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Could adult or juvenile dispersal shape geographical parthenogenesis? Evidence from the facultatively parthenogenetic phasmid *Megacrania batesii*

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Abstract

Despite their individual reproductive flexibility, populations of many facultatively parthenogenetic animals vary in sex ratio and reproductive mode. Sex-specific dispersal could contribute to such spatial variation. We asked if sex-specific dispersal by adults or nymphs occurs in the facultatively parthenogenetic phasmid *Megacrania batesii*, which forms a geographical mosaic of mixed-sex (mostly sexually reproducing) and all-female (parthenogenetic) populations. If sex-specific dispersal contributes to sex-ratio variation in this species, we would expect to observe greater dispersal by females than by males. We carried out a mark-resighting field study over three years to investigate adult dispersal in mixed-sex and all-female populations. To better understand how males affect female behaviour, we also investigated pairing and mate-guarding. In addition, we investigated dispersal by hatchling nymphs in a semi-natural enclosure. Mean nightly movement distances did not differ between unpaired (single) females and males in the mixed-sex population. However, unpaired females moved further in mixed-sex than in all-female populations. Many adult females in the mixed-sex population continually carried guarding males on their dorsum. Pairs often remained together for multiple days, and few females or males were observed pairing with multiple partners. Paired females moved shorter distances than unpaired females, and such females' movement increased following experimental removal of males. Hatchling nymphs rarely moved between plants. Our findings suggest that guarding males affect females' movement patterns, but nymph and adult dispersal is unlikely to shape spatial variation in sex ratio in *Megacrania batesii*.

Keywords Geographical parthenogenesis · Dispersal · Facultative parthenogenesis

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Introduction

Asexual and sexual lineages are often found to occupy distinctive habitats, typically along a geographical gradient—a phenomenon called geographical parthenogenesis (Vandel 1928; Glesener and Tilman 1978; Chaplin and Ayre 1997). Asexual lineages tend to inhabit disturbed areas, occur at higher altitudes or latitudes, and exhibit more widespread distributions when compared to sexual lineages (Vandel 1928; Glesener and Tilman 1978; Kearney 2005; Kearney et al. 2006). Such expansion of geographical range by asexual lineages could be facilitated by rapid population growth (Glesener and Tilman 1978; Hörandl 2006). Moreover, hybrid origin (Wright and Lowe 1968; Kearney 2005; Kearney et al. 2006) and polyploidy (Otto and Whitton 2000) could enable some asexual lineages to perform better in harsh environments by comparison with their sexual relatives (Peck et al. 1998). While some studies of geographical parthenogenesis have focused on reproductively isolated sexual and asexual lineages, spatial variation in sex ratio and reproductive mode also occurs in facultatively parthenogenetic animals (Buckley et al. 2009; Morgan-Richards et al. 2010). The causes of such demographic and reproductive variation in facultative parthenogens remain poorly understood.

In facultatively parthenogenetic species, every female can switch between asexual reproduction (which typically results in all-female broods) and sexual reproduction (which results in production of both sons and daughters), depending on whether mating occurs (Bedford 1978; Galis and van Alphen 2020). Yet, despite this individual reproductive flexibility, spatial gradients or mosaics of mixed-sex and all-female populations have been reported in facultatively parthenogenetic animals such as phasmids (Buckley et al. 2009; Morgan-Richards et al. 2010; Larose et al. 2023; Miller et al. 2024a), opilionids (Burns et al. 2018; Machado and Burns 2022), and ostracods (Chaplin and Ayre 1997). For example, in the New Zealand stick insect, *Clitarchus hookeri*, mixed-sex populations are mainly found on the north island whereas all-female populations are mainly found on the south island (Morgan-Richards et al. 2010), while the stick insect *Megacrania batesii* forms a mosaic of mixed-sex and all-female populations throughout its range in Queensland, Australia (Miller et al. 2024a). Such demographic variation in facultative parthenogens is especially intriguing and challenging to explain because it occurs within species.

Sex-biased dispersal has the potential to generate variation in population sex ratio (Gaines and McClenaghan 1980), but there has been little research on dispersal in facultatively parthenogenetic species (although see Chaplin and Ayre 1997). Dispersal could play an important role in facultatively parthenogenetic species because, just as a single seed of a self-compatible plant could colonize a new habitat patch (Baker 1967), a single facultatively parthenogenetic female could establish a new all-female population. Conversely, invading males could change all-female populations into mixed-sex populations by mating with the females and inducing the production of sons (Miller et al. 2024a).

Dispersal in insects can occur at different life stages: egg, nymph, and/or adult (Stanton et al. 2015; Keller et al. 2020). Sex-specific dispersal has been reported in some insects at the adult stage (Auckland et al. 2004; Asplen et al. 2016). However, in some hemipterans, juvenile stages can be more mobile and dispersive (Lee et al. 2014; Keller et al. 2020), and could therefore contribute to sex-specific dispersal. In some phasmids, where females flick their eggs actively from their ovipositor, drop eggs on the ground, or attach eggs to plants (Bedford 1978; Robertson et al. 2018), dispersal can occur at

the nymphal stage as hatchlings seek a suitable host plant to inhabit (Zeng et al. 2020). Zeng et al. (2020) described active movement strategies by hatchlings of the stick insect *Extatosoma tiaratum*, but whether dispersal differs between the sexes at the nymphal stage is unknown.

