Genetic and Phenotypic Consequences of Local Transitions between Sexual and Parthenogenetic Reproduction in the Wild

Soleille M. Miller,[1,*] Katarina C. Stuart,[1,2] Nathan W. Burke,[2,†] Lee A. Rollins,[1] and Russell Bonduriansky[1]

1. Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales, Australia; 2. School of Biological Sciences, University of Auckland, Auckland, New Zealand

Submitted December 6, 2022; Accepted July 11, 2023; Electronically published November 13, 2023

Online enhancements: supplemental PDF.

ABSTRACT: Transitions from sexual to asexual reproduction have occurred in numerous lineages, but it remains unclear why asexual populations rarely persist. In facultatively parthenogenetic animals, all-female populations can arise when males are absent or become extinct, and such populations could help to understand the genetic and phenotypic changes that occur in the initial stages of transitions to asexuality. We investigated a naturally occurring spatial mosaic of mixed-sex and all-female populations of the facultatively parthenogenetic Australian phasmid *Megacrania batesii*. Analysis of single-nucleotide polymorphisms indicated multiple independent transitions between reproductive modes. All-female populations had much lower heterozygosity and allelic diversity than mixed-sex populations, but we found few consistent differences in fitness-related traits between population types. All-female populations exhibited more frequent and severe deformities in their (flight-incapable) wings but did not show higher rates of appendage loss. All-female populations also harbored more ectoparasites in swamp (but not beach) habitats. Reproductive mode explained little variation in female body size, fecundity, or egg hatch rate. Our results suggest that transitions to parthenogenetic reproduction can lead to dramatic genetic changes with little immediate effect on performance. All-female *M. batesii* populations appear to consist of high-fitness genotypes that might be able to thrive for many generations in relatively constant and benign environments but could be vulnerable to environmental challenges, such as increased parasite abundance.

Keywords: asexual reproduction, heterozygosity, fitness consequences, facultative parthenogenesis, parasitism, deformities.

* Corresponding author; email: soleille.miller@unsw.edu.au.

† Present address: Institute of Cell and Systems Biology of Animals, Department of Biology, University of Hamburg, Hamburg, Germany.

ORCIDs: Miller, https://orcid.org/0000-0002-7880-6501; Stuart, https://orcid.org/0000-0002-0386-4600; Burke, https://orcid.org/0000-0002-9843-926X; Rollins, https://orcid.org/0000-0002-3279-7005; Bonduriansky, https://orcid.org/0000-0002-5786-6951.

Introduction

The pervasiveness of sexual reproduction in multicellular animals has remained a mystery for more than a century. Parthenogenetic strategies avoid physically costly sexual encounters, enjoy increased colonialization ability, and do not spend resources creating males that contribute minimally to offspring (Smith 1978). Yet sex is nearly ubiquitous across the animal kingdom.

Efforts to explain the prevalence of sexual strategies have sought to identify the advantages that sexual reproduction offers, particularly in the long term (reviewed in Barton and Charlesworth 1998; Otto and Gerstein 2006; Hadany and Comeron 2008; Neiman and Schwander 2011). Sex generates genotypic diversity, thereby potentially facilitating adaptation (Fisher-Muller effect: Fisher 1930; Muller 1932; Crow and Kimura 1965; Colegrave 2002; Cooper 2007; Becks and Agrawal 2012; Park and Krug 2013). Other hypotheses focus instead on the long-term consequences of asexuality (Bell 1982; Howard and Lively 1994; Agrawal 2006; Park et al. 2010). In particular, the accumulation of deleterious alleles in nonrecombining animals could result in a reduction of mean fitness due to increased mutation load (i.e., Muller's ratchet; Muller 1964), which can lead to population extinction (Chao 1990; Lynch and Gabriel 1990; Lynch et al. 1993; Loewe and Cutter 2008). Many studies have compared costs and benefits of sex and parthenogenesis between closely related sexual and asexual species (Kimmerer 1994; Tarkhnishvili et al. 2010; Bast et al. 2018). Consequently, most knowledge of shifts to asexuality comes from studying ancient transitions and sheds light on the features of successful ancient asexuals (e.g., Loewe and Lamatsch 2008; Schlupp 2010; Fontaneto et al. 2012; Larose et al. 2018). However, the initial stages

  https://doi.org/10.1086/727511

of such transitions and the features of young asexual lineages are less well known.

In animals, asexual lineages typically arise from sexual ancestors (Butlin 2002), but transitions to asexuality are thought to be impeded by constraints on the evolution of developmental mechanisms that decouple development from fertilization (reviewed in Engelstädter 2008). In mammals, asexual reproduction might be precluded by genomic imprinting (Reik and Walter 2001). In many animals, sperm activates eggs and provides centrioles to form centrosomes (Manandhar et al. 2005), and transitions to asexuality may depend on the evolution of sperm-independent development, diploidy restoration, and alterations to meiosis in the zygote. Even when only the maternal genome is transmitted to the offspring, the need for activation of the egg by the sperm is often retained (pseudogamy or gynogenesis; Beukeboom and Vrijenhoek 1998). Phasmids (order Phasmatodea, which includes the stick and leaf insects) have overcome this obstacle because they form centrosomes from exclusively maternal components (Marescalchi et al. 2002; Scali 2009). In clades such as Phasmatodea that have overcome sperm dependence, parthenogenetic reproduction usually occurs via one of two general mechanisms of diploidy restoration: apomictic parthenogens forego meiosis entirely and produce eggs through mitosis, whereas automictic parthenogens undergo a modified form of meiosis involving crossing over between maternal chromosomes.

Apomictic and automictic parthenogenesis are expected to have different genetic consequences for the lineage (reviewed in Stenberg and Saura 2009). Most studies on young asexual lineages and the consequences of transitions to asexuality have focused on apomictic parthenogens, which often arise via hybridization (Parker 1979; Kearney and Shine 2004; Kearney et al. 2022). In apomicts, theory predicts that heterozygosity levels will be retained over many generations of asexual reproduction and possibly even increase owing to mutations or polyploidy, as shown in *Timema* stick insects (Schwander and Crespi 2009). Apomictic lineages may therefore be expected to experience little or no reduction in performance relative to their sexual ancestors. By contrast, few studies have investigated the genetic or phenotypic consequences of transitions from sexual reproduction to automictic parthenogenesis. In automicts, heterozygosity is predicted to decrease at rates of 0%–100% per generation, depending on the meiotic mechanisms involved (reviewed in Stenberg and Saura 2009). This reduction in heterozygosity is expected to expose deleterious recessive alleles as well as to deplete allelic diversity, thereby resulting in reduced performance in the short term and reduced ability to adapt to environmental change on longer timescales. However, while the genomic consequences of automixis have been investigated in the laboratory (reviewed in Jaron et al. 2021) or modeled mathematically (Engelstädter 2017), much less is known about how such changes are manifested in wild populations, and even less is known about their phenotypic and fitness consequences in natural environments (but see Morgan-Richards et al. 2019; Jaron et al. 2022). A number of studies have compared the performance of sexual and asexual animals in the laboratory (Browne et al. 1988; Kenny 1996; Cullum 1997; Mee et al. 2011; Sukumaran and Grant 2013), but given the strong environment dependence of life history and fitness (Sæther and Engen 2015), there is a need for research on wild populations in fully natural environments. Do natural populations that have undergone recent transitions to automictic parthenogenesis experience the predicted declines in heterozygosity and allelic diversity? To what extent do these genomic changes affect performance in the wild?

In facultatively parthenogenetic animals, every female can engage in sexual or asexual reproduction depending on whether mating and fertilization occur (Normark 2003). Such animals therefore appear to have the potential to reap the benefits of both modes of reproduction while avoiding many of the costs (D'Souza and Michiels 2010). Facultative parthenogenesis can also be an intermediate stage between obligate sexuality and obligate asexuality (Schwander et al. 2010). Notably, many facultatively parthenogenetic animals exhibit a geographic pattern where some populations consist of both sexes and reproduce sexually while other populations consist of females only and reproduce asexually. This phenomenon, known as geographic parthenogenesis (GP), has been reported in Opiliones (Burns et al. 2018), mayflies (Tojo et al. 2006), phasmids (Law and Crespi 2002; Morgan-Richards et al. 2010), and others (reviewed in Glesener and Tilman 1978; Kearney 2005; Hörandl 2006; Vrijenhoek and Parker 2009). GP animals provide valuable opportunities to investigate the immediate effects that follow the establishment of asexual populations, a process that can involve unmated females colonizing new areas or males becoming locally extinct. Newly established asexual populations may show founder events and genetic bottlenecking due to the lack of sexual recombination, but the maintenance of heterozygosity in such populations is expected to depend largely on whether they engage in apomictic or automictic parthenogenesis. The short-term genetic and phenotypic effects of such transitions could determine whether asexual populations persist or suffer rapid extinction. Yet very little is known about the relative performance of all-female versus mixed-sex populations of such species.

The "peppermint stick insect," *Megacrania batesii*, is a flightless facultative parthenogen endemic to the wet tropics of far-north Queensland, Australia (Cermak and Hasenpusch 2000), as well as the Solomon Islands and other Pacific islands (Van Herwaarden 1998). When *M. batesii*

females mate, they create a mixed-sex brood of sexually produced offspring. However, females can also reproduce via thelytoky, whereby females that avoid mating produce all-female broods from unfertilized eggs. Some phasmids occasionally produce male offspring from unfertilized eggs (Pijnacker and Ferwerda 1980; Scali 2009; Brock et al. 2018), but it is not known whether *M. batesii* can do so. Cermak and Hasenpusch (2000) reported the existence of a mixed-sex population near Cape Tribulation, Queensland, and two isolated all-female populations ~150 km farther south. However, we recently discovered a complex geographic mosaic of populations with contrasting sex ratios throughout the species range. Females in all-female populations do not show obvious morphological differentiation from females in mixed-sex populations. Since sexual reproduction is believed to be the ancestral reproductive mode in phasmids (Schwander and Crespi 2009), we assume that all-female populations of this species are descended from mixed-sex sexual populations.

In this study we describe the geographic variation in sex ratio and reproductive mode in wild populations of *M. batesii*. We then use these populations to investigate the consequences of transitions to asexual reproduction in wild populations within their natural habitats. In particular, we aimed to determine (1) whether incipient transitions to asexuality (i.e., parthenogenetic reproduction) are associated with reduced allelic diversity and heterozygosity, as predicted by theory and shown by empirical studies on other species (Stenberg and Saura 2009), and (2) whether such genetic changes are associated with reduced performance in fitness-related traits, including female body size, fecundity, egg hatching success, appendage deformity and loss, and ectoparasite load. *Megacrania batesii* occurs in two main types of habitat: rainforest along beach margins (where it feeds on *Pandanus* sp. host plants) and swamp within closed-canopy rainforest (where it feeds on *Benstonea* sp. host plants). We therefore also asked whether phenotypic and genotypic parameters are affected by habitat type. As our focus is on the impact of transitions in reproductive mode on functionality in natural environments, we report phenotypic data collected from individuals observed in natural populations rather than from their lab-reared descendants.

