

# Positive feedback in the transition from sexual reproduction to parthenogenesis

Tanja Schwander<sup>1,\*</sup>, Séverine Vuilleumier<sup>2</sup>, Janie Dubman<sup>1</sup>  
and Bernard J. Crespi<sup>1</sup>

<sup>1</sup>Biological Sciences, Simon Fraser University, Burnaby, Canada V5A 1S6

<sup>2</sup>Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland

Understanding how new phenotypes evolve is challenging because intermediate stages in transitions from ancestral to derived phenotypes often remain elusive. Here we describe and evaluate a new mechanism facilitating the transition from sexual reproduction to parthenogenesis. In many sexually reproducing species, a small proportion of unfertilized eggs can hatch spontaneously ('tychoparthenogenesis') and develop into females. Using an analytical model, we show that if females are mate-limited, tychoparthenogenesis can result in the loss of males through a positive feedback mechanism whereby tychoparthenogenesis generates female-biased sex ratios and increasing mate limitation. As a result, the strength of selection for tychoparthenogenesis increases in concert with the proportion of tychoparthenogenetic offspring in the sexual population. We then tested the hypothesis that mate limitation selects for tychoparthenogenesis and generates female-biased sex ratios, using data from natural populations of sexually reproducing *Timema* stick insects. Across 41 populations, both the tychoparthenogenesis rates and the proportions of females increased exponentially as the density of individuals decreased, consistent with the idea that low densities of individuals result in mate limitation and selection for reproductive insurance through tychoparthenogenesis. Our model and data from *Timema* populations provide evidence for a simple mechanism through which parthenogenesis can evolve rapidly in a sexual population.

**Keywords:** parthenogenesis; sex ratio; mate limitation; reproductive insurance; positive feedback loop

## 1. INTRODUCTION

Understanding how new phenotypes evolve is often difficult because the intermediate stages in the transition from the ancestral to the derived phenotype remain elusive (Maynard Smith & Szathmari 1995). The causal bases of intermediate phenotypes may, however, elucidate why certain transitions are relatively more likely to occur than others, and may help in explaining patterns of phenotypic variation within and between species. One transition where the intermediate stages are poorly understood is the transition from sexual reproduction to thelytokous (female-producing) parthenogenesis. Sexual reproduction evolved in the early evolutionary history of eukaryotes, some 2.0–3.5 billion years ago (Miyamoto & Fitch 1996; Gu 1997); it is the predominant mode of reproduction in multicellular taxa, yet several hundred lineages have undergone the large-scale transition from sexuality to facultative or obligate thelytokous parthenogenesis (Bell 1982; Avise 2008; Schön *et al.* 2009).

The switch from sexual reproduction to thelytoky is theoretically associated with multiple advantages, most notably the twofold demographic advantage of producing all-female offspring (Maynard Smith 1978). The ubiquitous maintenance of sexual reproduction in spite of its demographic disadvantage has long been considered a paradox in evolutionary biology (Williams 1975; Maynard Smith 1978). However, depending on how the

transition from sexuality to thelytokous parthenogenesis occurs, an incipient parthenogenetic lineage may not necessarily be characterized by a twofold, if any, demographic advantage over its sexual ancestor (Templeton 1982).

It has been suggested that the transition from sexual reproduction to thelytoky could occur via tychoparthenogenesis (i.e. the spontaneous hatching of a small proportion of unfertilized eggs in a normally sexually reproducing species; White 1964, 1973; Suomalainen *et al.* 1987). Tychoparthenogenesis is widespread in sexual invertebrates (Bell 1982) and has also been reported in vertebrate species (Chapman *et al.* 2007; Lampert 2008; Schut *et al.* 2008); it is presumed to occur because errors during the meiotic divisions can result in the production of diploid instead of haploid oocytes. Diploidy in these oocytes is usually restored either by fusion of one of the three polar bodies with the oocyte or an additional step of chromosome duplication ('automixis'; White 1964, 1973; Suomalainen *et al.* 1987). Both mechanisms of diploidy restoration result in the production of females in species where males are the heterogametic sex. Given the cytological similarities with sexual reproduction, tychoparthenogenesis provides a probable first step towards obligate automictic parthenogenesis (White 1973). It is less clear whether tychoparthenogenesis may also give rise to apomictic parthenogenesis (Suomalainen *et al.* 1987), where eggs are produced mitotically and offspring are genetically identical to their mother. An automictic stage in the evolution of apomixis has been suggested because of meiosis-like patterns of chromosome pairing

\* Author for correspondence (tanja.schwander@gmail.com).