In this study, we asked whether unequal movement rates of adult or hatchling females and males could help explain sex ratio variation in the facultatively parthenogenetic stick insect *Megacrania batesii*. Cermak and Hasenpusch (2000) reported two isolated all-female populations at the southern edge of the species range in Australia. Further research showed that *Megacrania batesii* populations vary in sex ratio throughout their range, forming a spatial mosaic of mixed-sex populations (mostly sexually reproducing and exhibiting approximately equal sex ratios) and all-female populations (parthenogenetically reproducing and containing only females). It remains unclear how all-female populations form and persist given that these populations occur in close proximity to mixed-sex populations, with few obvious barriers to dispersal and no known differences in habitat (Miller et al. 2024b). Mark-recapture/resighting is a common method for investigating dispersal in wild animals (Auckland et al. 2004). However, mark-resighting studies are rarely done in invertebrate animals (Janks and Barker 2013; Zajitschek et al. 2019) and, to our knowledge, have never been performed in any phasmid. We used mark-resighting to address three main aims: (1) to compare movement of adult females and males, (2) to investigate female-male pairing, and effects of guarding males on female movement, and (3) to compare movement of females in all-female vs. mixed-sex populations. We also tracked marked *Megacrania batesii* hatchlings in a semi-natural enclosure to determine whether hatchlings are dispersive and whether movement is sex-specific. If dispersal after hatching contributes to the establishment of all-female populations in *Megacrania batesii* then we would expect to observe greater movement distances by female hatchlings or unpaired adult females than by males or paired (guarded) females.

Methods

Study populations

Australian *Megacrania batesii* populations are patchily distributed along the coastline between Mission Beach and the Bloomfield River in far-north Queensland (Cermak and Hasenpusch 2000; personal observations). Detailed, multi-year data on sex ratios in populations CO, CB and KB, as well as other wild populations of *M. batesii*, can be found in Miller et al. (2024a). The body length varies from 72 to 87 mm in males, and from 101 to 102 mm in females (Cermak and Hasenpusch 2000). Both sexes have the same bluish-green colour (Brock and Hasenpusch 2009) and neither sex can fly, although males have longer wings than females (Cermak and Hasenpusch 2000). We conducted our fieldwork between Cape Kimberley and Cape Tribulation, where *Megacrania batesii* is abundant, between February and March over three consecutive years (2020–2022). This time of the year corresponds to the rainy season, when *Megacrania batesii* adults reach peak numbers (Cermak and Hasenpusch 2000). *Megacrania batesii* adults are unlikely to survive more than approximately six months in the wild (JB, personal observation). Therefore, different individuals were marked each year. In selecting study sites (Fig. 1), we chose *Pandanus* and *Benstonea* host-plant patches that supported *Megacrania batesii* populations and were easy to access along beachfronts. We studied three populations (codes CO, CB
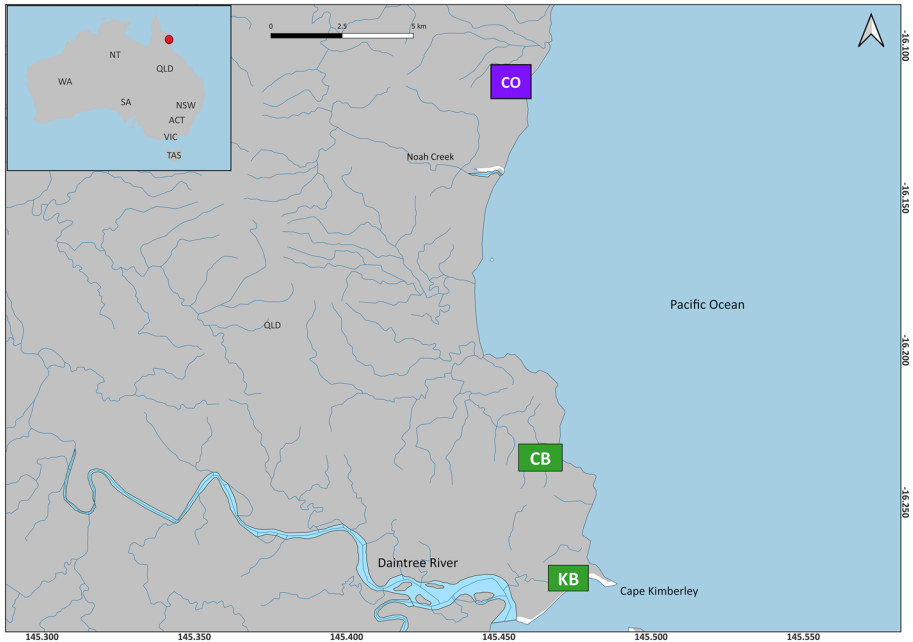
Fig. 1 Mark-resighting study sites: The general study area is indicated by a red point on the inset map. Mixed-sex population CO is shown in purple and all-female populations CB and KB are shown in green. Population codes correspond to Miller et al. (2024a)

and KB in (Miller et al. 2024a)): Mixed-sex population CO occurs on immature and mature *Pandanus tectorius* plants in beach-front forest at the side of a stream at the south end of Cape Tribulation village. Individuals at CO were categorised into three groups: unpaired females, unpaired males, and pairs (i.e., an adult female with a guarding male clinging to her dorsum). All-female population CB occurs on *Benstonea monticola* in a swampy area along a stream and on adjacent immature and mature *Pandanus tectorius* plants along the beach at the east end of Cow Bay village. All-female population KB occurs on *Pandanus tectorius*, *Pandanus spiralis* and *Pandanus cookii* in beach-front forest near Cape Kimberley. All individuals at CB and KB were females. Therefore, each year, we carried out a mark-resighting study in the mixed-sex population CO and one all-female population (CB or KB), as described below.

