On the Repeated Evolution of Parthenogenesis in Stick Insects

Tanja Schwander[1], Luca Soldini[1], Romain P. Boisseau[1], Vincent Mérel[1], William Toubiana[1], Guillaume Lavanchy[2]

[1]Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland

[2]Biodiversity and Evolution Unit, Biology Department, Lund University. Ekologihuset, Sölvegatan 37, 223 62 Lund, Sweden

Corresponding authors: tanja.schwander@unil.ch; guillaume.lavanchy@gmail.com

Abstract

A striking aspect of the biology of stick insects is the widespread occurrence of parthenogenesis, including rare, spontaneous events in sexual species, facultative parthenogenesis as well as obligately parthenogenetic species. This review synthesizes current knowledge on the origins, mechanisms, and evolutionary consequences of parthenogenesis in stick insects, with a particular focus on its hybrid and intra-specific origins. Hybrid-derived parthenogens are relatively rare but invariably obligate. They are often polyploid and produce eggs via endoduplication, a parthenogenesis mechanism maintaining genome-wide heterozygosity. Intra-specific cases of parthenogenesis (facultative and obligate) are much more frequent, are associated with diploidy, and frequently involve genome-wide homozygosity as a consequence of gamete duplication.

Facultative parthenogenesis allows females to reproduce both sexually and parthenogenetically, yet natural populations tend to either comprise equal numbers of males and females or consist entirely of females, with intermediate sex ratios being rare. In multiple species, mixed-sex and female-only populations are distributed in a mosaic pattern without clear ecological differences, suggesting that factors beyond environment shape their maintenance. Sexual conflict, including male harassment and female resistance, has been proposed as a potential driver of these patterns, but empirical evidence does not point to male-inflicted harm on females and instead indicates fitness benefits associated with mating and sexual reproduction.

Several negative evolutionary consequences of parthenogenesis, including reduced selection efficiency and slower adaptation, have been identified in the *Timema* genus. Multiple, mostly obligately parthenogenetic *Timema* species have independently evolved from different sexual ancestors, with a shared mechanism of gamete duplication. Because rare, spontaneous parthenogenesis in sexual species involves the same mechanism, the repeated evolution of parthenogenetic species likely occurred via recurrent selection for increased frequencies of parthenogenesis in different genomic backgrounds.

Overall, this review highlights the diversity and distribution of parthenogenesis in stick insects and their broader implications for understanding the evolution of asexual reproduction.

Introduction

Phasmids, commonly known as stick and leaf insects, are renowned for their exceptional camouflage, serving as an effective defense against visually hunting predators. This order of polyneopteran insects comprises over 3,500 described species distributed worldwide [1,2]. Due to their extraordinary morphological and behavioral crypsis, phasmids attract considerable interest from both researchers and insect breeders globally [2].

In addition to their cryptic adaptations, phasmids are also remarkable for their ability to reproduce via parthenogenesis (**Figure 1**). Many breeders have observed the spontaneous re-establishment of female-only cultures from a single nymph after the loss of a population. Thus, parthenogenesis in phasmids is almost always thelytokous, meaning it exclusively produces female progeny [3]. The presence of parthenogenesis is not merely anecdotal as extensive documentation exists for over 80 different species spanning the stick insect phylogeny, encompassing both facultative and obligate parthenogenetic lineages. This widespread occurrence of parthenogenesis makes phasmids a useful empirical model for investigating the factors that favor sexual versus asexual reproduction in natural populations [4–9] as well as identifying morphological, behavioral and life-history differences associated with different reproductive modes [6,10–13].

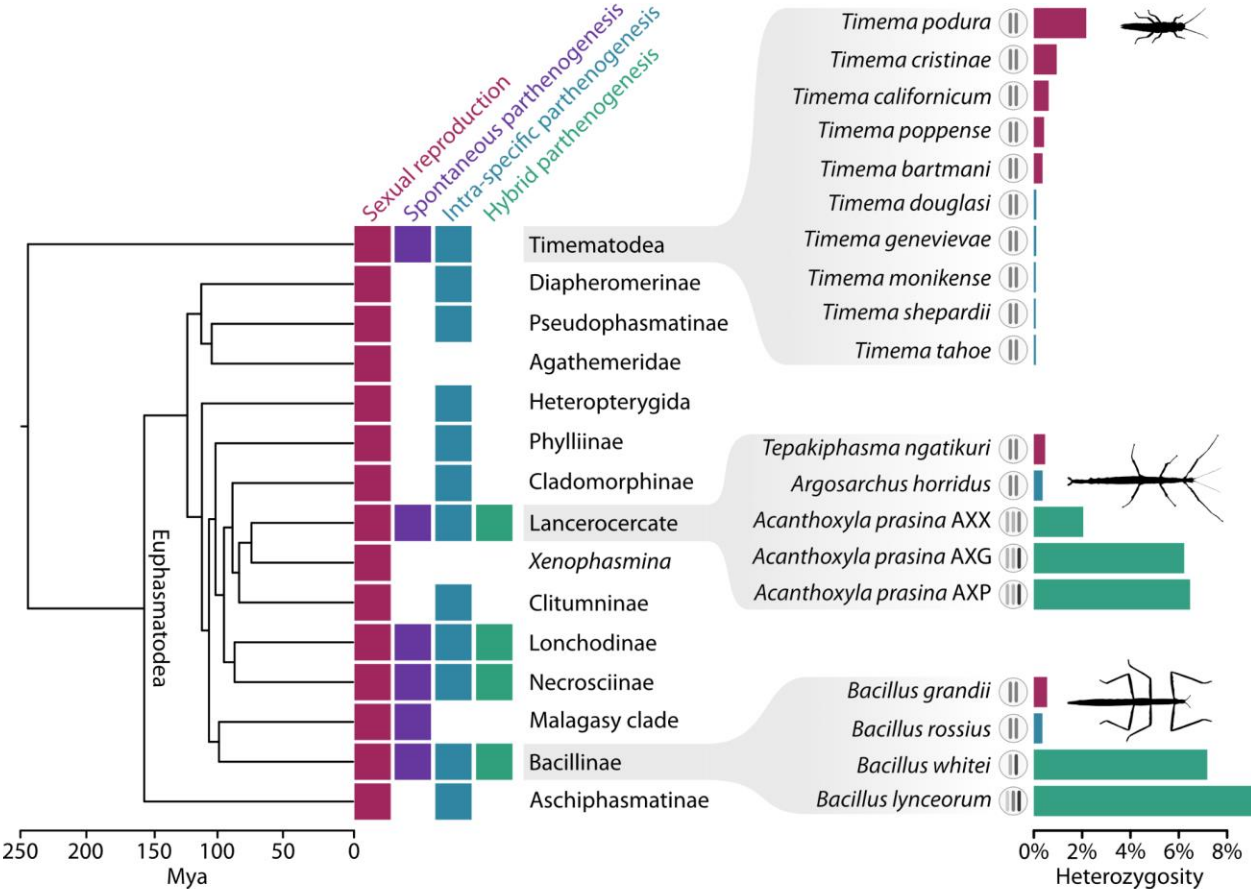

Figure 1: Repeated evolution of parthenogenesis across the stick and leaf insect phylogeny. From left to right: Tree topology and taxon names from [14]. Spontaneous parthenogenesis refers to rare hatching of unfertilized eggs in sexual species. Intra-specific origin refers to cases in which female-only populations can be maintained, either in natural populations or in culture, and mostly represent facultative parthenogens. Hybrid origin refers to cases in which hybridization led to obligate parthenogenetic species. The age of the group and clades within remain controversial, with a much more recent origin of Phasmatodea, dated to the Jurassic ~180 million years ago, in previous studies [15,16]. Right: Genome-wide heterozygosity for different parthenogenetic stick insect species, with related sexual species indicated for comparison. The ploidy for each species is indicated by the number of chromosomes (bars) in circles, chromosomes with different shading originate from different species. The indicated heterozygosity is estimated from sequencing reads using k-mer spectra analysis [17,18] and corresponds to the proportion of homologous 21-mers with more than one variant. Data combined from [9,19,20]. Stick insect silhouettes from https://www.phylopic.org/.

