colonies

The adults were identified using the taxonomic keys of Carvalho and Melo-Patiu (2008) and Mulieri et al. (2010), and the morphological details used in the identification of the specimens are shown in Fig. 1. The specimens studied were deposited in the entomological collection of the Universidade Federal de Pernambuco (UFPE).

The colonies of B. stalleni and S. ruficornis used in this study were established from larvae collected in traps containing decomposing bovine liver as baits, exposed in a fragment of dry forest in the municipality of Afogados da Ingazeira, Northeastern Brazil (7° 45’ 52’’S; 37° 35’ 46’’W), three months prior to the experiments. Insects were reared under controlled temperature (25 ± 2 °C) and relative humidity (50 ± 10%) and under a light:dark cycle of 12 h:12 h. Flies that emerged from field-collected larvae were maintained in wooden cages (40 cm × 60 cm × 40 cm) covered with fine mesh. A solution of water and sucrose and a mixture of minced beef and sardine (50:50) was offered to adults as source of food and site for egg/larva laying, all of which were changed daily. First instar larvae resulting from reproduction in the cages were transferred to fresh containers (400 mL) and also fed on a beef:sardine mixture. After pupariation, pupae of both species were transferred to plastic containers (100 mL) in groups of ten and monitored until the emergence of adults.

Two separate studies were performed to integrate information on behaviour and development of both species. Data on mating behaviour, instar determination, pupation, and emergence of adults were collected twice a day, in the morning (9:00–11:00) and in the afternoon (14:00–16:00).

Mating behaviour

The description of the reproductive behaviour was based on 50 pairs of each species, maintained in the experimental cages described above. We recorded behavioural acts related to precopulation, copulation and strategies of offspring deposition. An ethogram was built to depict the behavioural repertoire and confirmed by photography. Filming was performed using a digital camera (Sony HDR-CX130) fixed to one side of the cage. For each species, we quantified the time elapsed from oviposition until sexual maturation and the duration of copulation. We focused on three phases:
1. Pre-copulation: involved pre-nuptial behavioural acts, including courtship, observed from the moment the male approached the female (<5 cm distance) until the insertion of the aedeagus in the female genitalia. This observation had no limit of time and focused on a single female at a time.

2. Copulation: comprised the acts associated with the insertion of the aedeagus in the female until the pair separated spontaneously. A single female was observed continuously until the end of copulation.

3. Post-copulation: behaviour exhibited by male and female following separation of the pair (e.g., feeding, flying, landing and oviposition), observed continuously for 15 min.

**Time of development**

For the study of the developmental time, 250 larvae of each species were observed from egg (or first instar when applicable) until pupation. Larvae were transferred using a soft brush from the substrate to 200 mL cages containing 250 g of the beef/sardine mixture, where they were maintained in groups to mimic larval aggregation found in nature. Instar was determined twice a day until pupariation. Pupae were transferred to individual plastic containers and monitored until the emergence of the adult.

For each species, we quantified the mean time of development from egg/larvae to adult, the mean duration of each larval instar and of the pupal stage, and the sex and longevity of the emerged adults. Statistical analyses were performed using the Program Bio-stat 5.0®, with a 5% significance level. We used a Kruskal–Wallis test to compare means related to: duration of the mating process; time of development and adult longevity. The sex ratio of the adults was compared by applying a Chi-Square test.

**Results**

**Reproductive behaviour**

Throughout the experiment, we observed 55 copulations for *B. stallungi*. In the case of *S. ruficornis*, 87 (38%) out of 229 mating attempts were interrupted by competing males, that is, males that approached the couple forcing its separation before copulation, and thus 142 mating events were considered successful.

Pre-copulation acts included part of the courtship dynamics and involved sound signals, mechanical stimulation and a variety of body movements (Fig. 2). In both species, males approached females and performed repeated, short and rapid flights associated with strong wing vibration and intense sound. Immediately following the approach of the male (ca. 15 cm from the female), attempts at copulation took place, in which the male performed short-distance flights around the female until landing on her dorsum. For *B. stallungi*, no rejection behaviour by the female and no aggressive male-male competition were observed. All females were courted by a single male per event. Every attempt to mate was successful and the pair remained in copula until separation.