Mark-resighting methodology

Within each population, we used a mark-resighting method to track movements of *Megacrania batesii* adults (and a few final-instar female nymphs). Individuals were marked on the first day of the study each year, and additional unmarked individuals were marked on subsequent days. Resighting data were collected either daily or every other day, starting on the 2nd or 3rd day of the study (see below). Newly sighted individuals were marked with unique codes on their thorax and/or wings (Fig. 2) using black permanent marker (Artline xylene-free permanent marker, Shachihata, Japan) in situ on their host plants, without handling or restraining them. Host plants on which *Megacrania batesii* individuals

Fig. 2 Marked *Megacrania batesii* individuals and their host plants: **a** marked female at CB, **b** marked female-male pair at CO. Host plants where *Megacrania batesii* individuals were marked or resighted were tagged with numbered plastic key tags (indicated in the images with blue arrows): **c** *Pandanus tectorius* plant at CO, **d** *Benstonea monticola* plant at CB
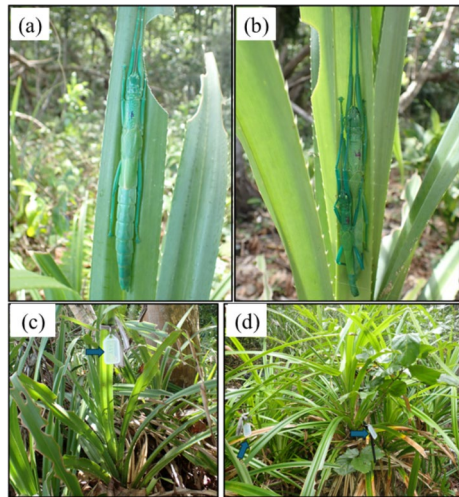
were marked or resighted were tagged with numbered plastic tags (Fig. 2). Both males and females can spray a defensive fluid when disturbed (Cermak and Hasenpusch 2000; Jones and Bulbert 2020), which can complicate marking by depositing moisture on the thorax. If a spraying response occurred, we waited a few minutes and then attempted to write the same code on the forewings. When an individual was found at the base of a leaf, we gently tapped the tip of its abdomen to trigger it to crawl upwards to become accessible for marking. When marking male–female pairs, the male's antennae sometimes blocked the female's thorax. In such cases, the antennae were pushed to the side or the male was moved gently to the side or backwards to expose the female's thorax.

Each year, we conducted mark-resighting over 11–12 days in February/March, the time of the season when *M. batesii* adults are most abundant (Table 1). In 2020, in addition to adult females, we also marked three final-instar female nymphs at CB. In 2020, almost no rain occurred during our mark-resighting study, and average maximum temperature was ~34.6 °C (Tables S1–S3). In 2021, there was considerable rainfall during our mark-resighting study, and the average maximum temperature was ~30.5 °C (Tables S1–S3). In 2022, there was little rainfall during our field-study, and average maximum temperature was ~34.7 °C (Tables S1–S3).

Dispersal

To estimate dispersal rates, we quantified how far individuals moved between successive sightings. We checked for previously marked individuals on each plant (absence or presence) on each resighting day, measured the distance between the previous sighting location and new location, and recorded the identity and pairing state (unpaired or paired) of each marked individual. We collected the Global Positioning System (GPS) coordinates of each individual at its point of initial sighting or resighting using a Garmin eTrex 20×GPS. Because GPS coordinates are imprecise over small distances, we also measured the linear distance between plants using a tape-measure and use these measurements in analyses of dispersal distance. The "nightly dispersal" distance was calculated for each individual each time it was resighted as the distance between the previous and current location divided by

Table 1 Mark-resighting period and duration across three years at all-female and mixed-sex populations. Each year, two populations were studied (one all-female, one mixed-sex). For each population, the habitat type and host-plants, start and end dates of mark-resighting, and the dates when mark-resighting was conducted are shown

Year	Population type	Location	Habitat type	Host-plants	Mark-resighting period	Resighting dates
2020	All-female	CB	Swampy forest	*Benstonea monticola*	3rd to 14th February	February: 4, 6, 8, 10, 12, 14
2020	Mixed-sex	CO	Beach-front forest	*Pandanus tectorius*	2nd to 14th February	February: 3, 4, 6, 8, 10, 12, 14
2021	All-female	KB	Beach-front forest	*Pandanus tectorius; Pandanus spiralis; Pandanus cookii*	25th of February to 8th March	February: 26, 28; March: 2, 4, 6, 8
2021	Mixed-sex	CO	Beach-front forest	*Pandanus tectorius*	26th of February to 8th March	February: 27, 28; March: 1, 2, 3, 4, 5, 6, 7, 8
2022	All-female	CB	Beach-front forest	*Pandanus tectorius*	23rd February to 5th March	February: 25, 27, 28; March: 1, 3, 5
2022	Mixed-sex	CO	Beach-front forest	*Pandanus tectorius*	22nd February to 4th March	February: 24, 26, 28; March: 2, 4

the number of nights between the current sighting and the previous sighting (since *Megacrania batesii* are mostly active at night). The mean nightly dispersal distance for each individual was calculated as the sum of the nightly dispersal distances divided by the number of nights between the initial sighting/marking and the final resighting.

Male removal experiment

To further investigate the effect of male guarding on female dispersal, we removed males from a patch of host plants at CO (Middle patch) on the 3rd of March 2021, thoroughly checking both during the day and at night and removing any males that we could find. Locations of all females that had been separated from guarding males were recorded daily until the 7th of March, and their nightly dispersals were measured. The removed males were kept in individual mesh cages in our field-laboratory, misted with water and provided fresh host plant leaves daily. The males were released in the same patch on 7th of March.

