Dispersal and foraging rates of the facultatively parthenogenetic stick insect Megacrania batesii

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DISPERSAL AND FORAGING RATES OF THE FACULTATIVELY PARTHENOGENETIC STICK INSECT *MEGACRANIA BATESII*

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Thesis Title

Dispersal and foraging rates of the facultatively parthenogenetic stick insect *Megacrania batesii*

Thesis Abstract

Sexual conflict could play an important role in shaping spatial variation in sex ratio in facultatively parthenogenetic species. This is because if females of such species avoid mating, they can establish all-female populations via parthenogenetic reproduction. By contrast, if females cannot avoid mating (i.e., males), they reproduce offspring of both sexes and establish mixed-sex populations in the wild. In Australia, natural populations of *Megacrania batesii* exhibit extreme spatial variation in sex ratio, with all-female and mixed-sex populations occurring over a small scale. However, it is unclear how facultatively parthenogenetic females avoid mating and establish all-female populations. Sex-specific patterns of dispersal could play a role because, depending on whether females are mated or unmated, they could establish mixed-sex and all-female populations if they successfully disperse to new areas. However, if males invade all-female populations, they could convert these populations into mixed-sex populations via mating. In chapter one, I therefore investigated dispersal rate of females and males from a mixed-sex population and single females from an all-female population. I found that females from the all-female population were less dispersive than females and males from the mixed-sex population. I also found that dispersal was limited to movement within habitat patches. Additionally, in chapter two, I examined whether costs associated with mate guarding (which is widespread and long-lasting in *M. batesii*) imposes energetic costs on females (i.e., reduction in foraging rate) because guarded females carry males. If females' foraging rates were impacted by guarding males, mate guarding could be a manifestation of sexual conflict in *M. batesii*. However, I found little evidence that laboratory-reared females from mixed-sex populations fed more while unpaired than while paired with males. Overall, these studies contribute insights into how sex-specific dispersal and costs of mating and guarding might contribute to sexual conflict and variation in sex ratio in natural populations of the facultative parthenogenetic species, *M. batesii*.

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ABSTRACT

Sexual conflict could play an important role in shaping spatial variation in sex ratio in facultatively parthenogenetic species. This is because if females of such species avoid mating, they can establish all-female populations via parthenogenetic reproduction. By contrast, if females cannot avoid mating (i.e., males), they reproduce offspring of both sexes and establish mixed-sex populations in the wild. In Australia, natural populations of *Megacrania batesii* exhibit extreme spatial variation in sex ratio, with all-female and mixed-sex populations occurring over a small scale. However, it is unclear how facultatively parthenogenetic females avoid mating and establish all-female populations. Sex-specific patterns of dispersal could play a role because, depending on whether females are mated or unmated, they could establish mixed-sex and all-female populations if they successfully disperse to new areas. However, if males invade all-female populations, they could convert these populations into mixed-sex populations via mating. In chapter one, I therefore investigated dispersal rate of females and males from a mixed-sex population and single females from an all-female population. I found that females from the all-female population were less dispersive than females and males from the mixed-sex population. I also found that dispersal was limited to movement within habitat patches. Additionally, in chapter two, I examined whether costs associated with mate guarding (which is widespread and long-lasting in *M. batesii*) imposes energetic costs on females (i.e., reduction in foraging rate) because guarded females carry males. If females' foraging rates were impacted by guarding males, mate guarding could be a manifestation of sexual conflict in *M. batesii*. However, I found little evidence that laboratory-reared females from mixed-sex populations fed more while unpaired than while paired with males. Overall, these studies contribute insights into how sex-specific dispersal and costs of mating and guarding might contribute to sexual conflict and variation in sex ratio in natural populations of the facultative parthenogenetic species, *M. batesii*.

Contents

GENERAL INTRODUCTION

Facultative parthenogenesis is the ability of individual females to reproduce sexually if they mate and asexually if they do not (Bedford, 1978; Galis & van Alphen, 2020). The ability to switch between two reproductive modes depending on whether mating takes place can have important effects on demography. In populations that consist of both males and females, sexual reproduction promotes the continued production of both sexes, whereas in populations where males are rare or absent, parthenogenesis promotes the production of mostly females (Schwander, Vuilleumier, Dubman, & Crespi, 2010). These dynamics can lead to spatial variation in sex ratio whereby certain populations are mixed-sex and others are female-only, and such spatial variation can occur over very small scales (i.e., distances that can be traversed by dispersing individuals over one or a few generations) (Buckley, Marske, & Attanayake, 2009; Cermak & Hasenpusch, 2000; Morgan-Richards, Trewick, & Stringer, 2010). It has been hypothesized that the presence of males plays an important role in maintaining sexual reproduction in mixed-sex populations by coercing females to mate, and that female-only populations may be maintained if females are selected to escape from coercive males (Burke & Bonduriansky, 2018). Although a number of recent studies have highlighted the role of sexual conflict in determining sexual versus parthenogenetic reproduction in facultatively parthenogenetic species (Burke & Bonduriansky, 2017; Burke & Bonduriansky, 2019), the extent to which conflicts between the sexes account for demographic variation in natural populations of facultative parthenogens remains unexplored.

Sexual conflict can be substantial in facultative parthenogenetic system where males are not required for the production of offspring. If sexual conflict over mating is responsible for the spatial mosaics of reproductive mode observed in facultative parthenogens, then sexual conflict should manifest differently in all-female populations, in which females might encounter male dispersers from neighbouring populations on occasion, versus mixed-sex populations where females frequently encounter males. This reflects differences in the level of male-imposed costs and female responses. Female-only populations might withstand subsequent invasion by neighbouring males if females in those populations show highly effective mate-avoidance strategies that promote the parthenogenetic production of all-female offspring, whereas mixed-sex populations might be maintained if females fail to escape from males and sexual reproduction is enforced through coercive mating (Burke & Bonduriansky, 2018; Kawatsu, 2013). Males might disperse into all-female populations and guard the females for prolonged durations. Such mate guarding could be more costly than costs related to mating only. Therefore, the females might be selected to avoid males due to the costs of prolonged guarding (whether or not mating itself is costly). Female resistance behaviours will be maintained in all-

female populations if male dispersal into such populations is fairly frequent. Female dispersal behaviour can be driven by male presence and harassment behaviour in the population. For instance, an empirical study on water striders found that females tend to disperse away from areas with male-biased sex ratios, perhaps to avoiding aggressive males (Eldakar, Wilson, Dlugos, & Pepper, 2010). However, the manifestation of sexual conflict in facultatively parthenogenetic populations, and the nature of mating interactions across such populations, is poorly understood. Several aspects of sexual conflict can impose net costs on females. For example, if males' mating interaction with females limits females' movements and foraging, mating could be costly for the females.

Phasmids, or stick insects, are an ideal group for investigating questions about the ecology of reproductive modes, since a large number of these insects reproduce both asexually and sexually and can switch between reproductive modes (Bedford, 1978; Bradler & Buckley, 2018; Burke, Crean, & Bonduriansky, 2015). Recently, some phasmids have received attention for their suitability in tackling questions related to geographical parthenogenesis which is a pattern of distribution where asexual lineages inhabit different habitats than their sexual relatives (Burke & Bonduriansky, 2018; Kearney, 2005; Morgan-Richards et al., 2010). Indeed, many stick insect species show spatial variation in sex ratio across their range, suggesting that in some populations females reproduce only via parthenogenesis (thus producing only female offspring) whereas females in other populations reproduce sexually (thus producing both male and female offspring) (Buckley et al., 2009; Morgan-Richards et al., 2010). In some instances, mixed-sex and female-only populations occur side-by-side in a mosaic pattern without any discernible geographic barrier. This occurs in the peppermint stick insect, *Megacrania batesii*—a phasmid from the tropical rainforests of North-Eastern Australia (Bonduriansky and Burke, unpublished data).

Sex-specific dispersal could be important in understanding spatial variation in sex ratio in facultative parthenogens. Both theoretical and empirical studies have suggested that males tend to disperse to find mating partners whereas females tend to disperse to seek better food source (Mishra, Tung, Shree Sruti, Srivathsa, & Dey, 2020; Shaw & Kokko, 2014). However, in *Megacrania batesii* the spatial patterns in sex ratio might be explained by sex-differences in dispersal whereby females disperse further distances than males and establish female-only populations because males might rarely disperse far enough to reach those populations. Cues (visual, tactile, or chemical) of male presence might induce females to move from one host plant to another to escape from males in order to avoid the costs of prolonged guarding and mating. Alternatively, it is possible that females disperse more than males because females require more food to provision their eggs and move more frequently between host plants in search of more

nutritious leaves. In either case, if females tend to disperse over longer distances than males, this pattern of sex-biased dispersal could lead to the establishment of all-female populations. However, the effect of sexual conflict on dispersal patterns in natural populations of facultatively parthenogenetic species has not been investigated empirically before.

In Chapter 1, I aimed to understand whether sex-specific dispersal might explain spatial sex ratio variation in the facultatively parthenogenetic phasmid *Megacrania batesii.* To investigate whether females of this species are generally more dispersive than males, or whether females from different populations disperse differently in response to the presence versus absence of males, I investigated female activity levels in situ in naturally-occurring all-female and mixed-sex populations. I also examined, under laboratory conditions, whether newly hatched nymphs can be dispersive and whether dispersal at that ontogenetic stage can contribute to spatial variation in sex ratio. This could provide insights on whether individual dispersal might explain current mixed-sex and all-female populations in *Megacrania batesii*. In Chapter 1, I found evidence that females' behaviour differs between all-female and mixed-sex populations. I also found that female dispersal behaviour was affected by male guarding. However, I did not find evidence that females disperse longer distances than males do.

In many species, females incur costs imposed by males, and such costs can reduce females' fitness due to the mating interaction. An important cost of mating in some species is increased risk of predation or pathogen transmission, which can reduce female fitness by increasing mortality risk (Daly, 1978; Hurst, Sharpe, Broomfield, Walker, Majerus, Zakharov, & Majerus, 1995; Strandberg & Tucker, 1974). Another type of cost that females can incur is reduced foraging ability. Males in many insects tend to mate with and guard females for a prolonged period of time. Prolonged mate guarding behaviour by males can conflict with female interests. In insects where males physically cling to females, guarding can potentially expose females to elevated predation risks (Cothran, 2004), or impose energetic costs on females (Watson, Stallmann, & Arnqvist, 1998). This is because females often have to carry the males around, as observed in stick insects, isopods, and water striders. In water striders, mate guarding can elevate predation risk to females (Han & Jablonski, 2010; Rowe, Arnqvist, Sih, & Krupa, 1994). Carrying the males can also be energetically costly for females in water striders (Watson et al., 1998), and further energetic costs can result from reduction in foraging rates (Eldakar, Dlugos, Wilcox, & Wilson, 2009; Rowe, 1992). This could lead to substantial fitness costs if females encounter many males or are paired with males for an extended duration, because females will have less energy to allocate to reproductive output. Yet, most past research on male mate guarding behaviour has focused on costs to males (Alberts, Altmann, & Wilson, 1996; Schubert, Schradin, Rödel, Pillay, & Ribble, 2009; Sparkes, Keogh, & Pary, 1996), while less work has examined costs to paired females (Amano & Hayashi, 1998; Rowe, 1992).

In Chapter 1, I report that pairs remained and dispersed together for extended periods in the mixed-sex population. I therefore wondered whether females' foraging behaviour was hindered due to mate guarding behaviour by males. Mate guarding is a common behaviour in phasmids where males cling to the female for extended durations ranging from days to months (Bedford, 1978). Investigating the economics of mate guarding behaviour can provide an opportunity to examine whether the interests of the paired females and males are in conflict. It is generally thought that mate guarding ensures a male's paternity by preventing the female from mating with rivals (Alberts et al., 1996). For males, mate guarding is subject to a trade-off between energetic costs and successful fertilisation but is expected to have net positive effects on male fitness through enhanced fertilisation success (Alberts et al., 1996; Alcock, 1994; Parker, 1974). However, if male guarding behaviour is costly for females, females might be selected to escape from males and disperse further away, thereby potentially establishing new female-only populations in the wild. Over many generations, if there are high costs of mating for females, selection will favour any kind of strategy that allows females to reduce those costs, including by increasing dispersal to avoid males.

Several studies have examined sexual conflict between paired individuals and found that mate-guarding can impose energetic costs on females (Arnqvist, 1989; Jormalainen, Merilaita, & Riihimäki, 2001). Guarded females can also be exposed to injury or stress (Dunbar, 1987) due to rival males attempting to dislodge the original male (Kelly, 2014) which may affect guarding females' dispersal and foraging activities (Arnqvist, 1989; Rowe, 1992). In Chapter 2, I aimed to examine whether guarding males or presence of males impose costs by reducing females' foraging behaviour. Due to males' presence or guarding behaviour, females may exhibit reduced foraging due to carrying males as an extra weight. My results suggest that guarding males can reduce guarded females' foraging rates in some circumstances, but do not appear to affect female foraging in other circumstances.

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CHAPTER ONE

ADULT AND JUVENILE DISPERSAL IN THE FACULTATIVELY PARTHENOGENETIC PHASMID *MEGACRANIA BATESII*

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ABSTRACT

In many facultatively parthenogenetic animals, populations exhibit sex ratio variation where reproduction is typically sexual (mixed-sex population) or typically parthenogenetic (all-female population). Sex-biased dispersal could contribute to such spatial variation in population sex ratio and reproductive mode, but dispersal patterns are poorly known in such species. We asked whether sex-biased dispersal occurs in *Megacrania batesii*, a facultatively parthenogenetic phasmid that occurs in mixed-sex populations and all-female populations in far-north Queensland, Australia. If sex-biased dispersal contributed to sex-ratio variation in *M. batesii* then we would expect to see greater dispersal by adult or juvenile females than by males. To address these questions, we carried out a mark-recapture field study over two weeks to estimate mean and maximum nightly dispersal distances for adult females and males in a mixed-sex population and for females in an all-female population. We also investigated dispersal by hatchling nymphs in the laboratory. We found no difference in mean nightly dispersal distance between unpaired males and unpaired females from the mixed-sex population. However, we found that unpaired females from the mixed-sex population dispersed greater distances than females from the all-female population. Because resighting probability was significantly lower for the all-female population when compared to the mixed-sex population, more research is needed to verify this result. We also found that paired females tended to disperse shorter distances than unpaired females in the mixed-sex population, suggesting that guarding males could impede female dispersal. We did not find any substantial dispersal by hatchlings in the laboratory study. Our results suggest that sex-specific dispersal is unlikely to contribute substantially to variation in sex ratio among populations of this species.

INTRODUCTION

The role of dispersal in shaping spatial sex ratio variation in facultatively parthenogenetic species remains poorly understood. In facultatively parthenogenetic animals, females can switch between asexual and sexual reproduction depending on whether they mate. This plastic reproductive capacity can result in natural populations of mixed-sex and all-female composition that co-exist within the same range in close proximity. This is because facultative parthenogenetic systems allow mated females to generate both sons and daughters from sexual reproduction and only daughters from asexual reproduction (Bedford, 1978; Galis & van Alphen, 2020). When investigating spatial variation in sex ratio, theoretical and empirical studies have tended to focus on the phenomenon of geographical parthenogenesis, where distinct asexual and sexual lineages occupy distinctive habitats, typically along a geographical gradient (Chaplin & Ayre, 1997; Glesener & Tilman, 1978; Vandel, 1928). Asexual lineages tend to inhabit previously disturbed areas, occur at higher altitudes or latitudes, and exhibit more widespread distributions when compared to sexual lineages (Glesener & Tilman, 1978; Michael Kearney, 2005; Michael Kearney, Blacket, Strasburg, & Moritz, 2006; Vandel, 1928). Such expansion of geographical range by asexual lineages has been attributed to the ability to reproduce via asexual reproduction, which allows asexual populations to increase more rapidly (Glesener & Tilman, 1978; Hörandl, 2006). Genetic explanations for geographic parthenogenesis, such as hybridization (Michael Kearney, 2005; Michael Kearney et al., 2006; Wright & Lowe, 1968) and polyploidization (Otto & Whitton, 2000), have been put forward as potential mechanisms that may enable asexual lineages to perform better in harsh environments than their sexual relatives (Peck, Yearsley, & Waxman, 1998).

While most studies have focused on the spatial distributions of reproductively isolated sexual and asexual lineages, explaining the demographic patterns of facultatively parthenogenetic species has received far less attention. Few studies have documented spatial variation in the sex ratio of facultatively parthenogenetic species (Buckley, Marske, & Attanayake, 2009; Morgan-Richards, Trewick, & Stringer, 2010). For example, the New Zealand stick insect, *Clitarchus hookeri*, exhibits geographical patterns where mixed-sex populations are mainly distributed on the north island whereas all-female populations are mainly found on the south island (Morgan-Richards et al., 2010). Classic ecological explanations have suggested several factors that may contribute to such sex ratio variation, including predation and competition with other species (Merilä & Wiggins, 1995; Post & Götmark, 2006), cyto-parasites that kill or feminize males (Perez-Ruiz, Martinez-Rodriguez, Herranz, & Bella, 2015), and sex-biased

dispersal (Chaplin & Ayre, 1997). Dispersal can have a particularly profound effect on sex ratio variation (Gaines & McClenaghan, 1980). Unfortunately, the role of dispersal in contributing to sex ratio variation in facultatively parthenogenetic species has been overlooked, and there has been little research on adult dispersal ability in facultative parthenogenetic species in their natural habitats. Understanding effects of dispersal ability in facultatively parthenogenetic species is important because a single egg or unmated female can establish an all-female population (Baker, 1967). Conversely, invading males can change all-female populations into mixed-sex populations by mating with the females, leading to the production of sons and ultimately replacing asexual reproduction with sexual reproduction.

In this study, we examined whether dispersal rate of females and males could help to explain the sex ratio variation of a facultatively parthenogenetic organism by clarifying whether one sex disperses over longer distances than the other in their natural environment. The facultatively parthenogenetic Australian stick insect, *Megacrania batesii*, is a suitable model to investigate how dispersal might contribute to variation in sex ratio. Cermak and Hasenpusch (2000) reported that mixed-sex *M. batesii* populations exist north of the Daintree River while all-female populations occur further south. Further research has shown that *M. batesii* populations also vary in sex ratio north of the Daintree River, where multiple mixed-sex and all-female populations coexist side-by-side between Cape Tribulation and Cape Kimberley, Queensland (Bonduriansky & Burke, unpublished data). It is unclear how all-female populations are established or why males are not able to invade these all-female populations given that these populations occur in close spatial proximity and with few obvious barriers to dispersal. Mark-recapture is a common method for investigating dispersal in wild animals (Auckland, Debinski, & Clark, 2004). However, mark-resighting studies have never been performed in *M. batesii* to our knowledge. We used this method to address three main aims: (1) to measure and compare nightly dispersal distances of adult females and males in a mixed-sex population, (2) to compare the dispersal rates between females in an all-female population and mixed-sex population, and (3) to determine whether guarding males affect female dispersal. If dispersal contributes to the establishment of all-female populations in *M. batesii*, then we would expect to observe greater dispersal by single females than by males or female-male pairs.

