Animal Behaviour 101 (2015) 117–127

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

The role of sexual conflict in the evolution of facultative parthenogenesis: a study on the spiny leaf stick insect

Nathan W. Burke*, Angela J. Crean, Russell Bonduriansky

Evolution & Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

ARTICLE INFO

Article history:
Received 24 July 2014
Initial acceptance 4 September 2014
Final acceptance 25 November 2014
Available online 28 January 2015
MS. number: 14-00593R

Keywords:
cost of sex
evolution of sex
Extatosoma tiaratum
facultative parthenogenesis
female resistance behaviours
pheromones
sexual conflict

Parthenogenesis is an asexual mode of reproduction that is rare in nature compared to sex. Parthenogenetic animals are derived from sexual progenitors, but little is known about how transitions to parthenogenesis evolve and persist. One hypothesis is that selection promotes transitions to parthenogenesis when males are rare or when mating is impossible (the mate scarcity hypothesis). However, we hypothesized that selection might also favour sex-to-parthenogenesis transitions when sexual interactions are costly (the sexual conflict hypothesis). These hypotheses lead to contrasting predictions about the nature of male–female interactions and their effects on female fitness. To test these hypotheses, we conducted a series of life history and behavioural experiments on the facultatively parthenogenetic spiny leaf stick insect, *Extatosoma tiaratum*. As predicted by sexual conflict theory, we found that females appeared to neutralize the costs of sex by utilizing counterevolved resistance traits: male-paired females resisted matings by curling their abdomens and kicking their legs during copulation attempts, prereproductive virgin females produced an antiaphrodisiac that repelled males, and parthenogenetic females made themselves inconspicuous to males by altering their pheromonal signals. Although measures of offspring viability point to possible advantages of sexual reproduction, females that were experimentally switched from a parthenogenetic to a sexual reproductive mode suffered elevated mortality and egg production costs that were not observed in exclusively parthenogenetic or exclusively sexual females. Our results suggest that females can benefit by avoiding mating in at least some circumstances, therefore supporting the hypothesis that sexual conflict mediated by female resistance could contribute to the evolution of facultative parthenogenesis.

Parthenogenesis occurs when embryos develop from unfertilized ova. Although rarer than sex (De Meeûs, Prugnolle, & Agnew, 2007), parthenogenetic reproduction has evolved multiple times in angiosperms (Richards, 2003) and in all major animal groups except mammals (Avise, Quattro, & Vrijenhoek, 1992). Phylogenetic evidence suggests that the vast majority of parthenogenetic species evolved recently from sexual ancestors (Butlin, 2002). But the selective forces that may favour or impede evolutionary transitions from sex to parthenogenesis remain poorly understood (Archetti, 2010; Corley, Blankenship, & Moore, 2001; D'Souza & Michiels, 2010; Engelstaedter, 2008; Hadany & Beker, 2007; Moore & Moore, 2003; Yamauchi & Kamite, 2003).

Facultative parthenogenesis is the capacity to reproduce either sexually or asexually (Normark, 2003) and is considered an important evolutionary stepping stone in transitions from obligate sex to obligate parthenogenesis (Schwander, Vuilleumier, Dubman, & Crespi, 2010). Although it is unclear how facultative parthenogenesis evolves, selection is thought to promote occasional parthenogenetic reproduction if mating is impossible or opportunities to mate are rare (Kramer & Templeton, 2001; Schwander et al., 2010; Stalker, 1956). This hypothesis (hereafter referred to as the mate scarcity hypothesis) assumes that sex is the selectively favoured or 'preferred' mode of reproduction, and that females that engage in parthenogenetic reproduction merely make the best of a bad (i.e. mateless) situation. Although the mate scarcity hypothesis has some empirical support (e.g. Kramer & Templeton, 2001; Schwander et al., 2010), its assumption that

* Correspondence: N. W. Burke, Evolution & Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia.
E-mail address: nathwilliamb@runbox.com (N. W. Burke).

http://dx.doi.org/10.1016/j.anbehav.2014.12.017
0003-3472/

sex invariably confers higher fitness than parthenogenesis in facultative species is problematic in light of theoretical and empirical evidence of the 'two-fold cost' of sex (e.g. D'Souza & Michiels, 2010; Jokela, Lively, Dybdahl, & Fox, 1997; Maynard Smith, 1978; Yamauchi & Kamite, 2003), and theoretical studies suggesting that rare sex can confer the same benefits as frequent sex (Green & Noakes, 1995; D'Souza & Michiels, 2010). The mate scarcity hypothesis also fails to account for the additional costs of sexual conflict (see Lehtonen, Jennions, & Kokko, 2012) and the important role that sexual conflict plays in life history evolution (see Connallon, Cox, & Calsbeek, 2009).

Sexual conflict may offer an additional or alternative set of conditions under which selection can favour facultative parthenogenesis. Kawatsu (2013) showed mathematically that the coexistence of sexual and asexual reproduction in facultative systems can be maintained by antagonistic coevolution between male coercion and female resistance. Following Kawatsu (2013), we propose that the benefits of parthenogenesis relative to sex in facultative species might be amplified when the costs to females that do not resist mating are large (hereafter referred to as the sexual conflict hypothesis). If parthenogenetically reproducing females incur large costs as a consequence of switching to sex, for example, then female resistance to mating could evolve and thereby maintain parthenogenetic reproduction, as long as the costs of resistance are not so great as to negate any benefits gained (see Rowe, Arnqvist, Sih, & Krupa, 1994).

The sexual conflict and mate scarcity hypotheses yield sharply contrasting predictions. According to the mate scarcity hypothesis, virgin and parthenogenetically reproducing females should generally benefit from mating, especially if they have been unable to find a mate for a long time. Virgin and parthenogenetically reproducing females should strongly attract mates if sex is beneficial, and should therefore show few signs of resistance when mating opportunities arise. Virgin females might also be expected to delay onset of parthenogenetic reproduction in anticipation of the arrival of mates. In contrast, the sexual conflict hypothesis predicts that females should exhibit a high degree of mating reluctance by either avoiding sex entirely, or avoiding switching to sex once parthenogenetic reproduction has commenced. Additionally, females that make themselves unattractive to potential mates or that stop signalling their reproductive status might be favoured by selection if mating is costly. If parthenogenesis is favoured over sex, females might also be expected to hasten the onset of parthenogenetic reproduction. Finally, in at least some contexts, females should achieve higher fitness through parthenogenetic reproduction than through sexual reproduction. The fitness benefits of mating avoidance may be direct (e.g. enhanced longevity or fecundity) and/or indirect (e.g. enhanced offspring quality).

The Australian spiny leaf stick insect, *Extatosoma tiaratum*, is a facultatively parthenogenetic species whose life history and ecology have been interpreted as being broadly consistent with the mate scarcity hypothesis (Schneider & Elgar, 2010). However, no one has yet tested for sexual conflict in this species. Very little is known about the sex ratio of wild populations, but it is assumed that parthenogenetic production of all-female offspring and the relatively short life span of males result in a female-biased operational sex ratio (Schneider & Elgar, 2010). Unlike males, which are fully capable of flight, *E. tiaratum* females possess vestigial wings and are flightless (Brock, 2001). Males possess clasping organs (modified cerci) that facilitate copulation (personal observation). The characteristic posture of females (curling the abdomen over the back) is thought to be an antipredator defence, although it also occurs during courtship and could therefore play a role in female resistance to mating. Both sexes produce a nontoxic repugnatorial secretion that is limited in its effectiveness as a predator repellent (Carlberg, 1985, 1987), but could play a role in sexual signalling (Strong, 1975).