Dispersal of hatchlings

We observed dispersal of *Megacrania batesii* hatchling nymphs from 25th July to 28th August 2020 in a semi-natural enclosure set up inside a controlled-temperature room at UNSW Sydney. Hatchlings were obtained from eggs collected in the field between Cow Bay and Cape Tribulation, Queensland, in February 2020. Each day, newly hatched nymphs were sexed based on the morphology of the terminal abdominal sternites (see Miller et al. 2024a), marked individually with distinctive patterns of coloured dots using permanent marker (Sharpie, Australia), and randomly placed on *Pandanus tectorius* plants distributed on the floor of a controlled-temperature room (maintained at 25 ± 2 °C and $60 \pm 20\%$ relative humidity; 12 h of cycle day/night with white and red lights). Six immature *Pandanus* plants (40 – 60 cm in height) were placed in the room, with one plant in
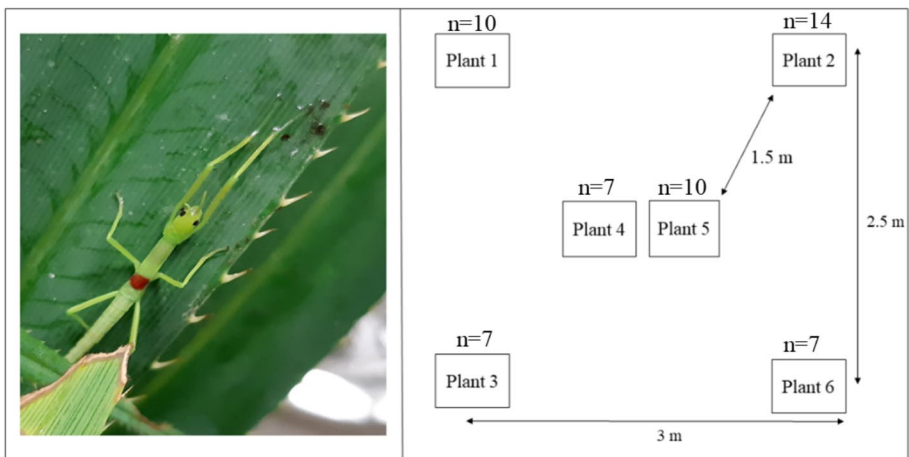

Fig. 3 Hatchling dispersal experiment: Left panel: a hatchling marked on the thorax with a red dot. Right panel: distribution of *Pandanus* plants in the controlled-temperature room. The total number of hatchlings introduced to each plant is indicated above each plant. The black arrows indicate the distances between the plants

each corner and two plants in the centre (Fig. 3). Each morning, we recorded the locations of all hatchlings. Random placement of hatchlings on the plants, and removal of individuals that moulted into the 2nd instar, led to an uneven distribution of hatchlings on the plants (Fig. 3). However, since all hatchlings had abundant food, we do not believe that this variation in numbers affected hatchling behaviour or biased our results. Each nymph was monitored until it underwent its first molt, after which we were not able to track individuals because their individual markings were shed along with their exoskeletons. In total, we marked and released 55 hatchlings (39 females, 16 males), and observed each hatchling over 10 ± 2 days.

Statistical analyses

We carried out analyses of nightly dispersal (excluding data from CO during the period of male removal) using linear mixed models (LMM) with Gaussian distributions in R version 4.2.0 (R Core Team 2024). Models were fitted using the lmer function from the lme4 package (Bates et al. 2015), and fixed effects (as described below) in LMMs were tested with F-tests based on Satterthwaite's degrees of freedom using the lmerTest package (Kuznetsova et al. 2017). To assess whether there were differences in nightly dispersal between unpaired females in the mixed-sex versus all-female populations, we used a LMM with population type (mixed-sex vs. all-female) and year as fixed effects, and nightly dispersal (individual values for each observation) used as the response variable.

To assess whether there were differences in nightly dispersal distance between paired versus unpaired females in the mixed-sex population, we used a LMM with pairing status (paired vs. unpaired) and year as fixed effects. Finally, to test whether unpaired males and unpaired females differed in nightly dispersal in the mixed-sex population, we used an LMM with individual sex (female vs. male) and year as fixed effects. Observation day (represented by a unique code for each day when we searched for marked individuals), and individual ID, were included as random effects in all nightly dispersal models. We did not include population identity or host-plant species in the models because our data do not allow us to differentiate effects of unique population attributes (genotype, host plant, habitat parameters) from effects of population type (mixed-sex vs. all-female).

In the analysis of data from the male removal experiment, we only used data from the Middle host-plant patch where male removal was carried out, from the day following male removal (4th March) until the final day prior to male release (6th March). Seven females in this patch were paired with a male at each resighting before male removal. For these seven females, we compared nightly dispersal distances before male removal (i.e., while paired) versus after male removal (i.e., while unpaired) using a LMM with nightly dispersal distance as the response variable, female status (before vs. after male removal) as the fixed effect, and female individual ID as a random effect. Day of observation was excluded from this model because its inclusion resulted in a singular fit warning, but results are qualitatively identical with and without this random effect in the model. To determine if there was any change in mean movement distances of unmanipulated females during the time interval when male removal was carried out in the Middle patch, we also analysed the dispersal of paired females ($N=15$) in the Southern and Northern patches (where no males were removed). We compared nightly dispersal distances of paired females in those patches before and including March 3, 2021 (when males were removed from the Middle patch) with nightly dispersal distances of those females after this date. We used a linear mixed

model (LMM) with nightly dispersal distance as the response variable, time interval before vs. after male removal as the fixed effect, and female individual ID as a random effect.

Finally, we used our resighting data to assess pairing and mate-guarding patterns by calculating the number of different partners that each female and male was observed with sequentially over the course of the observation period each year at CO. Male mating success (S) over the period of observation was calculated as the number of different females with which each male was found paired. Opportunity for sexual selection on males was estimated as the coefficient of variation in male mating success, $I_S = \frac{\sigma_S}{\mu_S}$ (Henshaw et al. 2016), where σ_S is the standard deviation of the distribution of individual male mating success (S) while μ_S is the mean of the distribution of S. If opportunity for sexual selection on males (I_S) is high, this indicates that sexual traits that contribute to successful mating can be strongly favoured in the population. Conversely, if I_S is low, male sexual traits are unlikely to experience strong sexual selection.