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during the mitotic production of eggs in several apomictic insect species, particularly certain chironomids, embiopterans and weevils (Porter 1971).

Tychoparthenogenesis is typically characterized by extremely low hatching success of unfertilized eggs (e.g. approx.  $10^{-5}$  in *Drosophila mercatorum*; Templeton *et al.* 1976). This low rate of tycho- parthenogenesis observed in natural populations can be increased by several orders of magnitudes through artificial selection (Carson 1967; Templeton *et al.* 1976), demonstrating considerable standing genetic variation for tycho- parthenogenetic ability. Obligate parthenogenesis could thus evolve gradually from tycho- parthenogenesis if selection favoured an increase in the proportion of tycho- parthenogenetic eggs. However, with such a low initial rate of tycho- parthenogenesis, it is not clear how a new parthenogenetic lineage could arise and persist in a sexual population (Kramer & Templeton 2001).

We hypothesize that an initially low rate of tycho- parthenogenesis could be favoured through a positive feedback loop. In low-density populations, or in populations with highly female-biased sex ratios owing to demographic stochasticity, some females may fail to find a mate. Because unmated females can only produce daughters through tycho- parthenogenesis, the population sex ratio becomes more female-biased, resulting in a yet larger proportion of females not finding mates. Thus, the strength of selection for tycho- parthenogenesis increases in concert with the proportion of tycho- parthenogenetic offspring in the sexual population. This effect might further be enhanced by the small number of offspring produced by unmated as compared with mated females, reducing the density of individuals in the population and the chance for females to successfully find a mate.

Our study has two aims. First, we quantitatively test whether our suggested feedback loop could result in the loss of males from a sexual population and favour the transition from sexual reproduction to parthenogenesis. To this end, we build an analytical model from which we derive the stable sex ratio in a population as a function of the tycho- parthenogenesis rate and female mating probability, with female mating probability depending on the population sex ratio. Second, we use data from *Timema* stick insects to evaluate whether our suggested feedback mechanism may contribute to female-biased sex ratios and high tycho- parthenogenesis rates in natural populations. Although it is not feasible to directly test for interactions between sex ratio, mate limitation and selection for tycho- parthenogenesis, we tested for three key conditions of the positive feedback: (i) whether females in some populations may be subject to mate limitation, (ii) whether increased mate limitation selects for increased rates of tycho- parthenogenesis and (iii) whether tycho- parthenogenesis generates female-biased sex ratios.

The genus *Timema* consists of 22 described species, five of which reproduce parthenogenetically (Sandoval & Vickery 1996; Vickery & Sandoval 1998, 1999). The remaining species reproduce sexually and have the capacity for tycho- parthenogenesis (Schwander & Crespi 2009). We measured the average rate of tycho- parthenogenesis in 41 populations comprising eight different sexual species, and examined how this rate varied according to the proportion of females and the density of

individuals in each population. We also evaluated whether females were indeed mate-limited in the low-density populations by measuring the proportion of adult females that were unseminated in low- and high-density populations. Finally, because putative tycho- parthenogenesis rate variation among populations and geographically heterogeneous sex ratios are more easily maintained if populations are genetically isolated, we estimated the level of population differentiation in two of the eight sexual *Timema* species, using microsatellite genotyping.