Hybrid vs. Intra-specific Origins of Parthenogenesis

The evolution of parthenogenesis in phasmids is particularly intriguing because it arises through multiple, mechanistically distinct pathways involving both hybrid and intra-specific origins. All parthenogenetic phasmids of hybrid origin studied thus far are obligately parthenogenetic. Furthermore, most if not all of them reproduce via premeiotic endoduplication as documented in the two triploid hybrids *Carausius morosus* [21] and *Bacillus lynceorum* [22,23], and the tetraploid hybrid *Sipyloidea sipylus* [24]. The same mechanism likely occurs in presumed triploid hybrids between the sexual species *S. nelida* and *S. similis* [25,26]. Under premeiotic endoduplication, chromosomes duplicate during meiosis, and recombination occurs between duplicates rather than homologs [27]. This process maintains high heterozygosity over evolutionary time and is widespread among hybrid parthenogens beyond stick insects [28,29] (**Figure 1**). However alternative mechanisms underlying parthenogenesis cannot be excluded in some species such as the diploid hybrid *B. whitei* [22,30] (see also **Figure 1**) and exist in phasmid species with suggested but unconfirmed hybrid origins such as *B. atticu*s. Parthenogenesis in the latter species involves an abortive meiosis I, but there is currently no data available to test for a hybrid or intra-specific origin of this species [31–33]. *B. atticu*s is further interesting because it comprises diploid and triploid parthenogenetic lineages [31–34]. Females from a subset of the diploid lineages retain the ability to produce offspring from rare, fertilized eggs in experimental crosses with males from sexual relatives [32]. Surprisingly, some of these sexually produced offspring are diploid (and not always triploid as would be expected under fertilization of parthenogenetic eggs). This implies that fertilization occasionally triggers the production of a haploid maternal nucleus which then fuses with a sperm nucleus [31–33].

Although hybrid-origin parthenogenesis is relatively rare among phasmids —and in animals more broadly [35,36] —it has traditionally been studied in greater detail, particularly due to its frequent association with polyploidy and distinctive chromosome morphologies observed in karyotypes [27,37]. Curiously, in groups where it occurs, multiple parthenogenetic hybrid species deriving from the same sexual ancestors are known. This is notably the case for the genera *Bacillus* (hybrid parthenogens *B. whitei* and *B. lynceorum* [4]), *Acanthoxyla* (at least three parthenogenetic hybrid lineages comprised in the species *A. inermis* and *A. prasina* [19]) and likely in the genera *Leptynia* and *Pijnackeria* [38–40]. However, attempts to re-create parthenogenetic lineages *de novo* via crosses between sexual species in these groups have thus far been unsuccessful [41]. This contrasts with findings in vertebrates, where most parthenogenetic species arose through hybridization and new parthenogenetic hybrid lines can be generated through experimental crosses in different taxa [42,43].

In phasmids, parthenogenetic populations and species are mostly of intra-specific origins, and emerge either from facultatively parthenogenetic or predominantly sexual populations. In the former case, it happens simply through local extinction of males. Such transitions likely started in specific populations, a pattern still observable today in facultatively parthenogenetic species like *Megacrania batesii*, *Clitarchus hookeri*, and *B. rossius* (described in the next section). Alternatively, parthenogenesis can emerge through the co-option of rare spontaneous parthenogenesis in predominantly sexual species, as demonstrated in *Timema* stick insects (see below). In both intra-specific origins, a frequently observed mechanism underlying parthenogenesis is referred to as “gamete duplication” [27]. This process begins with a typical meiosis, producing an egg that contains a haploid maternal nucleus. During early embryogenesis, this haploid nucleus undergoes division, generating a population of haploid nuclei within a syncytium, a shared cytoplasmic environment characteristic of early development. While normal diploid embryos inherit two parental chromosome sets, parthenogenetic embryos restore diploidy through an unknown mechanism—potentially involving endoreplication or nuclear fusion within the syncytium. As a result, the developing stick insect is diploid but fully homozygous. This distinctive developmental pathway has been directly documented or strongly suggested in species from at least six genera (**Figure 1**; *B. rossius* [44], *Medauroidea extradentata,* formerly known as *Clitumnus extradentatus* [37,45], *Menexenus* [46], *Phraortes elongatus* [47], *M. batesii* [48]*,* and *Timema* (see **Figure 2**)). Nevertheless, alternative mechanisms of parthenogenesis, associated heterozygosity maintenance, also exist, and are documented in the species *B. atticus* [31,32]*, Ramulus mikado* [49], as well as in *M. batesii*, where they co-occur with gamete duplication [48].

In combination, these different studies show that in phasmids, intraspecific origins of parthenogenesis, both facultative and obligate, occur only in diploid species, and often rely on gamete duplication, which generates complete homozygosity. By contrast, hybrid parthenogenesis is obligate, typically involves endoduplication, is associated with high heterozygosity that can be maintained genome-wide and is mostly documented in polyploid species.

A mosaic of female-only and sexual populations in facultative parthenogens

Facultative parthenogenesis corresponds to cases in which a single female is able to reproduce both sexually and asexually [50]. It is extremely widespread among phasmids, as for example revealed by many female-only stock cultures of otherwise sexually reproducing species [2]. It has further been characterized via experimental approaches in at least ten different genera across the phylogeny (*Argosarchus, Bacillus, Clitarchus, Extatosoma, Medauroidea, Megacrania,* *Megaphasma, Phraortes, Sipyloidea, Timema;* **Figure 1**). Because parthenogenesis produces females, populations of facultatively parthenogenetic species may exhibit a female-biased sex ratio, ranging from a slight bias to entirely female populations. However, natural populations typically do not show this full range; they either have equal numbers of males and females (hereafter "sexual populations") or consist exclusively of females ("parthenogenetic populations").