Results

Over three years (2020, 2021, 2022), we marked a total of 209 *Megacrania batesii* adults (112 females, 97 males) from the mixed-sex population (CO). Of these, 191 (91%) were resighted after marking, with an overall mean of 5.0 sightings per individual. Adult sex ratio at CO was approximately even each year, consistent with population sex ratios in most mixed-sex populations in *M. batesii* (Miller et al. 2024a). We also marked 71 females from all-female populations (CB, 54 females; KB, 17 females). Of these, 45 (63%) were resighted, with an overall mean of 2.6 sightings per individual. Our field mark-resighting data consist of 977 resightings of these marked *Megacrania batesii* individuals over the three years.

Dispersal in all-female populations

In population CB, we marked 18 females (15 adults and three final-instar nymphs) in a patch of *Benstonea monticola* host plants in 2020. Nine females moved between neighbouring (often entangled) host plants but were rarely observed dispersing over longer distances (Fig. 4), three other females remained on the same plants throughout, and six females were never resighted. The maximum distance travelled in one night was 1.15 m. We also marked 36 adult females in population CB in a patch of *Pandanus tectorius* host plants in 2022. Of these, 17 females dispersed to neighbouring plants, five females remained on the same plants throughout, and 14 females were never resighted. The maximum distance travelled in one night was 20 m. At KB, we marked 17 adult females on *Pandanus* spp. host plants in 2021. Of these, seven females dispersed between plants, four females remained on the same plant throughout (Fig. 4), and six females were never resighted. The maximum distance travelled in one night was 11 m.

Dispersal, pairing and mate-guarding in the mixed-sex population

In the mixed-sex population (CO), females, males, and pairs (i.e., females with guarding males) frequently dispersed between host plants, including non-neighbouring plants (Fig. 5). Of 209 individuals marked at CO, only five individuals (2%; 4 males, 1 female)
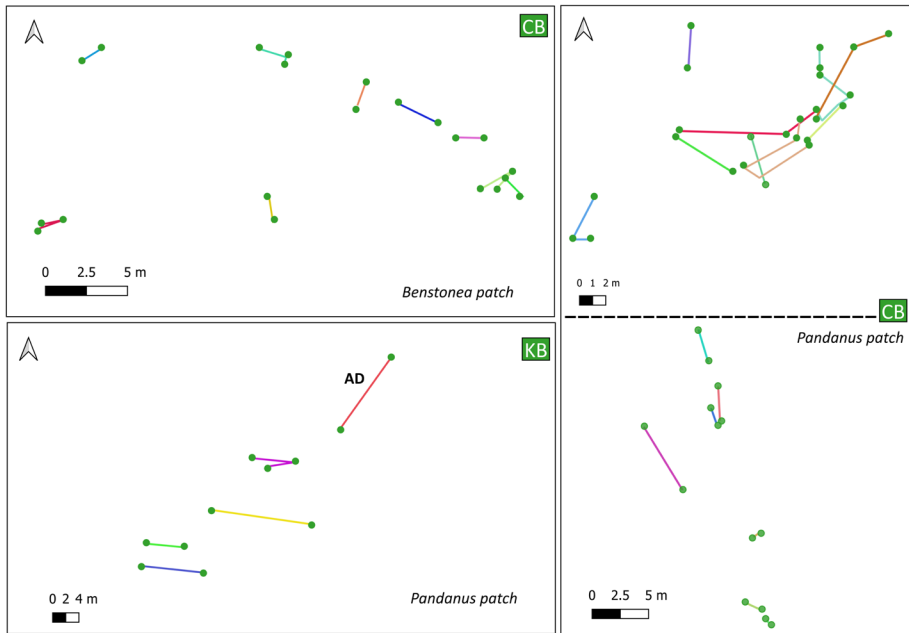
Fig. 4 Dispersal patterns of females in all-female populations CB and KB. Female dispersal between host plants (green points) is represented by coloured lines, with different colours representing different individuals within populations. At CB, mark-resighting studies were carried out in a swampy *Benstonea monticola* patch in 2020 and a beachfront *Pandanus tectorius* patch in 2022. The *Pandanus* patch at CB is subdivided by a small estuary (represented by the dashed line), and locations on different sides of this estuary are shown at different scales. At KB, a mark-resighting study was carried out on immature *Pandanus* spp. plants in 2021. Letters "AD" represent codes of two females that travelled between the same two host plants

were always resighted on the same plant (mean = 2.4 resightings per individual), and 18 were never resighted. The longest nightly dispersal distances were 30 m by an unpaired female, 19 m by an unpaired male, and 11 m by a pair.

When mate-guarding, the *Megacrania batesii* male typically sits on the female's dorsum (Fig. 2b). Pairs were usually not in genital contact when resighted, but guarding males were sometimes observed clasping the female's genitalia with their genitalic claspers, with or without intromission. Paired females dispersed between leaves and host plants by carrying guarding males. Females in the mixed-sex population were guarded ~ 57% of the time on average (344 of 603 sightings), but there was substantial variation among individual females: 44 females were always found with a guarding male (mean = 4.25 sightings per female), 43 females were found both with and without a guarding male (mean = 6.9 sightings per female), and 23 females were never guarded (mean = 5.2 sightings per female).

