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Asexual reproduction in a sexual population of the Brazilian yellow scorpion (*Tityus serrulatus*, Buthidae) as evidence of facultative parthenogenesis

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Abstract. Most Brazilian yellow scorpion (*Tityus serrulatus* Lutz & Mello, 1922) populations reproduce by parthenogenesis, and only a few sexually reproducing populations are known. It has been suggested that the parthenogenesis in *T. serrulatus* is related to bacterial endosymbionts, but this hypothesis was recently refuted, so the causes of parthenogenesis in this species are still unknown. In the present study, we report parthenogenetic reproduction in females from a sexual population, either isolated in laboratory since birth or collected at juvenile stages. Twelve females collected as juveniles became adult and reproduced without contact with males (thus, through parthenogenesis) in the laboratory. Five females collected already pregnant gave birth to litters (F1) composed only of females, which is suggestive of parthenogenesis in the field. Eight F1 females from those litters subsequently reproduced by parthenogenesis in the laboratory. Another female collected already pregnant gave birth to a litter composed of males and females (F1), indicating sexual reproduction in the field. However, one F1 female from that litter reproduced by parthenogenesis in the laboratory. These results suggest that asexual reproduction is facultative in this population.

Keywords: geographic parthenogenesis, sex ratio, sexual reproduction

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Parthenogenesis, the development of offspring from unfertilized eggs, although present in few species, occurs in diverse invertebrate and vertebrate taxa (Simon et al. 2003). If the fertility of parthenogenetic and sexually reproducing females were the same, asexual reproduction is considered to be more efficient than sexual reproduction, since the latter involves additional costs (Maynard-Smith 1978; Kawatsu 2013), such as the production of males, whose paternal investment in the offspring is often minimal (Maynard-Smith 1971). In addition, asexually reproducing animals are more efficient in the colonization of disturbed environments since reproduction may occur without the presence of a member of the opposite sex (Cuellar 1977; Lourenço 2008). However, the evolutionary consequences of parthenogenesis may involve reproductive isolation in relation to sexual forms and the emergence of lineages with low genotype diversity, which may lead to their extinction (Simon et al. 2003). Facultative parthenogenesis, the ability to reproduce both sexually and asexually, brings the advantages of sexual reproduction but with lower costs (D'Souza & Michiels 2010). Despite presenting the advantages of both forms of reproduction, facultative parthenogenesis is rare in higher eukaryotes, probably due to genetic and developmental constraints (Engelstädter 2008). Thus, the study of populations with mixed mode of reproduction may offer clues to the advantages and disadvantages of facultative parthenogenesis and its emergence from sexual reproduction (Burke & Bonduriansky 2017).

Although facultative parthenogenesis is rare, most asexual animal lineages can also reproduce sexually (Bengtsson 2009), whereas very few lineages, such as bdelloid rotifers, are exclusively parthenogenetic (Welch & Meselson 2000). In some invertebrates, sexual and asexual reproduction occur cyclically, such as in monogonont rotifers, cladoceran crustaceans, and aphids (Decaestecker et al. 2009; Loxdale 2009; Serra & Snell 2009), while in other groups, the presence of both forms of reproduction is an alternative strategy (Buřič et al. 2011). In arthropods, facultative parthenogenesis is observed in insects (Corley et al. 1999; Chang et al. 2014; Sekiné et al. 2015; Walker & Holwell 2015), crustaceans (Buřič et al. 2011) and arachnids, specifically some scorpion species (Toscano-Gadea 2005; Francke 2008; Lourenço 2008). According to criteria established by Francke (2008) for the identification of a parthenogenetic species, eleven scorpion species are currently known to reproduce asexually (Francke 2008; Ross 2010; Seiter 2012; Seiter et al. 2016; Seiter & Stockmann 2017), most through facultative parthenogenesis (Francke 2008; Seiter & Stockmann 2017). For example, although *Tityus trivittatus* Kraepelin, 1898 populations from southern Argentina appear to consist exclusively of parthenogenetic females, this scorpion has been reported reproducing sexual and asexually through parthenogenesis across several countries in South America (Maury 1970, 1997; Ojanguren-Affilastro 2005). The first evidence of parthenogenesis in scorpions came from observations regarding the sex ratio of populations of the Brazilian yellow scorpion, *Tityus serrulatus* Lutz & Mello, 1922, in which no male had been found (Piza 1940; Bücherl 1956). However, the demonstration of asexual reproduction in this species came after Matthiesen (1962) observed that captive females, isolated from other individuals, generated offspring. This was confirmed in subsequent studies (San Martin & Gambardella 1966; Matthiesen 1971). Parthenogenesis was thenceforth considered the only form of reproduction of this species, until the discovery of males from a few sexual populations in Brazil (Souza et al. 2009; Santos et al. 2014; Lima et al. 2020). Although *T. serrulatus* could be an interesting model to study parthenogenesis, the causes of asexual reproduction in this species are still unknown. A proposition of *Wolbachia*-induced parthenogenesis (Suesdek-Rocha et al. 2007) was recently refuted (Braga-Pereira et al. 2019), discarding the only