In some instances, the distribution of sexual and parthenogenetic populations follows a latitudinal or altitudinal gradient. Such distributions can reflect the broader benefits of parthenogenesis and other forms of unisexual reproduction for the colonization of new habitats [51]. In these environments, the growth of sexual populations is typically limited by low densities as a consequence because of mate limitation ("inverse Allee effects" [52]). Examples in stick insects include post-glaciation recolonization led by parthenogens in *Argosarchus* [53] and *Clitarchus hookeri* [54,55], or the observation that island and introduced populations are typically parthenogenetic, but often derive from native sexual populations [2,55,56]. In other instances, latitudinal or altitudinal variations point to potential ecological gradients shifting the relative benefits of sexual *vs* parthenogenetic reproduction (e.g., in *P. elongatus* [47]). However, in several species, parthenogenetic populations are distributed in a mosaic-fashion among sexual populations, without any evident variation in ecology, and reflect multiple independent local losses of males (e.g., *M. batesii* [57], *B. rossius* [58,59], *C. hookeri* [55]). The factors underlying these repeated losses as well as those maintaining a mosaic distribution of sexual and parthenogenetic populations are currently unclear.

It has been suggested that sexual conflict over the number of matings may promote either sexual or parthenogenetic reproduction depending on how fitness returns per mating differ between males and females [60–64]. Male-imposed costs on females indeed increase with sexual conflict levels [65,66]: for example, male coercion makes it more costly for females to avoid mating. However, it is only under extreme costs of mating for females that sexual conflict can sustain the co-existence of sexual and parthenogenetic populations [62,67]. Experiments aiming at identifying costs of mating in stick insects have thus far only revealed small, if any, costs of mating for females. Specifically, in an obligately sexual *Timema* species, singly and multiply mated females have similar fecundity and lifespan [68]. In facultative parthenogens, virgin and mated females also have similar fecundity (in *C. hookeri* [5] and *M. batesii* [57]) or even lifetime reproductive success (in *Extatosoma tiaratum* [69,70] and *S. larryi* [71]). Furthermore, mating in facultative parthenogens (or multiple mating in sexual species) increases the reproductive output of females (more eggs, higher egg hatching success and/or survival in *Megaphasma dentricus* [72]*, E. tiaratum* [69] but see [70], *M. batesii* [7], *Timema cristinae* [68], and *S. larryi* [71]). Finally, additional increases of reproductive outputs under sex relative to parthenogenesis are likely caused by inbreeding depression, given the large-scale or complete homozygosity of parthenogenetic offspring. Thus, while sexual conflict provides an interesting idea for explaining recurrent transitions to all-female populations in facultative parthenogens [60–64], there is little empirical support for such mechanisms in stick insects, where the lifetime reproductive output of females is generally improved by (multiple) mating. Consequently, the factors driving the mosaic-like distribution of parthenogenetic and sexual populations remain elusive.

A possible mechanism driving the described patterns is a trade-off between sexual vs parthenogenetic reproduction. Such a trade-off was previously suggested as an explanation for why mostly obligate parthenogenesis and sex prevail over facultative parthenogenesis in animals [36,73]. Females from parthenogenetic populations in at least three facultatively parthenogenetic stick insect species only produce few if any fertilized eggs after mating (*B. rossius* [74,75], *C. hookeri* [5,55], and *M. batesii* [7]). This suggests that they lost the ability to fertilize eggs. Such losses (or partial losses) could help explain why reversals from parthenogenesis to sex in all-female populations are not more frequent, even though they could easily be mediated by immigrant males from neighbouring sexual populations. Indeed, only two such cases have been documented (in *M. batesii,* [57] and *C. hookeri* [55]) with two additional ones likely stemming from males produced locally via parthenogenesis (*C. hookeri* [55] and *B. rossius*, see below). Given that these parthenogenetic populations only recently derived from sexual ones [55], losses of egg fertilization capacity (and other sexual traits) are unlikely to result from relaxed selection alone [11]. Instead, they could be linked to the evolution of efficient parthenogenesis, where parthenogenetic egg development proceeds in a way that directly prevents fertilization or reduces egg viability if fertilization occurs [11,76]. Such a trade-off could explain why intermediate sex ratios are rare and why facultatively parthenogenetic stick insects tend to have predominantly obligate parthenogenetic populations. The widespread persistence of facultative parthenogenesis in sexual stick insect populations, despite such putative trade-offs (**Figure 1**), then implies that conditions favoring parthenogenesis occur frequently enough to outweigh fertility costs under sexual reproduction. Metapopulation dynamics with frequent local extinctions or sudden extreme reductions of local population densities could provide such conditions. Finally, it is interesting to note that maternal effects could mediate trade-offs between sexual reproduction and parthenogenesis. In fact, females produced via parthenogenesis in *M. batesii* [7] and *E. tiaratum* [6] tend to fertilize fewer eggs than those produced sexually. Assessing whether the efficiency of parthenogenesis is indeed traded off against successful sexual reproduction would be an interesting focus for future research.

Recurrent Evolution of Parthenogenesis from sexual ancestors in *Timema* Stick Insects

Timema stick insects are a model system for studying the predicted consequences of parthenogenesis in natural populations. These consequences include reduced efficacy of selection, leading to an increased accumulation of deleterious mutations [77,78] and a slower rate of adaptive evolution [9]. *Timema* is one of the few taxa where multiple parthenogenetic species are described, providing key replication opportunities to distinguish the effects of reproductive mode from species-specific idiosyncrasies.

Specifically, five morphologically and ecologically distinct, female-only parthenogenetic species are taxonomically described, each with sexually reproducing relatives of similar morphology and ecology [79–81]. Phylogenetic analyses based on mitochondrial sequences indicate that these parthenogenetic species originate from different sexual ancestors [82–84], a finding corroborated by different whole-genome studies [9,78]. Parthenogenesis in these species was initially thought to be obligate, as mating with males from sexual species did not result in fertilized eggs [11].

However, signatures of occasional recombination later revealed rare instances of sexual reproduction in some populations of two species [85]. Additionally, one parthenogenetic species was found to comprise multiple lineages that had independently evolved from the same sexual ancestor [84]. The frequent evolution of parthenogenesis in a single taxon such as *Timema*, which is only ~30 million years old [86], raises fundamental questions about how and why (mostly) obligate parthenogenesis evolves recurrently in this group.

Unlike many stick insects, which often exhibit facultative parthenogenesis, sexual *Timema* species are largely incapable of reproducing asexually. Experiments testing many virgin females from different sexual species show that they either fail to produce parthenogenetic offspring or do so at very low frequencies [87,88] —comparable to the spontaneous parthenogenesis occasionally observed in sexual species from other invertebrate taxa [89]. Facultative parthenogenesis is only known in localized populations of the otherwise obligate parthenogenetic species *T. douglasi* [73]. Some females in these populations are sexually produced (reflected by elevated heterozygosity), others parthenogenetically (homozygosity) (**Figure 2D**). This differs from the patterns discussed for the facultative species above where individuals from mixed sex populations are almost exclusively produced sexually. This notably led to the suggestion that these populations may derive from ancestrally parthenogenetic populations [73], with reversals to sex mediated by rare or immigrant males as described for *C. hookeri* [55]. However, they may also represent vestiges of formerly sexual reproduction in *T. douglasi*. Both scenarios point to facultative parthenogenesis being a transient state towards obligate strategies in *Timema* [73]. Independently of the status of these exceptional populations, even a low baseline frequency of spontaneous parthenogenesis in sexual species could, in theory, be co-opted into facultative or obligate parthenogenesis under favorable ecological conditions [90].