Many pairs remained together for the entire duration of our annual observations, and few females or males were seen paired sequentially with multiple partners (Table 2). Across the three years, mean male mating success over the period of observation was $\mu_S = 1.15$ ($\sigma_S = 0.58$), and estimated opportunity for sexual selection on males was $I_S = 0.50$. This indicates that opportunity for sexual selection to act on males in mixed-sex population (CO) is moderate.
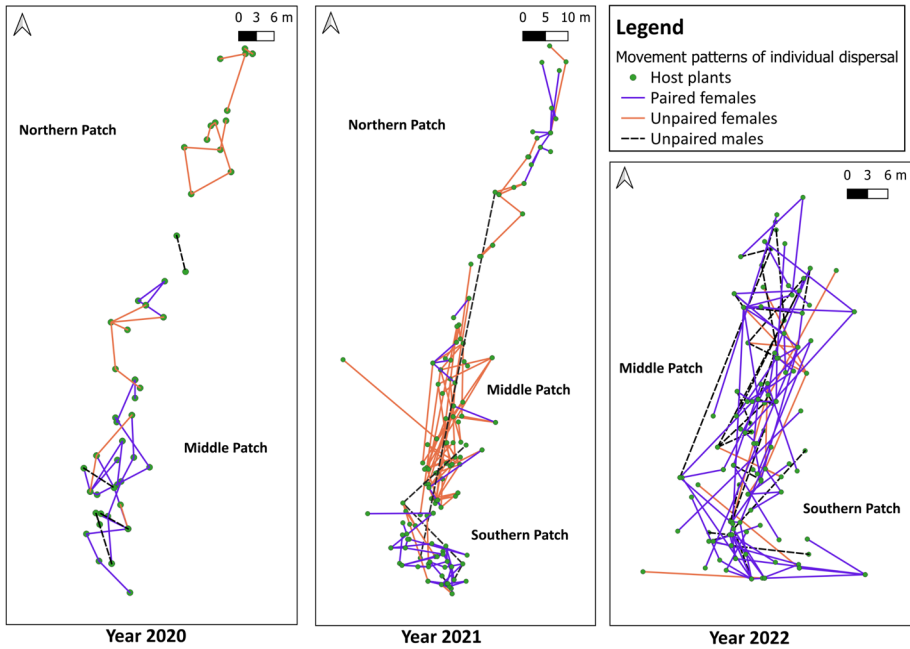
Fig. 5 Movement patterns of individuals in mixed-sex population CO across three years. Movements by pairs (solid purple lines), unpaired females (solid orange lines) and unpaired males (dashed black lines) are shown separately for each year. Green points indicate host plants where *Megacrania batesii* individuals were marked or resighted. The habitat where the mark-resighting studies were carried out consists of three patches of *P. tectorius* host plants (Southern, Middle, Northern), but some patches were not studied in some years

Table 2 Mate switching and male mating success at CO across three years. For each year, the data show the total number of females observed with a guarding male, the number of females seen paired sequentially with more than one male (including one female paired with three males in 2021 and one female paired with three males in 2022), the total number of males marked, the number of males seen guarding one, two or three females sequentially, and mean male mating success (i.e., total number of individual females guarded per male) over the duration of the mark-resighting study in each year

Year	Paired females marked	Females paired with 2 or more males	Total males marked	Males paired with at least 1 female	Males paired with 2 females	Males paired with 3 females	Mean male mating success
2020	8	1	10	9	0	0	0.9
2021	35	2	32	30	10	1	1.35
2022	45	13	55	48	10	0	1.07

Dispersal distance

In the mixed-sex population, we did not find a difference between mean nightly dispersal distances of unpaired females versus unpaired males (Fig. 6; Table 3). However, unpaired females tended to disperse further than paired females (Fig. 6; Table 3). There
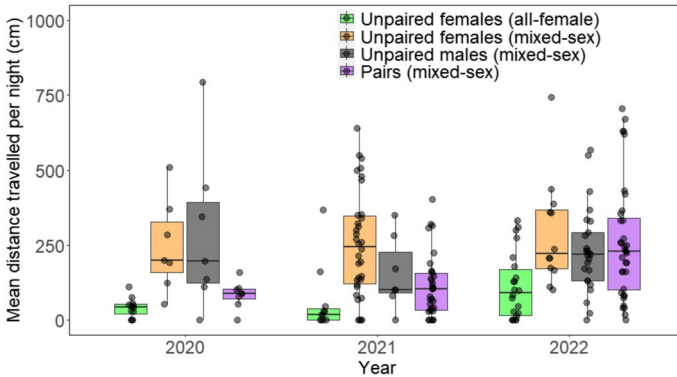
Fig. 6 Mean nightly dispersal distances (cm) of unpaired females in all-female populations (CB, KB) (green), unpaired females in mixed-sex population CO (orange), unpaired males in mixed-sex population CO (grey), and pairs (females with guarding males) in mixed-sex population CO (purple). The black line inside each box shows the median of individual values, the box shows the inter-quartile range, and the whisker shows the non-outlier range. Each point represents mean nightly dispersal distance for an individual. For females in the mixed-sex population that were observed both paired and unpaired, separate points represent means for paired and unpaired observations

was also a near-significant effect of year, and post-hoc Tukey tests showed that females' mean nightly dispersal distance was greater in 2022 than in 2021 and 2020 (Table S5). Unpaired females from the mixed-sex population dispersed further than unpaired females from all-female populations (Fig. 6; Table 3).

Male removal experiment

Females that had been paired prior to male removal dispersed longer distances after the guarding males were removed ($F = 8.1889$, df $= 42.372$, $p = 0.006$; Fig. 7a). These females travelled approximately 4 times farther per night after male removal than while they were paired with guarding males (Fig. 7a and b). In paired females in patches where males were not removed, no change occurred in nightly dispersal distance over the same time period ($F = 0.0581$, df $= 79$, $p = 0.81$; Fig. 7c).

Dispersal by hatchling nymphs

Of 55 hatchling nymphs, only three individuals (2 males, 1 female) dispersed from their initial plants in the semi-natural enclosure. One male nymph dispersed 1.5 m to a neighbouring plant two days after being released into the enclosure. Another male nymph dispersed 10 days after being released but did not locate a new plant. A female nymph 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 over 10 ± 2 days of observation.