In general, dispersal in insects can occur at different life stages: egg, nymph, and/or adult (Auckland et al., 2004; Keller, Johnson, Uyi, Wurzbacher, Long, & Hoover, 2020; Stanton, Dias, & O'Hanlon, 2015). Sex-specific dispersal has been reported in some insects at the adult stage (Asplen, Chacón, & Heimpel, 2016; Auckland et al., 2004). However, in some hemipterans, juvenile stages can be more mobile and dispersive (Keller et al., 2020; Lee,

Nielsen, & Leskey, 2014) and could therefore contribute to sex-specific dispersal. In some phasmids, where females flick their eggs passively from their ovipositor dropping the eggs on the ground or attach their eggs to plants (Bedford, 1978; Robertson, Bradler, & Whiting, 2018), dispersal can occur at the nymphal stage as hatchlings seek a suitable host plant to inhabit (Zeng, Chang, Williams, Nguyen, Tang, Naing, Kazi, & Dudley, 2020). But whether dispersal differs between the sexes at the nymphal stage is still unknown, especially in the wild. A few studies have reported hatchling dispersal of phasmids in the laboratory. Zeng et al. (2020) have described several active movement strategies by hatchlings of the stick insect, *Extatosoma tiaratum* (Macleay 1826) under laboratory conditions. We therefore also investigated the movement patterns of *M. batesii* hatchlings in the laboratory to determine whether nymphs are dispersive and whether this dispersal is sex-specific. Our findings provide initial answers to two main questions: does sex-specific dispersal occur in *M. batesii* hatchlings or adults, and can sex-specific dispersal explain sex ratio variation?

METHODS

Species description

Megacrania batesii Kirby (Phasmatodea: Phasmatidae), commonly known as the peppermint stick insect, has been recorded from several South Pacific islands and from northern Australia (Rentz, 1993). The body length ranges from 72-87 mm in males, and 101-102 mm in females (Brock & Hasenpusch, 2009). Both sexes have the same bluish-green colour (Brock & Hasenpusch, 2009) and neither sex can fly, although males are longer-winged whereas females have reduced wings (Cermak & Hasenpusch, 2000). Since *M. batesii* feed and live only on *Pandanus* and *Benstonea* plants from the family *Pandanaceae*, they are highly dependent on these plants (Cermak & Hasenpusch, 2000). Members of this plant family are commonly distributed along the coastal region of Queensland, Australia (Gallaher, Callmander, Buerki, & Keeley, 2015). *Pandanus tectorius* Parkinson and *Benstonea monticola* Callm. & Buerki (*Pandanaceae*) are dioecious plants with spirally-arranged long, spiky leaves (Toman, 2017). The stick insects usually leave distinctive chewing marks (longitudinal scars) on the leaf margin which helps in locating individuals and populations in the wild (Cermak & Hasenpusch, 2000). The stick insects are usually found in the groove that runs along the centre of the leaves, where they cling by stretching their forelegs and mid legs forward and their hind legs backwards during daytime (Fig. 1). This species appears to be almost sedentary during the day and becomes more active at night (JB, personal observation). This species is facultatively parthenogenetic: females can produce eggs both asexually (if they do not mate) and sexually (if they mate). Fertilized eggs hatch into females and males in equal sex ratio while unfertilized eggs all hatch into females (Bonduriansky et al., unpublished data).

Figure 1. Marked *M. batesii* individuals on their host plants. Left image: a marked female at Cow Bay Beach; Right image: marked female-male pair at Coconut Beach.

Study area and mark-resighting methodology

This study was conducted in the far-north region of Queensland, which includes Australia's largest area of tropical rainforest (Fig. 2). In this area, annual mean rainfall is approximately 2114.8 mm, and average maximum temperature is 29.4ºC (Bureau of Meteorology, 2020). During our field study in February 2020, almost no rain was observed and average daily maximum temperature was ~34.9ºC (Bureau of Meteorology, 2020). Australian *Megacrania batesii* populations are patchily distributed along the coast, primarily between Cape Tribulation and the Daintree River (Cermak & Hasenpusch, 2000). We carried out this study in February because adults reach high numbers from December to March (Cermak & Hasenpusch, 2000). In selecting the study sites, we chose *Pandanus* and *Benstonea* patches that were easy to access along beachfronts, including a mixed-sex population at Coconut Beach (~2.7 km south of Cape Tribulation village) and an all-female population at Cow Bay Beach (east of Cow Bay village). The road distance between these patches is around 28 km and the linear distance is 16 km. In terms of habitat type, the mixed-sex population in Coconut Beach occupies small *Pandanus tectorius* plants in beachfront rainforest at the side of a stream, whereas the all-female population at Cow Bay Beach occupies *Benstonea monticola* plants in a swampy beachfront rainforest area along a stream (Fig. 3). The study area (where individuals were marked and resighted) covered approximately 1196 m^2 at Cow Bay Beach and approximately 3745 m^2 at Coconut Beach.
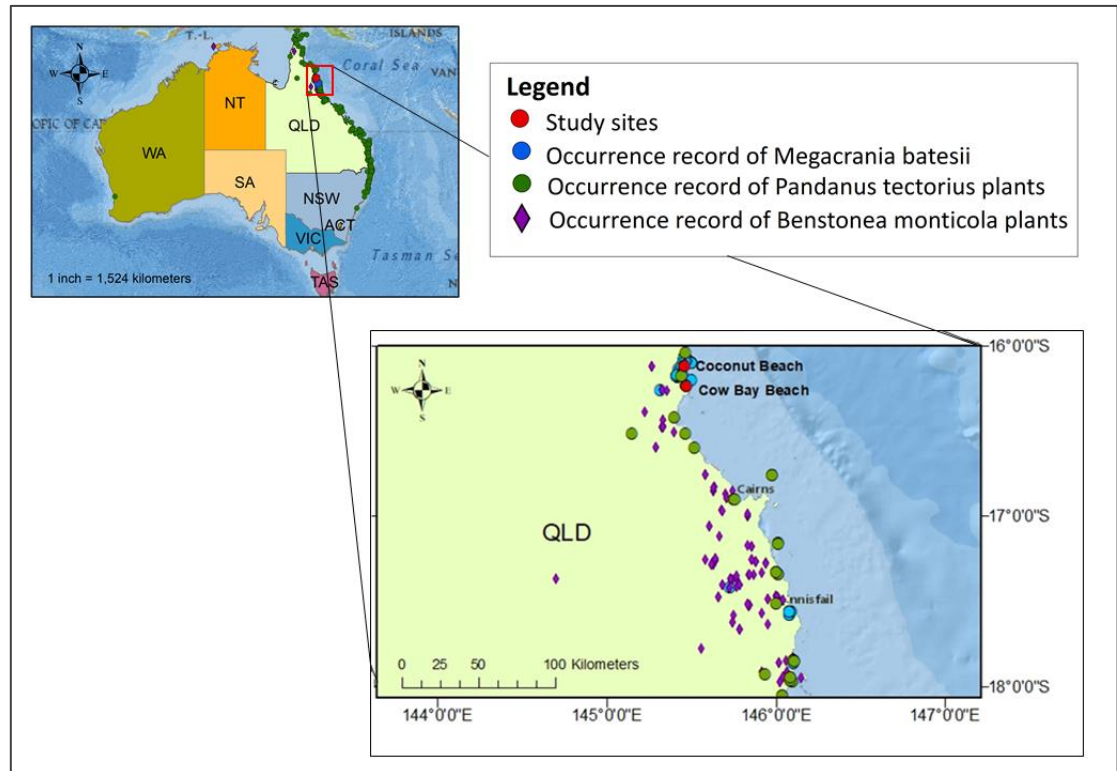
Figure 2. Distribution of *Pandanus tectorius* and *Benstonea monticola* in far-north Queensland, and a previous occurrence of *M. batesii* based on existing records from (Atlas of Living Australia, 12 April 2020a, 12 April 2020b). Our mark-resighting study sites are indicated by red circles.

Figure 3. Left image: *Pandanus tectorius* plants at Coconut Beach. Right image: *Benstonea monticola* plants at Cow Bay Beach. The plants at our study sites were tagged with numbered plastic key tags as shown in the images.

Macro-arthropod community on host plants

Pandanus and *Benstonea* plants not only provide habitat and food sources for *M. batesii* but also contain a range of other arthropod species that might prey on or compete with *M. batesii*. Therefore, while carrying out the mark-resighting study, we also recorded and collected voucher specimens of these other common, large-bodied arthropods on *Pandanus* and *Benstonea* host plants at the two locations as a preliminary assessment of the communities of macro-arthropods that might influence *M. batesii* populations. The arachnid samples were imaged through a microscope (Leica MS5) and identified by Dr. Graham Milledge (Australian Museum Research Institute). Most of the spiders that we collected were at juvenile stages, and therefore could only be identified to genus level. The orthopterans and cockroaches were examined and identified by Dr. David Rentz.

Mark-resight study

Within each population (Coconut Beach and Cow Bay Beach), we used a mark-resighting method to track movements of *M. batesii* individuals. Newly sighted individuals were marked with unique numbers either on their thorax or wings or both (Fig. 1). We marked individuals with permanent marker in situ on the host plants, without handling or holding them. Both males and females can spray a defensive fluid when disturbed (Cermak & Hasenpusch, 2000), which can make marking difficult. If a spraying response occurred, we waited a few minutes and then made another attempt at writing the same number on the forewings. During the marking process, if the permanent marker tip was sprayed with fluid, we wiped it dry with a tissue. When an individual was found at the base of a plant, we gently tapped the tip of its abdomen to trigger it to crawl upwards to become accessible on the leaves for marking. This method allowed us to mark the stick insects easily while they were sitting on the upper side of the leaves. Slightly different marking techniques were used in marking unpaired and paired individuals. Unpaired individuals were usually marked while sitting on the *Pandanus* leaves. But, for paired individuals, on some occasions, the males' antennae were blocking the female's thorax. In this situation, male's antennae were pushed to the side while marking the female thorax or, in some cases, the male was moved gently to the side or backwards to expose the female's thorax.

After the first day of marking, we checked daily for marked individuals for the first three days (from 2nd to 4th of February 2020) and then resighting data were collected every second day thereafter (from 4th to 14th of February 2020). At each sampling event, we checked for previously marked individuals on each plant (absence or presence), recorded the dispersal distance between the previous sighting location and new location, and pairing state (unpaired, paired with an individual of the opposite sex). On each occasion, we also marked any new

individuals that we found but that had not been marked before. We collected the Global Positioning System (GPS) coordinates of each individual at its point of capture/resighting using a Garmin eTrex 20x GPS unit. In addition, we tagged the host plants at the two study sites using numbered plastic key tags (removed at the end of the study) which allowed us to measure the accurate linear distance (using a tape-measure) between the previous plant where the individual had been encountered and the latest plant where the marked individual was found.

Nightly dispersal distance, displacement, and displacement ratio

We calculated mean nightly dispersal distance for all individuals and for each population by dividing the total dispersal distance (sum of distances) by the number of nights between that individuals' initial and final observations. We also calculated the mean nightly displacement for each individual as the linear distance in cm between the initial location where individuals were first sighted and the plant where that individual was last resighted divided by the number of nights between that individuals' initial and final observations. The linear displacement distance between the initial and final sighting locations was obtained using the distance measuring tool in ArcGIS software (ArcMap 10.6). Because we are dealing with small distances and GPS locations are not precise on that scale, our displacement distance estimates are only approximate. To examine the extent to which individual dispersal is directional, we also calculated the displacement ratio as the total displacement (distance between first land last locations sighted) divided by the total dispersal distance (sum of all nightly dispersal distances) (Fig. 4). The displacement ratio ranges between 0 and 1 (although some calculated values slightly exceeded 1 because of GPS location inaccuracy), we therefore changed such displacement values to 1. If an individual ultimately returned to the same plant where it was first sighted, the displacement ratio for that individual would equal 0. Conversely, if an individual moved in a straight line away from the plant where it was initially sighted, the displacement ratio would equal 1 (Turchin, Odendaal, & Rausher, 1991). Values between 0 and 1 therefore indicate that individuals are moving further from their initial location over time.
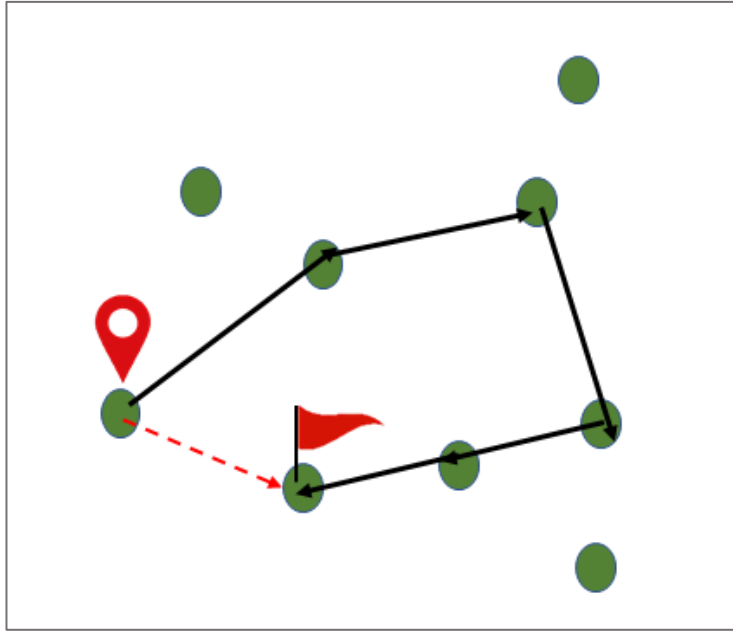
Figure 4. A diagram showing the method used to calculate individuals' dispersal distances and displacement distance between the host plants (green circles). The location where an individual was first seen is indicated by the red pointer whereas the location where the individual was last resighted is indicated by the red flag. The black arrows show nightly dispersals between host plants. The total dispersal distance is the sum of lengths of all the black arrows, the total displacement is the length of the red dashed arrow, and the displacement ratio is the length of the red dashed arrow divided by the length of the total black lines. To obtain mean nightly dispersal and displacement distances, the total dispersal and total displacement were divided by the number of nights between the initial and final observation for each individual.

Statistical analyses

To estimate population size at Cow Bay Beach and Coconut Beach, we used the Rcapture package in R version 4.0.4 (R Core Team, 2021), based on loglinear models that assume a closed population (Rivest & Baillargeon, 2019). For each individual, resighting history was represented as "0" if absent or not-resighted and "1" if marked or resighted on each occasion. Since we had a closed population data set, we used the function "closedp" in estimating population size at both sites (Table S2-S3). The Rcapture package compares several population size models based on the Akaike Information Criterion (AIC).

With regards to dispersal distance travelled per night, as our data contain two independent groups with small sample size and the distribution of dispersal distances does not follow a normal distribution, we compared nightly dispersal distances between groups using a nonparametric test (Mann-Whitney U test) in R version 4.0.4 (R Core Team, 2021). This test was used to conduct pairwise comparisons of groups defined by a categorical grouping variable

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(all-female vs. mixed-sex population; unpaired vs. paired individuals; females vs. males). The dependent variable was individual mean nightly dispersal distance and displacement. Descriptive statistics for the various groups are provided for nightly dispersal distance and displacement (Table 1-2). When calculating the mean and standard deviation among individuals, we excluded individuals that were never resighted after marking.

Nightly dispersal distance and displacement were visualised in R using violin plots. To compare females from the all-female population at Cow Bay Beach and the mixed-sex population at Coconut Beach, we used single females from Cow Bay (n = 9) and females' dispersal distances while unpaired from Coconut Beach (n = 8). Individuals at Coconut Beach were categorised into three groups: unpaired females, unpaired males, and female-male pairs. Pairs (n = 8) and unpaired females from the mixed-sex population (n = 8) were compared to determine whether there was any impact of male presence on female dispersal. We excluded one pair that was seen for the first time at the end of our study. We also compared unpaired females and males in the mixed-sex population (n = 8 females, n = 7 males). One other unpaired adult male was only found at the end of the study, and therefore excluded from analyses.

Experiment on dispersal of hatchling nymphs

We observed dispersal in hatchlings of *M. batesii* from 25th of July to 28th of August 2020. We used newly hatched nymphs from eggs collected in the field at seven locations between Cow Bay and Cape Tribulation, Queensland, in February 2020. Each day, newly hatched nymphs were checked and sexed (based on the morphology of the terminal abdominal sternites) during the study, marked individually with distinctive patterns of coloured dots using permanent marker, and released on *Pandanus tectorius* plants distributed on the floor of a controlled-temperature (CT) room (maintained at ~25 ± 2°C and ~60 ± 20% relative humidity). Six *Pandanus* plants (40 – 60 cm in height) were placed in the CT room, with one plant in each corner and two plants in the centre (Fig. 5). The hatchlings were added sequentially and randomly on the plants. In order to avoid creating a high density of hatchlings on one plant, the hatchlings were distributed evenly among the six Pandanus plants in the CT room. Each morning, we recorded the locations of all hatchlings in the CT room. In total, we marked and released 55 hatchlings (39 females, 16 males). We observed each of these newly hatched nymphs over 10 ± 2 days.
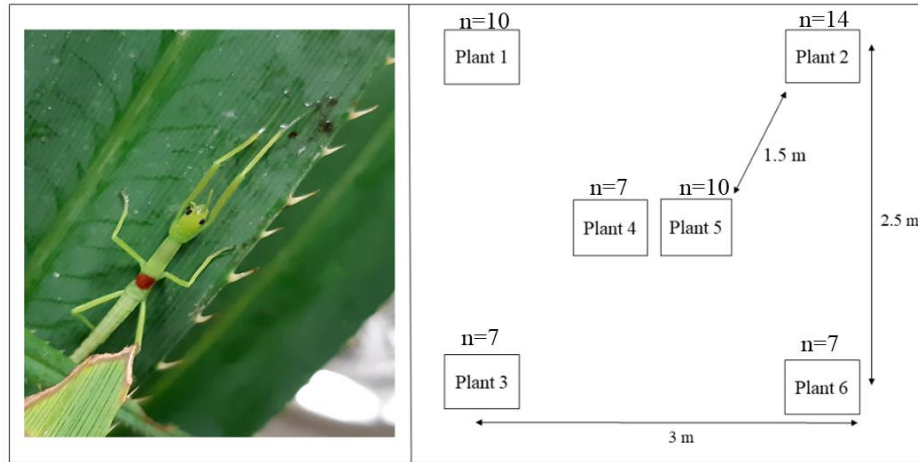
Figure 5. Hatchling dispersal experiment: Left image: Hatchling marked with a red dot; Right image: distribution of *Pandanus* plants in the controlled-temperature (CT) room. The total number of hatchlings introduced to each plant is indicated above each box.

RESULTS

The most common macro-arthropods that we found and collected from the plants that stick insects occupied were spiders (Araneidae, Salticidae, Tetragnathidae), crickets and grasshoppers (Gryllidae, Gryllacrididae, Acrididae, Tettigoniidae) and cockroaches. The macro-arthropod community associated with the all-female population at Cow Bay Beach was dominated by orthopterans, while that of the mixed-sex population at Coconut Beach was dominated by spiders (Table S1). Also, I did not witness any behavioural interactions between these arthropods and *M. batesii* individuals.

Over the two weeks of this study, we sighted and marked a total of 18 single females (N = 15 female adults; N = 3 final-instar female nymphs) from the all-female population at Cow Bay Beach, and 22 individuals (N = 11 females N = 11 males) from the mixed-sex population at Coconut Beach. Of these individuals, 66% were resighted at least once at Cow Bay Beach and 100% were resighted at least once at Coconut Beach. Population size at each location was estimated from mark-resighting data. At Cow Bay Beach, the Mbh model yielded the lowest AIC value, and provided an approximate population size of 40 ± 90.1 ($\pm$ SE). At Coconut Beach, the $M_{h\ Darroch}$ model yielded the lowest AIC value, and provided an approximate population size of 23 ± 1.7 (Table S2-S3; Fig. S1-S2).