We carried out an experimental investigation of sexual behaviour and reproductive performance in *E. tiaratum* to test the contrasting predictions of the sexual conflict and mate scarcity hypotheses. First, we compared fitness components (including egg output, hatching success and offspring juvenile viability) of unmated females that reproduced parthenogenetically and mated females exposed to males either prior to or following the start of parthenogenetic oviposition. Second, we tested for antagonistic pheromone signalling. Third, we looked for evidence of female behaviours and chemical defences that function to deter or repel male mating attempts.

METHODS

Animal Maintenance

Focal female and male individuals were obtained as hatchlings and young instar nymphs from a professional insect breeder (Insectpets, Gisborne, Victoria, Australia). A small number of supplementary individuals were also obtained from amateur breeders in the Greater Sydney Region, Australia. All individuals were the North Queensland race of *E. tiaratum tiaratum* (Brock, 2001). Nymphs were initially fed *Agonis flexuosa* leaves and housed in a single 12-litre plastic terrarium, but were later segregated by sex into groups of no more than 10 same-sex individuals. At maturity, males were housed in 40-litre plastic tubs each containing no more than 20 individuals at a time. Mature, focal females were housed individually in plastic cylinders with fibreglass fly screen lids (diameter: 20 cm; height: 40 cm; see mmc2 Supplementary Fig. S1). Stock females were housed communally in a large glass tank (76×47 cm and 50 cm high) covered with fly screen. Leaves of *A. flexuosa* and various species of *Eucalyptus* were fed to adult stick insects and replaced weekly. All leaves were sprayed once a day with tepid water. Female body length at time of death was determined by measuring abdomen tip to antennae base using a ruler.

Experiment Design

All experimental assays were conducted in the laboratory at room temperature (20–25 °C) from March to August 2013, and were based on three treatment groups, established as follows. Fifteen focal females of roughly equal size and age were haphazardly allocated 1 week after their final moult to each of three treatment groups: 'sexual', 'parthenogenetic' and 'interrupted'. Depending on the treatment, focal females were paired with either same- or opposite-sex individuals for 3 consecutive days per week: Sexual females were paired with males, parthenogenetic females were paired with stock females to control for density effects, and interrupted females were initially paired with stock females, but, following an initial 5 weeks of parthenogenetic reproduction, were paired with males. The switch to sex was initiated at 5 weeks to ensure that interrupted females had fully invested in the parthenogenetic pathway before males were introduced. At the end of the 3-day pairing period each week, males and stock females were removed from all focal-female enclosures. A subset of nine focal females from each of the three treatments was used for all assays except for those for pheromone

and defence gland secretions which utilized all 15 sexual and parthenogenetic females plus individuals from two additional treatments: 'male' and 'prereproductive virgin female'. These two extra treatments were included so that chemical and pheromonal attraction dynamics between opposite- and same-sex pairs could be comprehensively investigated. The prereproductive treatment group consisted of 15 newly mature females yet to start ovipositing, chosen haphazardly from female-only breeding stocks and housed individually in the same kinds of cylindrical enclosures as other focal females. Males were chosen haphazardly from male stocks as required.

Female Life History Responses

Focal females in this assay were paired with males or control females for a maximum period of 15 weeks, except for two 2-week periods late in the experiment when an unrelated assay not reported in this paper was performed on focal females. Mating rate, longevity and reproductive output were recorded for each female for 12 consecutive weeks from the onset of oviposition. The number of days from imaginal moult to first oviposition was also noted. Copulation was determined by counting spermatophores, which are dropped by females after mating (Clark, 1974). To avoid the confounding factor of eggs of control females, only eggs of focal females were counted and weighed during the 4-day period per week that females were unpaired. To quantify egg hatching, hatchling survival to second instar and offspring sex ratio, eggs of each female were placed into separate plastic hatcheries (diameter: 110 mm; height: 120 mm) containing damp coco-peat, and sprayed weekly with tepid water. Offspring data were collected over a period of approximately 6 months from first hatching (September 2013 to March 2014). Newly hatched nymphs were housed in their respective maternal lines in separate plastic cylindrical enclosures (diameter: 110 mm; height: 210 mm). All offspring were fed *A. flexuosa* leaves, and sexed upon moulting to second instar.

Behavioural Responses

Female abdominal curling

To determine whether abdominal curling is used by females to resist copulations, abdomen curling behaviours were recorded for parthenogenetic females following initial pairings with stock females, and for interrupted and sexual females following initial pairings with males. Observations were made 12 h before and 4, 28 and 52 h after the start of each pairing per week, over 13 weeks. Degree of abdominal curling was quantified using four abdomen shape categories: 0 = obtuse thorax–abdomen angle ($90° < x \leq 180°$); 1 = L-shaped thorax–abdomen angle ($45° < x \leq 90°$); 2 = U-shaped thorax–abdomen angle ($0° < x \leq 45°$); 3 = abdomen tightly coiled over thorax (see mmc3 Supplementary Fig. S2), with a greater degree of abdomen curling interpreted as a more defensive posture. All behavioural observations were carried out by N.W.B. at night (when insects were most active) under red light illumination.

Attractiveness of male and female pheromones

We measured the responses of males, parthenogenetic, sexual and prereproductive females to odours produced by individuals of each of these treatment groups. Four assays were performed in total, one for each responder class. Although trials in which signallers and responders belonged to different treatment groups were of primary interest, trials in which signallers and responders belonged to the same treatment group were included as controls (i.e. one control for each assay). Responder individuals were placed in glass tanks (76 × 47 cm and 50 cm high) whose bases had been subdivided into thirds using a black marker. *Eucalyptus* leaves that had been previously exposed to signaller individuals and therefore carried odours ('treated' leaves) were placed at one end of the tank, and control leaves of the same age that had not been exposed to any stick insects were placed at the other end (see mmc4 Supplementary Fig. S3). Responders were initially placed at the base of a bunch of bare *Eucalyptus* twigs positioned between the treated and control leaves, and left in the glass tanks for 24 h. Because the defecation rate in *E. tiaratum* is constant (Carlberg, 1988), the number of droppings under treated and control leaves was quantified as an index of the length of time spent on these leaves. Trials for each signal–response combination were performed weekly over 15 weeks using different responder individuals for each replicate. Some signaller individuals from treatment groups that experienced high mortality were used more than once. The sequence in which trials were conducted each week and the side of the glass tank in which treated leaves were placed were randomized. Glass tanks were wiped clean with soapy water and dried between trials.

Male response to repugnatorial secretions

Many phasmatids release repugnatorial fluids on to the surface of the body from glands in the prothorax when subjected to stress or attack (Scudder, 1876). Since previous work suggested that the repugnatorial fluids of *E. tiaratum* are ineffectual at deterring predators (Carlberg, 1985, 1987), an assay was performed to test whether females use repugnatorial fluids to ward off males. Leaves were taken from the enclosures of signaller individuals and split equally into two bunches. Signaller individuals were then placed on one of the leaf bunches ('treated' leaves) and their abdomens prodded with a finger for 1 min to initiate the secretion of repugnatorial fluids onto leaves. The other set of leaves was used as a control. Both sets of leaves thus carried signaller pheromones, but only the treated leaves carried repugnatorial secretions. Responses to treated and control leaves were then assessed using the same procedure as described for the pheromone assays, but only male responses were measured. Fifteen replicates were obtained for each type of signaller (one replicate per week) using different responder males for each replicate. Some signaller individuals from treatment groups that experienced high mortality were used more than once.

Data Analyses

Treatment effects on female longevity were investigated using survival analysis, with females that were alive at the end of the experiment treated as censored observations. Female 'age' was defined as time since imaginal moult. A single parthenogenetic female died before males were introduced to the interrupted treatment (i.e. when parthenogenetic and interrupted treatment females were still treated identically). Since this female's longevity did not contribute any information about differences between the parthenogenetic and interrupted treatments, we ran an additional analysis that excluded this female.