On the other hand, pre-copulatory behaviour of *S. ruficornis* differed markedly, with evident pre-copulatory selection and male-male competition. Males approached females faster and more aggressively than *B. stallungi* males. Successful copula in *S. ruficornis* only occurred after several failed courting attempts by different males. Females often showed non-receptivity behaviour, refusing the male’s attempt to mate in two ways: (1) taking away – female attempted to escape the male’s overtire by flying off to the vicinity of the male, or to the other side of the cage, and (2) repelling movements – when a male successfully mounted a female, she insistently fluttered her wings and kicked the male using her forelegs. This behaviour was effective to reject the male before the insertion of the aedeagus.

In both species, the copulating male remained behind and close to the dorsal surface of the female, while it curled its abdomen downward. The front legs tended to stay up or on the mesonotum of the female. At the same time, females grasped the evaginated cerci of the mounting male with their hindlegs tarsi, intermittently. During copulation, the male’s body remained at an angle of 45° in relation to the horizontal position of female’s body. Females performed slight lateral movements, with different intensities (Video 1).

In *S. ruficornis*, male-male aggressive behaviour was recorded and several attempts were performed by other males to disturb the mating pair (Video 2), which included: (1) another male flying...
around and close to the mating pair, (2) another male trying to engage copula with a mating male, and (3) other males crawling constantly around the mating pair. When the interruption process was successful, the female usually did not engage in a second copula with the aggressive male.

Mating behaviour are summarized in Table 1. B. stallengi preferred to copulate in the morning (92.7% of the cases; p < 0.001), whereas S. ruficornis copulated slightly more frequently in the afternoon – 59.8% of the successful mating (p = 0.05). Mean duration of copulation was 64.2 min for B. stallengi and 69.8 min for S. ruficornis and this difference was not significant (H = 0.013; d.f. = 1; p = 0.987).

In both species, the female took the initiative in ending copulation, by kicking the male with the hindlegs until separation, simultaneously to a rotatory movement (360°) to help disengaging. Near the copulation ending, males cleaned the front legs and genitalia and females walked towards the available food.

The species differed in offspring deposition: B. stallengi is ovo-viviparous and S. ruficornis is viviparous, laying first instar larvae. Females of S. ruficornis deposited the majority of larvae (ca. 90%) in protected parts of the substrate, such as crevices and orifices whereas B. stallengi typically laid their eggs in arrangements (packets) on the surface of the substrate. Eggs of B. stallengi were whitish and cylindrical, and laid in packs of 5–8 eggs scattered on the substrate. Both species demonstrated a significantly more frequent ovi/larviposition in the morning (Table 2) which reached 90% in the case of B. stallengi.

### Table 1
Behavioural acts associated with mating in two species of Sarcophagidae.

<table>
<thead>
<tr>
<th>Behavioural acts in courtship and mating</th>
<th>Blaesoxipha stallengi</th>
<th>Sarcophaga ruficornis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abundance of males around the female (15 cm radius) in attempt courtship</td>
<td>Low (1–2 males)</td>
<td>High (&gt;10 males)</td>
</tr>
<tr>
<td>Interference of males (e.g., attempt to prevent approach of competitors towards female)</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Initial/preliminary rejection of males by the female, by pushing away males with hind legs</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Sound production (intense wing vibration) by males</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Female accepts male by allowing physical contact</td>
<td>Immediate/easy</td>
<td>Hesitant/difficult</td>
</tr>
<tr>
<td>Mating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female opens wings prior to mating</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Body position of the male–female association</td>
<td>At a 45° angle</td>
<td>At a 45° angle</td>
</tr>
<tr>
<td>Post-abdomen stimulus (male and female) during mating</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Movements of the body (latero-lateral) during mating</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Intense competition between males during mating</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Mating disruption by competitor males</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Mean duration of mating (min)</td>
<td>64.2 ± 8</td>
<td>69.8 ± 5</td>
</tr>
<tr>
<td>Retraction of aedeagus for copulation completion.</td>
<td>Rapid</td>
<td>Rapid</td>
</tr>
<tr>
<td>Post-copulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male grooming (legs and aedeagus)</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Displacement of the female to the after mating</td>
<td>Present/mediate</td>
<td>Present/mediate</td>
</tr>
<tr>
<td>Reproductive strategy</td>
<td>Oviposition</td>
<td>Larviposition</td>
</tr>
</tbody>
</table>

### Table 2
Preferential time for mating and ovi/larviposition of two forensically relevant species, Blaesoxipha stallengi (Lahille) and Sarcophaga ruficornis (Fabricius).