Movement patterns at Cow Bay Beach (all-female population)

Nine single females (F1-F9) at Cow Bay Beach moved between neighbouring (often entangled) host plants but were rarely observed dispersing over longer distances (Fig. 6). Only three other females (F10, F11, F12) remained throughout the study on the same plants where they were marked, and six females (F13-F18) were never resighted after marking. We found that the longest mean nightly dispersal distance of a single female (F1) was 1 m and the highest maximum dispersal distance over a single night by a single female was 115 cm (F3) at Cow Bay Beach (Fig. 6).
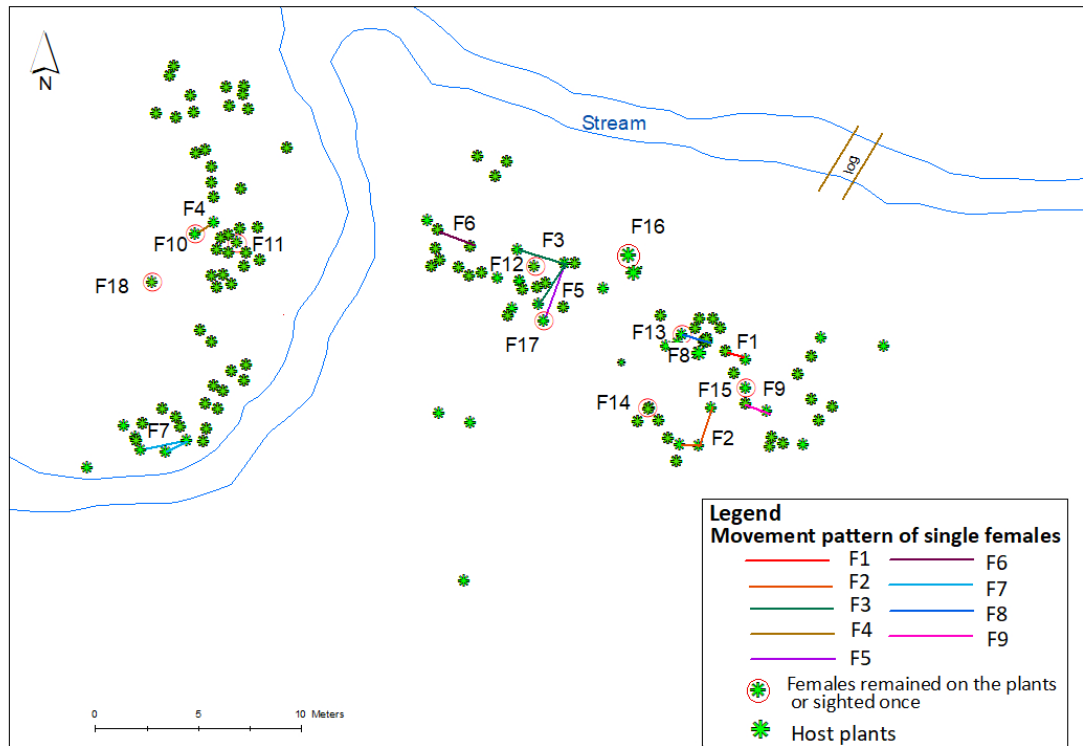
Figure 6. Dispersal patterns of females in the all-female population at Cow Bay Beach. The females that moved between plants (n = 9) are represented by coloured thick lines. Green small stars represent distribution of *Benstonea monticola* plants in the patch. Green small stars surrounded by red circles represent locations of six other females (F13-F18) that were never resighted after the initial sighting, females (F10, F12) that remained on the host plants over two nights, and a female (F11) that remained at the same location over nine nights of observation.

Movement patterns at Coconut Beach

In the mixed-sex population at Coconut Beach, we found that females, males, and pairs frequently dispersed between host plants, including non-neighbouring plants.

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The highest maximum dispersal distance over a single night by an unpaired female was 9 m (female F26), and the highest maximum dispersal distance over a single night by an unpaired male was 8 m (male M11). The highest maximum dispersal distance travelled over a single night by a pair was 3 m (pair C). As for the average dispersal distance travelled per night, the longest in unpaired males was 8 m (M2), the longest in unpaired females was 5 m (F26), and the longest mean nightly dispersal by a pair was only 160 cm (pair E).

Paired individuals' behaviour and mate switching

The site at Coconut Beach consisted of three smaller patches of *Pandanus tectorius* plants (southern, middle, and northern), and stick insects mostly dispersed within each of those patches over the two weeks of the study. Paired females dispersed by carrying male (which is smaller and guards the female by sitting on her back, with or without clasping the underside of her abdomen with his genital claspers). We resighted a total of 8 female-male pairs during our study, all consisting of adult individuals engaged in mate guarding and copulation. Among these, we observed several transitions from pairs to single individuals and vice versa (Fig. 7).

The southern patch contained six female-male pairs (A, B, C, E, F, H) and five unpaired males (D, G, O, P, Q). While five pairs remained together (A, C, H, I, F) over the two weeks of observations (i.e., no male switching), one female-male pair (B) switched males. The female (F20) from pair B was found paired with a new male (male M12). This new male dispersed about 12 m over the two nights to find the female on our last observation day. The original male (male M2) of pair B travelled around 16 m over two nights after separating from the female (female F20) and was found unpaired on our last observation day. Female F22 from pair E was not found on the last observation day but her former male partner was found individually on that day. Pair H (M7 + F24) dispersed from the middle patch to the southern patch, while all the pairs marked in the southern patch stayed within that patch throughout the study.

The middle patch initially contained two unpaired females, female F25 (line I in Fig. 7) and female F29 (line N in Fig. 7) and one unpaired male (line J in Fig. 7). That male M9 (line J) dispersed about 9 meters over four nights and was then found paired with female F25 (pair I in Fig. 7).

In the northern patch, we observed two unpaired adult females (L, M in Fig. 7) and one pair K (M10 + F26). Pair K was initially found as a pair and stayed together over four nights but then separated and female F26 dispersed ~18 meters over two nights and remained unpaired till the last day of observation whereas male M10 was never resighted after separating from the female.

Also, in this northern patch one male remained unpaired on the same plant where he was first marked throughout the study (M14).
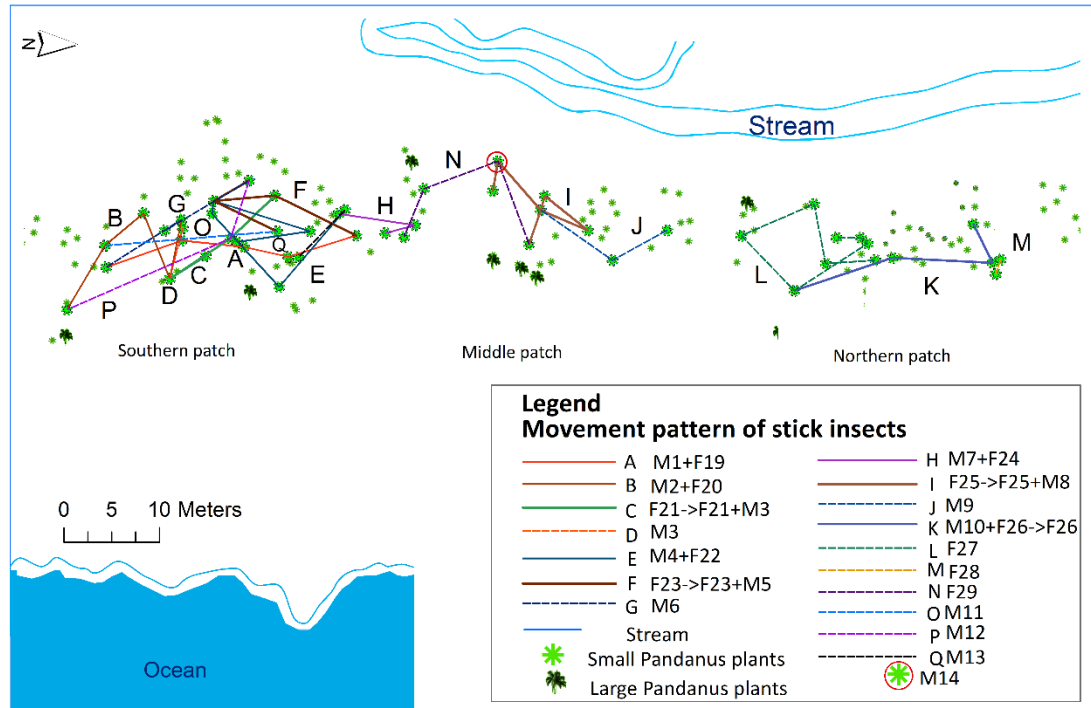

Figure 7. Dispersal patterns of individuals and pairs (A-Q) at Coconut Beach. Female-male pairs' dispersal is shown with thick lines whereas unpaired individuals' dispersal is shown with dashed lines. The letters (M, F) with numbers next to the letters (A-Q) in the legend indicate individuals' codes for female-male pairs vs. unpaired females and males. Arrows between the symbols indicate that transitions between unpaired and paired or vice versa. Green small stars indicate that host plants and a green star surrounded by a red circle is a male remained on the same host plant throughout the study (male M14).

Comparison of dispersal distances

Nightly median distances travelled by individuals and pairs are shown in Fig. 8. We found that unpaired females from the mixed-sex population dispersed further than single females from the all-female population (Mann-Whitney U test: $W = 8$, $p = 0.008$; Table 1; Fig. 8).

The female-male pairs dispersed shorter distances than unpaired females within the mixed-sex population (Mann-Whitney U test: $W = 9$, $p = 0.01$; Table 1; Fig. 8). We did not detect a difference in nightly median dispersal distance between unpaired males and unpaired females from the mixed-sex population (Mann-Whitney U test: $W = 21$, $p = 0.45$; Table 1; Fig. 8), although this might reflect the low sample size resulting from the small numbers of unpaired adult individuals in the mixed-sex population.
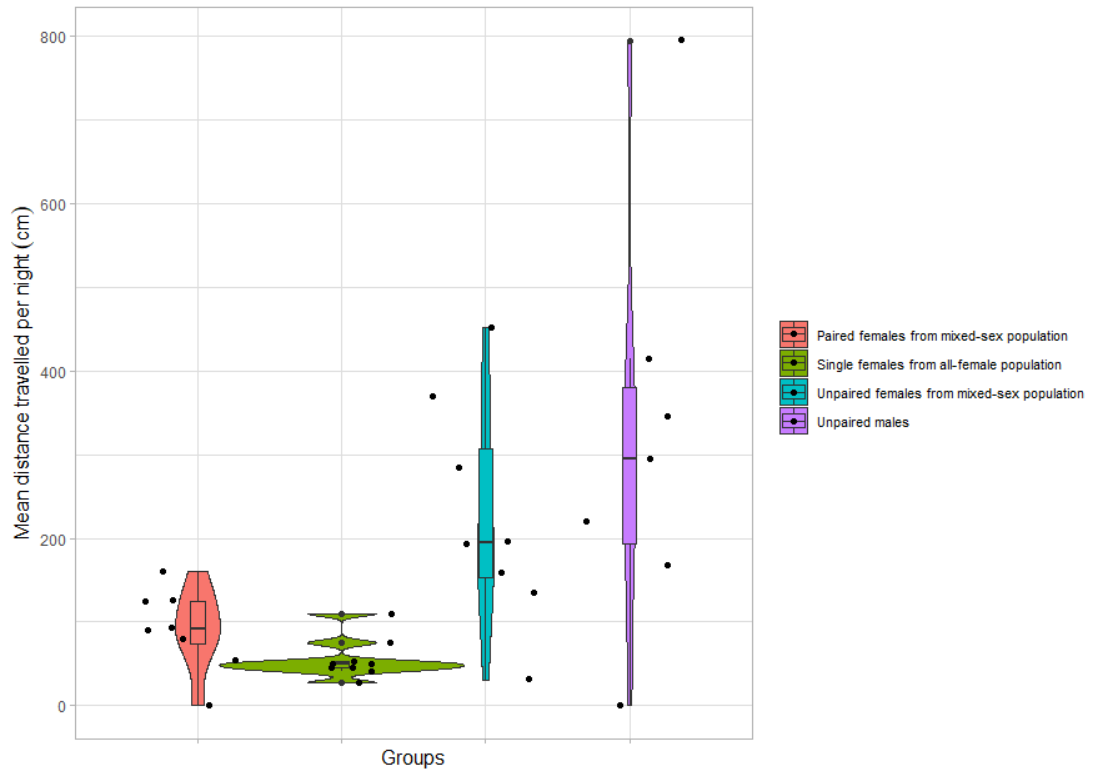
Figure 8. The violins show the distribution of the data within each group. The y-axis shows distance moved per night (cm). The centre black line inside each boxplot shows the median, and the box inside the violin shows the inter-quartile-distance and the whiskers (the vertical thin lines) show the range of values outside the inter-quartile range. Black dots represent nightly mean dispersal distances for individuals or pairs.

Table 1. Descriptive statistics for nightly dispersal distance (cm) for *M. batesii* individuals at Cow Bay Beach (all-female population) and individuals and pairs at Coconut Beach (mixed-sex population). N = Sample size, SD = Standard deviation, SE = Standard error.

Groups	N	Mean	SD	Median	Min	Max	SE
Single females from all-female population	9	55	24	50	27	110	8
Paired females from mixed-sex population	8	91	49	92	0	160	17
Unpaired females from mixed-sex population	8	228	135	194	31	452	48
Unpaired males	7	320	249	295	0	795	94

Displacement distance

Displacement provides information on how far each individual or pair moved from the initial sighting location during our study. We found that maximum displacement observed between the initial and final locations over the duration of the study by a female individual was approximately 19 m over ten nights (female F26, indicated by line K in Fig. 7) and maximum displacement distance travelled by a male was 23 m over four nights (M12, indicated by line P in Fig. 7). The resighted single females at Cow Bay Beach exhibited a tendency to remain on the same host plant and maximum displacement was only 3 m at this location. Mean nightly displacement (i.e., total displacement divided by the number of nights between the first and last observation) for individuals in the various groups is shown in Fig. 9. We found that paired females from the mixed sex population exhibited a greater mean nightly displacement than single females from the all-female population (Mann-Whitney U test: $W = 67$, $p = 0.003$; Fig. 9; Table 2). There was no sex-specific displacement found between the unpaired sexes in the mixed-sex population (Mann-Whitney U test: $W = 17$, $p = 0.2$; Fig. 9; Table 2), although the highest displacement value was by unpaired males.

Ratio of mean nightly displacement to mean nightly dispersal distance was calculated for each individual. A ratio of zero indicates that individuals ultimately return to the location of origin, whereas a ratio of one indicates that individuals move linearly away from the location of first sighting. The mean displacement ratio was 0.7 at Coconut and 0.6 at Cow Bay Beach. This suggests that, at both locations, individuals gradually move away from the location of first sighting over time, perhaps by moving semi-randomly through their habitat. They do not appear to be travelling in a specific direction, but rather tend to move back and forth between a set of adjacent plants (see Fig. 6, 7).
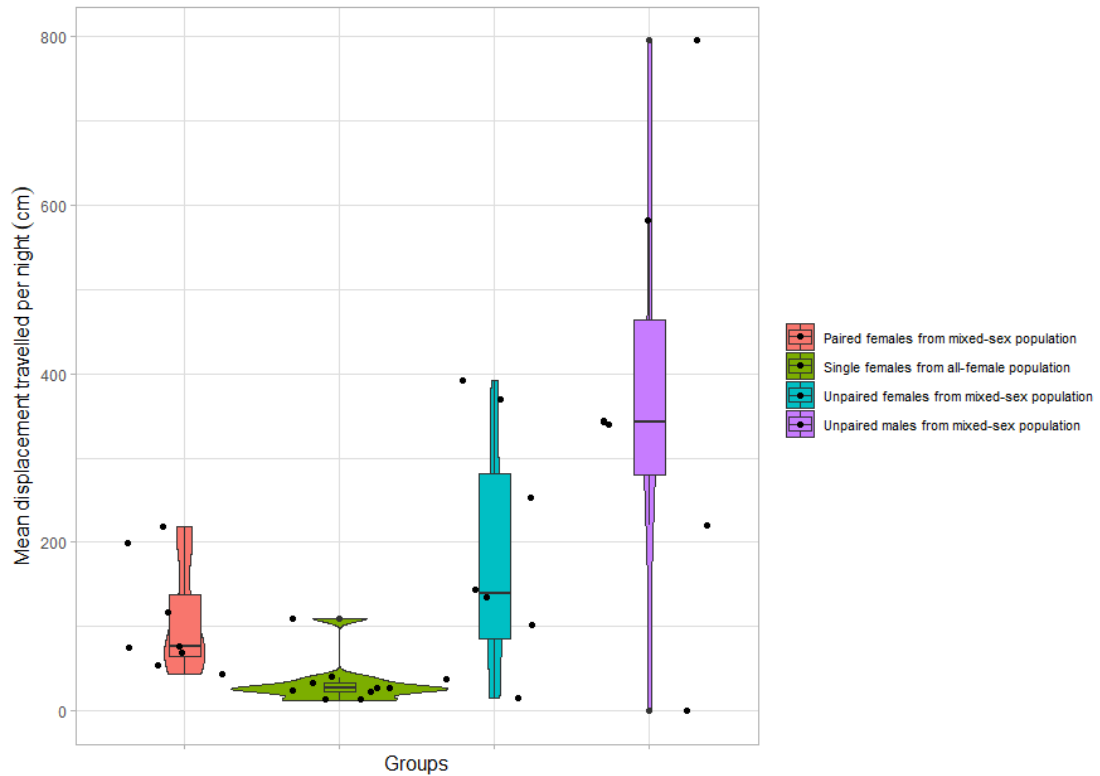
**Figure 9.** Displacement per night away from the location of first observation. The violins show the distribution of the data within each group. The y-axis shows displacement per night (cm). The centre black line inside each boxplot shows the median, and the box inside the violins shows the inter-quartile-distance and the whiskers (the vertical thin lines) show the range of values outside the inter-quartile range. Black dots show nightly mean displacement distances for individuals or pairs.

Table 2. Descriptive statistics for nightly displacement distance (cm) and displacement ratio for groups. N = sample size, SD = standard deviation, SE = standard error, and D = displacement ratio.

Groups	N	Mean	SD	Median	Min	Max	SE	D
Single females from all-female population	9	35	30	27	13	110	9.9	0.6
Paired females from mixed-sex population	8	107	67	76	44	219	23.6	0.7
Unpaired females from mixed-sex population	8	181	143	140	16	392	50.5	0.7
Unpaired males	7	375	254	343	0	795	96	0.9

Dispersal by hatchlings in the laboratory

In the laboratory, hatchlings were observed within the plants but only three hatchlings (n = 2 males, n = 1 female) dispersed to neighbouring plants. A male hatchling (M14) dispersed 1.5 m to a neighbouring plant two days after being released into the temperature-controlled room. Another male hatchling (M54) dispersed 10 days after being released and was found off the plant. A female hatchling (M49) dispersed 1.5 m to a neighbouring plant eight days after release. Remaining individuals (n = 52 nymphs) stayed on the same plants where they were released (10 ± 2 days). We did not detect any reluctance by the former or latter introduced hatchlings in sharing the same host plant; dispersal between plants was uncommon in all cases.