One such condition is mate limitation, particularly at range margins or in low-density populations, where even inefficient parthenogenesis can be favored by selection. Consistent with this hypothesis, the ability of sexual *Timema* females to produce parthenogenetic offspring is higher in low-density populations, where many adult females collected in the field are unmated [87]. This finding suggests that the recurrent evolution of obligate parthenogenesis in *Timema* may have been driven by selection for increased parthenogenetic capacity in different sexual populations.

Initially, this scenario was dismissed based on the presumed mechanisms underlying parthenogenesis in obligate parthenogens, which appeared to differ fundamentally from those associated with spontaneous parthenogenesis in sexual species [88]. Microsatellite-genotype data suggested that parthenogenetic species maintained conserved heterozygosity and reproduced clonally via apomixis, whereas spontaneous parthenogenesis in sexual species was linked to large-scale heterozygosity loss [88]. However, whole-genome analyses later revealed that the apparent heterozygosity in parthenogenetic species was an artifact caused by paralogous loci [9]. In reality, all parthenogenetic *Timema* species exhibit genome-wide homozygosity. Moreover, genome-wide analyses of heterozygosity loss in parthenogenetic offspring produced by heterozygous females of facultatively parthenogenetic *T. douglasi* [73] point to gamete duplication as the underlying mechanism—identical to the mechanism driving parthenogenesis in obligate *Timema* parthenogens (**Figure 2**) and documented in other phasmids (see main text).

In summary, obligate parthenogenesis in *Timema* evolved repeatedly from different sexual ancestors, with a conserved proximate mechanism (gamete duplication). However, an open question remains: which genetic loci increase the capacity for parthenogenesis in different *Timema* lineages? One possibility is the recurrent selection of ancestral polymorphisms in different genomic backgrounds. Such polymorphisms could involve loci such as those enhancing the likelihood of the spontaneous development of unfertilized eggs in *Drosophila* fruit flies as revealed in recent experimental work [91]. Whether similar loci contribute to gamete-duplication parthenogenesis in *Timema* and other phasmids can now be investigated, thanks to the recent development of CRISPR technology for this insect order [92]. Another possibility is the introgression of "parthenogenesis alleles" from facultatively parthenogenetic strains into largely sexual populations via crosses with males produced by parthenogenetic females—a phenomenon known as "contagious parthenogenesis” [93]. Notably, both introgression and recurrent selection of ancestral polymorphisms have been implicated in classic cases of convergent evolution, such as freshwater adaptations in threespine sticklebacks [94] and mimicry in *Heliconius* butterflies [95].

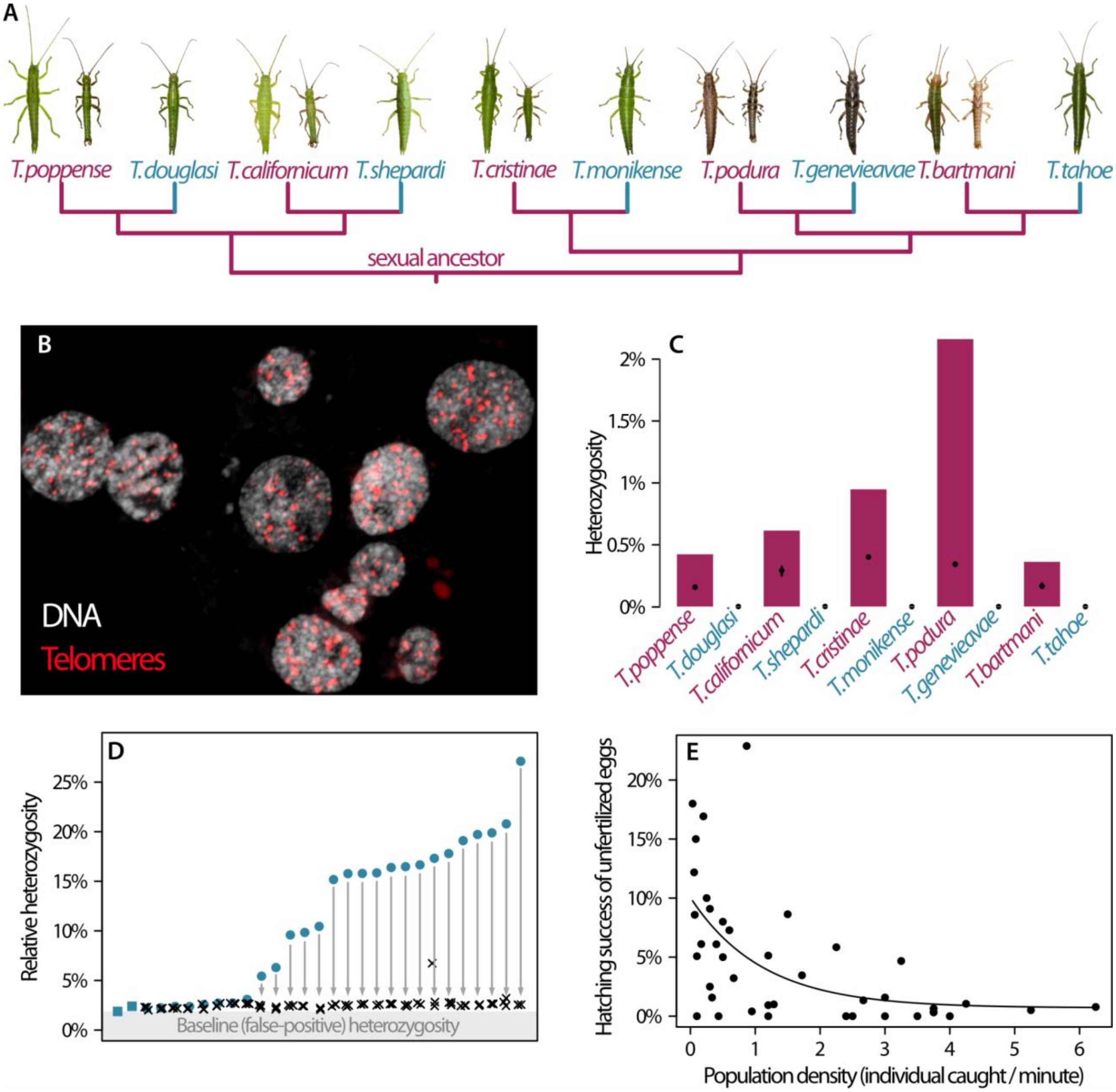

Figure 2: Recurrent evolution of parthenogenesis in *Timema*. **A** Phylogeny of parthenogenetic *Timema* species (in blue) and their sister sexual species (red). Other sexual species are omitted for display. **B** Diploidization in action: stacked images of an unfertilised egg of *T. douglasi* (8 to 12 days post laying). DNA is stained with DAPI (white), telomeres with a specific FISH probe (red; against the AACCT motif as described in [96]). Some nuclei are still haploid (n = 12; 24 telomeres) while others have undergone diploidization (2n = 24; 48 telomeres). **C** Heterozygosity variation among sexual *Timema* species and genome-wide homozygosity in obligate parthenogens. Barplots are k-mer based estimations of heterozygosity, black circles and lines represent SNP-based estimations of heterozygosity and range in five individuals (redrawn from [9]). **D** Heterozygosity reduction within a single generation in facultatively parthenogenetic populations of *T. douglasi*. Relative heterozygosity refers to the proportion of heterozygous SNPs among polymorphic RAD-seq loci, which includes false-positive heterozygosity (e.g. as a consequence

of mis-mapping and structural variation [73]). Colored dots represent values for a female, black crosses on the same column are values for her parthenogenetically-produced daughters. Heterozygosity falls to a baseline (i.e. false-positive) value in one generation, regardless of maternal heterozygosity. The two squares on the left are heterozygosity values obtained with the same data and method for two obligately parthenogenetic females also included in **C** (data from [73]). **E** Mate limitation under low population density can select for increased parthenogenesis capacity in sexual populations. Each point is a different sampled population of sexual *Timema* species (data from [87]).