Table 3 Type III Analysis of Variance (ANOVA) with Satterthwaite's method comparing nighly dispersal distances of (a) unpaired females versus unpaired males in the mixed-sex population (CO), (b) paired versus unpaired females in the mixed-sex population (CO), and (c) unpaired females from all-female and mixed-sex populations in years 2020, 2021, and 2022. Individual ID and observation day were modeled as random effects in all models. DF represents degrees of freedom

| Comparison	Source of variation	DF	F ratio	P value
(a) Unpaired females vs unpaired males in mixed-sex population (CO)	Sex	82.755	0.734	0.3939
	Year	34.613	0.624	0.5416
	Sex×Year	83.631	0.213	0.8087
(b) Paired vs unpaired females in mixed-sex population (CO)	Status	216.572	17.464	<0.0001
	Year	36.198	2.899	0.0680
	Status×Year	220.274	1.320	0.2692
(c) Unpaired females from the mixed-sex vs all-female populations	Population type	85.469	23.604	<0.0001
	Year	20.276	1.065	0.3634
	Year×Population type	83.606	0.206	0.8143
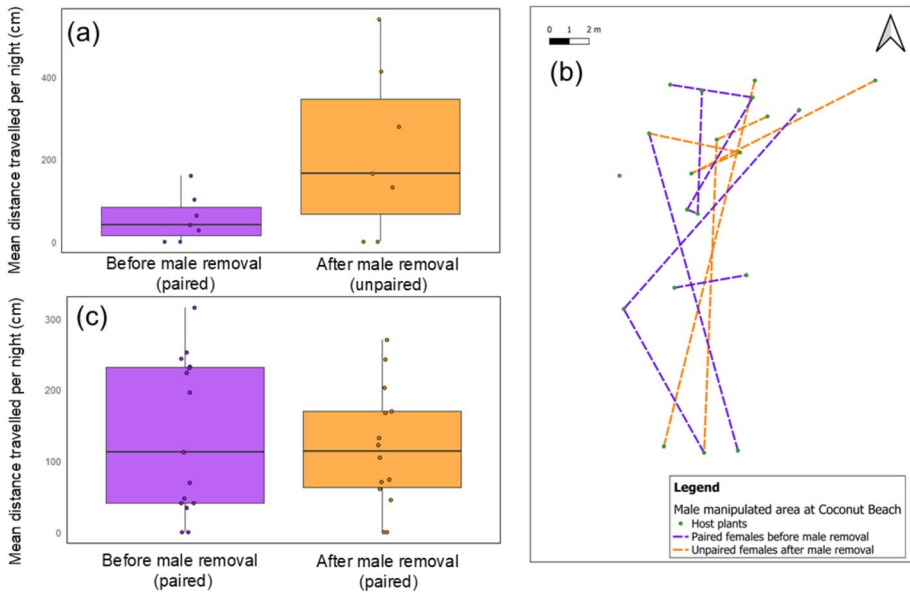
Fig. 7 (**a**) Mean nightly distances travelled by the same individual females ($N = 7$) before male removal (purple) vs. after male removal (orange) in the male removal patch at CO (2021). The centre black line inside each box shows the median, the box shows the inter-quartile distance and the whiskers show the non-outlier range. Points represent nightly mean displacement distances for individual females. (**b**) Dispersal patterns for the same set of females before male removal (purple dashed lines) and vs. after male removal (orange dashed lines). (**c**) Mean nightly distances travelled during the same time intervals by paired females in other patches where male removal did not occur

Discussion

Our mark-resighting data indicate that, on average, unpaired females in the mixed-sex population dispersed greater distances than paired females, but unpaired males dispersed similar distances to unpaired females. Females in all-female populations appeared to disperse shorter distances than unpaired females in the mixed-sex population. This finding suggests that new populations might be established most frequently by females dispersing from mixed-sex populations. The likelihood of such newly established populations being all-female would therefore depend on how often the founding females are unmated. We observed little dispersal by nymphs of either sex in a semi-natural enclosure. Thus, our results provide little evidence that dispersal by nymphs or adults could contribute to observed variation in population sex ratio in this species.

We found that both males and females typically moved short distances between adjacent or nearby plants, and rarely traversed areas devoid of host plants. Across all populations and individuals, mean nightly distance moved was < 2 m. However, both males and females were occasionally observed to move longer distances. Such occasional long-distance dispersal by adults could contribute to gene flow or the establishment of new populations. Long-distance dispersal events also have the potential to bias mark-resighting data, potentially resulting in under-estimation of true dispersal propensity. If any individuals dispersed from our study areas to other habitat patches, we would have failed to re-sight them. Such detection bias could be especially important in the all-female populations, given the

relatively low resighting rate. However, population-genomic analysis suggests that rates of gene flow are very low in *Megacrania batesii* (Miller et al. 2024a), consistent with the low dispersal propensity suggested by our mark-resighting data.

We only investigated post-hatching dispersal, but research on the congeneric species *Megacrania tsudai* shows that eggs can survive immersion in sea water for several months, suggesting that rare long-distance dispersal events could involve eggs carried by water (Kobayashi et al. 2014). Eggs could also be transported by animals such as ants or birds (Wu et al. 2020). Egg dispersal is unlikely to be sex-specific, but egg dispersal might contribute to sex ratio variation in *Megacrania batesii* because a single egg containing a female embryo could establish an all-female population, which could persist unless eggs containing male embryos are subsequently transported to the same habitat patch. More research is needed to understand the role of dispersal by eggs versus post-hatching stages.

In the mixed-sex population, unpaired females moved longer distances than paired females, suggesting that the presence of guarding males reduced females' dispersal. Moreover, paired females moved more after the guarding males were removed, supporting the causal role of guarding males in reducing female movement. By contrast, we found no change in mean movement distances of paired females in other habitat patches over the same time period. Thus, the increased movement distances of paired females after the removal of guarding males cannot be attributed to seasonality or weather changes. We cannot be certain whether unpaired females moved more because of lower constraints on movement, or because they were searching for mates. It is also possible that the removal of guarding males as part of this experiment disturbed or frightened the females, and that this caused those females to move longer distances. A follow-up study with a larger sample size and conducted over a longer time-period would be valuable.