DISCUSSION

We posed the questions of whether sex-specific dispersal could contribute to the establishment of all-female populations in the facultatively parthenogenetic stick insect, *Megacrania batesii*. In particular, if single females disperse greater distances or more directionally than males or paired females, dispersal by unmated females or nymphs to new habitat patches could lead to the establishment of all-female populations and thus potentially contribute to observed sex-ratio variation in this species. We found no strong evidence of differences in dispersal between unpaired females and males in the mixed-sex population. Importantly, we found no evidence that adult females or female nymphs are more dispersive than males. Single females in the mixed-sex population dispersed greater distances than pairs on average, suggesting that unmated females might occasionally disperse to new habitat patches. However, single males dispersed similar distances to single females. It is likely that *M. batesii* individuals occasionally leave their habitat patch and traverse terrain devoid of host plants, and such rare dispersal events might be important in shaping patterns of sex-ratio variation.

Although we were able to find only a few unpaired males, some of these males were observed to disperse substantial distances, perhaps while searching for females. For example, we found that male M12 dispersed about 12 m over the two nights prior to pairing up with female F20. It is possible that males of *M. batesii* are intrinsically more dispersive than females because of their mate-searching behaviour, but our observations suggest that males' long-distance dispersal might usually be limited to movement within a habitat patch. All marked individuals except for one male were only observed dispersing within their habitat patch, and their movement patterns appeared to involve moving between adjacent or nearby plants rather than moving linearly away

from the point of first sighting. Likewise, females tended to move between plants within patches.

Comparison between paired versus unpaired females in the mixed-sex population suggests that males can reduce females' dispersal. Our observations indicate that males guard females for prolonged durations in the wild, and the extra weight might elevate the energetic costs of movement for guarded females and reduce dispersal rates of females that are carrying males. Such reduction in paired individuals' dispersal has also been found in several studies on other arthropods, such as water striders, *Aquaris remigis* (Fairbairn, 1993) and crustaceans, *Gammarus duebeni*, where paired individuals experience costs associated with weight loading (Naylor & Adams, 1987). Therefore, costs associated with the presence of males may hinder dispersal of *M. batesii* females, and could potentially lead to sexual conflict in this facultatively parthenogenetic species by imposing costs on females (Burke & Bonduriansky, 2017). However, more research is needed to clarify the role of sexual conflict in this species.

The nature and role of sexual conflict is poorly understood in facultatively parthenogenetic animals (although see Burke et al., 2015 etc). This chapter highlighted how *M. batesii* could be used to investigate many aspects of sexual conflict in such a species. To understand establishment of spatial variation in sex ratio in *M. batesii* and other facultative parthenogens, the mating behaviours and costs associated with guarding and mating should be investigated. In particular, it is possible that females may benefit from mating but not from prolonged mate guarding, which might impose a variety of costs by interfering with female foraging and subjecting females to potential injury during take-over attempts by rival males. The *M. batesii* system offers a valuable opportunity to investigate the costs and benefits of mating and mate guarding in both natural populations and controlled laboratory environments.

This study was subject to some limitations, such as small sample size and relatively short duration of sampling in the field. In particular, the small sample of unpaired females and males in the mixed-sex population limited our ability to determine test for sex-specific dispersal in such populations. We checked marked individuals during the day only, even though this species is more active at night. Although we did not see any instances of unpaired females trying to escape from males or attempting to avoid mating in the wild, observations during the night might reveal aspects of sexual behaviour that we did not see during the daytime. In addition, our mark-resighting study was limited to a two-week period, and we were able to mark only a relatively small number of individuals. To clarify whether sexual conflict occurs in this species, further research to examine females' behaviour during encounters with males would be useful. Given the small number of females that switched between paired and unpaired status during the study, we were unable to compare dispersal patterns within individual females. Such an analysis

would provide more power and would be interesting to do in the future. It would also be interesting to remove males from small habitat patches and examine the behaviour of the same females while paired and unpaired.

Our observations also provide some insight on the frequency of take-over behaviour by males in the wild. Male-male combat over females has been observed occasionally in the field in this species (Bonduriansky & Burke, unpublished observations), and could lead to take-overs. However, we observed that seven pairs remained together throughout our two-week study, and only one guarding male was replaced by a different male in the mixed-sex population. It is likely that males guard females for prolonged durations because, if left unguarded, females would remate quickly with other males. Our mark-resighting results and observations therefore suggest that mate-switching and take-overs are relatively infrequent in this species. One paired female switched her initial partner at the end of our study. However, it is not clear how the male (M2) separated from the female (F20) and whether he was displaced by the new male (M12) through a take-over or separated for some other reason from the female (e.g., as a result of predator attack).

We found that single females from the mixed-sex population dispersed greater distances than females from the all-female population. Perhaps encountering males and mating or being guarded is costly for females, and females might disperse to escape from males. However, comparison between the Cow Bay and Coconut Beach populations is complicated by the lower resighting probability for marked females in the all-female Cow Bay population. It is possible that individuals at Cow Bay that we never resighted after marking dispersed long distances and left the habitat patch as has been suggested in other systems (Krebs, 1999), but it is more likely that we simply failed to locate these individuals. The habitat at Cow Bay Beach is covered with taller and denser host plants (*Benstonea monticola*) which might have restricted our resighting ability and therefore biased our observations. If individuals that dispersed longer distances were less likely to be resighted at Cow Bay Beach, then our data would tend to underestimate dispersal of females in that population.

Overall, neither lab-reared hatchlings nor wild-observed nymphs (3 late-instar nymphs marked at Coconut Beach) showed much willingness to disperse based on our field and laboratory studies. In our hatchling laboratory experiment, we found that 95% of all newly hatched individuals remained on the plants where they were initially introduced. Thus, *M. batesii* hatchlings may disperse actively in finding a host plant, but, once they find a suitable plant, they might remain on that plant for an extended period of time. Other studies have found that substantial hatchling dispersal or movement may occur if hatching takes place on the ground,

and that dispersal at this life-stage can be impacted by abiotic factors. An empirical study conducted on another phasmid species, *Extatosoma tiaratum*, under laboratory conditions, found that first instar nymphs actively dispersed by climbing, and that such active dispersal behavior might be associated with finding host plants from the ground where eggs are usually dropped in the wild (Zeng et al., 2020). However, in our study, we deposited newly hatched individuals on host plants instead of on the ground and they may have preferred to remain on the initial plant rather than dispersing to another plant. Such behaviour might help to decrease mortality risk because dispersing might be very dangerous for hatchlings that are small and vulnerable to predators. Each newly hatched individual was monitored for at least 10 days until molting occurred (once molting occurred we were not able to track individuals because their individual markings were shed along with their exoskeletons). It is possible that hatchlings may become more active in dispersing after undergoing several molts. It would therefore be interesting to investigate the dispersal behaviour of older nymphs. Furthermore, the laboratory conditions (temperature, humidity) might not have provided natural conditions and could have affected willingness to disperse. It is possible that juveniles may behave differently in response to external perturbations including windstorms, heavy rain, or temperature variation in the wild. Other studies have suggested that external factors such as weather patterns play an important role in affecting habitat and insect dispersal (Dale, Joyce, McNulty, Neilson, Ayres, Flannigan, Hanson, Irland, Lugo, & Peterson, 2001; McCay, 2003).

A previous study and pilot observations have reported several arthropods, namely huntsman spiders (Sparassidae), jumping spiders (Salticidae), myriapods (Scolopendrida), ants and orthopteroids, that might prey on or compete with peppermint stick insects (Cermak and Hasenpusch, 2000; Bonduriansky & Burke, unpublished data). Generally, predation and competition with other species can have stronger effects on one sex and exclude the other (Merilä & Wiggins, 1995; Post & Götmark, 2006). However, based on our observation these macro-arthropods may not have such influence on *M. batesii* in excluding one sex but not the other, or preying on them, at least at the adult stage. In the wild, there is also a possibility of exposure to other arthropods sharing the same habitat as *M. batesii*, and it is possible that encounters with some species induce *M. batesii* to disperse to other plants. However, we did not observe any behavioural interactions between other arthropods and *M. batesii* individuals inhabiting the same plants at Cow Bay Beach or Coconut Beach. We observed substantial numbers of crickets (Orthoptera: Gryllidae) and spiders (Araneae: Salticidae), which are potential competitors and predators for *M. batesii* (Cermak & Hasenpusch, 2000). However, *M. batesii* individuals did not appear to avoid individuals of these species since we found that *M. batesii* remained on the same plants as the spiders and crickets. If predation risk was high in this

species, *M. batesii* would not disperse and forage at night. However, such behaviour could suggest that animals active during the day, such as birds, might be more dangerous than nocturnal animals. Perhaps *M. batesii* can defend itself effectively against spiders and orthopterans by using its defensive spray (Ho & Chow, 1993; Jones & Bulbert, 2020). Such chemical odours and components may act as a repellent against other arthropods and predators. Eisner, Morgan, Attygalle, Smedley, Herath, and Meinwald (1997) reported that both juveniles and adults of the Peruvian stick insect, *Oreophoetes peruana*, contain chemical substances (i.e., quinoline) in their defensive glands. The authors found that this chemical compound acts as a repellent against predators including spiders, and cockroaches. In *M. batesii*, even newly hatched nymphs can spray or emit such chemical substances immediately when disturbed (Bonduriansky, unpublished observation). Perhaps this chemical defensive mechanism protects both hatchlings and adults of *M. batesii* in the wild. Although we spotted a few hatchlings that were stuck in a spider's web during the mark-resighting study at Cow Bay beach, the major predators of *M. batesii* remain unknown.

To sum up, neither adult nor hatchling dispersal are likely to explain sex ratio variation and the occurrence of all-female populations in *M. batesii*. Adult males of *M. batesii* appear to be at least as dispersive as females. Mate searching behaviour may induce dispersal by males in this species, occasionally enabling males to disperse to and invade all-female populations that are not isolated by geographical barriers. Streams and estuaries, as well as habitat patches devoid of suitable host plants, could limit dispersal over long distances. Our observations suggest that adult *M. batesii* mostly disperse over short distances within habitat patches, and we also found evidence that guarding by males can reduce females' dispersal. Long-distance dispersal could occur via eggs that can be transported by other species such as ants or birds, or abiotic factors such as streams or ocean currents (Wu, Liu, Chen, Tsai, Yu, Hsiao, & Yeh, 2020), but egg dispersal is even less likely to be sex-specific. However, egg dispersal might contribute to sex ratio variation in *M. batesii* because eggs containing female embryos can establish all-female populations on their own. Thus, for example, eggs from an existing all-female population might be carried by a stream or animal vectors such as ants to new locations, hatch into female nymphs, and thus establish new all-female populations. Such populations could then persist unless eggs containing male embryos are subsequently transported to these populations.

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Table S1. Large arthropod species observed on *Pandanus tectorius* and *Benstonea monticola* plants at Cow Bay Beach and Coconut Beach.

Order	Family Name	Species Name	Common English Name	Location Name
Araneae	Salticidae	*Bavia aericeps* (Simon, 1877)	Jumping spiders	Coconut Beach
Araneae	Sparassidae	*Heteropoda* sp.	Huntsman spiders	Coconut Beach
Araneae	Salticidae	*Omoedus* sp.	Ant Eating Jumping spiders	Coconut Beach
Araneae	Salticidae	*Cytaea* sp.	Jumping spiders	Coconut Beach
Araneae	Salticidae	*Omoedus* sp.	Ant Eating Jumping spiders	Coconut Beach
Araneae	Tetragnathidae	*Tetragnatha* sp.	Long Jawed spiders	Coconut Beach
Orthoptera	Gryllacradidae	*Xanthogryllacris punctipennis* (Walker, 1869)	Raspy Cricket	Coconut Beach
Araneae	Araneidae	*Argiope aethera* (Walckenaer, 1842)	St Andrews Cross spider	Coconut Beach
Orthoptera	Gryllidae	*Cardiodactylus novaeguineae* (Haan, 1842)	Cricket	Coconut Beach
Blattodea	Ectobiidae	*Megamareta phaneropyga* (Chopard, 1924)	Cockroach	Coconut Beach
Blattodea	Blattidae	*Melanozosteria* sp.	Black cockroach	Cow Bay Beach
Orthoptera	Acrididae	*Rectitropis australis* (Sjöstedt, 1936)	Grasshopper	Cow Bay Beach
Orthoptera	Gryllidae	*Cardiodactylus* sp.	Crickets	Cow Bay Beach
Orthoptera	Tettigoniidae	*Austrosalomona destructor* (Rentz & Su, 2019)	Green katydid	Cow Bay Beach
Orthoptera	Tettigoniidae	*Phricta spinosa* (Redtenbacher, 1892)	Giant Spiny Forest katydid	Cow Bay Beach
Araneae	Araneidae	*Argiope* sp.	NA	Cow Bay Beach

Table S2. Models used to estimate population size at Coconut Beach, ranked by AIC: *M0 = the* constant capture probability, *Mt* = the capture probability affected by time, *Mh* = the capture probability affected by heterogeneity, *Mb* = the capture probability affected by animal behaviour, *Mbh* = the capture probability affected by behaviour and heterogeneity, *Mth* = the capture probability affected by time and heterogeneity. Four loglinear heterogeneity models are given: *Mh* and *Mth* (*Chao, Darroch, Poisson2*, and *Gamma3.5)*. The model with the lowest AIC value is highlighted in bold.

Models	Abundance	Standard error	Abundance estimations and model fits			
			Deviance	df	AIC	BIC
M0	22	0	86.417	253	115.282	117.464
Mt	22	0	79.578	246	122.442	132.262
Mh Chao (LB)	22.7	1.9	56.694	249	93.559	100.105
Mh Poisson2	22	0.2	60.035	252	90.9	94.173
Mh Darroch	**23**	**1.7**	**59.348**	**252**	**90.212**	**93.486**
Mh Gamma3.5	35.2	20.5	61.368	252	92.232	95.505
Mth Chao (LB)	22.6	1.5	46.445	242	97.309	111.493
Mth Poisson2	22	0.1	50.619	245	95.484	106.394
Mth Darroch	22.8	1.4	49.173	245	94.038	104.948
MthGamma3.5	35	21.1	51.388	245	96.252	107.163
Mb	22	0.1	72.722	252	103.586	106.859
Mbh	22.9	9.7	64.551	251	97.415	101.779

Table S3. Models used to estimate population size at Cow Bay Beach, ranked by AIC: M0 = the constant capture probability, Mt = the capture probability affected by time, Mh = the capture probability affected by heterogeneity, Mb = the capture probability affected by animal behaviour, Mbh = the capture probability affected by behaviour and heterogeneity, Mth = the capture probability affected by time and heterogeneity. Four loglinear heterogeneity models are given: Mh and Mth (Chao, Darroch, Poisson2, and Gamma3.5). The model with the lowest AIC value is highlighted in bold.

Models	Abundance	Standard error	Abundance estimations and model fits			
			Deviance	df	AIC	BIC
M0	19.8	1.7	59.814	125	95.42	97.201
Mt	19.6	1.6	51.08	119	98.686	105.809
Mh Chao (LB)	20.7	2.4	58.955	124	96.561	99.232
Mh Poisson2	20.2	2.1	59.541	124	97.146	99.818
Mh Darroch	21.4	3.7	59.236	124	96.842	99.513
Mh Gamma3.5	22.6	6.2	59.204	124	96.81	99.481
Mth Chao (LB)	20.5	2.3	49.886	118	99.492	107.505

Mth Poisson2	20.1	1.9	50.619	118	100.224	108.238
Mth Darroch	21.5	3.8	50.201	118	99.806	107.819
MthGamma3.5	23.1	6.8	50.169	118	99.775	107.788
Mb	19.5	2	59.753	124	97.358	100.029
Mbh	**40**	**90.1**	**55.231**	**123**	**94.837**	**98.398**

Figure S1. Distribution of captures of single females at the all-female population at Cow Bay Beach. The upper plot (*fi*) indicates the number of individuals captured (resighted) *i* times, showing that individuals in our study were captured between one and five times ($N_{obs.\ day} = 7$). The lower plot (*ui*) indicates that number of females captured for the first time on day *i*, indicating that half of all marked individuals were captured for the first time on observation day 1 (N = 9) and remaining new individuals (N = 9) were captured for the first time on observation days 3 (n = 2), 4 (n = 3), 5 (n = 3) and 6 (n = 1), respectively.

Exploratory Heterogeneity Graph

fi: number of units captured i times

$\log\left(\dfrac{fi}{\binom{t}{i}}\right)$

i: number of captures

ui: number of units captured for the first time on occasion i

$\log(ui)$

i: capture occasion identification number
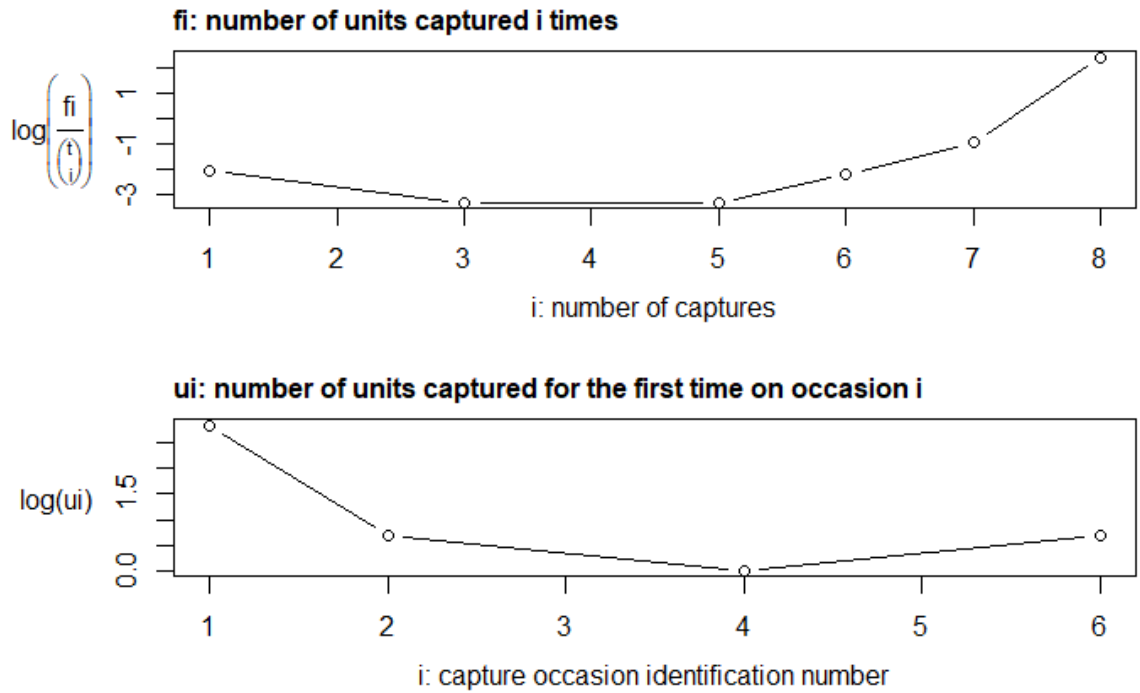
Figure S2. Distribution of captures of single individuals and pairs at the mixed-sex population at Coconut Beach. The upper plot (*fi*) indicates the number of individuals captured *i* times, showing that individuals in our study were captured (resighted) between one and eight times ($N_{obs.\ day}=8$). Most individuals were resighted seven or eight times. The lower plot (*ui)* indicates the number of individuals captured for the first time on day *i*, indicating that most of the individuals were captured for the first time on observation day 1 (N = 17), and remaining individuals (n = 4) were captured for the first time on observation day 2 (n = 2), 4 (n = 1) and 6 (n = 2), respectively.