Although raw data from the abdominal curling assay were ordinal, parametric *t* tests were performed on means for individual females (which were continuously distributed) to assess treatment effects. One-way ANOVAs were used to assess differences between treatments in total egg output per female, mean mass of all eggs produced per female, total number of hatched

eggs per female and total number of second-instar nymphs per female. In addition, to examine age-specific treatment effects, weekly egg output per female and weekly mean egg mass per female were analysed using linear mixed models that incorporated Poisson and normal error structures, respectively. For females that died before the end of the experiment, data were included up to the point of death, such that the mean weekly values did not reflect variation in female longevity. Treatment was modelled as a categorical fixed effect, number of egg collection days per week was added as a covariate, and female identity and week of oviposition were modelled as random effects to account for repeated measures (see Crawley, 2013). In addition, a binary dummy variable (before week 5 versus after week 5) was included in the models as a fixed effect to assess whether the point in time that males were introduced to interrupted females corresponded to changes in weekly egg output and egg mass. In these analyses, a treatment * dummy interaction effect would indicate that changes in reproductive output or egg mass were unequal in the three treatment groups. Because we detected a significant interaction for weekly egg output, we then compared egg output before versus after week 5 separately within each treatment group to determine which treatment(s) were driving the observed interaction. In egg output analyses, a unique code for each observation was modelled as an additional random effect to control for overdispersion.

Data from pheromone and repugnatorial secretion assays were analysed using linear mixed models with Poisson error structures to test for leaf treatment effects within each signaller–responder combination, with signaller type modelled as a fixed categorical effect, and signaller identity, trial, date of trial and a dummy variable for overdispersion included as random effects. Generalized linear fixed-effects models with quasi-Poisson error structures were used to analyse egg hatching and nymph survival data, with egg output per female included as a covariate in the model of egg hatching, and number of eggs hatched per female included as a covariate in the model of nymph survival.

Linear mixed model analyses were performed using the glmer function (lme4 package) in R (version 3.0.2; R Core Team, 2013). The statistical significance of fixed and interaction effects was tested by excluding each effect from mixed models and conducting likelihood ratio tests. Generalized linear fixed-effects model analyses were performed in R using the glm function, with analyses of deviance performed using the anova.glm function. Other analyses were computed using STATISTICA 7 version 7.1 (StatSoft, Inc., 2005). Descriptive data in the Results are reported as means ± SEs and all P values for statistical tests are two tailed unless otherwise stated.

RESULTS

Female Life History Responses

There was a marked difference in the latency of sexually and parthenogenetically reproducing females to oviposit after pairing. Mated females took significantly longer to start ovipositing than unmated females (mated: 51.78 ± 3.733 days, $N = 9$; unmated: 39. ± 1.598 days, $N = 18$; t test: $t_{25} = -3.67$, $P = 0.001$). All females started ovipositing by 70 days after their final moult (range 32–70 days).

More than half the females in the interrupted treatment group died soon after being paired with males (Fig. 1). The difference in survival rate between interrupted and parthenogenetic females was marginally nonsignificant (Cox–Mantel test: $C = -1.79$, $P = 0.074$). However, when a female that died prior to the introduction of males to the interrupted treatment was excluded, interrupted females exhibited a significantly lower survival rate than parthenogenetic females (Cox–Mantel test: $C = -2.42$, $P = 0.015$). There was no significant difference in survival rate between sexual and parthenogenetic females when the above-mentioned female was included (Cox–Mantel test: $C = -0.544$, $P = 0.587$) or excluded (Cox–Mantel test: $C = 1.374$, $P = 0.170$), nor was there a difference in survival rate between sexual and interrupted females (Cox–Mantel test: $C = -1.430$, $P = 0.153$).

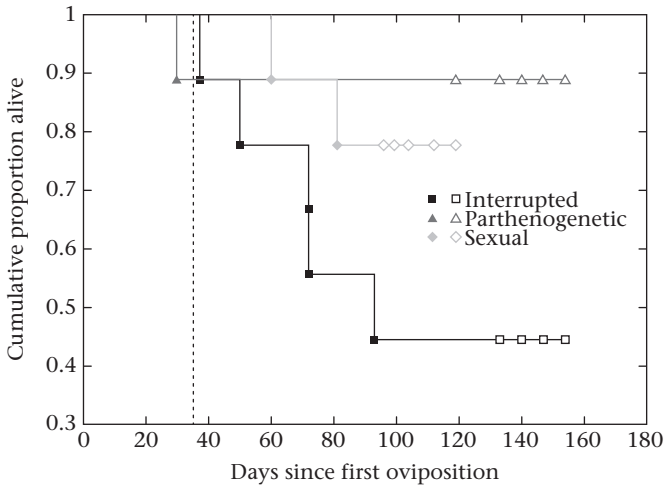


Figure 1. Survival curves showing the cumulative proportion of females alive over time in each treatment. Closed data points indicate mortalities; open data points indicate females that were still alive at the end of the experiment. Females in the interrupted group reproduced parthenogenetically during the first 5 weeks of oviposition but switched to sexual reproduction (indicated by the vertical dashed line) when males were introduced.

There was no significant treatment effect on the mean mass of all eggs produced per female (sexual: 0.038 ± 0.002 g; interrupted: 0.041 ± 0.001 g; parthenogenetic: 0.038 ± 0.002 g; ANOVA: $F_{2,24} = 0.622$, $P = 0.546$) or on weekly mean egg mass per female (likelihood ratio test: $\chi^2_2 = 1.443$, $P = 0.486$). Likewise, treatment had no significant effect on total egg output (i.e. total number of eggs laid in the first 12 weeks of oviposition) per female (sexual: 74.556 ± 7.645; interrupted: 66.444 ± 5.838; parthenogenetic: 60.778 ± 9.058; ANOVA: $F_{2,24} = 0.824$, $P = 0.451$) or on weekly egg output per female (likelihood ratio test: $\chi^2_2 = 3.026$, $P = 0.220$).

Changes in weekly egg mass after week 5 (when males were introduced to the interrupted treatment) did not differ between treatments (treatment * pre versus post week 5 dummy variable: likelihood ratio test: $\chi^2_2 = 3.651$, $P = 0.161$). Thus, there was no evidence that mating affected egg mass of interrupted females. However, egg output changed unequally among the three treatment groups after the fifth week of oviposition (treatment * pre versus post week 5 dummy variable: likelihood ratio test: $\chi^2_2 = 7.640$, $P = 0.022$). This interaction effect was driven by the sharp decline in egg production of interrupted females (Fig. 2): weekly egg output of interrupted females during the first 5 weeks of reproduction was significantly higher than during the following 7 weeks of reproduction (likelihood ratio test: $\chi^2_1 = 5.776$, $P = 0.016$), but weekly egg production did not differ between the

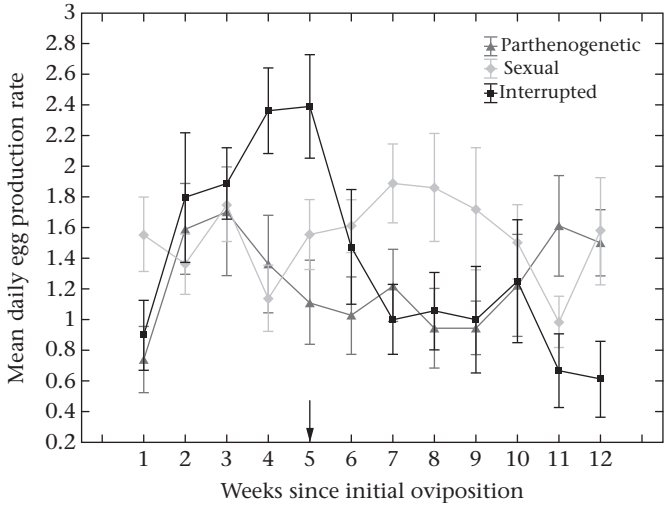


Figure 2. Mean daily egg production rates ± SE of parthenogenetic, interrupted and sexual females during the first 12 weeks of oviposition. The decline in egg output in the interrupted treatment from week 5 coincides with reproductive switching (indicated by the arrow).

first 5 weeks and the following 7 weeks within sexual or parthenogenetic treatments (likelihood ratio test: parthenogenetic: $\chi^2_1 = 0.600$, $P = 0.439$; sexual: $\chi^2_1 = 2.230$, $P = 0.135$). Because egg output did not differ between treatments during the first 5 weeks of oviposition (likelihood ratio test: $\chi^2_2 = 3.056$, $P = 0.217$), the sharp decline in egg output of interrupted females after the introduction of males cannot be attributed to higher egg output prior to male introduction.