Our observations indicate that males guard females for prolonged durations in the wild and therefore suggest that mate-guarding could impose substantial costs on females. Guarding males' weight is likely to directly elevate the energetic costs of movement for paired females. Reduction in paired females' dispersal has also been found in other arthropods, such as the crustacean *Gammarus duebeni* (Naylor and Adams 1987) and the water strider *Aquarius remigis* (Fairbairn 1993). In *A. remigis*, it has been shown that males' added weight increases females' energetic costs (Watson et al. 1998). Guarding males might also impose other costs, for example by increasing visibility to predators (Han and Jablonski 2010), or interfering with female foraging (although see Galipaud et al. 2011). Therefore, costs associated with the presence of males may hinder dispersal of *Megacrania batesii* females, and could thereby contribute to sexual conflict (Watson et al. 1998; Burke and Bonduriansky 2017).

Our observations provide some insight on the rate of polyandry, frequency of takeover, and opportunity for sexual selection on males in natural populations of *Megacrania batesii*. It is likely that males guard females for prolonged durations because, if left unguarded, females would remate quickly with other males. Indeed, male-male combat over females has been observed occasionally in the field in this species (D. Wilner, R. Bonduriansky & N. Burke, unpublished observations). However, we found that only ~20% (18/89) of initially paired males were replaced by rival males during our mark-resighting studies in the mixed-sex population across three years. It is not clear how often male replacement involved a take-over versus other causes, such as predator attack. Nonetheless, our results suggest that successful take-overs are infrequent in *Megacrania batesii*. The low rate of male replacement may be associated with relatively low rates of polyandry in *Megacrania batesii*. Across all females that were found paired, the average number of partners was 1.25, while 21% of females were never found paired,

despite a near-even adult sex ratio. Likewise, we found that, on average, males in the mixed-sex population were paired with only 1.15 females, and no male was seen pairing with more than three females. Estimated opportunity for sexual selection over the three years of the study was 0.50, which is moderate to low by comparison with other polyandrous species (see Vanpé et al. 2008). However, these values are based on relatively brief observation periods (11–12 days each year) and therefore under-estimate lifetime polyandry and male mating success. Although the lifespan of *Megacrania batesii* in the wild is not known, adults can survive for several months in the laboratory (R. Bonduriansky, unpublished data). Mark-resighting studies over longer periods of time are needed to obtain lifetime estimates of male mating success and female polyandry. Likewise, the duration of our field studies each year (11–12 days) was too short to estimate survival rates in a meaningful way in these long-lived stick insects. Field-studies spanning several months are required to obtain survival rate estimates, which could allow for comparison of female longevity in all-female vs. mixed-sex population and provide insight into the effects of male–female interactions on female survival. In addition, we searched for marked individuals during the day, but *Megacrania batesii* is more active at night. Field observations at night might provide additional insight into sexual behaviours.

We found that unpaired females in the mixed-sex population dispersed greater distances than females in all-female populations (Fig. 6). If encountering males and mating or being guarded are costly for females, females might disperse to escape from males. However, comparison between population types is complicated by the substantially lower resighting probability for females in the all-female populations (mean of 1.6 resightings) vs. mixed-sex population (mean of 4.3 resightings). It is possible that individuals at CB and KB 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. Marked individuals might be more difficult to resight at CB because the *P. tectorius* patch includes several mature trees with canopies that are challenging to inspect, while the *B. monticola* patch consists of very dense host plants where *Megacrania batesii* individuals are difficult to locate. It is therefore possible that our data underestimate movement of females in population CB. However, mean nightly dispersal distances were similarly short at KB (Fig. 6), where the habitat consists mostly of young *Pandanus* spp. plants that do not seem to present greater challenges to resighting than those at the mixed-sex population. Thus, the difference in mean nightly dispersal distance between mixed-sex and all-female populations appears to be genuine.

Lab-reared hatchlings rarely dispersed in the semi-natural enclosure. We found that 95% of all hatchlings remained on the plants where they were initially placed. Thus, *Megacrania batesii* hatchlings may disperse actively to find a host plant (perhaps typically the plant from which their mother dropped her eggs), but, once they find a suitable plant, they might remain on that plant for an extended period of time. It is possible that hatchling nymphs disperse more when food becomes limited. A study on the phasmid *Extatosoma tiaratum*, which also drops eggs to the ground, found that hatchling nymphs actively dispersed by climbing up from the ground (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 behavior might help to decrease mortality risk because dispersing might be very dangerous for hatchlings that are small and vulnerable to predators. It is possible that hatchlings may become more active in dispersing after undergoing several molts (i.e., at later nymphal instars). Furthermore, external factors such as weather patterns can affect insect dispersal (Dale et al.

2001; McCay 2003). Ambient conditions (e.g. temperature, humidity) in the semi-natural enclosure might not have provided natural conditions and could have affected willingness to disperse.

To sum up, our findings suggest that neither adult nor hatchling dispersal are sufficient to explain the occurrence of all-female populations in *Megacrania batesii*. Our observations suggest that *Megacrania batesii* adults mostly disperse over short distances within habitat patches. Adult males of *Megacrania batesii* appear to be as dispersive as females, and females in all-female populations disperse less than females in mixed-sex populations. Hatchling nymphs rarely move between plants. We also found evidence that prolonged mate-guarding by males can reduce females' dispersal, and might impose substantial costs on females, although rates of polyandry and multiple mating by males appear to be low (at least over the duration of our observations). Further research integrating dispersal by eggs, nymphs and adults could provide additional insights into sex ratio variation in *Megacrania batesii.*

Declarations

Conflict of interests The authors declare no competing interests.

Ethical approval Not applicable.

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