## 2. MATERIAL AND METHODS

### (a) *Positive feedback model for the transition to parthenogenesis*

To test whether the positive feedback loop could result in the loss of males from a sexual population, we predicted the proportion of females in a population  $p_F$  as a function of the tycho- parthenogenesis rate  $v$  and female mating probability. Females mate with a probability  $\alpha$ , which increases linearly with the proportion of males  $p_M = (1 - p_F)$  in the population:  $\alpha = \beta p_M$ . The constant  $\beta$  can be interpreted as the probability of encounters between individuals, which should decrease in concert with the density of individuals in a population. Mated females produce 50 per cent male and 50 per cent female offspring, whereas unmated females produce a proportion of diploid eggs  $v$  which develop into females (tycho- parthenogenetic offspring). We further assume that tycho- parthenogenetic eggs are not viable if females are inseminated (i.e. there is a cost to tycho- parthenogenesis, which is consistent with empirical data from *Timema*; see §3). Generations are non-overlapping. Thus, after one generation, the proportion of females,  $p'_F$ , in the population becomes

$$p'_F = \frac{\alpha/2(1-v) + v(1-\alpha)}{\alpha(1-v) + v(1-\alpha)}. \quad (2.1)$$

From equation (2.1), we derived the stable proportion of females in a population as a function of the tycho- parthenogenesis rate  $v$  and the constant  $\beta$  (see appendix A) to evaluate what conditions could lead to a complete loss of males in *Timema* stick insect populations.

### (b) *Empirical tests using Timema stick insects*

To test whether mate limitation could select for tycho- parthenogenesis, we determined the density of individuals, the sex ratio and rate of tycho- parthenogenesis in 41 populations comprising eight sexual *Timema* species: *T. poppensis* (10 locations), *T. cristinae* (9), *T. californicum* (4), *T. chumash* (6), *T. landelsensis* (1), *T. knulli* (5), *T. petita* (3) and *T. podura* (3). Individuals were collected between March and May of 2007 and 2008, by shaking branches of the appropriate host plant into a sweep net. Populations were sampled only after the majority of individuals in the population were in the third instar. To quantify the density of individuals, we sampled during fixed time intervals and divided the number of individuals caught during each interval by its length in minutes. We had to vary the length of the interval (2, 10 or 40 min) because the density was extremely variable, so that individuals would start escaping from the sweep net after 2 min of sampling in high-density populations, whereas in low-density populations we often needed more than 20 min to catch even a single individual. The density estimate in each population used for all analyses is an average across at least four intervals (range 4–12).

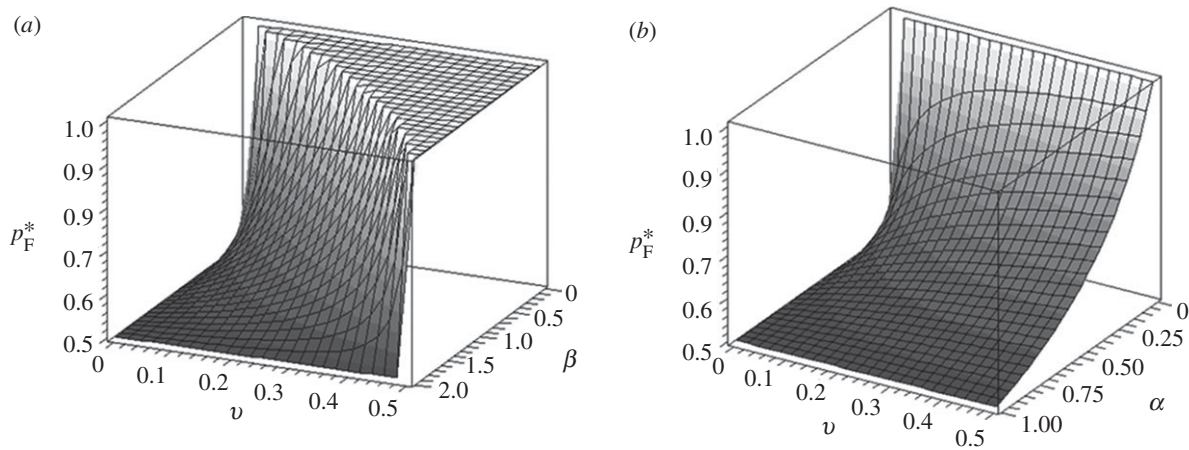


Figure 1. The stable proportion of females,  $p_F^*$ , depending on the tycho-parthenogenesis rate (proportion  $v$  of unfertilized eggs that develop into females) and the mating probability of females,  $\alpha$ , as predicted by our analytical model. (a) Including the feedback between population sex ratio and female mating probability,  $\alpha = \beta p_M$  in equation (2.1); (b) with female mating probability independent of the population sex ratio,  $\alpha = \beta$  in equation (2.1).