Spontaneous production of rare males by parthenogenesis

Another shared feature across stick insects is that parthenogenetic reproduction can generate males. This has been reported for cases of spontaneous, facultative, and obligate parthenogenesis, whereby the frequency of males is typically in the order of 1 every 1000 or rarely up to every 100 females [11,21,57,97–103]. All stick insects investigated thus far have XX/XO or, more rarely, XX/XY sex chromosome systems [104]. The karyotypes of parthenogenetically produced males, where studied, are always XO as under sexual reproduction, implying rare losses of one of the X chromosomes during meiosis or early mitotic divisions. In the species *Anchiale austrotessulata*, such losses are much more frequent and result in approximately 16% males among the parthenogenetically produced progeny [105]. Whether these frequent losses of the X involve a targeted elimination would be an interesting avenue for future research. It could perhaps reveal convergent mechanisms with the targeted X chromosome elimination in aphids. In the latter species, the production of males always involves a targeted elimination of the X chromosome [106,107], which marks the transition to the sexual generation.

The rare spontaneous loss of the X in some parthenogenetic individuals typically results in fertile males. This is the case in *Bacillus* [108], *Clitarchus* [55] and *Timema* [11], though not in *Ramulus mikad*o [101] or *Clonopsis gallica* [97]. When spontaneously produced males are fertile, they could potentially drive reversals from parthenogenesis to sexuality in female-only populations of facultative parthenogens [55]. This has notably been suggested for a population of *C. hookeri*. A recently arisen sexual population has been identified within the natural range of the lineage otherwise associated with parthenogenesis in this species and microsatellite genotypes could not identify traces of admixture mediated by immigrant males from sexual populations. Males therefore most likely resulted from the spontaneous loss of an X chromosome, producing a male phenotype *in situ* [55]. A similar reversal to sex mediated by a parthenogenetically produced male is also the most likely explanation for the presence of an isolated (though not genetically characterized) sexual population of *B. rossius* in Southern France [109], where otherwise only parthenogenetic populations are known.

Box1: Open questions.

- Which cell-biological or developmental traits enable parthenogenesis to evolve so frequently among phasmids?
- Are there any life-history traits in phasmids, specific or not to this group, that make parthenogenesis particularly beneficial?
- What factors drive the mosaic-like distribution of parthenogenetic and sexual populations in facultative parthenogens?
- Why do some facultative parthenogens rapidly lose the ability to fertilize eggs in all-female populations, and is this linked to maternal effects?
- What role do ecological gradients (e.g., altitude, latitude) play in shaping the distribution of sexual and parthenogenetic populations?
- To what extent does inbreeding depression influence the relative fitness of parthenogenetic vs. sexual reproduction?
- What are the precise cellular and molecular mechanisms underlying different forms of parthenogenesis in phasmids? Are there additional, yet undiscovered, mechanisms contributing to parthenogenesis in phasmids?
- What are the genetic bases of parthenogenesis, and to what degree (if any) are they shared across phasmids and beyond?
- Why do hybrid-origin parthenogenetic phasmids tend to be obligate, whereas intra-specific parthenogens often retain some sexual reproduction?
- What factors determine whether hybridization leads to parthenogenesis or sterility?

Conclusion

In combination, studies in phasmids suggest that sexual species possess an ancestral ability to produce parthenogenetic eggs spontaneously via gamete duplication and perhaps other mechanisms, and that the spontaneous production of males via X-chromosome losses could potentially mediate reversals from parthenogenesis to sex. Furthermore, a spontaneous capacity for rare gamete duplication in sexual species can be co-opted into obligate parthenogenesis. Such co-option is particularly likely under specific ecological conditions. For example, mate limitation can generate selection for reproductive assurance via parthenogenesis [110]. This phenomenon is particularly relevant in wingless species, where mate limitation is more likely to occur [87,111]. With stick insects being renowned for their repeated losses of wings and many wingless species [15], the repeated emergence of parthenogenetic lineages could involve shared ancestral polymorphisms for parthenogenetic egg development which may have been selected recurrently in different sexual lineages when exposed to similar selection pressures.

References

1. Bradler S, Buckley TR. 2018 Biodiversity of Phasmatodea. In *Insect Biodiversity*, pp. 281–

313. Chichester, UK: John Wiley & Sons, Ltd.

2. Brock PD, Büscher TH, Baker E. 2023 Phasmida Species File. *https://phasmida.speciesfile.org/*. See https://phasmida.speciesfile.org/ (accessed on 3 March 2025).

3. Normark BB. 2014 Modes of reproduction. In *The Evolution of Insect Mating Systems*, pp. 1–19. Oxford University Press.

4. Scali V, Passamonti M, Marescalchi O, Mantovani B. 2003 Linkage between sexual and asexual lineages: genome evolution in *Bacillus* stick insects. *Biol. J. Linn. Soc. Lond.* **79**, 137–150.

5. Morgan-Richards M, Trewick SA, Stringer IAN. 2010 Geographic parthenogenesis and the common tea-tree stick insect of New Zealand. *Mol. Ecol.* **19**, 1227–1238.

6. Burke NW, Bonduriansky R. 2022 Sexually but not parthenogenetically produced females benefit from mating in a stick insect. *Funct. Ecol.* **36**, 2001–2014.

7. Wilner D, Boldbaatar J, Miller SM, Burke NW, Bonduriansky R. 2024 Can sexual conflict drive transitions to asexuality? Female resistance to fertilization in a facultatively parthenogenetic insect. *Evolution* (doi:10.1093/evolut/qpae187)

8. Larose C, Parker DJ, Schwander T. 2018 Fundamental and realized feeding niche breadths of sexual and asexual stick insects. *Proc. Biol. Sci.* **285**. (doi:10.1098/rspb.2018.1805)

9. Jaron KS *et al.* 2022 Convergent consequences of parthenogenesis on stick insect genomes. *Sci Adv* **8**, eabg3842.

10. Boldbaatar J, Vasconcelos ACO, Burke NW, Bonduriansky R. 2024 Could adult or juvenile dispersal shape geographical parthenogenesis? Evidence from the facultatively parthenogenetic phasmid *Megacrania batesii*. *Evol. Ecol.* (doi:10.1007/s10682-024-10327-y)

11. Schwander T, Crespi BJ, Gries R, Gries G. 2013 Neutral and selection-driven decay of sexual traits in asexual stick insects. *Proc. Biol. Sci.* **280**, 20130823.