Female body length did not differ between treatments (sexual: 11.211 ± 0.237 cm; interrupted: 11.200 ± 0.286 cm; parthenogenetic: 11.211 ± 0.178 cm; ANOVA: $F_{2,24} = 0.001$, $P = 0.999$). Oviposition rate covaried positively with body size in sexual females (linear regression: $r = 0.676$, $F_{1,7} = 5.883$, $P = 0.046$), but not in interrupted or parthenogenetic females (linear regression: interrupted: $r = 0.192$, $F_{1,7} = 0.269$, $P = 0.620$; parthenogenetic: $r = -0.504$, $F_{1,7} = 2.383$, $P = 0.167$). Egg mass was not correlated with female body size in any treatment (linear regression: sexual: $r = -0.222$, $F_{1,7} = 0.363$, $P = 0.566$; interrupted: $r = -0.164$, $F_{1,7} = 0.192$, $P = 0.674$; parthenogenetic: $r = -0.143$, $F_{1,7} = 0.146$, $P = 0.714$).

There was no significant treatment effect on the total number of hatched nymphs produced per female (sexual: 48.222 ± 10.873; interrupted: 36.111 ± 6.925; parthenogenetic: 22.556 ± 9.971; ANOVA: $F_{2,24} = 1.862$, $P = 0.177$), nor did treatment significantly affect the number of hatched nymphs when egg output per female was accounted for (analysis of deviance: $\chi^2_2 = 3.084$, $P = 0.214$), although there was a trend towards lower hatching in the parthenogenetic treatment (Fig. 3). The interval of time between initial oviposition and first hatching of parthenogenetically produced eggs was longer than for sexually produced eggs (sexual median: 153.5 days; interrupted median: 188 days; parthenogenetic median: 207 days; Kruskal–Wallis test: $H_2 = 13.713$, $P = 0.001$), suggesting that parthenogenetic eggs take longer to hatch.

There was no significant effect of treatment on total number of offspring per female surviving to the second instar (sexual: 14.00 ± 2.920; interrupted: 15.111 ± 2.946; parthenogenetic: 7.222 ± 3.811; ANOVA: $F_{2,24} = 1.724$, $P = 0.200$). However, there

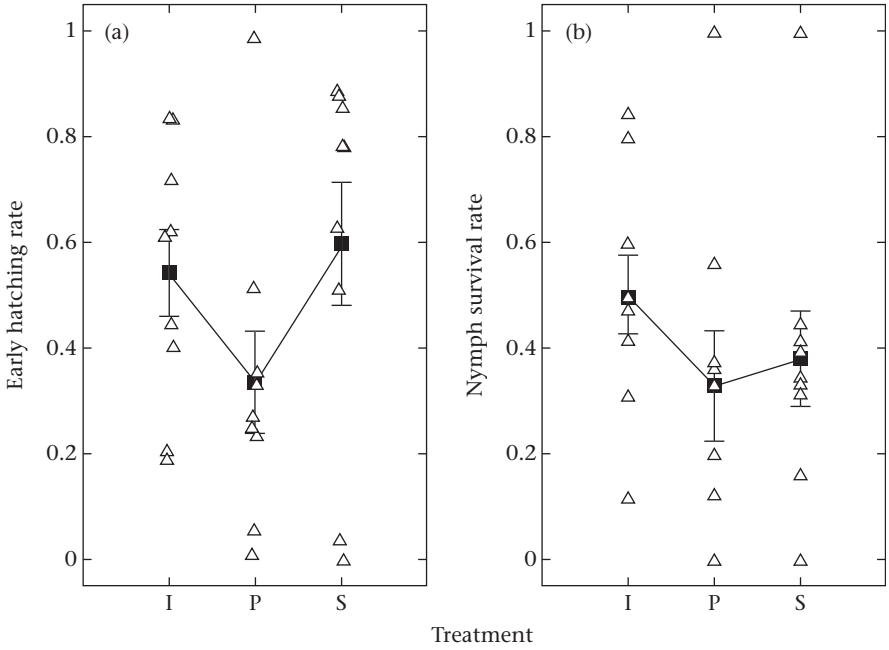


Figure 3. Mean (a) early egg hatching rate ± SE and (b) nymph survival to second instar ± SE of offspring produced by sexual (S), interrupted (I) and parthenogenetic (P) females. Note that the trend towards lower early hatching rate in the parthenogenetic treatment is likely to be negatively biased by the tendency for long periods of diapause in parthenogenetically produced eggs. Triangles represent data points for individual females; black squares and error bars indicate treatment means and SEs, respectively.

was a significant effect of treatment on the number of offspring surviving to the second instar when the number of hatched nymphs was accounted for (analysis of deviance: $\chi^2_2 = 6.607$, $P = 0.038$). This effect was driven by differences in hatchling-to-second-instar survival between parthenogenetic and interrupted treatments (analysis of deviance: $\chi^2_1 = 5.141$, $P = 0.023$; Fig. 3), whereas hatchling-to-second-instar survival did not differ between sexual and parthenogenetic treatments (analysis of deviance: $\chi^2_1 = 0.250$, $P = 0.617$), or between sexual and interrupted treatments (analysis of deviance: $\chi^2_1 = 2.290$, $P = 0.130$).

The sex ratio of sexually produced second-instar nymphs was even (50.4% male), whereas all the parthenogenetically produced second-instar nymphs were female. As expected, interrupted females produced an offspring sex ratio intermediate between the sexual and parthenogenetic treatments (21% male). It is not clear whether interrupted females continued to produce some offspring parthenogenetically after switching to sex.

Behavioural Responses

Female abdominal curling

During copulation, females clung onto leaves with their legs while males held on to the dorsal side of the female abdomen (see Fig. 4). Males used their modified cerci to clasp onto the ventrodistal end of female abdomens, effectively preventing females from curling their abdomens during spermatophore transfer (see Fig. 4). In both the sexual and interrupted treatments, female mating rates were highest during the first 24 h of pairing each week. However, males never achieved more than one copulation per 3-day weekly pairing. Sexual and interrupted females exhibited strong resistance behaviours when paired with males, resisting matings by curling their abdomens tightly over their back out of reach of male clasping organs, and kicking their mid- and hindlegs to try to dislodge males (see mmc1 Supplementary video).