CHAPTER TWO
EFFECTS OF MALES ON FEMALE FEEDING BEHAVIOR IN
MEGACRANIA BATESII

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ABSTRACT

Across animal taxa, mate guarding by a male is a common behaviour to prevent a mating partner from remating. While such behaviour can benefit males by assuring their paternity, mate guarding can impose substantial costs on females by interfering with foraging. To examine effects of mate guarding on female foraging behaviour, we used the facultatively parthenogenetic stick insect, *Megacrania batesii*. Facultatively parthenogenetic females can reproduce both sexually and asexually. In the wild, mixed-sex populations coexist alongside all-female populations that possess no males. The ability to reproduce parthenogenetically means that females can potentially reproduce without incurring any of the costs of mating, including costs of mate guarding. Therefore, this species is a perfect model to examine costs of reproductive behaviour. *Megacrania batesii* males guard their female partners by physically clinging to them for days or weeks, and such prolonged guarding could affect feeding rates. We carried out experiments using both wild-caught and laboratory-reared individuals to quantify foraging rates by females and males when housed in pairs versus individually. We measured nightly amount of leaves those individuals consumed. We found that laboratory-reared females from mixed-sex populations fed more while housed individually than while housed together with males, but we did not detect such an effect in field-collected females from mixed-sex or all-female populations. This suggests that mate guarding by males may impose constraint on female foraging under some circumstances, but further research is needed to examine if that effect is context-dependent.

INTRODUCTION

Mate guarding is an integral part of male reproductive success in the animal kingdom. Guarding behaviour is often performed by a male to ensure reproductive success by preventing other males from mating with the guarded female (Birkhead, 1995; Parker, 1970). This is because the last male that mates with a female often has a higher probability of fertilizing her eggs (last male precedence) (Mclain, 1989; Parker, 1970). Mate guarding behaviour can be seen in a broad range of animal taxa such as insects (Baxter, Barnett, & Dukas, 2015), reptiles (Cuadrado, 1998; Gullberg, Olsson, & Tegelström, 1996), birds (Björklund & Westman, 1986), and mammals (Alberts, Altmann, & Wilson, 1996; Girard-Buttoz, Heistermann, Rahmi, Marzec, Agil, Fauzan, & Engelhardt, 2014; Poole, 1989) but varies in how it is expressed in different species. In birds and mammals, males typically guard female partners by maintaining close spatial proximity without physical contact (Björklund & Westman, 1986; Schubert, Schradin, Rödel, Pillay, & Ribble, 2009). However, in some invertebrates such as water striders and phasmids, males often guard by grasping females and maintaining close contact (Arnqvist & Rowe, 1995; Sivinski, 1979).

The role of guarding as part of the male reproductive strategy, as well as its impact on females, vary in different species. While mate guarding can improve male fertilization rates, it can also trade off with other components of male fitness. Studies on baboons and lizards suggest that mate guarding can be costly for males in terms of reduced feeding opportunities or increased risk of agonistic interactions with male rivals (Alberts et al., 1996; Ancona, Drummond, & Zaldívar-Rae, 2010). However, in isopods, guarding males fed less and spent more of their energetic reserves (i.e. glycogen) than non-guarding males (Sparkes, Keogh, & Pary, 1996), but males are still able to achieve enhanced fertilization success (Alberts et al., 1996; Alcock, 1994; Parker, 1974). This suggests that male's trade-off food intake for reproductive success while mate guarding. By contrast with males, mate guarding could reduce females' net fitness. A couple of studies on water striders are classical examples that showed mating interaction that conflicts with females' foraging and movement (Rowe, 1992; Rowe, Arnqvist, Sih, & Krupa, 1994). Parker (1970) suggested that mate guarding by males could impose energetic costs on females and thereby reduce female fitness. This suggests that mating may reduce access to suitable habitat or better food resources. Such costs could have important implications for sexual conflict but have rarely been assessed in guarded females.

Costs of guarding are typically investigated in obligately sexual species (Alberts et al., 1996; Schubert, Schradin, Rödel, Pillay, & Ribble, 2009), although non-obligately sexual species also experience mate guarding. In facultatively parthenogenetic systems, females can reproduce both

sexually and asexually depending on whether they mate: fertilised eggs develop into sons and daughters, whereas unfertilized eggs develop into daughters only (Bedford, 1978; Simon, Delmotte, Rispe, & Crease, 2003). This unique reproductive plasticity can generate spatial variation in the relative incidence of sex and parthenogenesis such that some populations reproduce mostly sexually and are mixed-sex, while other populations reproduce mostly parthenogenetically and are all-female (Kearney, 2005; Morgan-Richards, Trewick, & Stringer, 2010). How these population differences affect the economics of mate guarding in facultative parthenogens remains unknown. Mate guarding could be costly regardless of the population from which females are descended. However, because males are typically absent from all-female populations for multiple generations, it is possible that females from all-female populations respond to guarding males differently than females from mixed-sex populations, and experience greater costs due to their lost familiarity with mating interactions.

If guarding males employ coercive strategies and tend to guard females for extended periods, guarding could be subject to sexual conflict. Precopulatory mate guarding in crustaceans provides clear examples of sexual conflict and shows that mate guarding varies in duration depending on duration of females' copulation receptivity or how close females are to being in the receptive stage (Birkhead & Clarkson, 1980; Jormalainen, 1998). In some insects, guarding duration is extremely prolonged: Males physically guard the female partner to ensure that his sperm gets to fertilize the eggs (Alcock, 1994; Parker, 1970, 1974), as the last male that mates with a female often has a higher probability of fertilization (Mclain, 1989; Parker, 1970). Many phasmids are facultative parthenogens and males guard the females for a prolonged period of time ranging from days to months (Bedford, 1978). For example, the empirical study on New Zealand stick insect, *Micrarchus hystriculeus*, showed prolonged guarding duration which lasted up to 29 days (Kelly, 2015). However, the costs of guarding to females, and female resistance to mate guarding, are poorly known in phasmids. It remains unclear whether females achieve higher fitness through sexual or asexual reproduction in *M. batesii*. While sexual reproduction imposes a variety of costs, females could also benefit from sexual reproduction (e.g., by producing sons). However, even if mating and sexual reproduction are beneficial, prolonged guarding could be costly for females. Prolonged guarding could interfere with female foraging, increase vulnerability to predation, or expose females to injury during take-over attempts by rival males (Cothran, 2004; Eldakar, Dlugos, Wilcox, & Wilson, 2009; Sivinski, 1979). Females could also benefit from being guarded by males, if male presence protects females (Alcock, 1994), but the costs could exceed the benefits.

The facultatively parthenogenetic stick insect species, *Megacrania batesii*, is native to the tropical rainforests of northern Queensland, Australia (Cermak & Hasenpusch, 2000). Males are much smaller and have longer wings than females, but both sexes cannot fly. In the wild, mixed-sex populations (both males and females present) and all-female populations (no males at all) have been observed (Cermak & Hasenpusch, 2000). Consequently, mate guarding only occurs in the mixed-sex populations (Cermak & Hasenpusch, 2000). Mate guarding duration in *M. batesii* is quite substantial, often lasting for weeks based on our field observations (Chapter 1). Such prolonged mate guarding behaviour can be costly for the female if guarding by the male interferes with the female's dispersal or reduces foraging opportunities. It is also unknown whether females originally descended from all-female (AF) and mixed-sex (MS) populations may respond to guarding males differently.

Our field observations (Chapter 1) suggested that guarded females usually become active at night, dispersing, and foraging while carrying the guarding male. Like many phasmids (Bedford, 1978), male *M. batesii* can feed on host plants while guarding by sitting on top of the female. *M. batesii* males use several strategies to guard their female partners: a) holding the female with his legs while also grasping the female's ovipositor with his genitalia (with or without intromission of the aedeagus); b) holding the female with his legs without genital contact, usually while facing in the same direction; or c) remaining close to the female by sitting beside the female on the same leaf or host plant. We found that, in most cases, females remained paired with the same male partner over the two weeks of observations, and most females in the mixed-sex population were guarded throughout the study (see Chapter 1). If females are guarded for several weeks or months, and if guarding interferes with females' ability to forage, this male behavioural strategy might have negative effects on females' overall performance. It is unknown in *M. batesii* whether females' foraging might be hindered due to prolonged guarding by males.

The specific aim of this research was to determine how mate guarding affects female feeding rates. To achieve this, we quantified (1) how much females and males feed while housed together versus while housed individually; (2) whether feeding rate and the effect of males on female feeding differ between females from all-female populations versus mixed-sex populations. We also investigated whether female responses differed between wild-caught individuals (Experiment 1) and laboratory-reared individuals (Experiment 2).

METHODS

We conducted two experiments, one using wild-caught individuals (Experiment 1) and the other using laboratory-reared individuals (Experiment 2). We used the data from Experiment 1 to compare two different treatment groups (i.e., "pairing treatment" and "housing treatment") (Fig. 1 & 2). The pairing treatment compared the same females that were paired together with males vs. unpaired. However, results from the "paired" treatment in Experiment 1 did not provide a clear answer to our question about how male guarding affects female feeding because a paired male can also feed on the leaves during the pairing. We therefore also carried out a "housing" comparison in which we summed the amount eaten by individual males and females when housed individually (HI) and compared these values to the total amounts eaten by the same individuals when housed together (HT). In Experiment 1, we could not pair females from all-female populations and allow them to mate, because we released all wild-caught individuals in their natural source populations following the experiment. We therefore examined the effects of male presence on feeding rates of females descended from all-female populations using lab-reared individuals in Experiment 2 only.

Experiment 1: wild-caught individuals

This empirical study was carried out in a field laboratory set up in a house in Cow Bay Village within the natural range of *M. batesii* in far-north Queensland, Australia from 4th to 13th of February 2020. The objective of this study was to determine whether the presence of males affects females' feeding activity. Two researchers (RB and NB) collected *M. batesii* individuals from three different locations where all-female populations of *M. batesii* occur (Thornton Beach, Cow Bay Beach, Noah Beach) and three other locations where mixed-sex populations occur (Coconut Beach, Myall Boardwalk, Myall Beach) in the first two days of February 2020. These stick insects were housed and used in experiments for several days, and then released at the locations of capture.

All collected insects were initially kept in separate mesh cages (30*30*30 cm) and were fed *Benstonea* host plant leaves (2 × 44 cm long leaves per cage per day). The mesh cages were kept at ambient temperature in the shade during the experiment. Throughout Experiment 1, no rain was observed at Cow Bay Village and mean maximum daily and minimum temperature recorded at Low Isles Lighthouse QLD weather station were ~35.9°C and ~28 °C (Bureau of Meteorology, 2020). Each cage was sprayed with fresh water twice per day. Data collected between the fourth and sixth day of the experiment (February 7th and 9th) was excluded because high ambient temperature (~39°C) resulted in shrivelling of the leaves provided to the experimental insects. One paired male was found dead at the end of our experiment, but data collected on that male prior to its death are included in the analysis.

In Experiment 1, we used a total of 10 pairs from mixed-sex populations. Each female's feeding was quantified while she was paired with a male ("paired" treatment) and while she was separated from the male ("unpaired" treatment), such that each individual experienced both treatments. Individuals of each pair were from the same location. Each paired cage contained one female and one male. We set up four pairing treatment groups as follows: (i) 5 females from mixed-sex populations paired with 5 males (hereafter referred to as the 'pairs from MS'), (ii) another 5 females from mixed-sex populations without males (hereafter referred to as the 'unpaired females from MS'), (iii) 5 males without female counterparts (hereafter referred to as the 'unpaired males'), and (iv) 10 unpaired females from all-female populations (hereafter referred to as the 'unpaired females from AF') (Fig. 1). On the first night, 5 females from mixed-sex populations were placed with male counterparts. Each pair was kept in a separate cage overnight (4th -5th Feb). Meanwhile, another 5 females from mixed-sex populations were kept individually in 5 cages without any males. Likewise, 5 unpaired males were also kept in 5 separate cages. On the next day, paired individuals from mixed-sex populations from the first night were separated and placed individually into 10 cages whereas both unpaired females and males from first night were paired together into five female-male pairs in separate cages. We repeated this procedure over 6 sets of nightly feeding observations. Each night, we collected fresh leaves of *Benstonea monticola* from Cow Bay Village and Coconut Beach, cut the leaves into sections ~44 cm in length, photographed the leaf sections (see below), and placed two leaf sections into each cage. Leaf sections of similar width and shape were provided to all experimental individuals. We removed the leaves from each cage after 22 ± 3 hrs and photographed them for later quantification of the amount eaten (see below). 10 unpaired females from AF were placed individually in cages without any males over the 6 sets of nightly feeding observations and provided with two fresh leaves each night as described above.

(i). n=5 pairs

(ii). n=5 unpaired females from MS

(iii). n=5 unpaired males

(iv). n=10 unpaired females from AF
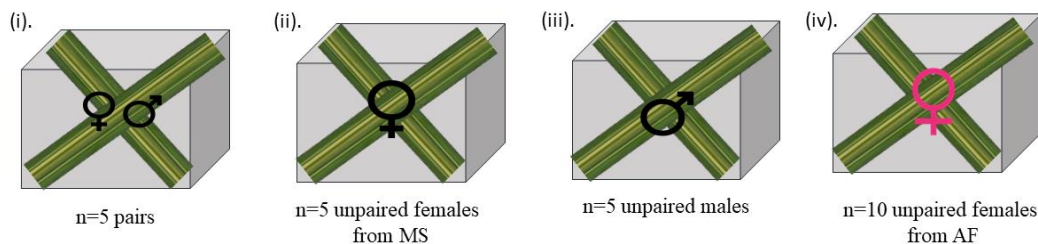
Figure 1. Pairing treatment for Experiment 1 setup: The diagram shows each treatment group including pairs and unpaired individuals from mixed-sex populations (i, ii, iii), and unpaired females from all-female populations (iv). The pairs shown in (i) were housed together while the other 5 females and 5 males were housed individually for one night as shown in (ii) and (iii). These individuals were then switched between individual and paired treatments on the

next day. The unpaired females from all-female populations were maintained without any males throughout the study.
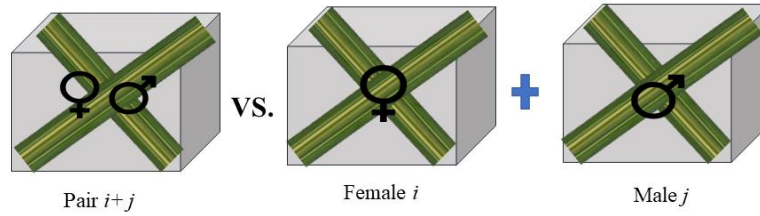
Figure 2. Housing treatment for Experiment 1: To determine whether feeding by females from mixed-sex populations was affected by male presence, we compared the amounts eaten by pairs housed together with summed amounts eaten by the same individuals when housed individually. Amount eaten by pair consisting of female *i* and male *j* versus amount eaten by female *i* plus amount eaten by male *j*. The individuals were from mixed-sex populations only.

Experiment 2: laboratory-reared individuals
Eggs collected from the individuals used in Experiment 1 were hatched in the laboratory. Hatchlings were maintained in same-sex full-sibling pairs in 20×40 cm containers and fed on *Pandanus tectorius* plants until adulthood. Adult females (aged 73 ± 2 d) and males aged 98 ± 2 d) were used in the experiment as described below. All individuals were watered daily and kept at a temperature of $25 \pm 2°$ Celsius and relative humidity of $50 \pm 10\%$. Adults were kept individually in cylindrical containers (20×40 cm) and were fed *Pandanus tectorius* leaves until the start of the experiment. We used glasshouse-grown *Pandanus tectorius* plants, and each individual received leaves from the same plant throughout the experiment. All individuals received ~15-20 cm long leaf pieces. Fresh and eaten leaves were photographed each day.
In Experiment 2, a total of 20 females and 15 males were used: same 5 males from MS used for pairing with 10 females (Fig. 3). Another 10 males were paired with 10 different females from MS and AF respectively (Fig. 3). Females descended from mixed-sex populations were housed with males (one female-male pair per cylindrical container) for 2 nights and then housed individually without males another 2 nights. We then carried out a similar manipulation with females descended from all-female populations paired (i.e., housed with males) and then unpaired (i.e., housed without males). We also quantified feeding by the males when housed in separate containers without females. Female-male pairs were created using individuals originating from different locations.
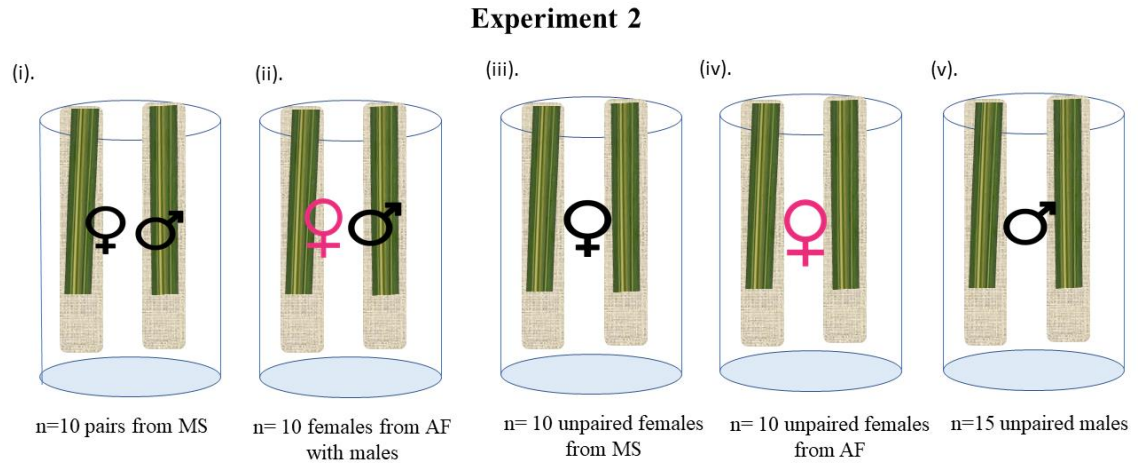
Experiment 2

Figure 3. In Experiment 2, (i) 10 females from mixed-sex populations (MS) were paired with males and (iii) the same females MS were maintained without males (unpaired). (ii) The 10 females from all-female populations (AF) were also paired with males and (iv) the same females from AF were maintained without males (unpaired). (v) In total, 15 males were used throughout the Experiment 2: the same 5 males were used twice in pairing with 5 females from AF and 5 females from MS and another 10 males were paired with the other 5 females from MS and 5 females from AF, respectively.

Measurement of amount eaten

Each night the 2 leaves from each container were removed and were replaced with fresh leaves. Both fresh and eaten leaves were photographed alongside a ruler for scale. We used ImageJ software (Schindelin, Arganda-Carreras, Frise, Kaynig, Longair, Pietzsch, Preibisch, Rueden, Saalfeld, & Schmid, 2012) to measure leaf area (cm²) before and after the trials. All fresh and eaten leaves were measured twice using the ImageJ program to estimate the area and proportion eaten and calculate measurement repeatability.