Methods

Study Locations

We investigated *Megacrania batesii* populations along ~33 km of coastline north of the Daintree River in Queensland, Australia. We also investigated two isolated all-female populations (reported previously by Cermak and Hasenpusch 2000) located ~160–180 km south of the Daintree River at Etty Bay and Bingil Bay. *Megacrania batesii* were observed and sampled from habitat along Cape Tribulation Road and Kimberley Road; in Forest Creek Village, Cow Bay Village, and Cape Tribulation Village and adjacent beaches; and at Emmagen Creek and Etty Bay and Bingil Bay beaches. Our study encompassed nearly all of the known range of *M. batesii* in Australia. Aside from these locations, *M. batesii* has been reported in the Atlas of Living Australia or on iNaturalist at a few isolated locations south of the Daintree River, but we were unable to locate any *M. batesii* individuals there. Potential *M. batesii* habitat also occurs in adjacent mountainous rainforest areas within ~30 km of the coast, but most of these areas are inaccessible and were not included in our study.

Population Sex Ratio and Phenotyping

A total of 1,324 wild *M. batesii* individuals were photographed on their host plants at multiple locations in 2019 ($N = 258$), 2020 ($N = 283$), 2021 ($N = 259$), and 2022 ($N = 524$) to quantify sex ratio and collect phenotypic data. Fieldwork was carried out each year between late January and early March, which corresponds to the summer/wet season when *M. batesii* adults and final-instar nymphs are abundant but early-instar nymphs are rare (R. Bonduriansky, unpublished data). Surveys were done during daylight hours at all locations. However, at locations NN and NS, where taller *Pandanus* sp. trees grow in clusters along the beach, we also conducted nightime surveys (between 9 p.m. and midnight), using flashlights to spot and photograph *M. batesii* individuals in the canopies. During the day, *M. batesii* individuals typically sat motionless in the grooves of host plant leaves (fig. 1); after dark, *M. batesii* individuals were often observed feeding on host plant leaves and were therefore more easily seen in taller *Pandanus* sp. trees. At each location, we scanned all of the host plants (*Pandanus* sp. or *Benstonea* sp.) that had the characteristic chew marks produced by *M. batesii* (see Cermak and Hasenpusch 2000) and recorded the presence, developmental status (nymph or adult), sex (for adults and some nymphs that were developed enough to determine sex without microscopic examination), and pairing status (single female, single male, or male-female pair) of each *M. batesii* individual seen.

Although it is likely that we failed to spot some individuals, our surveys should nonetheless represent differences between locations in the relative abundance, sex ratio, and phenotype of *M. batesii*. The *M. batesii* populations surveyed were classified as all female if only females were seen and as mixed sex if both sexes were seen. Localities with numerous individuals could be differentiated as all female or mixed sex because males, when present, are easy to distinguish from females by their body shape and wing

Figure 1: *Megacrania batesii* on its host plants. *A*, Adult female from an all-female population on *Pandanus* sp. *B*, Adult female from a mixed-sex population on *Pandanus* sp. *C*, Adult male on *Benstonea* sp. *D*, Female guarded by male on *Benstonea* sp. A color version of this figure is available online.

length (see fig. 1). In mixed-sex populations, most adult females are continuously guarded by males (Boldbaatar 2022). One location (B1) where a few females (but no males) were found and eggs collected from plants produced only female hatchlings was designated a putative all-female population. One population (NN) was consistently all female in 2019–2021, but in 2022 males were found there. This transitional population was excluded from phenotypic analyses comparing sexual and asexual populations.

Each adult and nymph seen in the field was imaged in situ on its host plant using a Sony RX10-IV camera. When possible, a transparent plastic scale attached to a thin metal

rod was held next to the adult insect so that body size could be measured from the image (fig. S1; figs S1–S7 are available online). In most cases this procedure did not appear to disturb the insects, since no movement or defensive reaction (retreating, jumping off the leaf, or spraying of defensive fluid) was observed. From the images, ImageJ (Schneider et al. 2012) was used to measure the length of the prothoracic notum (pronotum) and scale. Pronotum length was then calculated in centimeters as a measure of body size. We measured pronotum length because this part of the body was consistently visible in the photos, including photos of females being guarded by males. The photos were also used to record the number of missing legs and antennae (fig. 2). Leg and antennae loss in arthropods is an indication of poor condition that could result from predator attack or problems with molting (Maruzzo et al. 2005). We also recorded the presence of black spots indicative of fungal/bacterial infection and the presence of arthropod parasites (mites or biting midges) on the cuticle (fig. 2). Finally, we recorded the presence and severity of wing deformities (fig. 3). Normal wings are held flat over the dorsal thorax of the insect, with one wing overlapping the other. Mild wing deformities were defined as normally shaped wings held apart (not overlapping), whereas severe wing deformities were defined as wings that were highly abnormal in shape or position.

In 2019 and 2020 we collected 1,418 eggs from several wild-collected *M. batesii* females and sexed 670 hatchlings from those eggs to verify that females in all-female populations were reproducing asexually while females in mixed-sex populations were reproducing sexually. Wild-collected females were kept in mesh cages for 7–12 days, fed on *Benstonea* sp. or *Pandanus* sp. leaves, and then released at the site of capture. Females that were mating or guarded by males when collected were housed together with the male. Eggs laid by wild-collected females were counted and then kept in containers with moist coco peat at ~27°C until hatching in controlled-temperature rooms at University of New South Wales (UNSW), Sydney. Fecundity was quantified for each wild-collected female as the number of eggs laid per day over 7–12 days, and egg viability was quantified as the hatching rate of these eggs. Because *M. batesii* adults can live for many months (R. Bonduriansky, unpublished data) and we wanted to avoid collecting adults from the field to minimize impact on the natural populations, our estimates of fecundity and egg hatch rate are short-term snapshots that we assume to be representative of variation in female lifetime reproductive performance. Hatchlings were sexed according to the morphology of the eighth and ninth abdominal sternites (fig. S2), and hatchling sex ratio was used to assess reproductive mode in the natural source populations. Fertilized eggs hatch into either males or females and result in mixed-sex broods (indicative of sexual reproduction), whereas unfertilized eggs typically hatch into females only (indicative of parthenogenetic reproduction).

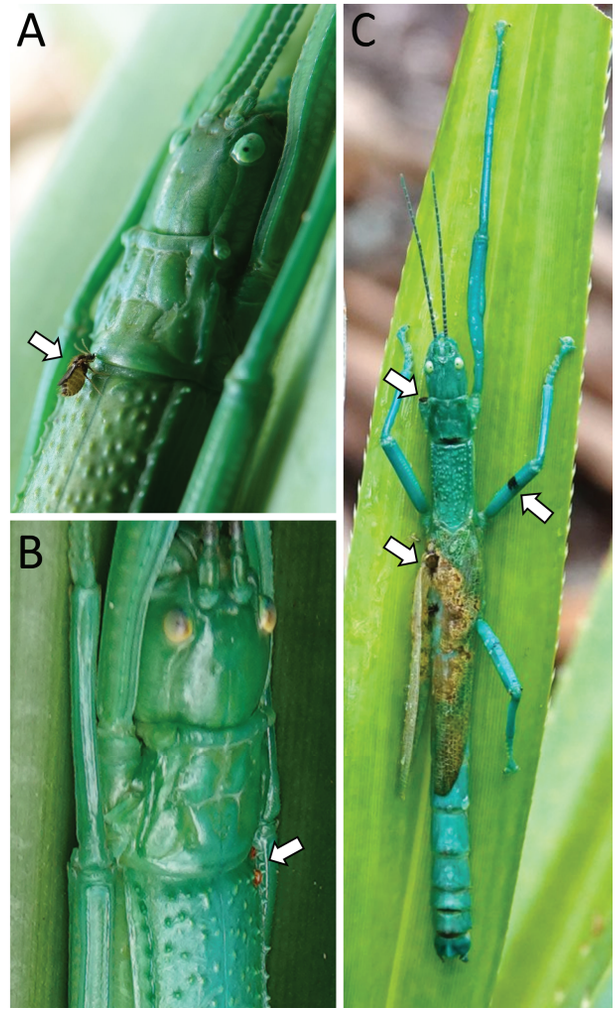


Figure 2: *Megacrania batesii* individuals with ectoparasites or missing appendages. *A*, Female with an engorged biting midge (arrow). *B*, Female with mites (arrow). *C*, Male with fungal/bacterial infections on his legs and wings (arrows) and two legs missing. A color version of this figure is available online.

DNA Sampling, Extraction, and Purification

In 2020 and 2021 midlegs were collected from wild nymphs or adults for DNA sequencing by pinching the leg with sterile tweezers, causing the insect to autotomize the pinched leg. A few additional samples were obtained from adults that died in cages during egg collection or from whole first-instar nymphs collected in the field. Samples were stored in pure ethanol at 4°C in the field and then at −80°C at UNSW until DNA extraction.

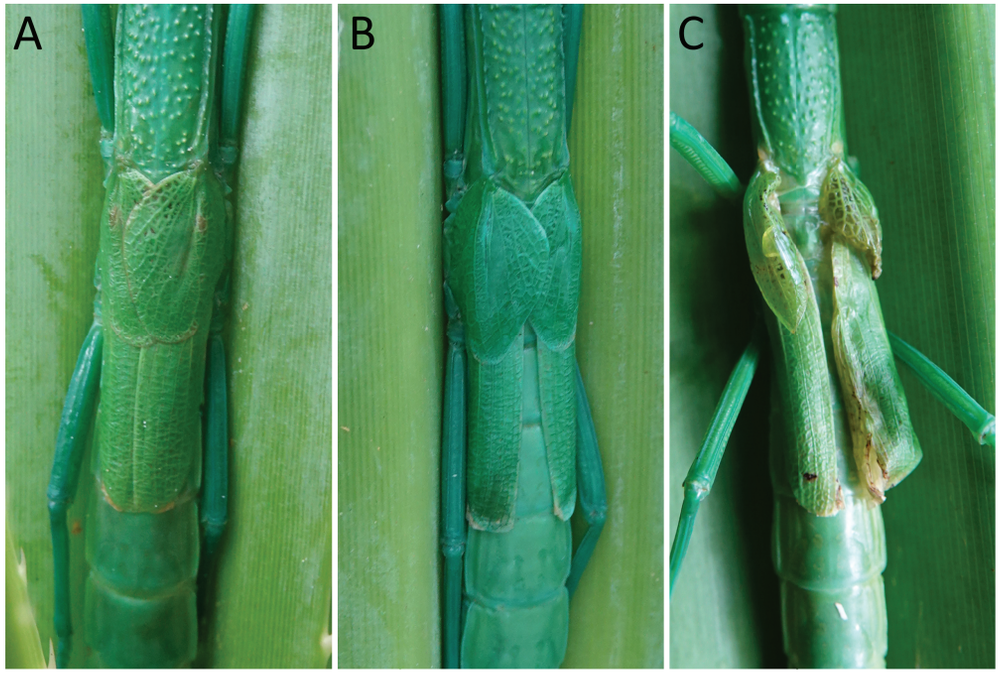


Figure 3: *Megacrania batesii* females with normal wings (*A*), slightly deformed wings (*B*), and severely deformed wings (*C*). A color version of this figure is available online.