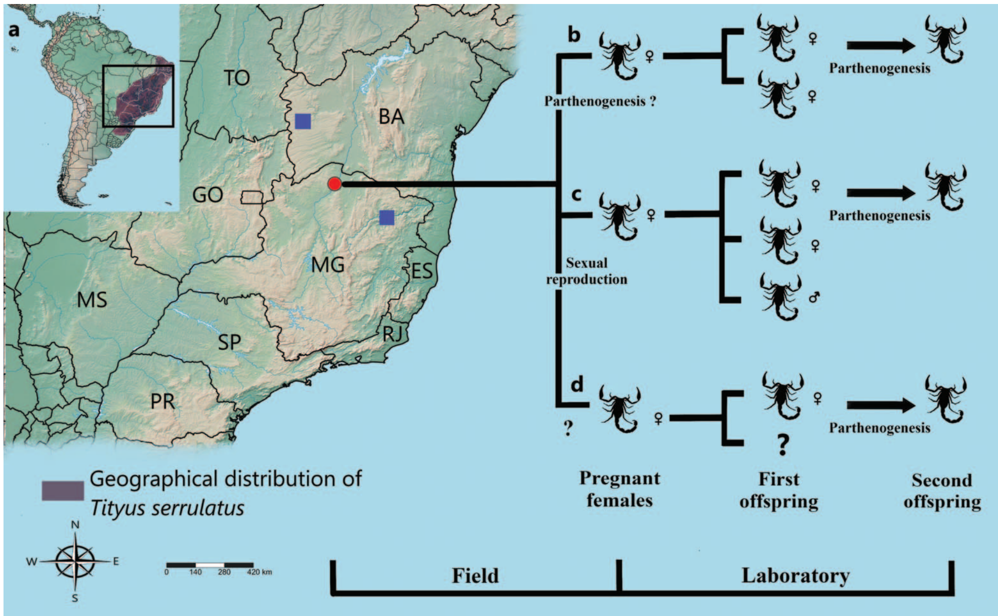

Figure 1.—Reproductive history of female Brazilian yellow scorpions (*Tityus serrulatus*) in laboratory: a. Geographic distribution of *Tityus serrulatus* specimens sampled in this study. The red circle represents the sexual population analyzed in the present study and blue squares show other sexual populations. b. Pregnant females gave rise to offspring containing only females; eight of them reproduced by parthenogenesis. c. Pregnant female gave rise to offspring containing male and females; one of them reproduced by parthenogenesis. d. Pregnant females gave rise to offspring containing females; five of them reproduced by parthenogenesis. Sex of the other individuals of the second offspring was not determined.

proposed mechanism of asexual reproduction suggested for this species. In this study, we demonstrate through captive rearing that females from a sexual population are capable of reproducing parthenogenetically, indicating that the asexual reproduction in *T. serrulatus* is actually facultative.

METHODS

We collected 174 individuals of *T. serrulatus*, including 59 adult females, 40 adult males and 75 second to fifth instar juveniles in urban and suburban habitats in Itacarambi, Minas Gerais, Brazil (15.1730° S, 44.1787° W, Fig. 1a) during two collection expeditions in March 2016 and January 2017. In the second expedition we also recorded the environment in which they were found: urban areas (backyards of houses and an abandoned sawmill) or suburban areas (farms and a coconut plantation). We captured the scorpions in the morning, under trunks, stones and bricks and inside termite mounds. During the night, we collected active animals using ultraviolet light. In the laboratory, we identified each specimen with a number and kept them individually in plastic containers (juveniles: 5.6 cm diameter X 6.0 cm height; subadults and adults: 8.5 cm X 6.5 cm), with a wet cotton ball to provide water and a piece of cardboard as a refuge. We fed the juveniles one cockroach (*Nauphoeta cinerea* (Olivier, 1789)) per week and the adults every 15 days, and inspected the containers four times a week to check for molting or birth events. Prey remnants were taken away one day after scorpion feeding to avoid contamination by fungi. Scorpions were kept in the laboratory at a mean temperature of 25 ± 3 °C, 58 ± 11% relative humidity and natural illumination providing a photoperiod of approximately 12:12 hours light:dark, varying seasonally from 10:55 to 13:20 hours of light.