Statistical analyses for Experiments 1 and 2

All analyses were carried out in R version 4.0.4 (R Core Team, 2021). All the figures were created using the package tidyverse (Wickham, Averick, Bryan, Chang, McGowan, François, Grolemund, Hayes, Henry, & Hester, 2019). We estimated measurement repeatability from leaves eaten by females from MS and AF populations that were housed individually on Day 1 of Experiment 1. To estimate leaf area measurement repeatability (R) for our Gaussian data, we used the rptR package (Stoffel, Nakagawa, & Schielzeth, 2017). To examine and compare amount eaten of various treatment groups (paired vs. unpaired), linear mixed models (LMM) were used (Bates, Sarkar, Bates, & Matrix, 2007) and effects were tested using F-tests with Satterthwaite degrees of freedom using the lmerTest package (Kuznetsova, Brockhoff, &

Christensen, 2017). The response variable in the analyses was the amount eaten (quantified as the average of the two estimates of leaf area eaten in cm^2) per night. We used the multcomp package (Bretz, Hothorn, & Westfall, 2008) to examine pair-wise comparisons between treatment groups in Experiment 1.

In Experiment 1, we could not pair females from all-female populations with males, and therefore population type was not included as a fixed effect in the model. First, we used a linear mixed effects model with all four groups as the fixed effect and cage ID as a random effect (Table S1; Fig. 4). We then did multiple comparisons with "unpaired males" excluded using the multcomp package (Bretz et al., 2008) (Table S2). In a subsequent analysis, "housing treatment" (the sum of amounts eaten by the female and male when housed individually versus the amount eaten by the same individuals when housed together as a pair) was the fixed effect, whereas cage ID was included as a random effect in the model. Because of high ambient temperature during Experiment 1, two females from all-female populations died during the study on sampling days 7-8, and these individuals were excluded from analyses.

In Experiment 2, we compared the treatment groups for females only, excluding the "unpaired males". In these linear mixed effects model, the fixed effects were pairing treatment (paired vs. unpaired), population type (mixed-sex vs. all-female populations) and their interaction, and the random effect was pair ID (Table S3; Fig. 5). We also did multiple comparisons between the groups (females from MS and AF while they were paired and while they were unpaired) (Table S4). One male was dead at the end of experiment and therefore his unpaired data was excluded. But data obtained in his prior pairings with females were used in the analysis.

RESULTS

Experiment 1: Wild-caught individuals

Measurement repeatability for the leaf area eaten was high (R = 0.959; CI = 0.887-0.986) which suggests that our measurements reliably capture variation in amounts eaten by our focal individuals.

In general, females of *Megacrania batesii* heavily fed on the *Pandanus tectorius* leaves when compared to males (Table S1; Fig. 4). Unpaired females from mixed-sex populations and unpaired females from all-female populations consumed similar amounts on average (Table S7; Fig. 4). Unpaired females from mixed-sex populations were found to consume almost three times as much as unpaired males (Table S7; Fig. 4)**.** On average, amounts eaten by unpaired females from mixed-sex versus all-female populations did not differ (Table S2; Fig. 4). We

detected a near-significant difference within the mixed-sex populations where female-male pairs consumed more than unpaired females (Table S1-S2; Fig. 4). Yet, it is not clear whether this difference reflected increased feeding by paired females, or the amount that males fed while housed in the same container with females. To investigate this, we combined the amounts eaten by the male and female while they were housed individually and compared this to the amount eaten by the same pairs of individuals when housed together. This comparison showed no difference between the amounts eaten by the same female-male pairs when housed together versus when housed individually (ANOVA: $F = 0.016$, $p = 0.89$; Fig. 5).

Figure 4. Experiment 1: Violin plots showing amount eaten (leaf area, cm^2) by wild-caught individuals as a function of pairing treatment. The centre black line inside each boxplot shows the median, and the box inside the violins shows the inter-quartile-distance and the whiskers (the vertical thin lines) show the range of values outside the inter-quartile range. Black dots show nightly values for individuals or pairs.
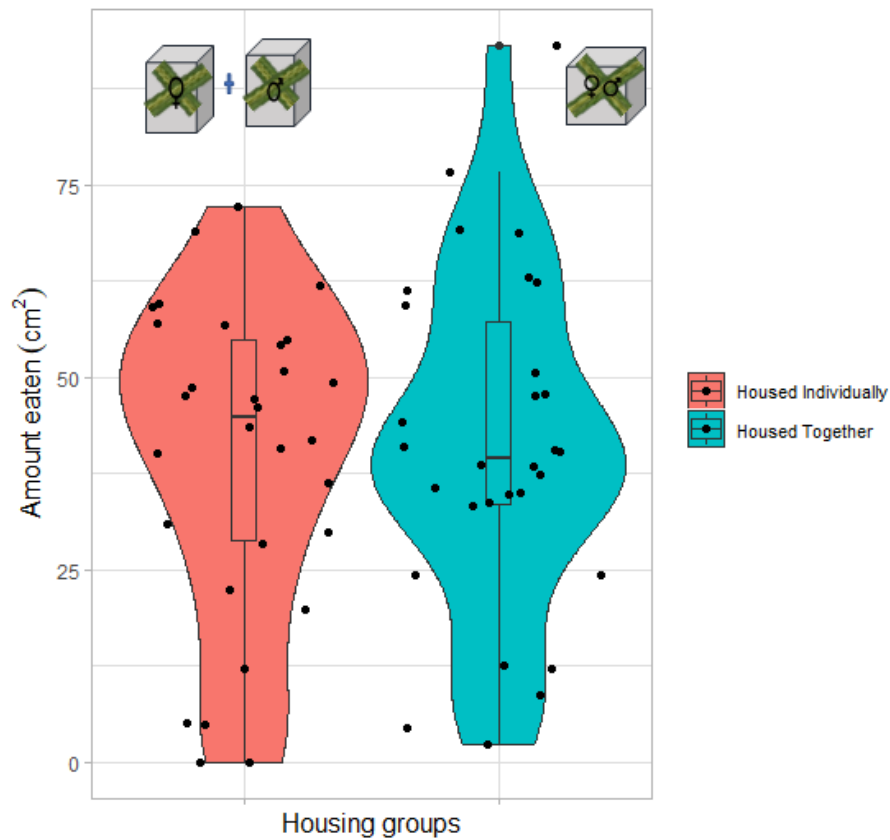
Figure 5. Experiment 1: Violin plots showing amounts eaten (leaf area, cm^2) by wild-caught females and males from mixed-sex populations while housed individually (HS) and housed together (HT). The centre black line inside each boxplot shows the median, and the box inside the violins shows the inter-quartile-distance and the whiskers (the vertical thin lines) show the range of values outside the inter-quartile range. Black dots show nightly values for individuals or pairs.

Experiment 2: Laboratory-reared individuals

We collected additional data on the feeding behaviour of laboratory-reared individuals of *M. batesii* to test whether male presence interacted with population type (AF vs. MS). There was a near-significant interaction of population type with pairing treatment (F = 3.2115; p = 0.078). Post-hoc comparisons showed that, for females descended from all-female populations, there was no difference in the amount eaten by unpaired females vs. female-male pairs (Table S4; Fig. 6). However, for females from mixed-sex populations, the amount eaten by the females when unpaired was significantly greater than the amount eaten by the female-male pairs (Table S4; Fig. 6). This indicates that females descended from MS populations fed less when paired with males

and suggests that males interfere with female foraging. Clearly, unpaired males fed significantly less than unpaired females from both populations (Table S7; Fig. 6).
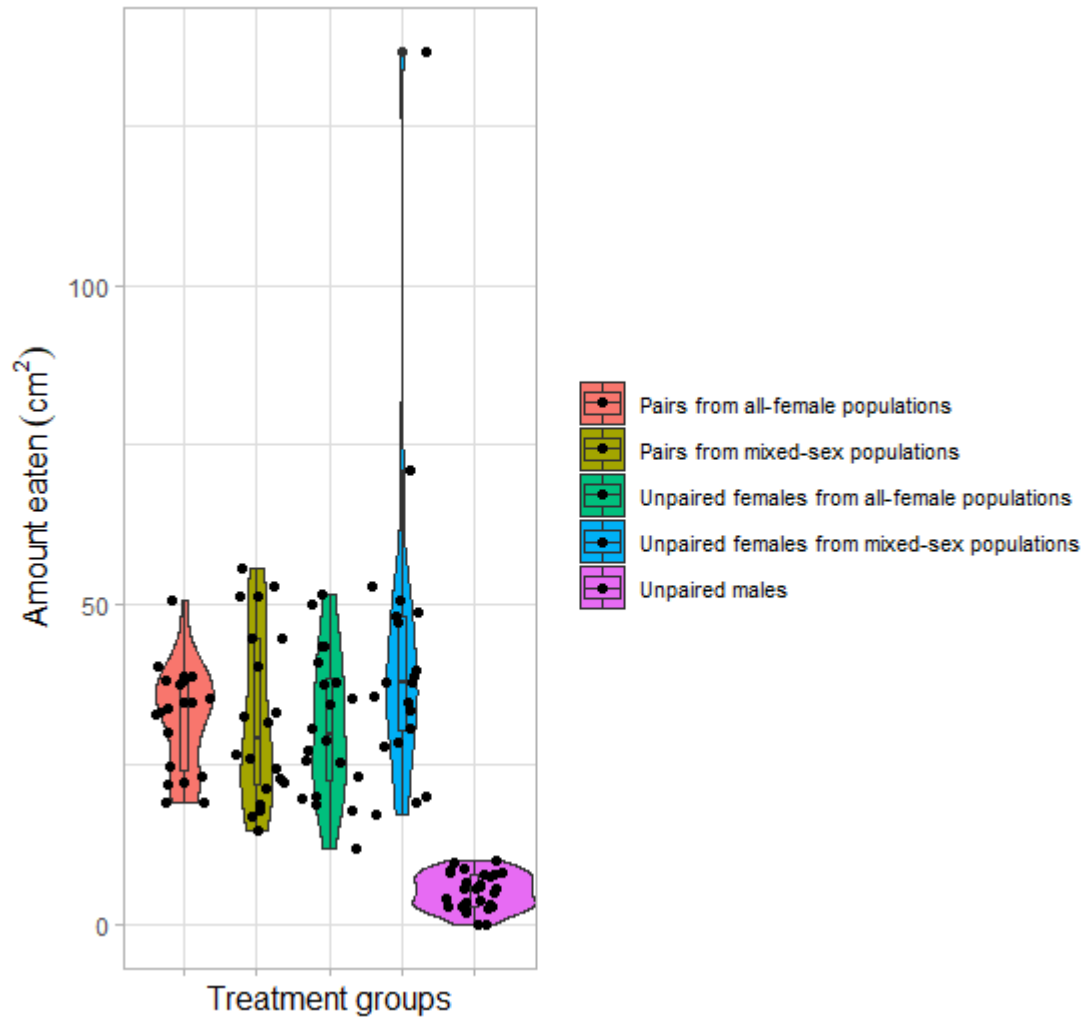
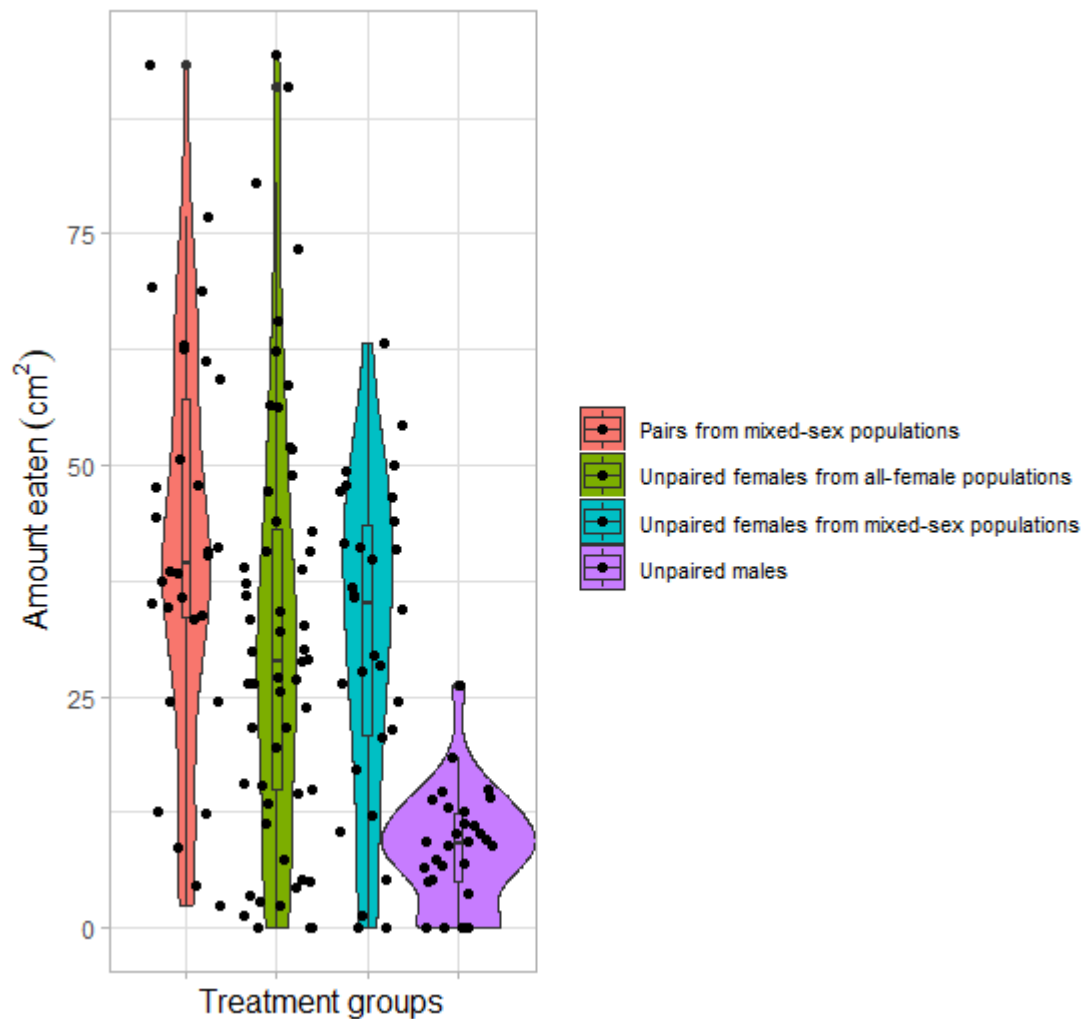**Figure 6.** Experiment 2: Violin plots showing amount eaten (leaf area, cm^2) by lab-reared individuals as a function of pairing treatment and population type. The centre black line inside each boxplot shows the median, and the box inside the violins shows the inter-quartile-distance and the whiskers (the vertical thin lines) show the range of values outside the inter-quartile range. Black dots show nightly values for individuals or pairs.

DISCUSSION

This study is the first to investigate whether guarding by males interferes with feeding rates of females in the facultatively parthenogenetic stick insect, *M. batesii*. Overall, our results suggest that males can interfere with female foraging, but more research is needed to understand the circumstances when this occurs. Very little research has been done on sexual conflict and male-female interactions in phasmids, but the *M. batesii* system offers a valuable opportunity to investigate how male behaviour affects females in both natural populations and laboratory experiments. This study revealed that the effects of male presence or guarding behaviour on females can be context-dependent. Overall, foraging rates of females from mixed-sex vs. all-female populations were different, as were effects of males on female foraging in these populations. This suggests that males can interfere more with females' foraging behaviour in some circumstances than in others. Therefore, it is important to examine the potential factors that influence male sexual behaviour and coercion towards the female. This will help to determine how important sexual conflict is in this system, and in what contexts it is especially pronounced.

Overall, the feeding rate in the Experiment 2 was higher than in the Experiment 1. Such differences might have occurred due to differences between experiments in the mean age of females or males, mean temperature, or other factors. The amount that females fed while paired versus unpaired was not consistent between wild-caught and lab-reared individuals. In Experiment 1, feeding by wild-caught females from the mixed-sex population was not affected by pairing with males (housing analysis). This is in contrast to Experiment 2, which showed that lab-reared females from mixed-sex populations fed more when they were unpaired than when they were paired (Fig. 4 & 6). The average temperature during Experiment 1 in the field was ~35°Celsius and no rain fell during that period. Thus, our wild-caught male individuals may have been stressed by these conditions, causing males to feed more than usual so as to allocate more energy to survival.

A study on greater kudus (*Tragelaphus strepsiceros*) examined the correlation between higher temperature and foraging time, with seasonal changes. It was found that individuals tend to feed more as a strategy for coping with stressful conditions involving hot weather with limited food sources (Owen-Smith, 1998). Differences in food supply might also be responsible for our contrasting results. Studies on other stick insect species reported that behaviours differed on different types of host plants (Nosil, Crespi, & Sandoval, 2002), and that stick insects tend to prefer younger leaves over older leaves due to chemical compounds present within the host plant (Blüthgen & Metzner, 2007). In our experiments, lab-reared individuals might have behaved differently because the varieties of host plants that we provided were not the same as

those available in the field. In Experiment 1, the same species of host plant *Benstonea monticola* was used to feed all wild-caught individuals. However, several varieties of *Pandanus tectorius* were used to feed all individuals in Experiment 2. Although these plants were randomized across replicate individuals and treatments, this variation in food may have affected feeding behaviour in Experiment 2. It is possible that variation in leaf thickness of the different varieties of *Pandanus tectorius* might have introduced some effects on females' foraging rates. The age differences of individuals between Experiment 1 and Experiment 2 could also be responsible for the different results. A number of empirical studies on different species have found that foraging rates tend to decrease with age (Morley, Kumar, Mattammal, Farr, Morley, & Flood, 1996; Peng, Jiang, & Hsü, 1980) and therefore foraging behaviour in *M. batesii* may vary throughout the lifespan. Unfortunately, the ages of the wild-caught individuals of *M. batesii* used in Experiment 1 were unknown whereas the average age of lab-reared females and males in Experiment 2 was 73 ± 2 and 98 ± 2 days from the adult moult (which can be considered relatively young, given that females take ~ 20 days to start ovipositing after their adult moult and can live for > 6 months; JB and RB, unpublished data). Probably, the wild caught individuals varied in age because we found several last instar nymphs in each location where individuals were collected. Therefore, it is worth examining how foraging behaviour changes with aging in *M. batesii* to clarify whether differences in age could explain different results in Experiment 1 vs. Experiment 2 in our study.

We found that the amounts eaten by females from mixed-sex and all-female populations were the same when paired with males in Experiment 2 (Fig. 6). This might suggest that *M. batesii* females from all-female populations may respond similarly to females from mixed-sex populations when males are present. Alternatively, males might have behaved differently towards females from all-female vs. mixed-sex populations. The amount of time spent guarding females from all-female vs. mixed-sex populations might have been different. It is possible that females from mixed-sex populations were more attractive to males, and guarding might therefore have started sooner or lasted longer. Moreover, because all-female populations consist of clonal lineages that rarely, if ever, encounter males, females from such populations may be less likely to produce male-attracting signals. Indeed, reduced attractiveness of parthenogenetically reproducing females has been observed in another facultatively parthenogenetic species, *Extatosoma tiaratum* (Burke, 2018). The possibility of differences in male responses to females complicates the interpretation of female feeding rates and requires further research on behavioural interactions between the sexes in relation to feeding.