12. Nakano M, Morgan-Richards M, Godfrey AJR, Clavijo McCormick A. 2019 Parthenogenetic females of the stick insect *Clitarchus hookeri* maintain sexual traits. *Insects* **10**. (doi:10.3390/insects10070202)

13. Nakamura K, Fukushima Y. 2022 Seasonal and geographical adaptations in the parthenogenetic stick insect, *Ramulus mikado* (Phasmatodea: Phasmatidae). *Eur. J. Entomol.* **119**, 354–361.

14. Tihelka E, Cai C, Giacomelli M, Pisani D, Donoghue PCJ. 2020 Integrated phylogenomic and fossil evidence of stick and leaf insects (Phasmatodea) reveal a Permian-Triassic co-origination with insectivores. *R. Soc. Open Sci.* **7**, 201689.

15. Bank S, Bradler S. 2022 A second view on the evolution of flight in stick and leaf insects (Phasmatodea). *BMC Ecol Evol* **22**, 62.

16. Simon S *et al.* 2019 Old world and new world Phasmatodea: Phylogenomics resolve the

evolutionary history of stick and leaf insects. *Front. Ecol. Evol.* **7**. (doi:10.3389/fevo.2019.00345)

17. Ranallo-Benavidez TR, Jaron KS, Schatz MC. 2020 GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat. Commun.* **11**, 1432.

18. Kokot M, Dlugosz M, Deorowicz S. 2017 KMC 3: counting and manipulating k-mer statistics. *Bioinformatics* **33**, 2759–2761.

19. Choi S-S, Mc Cartney A, Park D, Roberts H, Brav-Cubitt T, Mitchell C, Buckley TR. 2024 Multiple hybridization events and repeated evolution of homoeologue expression bias in parthenogenetic, polyploid New Zealand stick insects. *Mol. Ecol.* , e17422.

20. Brandt A, Lavanchy G, Merel V, Dumas Z, Labedan M, Soldini L, Schwander T. 2025 Asexual diversification with hybridogenesis as stepping stone in a stick insect.

21. Pijnacker LP. 1966 The maturation divisions of the parthenogenetic stick insect *Carausius morosus* Br. (Orthoptera, Phasmidae). *Chromosoma* **19**, 99–112.

22. Marescalchi O, Pijnacker LP, Scali V. 1991 Cytology of parthenogenesis in *Bacillus whitei* and *Bacillus lynceorum* (Insecta Phasmatodea). *Invertebr. Reprod. Dev.* **20**, 75–81.

23. Bullini L, Nascetti G, Bianchi Bullini AP. 1983 A new stick-insect of hybrid origin: *Bacillus lynceorum* n. sp. (Cheleutoptera: Bacillidae). *Atti Accad. Naz. Lincei Cl. Sci. Fis. Mat. Nat. Rend.* **75**, 169–176.

24. Pijnacker LP. 1967 Oogenesis in the parthenogenetic stick insect *Sipyloidea sipylus* Westwood (Orthoptera, Phasmidae). *Genetica* **38**, 504–515.

25. John B, Rentz DCF, Contreras N. 1987 Extensive chromosome variation in the stick insect genus *Sipyloidea* Brunner von Wattenwyl (Phylliidae : Necrosciinae) within Australia, and descriptions of three new species. *Invertebr. Syst.* **1**, 603.

26. Kearney M, Moussalli A. 2003 Geographic parthenogenesis Australian arid zone: II. Climatic analyses orthopteroid insects genera *Warramaba Sipyloidea*. *Evol. Ecol. Res.* **5**, 977–997.

27. Suomalainen E, Saura A, Lokki J. 1987 *Cytology and evolution in parthenogenesis*. CRC Press.

28. Jaron KS, Bast J, Nowell RW, Ranallo-Benavidez TR, Robinson-Rechavi M, Schwander T. 2021 Genomic features of parthenogenetic animals. *J. Hered.* **112**, 19–33.

29. Dedukh D, Altmanová M, Klíma J, Kratochvíl L. 2022 Premeiotic endoreplication is essential for obligate parthenogenesis in geckos. *Development* **149**. (doi:10.1242/dev.200345)

30. Marescalchi O, Scali V. 2001 New DAPI and FISH findings on egg maturation processes in related hybridogenetic and parthenogenetic *Bacillus* hybrids (Insecta, Phasmatodea). *Mol. Reprod. Dev.* **60**, 270–276.

31. Marescalchi O, Pijnacker LP, Scali V. 1993 Automictic parthenogenesis its genetic consequence *Bacillus atticus atticus* (Insecta Phasmatodea). *Invertebr. Reprod. Dev.* **24**,

7–12.

32. Marescalchi O, Scali V. 2003 Automictic parthenogenesis in the diploid-triploid stick insect *Bacillus atticus* and its flexibility leading to heterospecific diploid hybrids. *Invertebr. Reprod. Dev.* **43**, 163–172.

33. Mantovani B, Scali V. 1993 Genetic structure and phyletic relationships of eastern Mediterranean *Bacillus atticus* Brunner (Insecta Phasmatodea): a biochemical study. *Biochem. Genet.* **31**, 343–362.

34. Goday C, Bianchi Bullini AP, Nascetti G, Bullini L. 1981 Chromosome studies on *Bacillus atticus*, *B. rossius* and their hybrids (Cheleutoptera, Bacillidae). *Atti Accad. Naz. Lincei Cl. Sci. Fis. Mat. Nat. Rend.* **71**, 126–133.

35. van der Kooi CJ, Matthey-Doret C, Schwander T. 2017 Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. *Evol Lett* **1**, 304–316.

36. Liegeois M, Sartori M, Schwander T. 2021 Extremely widespread parthenogenesis and a trade-off between alternative forms of reproduction in mayflies (Ephemeroptera). *J. Hered.* **112**, 45–57.

37. White MJD. 1977 *Animal Cytology and Evolution*. 3rd edn. Cambridge, England: Cambridge University Press.

38. Ghiselli F, Milani L, Scali V, Passamonti M. 2007 The *Leptynia hispanica* species complex (Insecta Phasmida): polyploidy, parthenogenesis, hybridization and more. *Mol. Ecol.* **16**, 4256–4268.

39. Scali V, Milani L, Passamonti M. 2013 Description and ecology of new *Pijnackeria* stick insects: four bisexual species and a triploid parthenogen with their phyletic relationships. *J. Zoolog. Syst. Evol. Res.* **51**, 213–226.

40. Milani L, Scali V, Punzi E, Luchetti A, Ghiselli F. 2020 The puzzling taxonomic rank of *Pijnackeria hispanica*, a chimerical hybrid androgen (Insecta, Phasmida). *Org. Divers. Evol.* **20**, 285–297.

41. Mantovani B, Tinti F, Barilani M, Scali V. 1996 Current reproductive isolation between ancestors of natural hybrids in *Bacillus* stick insects (Insecta: Phasmatodea). *Heredity* **77**, 261–268.