Isolation of DNA was conducted using the Gentra Puregene tissue kit (4 g) from Qiagen according to the manufacturer's protocol ("DNA Purification from Mouse Tail Tissue Using the Gentra Puregene Mouse Tail Kit") with slight modifications to suit the study species (http://www.qiagen.com). Specifically, before adding 1.5 μL of Puregene Proteinase K to the lysate, the samples were subsampled and placed in 2 mL of free-standing screw-capped tubes. Then three 5-mm glass beads and approximately five to ten 1-mm silicon beads were placed in the tubes with the samples. The tubes were placed in a FastPrep-24 (MP Biomedicals) homogenizer and run on two 25-s cycles to break down the sample exoskeleton. Following homogenization, 1.5 μL of Puregene Proteinase K was added to the lysate, and the rest of the manufacturer's protocol was followed.

Isolates from a total of 178 field-collected *M. batesii* individuals were sent to Diversity Arrays Technology (Canberra, Australia) for whole-genome reduced-representation genotyping using the DArTseq protocol. Genome complexity reduction was performed using a restriction enzyme double digest of PstI and HpaII. Next-generation sequencing of amplification fragments was conducted on the Illumina Hiseq 2500 (http://www.illumina.com), producing 1,384,779 single-end reads of raw data. Single-nucleotide polymorphisms (SNPs) were then called using the DArTsoft analytical pipeline (Kilian et al. 2012).

Quality Control Filtering

The DArTsoft pipeline was used to calculate quality parameters, including call rate, reproducibility, and polymorphic information content (PIC). The initial dataset provided by DArTseq consisted of 12,977 SNPs across the 178 samples. Thirteen samples were omitted from analysis because of extensive missing data (>30%). An additional five samples were excluded because of apparent mislabeling or because a single individual was sampled from a location and its genetic affinity could not be determined with confidence. A total of 12,518 polymorphic DArT markers were generated for the retained 155 samples. Before quality control filtering, the mean repeatability was 0.987, and more than 50% of SNPs had reproducibility over 99% (see fig. S3). The mean minor allele frequency by locus was 0.18 but varied across locations, with all-female population samples having higher levels of minor alleles compared with mixed-sex population samples. The mean call rate was 0.95, and 95% of SNPs had a call rate above 75%. The PIC value of SNP markers ranged from 0.0 to 0.5, with a mean of 0.239 and median of 0.228.

DArTseq sequences were filtered using dartR (Gruber et al. 2018). Various filter parameters were tested to determine suitable filters, and SNP sequences matching the following parameters were removed from the dataset: average reproducibility < 95%, call rate < 75%, minor allele frequency (MAF) < 0.05, and a read depth < 3. Quality control filtering removed 4,151 SNPs, or 33.16% of SNPs (see table S1; tables S1–S9 are available online). After quality control, 8,367 high-quality SNPs were identified for further analysis.

Phenotypic Analysis

All phenotypic analyses were conducted in R (ver. 4.0.2; R Core Team 2019). The package MCMCglmm was used to model variation in phenotypic traits using a mixed model approach (Hadfield 2010). We carried out a separate analysis for each response variable (untransformed), with reproductive mode (assumed to be sexual for mixed-sex populations and asexual for all-female populations), habitat type (*Benstonea* swamp or *Pandanus* beach), and their interaction modeled as fixed effects. Model specifications are shown in table S2. All models also included year and a matrix representing genetic structure as random effects.

Sample sizes differed for different phenotypic variables (for all summary statistics, see table S3). Pronotum length was analyzed with a Gaussian model. Presence of fungal/bacterial infections and arthropod parasites were combined into one response variable (ectoparasites) analyzed with a categorical (binary) model. The summed number of missing legs and antennae was modeled as a single response variable. This was analyzed with a Poisson model because there were no individuals with all appendages missing, and thus, in practice, there was no upper bound on the variable. Presence/absence of mild and severe wing deformities was analyzed with categorical (binary) models. Hatching success of eggs collected from wild females was also analyzed as a binary variable representing success or failure to hatch for each egg. Fecundity (number of eggs laid per day) was analyzed using a Gaussian model.

Host plant type is strongly correlated with habitat type, with *Pandanus* sp. host plants typically growing along beach margins and *Benstonea* sp. host plants growing along margins of streams and swamps in closed-canopy rainforest. We therefore characterized habitat type as *Pandanus* beach or *Benstonea* swamp. All models initially included the interaction between reproductive mode and habitat, but this interaction was dropped if its inclusion increased the deviance information criterion. However, the main effects of reproductive mode and habitat type were retained in all models (see table S4). For each phenotypic variable, we also included the matrix representing population-level phylogenetic information (i.e., genetic structure, determined via analysis of SNP data) and used this to calculate the broad-sense phylogenetic heritability (H^2). The H^2 represents the contribution of phylogeny (and therefore genotype and any shared plastic responses resulting from common environments shared by related populations) to variation in a phenotypic trait and is calculated as the ratio of the variance in trait values among individuals that is due to their phylogenetic relationship (i.e., their belonging to a particular population or group of related populations) to the total variance in that trait (Housworth et al. 2004; Hadfield 2010). A high H^2 indicates that much of the phenotypic variation is due to the phylogenetic relatedness of the samples. Phylogenetic information used in the models was collected using the gl.dist.pop() function in the R package dartR, and inverse phylogenetic covariance matrices were created using the function inverseA().

Population Structure and Genetic Diversity

We investigated the genetic relationships between population samples by calculating a dissimilarity matrix using the bitwise.dist() function in the R package poppr (Kamvar et al. 2014) based on Hamming distances (see Wang et al. 2015). This matrix was then used with pvclust() to create a dendrogram based on correlation distance between individuals, and 10,000 bootstrap iterations were done to determine the level of support for each node (Suzuki and Shimodaira 2006). Several clustering methods were compared (see supplemental file testing_clustering_methods.html in the Dryad Digital Repository [https://doi.org/10.5061/dryad.n02v6wx1g; Miller et al. 2023]), but all methods yielded similar genetic structure. Pairwise F_{ST} values were calculated using the function stamppFst() with 10,000 bootstrap iterations used to obtain confidence limits for each pairwise difference (Pembleton et al. 2013). Nonmetric multidimensional scaling (NMDS) using metaMDS() was then used to visualize the genetic structure as a complement to the cluster analysis (Dixon 2003).

We calculated the observed SNP heterozygosity (H_O) of each individual using the function gl.report.heterozygosity() from the package dartR with the argument method = "ind" (Gruber et al. 2018; table S5). SNP heterozygosity is based on variable genomic regions only and can be biased when sample size is small, whereas whole-genome heterozygosity is less subject to bias (Schmidt et al. 2021). For comparison, we therefore also estimated observed whole-genome heterozygosities based on monomorphic as well as polymorphic sites in our reduced-representation sequence data (see Schmidt et al. 2021). We also calculated expected heterozygosities (H_E) based on observed allelic diversity and the assumption of Hardy-Weinberg equilibrium and F_{IS} of each population sample (see table S6).

To test whether observed heterozygosity levels differed between mixed-sex and all-female populations, we fitted a linear mixed model using the lme4 package (ver. 1.1-29; Bates et al. 2015) to individual SNP H_O values, with population type (mixed sex or all female; transitional population samples were removed) as a fixed effect and the location as a random effect. Whole-genome H_O estimates were very strongly correlated with SNP H_O estimates (Pearson correlation > 0.99), and analysis of whole-genome estimates yielded qualitatively identical results (not shown).

Shannon's diversity metric (hereafter, "allelic diversity") was quantified using the function gl.report.diversity(), as described by Sherwin et al. (2017) to quantify differences in allelic diversity between populations. Shannon's diversity (^1H) is an abundance-sensitive measure of allelic diversity based on the summed proportional abundances of each allele present in a population. A higher ^1H value means that there is greater uncertainty about the identity of a randomly sampled allele.

Isolation by distance was determined using the function gl.ibd() from the package dartR (Gruber et al. 2018). Gene flow between populations was estimated using discriminant analysis of principal components (DAPC). DAPC uses *K*-means and model selection to determine genetic clusters in lieu of known populations or genetic groups (Jombart et al. 2010). The optimal model had 17 genetic clusters, identified using the Bayesian information criterion (BIC; see fig. S4) and DAPC cross validation using the function xvalDapc(). Output based on 16 and 21 genetic clusters (corresponding to the second- and third-lowest BIC values) is shown for comparison in the files assignment_probabilitiess_k16.csv and assignment_probabilitiess_k21.csv in the Dryad Digital Repository (https://doi.org/10.5061/dryad.n02v6wx1g; Miller et al. 2023). Six discriminant analysis axes were retained based on the α score. Membership probabilities were estimated for each individual in each identified genetic cluster, and ggplot() was then used to visualize the admixture proportions of each individual's genotype. Populations with fewer than two sampled individuals were excluded from these analyses. The data that support the findings of this study are openly available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.n02v6wx1g; Miller et al. 2023).

Results

Sex Ratio and Reproductive Mode

Sex ratio was bimodally distributed, with either only females or an approximately equal number of females and males observed at nearly all locations (see table 1). With one exception (see below), we did not find any locations where adult males were present but rare (and the absence of such locations is supported by population genomic analysis, described below). In several cases, all-female and mixed-sex populations were found to occur in close proximity and with no obvious barriers to dispersal. For example, mixed-sex populations B4 and VR are separated by <2 km of contiguous rainforest from all-female populations B1 and CB, and the all-female population NS is separated by <1 km of rainforest from adjacent mixed-sex populations (see fig. 4). Despite the close proximity of males to some all-female populations, sex ratios were consistent over 4 years of surveys at nearly all populations (see the file sex_ratio_by_year.csv in the Dryad Digital Repository [https://doi.org/10.5061/dryad.n02v6wx1g; Miller et al. 2023]).

Eggs collected from most all-female populations typically produced 100% female hatchlings, whereas eggs collected from mixed-sex populations produced approximately equal numbers of female and male hatchlings (see table S7). We observed only two exceptions to this pattern. A sample of eggs from the all-female population TB produced one hatchling that was phenotypically male-like, and genotyping showed that this hatchling was not a hybrid between locations. This hatchling was therefore either a rare spontaneous male (see "Discussion") or a phenotypically atypical female. In addition, at one location (NN) where only females had been observed before 2022, eggs collected from one female in 2021 produced several male hatchlings, and genotyping indicated either that this female was a migrant from an adjacent, genetically similar mixed-sex location (BK or NW) or that this female had mated with a male migrant. The following year adult males were observed at NN, and this population therefore appears to be undergoing a transition from all female to mixed sex.