<table>
<thead>
<tr>
<th>Species</th>
<th>Morning</th>
<th>Afternoon</th>
<th>X²</th>
<th>d.f.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. stallengi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating (n = 55)</td>
<td>92.7%</td>
<td>7.3%</td>
<td>71.23</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oviposition (n = 30)</td>
<td>90.0%</td>
<td>10.0%</td>
<td>62.41</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. ruficornis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating (n = 229)</td>
<td>40.2%</td>
<td>59.8%</td>
<td>3.84</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Larviposition (n = 75)</td>
<td>77.3%</td>
<td>22.7%</td>
<td>28.73</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Discussion
This study reveals similarities (e.g., preferential time for ovi/larviposition) in the reproductive behaviour of the flesh flies B. stallengi and S. ruficornis. Some peculiarities, however, distinguish their pre-copulatory behaviour (Fig. 2), such as interruptions caused by male competition in the case of S. ruficornis and differences in the mating strategy – eggs for B. stallengi and larvae for S. ruficornis. Males take the initiative for mating without noticeable signs from the female that lead to encouragement and produce sound by vibrating the wings fiercely during courtship, a behaviour previously described for Sarcophaga species (Thomas, 1956).

The complexity of pre-copulatory behaviour in Sarcophagidae encompasses aggression between competing males (Moore et al., 2014; Shropshire et al., 2015) and elaborate courtship displays (Spofford and Kurczewski, 1985). The intense and aggressive male-male competition and the rejection of mating attempts by females suggest that S. ruficornis shows strong pre-copulatory sexual selection. This strategy is characterized by females being highly selective to specific physical and behavioural male traits prior to mating, and a second mating is usually rare or nonexistent (Kvarnemo and Simmons, 2013). On the other hand, B. stallengi showed no apparent pre-copulatory selection, given the absence of male competitors in
the vicinity of the available female, the female’s acceptance of each courting male and the lack of mating disruption by another male (Thornhill and Alcock, 1983).

A common ecological factor shared by S. ruficornis and B. stalleni is the unpredictability and scarcity of the resource (e.g., carcass or cadaver). The brief period of availability of the carcass imposes a narrow window of opportunity for mating events, offspring deposition and larval development. In this situation, mating strategies may evolve differently. For example, in species depending of a time-limited resource, such as a rotting carcass, females may be less selective for available males, mating as soon as possible to avoid the risk to remain unpaired at the end of the resource, so that the progeny can utilize the decomposing resource while it remains suitable. This explains why females with limited time to copulate are usually less discriminating for mates (Moore and Moore, 2001). On the other hand, a strong pre-copulatory sexual selection can also be associated with an ephemeral resource. Females must be more selective for high-quality males to ensure a greater survivorship of the offspring in a food resource exposed to intense competition, which is the case of carrion.

The precopulatory behaviour shown in S. ruficornis may be a result of a female sexual mating system such as monandry (Thornhill and Alcock, 1983), or a consequence of ecological factors such as a biased operational sex ratio (Weir et al., 2011; Painting et al., 2014). On the other hand, if the sex ratio tends to be female biased, as observed for B. stalleni, we expect that the number of available females overcoming the number of males may reduce male–male competition, resulting in low or absence of precopulatory sexual selection (Emlen and Oring, 1977; Weir et al., 2011).

For the highly competitive S. ruficornis, when copulation is interrupted early, females tend to mate again. A successful copula requires a minimum time for the male to completely inseminate the female (Thornhill, 1980), and when the mating pair is interrupted, females still tend to be receptive for a second male, if the interrupted copulation did not allow for the transfer of a satisfactory amount of sperm (Gilchrist and Partridge, 2000).

The duration of copulation in both species is similar to what has been observed for other sarcophagid species (Thomas, 1950). Longer copula may arise as a means of mate guarding, when males must ensure that the transferred ejaculate will be used to fertilize the egg clutches before the female is ready to copulate with a rival male (Simmons, 2001). In Drosophila melanogaster Meigen, 1830, for example, a long copula allows the male to transfer chemical substances along with the seminal fluid, which increase refractory period and stimulate oviposition (Chen et al., 1988). Curiously, the period of copulation differed between the species, despite similarities in their period of activity.

According to Pape (1987), all sarcophagids deposit incubated eggs with mature first instar larvae ready to hatch, or newly-hatched first instar larvae, or, additionally, second instar larvae that have been nourished by the maternal accessory glands. The ovoviviparity in B. stalleni, as opposed to the majority of sarcophagid flies, which are viviparous (Pape, 1996), has been recorded for Sarcophaga (Liosarcophaga) aegyptica (Salem, 1935) (Saloná Bordas et al., 2007) and Blaesoxipha (Gigantotheca) plinthopyga (Wiedemann, 1830) (Pimslr et al., 2014), which suggests that oviposition has been largely overlooked for the family.