All captured nymphs were sexed and 4–43 females per population were raised to maturity to determine the rate of tycho-parthenogenesis. The large variation in sample size was due to the populations with the lowest density, where we were unable to increase the sample despite sustained efforts to collect additional individuals. The females were maintained individually in Petri dishes, and fed with *Ceanothus* cuttings and a mixture of wheat gluten, sugar and vitamins until they had laid approximately 50 eggs. We used the average proportion of unfertilized eggs that hatched per female as an estimate of the population-level tycho-parthenogenesis rate in all analyses. Preliminary experiments revealed that the capacity for tycho-parthenogenesis was independent of whether females were reared to sexual maturity with or without males; in two different populations, the tycho-parthenogenesis rates estimated from eggs laid by virgin females reared in isolation did not differ from the tycho-parthenogenesis rates estimated from eggs laid by virgin females reared up to maturity at high densities and a 50 : 50 sex ratio (results not shown).

We attempted to estimate the proportion of females that fail to find a mate as a function of the density of individuals, in a subset of the populations, by sampling adult females in June 2008 and 2009 and inspecting their spermatheca for the presence of sperm. However, the small number of adult females (one to two per population) caught in six low-density populations precluded a population-level estimate of mate limitation. To validate our assumption that females in low-density populations may be mate-limited, we therefore compared the overall proportion of non-inseminated adult females between low- and high-density populations.

To test whether females also produce tycho-parthenogenetic eggs when they are inseminated, we used microsatellite genotyping to distinguish between offspring produced via sexual reproduction and tycho-parthenogenesis in *T. cristinae*. We genotyped 30 offspring from each of 25 females ( $n = 750$  offspring) at four microsatellite markers (Tim1, Tim4, Tim6 and Tim7), as described in Schwander & Crespi (2009), and compared each offspring's genotype with its mother's genotype to determine whether it was produced by tycho-parthenogenesis. Thus, tycho-parthenogenesis would be indicated if an individual's genotype exclusively comprised alleles present in its mother at each of the four markers.

Mate limitation and positive feedback loop dynamics are more likely to contribute to female-biased sex ratios if there is relatively little migration between populations, because locally adapted tycho-parthenogenesis rates are less likely to be swamped by gene flow, and dispersal would not tend to homogenize sex ratios across populations. We used the same four microsatellite markers as for the offspring genotyping and two additional markers (Tim5 and Tim8) to measure the levels of gene flow among a subset of populations in the two species for which we had the largest number of samples. Thus, we analysed six of the nine *T. cristinae* populations and seven of the ten *T. poppensis* populations. We only genotyped females (average 18.6, range 8–45 per population), so that we could further test whether populations with more female-biased sex ratios were also characterized by relatively higher levels of inbreeding (as measured by the statistic  $F_{is}$ ). Such a pattern would be expected if female-biased sex ratios largely stemmed from offspring production via tycho-parthenogenesis, because, as shown previously in *Timema* (Schwander & Crespi 2009), the fusion of the polar body with the egg results in females heterozygous at the microsatellite loci Tim4–Tim8 producing offspring homozygous at the same loci. Thus, similar to inbreeding, tycho-parthenogenesis in *Timema* should generate an excess of homozygotes relative to a population with random mating (Hood & Antonovics 2004; Pearcy et al. 2006).