After adult females (parental generation, P) gave birth, we separated the second instar juveniles (F1 generation) from the mother after the first molt. We kept each juvenile in an individualized container, under the same conditions described above. We recorded the date of each juvenile molt until they reached adulthood, and afterwards the date of their own offspring birth (F2 generation; see Supplemental Tables S1, S2 online at https://doi.org/10.1636/JoA-S-20-001.s1). Adult individuals, both found in field and grown in laboratory, were sexed based on the width of metasomal segments and length of pedipalp segments (following Souza et al. 2009). Males that showed no unambiguous morphological differences from females were identified through their behavior when exposed to females: males exhibit tremors (“Vibración”, as in Peretti 1991) and females tend to tilt the metasoma towards the male (Braga-Pereira & Santos, personal observations). Dead specimens were fixed in 80% ethanol, or discarded if they were in decay. After the end of the study, we fixed the surviving specimens in 80% ethanol and deposited them in the Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais. We made observations between March 2016 and March 2019.

RESULTS

In the first expedition, we collected 11 adult females, 6 adult males, and 35 juveniles. In the second expedition, we collected

6 adult females, 0 adult males, and 9 juveniles from urban areas and 42 adult females, 34 adult males and 31 juveniles from suburban areas. Forty-four percent of the 75 juveniles collected during the first and second expeditions reached adulthood, producing 21 females and 12 males. Thus, the juveniles and adults collected in the field on both collection expeditions totaled 80 females (61%) and 52 males (39%). In the second collection expedition, we obtained from urban environments nine females (60% of collected specimens) and no males (0%); none of the six juveniles (40%) survived to adulthood. A total of 49 females (46.22%) and 43 males (40.57%) were obtained in suburban environments, with 15 juveniles that did not survive to adulthood (13.2%) (see Supplemental Figure S3, online at https://doi.org/10.1636/JoA-S-20-001.s1).

Among the 21 females that became adult in the laboratory and never had contact with males, 12 gave birth through parthenogenesis, once or twice (Supplemental Table S1). We raised to adulthood all offspring (F1) of six females (P) that were collected already pregnant. Five of these females gave birth to a total of 39 individuals (F1), all females, which is suggestive of parthenogenesis in the field. Eight of those 39 females (F1) reproduced by parthenogenesis in the laboratory (Fig. 1b). Another female collected already pregnant (P) gave birth to five males and nine females (F1), an indication of sexual reproduction in the field. One (F1) of those nine females reproduced by parthenogenesis in the laboratory (Fig. 1c). Furthermore, we also raised part (15 individuals, F1) of the litters born from three additional females collected already pregnant. Six of those F1 did not survive to adulthood. The remaining nine specimens became adult females, and five of them reproduced parthenogenetically in the laboratory (Fig. 1d). (Supplemental Table S2).

DISCUSSION

Our results demonstrate that asexual reproduction is facultative in the Brazilian yellow scorpion populations in which males are found. This result reinforces our previous conclusion that the parthenogenesis in *T. serrulatus* is not induced by endosymbiont bacteria (Braga-Pereira et al. 2019), as suggested for *Wolbachia* (Suesdek-Rocha et al. 2007). This hypothesis was refuted due to negative PCR tests for *Wolbachia* in several parthenogenetic populations (Braga-Pereira et al. 2019). *Wolbachia* is an endosymbiont microorganism that reproduces mainly by vertical transmission (Hoffman et al. 1990), usually inducing parthenogenesis by converting unfertilized haploid eggs into diploid embryos via gamete duplication (Stouthamer & Kazmer 1994). Within the same lineage, we observed that one female (P) gave rise to offspring (F1) consisting of both sexes, evidence of field mating and sexual reproduction. One F1 female from this litter, which was kept isolated in the laboratory, reproduced by parthenogenesis. As far as we know, there is no report of *Wolbachia* infection suppressed in one generation and expressed in the subsequent.

Our results suggest that the absence of males may trigger the parthenogenesis in *T. serrulatus* females. The field sampling suggests that females outnumber males in the Itacarambi population, a condition that may favor parthenogenesis, as asexual reproduction may be an advantageous strategy under low male availability (Markow 2013; Burke & Bonduriansky 2019). A similar pattern was reported for *Drosophila* species that, depending on male availability, can alternately use both reproductive modes (Chang et al. 2014). In fact, females of some species can maintain their ability to reproduce asexually for generations, even after mating with males (Kramer & Templeton 2001). In populations of stick insects, which reproduce by facultative parthenogenesis, this form of reproduction can generate female-biased sex ratios, increasing mating limitation and thus leading to male scarcity in a positive feedback mechanism (Schwander et al. 2010). Within this scenario, the absence of males may lead to obligatory parthenogenesis (Schwander & Crespi 2009), an event that may have occurred in most Brazilian yellow scorpion populations. Further laboratory experiments are needed to assess whether obligatory parthenogenesis is present in populations composed only by females.