Morphology, Fecundity, and Egg Hatching Success

We did not find clear and consistent evidence of an effect of reproductive mode across the fitness-related phenotypic traits measured (see fig. 5*B*, 5*C*; for all summary statistics, see table S3). Mean fecundity and egg hatching success were ~12% and 19% lower, respectively, in all-female than in mixed-sex populations, but there was a great deal of variation in these variables among both mixed-sex and all-female populations (fig. S5). Adult females from all-female populations were more likely to exhibit mild (pMCMC $<$ 0.001; fig. 5*G*) and severe (pMCMC $<$ 0.005; fig. 5*H*) deformities in their wings than were females from mixed-sex populations. Relative to females in mixed-sex populations, adult females in all-female populations had more ectoparasites in *Benstonea* swamp habitats but not in *Pandanus* beach habitats (population type × habitat interaction: pMCMC $<$ 0.005; see fig. 6). Post hoc Tukey tests

Table 1: Sample sizes and sex of *Megacrania batesii* adults and nymphs that could be sexed found in the field at each of the surveyed locations (summed over the 4 years of the study)

Location	Population type	No. males	No. females	Total	Sex ratio	*P*
EC	All female	0	27	27	1	<.001
MS	Mixed sex	85	94	179	.53	.10
MK	Mixed sex	69	58	127	.46	.09
MB	Mixed sex	24	34	58	.59	.09
CO	Mixed sex	105	116	221	.52	.08
RP	Mixed sex	20	37	57	.65	.02
BK	Mixed sex	21	36	57	.63	.03
NW	Mixed sex	21	33	54	.61	.06
NN	Transitional	4	30	34	.88	<.001
NS	All female	0	65	65	1	<.001
TB	All female	0	46	46	1	<.001
TS	All female	0	12	12	1	<.001
B1	Putative all female	0	3	3	1	.125
B4	Mixed sex	2	2	4	.50	.75
CB	All female	0	223	223	1	<.001
CN	All female	0	62	62	1	<.001
VR	Mixed sex	13	15	28	.54	.28
VS	Mixed sex	6	10	16	.63	.24
MA	All female	0	16	16	1	<.001
KB	All female	0	31	31	1	<.001
KR	All female	0	24	24	1	<.001
FC	All female	0	7	7	1	<.01
EB	All female	0	27	27	1	<.001
BL	All female	0	18	18	1	<.001

Note: At locations where we found only females, a binomial two-tailed test was used to determine the probability that a population is a mixed-sex population (even sex ratio) based on the number of individuals seen. At locations where males and females have been seen, the population is mixed sex by definition, so we used the binomial test to calculate the probability that the sex ratio of individuals photographed at each location deviated significantly from 50%. At the transitional location (NN), only females were seen in 2019–2021, but males were found in 2022.

showed that this interaction is driven by a near-significant difference between parasitism rates between all-female and mixed-sex populations within *Benstonea* swamp habitats ($P = .054$), whereas no other pairwise comparisons approached statistical significance (all $P > .2$; see table S8). Overall, mean rates of ectoparasitism and wing deformities were ~19% and 91% higher, respectively, in females from all-female locations. Additionally, mean numbers of missing legs and antennae were ~40% higher in adult females in all-female populations, but among-population variation was considerable, and there was no support for an overall difference in rate of appendage loss between mixed-sex and all-female populations (pMCMC = 0.34).

Estimated phylogenetic heritability (H^2) values suggested effects of phylogenetic relatedness (i.e., genotype and any environmental variation shared by related populations) for the presence of ectoparasites ($H^2 = 0.64$) and egg hatch rate ($H^2 = 0.87$). There was moderate support for an effect of genotype on missing antennae and legs ($H^2 = 0.41$) and fecundity ($H^2 = 0.49$) but little support for effects of genotype for mild ($H^2 = 0$) or severe ($H^2 = 0$) wing deformities or for pronotum length ($H^2 = 0.12$; see table S4).

Population Structure

Genotypes clustered by geographical location rather than by sex ratio. Two major genetic clusters were observed, a northern cluster and a southern cluster separated by the Noah Creek mouth. The mouth of Noah Creek (~150 m wide at its widest point) therefore appears to serve as a barrier to *Megacrania batesii* dispersal and gene flow. The northern cluster consists mostly of mixed-sex populations, while the southern cluster consists mostly of all-female populations, but both genetic clusters contain both population types (see fig. 7). Pairwise F_{ST} values likewise showed that population samples separated by greater geographic distances tended to be less closely related (see table S9), and this pattern was supported by isolation-by-distance analysis (Mantel test based on Pearson's product-moment correlation: Mantel $r = 0.366$, $P < .001$). The F_{ST} values between the populations varied between 0.19 and 0.95, with lower

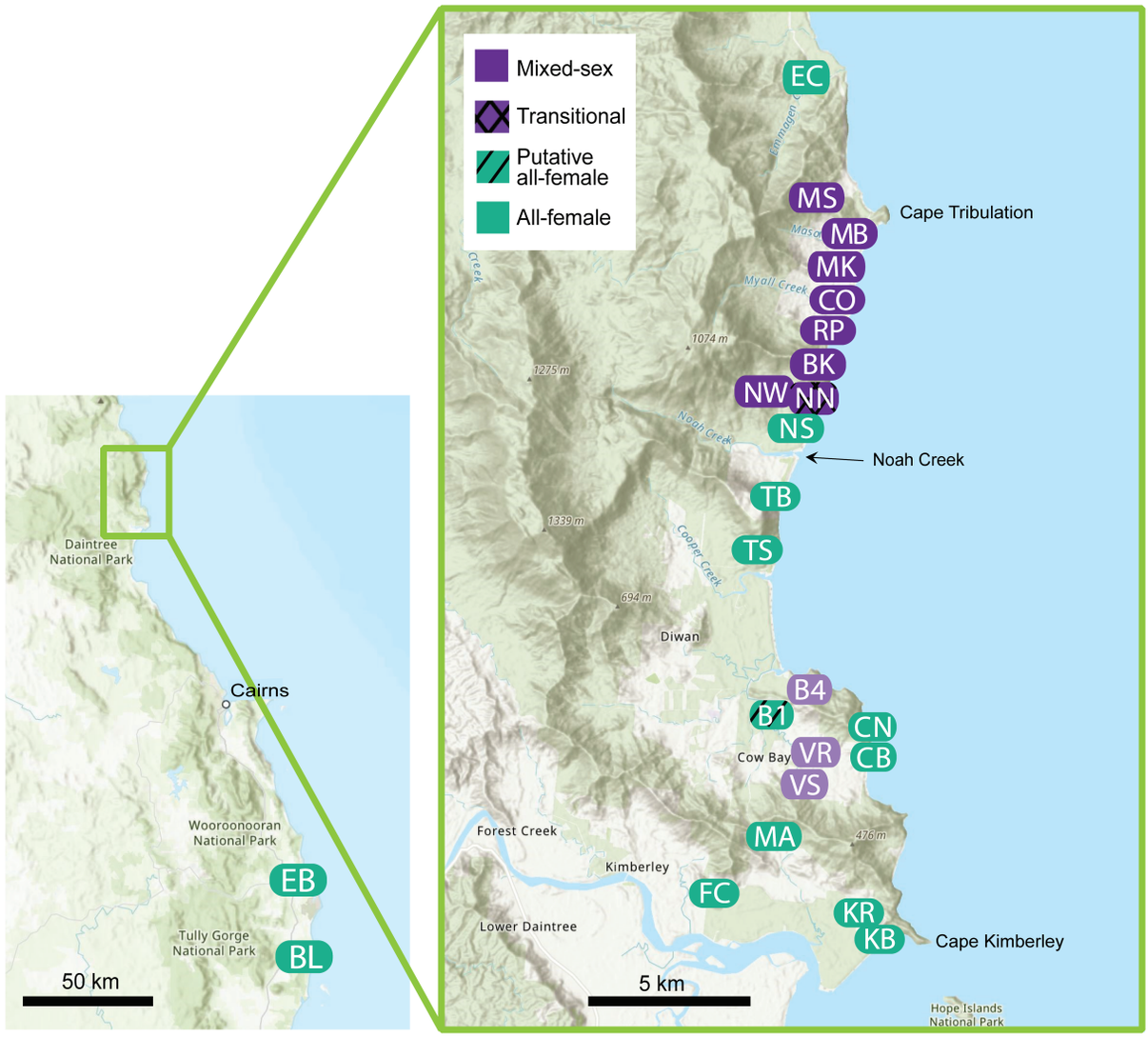


Figure 4: Geographical variation in sex ratio in *Megacrania batesii*. Two isolated all-female populations occur at the southern limit of the known range of *M. batesii* in Australia, and a spatial mosaic of all-female and mixed-sex populations occurs ~160–180 km north of this area between Forest Creek and Emmagen Creek. Noah Creek is inferred to act as a gene flow barrier separating the northern and southern genetic clusters (see fig. 7). Locations are characterized as mixed sex, putative all female, or all female based on the sex of adults and nymphs photographed at each location (see table 1). The transitional location was all female in 2019–2021, but males were found at this location in 2022. Map source: National Base Map (Geoscience Australia). A color version of this figure is available online.

values between mixed-sex populations in the northern cluster and higher values between all-female populations in the southern cluster. NMDS analysis (fig. S6) produced a pattern consistent with the population structure obtained from genetic cluster analysis, with the biggest separation found between populations north versus south of the Noah Creek mouth.

Admixture analysis revealed that the 155 individuals sampled across the study area belong to 17 genetic populations, with little gene flow detected between populations. Most individuals had 1.0 membership probability in a single population (fig. 7*B*). Two sets of population samples found near one another (<1,000 m) had the same genotype but differing reproductive modes and heterozygosity levels (B1-B4 and NN-NS-NW).

Allelic Diversity and Heterozygosity

All-female population samples had nearly fivefold lower allelic diversity (Welch's two-sample *t*-test: $P < .001$; fig. S7) relative to mixed-sex population samples. However, there