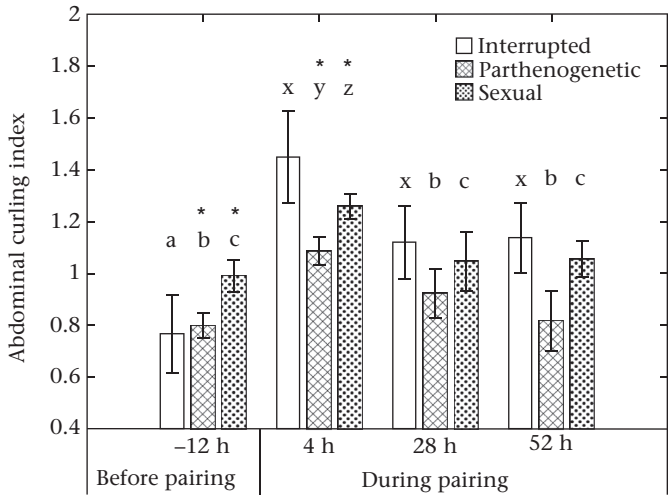


Figure 5. Mean ± SE abdominal curling indices of parthenogenetic, interrupted and sexual females before and during pairings with males or stock females. Higher scores denote a higher degree of abdomen curling (see Methods for details). Dissimilar letters above columns indicate significant differences at $P < 0.05$, based on independent-sample t tests, between observation times within treatment. Columns with asterisks denote treatments within observations that are significantly different from each other at $P < 0.05$.

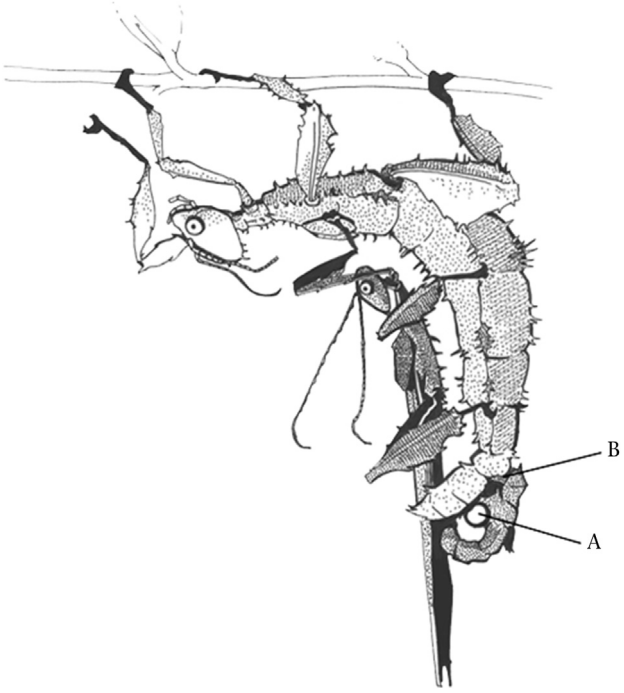


Figure 4. Copulating spiny leaf stick insects. Copulation involves the transfer of a large white spermatophore (labelled A). The male stays mounted on top of the female and grips onto the ventrodistal end of the female's abdomen with his genital clasping organ (labelled B). In this position, the female is unable to curl her abdomen to resist mating. Drawing: David Sindel.

Table 1
Results of likelihood ratio tests for the effect of signaller pheromone on responder behaviour

Signaller	Responder	Chi-square	*df*	Estimate	*P*
Male	**F(PRV)**	**4.727**	**1**	**−1.533**	**0.030**
F(sex)	F(PRV)	0.873	1	−0.635	0.350
F(parth)	F(PRV)	0.297	1	0.421	0.586
F(PRV)	F(PRV)	2.067	1	1.057	0.151
F(sex)	**Male**	**9.032**	**1**	**1.668**	**0.003**
F(PRV)	**Male**	**9.455**	**1**	**1.283**	**0.002**
F(parth)	Male	0.634	1	0.634	0.426
Male	Male	0.049	1	0.116	0.826
Male	F(sex)	0.059	1	0.193	0.809
F(PRV)	F(sex)	1.666	1	0.927	0.197
F(parth)	F(sex)	0.060	1	0.175	0.806
F(sex)	F(sex)	1.308	1	−0.699	0.253
Male	F(parth)	3.657	1	3.121	0.056
F(sex)	F(parth)	0.001	1	−0.020	0.973
F(PRV)	F(parth)	0.036	1	0.146	0.849
F(parth)	F(parth)	0.280	1	−0.341	0.596

For each signaller–responder combination, mixed models with/without leaf treatment were compared. Signaller–responder combinations highlighted in bold are those for which the number of droppings beneath treatment and control leaves was significantly different, with positive fixed-effect estimates indicating attraction of responders to signaller pheromones, and negative estimates indicating repulsion. Female treatment groups are abbreviated as follows: F(PRV) = prereproductive virgin females; F(sex) = sexual females; F(parth) = parthenogenetic females.

Before pairings, sexual females exhibited a higher mean degree of abdominal curling than parthenogenetic females (t test: $t_{15} = -2.394$, $P = 0.030$; Fig. 5). However, abdominal curling before pairings did not differ between interrupted and sexual females (t test: $t_{15} = -1.431$, $P = 0.173$), or between interrupted and parthenogenetic females (t test: $t_{14} = -2.03$, $P = 0.842$). Abdominal curling scores of all treatment groups before pairings were significantly lower than scores obtained 4 h into pairings (paired t tests: sexual: $t_8 = -4.900$, $P = 0.001$; parthenogenetic: $t_7 = -3.522$, $P = 0.010$; interrupted: $t_7 = -3.203$, $P = 0.015$). However, only interrupted females sustained elevated abdominal curling scores over the entire pairing period: scores obtained 28 h (paired t test: $t_7 = -3.542$, $P = 0.009$) and 52 h into pairings (paired t test: $t_7 = -4.080$, $P = 0.005$) were significantly higher than scores before pairings. In contrast, abdomen curling scores in the sexual and parthenogenetic treatments declined to levels before pairings over the same period, such that there was no difference between abdomen curling scores before pairings and either 28 or 52 h into pairings (paired t tests: sexual: both $t_8 < -0.719$, both two-tailed $P > 0.492$; parthenogenetic: both $t_7 < -2.225$, both two-tailed $P > 0.061$).

Attractiveness of male and female pheromones

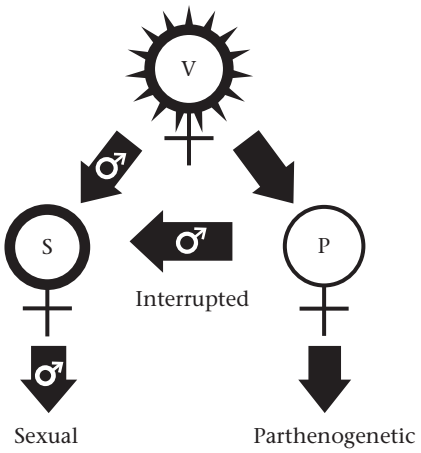
Analyses of dropping counts from pheromone assays identified significant leaf treatment effects (Table 1, Appendix Fig. A1). Males spent significantly more time on leaves that carried pheromonal scents of sexual females and prereproductive females than on control leaves that carried no scents, indicating that males were attracted to leaves that carried sexual and prereproductive female scents. However, males did not spend significantly more or less time on scented leaves that had been exposed to parthenogenetic females than on control leaves. Males were also unaffected by the scents of other males compared to controls (Table 1, Appendix Fig. A1).

Male pheromonal scents did not significantly attract or repel sexual females, but parthenogenetic females exhibited a near-significant preference for leaves bearing male scents. The odours of other females had no appreciable effect on the amount of time that sexual and parthenogenetic females spent on leaves. Prereproductive females spent significantly more time on control leaves than on leaves that carried male pheromonal odours, indicating that prereproductive females were repelled by male scents. Prereproductive females were unaffected by the pheromonal scents of other females (Table 1, Appendix Fig. A1).

Table 2
Results of likelihood ratio tests for the effect of signaller repugnatorial secretion on responder behaviour

Signaller	Responder	Chi-square	*df*	Estimate	*P*
F(PRV)	**Male**	**10.181**	**1**	**−1.876**	**0.001**
F(sex)	Male	0.752	1	0.488	0.386
F(parth)	Male	0.087	1	−0.166	0.768
Male	Male	1.204	1	0.615	0.273

For each signaller–responder combination, mixed models with/without leaf treatment were compared. The signaller–responder combination highlighted in bold indicates that the number of droppings beneath treatment and control leaves was significantly different, with negative fixed-effect estimates indicating that responders were repelled by signaller secretions. Female treatment groups are abbreviated as follows: F(PRV) = prereproductive virgin females; F(sex) = sexual females; F(parth) = parthenogenetic females.