Populations that reproduce only asexually have some characteristics that tend to lead a lineage to extinction (Simon et al. 2003), as the accumulation of deleterious allele combinations (Charlesworth et al. 1993), the absence of fixation of beneficial mutations (Peck 1994) and developmental constraints (Corley et al. 1999). Although we observed a slightly higher laboratory mortality rate to adulthood in individuals from urban environments, where the number of females exceeds the number of males (thus females are likely to reproduce by parthenogenesis), our small sample size prevents reliable conclusions. Thus, a comparative study specifically designed to compare mortality rates between sexual and asexual population would be welcome. Mortality rate differences apart, the ability to reproduce through parthenogenesis seems to facilitate *T. serrulatus* invasion to disturbed environments. Female-only populations have a wide geographic distribution and may have dispersed from facultative parthenogenetic populations. Originally, *T. serrulatus* was known only from Minas Gerais, a state in the Southeast region of Brazil. However, the species has been increasingly found in regions where its presence was not reported earlier (Lourenço et al. 1996; Lourenço & Cloudsley-Thompson 1999; Bortoluzzi et al. 2007; Mario da Rosa et al. 2015). Lourenço (2008) proposed that the wide distribution of *T. serrulatus* is the result of recent dispersal events, since a medically important species would be recorded as soon as it became abundant in urban areas.

Furthermore, Lourenço (2008) proposed geographic parthenogenesis in *T. serrulatus,* a phenomenon in which parthenogenetic organisms have different geographical distributions than their closest sexual relatives (Vandel 1928; Tilquin & Kokko 2016). Indeed, the Brazilian yellow scorpion presents traits associated to geographic parthenogenesis, such as the parthenogenetic populations with wider geographic distribution compared to sexual populations (Hörandl 2009), and high abundance in disturbed environments, either natural or those created by human action (Hoffmann et al. 2008; Vrijenhoek & Parker 2009). The explanation for these two characteristics is due to the easy dispersal of parthenogenetic organisms, since a single individual can start a new population (White 1954). However, during our field work in an area where the sexual population is present, we observed a female-biased sex-ratio in disturbed environments, such as backyards and the abandoned sawmill, where vegetation was sparse and composed only of small plants and predominance of human constructions, if compared to less disturbed environments, such as farms and the coconut plantation, where we find dense vegetation, including large trees. Apparently, the both forms of reproduction of the Brazilian yellow scorpion may occur in the same region, with small differences related to the habitat. This indicates that ecological conditions may favor asexual reproduction in *T. serrulatus*.

Finally, in order to understand other factors that may have led to positive selection of parthenogenesis in the Brazilian yellow scorpion, it is necessary to observe some reproductive characteristics of the species. Is there any behavior in *T. serrulatus* mating that may favor parthenogenesis? For example, traits associated to sexual conflict may be a factor that favor the presence of parthenogenesis in a species, such as male coercive behaviors and female resistance responses (Burke & Bonduriansky 2017, 2019). For instance, in the scorpion *Zabius fuscus* Thorell, 1876, males show little or no coercion during courtship and copulation. Females, on the other hand, though receptive to first male courtship attempts, tend to resist subsequent mating attempts (Peretti & Carrera 2005). However, parthenogenesis has never been observed in *Z. fuscus*, thus it would be interesting to verify if coercive or resistance behaviors are present in asexually-reproducing species. Is the fertility of sexually reproducing females equal to the fertility of asexual reproducing ones? In the facultatively parthenogenetic cockroack *Nauphoeta cinerea*, developmental constraints limit the success of asexual reproduction, generating extreme clutch size variations (Corley et al. 1999). Do females of obligatory parthenogenetic populations copulate with males of sexual populations? In the facultative parthenogenetic *Drosophila albomicans*, females isolated from males for 20 years in the laboratory have reduced mating propensity, a process that may lead to obligatory parthenogenesis (Chang et al. 2014). Answers to these questions may not only help to understand the presence of parthenogenesis in the Brazilian yellow scorpion, but also the factors that led to the transition from facultative to obligatory asexual reproduction.

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SUPPLEMENTAL MATERIALS

Supplementary Files, available online at https://doi.org/10.1636/JoA-S-20-001.s1.

Table S1.—Reproductive history of female yellow scorpions (*Tityus serrulatus*), which reproduced by parthenogenesis in laboratory. Females were collected at juvenile stage, were kept until adulthood in captivity and never had contact with males.

Table S2.—Reproductive history of female yellow scorpions (*Tityus serrulatus*) born in laboratory from females collected already pregnant. Abbreviations: P, females collected in the field; F1, individuals born in laboratory, from the P females; F2, offspring born in laboratory from F1 females, by parthenogenesis.

Figure S3.—Individuals of *Tityus serrulatus* (Buthidae) collected in the first and second collection expeditions. The total number of individuals of each gender collected in each expedition is shown within the rectangles.

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