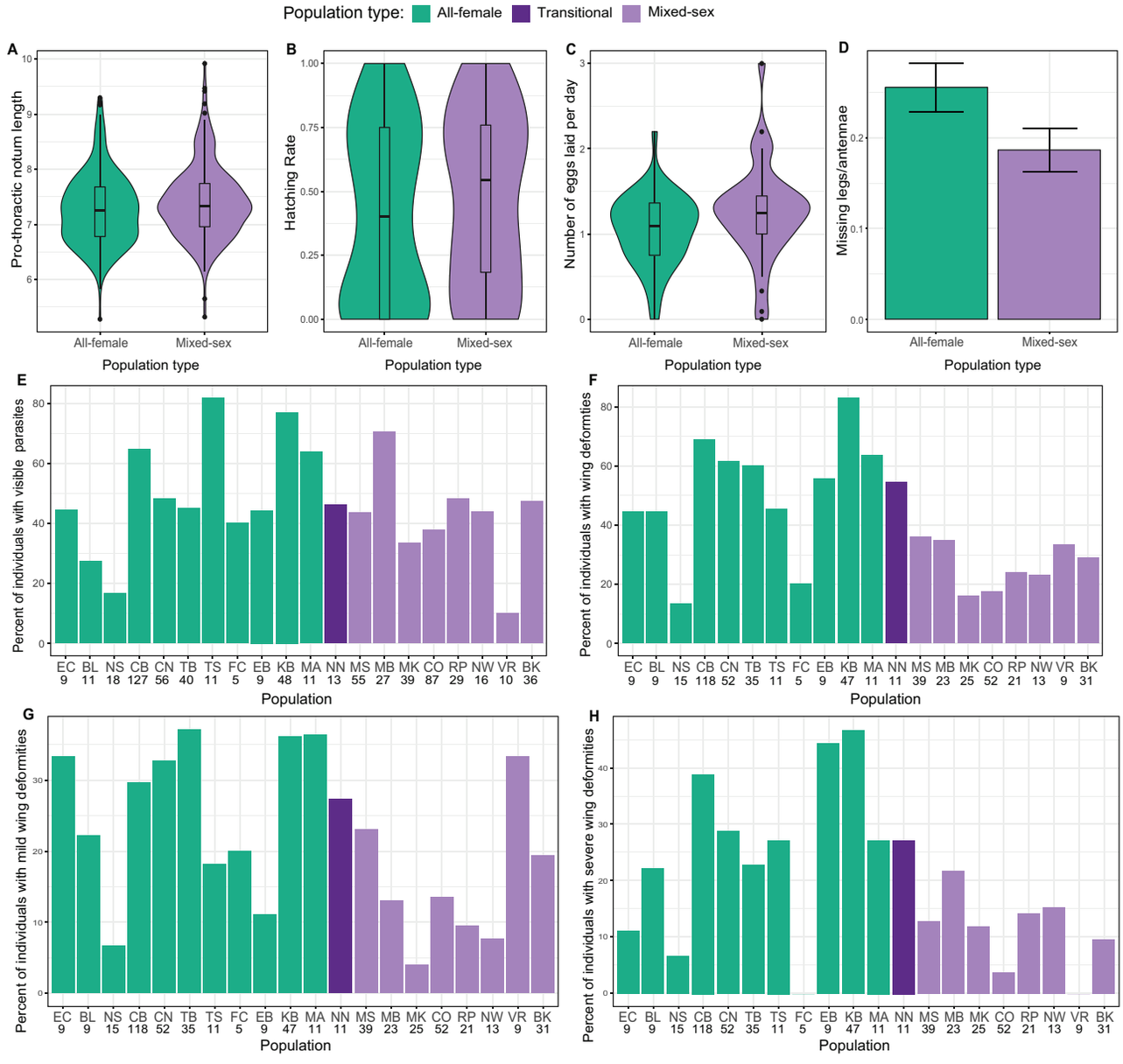


Figure 5: Phenotypic values for *Megacrania batesii* females in mixed-sex and all-female populations. *A*, Pronotum length as a measure of body size. *B*, Hatching rate of eggs. *C*, Number of eggs laid per day as a measure of fecundity. *D*, Number of missing legs and antennae per individual. *E*, Percentage of adult females observed at each location with visible ectoparasites (fungus/bacteria, biting midges, or mites). *F*, Percentage of adult females from each location with wing deformities (mild or severe). *G*, *H*, Percentage of wild *M. batesii* individuals with mild (*G*) and severe (*H*) wing deformities at each of the sampling locations. Data shown in *G* and *H* are derived from *F*. In *A*–*D*, means (bars), interquartile ranges (boxes), and nonoutlier ranges (whiskers) of individual values are shown. Violins indicate the distribution of individual values. Populations with information from fewer than three individuals were excluded from this analysis. A color version of this figure is available online.

was substantial variation in allelic diversity between mixed-sex population samples: for example, allelic diversity was more than fivefold higher at CO than at B4 (fig. S7). Likewise, observed individual SNP (i.e., variable-region) heterozygosity estimates were nearly fivefold lower, on average, in all-female population samples than in mixed-sex population samples (linear mixed model: $P < .001$; fig. 7*C*). Whole-genome heterozygosity estimates were less than SNP estimates for every individual, with a mean within-individual difference of 8.4% in all-female population

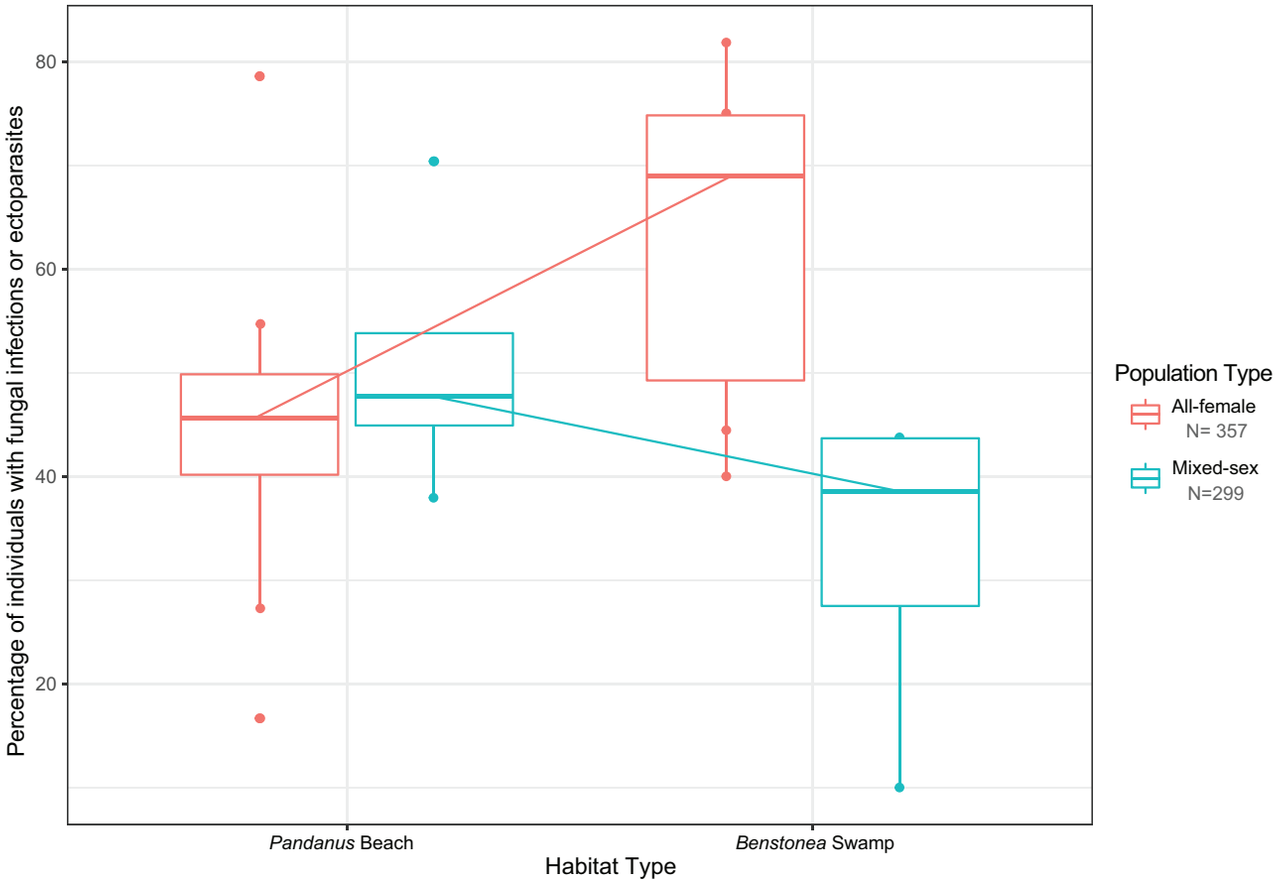


Figure 6: Effects of habitat type and population type on the rate of visible ectoparasites (fungus/bacteria, mites, or biting midges) in *Megacrania batesii* females in all-female and mixed-sex populations. Each point represents a population mean. Bars indicate means across all populations in each group, boxes represent interquartile ranges, and whiskers show nonoutlier ranges. A color version of this figure is available online.

samples and 8.1% in mixed-sex population samples. A few individuals sampled from mixed-sex populations had observed heterozygosities comparable to individuals from all-female populations, suggesting that these individuals were produced asexually (see fig. 7*C*). Additionally, three individuals from all-female populations CB, TB, and TS had relatively high heterozygosity, comparable to heterozygosity values in the least heterozygous mixed-sex populations (VR, VS, and B4; fig. 7*C*). This suggests that parthenogenesis in *M. batesii* can produce variable genetic outcomes (see "Discussion").

Discussion

Genetic relatedness and admixture analysis indicated multiple independent transitions from sexual reproduction to parthenogenesis in wild *Megacrania batesii*. At one location we also observed an ongoing invasion of males into a previously all-female population. We found that local transitions to asexual reproduction resulted in dramatic losses of allelic diversity and heterozygosity, but the consequences of these genomic changes for fitness appeared to be inconsistent and context dependent. Asexual reproduction promoted wing deformities but not appendage loss and resulted in elevated ectoparasite loads in *Benstonea* swamp but not in *Pandanus* beach environments. We found no statistical support for an overall effect of reproductive mode on body size, fecundity (measured as egg output over 7–12 days), or egg hatching success. Rather, all phenotypic traits varied substantially among both all-female and mixed-sex populations. Our findings suggest that genotype (or unmeasured, site-specific environmental parameters) is more important than reproductive mode in determining phenotype and fitness in *M. batesii* and that some all-female populations possess high-fitness