### 3. RESULTS

#### (a) Positive feedback model for the transition to parthenogenesis

Our analytical model revealed that a positive feedback between sex ratio and female mating probability may indeed result in the loss of males from the population (figure 1a). Relative to a model where tycho-parthenogenesis also influences the sex ratio, but where female mating probability is independent of the sex ratio (figure 1b), the feedback mechanism increases the range of conditions under which males can be lost. It also predicts overall more female-biased sex ratios for a given combination of tycho-parthenogenesis rate and female mating probability relative to the simpler model. However, even with the feedback, males are lost only if both the rate of

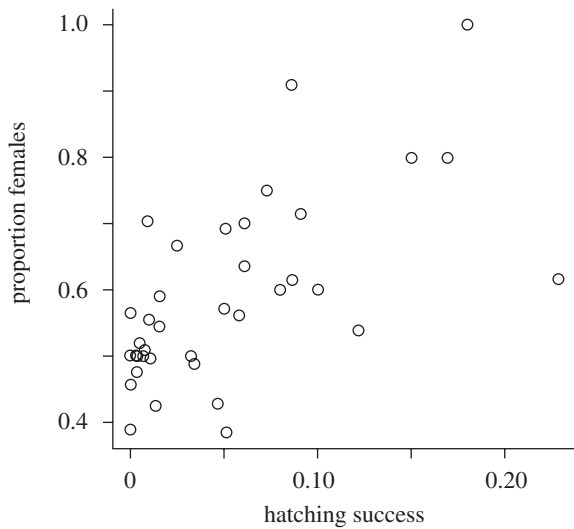


Figure 2. The rate of tycho-parthenogenesis is positively correlated with female bias across 41 populations of sexually reproducing *Timema* stick insects.

tycho-parthenogenesis and the proportion of females that fail to find a mate are large. For example, a rate of tycho-parthenogenesis close to 0.2 would result in the loss of males only if fewer than half of the females mate. In most of the examined parameter space, at least a small proportion of males are maintained in the population.

#### (b) Empirical tests using *Timema* stick insects

Although the small number of adult females (one to two per population) collected in six low-density populations precluded a population-level estimate of mate limitation, the overall proportion of adult females that were uninseminated was significantly higher in the low-density than in the high-density populations: of the ten adult females from the low-density populations, three were not inseminated; by contrast, all 45 adult females collected from four high-density populations were inseminated (Fisher's exact probability test,  $p = 0.009$ ).

The positive feedback loop model predicts that (i) females in some populations are subject to mate limitation, (ii) increased mate limitation selects for increased rates of tycho-parthenogenesis and (iii) tycho-parthenogenesis generates female-biased sex ratios. In line with these predictions, we found that the rate of tycho-parthenogenesis and the sex ratio (proportion of females) were significantly positively correlated across 41 *Timema* populations ( $\rho = 0.65$ ,  $t_{39} = 5.4$ ,  $p < 0.0001$ ; figure 2). Although the sex ratio in the majority of populations was relatively balanced, some populations had highly female-biased sex ratios, and in one extreme case all four individuals collected were female (figures 2 and 3a). The capacity for tycho-parthenogenesis also varied widely among populations, from no unfertilized egg hatching in eight populations to almost 23 per cent of unfertilized eggs hatching in one *T. poppensis* population.

Visual inspection of the variation in tycho-parthenogenesis rates and the proportion of females among populations suggested that these two variables may increase exponentially as the density of individuals decreases (figure 3a,b). We therefore log-log transformed the data (after adding 0.01 to each tycho-parthenogenesis