Total development time did not differ much between the two species (22.9 days for B. stalleni and 21.3 for S. ruficornis), and this variation derives from the additional time of egg development in B. stalleni. Bionomic data for B. stalleni is limited to those presented in this paper, while S. ruficornis takes an average of 420h to complete its life cycle at 25 °C (Nassu et al., 2014). Nevertheless, in the present study the species had a longer development time, requiring 512h at 25 °C.

Development time for B. stalleni was similar to other flesh flies with ability to lay eggs under laboratory conditions, for example, Sarcophaga (Liapygia) argyrostoma (Robineau-Desvoidy, 1830) (Grassberger and Reiter, 2002). The life cycle of S. argyrostoma takes 22 days at 25 °C, similar to our data for B. stalleni. In both species, pupal stage has the longest duration, which corroborates previous studies (Grassberger and Reiter, 2002; Nassu et al., 2014). In fact, duration of pupal stage varied little among the species (15.5–16.8 days), and was similar to that recorded for another species associated with cadaveric colonization such as Peckia (Peckia) chrysostoma (Wiedemann, 1830) (Ferraz, 1992). Mean longevity also varies little – both live for ca. 16 days – and is lower than that observed for other species of Sarcophagidae (Kamal, 1958).

Eggs of B. stalleni resemble – and are laid in a similar way – as those of S. aegyptica and B. plinthopyga (Saloná Bordas et al., 2007; Pimslr et al., 2014), as whitish, elliptical and cylindrical egg mass of no more than ten eggs. Both B. stalleni and S. ruficornis deposit their progeny preferentially in the morning, which supports data by Oliveira and Vasconcelos (2018), who demonstrated that over 90% of the sampled flesh flies in a fragment of Caatinga were active during the morning.

Due to the absence of studies on the life cycle and reproductive behaviour of flesh flies endemic to the Brazilian Caatinga we present here the first integrated data on the reproductive behaviour and development of B. stalleni, a species attracted to carcasses at early and intermediate stages of decomposition (Oliveira and Vasconcelos, 2018). Data on the life cycles of species are crucial for the consolidation of forensic entomology in South America, in which several cities exhibit extremely high rates of homicide. The pronounced similarities in the morphology of both species – combined with their similar time of development – may act as confounding factors for forensic entomologists and stress out the need for an accurate taxonomical identification. Furthermore, since both species studied here develop in carrion, bionomical data are of the utmost importance to subsidize reliable estimation of the post-mortem interval.

### Table 3

Duration of immature stages, sex ratio and longevity of the adult of Blaesoxipha stalleni (Lahille) and Sarcophaga ruficornis (Fabricius) under laboratory conditions (25 ± 5 °C, 50 ± 10% RH with 12 h of photophase).

<table>
<thead>
<tr>
<th>Duration of each phase, in hours (mean ± SD)</th>
<th>Blaesoxipha stalleni</th>
<th>Sarcophaga ruficornis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>18.2 ± 3.7</td>
<td>24 (n = 250)</td>
</tr>
<tr>
<td>1st instar larva</td>
<td>24 (n = 250)</td>
<td>43 ± 9.3 (n = 250)</td>
</tr>
<tr>
<td>2nd instar larva</td>
<td>82.2 ± 17 (n = 200)</td>
<td>72.0 ± 2 (n = 220)</td>
</tr>
<tr>
<td>Pupa</td>
<td>403.5 ± 20 (n = 200)</td>
<td>372.5 ± 12.8 (n = 220)</td>
</tr>
<tr>
<td>Total time of development (from egg/1st instar to adult)</td>
<td>551.0 ± 27.6 (n = 180)</td>
<td>512.1 ± 15.3 (n = 200)</td>
</tr>
<tr>
<td>Sex ratio (female/male + female)</td>
<td>0.68</td>
<td>1</td>
</tr>
<tr>
<td>Longevity of the adult (h)</td>
<td>378.0 ± 19.0</td>
<td>391.7 ± 17.2</td>
</tr>
</tbody>
</table>
Conflicts of interest

The authors declare no conflicts of interest.

Supplementary material

Video 1. Position of Blaesoxipha stalleni (Lahille) adults during copulation, with emphasis on body movements.

Video 2. Attempts of copulation dispute by competing males of Sarcophaga ruficornis (Fabricius).

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Appendix A. Supplementary data


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