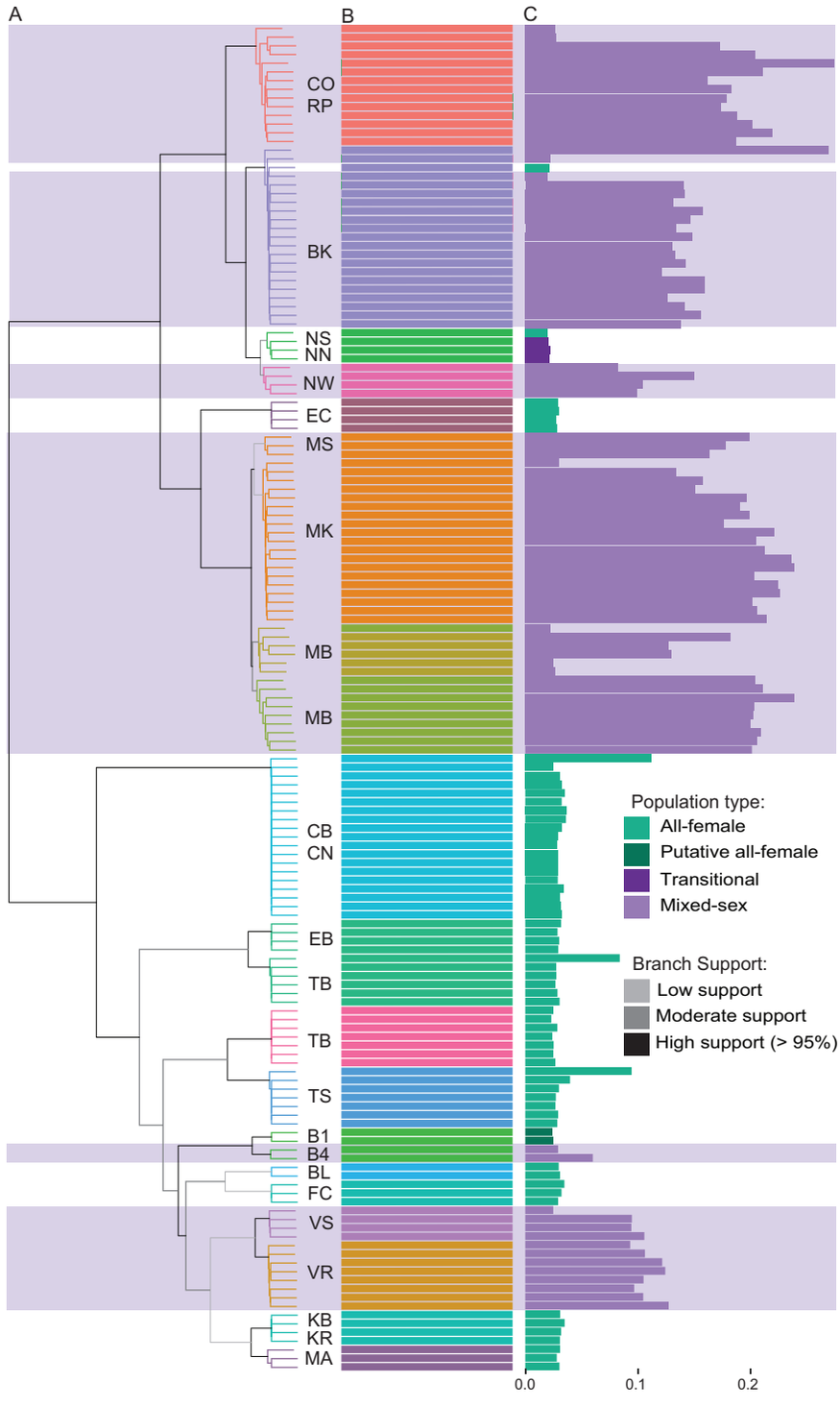


Figure 7: Population structure and individual single-nucleotide polymorphism (SNP) heterozygosity of *Megacrania batesii* populations based on 8,367 high-quality SNPs from 155 individuals sampled from the field. *A*, Dendrogram showing relatedness of sampled individuals, with branches colored according to the level of bootstrapped support (based on 10,000 bootstrap iterations) and tips colored to match their highest membership probability. *B*, Admixture plot showing percent membership in the 17 genetic populations supported by the analysis (fig. S4). *C*, Observed individual SNP heterozygosity values. In *A* and *B*, different colors represent the primary assigned cluster, as indicated by admixture analysis. In *C*, colors represent the observed sex ratio and inferred reproductive mode for each location. Areas shaded in purple show mixed-sex population samples.

genotypes that might enable them to thrive asexually for many generations.

Cluster analyses showed that *M. batesii* population samples grouped together according to geographic location rather than reproductive mode. With one exception (the transitional location NN), this pattern remained stable over 4 years of sampling. We can infer at least four independent transitions from sexual to parthenogenetic reproduction, resulting in all-female populations: the assumed transition from a sexually reproducing ancestral population to an all-female population that gave rise to the genetic cluster south of Noah Creek and three more recent transitions resulting in all-female populations EC, NS, and B1, all of which are close spatially and genetically to mixed-sex populations. Admixture analysis showed little gene flow between populations despite geographical proximity (see fig. 7*B*). It is not known how long ago population divergence and reproductive mode transitions occurred. However, the close geographical proximity and genetic relatedness of some mixed-sex (sexual) and all-female (asexual) populations suggest recent establishment. For example, population samples B1 and B4 as well as NW and NS exhibit differing reproductive modes but belong to the same genetic population.

We identified two main genetic groups separated by a large estuary (Noah Creek) that appears to act as a barrier to dispersal. The northern group consists of mainly sexual populations, and the southern group consists of mainly asexual populations. Results of interpopulation crosses (to be presented in a separate article) show that individuals from southern, northern, sexual, and asexual populations are interfertile and can therefore be regarded as a single species. This geographical pattern partially resembles the pattern of geographic parthenogenesis observed in other phasmids, whereby parthenogenetic populations occur at higher latitudes than sexual ones (Law and Crespi 2002; Buckley et al. 2009; Morgan-Richards et al. 2010). However, the northernmost *M. batesii* population (EC) is all female, while some southern populations (VS, VR, B4) are mixed sex. Thus, rather than a clear latitudinal gradient, *M. batesii* appears to exhibit a mosaic of sexual and asexual populations or, alternatively, a pattern of peripheral parthenogenesis with a central mixed-sex area near Cape Tribulation surrounded by all-female populations. In species that display geographical parthenogenesis, asexual populations are often found in marginal habitats, typically at a higher elevation or latitude than sexually reproducing populations (Lynch 1984; Kearney 2005; Tilquin and Kokko 2016). However, all Australian *M. batesii* populations inhabit similar tropical rainforest habitats spanning a small latitudinal range (0.24° for our main study area between Forest Creek and Emmagen Creek; 1.8° for the total species range including isolated southern populations EB and BL), with little variation in temperature, humidity, or precipitation between locations. Additionally, sexual and asexual populations were found with approximately equal frequency in each of the two major habitat types (*Pandanus* beach and *Benstonea* swamp). Thus, climate and habitat differences do not appear to explain the variation in reproductive mode.

The lack of obvious environmental barriers or differences in habitat between sexual and asexual populations suggests that spatial variation in reproductive mode results from factors intrinsic to the biology of *M. batesii*. Four processes might have contributed to the observed spatial pattern.

First, if females disperse more than males, they could travel farther from the mixed-sex populations and establish all-female populations that could avoid male invasion for many generations. Moreover, all-female populations could persist once established if parthenogenetically produced females exhibit reduced propensity to mate and reduced benefits of mating, as reported for the related phasmid *Extatosoma tiaratum* (Burke and Bonduriansky 2022). However, mark-resighting studies show that *M. batesii* adults of both sexes disperse similar distances (Boldbaatar 2022). Nonetheless, occasional random dispersal of eggs, nymphs, or adults (e.g., on rafting vegetation) could contribute to this process because a single unmated female could found a new (albeit perhaps transient) all-female population. The isolated all-female population EB may have originated in this way, since EB clusters genetically with TB despite being located ~175 km away (fig. 4).

Second, sexual populations south of Noah Creek (VR, VS, B4) could have arisen as a result of males from the north invading southern all-female populations. However, none of these populations show evidence of genetic admixture from northern populations. Nonetheless, we observed an apparent ongoing transition from all female to mixed sex at a northern population (NN), showing that male invasion of all-female populations is possible when all-female and mixed-sex populations occur in close proximity.

Third, spontaneous (parthenogenetic) production of males could have given rise to southern sexual populations (VS, VR, B4). Spontaneous male genesis is possible in diploid species with XX XO sexual karyotypes, where meiotic/developmental error results in the loss of one X chromosome in parthenogenically produced XX offspring (Pijnacker and Ferwerda 1980; Scali 2009). The viability and reproductive functionality of spontaneous males have not been studied in any phasmid (see Morgan-Richards et al. 2019). However, in the obligate asexual snail *Potamopyrgus antipodarum*, low rates of male production and reduced functionality in asexually produced males were found to make male invasion in asexual populations unlikely (Neiman et al. 2012; Jalinsky

et al. 2020). The karyotype of *M. batesii* is unknown, but diploid phasmids typically have either XX XY or XX XO sexual karyotypes (Scali 2009; Schwander and Crespi 2009). If spontaneous males are possible in *M. batesii*, data from our laboratory experiments (not shown) show that spontaneous males are extremely rare (<1/1,000 parthenogenetic eggs). From 533 field-collected eggs from all-female populations, we found one morphologically male-like hatchling. Morphologically ambiguous hatchlings are occasionally found, and this hatchling was not reared to adulthood, so it was not conclusively determined to be a functional male. From 1,083 eggs produced parthenogenetically in the lab, no male hatchlings were obtained. Thus, spontaneous emergence of sex in all-female populations seems unlikely in *M. batesii*.

Fourth, all-female populations could result from local extinction of males. Northern all-female populations (EC, NS) could have resulted from male extinction, while southern mixed-sex populations (VS, VR, B4) could represent remnants of an ancestral mixed-sex population. Just as small, isolated sexual populations are susceptible to stochastic extinction (see Pianka 2000), small, isolated mixed-sex populations of facultative parthenogens are susceptible to stochastic extinction of males. In a small *M. batesii* population, total failure of males to survive or fertilize eggs in a single year would result in local loss of sex. Male extinction might be even more likely if female resistance to mating evolves. Sexual reproduction is costly (Otto and Lenormand 2002; Lehtonen et al. 2012), and in facultative parthenogens such costs could generate sexual conflict and favor parthenogenetic reproduction (Kawatsu 2013; Gerber and Kokko 2016; Burke and Bonduriansky 2019). In mixed-sex *M. batesii* populations, continuous guarding of adult females by males could interfere with female foraging (Boldbaatar 2022). Gerber and Kokko (2016) found that female resistance is most beneficial at low population densities, such as those observed in many *M. batesii* populations. Moreover, we found that some individuals from mixed-sex populations have heterozygosities comparable to those of asexual populations, suggesting that females in mixed-sex populations sometimes avoid mating or fertilization. If selection favors asexual reproduction, this could potentially lead to the evolution of behavioral or physiological mechanisms of female resistance (Burke and Bonduriansky 2022).

Individuals from asexual populations had on average 78% lower heterozygosity than did individuals in mixed-sex populations, and asexual populations had 78% less allelic diversity than sexual populations. The cytological mechanism of asexual reproduction determines how much heterozygosity is maintained across generations (Moritz et al. 1990; Stenberg and Saura 2009). Preliminary results (S. M. Miller, unpublished data) suggest that parthenogenesis in *M. batesii* results in substantial (but not complete) loss of heterozygosity over a single generation of parthenogenetic reproduction, consistent with automixis via either terminal fusion or central fusion (Stenberg and Saura 2009). Both terminal fusion and central fusion allow for variation in the amount of heterozygosity maintained in parthenogenetically produced offspring, possibly explaining the presence of a few individuals with relatively high heterozygosity levels in all-female populations (fig. 7*C*). However, some phasmids exhibit multiple mechanisms of parthenogenesis within species (Craddock 1972). If diverse mechanisms of parthenogenesis are present in *M. batesii*, this could also explain the observed variation in heterozygosity within all-female populations.

Despite the dramatic loss of genetic diversity in asexual populations, we found little evidence of a consistent cost of asexual reproduction in fitness-related traits, including body size, number of missing appendages, and female reproductive output (measured over 7–12 days). Although mean phenotypic values were inferior in all-female populations relative to mixed-sex populations, there was considerable variation between populations within each reproductive mode and no statistical support for an overall effect of reproductive mode on these traits.

We observed a consistent effect of reproductive mode only on wing development: *M. batesii* females from asexual populations had higher rates of both mild and severe wing deformities (see figs. 3, 5*G*, 5*H*). Low heterozygosity could explain these findings. Parthenogenetically produced *E. tiaratum* females exhibit higher rates of wing deformities than do sexually produced females (Burke and Bonduriansky 2022). Likewise, multiple homozygous loss-of-function mutations cause wing defects in *Drosophila* (Terriente-Félix et al. 2010; George et al. 2019). If *M. batesii* shares any of these loci, homozygous genotypes of asexually produced females may result in the loss of function of genes involved in wing development. This could cause developmental instability, as seen with wing asymmetry in pea aphids (Hammelman et al. 2020). However, given that both sexes of *M. batesii* are flightless, wing deformities might have little or no effect on fitness.