In both experiments, unpaired males fed significantly less than unpaired females (Table S5). This is probably because males are smaller in body size and do not produce eggs, and therefore probably experience lower costs of somatic maintenance and gamete production than females

do. Males might also sacrifice feeding opportunities for opportunities to mate and fertilize eggs: it is possible that males could live longer if they fed more, but selection instead favours investment in competition and mating at the expense of foraging.

Interestingly, there was a clear separation between amounts fed by unpaired males and unpaired females in Experiment 2 (Fig. 6). However, there was some overlap in the amounts eaten by unpaired females and males in Experiment 1 (Fig. 4). Overall, the amount eaten by unpaired wild-collected males was slightly higher than that of unpaired lab-reared males. This difference suggests that male feeding in Experiment 1 might have been impacted by external factors. Because of high ambient temperature during Experiment 1, it is possible that males fed more while unpaired. Sexual size dimorphism could also explain the reason why we found no evidence of negative effects of mate guarding on female foraging rates in Experiment 1 where males developed in their natural environment. There could be differences in weight between wild-caught and lab-reared males. Males do not necessarily hinder females that carry them around (Fairbairn, 1990). Guarded *M. batesii* females might be able to feed and disperse effectively if guarded by males that are small and light. The males we reared in the laboratory were raised in pairs and fed ad libitum before we introduced and paired them with the females. We did not measure the body size of males and females. However, lab-reared males might have been heavier and, consequently, pairing with males might have hindered female foraging to a greater extent in Experiment 2 than in Experiment 1.

Our study has several limitations. First, it is possible that our sample size did not provide sufficient statistical power to detect effects of guarding males on females' foraging activity. Second, with our design, we could not quantify how much was eaten by the female vs. the male when paired in the same container. To clarify paired males' feeding contribution to the total amount eaten by female-male pairs, an approach that could be used in future studies would be to quantify the amount of frass produced by each sex. Observations we made during this study indicate that male and female frass is also noticeably different in size in *M. batesii*. Future studies could therefore compare the amount of frass produced by males and females during mate guarding to estimate the amounts eaten by each sex.

Lastly, since we focused on how female feeding is affected by the presence of single males while housed in cages, it could be problematic to conclude that guarding males do not hinder females from foraging effectively. In these female-male pairs, males could mate with and guard females without competing with other males, and females were not able to escape from males. However, in the wild, we observed that guarding males were sometimes replaced by others,

perhaps following a take-over (see Chapter 1). It is possible that over their lifespan, *M. batesii* females might be exposed to injures or disturbance due to male-male competition between guarding males and rivals in the wild. Male phasmids show defensive aggressiveness towards rival males (i.e. fighting) (Myers, Buckley, & Holwell, 2015; Sivinski, 1979). The former two studies showed that the incidence of such take-overs could negatively affect females, and that females could also be injured when a guarding male inserts his genitalia and injures the female partners' copulatory opening. During our field study, two other researchers (RB and NB) observed *M. batesii* males fight each other using their front legs (i.e., boxing) and mandibles (biting) on top of guarded females (Bonduriansky & Burke, unpublished data). This suggests that females experience sexual harassment and male-male competition in the wild, which could reduce the guarded females' foraging and might lead to strong sexual conflict between the mating partners. Therefore, future studies could investigate whether costs associated with guarding and mating in the presence of multiple males and for extended periods of time reveal sexual conflict in this facultatively parthenogenetic species.

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Table S1. Linear mixed model output with Gaussian distributions from Experiment 1. Effects of male presence/absence on female foraging rates (amount eaten) are shown for wild-caught individuals from mixed-sex (MS) and all-female (AF) populations. All four treatment groups were included as the fixed effect and cage ID was included as a random effect. Values in bold indicate significant effects ($p < 0.05$).

Treatment groups	Amount eaten (cm^2)		
	Estimates	CI	p value
Intercept: Pairs from MS	40.96	33.14 – 48.78	**<0.001**
Unpaired females from AF	-9.70	-19.80 – 0.40	0.060
Unpaired females from MS	-9.37	-18.76 – 0.01	**0.050**
Unpaired males	-31.90	-41.28 – -22.52	**<0.001**

Table S2. Pair-wise comparisons from Experiment 1. Pairs and unpaired females from mixed-sex (MS) populations, and unpaired female from all-female (AF) populations, are compared using Tukey post-hoc tests.

Multiple pair-wise groups	Estimate	SE	z value	p value
Unpaired females from AF - Pairs from MS	-9.7211	5.7749	-1.683	0.0923
Unpaired females from MS - Pairs from MS	9.3702	5.2934	-1.77	0.0767
Unpaired females from MS - Unpaired females from AF	0.3509	5.7749	0.061	0.9515

Table S3. Linear mixed model output with Gaussian distributions from Experiment 2. Effects of male presence/absence on female foraging rates (amount eaten) are shown for lab-reared individuals from mixed-sex (MS) and all-female (AF) populations. Population type (AF vs. MS) and paired versus unpaired treatments are included as the fixed effects, and pair ID is included as a random effect. Values in bold indicate significant effects ($p < 0.05$).

Treatment groups	Amount eaten (cm^2)		
	Estimates	CI	p value
Population type (MS)	0.16	-10.79 – 11.11	0.977

Pairing treatment (Unpaired)	-1.19	-10.14 – 7.77	0.795
Population type (MS) * Pairing treatment (Unpaired)	11.58	-1.08 – 24.24	0.073

Table S4. Pair-wise comparisons from Experiment 2. Pairs and unpaired females from mixed-sex (MS) and all-female (AF) populations are compared using Tukey post-hoc tests. Values in bold with single asterisk indicate significant effects (p<0.05).

Multiple pair-wise groups	Estimate	SE	z value	p value
Pairs from MS - Pairs from AF	0.1604	5.5863	0.029	0.9771
Unpaired females from AF - Pairs from AF	-1.1865	4.5683	-0.26	0.7951
Unpaired females from MS - Pairs from AF	10.5515	5.5863	1.889	0.0589
Unpaired females from AF - Pairs from MS	-1.3469	5.5863	-0.241	0.8095
Unpaired females from MS - Pairs from MS	10.3911	4.5683	2.275	**0.0229** *
Unpaired females from MS - Unpaired females from AF	11.738	5.5863	2.101	**0.0356** *

Table S5. Amounts eaten by pairs and unpaired individuals in Experiment 1. The proportion and area of leaf eaten is the average of two measurements of the same leaves.

Pairing Treatment	Population type	Location (Female origin)	Cage ID	Day of experiment	Proportion Eaten (%)	Area Eaten (cm^2)
Unpaired female	All-female	Noah Beach	A1	1	0.21	58.54
Unpaired female	All-female	Thornton Beach	A2	1	0.20	80.54
Unpaired female	All-female	Cow Bay Beach	A3	1	0.21	73.38
Unpaired female	All-female	Thornton Beach	A4	1	0.15	51.81
Unpaired female	All-female	Cow Bay Beach	A5	1	0.26	94.43
Unpaired female	All-female	Cow Bay Beach	A6	1	0.01	3.39
Unpaired female	All-female	Thornton Beach	A7	1	0.10	40.63
Unpaired female	All-female	Cow Bay Beach	A9	1	0.17	65.47
Unpaired female	All-female	Thornton Beach	A10	1	0.22	90.86
Unpaired female	All-female	Noah Beach	A1	4	0.12	28.80
Unpaired female	All-female	Thornton Beach	A2	4	0.13	34.14
Unpaired female	All-female	Cow Bay Beach	A3	4	0.14	47.11

Unpaired female	All-female	Thornton Beach	A4	4	0.13	33.34
Unpaired female	All-female	Cow Bay Beach	A5	4	0.21	56.23
Unpaired female	All-female	Cow Bay Beach	A6	4	0.04	11.35
Unpaired female	All-female	Thornton Beach	A7	4	0.10	21.64
Unpaired female	All-female	Cow Bay Beach	A8	4	0.15	36.01
Unpaired female	All-female	Cow Bay Beach	A9	4	0.10	23.79
Unpaired female	All-female	Thornton Beach	A10	4	0.12	29.83
Unpaired female	All-female	Noah Beach	A1	5	0.07	19.58
Unpaired female	All-female	Thornton Beach	A2	5	0.17	52.01
Unpaired female	All-female	Cow Bay Beach	A3	5	0.08	21.66
Unpaired female	All-female	Thornton Beach	A4	5	0.14	38.72
Unpaired female	All-female	Cow Bay Beach	A5	5	0.16	48.94
Unpaired female	All-female	Cow Bay Beach	A6	5	0.03	7.40
Unpaired female	All-female	Thornton Beach	A7	5	0.15	42.89
Unpaired female	All-female	Cow Bay Beach	A8	5	0.11	32.09
Unpaired female	All-female	Cow Bay Beach	A9	5	0.08	26.94
Unpaired female	All-female	Thornton Beach	A10	5	0.08	25.50
Unpaired female	All-female	Noah Beach	A1	6	0.10	26.36
Unpaired female	All-female	Thornton Beach	A2	6	0.20	62.23
Unpaired female	All-female	Cow Bay Beach	A3	6	0.02	5.20
Unpaired female	All-female	Thornton Beach	A4	6	0.00	0.00
Unpaired female	All-female	Cow Bay Beach	A5	6	0.00	0.00
Unpaired female	All-female	Cow Bay Beach	A6	6	0.00	1.25
Unpaired female	All-female	Thornton Beach	A7	6	0.00	0.00
Unpaired female	All-female	Cow Bay Beach	A8	6	0.04	15.49
Unpaired female	All-female	Cow Bay Beach	A9	6	0.02	4.93
Unpaired female	All-female	Thornton Beach	A10	6	0.01	4.40
Unpaired female	All-female	Noah Beach	A1	7	0.11	26.46
Unpaired female	All-female	Thornton Beach	A2	7	0.20	56.55
Unpaired female	All-female	Cow Bay Beach	A3	7	0.01	2.88
Unpaired female	All-female	Thornton Beach	A4	7	0.16	43.95
Unpaired female	All-female	Cow Bay Beach	A5	7	0.17	38.94
Unpaired female	All-female	Thornton Beach	A10	7	0.06	14.92
Unpaired female	All-female	Cow Bay Beach	A9	7	0.05	13.36
Unpaired female	All-female	Cow Bay Beach	A8	7	0.11	30.02

Unpaired female	All-female	Thornton Beach	A7	7	0.05	15.27
Unpaired female	All-female	Noah Beach	A1	8	0.09	27.01
Unpaired female	All-female	Thornton Beach	A2	8	0.14	37.29
Unpaired female	All-female	Cow Bay Beach	A3	8	0.01	2.43
Unpaired female	All-female	Thornton Beach	A4	8	0.13	32.66
Unpaired female	All-female	Cow Bay Beach	A5	8	0.15	40.62
Unpaired female	All-female	Thornton Beach	A7	8	0.11	26.43
Unpaired female	All-female	Cow Bay Beach	A8	8	0.12	28.91
Unpaired female	All-female	Cow Bay Beach	A9	8	0.05	14.61
Pair	Mixed-sex	Myall Boardwalk	B	1	0.19	47.79
Pair	Mixed-sex	Myall Boardwalk	C	1	0.35	93.20
Pair	Mixed-sex	Myall Beach	E	1	0.15	38.61
Pair	Mixed-sex	Coconut Beach	D	1	0.23	59.39
Pair	Mixed-sex	Coconut Beach	A	1	0.22	62.95
Unpaired female	Mixed-sex	Coconut Beach	F	1	0.10	27.63
Unpaired female	Mixed-sex	Coconut Beach	G	1	0.13	36.78
Unpaired female	Mixed-sex	Myall Beach	H	1	0.16	47.23
Unpaired female	Mixed-sex	Coconut Beach	J	1	0.17	44.04
Unpaired female	Mixed-sex	Myall Beach	I	1	0.04	12.10
Unpaired male	Mixed-sex	Coconut Beach	J	1	0.06	10.96
Unpaired male	Mixed-sex	Coconut Beach	G	1	0.05	12.58
Unpaired male	Mixed-sex	Myall Beach	H	1	0.00	0.00
Unpaired male	Mixed-sex	Myall Beach	I	1	0.00	0.00
Unpaired male	Mixed-sex	Coconut Beach	F	1	0.07	18.42
Pair	Mixed-sex	Coconut Beach	A	4	0.12	35.77
Pair	Mixed-sex	Myall Boardwalk	B	4	0.14	50.65
Pair	Mixed-sex	Myall Boardwalk	C	4	0.22	69.19
Pair	Mixed-sex	Coconut Beach	D	4	0.15	47.67
Pair	Mixed-sex	Myall Beach	E	4	0.21	76.73
Unpaired female	Mixed-sex	Coconut Beach	F	4	0.17	49.98
Unpaired female	Mixed-sex	Coconut Beach	G	4	0.11	34.42
Unpaired female	Mixed-sex	Myall Beach	H	4	0.20	63.27
Unpaired female	Mixed-sex	Myall Beach	I	4	0.03	10.43
Unpaired female	Mixed-sex	Coconut Beach	J	4	0.13	41.66
Unpaired male	Mixed-sex	Coconut Beach	F	4	0.03	6.97

Unpaired male	Mixed-sex	Coconut Beach	G	4	0.03	7.43
Unpaired male	Mixed-sex	Myall Beach	H	4	0.03	8.90
Unpaired male	Mixed-sex	Coconut Beach	J	4	0.03	9.47
Unpaired male	Mixed-sex	Myall Beach	I	4	0.03	9.25
Unpaired female	Mixed-sex	Coconut Beach	A	5	0.13	39.91
Unpaired female	Mixed-sex	Myall Boardwalk	B	5	0.07	24.40
Unpaired female	Mixed-sex	Myall Boardwalk	C	5	0.13	41.21
Unpaired female	Mixed-sex	Coconut Beach	D	5	0.08	26.34
Unpaired female	Mixed-sex	Myall Beach	E	5	0.15	35.84
Pair	Mixed-sex	Coconut Beach	J	5	0.13	38.37
Pair	Mixed-sex	Myall Beach	I	5	0.08	24.43
Pair	Mixed-sex	Myall Beach	H	5	0.17	62.44
Pair	Mixed-sex	Coconut Beach	G	5	0.13	44.27
Pair	Mixed-sex	Coconut Beach	F	5	0.14	40.34
Unpaired male	Mixed-sex	Coconut Beach	A	5	0.03	8.88
Unpaired male	Mixed-sex	Myall Boardwalk	B	5	0.03	6.54
Unpaired male	Mixed-sex	Myall Boardwalk	C	5	0.05	13.08
Unpaired male	Mixed-sex	Coconut Beach	D	5	0.04	13.86
Unpaired male	Mixed-sex	Myall Beach	E	5	0.02	5.02
Pair	Mixed-sex	Coconut Beach	A	6	0.04	12.27
Pair	Mixed-sex	Myall Boardwalk	B	6	0.02	4.57
Pair	Mixed-sex	Myall Boardwalk	C	6	0.18	68.75
Pair	Mixed-sex	Coconut Beach	D	6	0.04	12.65
Pair	Mixed-sex	Myall Beach	E	6	0.01	2.32
Unpaired female	Mixed-sex	Coconut Beach	F	6	0.00	0.00
Unpaired female	Mixed-sex	Coconut Beach	G	6	0.00	0.00
Unpaired female	Mixed-sex	Myall Beach	H	6	0.00	1.31
Unpaired female	Mixed-sex	Myall Beach	I	6	0.07	17.17
Unpaired female	Mixed-sex	Coconut Beach	J	6	0.02	5.15
Unpaired male	Mixed-sex	Coconut Beach	F	6	0.00	0.00
Unpaired male	Mixed-sex	Coconut Beach	G	6	0.00	0.00
Unpaired male	Mixed-sex	Myall Beach	H	6	0.01	3.63
Unpaired male	Mixed-sex	Myall Beach	I	6	0.01	5.30
Unpaired male	Mixed-sex	Coconut Beach	J	6	0.00	0.00
Unpaired male	Mixed-sex	Coconut Beach	A	7	0.00	0.00

Unpaired male	Mixed-sex	Myall Boardwalk	B	7	0.04	10.23
Unpaired male	Mixed-sex	Myall Boardwalk	C	7	0.06	14.72
Unpaired male	Mixed-sex	Coconut Beach	D	7	0.10	26.17
Unpaired male	Mixed-sex	Myall Beach	E	7	0.04	10.15
Unpaired female	Mixed-sex	Coconut Beach	A	7	0.10	28.46
Unpaired female	Mixed-sex	Myall Boardwalk	B	7	0.19	49.27
Unpaired female	Mixed-sex	Myall Boardwalk	C	7	0.20	54.31
Unpaired female	Mixed-sex	Coconut Beach	D	7	0.12	35.81
Unpaired female	Mixed-sex	Myall Beach	E	7	0.16	46.57
Pair	Mixed-sex	Coconut Beach	F	7	0.12	34.73
Pair	Mixed-sex	Coconut Beach	G	7	0.08	24.37
Pair	Mixed-sex	Myall Beach	H	7	0.21	61.21
Pair	Mixed-sex	Myall Beach	I	7	0.10	33.37
Pair	Mixed-sex	Coconut Beach	J	7	0.14	40.59
Pair	Mixed-sex	Coconut Beach	A	8	0.12	33.68
Pair	Mixed-sex	Myall Boardwalk	B	8	0.15	41.06
Pair	Mixed-sex	Myall Boardwalk	C	8	0.17	37.39
Pair	Mixed-sex	Coconut Beach	D	8	0.03	8.75
Pair	Mixed-sex	Myall Beach	E	8	0.16	35.05
Unpaired female	Mixed-sex	Coconut Beach	F	8	0.18	47.88
Unpaired female	Mixed-sex	Coconut Beach	G	8	0.07	20.59
Unpaired female	Mixed-sex	Myall Beach	H	8	0.16	40.87
Unpaired female	Mixed-sex	Myall Beach	I	8	0.10	21.53
Unpaired female	Mixed-sex	Coconut Beach	J	8	0.10	29.52
Unpaired male	Mixed-sex	Coconut Beach	F	8	0.04	11.32
Unpaired male	Mixed-sex	Coconut Beach	G	8	0.04	9.33
Unpaired male	Mixed-sex	Myall Beach	H	8	0.03	6.75
Unpaired male	Mixed-sex	Myall Beach	I	8	0.04	14.85
Unpaired male	Mixed-sex	Coconut Beach	J	8	0.06	14.08

Table S6. Leaves eaten by paired and unpaired individuals in the Experiment 2. The proportion and area of leaf eaten is the average of two measurements of the same leaves.