Egg output	NS	Decreased	NS
Hatching success	NS	NS	NS
Nymph survival	NS	Increased	NS
Longevity	NS	Decreased	NS

Figure 6. Schematic diagram of the potential reproductive pathways of *E. tiaratum*. Bold circles indicate females that attract males with their scents. Spikes around circles indicate females that can repel males with repugnatorial secretions. Arrows with and without male symbols indicate reproduction via sex and parthenogenesis, respectively. The box summarizes treatment effects on estimated fitness components. Prereproductive virgins (V) that are inseminated become sexual reproducers (S) and continue to attract males (left branch). If prereproductive virgins avoid mating, they become parthenogenetic reproducers (P) and no longer produce male-attracting scents (right branch). But if females that have already started reproducing parthenogenetically subsequently mate with a male, they switch from parthenogenetic to sexual reproduction (horizontal branch), which results in reduced egg production and shortened life span, but higher nymph survival. ('NS' indicates that no effect of female reproductive pathway (sexual, parthenogenetic or interrupted) was detected).

Male response to repugnatorial secretions

Analyses of dropping counts from the repugnatorial secretion assay (Table 2, Appendix Fig. A2) showed that males were significantly repelled by leaves that carried repugnatorial secretions of prereproductive virgin females. However, the amount of time that males spent on leaves that carried sexual or parthenogenetic female repugnatorial secretions was not significantly different from the amount of time that they spent on control leaves. Similarly, males did not spend more or less time on treated leaves that carried repugnatorial secretions of other males than on control leaves that did not carry repugnatorial secretions (Table 2, Appendix Fig. A2).

DISCUSSION

Our findings are broadly consistent with the predictions of the sexual conflict hypothesis that facultative parthenogenesis may be maintained by sexual conflict rather than mate scarcity. We found that females that were forced to switch from parthenogenesis to sex (interrupted females) incurred costs of reduced egg output and increased mortality (see Fig. 6). However, eggs of interrupted females showed a trend towards higher early hatching success, and the offspring of interrupted females were more likely to survive to the second instar than offspring of parthenogenetic females, suggesting that indirect benefits might

compensate to some extent for the direct costs of reproductive switching. None the less, parthenogenetic females stopped signalling their reproductive status to males, and females forced to switch from parthenogenesis to sex exhibited exaggerated and sustained defensive behaviour (abdomen curling) in the presence of males. These results suggest that parthenogenetic females are selected to avoid males and, if they encounter males, to minimize mating rate.

Further evidence for the sexual conflict hypothesis was found in the behaviour of prereproductive females, which appeared to possess adaptations to reduce the likelihood of encountering males. Although males were strongly attracted to prereproductive female pheromones, prereproductive females were repelled by male odours and, in addition, produced a repugnatorial secretion that was capable of repelling males. Although many phasmatids are known to secrete toxic chemicals from prothoracic glands in order to ward off predator attacks (Eisner, 1965; Scudder, 1876), the effect of the prereproductive female repugnatorial secretion on males in this case cannot be explained simply as male avoidance of an alarm pheromone. Such an explanation is inconsistent with our results because males were not repelled by the repugnatorial secretions of other males or reproductive females. This suggests that the repugnatorial secretion produced by prereproductive virgins could function as an antiaphrodisiac, although prereproductive females were not assessed as to whether they produce their secretion during actual intersexual encounters. These results suggest that while males are selected to be eager to pursue mating with prereproductive females, such females may be selected to evade males.

Little evidence was found in support of the predictions of the mate scarcity hypothesis. This hypothesis predicts that females reared without males should be eager to mate when given the opportunity to do so, and should delay parthenogenetic oviposition to increase their chances of locating a mate (e.g. see Schneider & Elgar, 2010). Instead, we found that *E. tiaratum* females appeared to resist mating by curling their abdomens away from male clasping organs and kicking males with their legs, and mated females took significantly longer to start ovipositing than females reproducing parthenogenetically. In addition, parthenogenetic females did not engage in leg-kicking behaviours when paired with stock females, and exhibited less pronounced abdomen curling behaviour, supporting the interpretation of female abdomen curling and leg kicking as sexually antagonistic behaviours.

Evidence of antagonistic pheromone signalling in *E. tiaratum* also contradicted the prediction of the mate scarcity hypothesis that selection should favour females that attract mates. Males were significantly attracted to the pheromones of prereproductive virgins and sexual females, but not the pheromones of parthenogenetic females. This suggests that parthenogenetic females stop signalling their reproductive state. A similar result was obtained by Schneider and Elgar (2010) in their life history study of *E. tiaratum*. However, our study suggests that males also produce scents and that prereproductive virgins are repelled by these scents. Although these findings are consistent with sexual conflict, it is unclear why prereproductive females should attract males only to repel them. One explanation might be that prereproductive females produce a pheromone detectable by males not because selection favours the sexual reproductive mode in females, but as a pleiotropic side-effect of selection for secretion of high levels of fertility-promoting hormones (such as juvenile hormone; Barth, 1962; Wyatt & Davey, 1996). Alternatively, mating might be advantageous in certain circumstances, and so females might be selected to produce pheromones when the costs of mating are lowest (e.g. prior to the onset of parthenogenetic reproduction).

While prereproductive virgin females were repelled by male scents, and sexual females were indifferent to male scents, parthenogenetic females exhibited a near-significant attraction to male scents. If confirmed by further research, such a response by parthenogenetic females would be surprising, given that parthenogenetic females do not signal their reproductive status to males, and mating appears to be highly costly for such females. Further investigation is required to verify this response and to determine its consequences for the probability of switching from parthenogenetic to sexual reproduction.

While our findings suggest that mating is costly for females that have already commenced parthenogenetic reproduction, it is not clear whether selection on prereproductive females favours the sexual or parthenogenetic reproductive mode (Fig. 6). Although prereproductive females appear to possess a suite of adaptations that function to reduce the probability of mating, and sexually reproducing females resist male advances, we did not find evidence of a fitness cost of sex when mating commenced prior to the onset of parthenogenetic reproduction. Over the first 12 weeks of adult life, sexual females did not exhibit significantly reduced longevity, egg hatching or nymph survival to the second instar. Indeed, we observed a trend towards better offspring performance in sexual females than in parthenogenetic females, although egg-hatching results for parthenogenetic females may have been negatively biased by the long diapause of parthenogenetically produced eggs, some of which would have been incorrectly scored as unviable when the experiment was terminated (Hadlington, 1966). However, such a delay in egg hatching could potentially represent a cost of parthenogenetic reproduction. Further research is required to achieve a more comprehensive comparison of the fitness of sexual and parthenogenetic reproductive strategies. In particular, it is important to obtain estimates of fecundity throughout the entire female life span. For example, sexual reproduction could impose latent survival costs that only become apparent at older ages. It is also important to assess offspring viability and growth rate throughout nymphal development as well as the mean performance of adults produced by sexual and parthenogenetic reproductive modes. Assessment of adult males is essential in this regard. Because parthenogenetic females produce all-female broods whereas sexual females produce offspring of both sexes, the relative net fitness of the sexual strategy will depend in part on the contribution of sons to maternal fitness in natural populations.