Our finding of considerable among-population variation in performance suggests that some asexual *M. batesii* lineages are well adapted to their environment. The absence of mating and sexual recombination could promote the evolution of coadapted gene complexes in asexual populations, potentially resulting in "general-purpose" genotypes that are well adapted to the full range of conditions experienced by those lineages over many generations (Lynch 1984). For example, such genotypes might enable mechanisms of adaptive plasticity that promote survival and reproduction under a range of conditions. The evolution of general-purpose genotypes might contribute to the success

of asexual *M. batesii* populations despite low heterozygosity and allelic diversity.

However, while locally adapted or general-purpose genotypes might allow asexual populations to thrive in relatively benign and stable habitats, our data also suggest that such genotypes might not cope as well with the added challenge of high parasite abundance. We found that some *M. batesii* individuals had brown or black spots on their head, thorax, abdomen, or legs that appeared to be a fungal and/or bacterial infection, and some individuals carried mites and/or biting midges (fig. 2). Theory suggests that no single genotype will confer long-term resistance to parasites; rather, success will depend on the ability to keep up in the coevolutionary chase against parasites. Sexual reproduction is predicted to confer an advantage in such Red Queen arms races because sex promotes genotypic diversity (Hamilton and Zuk 1982; Hamilton et al. 1990). Indeed, Hite et al. (2017) found higher frequencies of males and sexually produced offspring in *Daphnia dentifera* during natural and artificial fungal epidemics, and Lively (1987) found that male abundance was correlated with parasite infections in the New Zealand mud snail, *P. antipodarum*. We found no overall effect of reproductive mode on ectoparasite load. Instead, we observed an interaction between reproductive mode and habitat type, whereby asexual populations had higher rates of infection on *Benstonea* host plants in closed-canopy rainforest swamps but not on *Pandanus* host plants along beach margins. This suggests that asexual *M. batesii* populations are disadvantaged in their ability to cope with ectoparasites only in certain parts of their range, such as areas with very high humidity that might promote parasite reproduction.

Alternatively, it is possible that successful all-female populations owe their success not to their genotypes but to the habitat patches where they occur. Because we quantified phenotypes of wild *M. batesii* individuals, our analysis cannot differentiate between genotypic variation and unmeasured variation among habitat patches as sources of variation in phenotype. However, environmental variation is unlikely to account for the lack of overall effects of reproductive mode on phenotypic traits because we sampled multiple all-female and mixed-sex populations across the species range and in both major habitat types.

In conclusion, our findings show that transitions to parthenogenetic reproduction lead to rapid and dramatic loss of allelic diversity and heterozygosity in *M. batesii* but also suggest that these genomic changes might have little impact on fitness. Our results suggest that some locally adapted asexual lineages (such as the southern all-female populations) can persist for many generations, but some all-female populations might quickly revert to sexual reproduction (as suggested by the ongoing male invasion in a formerly all-female northern population). Reversions to sex could reintroduce allelic and genotypic diversity. However, the all-female populations examined in this study showed very low levels of allelic diversity and heterozygosity, suggesting that such reversions are rare or have only transient effects. Our findings also suggest that, in the long run, environmental changes—such as increased parasite abundance or diversity—might push all-female populations toward extinction as a result of failure to adapt. Large mixed-sex populations are expected to have a greater capacity for adaptive evolution in response to parasites and environmental change and might therefore fare better than all-female populations in the long run. By contrast, small mixed-sex populations could fail to reap substantial benefits from sexual reproduction because of low allelic diversity and inbreeding depression. Indeed, in small mixed-sex populations of facultatively parthenogenetic animals, it is possible that asexual reproduction both confers individual-level advantages for females (thereby generating sexual conflict) and enhances population viability.

Nonetheless, we do not yet know how all-female populations are typically established in *M. batesii* or other facultative parthenogens or how such populations avoid invasion by males. We also do not know what processes generate the highly bimodal distribution of sex ratios among populations—a pattern that suggests that sex ratios between 0% and ~50% male are unstable in *M. batesii*. Understanding the processes that shape variation in reproductive mode in facultative parthenogens could help to answer fundamental questions about the evolution of sexual and asexual reproduction.

Acknowledgments

We thank Jigmidmaa Boldbaatar, Ana Vasconcelos, and Braxton Jones for their assistance in the field. We also thank Holly Trim for assistance with DNA extractions. This research was funded by the Australian Research Council through Discovery Grant DP200101971 to R.B.

Statement of Authorship

R.B., N.W.B., and L.A.R. conceptualized the study; R.B. acquired funding; R.B., N.W.B., and S.M.M. collected the data; S.M.M. and K.C.S. analyzed the data; S.M.M. visualized the data; R.B. and L.A.R. supervised the project; S.M.M. wrote the original draft; and R.B., L.A.R., K.C.S., and N.W.B. reviewed and edited the manuscript.

Data and Code Availability

All code is publicly available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.n02v6wx1g; Miller et al. 2023).

Literature Cited

Agrawal, A. F. 2006. Similarity selection and the evolution of sex: revisiting the red queen. PLoS Biology 4:e265.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science 281:1986–1990.

Bast, J., D. J. Parker, Z. Dumas, K. M. Jalvingh, P. Tran Van, K. S. Jaron, E. Figuet, et al. 2018. Consequences of asexuality in natural populations: insights from stick insects. Molecular Biology and Evolution 35:1668–1677.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Becks, L., and A. F. Agrawal. 2012. The evolution of sex is favoured during adaptation to new environments. PLoS Biology 10:e1001317.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. University of California Press, Berkeley.

Beukeboom, L. W., and R. C. Vrijenhoek. 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. Journal of Evolutionary Biology 11:755.

Boldbaatar, J. 2022. Dispersal and foraging rates of the facultatively parthenogenetic stick insect *Megacrania batesii*. MS thesis. University of New South Wales, Syndey.

Brock, P., M. Lee, M. Morgan-Richards, and S. Trewick. 2018. Missing stickman found: the first male of the parthenogenetic New Zealand phasmid genus *Acanthoxyla* Uvarov, 1944 discovered in the United Kingdom. Atropos 60:16–23.

Browne, R. A., L. E. Davis, and S. E. Sallee. 1988. Effects of temperature and relative fitness of sexual and asexual brine shrimp *Artemia*. Journal of Experimental Marine Biology and Ecology 124:1–20.

Buckley, T. R., K. A. Marske, and D. Attanayake. 2009. Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: the New Zealand stick insect *Argosarchus horridus* (White). Molecular Ecology 18:4650–4663.

Burke, N. W., and R. Bonduriansky. 2019. The paradox of obligate sex: the roles of sexual conflict and mate scarcity in transitions to facultative and obligate asexuality. Journal of Evolutionary Biology 32:1230–1241.

———. 2022. Sexually but not parthenogenetically produced females benefit from mating in a stick insect. Functional Ecology 36:2001–2014.

Burns, M., M. Hedin, and N. Tsurusaki. 2018. Population genomics and geographical parthenogenesis in Japanese harvestmen (Opiliones, Sclerosomatidae, Leiobunum). Ecology and Evolution 8:36–52.

Butlin, R. 2002. The costs and benefits of sex: new insights from old asexual lineages. Nature Reviews Genetics 3:311–317.

Cermak, M., and J. W. Hasenpusch. 2000. Distribution, biology and conservation status of the peppermint stick insect, *Megacrania batesii* (Kirby) (Phasmatodea: Pasmatidae), in Queensland. Memoirs of the Queensland Museum 46. Queensland Museum, Brisbane.

Chao, L. 1990. Fitness of RNA virus decreased by Muller's ratchet. Nature 348:454–455.

Colegrave, N. 2002. Sex releases the speed limit on evolution. Nature 420:664–666.

Cooper, T. F. 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. PLoS Biology 5:e225.

Craddock, E. 1972. Chromosomal diversity in the Australian Phasmatodea. Australian Journal of Zoology 20:445.

Crow, J. F., and M. Kimura. 1965. Evolution in sexual and asexual populations. American Naturalist 99:439–450.

Cullum, A. J. 1997. Comparisons of physiological performance in sexual and asexual whiptail lizards (genus *Cnemidophorus*): implications for the role of heterozygosity. American Naturalist 150:24–47.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 14:927–930.

D'Souza, T. G., and N. K. Michiels. 2010. The costs and benefits of occasional sex: theoretical predictions and a case study. Journal of Heredity 101:S34–S41.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–1150.

———. 2017. Asexual but not clonal: evolutionary processes in automictic populations. Genetics 206:993–1009.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Fontaneto, D., C. Q. Tang, U. Obertegger, F. Leasi, and T. G. Barraclough. 2012. Different diversification rates between sexual and asexual organisms. Evolutionary Biology 39:262–270.

George, L. F., S. J. Pradhan, D. Mitchell, M. Josey, J. Casey, M. T. Belus, K. N. Fedder, et al. 2019. Ion channel contributions to wing development in *Drosophila melanogaster*. G3: Genes, Genomes, Genetics 9:999–1008.

Gerber, N., and H. Kokko. 2016. Sexual conflict and the evolution of asexuality at low population densities. Proceedings of the Royal Society B 283:20161280.

Glesener, R. R., and D. Tilman. 1978. Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis in terrestrial animals. American Naturalist 112:659–673.

Gruber, B., P. J. Unmack, O. F. Berry, and A. Georges. 2018. dartR: an R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular Ecology Resources 18:691–699.

Hadany, L., and J. M. Comeron. 2008. Why are sex and recombination so common? Annals of the New York Academy of Sciences 1133:26–43.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software 33:1–22.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proceedings of the National Academy of Sciences of the USA 87:3566–3573.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

Hammelman, R. E., C. L. Heusinkveld, E. T. Hung, A. Meinecke, B. J. Parker, and J. A. Brisson. 2020. Extreme developmental instability associated with wing plasticity in pea aphids. Proceedings of the Royal Society B 287:20201349.

Hite, J. L., R. M. Penczykowski, M. S. Shocket, K. A. Griebel, A. T. Strauss, M. A. Duffy, C. E. Cáceres, et al. 2017. Allocation, not male resistance, increases male frequency during epidemics: a case study in facultatively sexual hosts. Ecology 98:2773–2783.

Hörandl, E. 2006. The complex causality of geographical parthenogenesis. New Phytologist 171:525–538.

Housworth, E. A., E. P. Martins, and M. Lynch. 2004. The phylogenetic mixed model. American Naturalist 163:84–96.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554–557.

Jalinsky, J., J. M. Logsdon, and M. Neiman. 2020. Male phenotypes in a female framework: evidence for degeneration in sperm produced by male snails from asexual lineages. Journal of Evolutionary Biology 33:1050–1059.

Jaron, K. S., J. Bast, R. W. Nowell, T. R. Ranallo-Benavidez, M. Robinson-Rechavi, and T. Schwander. 2021. Genomic features of parthenogenetic animals. Journal of Heredity 112:19–33.