Pairing Treatment	Population type	Location (Female origin)	Cage ID	Day of experiment	Proportion Eaten (%)	Area Eaten (cm^2)
Pair	Mixed-sex	Burke's Beach	S1	1	0.16	26.58
Pair	Mixed-sex	Myall Boardwalk	S4	1	0.11	17.90
Pair	Mixed-sex	Myall Boardwalk	S5	1	0.20	26.08
Pair	Mixed-sex	Myall Boardwalk	S3	1	0.12	22.18
Pair	Mixed-sex	Myall Boardwalk	S2	1	0.13	21.19
Pair	Mixed-sex	Myall Boardwalk	S4	2	0.24	32.50
Pair	Mixed-sex	Myall Boardwalk	S5	2	0.22	44.74
Pair	Mixed-sex	Myall Boardwalk	S3	2	0.12	31.59
Pair	Mixed-sex	Myall Boardwalk	S2	2	0.23	40.26
Pair	Mixed-sex	Burke's Beach	S1	2	0.12	23.00
Unpaired female	Mixed-sex	Burke's Beach	S1	3	0.19	33.66
Unpaired female	Mixed-sex	Myall Boardwalk	S2	3	0.92	136.63
Unpaired female	Mixed-sex	Myall Boardwalk	S3	3	0.18	30.82
Unpaired female	Mixed-sex	Myall Boardwalk	S4	3	0.29	48.98
Unpaired female	Mixed-sex	Myall Boardwalk	S5	3	0.26	47.19
Unpaired female	Mixed-sex	Burke's Beach	S1	4	0.24	37.92
Unpaired female	Mixed-sex	Myall Boardwalk	S2	4	0.31	52.96
Unpaired female	Mixed-sex	Myall Boardwalk	S3	4	0.21	38.76
Unpaired female	Mixed-sex	Myall Boardwalk	S4	4	0.36	38.06
Unpaired female	Mixed-sex	Myall Boardwalk	S5	4	0.45	71.24
Unpaired male	Mixed-sex	Coconut Beach	S5	3	0.01	1.78
Unpaired male	Mixed-sex	Burke's Beach	S3	3	0.05	7.66
Unpaired male	Mixed-sex	Burke's Beach	S1	3	0.02	4.94
Unpaired male	Mixed-sex	Myall Boardwalk	S2	3	0.07	6.66
Unpaired male	Mixed-sex	Myall Boardwalk	S4	3	0.05	6.28
Unpaired male	Mixed-sex	Burke's Beach	S1	4	0.02	3.88
Unpaired male	Mixed-sex	Myall Boardwalk	S2	4	0.11	8.77

Unpaired male	Mixed-sex	Burke's Beach	S3	4	0.10	9.79
Unpaired male	Mixed-sex	Myall Boardwalk	S4	4	0.02	2.62
Unpaired male	Mixed-sex	Coconut Beach	S5	4	0.02	2.87
Pair	All-female	Thornton Beach	L1	1	0.19	38.17
Pair	All-female	Cow Bay Beach	L2	1	0.38	40.48
Pair	All-female	Thornton Beach	L3	1	0.22	33.70
Pair	All-female	Cow Bay Beach	L4	1	0.12	22.06
Pair	All-female	Cow Bay Beach	L5	1	0.12	24.62
Pair	All-female	Thornton Beach	L1	2	0.17	33.08
Pair	All-female	Cow Bay Beach	L2	2	0.48	50.64
Pair	All-female	Thornton Beach	L3	2	0.12	19.00
Pair	All-female	Cow Bay Beach	L4	2	0.23	38.70
Pair	All-female	Cow Bay Beach	L5	2	0.19	35.46
Unpaired female	All-female	Cow Bay Beach	L5	3	0.09	11.84
Unpaired female	All-female	Cow Bay Beach	L4	3	0.48	37.75
Unpaired female	All-female	Cow Bay Beach	L2	3	0.27	27.31
Unpaired female	All-female	Thornton Beach	L1	3	0.17	30.69
Unpaired female	All-female	Thornton Beach	L3	3	0.30	37.45
Unpaired female	All-female	Thornton Beach	L1	4	0.18	35.38
Unpaired female	All-female	Cow Bay Beach	L2	4	0.53	51.71
Unpaired female	All-female	Thornton Beach	L3	4	0.47	49.99
Unpaired female	All-female	Cow Bay Beach	L4	4	0.31	43.39
Unpaired female	All-female	Cow Bay Beach	L5	4	0.11	18.75
Pair	All-female	Thornton Beach	L6	1	0.28	34.90
Unpaired female	All-female	Thornton Beach	L7	1	0.18	23.21
Pair	All-female	Cow Bay Beach	L8	1	0.32	22.27
Unpaired female	All-female	Cow Bay Beach	L9	1	0.18	20.11
Pair	All-female	Thornton Beach	L10	1	0.18	23.18
Pair	All-female	Thornton Beach	L6	2	0.34	37.49
Unpaired female	All-female	Thornton Beach	L7	2	0.26	25.46
Pair	All-female	Cow Bay Beach	L8	2	0.51	38.77
Unpaired female	All-female	Cow Bay Beach	L9	2	0.29	28.69
Pair	All-female	Thornton Beach	L10	2	0.34	38.20
Unpaired female	All-female	Thornton Beach	L6	3	0.21	34.52
Pair	All-female	Thornton Beach	L7	3	0.23	29.94

Unpaired female	All-female	Cow Bay Beach	L8	3	0.24	19.64
Pair	All-female	Cow Bay Beach	L9	3	0.32	34.84
Unpaired female	All-female	Thornton Beach	L10	3	0.16	17.91
Unpaired female	All-female	Thornton Beach	L6	4	0.37	40.98
Pair	All-female	Thornton Beach	L7	4	0.15	18.99
Unpaired female	All-female	Cow Bay Beach	L8	4	0.58	43.40
Pair	All-female	Cow Bay Beach	L9	4	0.20	33.02
Unpaired female	All-female	Thornton Beach	L10	4	0.16	25.62
Unpaired male	Mixed-sex	Myall Beach	S6	1	0.04	5.55
Unpaired male	Mixed-sex	Coconut Beach	S8	1	0.06	7.72
Unpaired male	Mixed-sex	Coconut Beach	S9	1	0.07	10.10
Unpaired male	Mixed-sex	Coconut Beach	S10	1	0.09	7.98
Unpaired male	Mixed-sex	Coconut Beach	L8	1	0.05	2.71
Unpaired male	Mixed-sex	Coconut Beach	L7	1	0.02	2.09
Unpaired male	Mixed-sex	Coconut Beach	L6	1	0.04	5.83
Unpaired male	Mixed-sex	Coconut Beach	L9	1	0.02	2.68
Unpaired male	Mixed-sex	Burke's Beach	L10	1	0.05	5.56
Unpaired male	Mixed-sex	Coconut Beach	S6	2	0.04	5.66
Unpaired male	Mixed-sex	Coconut Beach	S8	2	0.03	3.30
Unpaired male	Mixed-sex	Coconut Beach	S9	2	0.03	3.45
Unpaired male	Mixed-sex	Coconut Beach	S10	2	0.05	3.96
Unpaired male	Mixed-sex	Coconut Beach	L7	2	0.08	8.29
Unpaired male	Mixed-sex	Coconut Beach	L6	2	0.05	8.42
Unpaired male	Mixed-sex	Coconut Beach	L8	2	0.12	8.02
Unpaired male	Mixed-sex	Burke's Beach	L10	2	0.00	0.00
Unpaired male	Mixed-sex	Coconut Beach	L9	2	0.00	0.00
Pair	Mixed-sex	Coconut Beach	S6	1	0.14	18.91
Unpaired female	Mixed-sex	Myall Boardwalk	S7	1	0.31	35.72
Pair	Mixed-sex	Burke's Beach	S8	1	0.13	14.86
Unpaired female	Mixed-sex	Coconut Beach	S9	1	0.16	17.11
Pair	Mixed-sex	Coconut Beach	S10	1	0.51	51.45
Pair	Mixed-sex	Coconut Beach	S6	2	0.40	52.78
Unpaired female	Mixed-sex	Myall Boardwalk	S7	2	0.41	39.74
Pair	Mixed-sex	Burke's Beach	S8	2	0.39	33.20
Unpaired female	Mixed-sex	Coconut Beach	S9	2	0.15	20.16

Pair	Mixed-sex	Coconut Beach	S10	2	0.50	51.45
Unpaired female	Mixed-sex	Coconut Beach	S6	3	0.11	19.08
Pair	Mixed-sex	Myall Boardwalk	S7	3	0.44	55.66
Unpaired female	Mixed-sex	Burke's Beach	S8	3	0.29	28.65
Pair	Mixed-sex	Coconut Beach	S9	3	0.13	16.93
Unpaired female	Mixed-sex	Coconut Beach	S10	3	0.56	48.09
Unpaired female	Mixed-sex	Coconut Beach	S6	4	0.16	28.03
Pair	Mixed-sex	Myall Boardwalk	S7	4	0.41	44.87
Unpaired female	Mixed-sex	Burke's Beach	S8	4	0.24	34.90
Pair	Mixed-sex	Coconut Beach	S9	4	0.18	24.57
Unpaired female	Mixed-sex	Coconut Beach	S10	4	0.50	50.84

Table S7. Mean proportions of leaves eaten in Experiments 1 and 2.

Groups	Experiment 1 (% eaten)	Experiment 2 (% eaten)
Unpaired females AF	11%	28%
Unpaired females MS	11%	32%
Pairs from AF	NA	25%
Pairs from MS	14%	24%
Unpaired males	3%	5%

GENERAL CONCLUSION

Theory predicts that sexual conflict can affect population dynamics and persistence (Burke & Bonduriansky, 2017, 2018; Rankin, Dieckmann, & Kokko, 2011). In facultatively parthenogenetic species, sexual conflict also has the potential to generate spatial sex ratio variation (Burke & Bonduriansky, 2018). Facultative parthenogenesis allows females to produce offspring both sexually and asexually depending on whether mating occurs. Facultatively parthenogenetic females can avoid costs associated with mating because of their capacity to reproduce on their own via parthenogenesis. This could be influential in determining spatial variation in sex ratio and could lead to different economics of mating interactions. This is because, as a consequence of females' ability to reproduce sexually if they mate and asexually if they avoid mating, facultative parthenogenesis can generate mixed-sex populations that consist of both males and females and all-female populations that consist of only female individuals in the wild. In principle, such sex ratio variation and establishment of populations that differ in reproductive modes could be explained by costs to females associated with mating and mate guarding, such as a reduced ability to forage. The costs of prolonged mate guarding could drive single females or unmated female nymphs to avoid such costs by dispersing to new areas, where they could produce all-female broods. If females tend to disperse more than males do, this could help to explain the existence of natural populations of all-female composition in the wild alongside mixed-sex populations. However, the costs of mating and mate guarding are poorly understood in facultatively parthenogenetic species.

The aim of this thesis was to explore whether the presence of males interferes with females' foraging behaviour, and whether the sexes differ in dispersal rates, in facultatively parthenogenetic peppermint stick insect, *Megacrania batesii*. In Chapter 1, I investigated whether there is any sex-specific dispersal and, in particular, whether adult females disperse more than males in a natural mixed-sex population. I also examined whether hatchlings are dispersive in travelling between host plants under laboratory conditions, which could clarify whether hatchling dispersal plays a role in spatial sex ratio variation. In Chapter 2, I examined whether males of *M. batesii* affect females' foraging behaviour, and whether such effects (if any) differ between females from all-female vs. mixed-sex populations.

In Chapter 1, I found that neither sex-specific adult dispersal nor hatchling dispersal could explain sex ratio variation in *Megacrania batesii*. I did not find any evidence that one sex disperses more than the other based on a comparison between unpaired females and males in the mixed-sex population. In the mixed-sex population, my observations suggest that both males and females may disperse to close neighbouring patches that are not separated by any

geographical barriers (i.e., streams, estuaries). Thus, females from the mixed-sex population could establish new populations of all-female composition only if those females are unmated and males cannot subsequently invade such populations. Both paired and unpaired individuals dispersed within the habitat patch throughout the study, but pairs moved substantially less than single individuals did. My laboratory study on hatchling dispersal revealed a reluctance to disperse from the initial plant where hatchlings were introduced, suggesting that *M. batesii* hatchlings are less likely to disperse over large distances than adults are, and that hatchling behaviour is unlikely to make a substantial contribution to spatial sex ratio variation.

Comparison of dispersal rates between populations that differed in sex ratio in the field suggested that single females in the mixed-sex population were more dispersive when compared to single females in the all-female population. The presence of males might be the key to explaining why nightly female movement is higher in the mixed-sex population than in the all-female population. However, the difference in dispersal distance between unpaired females in all-female vs. mixed-sex populations was small, and it is not clear whether this difference in behaviour has consequences for rates of female dispersal from the two types of populations. Male-female pairs of *M. batesii* might be more dispersive when disturbed by other pairs or competing rival males inhabiting the same patches when they become active at night, and unpaired females might be more dispersive to avoid males' mating attempts in the mixed-sex population, since such behaviour has been found commonly in other insects (Fairbairn, 1993; Naylor & Adams, 1987). Additionally, hatchlings remained on the initial host plants where they were introduced over 10 days under laboratory conditions. Thus, if adult and hatchling dispersal cannot explain the observed sex ratio variation in *M. batesii* and dispersal after hatching is generally limited to movements within patches, then it is possible that long-distance dispersal occurs by the transport of eggs. For example, a previous study on another species of stick insect suggested that long-distance dispersal can occur via bird predation, potentially leading to the establishment of new populations when predators consumed adult females along with eggs that subsequently hatched (Suetsugu, Funaki, Takahashi, Ito, & Yokoyama, 2018). In addition, several studies suggested that egg dispersal via streams or currents could explain long-distance dispersal of phasmids and the establishment of isolated populations (Kobayashi, Usui, Nomoto, Ushirokita, Denda, & Izawa, 2014; O'Hanlon, Jones, & Bulbert, 2020). Similarly, populations of *M. batesii* might be established via egg dispersal: eggs containing female embryos could establish new all-female populations if transported to new locations. The eggs of male embryos cannot establish new mixed-sex populations on their own unless transported to all-female populations and mating occurs. The persistence of all-female populations therefore suggests that dispersal of eggs

containing male embryos does not introduce males and sexual reproduction into those populations.

One main limitation to the study reported in Chapter 1 was that the majority of females in the mixed-sex population were paired, and there were few single females that remained unpaired throughout the study. This limited our ability to compare dispersal of paired versus unpaired females and unpaired females versus unpaired males in the wild. To address this limitation, it would be useful to carry out a manipulative experiment by removing all males from a habitat patch and to determine whether females' dispersal behaviour changes. Another limitation is the lower resighting probability for single females from the all-female population, which limited our ability to compare dispersal rates between females in the two populations. Larger sample sizes from different locations are needed, and it would be useful to conduct mark-recapture studies over a longer duration. This could provide further insight on the duration of mate guarding, and also potentially reveal rare, long-distance dispersal events that we did not observe over the two-week period.

In Chapter 2, I found inconsistent evidence that guarding males reduce guarded females' foraging rates in *M. batesii*. Results from field-reared and lab-reared individuals and from all-female and mixed-sex populations were somewhat different, suggesting that male effects on females could be context-dependent. In our study, potential factors affecting our results could be age differences between wild-caught individuals in Experiment 1 and lab-reared individuals in Experiment 2. This could explain why foraging rates between the two experiments differed. Other studies have found a negative correlation between aging and food consumption in other animal species such as rats and mice, with less food consumption by older individuals (Gosnell, Levine, & Morley, 1983; Morley, Kumar, Mattammal, Farr, Morley, & Flood, 1996). Additionally, the host plant varieties used to feed experimental animals in Experiment 2 may have affected the lab-reared individuals' foraging behaviour in comparison with wild-caught individuals in Experiment 1, which all fed on the same host plants. In Experiment 2, females from the mixed-sex populations fed more when unpaired than when paired with males, which suggests that male presence can reduce females' foraging rates. However, in Experiment 1, there was no difference in foraging of females from mixed-sex populations when paired versus unpaired. The amounts eaten by the females and males in separate containers, when added together, was not different from the amount eaten by the female and male when in the same container. Therefore, this suggests that females from MS fed the same regardless of whether unpaired or paired with males. Differences between field versus lab conditions (such as ambient temperature) could also have contributed to this difference in results between experiments. Nonetheless, our results suggest that males could interfere with females' foraging behaviour under some circumstances.

Overall, by comparing results from both chapters, I conclude that presence of males can affect females' behaviour in terms of dispersal and foraging activities. In Chapter 1, specifically, I found that the paired females showed shorter dispersal when compared to unpaired females within the mixed-sex population. Also, over the two weeks of our study, guarding males usually remained paired with the same females by clinging to the female partners for prolonged duration, which clearly indicates the potential for strong influence on females' overall activities. However, it would be interesting to examine whether dispersal of *M. batesii* varies over the course of the season, or depending on changes in temperature or precipitation. In Chapter 2, I found that females descended from mixed-sex populations (and reared in the laboratory) fed more when unpaired than when paired with males.

Our empirical findings suggest the possibility that males can be costly for females in impacting females' overall fitness, but this requires clarification through future research. While the presence versus absence of males might be responsible for the population differences observed in females' overall activity levels, whether females in mixed-sex populations disperse more to avoid males or to seek better quality mates is yet to be tested. One of the important insights of this thesis is the potential for context dependence of sexual conflict in *Megacrania batesii*. This is because many aspects of environment can shape sex-specific selection on individuals. Sex ratio variation might have a big influence on how males and females are selected in behaving during mating interaction. Plesnar-Bielak and Łukasiewicz (2021) reported that environmental changes and their varied effects play an important role in driving conflict between the sexes, and that individuals behave differently in response to different environmental conditions. Population sex ratio could be a key influence on sexual conflict in *M. batesii*.

This thesis explored questions related to how sexual conflict can drive demographic patterns in facultative systems in the wild. But there are still many gaps in knowledge yet to be filled. The natural populations of such facultative parthenogens need more studies to examine mating behaviours, particularly the ability of males to cause asexual populations to transition to sexuality and whether females from all-female populations resist mating attempts in the wild. Another interesting aspect to investigate is whether facultative parthenogenesis may lead males to expend more energy in mating because more energy is required to due to females' increased mating resistance, causing males to die sooner. The reasons and consequences for males of feeding at much lower rates than females (Chapter 2) are yet to be fully understood. Therefore, it is crucial to identify whether guarding males die sooner than non-guarding males because they allocate more time and energy to guarding. The empirical studies in this thesis also highlight the need to examine whether paired females experience reduction in offspring, which reflects whether females accumulate costs of guarding throughout the lifespan. How

sexual conflict can manifest could depend on sex ratios (all-female versus mixed-sex population) (Burke & Bonduriansky, 2018), quality of food (i.e., host plant) (Łukasiewicz, 2020), and environmental (e.g. field versus lab) conditions (Plesnar-Bielak & Łukasiewicz, 2021). In addition, theory predicts that males can impede asexual reproduction by being coercive towards females and force them to reproduce sexually (Burke & Bonduriansky, 2017; Kawatsu, 2013). The effects of sexual conflict can differ between populations when those populations differ in sex ratio. During such extended periods of guarding, the females of *Megacrania batesii* can be exposed to physical damage while carrying the males around. Such costs have been found in some animals where mating interactions impose injures on females (Johnstone & Keller, 2000; Rönn, Katvala, & Arnqvist, 2007). Further questions such as the effects of male presence and sexual conflict on population fitness would be interesting to explore with this system in future studies.

Understanding the evolution of facultative parthenogenesis and the factors responsible for demographic variation in facultative populations is important for understanding the economics of sex. The aim of this thesis was to test whether mate guarding imposes costs on female foraging, and whether sex-specific dispersal behaviour could explain the mosaic sex-ratio patterns seen in this species and other facultatively parthenogenetic animals. Overall, this thesis highlights that sexual conflict could be pronounced in *Megacrania batesii*, and that costs associated with mate guarding might contribute to the maintenance of female-only populations by inducing greater female dispersal. However, more investigations are needed to clarify how biotic and abiotic conditions favour or disfavour mating, whether males impose substantial costs on females, are whether such costs are constant or change over females' lifespan. Future studies that investigate lifetime costs and benefits will be key to understanding the link between sexual conflict and facultative parthenogenesis.

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