One limitation of our study is that it is unclear whether the extent of the sexual conflict we observed reflects the level of antagonistic interactions that actually occur in the wild. Nevertheless, our results are consistent with the hypothesis that sexually antagonistic signaller–receiver coevolution may play a role in the evolution and maintenance of facultative parthenogenesis. Our results suggest that prereproductive virgin females can potentially avoid sex via secretion of antiaphrodisiacs and avoidance of male odours. Since females that have started reproducing parthenogenetically are no longer attractive to males, such females appear to have the opportunity to continue to reproduce exclusively via parthenogenesis. Thus, parthenogenesis may persist in natural populations of *E. tiaratum* as a consequence of sexual conflict mediated by antagonistic signalling and female resistance. Further studies are required to establish how effective female resistance behaviours are under more ecologically relevant conditions.

Although it has been estimated that less than 1% of higher eukaryotic species reproduce parthenogenetically (De Meeûs et al., 2007), the actual incidence of parthenogenesis is poorly known (D'Souza & Michiels, 2010). If sexual conflict can promote the evolution and maintenance of facultative parthenogenesis, then, given the ubiquity of sexual conflict (Arnqvist & Rowe, 2005), the capacity to reproduce parthenogenetically might actually be more common than currently thought. Parthenogenesis might be possible in a range of taxa, but only rarely observed because males typically force females to mate. This might explain, for example, why parthenogenesis occurs in many *Drosophila* species, but the incidence of parthenogenetic reproduction within most of these species is so low (Markow, 2013).

It is also possible that facultative parthenogenesis is rare because males tend to 'win' sexual conflicts more frequently than females, by achieving matings despite female resistance, as the modelling by Kawatsu (2013) suggests. A parthenogenetic strategy might only persist if it actually allows females to avoid the costs of sex. If harassment by males cannot be avoided, then females might gain very little from parthenogenesis and might actually incur greater costs if resistance results in increased harassment, or if it fails to prevent matings. Thus, sexually antagonistic coevolution might help to explain why obligate sex is so widespread compared to parthenogenesis. It has been suggested that sex is a kind of evolutionary trap because adaptations associated with sex and recombination greatly reduce the probability of successful parthenogenetic reproduction (Corley et al., 2001; Engelstaedter, 2008; Moore & Moore, 2003; Neiman, 2004). Male coercion may contribute to this trap: once sex evolves, it might continue to persist simply because coercive males prevent females from initiating or continuing parthenogenetic reproduction.

Acknowledgments

We thank the volunteers who helped sort eggs, clean cages and replace leaves: Belden Lai, Tsai-Ming Lu, Ronda Lau, Erin Macartney and Lára Hallsson. Special thanks also to David Sindel for providing the illustration of the copulating stick insects. Funding was provided by the Australian Research Council through a Future Fellowship to R.B.

Supplementary Material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anbehav.2014.12.017.

References

Archetti, M. (2010). Complementation, genetic conflict, and the evolution of sex and recombination. *Journal of Heredity, 101*(Suppl. 1), S21–S33.
Arnqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton, NJ: Princeton University Press.
Avise, J. C., Quattro, J. M., & Vrijenhoek, R. C. (1992). Molecular clones within organismal clones: mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. *Evolutionary Biology, 26*, 225–246.
Barth, R. H., Jr. (1962). The endocrine control of mating behavior in the cockroach *Byrsotria fumigata* (Guérin). *General and Comparative Endocrinology, 2*, 53–69.
Brock, P. D. (2001). Studies on the Australasian stick-insect genus *Extatosoma* Gray (Phasmida: Phasmatidae: Tropoderinae: Extatosomatini). *Journal of Orthoptera Research, 10*, 303–313.
Butlin, R. (2002). The costs and benefits of sex: new insights from old asexual lineages. *Nature Reviews Genetics, 3*, 311–317.
Carlberg, U. (1985). Chemical defence in *Extatosoma tiaratum* (MacLeay) (Insecta: Phasmida). *Zoologischer Anzeiger, 214*, 185–192.
Carlberg, U. (1987). Chemical defence in Phasmida vs Mantodea (Insecta). *Zoologischer Anzeiger, 218*, 369–373.
Carlberg, U. (1988). Food consumption in *Extatosoma tiaratum* (MacLeay) (Insecta: Phasmida). *Zoologischer Anzeiger, 220*, 195–202.
Clark, J. T. (1974). A conspicuous spermatophore in the phasmid *Extatosoma tiaratum*. *Entomologist's Monthly Magazine, 110*, 81–82.
Connallon, T., Cox, R. M., & Calsbeek, R. (2009). Fitness consequences of sex-specific selection. *Evolution, 64*, 1671–1682.
Corley, L., Blankenship, J., & Moore, A. (2001). Genetic variation and asexual reproduction in the facultatively parthenogenetic cockroach *Nauphoeta cinerea*: implications for the evolution of sex. *Journal of Evolutionary Biology, 14*, 68–74.
Crawley, M. J. (2013). *The R book* (2nd ed.). Chichester, U.K.: John Wiley.
D'Souza, T. G., & Michiels, N. K. (2010). The costs and benefits of occasional sex: theoretical predictions and a case study. *Journal of Heredity, 101*(Suppl. 1), S34–S41.
De Meeûs, T., Prugnolle, F., & Agnew, P. (2007). Asexual reproduction: genetics and evolutionary aspects. *Cellular and Molecular Life Sciences, 64*, 1355–1372.
Eisner, T. (1965). Defensive spray of a phasmid insect. *Science, 148*, 966–968.
Engelstaedter, J. (2008). Constraints on the evolution of asexual reproduction. *BioEssays, 30*, 1138–1150.
Green, R. F., & Noakes, D. L. (1995). Is a little bit of sex as good as a lot? *Journal of Theoretical Biology, 174*, 87–96.
Hadany, L., & Beker, T. (2007). Sexual selection and the evolution of obligatory sex. *BMC Evolutionary Biology, 7*, 245.
Hadlington, P. (1966). Parthenogenesis and diapause in the eggs of the phasmatid *Extatosoma tiaratum* (MacLeay). *The Journal of the Entomological Society of Australia (N.S.W.), 3*, 59–65.
Jokela, J., Lively, C. M., Dybdahl, M. F., & Fox, J. A. (1997). Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. *Ecology, 78*, 452–460.
Kawatsu, K. (2013). Sexual conflict over the maintenance of sex: effects of sexually antagonistic coevolution for reproductive isolation of parthenogenesis. *PLoS One, 8*, e58141.
Kramer, M. G., & Templeton, A. R. (2001). Life-history changes that accompany the transition from sexual to parthenogenetic reproduction in *Drosophila mercatorum*. *Evolution, 55*, 748–761.
Lehtonen, J., Jennions, M. D., & Kokko, H. (2012). The many costs of sex. *Trends in Ecology & Evolution, 27*, 172–178.
Markow, T. A. (2013). Parents without partners: *Drosophila* as a model for understanding the mechanisms and evolution of parthenogenesis. *G3: Genes, Genomes, Genetics, 3*, 757–762.
Maynard Smith, J. (1978). *The evolution of sex*. Cambridge, U.K.: Cambridge University Press.
Moore, P. J., & Moore, A. J. (2003). Developmental flexibility and the effect of social environment on fertility and fecundity in parthenogenetic reproduction. *Evolution and Development, 5*, 163–168.
Neiman, M. (2004). Physiological dependence on copulation in parthenogenetic females can reduce the cost of sex. *Animal Behaviour, 67*, 811–822.
Normark, B. B. (2003). The evolution of alternative genetic systems in insects. *Annual Review of Entomology, 48*, 397–423.
R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.
Richards, A. (2003). Apomixis in flowering plants: an overview. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 358*, 1085–1093.
Rowe, L., Arnqvist, G., Sih, A., & Krupa, J. (1994). Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology & Evolution, 9*, 289–293.
Schneider, A., & Elgar, M. A. (2010). Facultative sex and reproductive strategies in response to male availability in the spiny stick insect, *Extatosoma tiaratum*. *Australian Journal of Zoology, 58*, 228–233.
Schwander, T., Vuilleumier, S., Dubman, J., & Crespi, B. J. (2010). Positive feedback in the transition from sexual reproduction to parthenogenesis. *Proceedings of the Royal Society of London B: Biological Sciences, 277*, 1435–1442.
Scudder, S. H. (1876). Odoriferous glands in Phasmidae. *Psyche: A Journal of Entomology, 1*, 137–140.
Stalker, H. D. (1956). On the evolution of parthenogenesis in Lonchoptera (Diptera). *Evolution, 10*, 345–359.
StatSoft, Inc.. (2005). *STATISTICA data analysis software system, version 7.1*. www.statsoft.com.
Strong, L. (1975). Defence glands in the giant spiny phasmid *Extatosoma tiaratum*. *Journal of Entomology Series A, General Entomology, 50*, 65–72.
Wyatt, G. R., & Davey, K. G. (1996). Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. *Advances in Insect Physiology, 26*, 1–155.
Yamauchi, A., & Kamite, Y. (2003). Facultative sexual reproduction under frequency-dependent selection on a single locus. *Journal of Theoretical Biology, 221*, 411–424.