Jaron, K. S., D. J. Parker, Y. Anselmetti, P. Tran Van, J. Bast, Z. Dumas, E. Figuet, et al. 2022. Convergent consequences of parthenogenesis on stick insect genomes. Science Advances 8:eabg3842.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Kamvar, Z. N., J. F. Tabima, and N. J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Kawatsu, K. 2013. Sexual conflict over the maintenance of sex: effects of sexually antagonistic coevolution for reproductive isolation of parthenogenesis. PLoS ONE 8:e58141.

Kearney, M. 2005. Hybridization, glaciation and geographical parthenogenesis. Trends in Ecology and Evolution 20:495–502.

Kearney, M., M. E. Jasper, V. L. White, I. J. Aitkenhead, M. J. Blacket, J. D. Kong, S. L. Chown, et al. 2022. Parthenogenesis without costs in a grasshopper with hybrid origins. Science 376:1110–1114.

Kearney, M., and R. Shine. 2004. Morphological and physiological correlates of hybrid parthenogenesis. American Naturalist 164:803–813.

Kenny, N. T. 1996. A test of the general-purpose genotype hypothesis in sexual and asexual *Erigeron* species. American Midland Naturalist 136:1–13.

Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia, H. Blois, V. Caig, et al. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. Pages 67–89 *in* F. Pompanon and A. Bonin, eds. Data production and analysis in population genomics, methods in molecular biology. Vol. 888. Humana, Totowa, NJ.

Kimmerer, R. W. 1994. Ecological consequences of sexual versus asexual reproduction in *Dicranum flagellare* and *Tetraphis pellucida*. Bryologist 97:20.

Larose, C., D. J. Parker, and T. Schwander. 2018. Fundamental and realized feeding niche breadths of sexual and asexual stick insects. Proceedings of the Royal Society B 285:20181805.

Law, J. H., and B. J. Crespi. 2002. The evolution of geographic parthenogenesis in *Timema* walking-sticks. Molecular Ecology 11: 1471–1489.

Lehtonen, J., M. D. Jennions, and H. Kokko. 2012. The many costs of sex. Trends in Ecology and Evolution 27:172–178.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519–521.

Loewe, L., and A. D. Cutter. 2008. On the potential for extinction by Muller's ratchet in *Caenorhabditis elegans*. BMC Evolutionary Biology 8:125.

Loewe, L., and D. K. Lamatsch. 2008. Quantifying the threat of extinction from Muller's ratchet in the diploid Amazon molly (*Poecilia formosa*). BMC Evolutionary Biology 8:88.

Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. Quarterly Review of Biology 59:257–290.

Lynch, M., R. Bürger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. Journal of Heredity 84:339–344.

Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. Evolution 44:1725–1737.

Manandhar, G., H. Schatten, and P. Sutovsky. 2005. Centrosome reduction during gametogenesis and its significance. Biology of Reproduction 72:2–13.

Marescalchi, O., C. Zauli, and V. Scali. 2002. Centrosome dynamics and inheritance in related sexual and parthenogenetic *Bacillus* (Insecta Phasmatodea). Molecular Reproduction and Development 63:89–95.

Maruzzo, D., L. Bonato, C. Brena, G. Fusco, and A. Minelli. 2005. Appendage loss and regeneration in arthropods: a comparative view. Pages 1–32 *in* S. Koenemann and R. Jenner, eds. Crustacea and arthropod relationships. CRC, Boca Raton, FL.

Mee, J. A., C. J. Brauner, and E. B. Taylor. 2011. Repeat swimming performance and its implications for inferring the relative fitness of asexual hybrid dace (Pisces: Phoxinus) and their sexually reproducing parental species. Physiological and Biochemical Zoology 84:306–315.

Miller, S., K. Stuart, N. Burke, L. Rollins, and R. Bonduriansky. 2023. Data from: Genetic and phenotypic consequences of local transitions between sexual and parthenogenetic reproduction in the wild. American Naturalist, Dryad Digital Repository, https://doi.org/10.5061/dryad.n02v6wx1g.

Morgan-Richards, M., S. S. Langton-Myers, and S. A. Trewick. 2019. Loss and gain of sexual reproduction in the same stick insect. Molecular Ecology 28:3929–3941.

Morgan-Richards, M., S. A. Trewick, and I. A. N. Stringer. 2010. Geographic parthenogenesis and the common tea-tree stick insect of New Zealand. Molecular Ecology 19:1227–1238.

Moritz, C., E. Suomalainen, A. Saura, and J. Lokki. 1990. Cytology and evolution in parthenogenesis. Evolution 44:1120.

Muller, H. J. 1932. Some genetic aspects of sex. American Naturalist 66:118–138.

———. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1:2–9.

Neiman, M., K. Larkin, A. R. Thompson, and P. Wilton. 2012. Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snail. Heredity 109:57–62.

Neiman, M., and T. Schwander. 2011. Using parthenogenetic lineages to identify advantages of sex. Evolutionary Biology 38:115–123.

Normark, B. B. 2003. The evolution of alternative genetic systems in insects. Annual Review of Entomology 48:397–423.

Otto, S. P., and A. C. Gerstein. 2006. Why have sex? the population genetics of sex and recombination. Biochemical Society Transactions 34:519–522.

Otto, S. P., and T. Lenormand. 2002. Resolving the paradox of sex and recombination. Nature Reviews Genetics 3:252–261.

Park, A. W., J. Jokela, and Y. Michalakis. 2010. Parasites and deleterious mutations: interactions influencing the evolutionary maintenance of sex. Journal of Evolutionary Biology 23:1013–1023.

Park, S.-C., and J. Krug. 2013. Rate of adaptation in sexuals and asexuals: a solvable model of the Fisher-Muller effect. Genetics 195:941–955.

Parker, E. D. 1979. Phenotypic consequences of parthenogenesis in *Cnemidophorus* lizards. II. Similarity of *C. tesselatus* to its sexual parental species. Evolution 33:1167.

Pembleton, L. W., N. O. I. Cogan, and J. W. Forster. 2013. StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Molecular Ecology Resources 13:946–952.

Pianka, E. R. 2000. Evolutionary ecology. 6th ed. Benjamin Cummings, San Francisco.

Pijnacker, L. P., and M. A. Ferwerda. 1980. Sex chromosomes and origin of males and sex mosaics of the parthenogenetic stick insect *Carausius morosus* Br. Chromosoma 79:105–114.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Reik, W., and J. Walter. 2001. Genomic imprinting: parental influence on the genome. Nature Reviews Genetics 2:21–32.

Sæther, B.-E., and S. Engen. 2015. The concept of fitness in fluctuating environments. Trends in Ecology and Evolution 30:273–281.

Scali, V. 2009. Metasexual stick insects: model pathways to losing sex and bringing it back. Pages 317–345 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex. Springer, Dordrecht.

Schlupp, I. 2010. Mate choice and the Amazon molly: how sexuality and unisexuality can coexist. Journal of Heredity 101:S55–S61.

Schmidt, T. L., M. Jasper, A. R. Weeks, and A. A. Hoffmann. 2021. Unbiased population heterozygosity estimates from genome-wide sequence data. Methods in Ecology and Evolution 12:1888–1898.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Schwander, T., and B. J. Crespi. 2009. Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in *Timema* stick insects. Evolution 63:84–103.

Schwander, T., S. Vuilleumier, J. Dubman, and B. J. Crespi. 2010. Positive feedback in the transition from sexual reproduction to parthenogenesis. Proceedings of the Royal Society B 277:1435–1442.

Sherwin, W. B., A. Chao, L. Jost, and P. E. Smouse. 2017. Information theory broadens the spectrum of molecular ecology and evolution. Trends in Ecology and Evolution 32:948–963.

Smith, M. J. 1978. The evolution of sex. Cambridge University Press, Cambridge.

Stenberg, P., and A. Saura. 2009. Cytology of asexual animals. Pages 63–74 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex. Springer, Dordrecht.

Sukumaran, S., and A. Grant. 2013. Differential responses of sexual and asexual *Artemia* to genotoxicity by a reference mutagen: is the comet assay a reliable predictor of population level responses? Ecotoxicology and Environmental Safety 91:110–116.

Suzuki, R., and H. Shimodaira. 2006. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. Bioinformatics 22:1540–1542.

Tarkhnishvili, D., A. Gavashelishvili, A. Avaliani, M. Murtskhvaladze, and L. Mumladze. 2010. Unisexual rock lizard might be outcompeting its bisexual progenitors in the Caucasus: ecological interactions between rock lizards. Biological Journal of the Linnean Society 101:447–460.

Terriente-Félix, A., A. López-Varea, and J. F. de Celis. 2010. Identification of genes affecting wing patterning through a loss-of-function mutagenesis screen and characterization of med15 function during wing development. Genetics 185:671–684.

Tilquin, A., and H. Kokko. 2016. What does the geography of parthenogenesis teach us about sex? Philosophical Transactions of the Royal Society B 371:20150538.

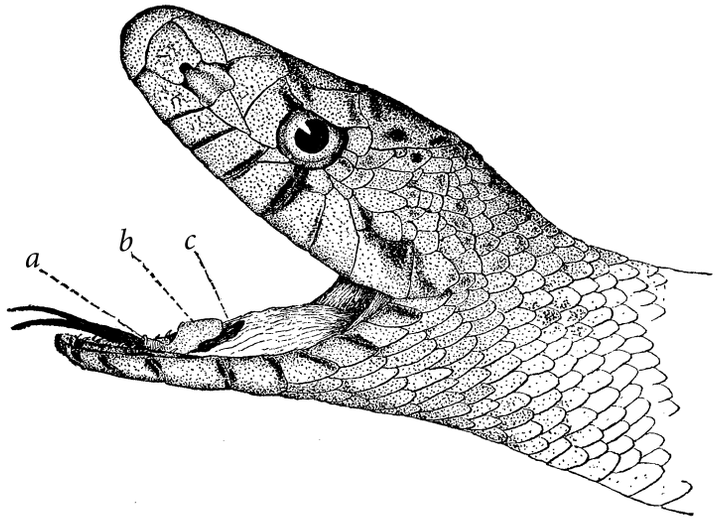
Tojo, K., K. Sekiné, and A. Matsumoto. 2006. Reproductive mode of the geographic parthenogenetic mayfly *Ephoron shigae*, with findings from some new localities (Insecta: Ephemeroptera, Polymitarcyidae). Limnology 7:31–39.

Van Herwaarden, H. C. M. 1998. A guide to the genera of stick- and leaf-insects (Insecta: Phasmida) of New Guinea and the surrounding islands. Science in New Guinea 24:55–117.

Vrijenhoek, R. C., and E. D. Parker. 2009. Geographical parthenogenesis: general purpose genotypes and frozen niche variation. Pages 99–131 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex. Springer, Dordrecht.

Wang, C., W.-H. Kao, and C. K. Hsiao. 2015. Using Hamming distance as information for SNP-sets clustering and testing in disease association studies. PLoS ONE 10:e0135918.

Associate Editor: Roger K. Butlin
Editor: Erol Akçay



"The tongue-sheath is represented at *a*; the epiglottis at *b*; and the rima-glotidis (aperture of the windpipe) at *c*." From "On the Character and Function of the Epiglottis in the Bull-Snake (Pityophis)" by Charles A. White (*The American Naturalist*, 1884, 18:19–21).