42. Schultz RJ. 1973 Unisexual fish: laboratory synthesis of a ‘species’. *Science* **179**, 180–181.

43. Choleva L, Janko K, De Gelas K, Bohlen J, Šlechtová V, Rábová M, Ráb P. 2012 Synthesis of clonality and polyploidy in vertebrate animals by hybridization between two sexual species. *Evolution* **66**, 2191–2203.

44. Pijnacker LP. 1969 Automictic parthenogenesis in the stick insect *Bacillus rossius* Rossi (Cheleutoptera, phasmidae). *Genetica* **40**, 393–399.

45. Bergerard J. 1955 Cytological mechanics of facultative telythocous parthenogenesis of *Clitumnus extradentatus* br. (Phasmidae). *C. R. Hebd. Seances Acad. Sci.* **240**, 1143–1145.

46. Cappe de Baillon P, Favrelle M, de Vichet G. 1934 La parthénogenèse des Phasmes. *Comptes rendus hebd. séances acad. sci. Paris* **199**, 1069–1070.

47. Nozaki T *et al.* 2021 Development of microsatellite markers for the geographically parthenogenetic stick insect *Phraortes elongatus* (Insecta: Phasmatodea). *Genes Genet. Syst.* **96**, 199–203.

48. Miller S. 2025 The Dawn of Asexuality: Investigating the early stages of parthenogenesis in wild populations of *Megacrania batesii*. PhD thesis, University of New South Wales, Sydney.

49. Suetsugu K, Nozaki T, Hirota SK, Funaki S, Ito K, Isagi Y, Suyama Y, Kaneko S. 2023 Phylogeographical evidence for historical long-distance dispersal in the flightless stick insect *Ramulus mikado*. *Proc. Biol. Sci.* **290**, 20231708.

50. Normark BB. 2003 The evolution of alternative genetic systems in insects. *Annu. Rev. Entomol.* **48**, 397–423.

51. Baker HG. 1967 Support for baker's law-as a rule. *Evolution* **21**, 853.

52. Courchamp F, Clutton-Brock T, Grenfell B. 1999 Inverse density dependence and the Allee effect. *Trends Ecol. Evol.* **14**, 405–410.

53. Buckley TR, Marske KA, Attanayake D. 2009 Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: the New Zealand stick insect *Argosarchus horridus* (White). *Mol. Ecol.* **18**, 4650–4663.

54. Buckley TR, Marske K, Attanayake D. 2010 Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. *J. Biogeogr.* **37**, 682–695.

55. Morgan-Richards M, Langton-Myers SS, Trewick SA. 2019 Loss and gain of sexual reproduction in the same stick insect. *Mol. Ecol.* **28**, 3929–3941.

56. Trewick SA, Goldberg J, Morgan-Richards M. 2005 Fewer species of *Argosarchus* and *Clitarchus* stick insects (Phasmida, Phasmatinae): evidence from nuclear and mitochondrial DNA sequence data. *Zool. Scr.* **34**, 483–491.

57. Miller SM, Stuart KC, Burke NW, Rollins LA, Bonduriansky R. 2024 Genetic and phenotypic consequences of local transitions between sexual and parthenogenetic reproduction in the wild. *Am. Nat.* **203**, 73–91.

58. Mantovani B, Scali V. 1991 Allozymic characterization of Sardinian *Bacillus rossius* (Rossi) and *B. atticus* Brunner (Insecta Phasmatodea). *Genetica* **83**, 275–287.

59. Scali V, Mantovani B. 1989 Updating of systematics and speciation mechanisms of *Bacillus* (Insecta, Phasmatodea). *Boll. Zool.* **56**, 87–98.

60. Gerber N, Kokko H. 2016 Sexual conflict and the evolution of asexuality at low population densities. *Proc. Biol. Sci.* **283**, 20161280.

61. Burke NW, Bonduriansky R. 2017 Sexual conflict, facultative asexuality, and the true paradox of sex. *Trends Ecol. Evol.* **32**, 646–652.

62. Burke NW, Bonduriansky R. 2019 The paradox of obligate sex: The roles of sexual conflict and mate scarcity in transitions to facultative and obligate asexuality. *J. Evol. Biol.* **32**, 1230–1241.

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

64. Kawatsu K. 2013 Sexually antagonistic coevolution for sexual harassment can act as a barrier to further invasions by parthenogenesis. *Am. Nat.* **181**, 223–234.

65. Edward DA, Fricke C, Chapman T. 2010 Adaptations to sexual selection and sexual conflict: insights from experimental evolution and artificial selection. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**, 2541–2548.

66. Lehtonen J, Jennions MD, Kokko H. 2012 The many costs of sex. *Trends Ecol. Evol.* **27**, 172–178.

67. Burke NW, Bonduriansky R. 2018 The geography of sex: sexual conflict, environmental gradients and local loss of sex in facultatively parthenogenetic animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373**. (doi:10.1098/rstb.2017.0422)

68. Arbuthnott D, Crespi BJ, Schwander T. 2015 Female stick insects mate multiply to find compatible mates. *Am. Nat.* **186**, 519–530.

69. Schneider A, Elgar MA. 2010 Facultative sex and reproductive strategies in response to male availability in the spiny stick insect, *Extatosoma tiaratum*. *Aust. J. Zool.* **58**, 228–233.

70. Burke NW, Crean AJ, Bonduriansky R. 2015 The role of sexual conflict in the evolution of facultative parthenogenesis: a study on the spiny leaf stick insect. *Anim. Behav.* **101**, 117–127.

71. Burke NW, Bonduriansky R. 2018 The fitness effects of delayed switching to sex in a facultatively asexual insect. *Ecol. Evol.* **8**, 2698–2711.

72. Maginnis T, Redmond CR. 2013 Sexual vs asexual reproduction stick insect (*Megaphasma dentricus*). *Tex. J. Sci.* **65**, 3–13.

73. Larose C, Lavanchy G, Freitas S, Parker DJ, Schwander T. 2023 Facultative parthenogenesis: a transient state in transitions between sex and obligate asexuality in stick insects? *Peer Community* **3.** (doi:10.24072/pcjournal.283)

74. Cappe de Baillon P, Favrelle M, De Vichet G. 1937 Parthénogenèse et variation chez les Phasmes. III. *Bacillus rossii* Rossi, *Epibacillus lobi*pes Luc., *Phobaeticus sinetyi* Br. *Parasosibia parva* Redt., *Carausius rotundato-lobatus* Br. *Bull. Biol. Fr. Belg.* **71**, 129–189.

75. Scali V. 1970 Obligatory parthenogenesis in the stick insect *Bacillus rossius* (Rossi). *Atti Accad. Naz. Lincei Cl. Sci. Fis. Mat. Nat. Rend.* **49**, 307–314.

76. van der Kooi CJ, Schwander T. 2014 On the fate of sexual traits under asexuality. *Biol. Rev. Camb. Philos. Soc.* **89**, 805–819.

77. Henry L, Schwander T, Crespi BJ. 2012 Deleterious mutation accumulation in asexual

Timema stick insects. *Mol. Biol. Evol.* **29**, 401–408.