APPENDIX

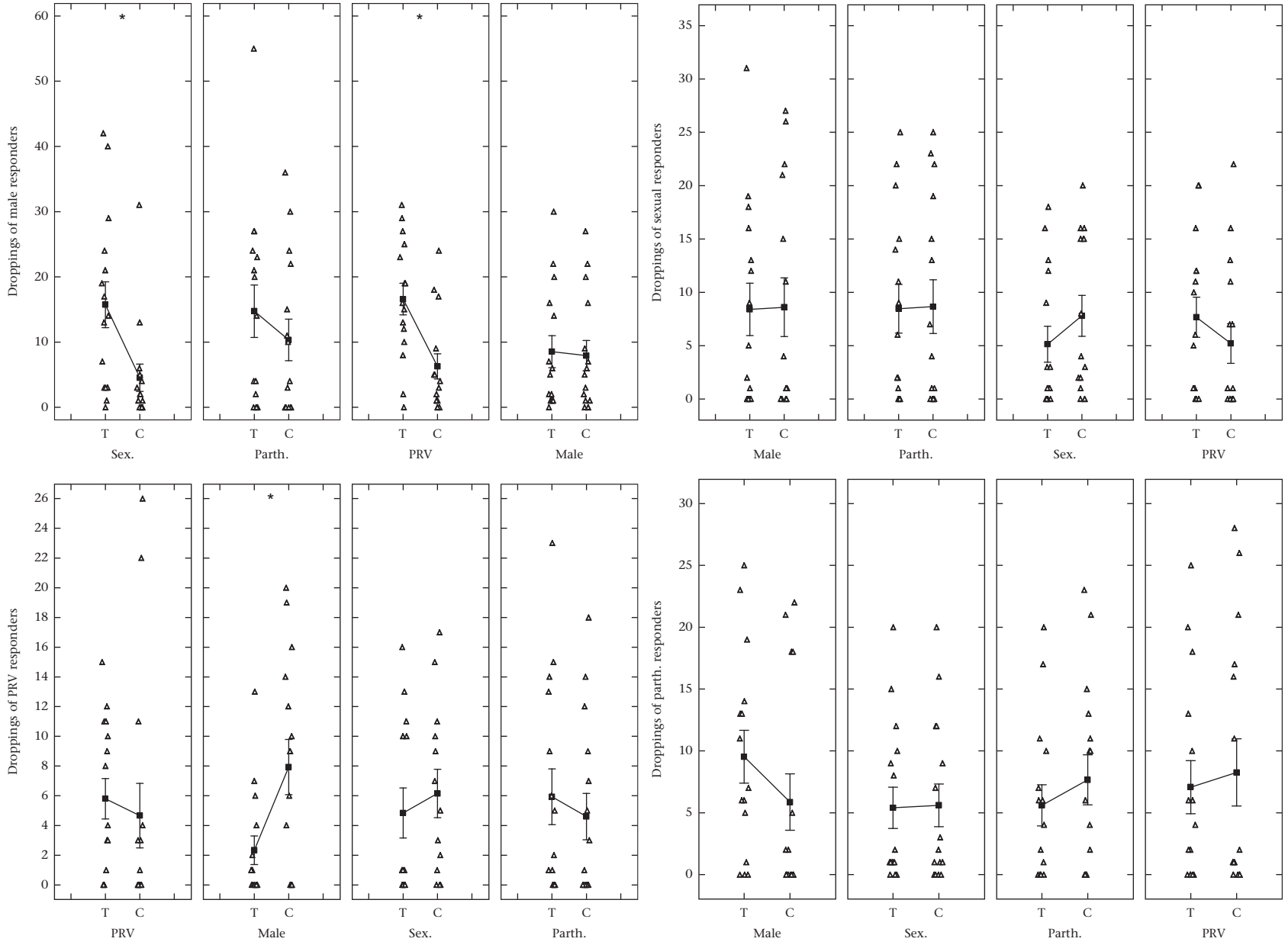


Figure A1. Mean dropping counts ± SE of males, sexual females, parthenogenetic females and prereproductive virgin females in response to signaller pheromones. Treated leaves (T) carried signaller odours; control leaves (C) carried no odours. Asterisks denote significant differences at $P < 0.05$ between treatment and control dropping counts.

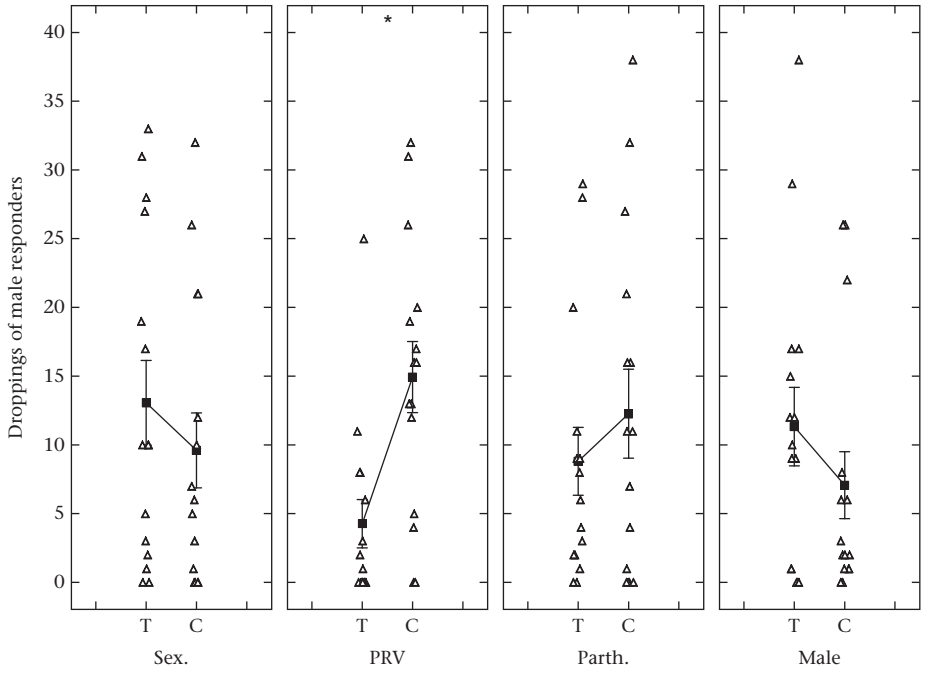


Figure A2. Mean dropping counts ± SE of males in response to the repugnatorial secretions of signallers. Treated leaves (T) carried signaller secretions; control leaves (C) carried no secretions. Asterisk denotes significant difference at $P < 0.05$ between treatment and control dropping counts.