78. Bast J *et al.* 2018 Consequences of asexuality in natural populations: Insights from stick insects. *Mol. Biol. Evol.* **35**, 1668–1677.

79. Vickery VR, Sandoval CP. 1999 Two new species of *Timema* (Phasmatoptera: Timematodea: Timematidae), one parthenogenetic, in California. *J. Orthoptera Res.* , 45–47.

80. Vickery VR, Sandoval CP. 2001 Descriptions of three new species of *Timema* (Phasmatoptera: Timematodea: Timematidae) and notes on three other species. *orth* **10**, 53–61.

81. Sandoval CP, Vickery VR. 1996 *Timema douglasi* (Phasmatoptera: Timematodea), a new parthenogenetic species from Southwestern Oregon and Northern California, with notes on other species. *Can. Entomol.* **128**, 79–84.

82. Law JH, Crespi BJ. 2002 Recent and ancient asexuality in *Timema* walkingsticks. *Evolution* **56**, 1711–1717.

83. Sandoval C, Carmean DA, Crespi BJ. 1998 Molecular phylogenetics of sexual and parthenogenetic *Timema* walking–sticks. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **265**, 589–595.

84. Schwander T, Henry L, Crespi BJ. 2011 Molecular evidence for ancient asexuality in *Timema* stick insects. *Curr. Biol.* **21**, 1129–1134.

85. Freitas S, Parker DJ, Labédan M, Dumas Z, Schwander T. 2023 Evidence for cryptic sex in parthenogenetic stick insects of the genus *Timema*. *Proc. Biol. Sci.* **290**, 20230404.

86. Riesch R *et al.* 2017 Transitions between phases of genomic differentiation during stick-insect speciation. *Nat Ecol Evol* **1**, 82.

87. Schwander T, Vuilleumier S, Dubman J, Crespi BJ. 2010 Positive feedback in the transition from sexual reproduction to parthenogenesis. *Proc. Biol. Sci.* **277**, 1435–1442.

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

89. White MJD. 1964 Cytogenetic mechanisms in insect reproduction. In *Insect Reproduction* (ed KC Highnam), pp. 1–12. Royal Entomological Society of London.

90. Templeton AR. 1982 The Prophecies of Parthenogenesis. In *Evolution and Genetics of Life Histories* (eds H Dingle, J Hegmann), pp. 75–101. Springer US.

91. Sperling AL, Fabian DK, Garrison E, Glover DM. 2023 A genetic basis for facultative parthenogenesis in *Drosophila*. *Curr. Biol.* **33**, 3545–3560.e13.

92. Di Cristina G, Dirksen E, Altenhein B, Büschges A, Korsching SI. 2025 Pioneering genome editing in parthenogenetic stick insects: CRISPR/Cas9-mediated gene knockout in *Medauroidea extradentata*. *Sci. Rep.* **15**, 2584.

93. Simon J-C, Delmotte F, Rispe C, Crease T. 2003 Phylogenetic relationships between

parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biol. J. Linn. Soc. Lond.* **79**, 151–163.

94. Aguirre WE, Reid K, Rivera J, Heins DC, Veeramah KR, Bell MA. 2022 Freshwater colonization, adaptation, and genomic divergence in Threespine Stickleback. *Integr. Comp. Biol.* **62**, 388–405.

95. Morris J, Hanly JJ, Martin SH, Van Belleghem SM, Salazar C, Jiggins CD, Dasmahapatra KK. 2020 Deep convergence, shared ancestry, and evolutionary novelty in the genetic architecture of *Heliconius* mimicry. *Genetics* **216**, 765–780.

96. Toubiana W *et al.* 2025 Functional monocentricity with holocentric characteristics and chromosome-specific centromeres in a stick insect. *Sci. Adv.* **11**, eads6459.

97. Cappe de Baillon P, De Vichet G. 1935 Le mâle du *Clonopsis gallica* Charp. *Orthopt. Phasmidae) Annales de la Société Entomologique de France* **104**, 259–272.

98. Cappe de Baillon P, De Vichet G. 1940 La parthénogenèse des espèces du genre *Leptynia* Pant. (Orthopt. Phasmidae). *Bulletin Biologique de la France et de la Belgique* **74**, 43–87.

99. Pijnacker LP, Ferwerda MA. 1980 Sex chromosomes and origin of males and sex mosaics of the parthenogenetic stick insect *Carausius morosus* Br. *Chromosoma* **79**, 105–114.

100. Scali V. 2013 A ‘ghost’ phasmid appearance: the male *Bacillus atticus* (Insecta: Phasmatodea). *Ital. J. Zool.* **80**, 227–232.

101. Nozaki T, Chikami Y, Yano K, Sato R, Suetsugu K, Kaneko S. 2025 Lack of successful sexual reproduction suggests the irreversible parthenogenesis in a stick insect. *Ecology* **106**, e4522.

102. Wu I-H, Liu H-H, Chen Y-Y, Tsai C-L, Yu Y-C, Hsiao C-Y, Yeh W-B. 2020 Life cycles, phenology and genetic structure of endangered *Megacrania tsudai* Shiraki (Phasmatodea: Phasmatidae): Male individuals from a geographic parthenogenesis species. *Entomol. Sci.* **23**, 183–192.

103. Kuyten P. 1962 Duration of life, egg number and parthenogenesis in the stick insect *Orxines macklotti* DE Haan. *Nature* **195**, 1124–1125.

104. Sylvester T, Hjelmen CE, Hanrahan SJ, Lenhart PA, Johnston JS, Blackmon H. 2020 Lineage-specific patterns of chromosome evolution are the rule not the exception in Polyneoptera insects. *Proc. Biol. Sci.* **287**, 20201388.

105. Hadlington P, Shipp ME. 1962 Diapause parthenogenesis eggs three species of Phasmatodea. *Proceedings Linnean Society New South Wales* **86**, 268–279.

106. Blackman RL, Hales DF. 1986 Behaviour of the X chromosomes during growth and maturation of parthenogenetic eggs of Amphorophora tuberculata (Homoptera, Aphididae), in relation to sex determination. *Chromosoma* **94**, 59–64.

107. Wilson ACC, Sunnucks P, Hales DF. 1997 Random loss of X chromosome at male determination in an aphid, Sitobion near fragariae, detected using an X-linked polymorphic microsatellite marker. *Genet. Res. (Camb.)* **69**, 233–236.

108. Bullini L. 1964 Ricerche sul rapporto sessi in *Bacillus Rossii* (Fab.). *Atti Accad. Naz. Lincei Cl. Sci. Fis. Mat. Nat. Rend.* **8**, 897–902.

109. De Vichet G. 1944 Découverte dans le sud de la France d'une station de mâles de *Bacillus rossii* (Fabr.) (Orthoptère, Phasmidae). *Bull. Mens. Soc. Linn. Lyon* **13**, 92–94.

110. Gerritsen J. 1980 Sex and parthenogenesis in sparse populations. *Am. Nat.* **115**, 718–742.

111. Blackmon H, Ross L, Bachtrog D. 2017 Sex Determination, Sex Chromosomes, and Karyotype Evolution in Insects. *J. Hered.* **108**, 78–93.