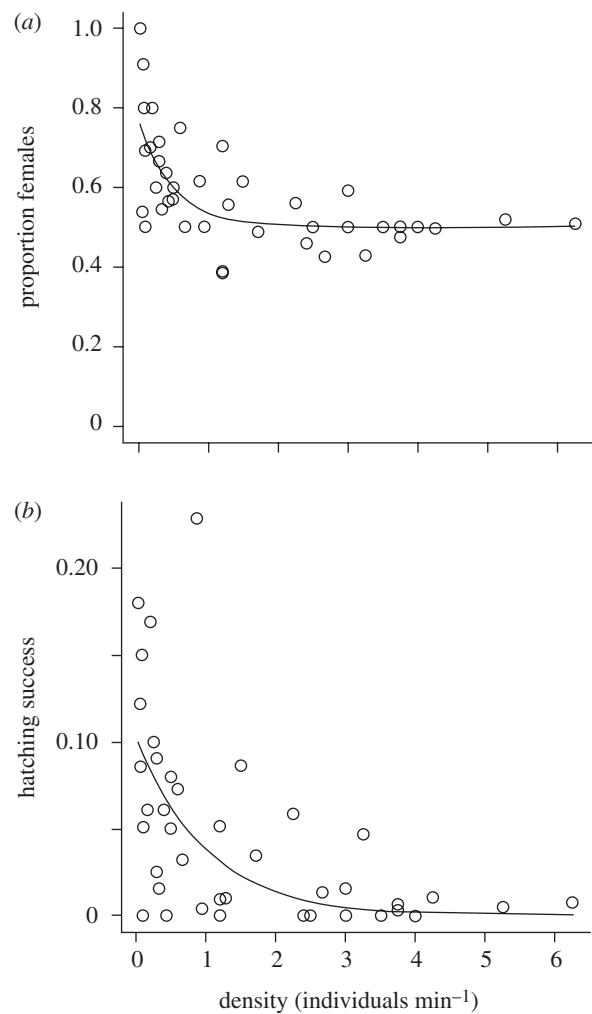


Figure 3. (a) Sex ratios (proportion of females) and (b) tycho-parthenogenesis rates across *Timema* populations with variable densities of individuals. The level of mate limitation is assumed to increase as the density of individuals in the population decreases.

value to avoid  $\log(0)$  values) to test, while adjusting for possible species effects, whether the tycho-parthenogenesis rates and the sex ratio significantly increased as the density of individuals decreased. An analysis of covariance (ANCOVA) on the transformed data revealed that both the tycho-parthenogenesis rates (species effect:  $F_{7,26} = 2.9$ ,  $p = 0.022$ ; density effect:  $F_{1,26} = 6.2$ ,  $p = 0.019$ ) and the proportion of females (species effect:  $F_{7,26} = 2.3$ ,  $p = 0.059$ ; density effect:  $F_{1,26} = 14.9$ ,  $p < 0.001$ ) significantly increased across populations as the density of individuals decreased. In each case, we found no significant interaction effect between species and density (tycho-parthenogenesis:  $F_{6,26} = 0.7$ ,  $p = 0.64$ ; sex ratio:  $F_{6,26} = 0.65$ ,  $p = 0.69$ ), suggesting that the significant (or marginally non-significant) species effects in our analyses resulted from different species being sampled at different densities rather than from some intrinsic variation among species in tycho-parthenogenesis rates or sex ratio. We therefore also fitted an exponential function to the untransformed data, unpartitioned by species, which revealed highly significant exponential effects of density on hatching success ( $r^2 = 0.306$ ,  $p < 0.0001$ ) and sex ratio ( $r^2 = 0.462$ ,  $p < 0.0001$ ; table 1 and figure 3a,b). The nonlinear effect of density may indicate

Table 1. Model coefficients and test values for the effect of density on tycho-parthenogenesis rates and sex ratio in *Timema* populations. For each model, we determined the nonlinear (weighted) least-squares parameter estimates for the function  $a \times (\exp(-b \times D)) + c$ , where  $D$  is the density of individuals. Because the parameters  $b$  and  $c$  were not significant in the tycho-parthenogenesis rate analysis, we re-estimated the parameter  $a$  for the simplified function  $a \times (\exp - D)$ .

	parameter	estimate	standard error	<i>t</i> value	Pr(>  <i>t</i>  )
tycho-parthenogenesis rate: function $\{a \times (\exp(-D))\}$	<i>a</i>	0.104	0.014	7.7	<0.0001
sex ratio: function $\{a \times (\exp(-b \times D)) + c\}$	<i>a</i>	0.277	0.055	5.1	<0.0001
	<i>b</i>	2.142	1.027	2.1	0.044
	<i>c</i>	0.502	0.025	20.2	<0.0001

accelerating selection for tycho-parthenogenesis as the level of mate limitation increases, or may derive from a nonlinear relation between the level of mate limitation and our density estimate.

The microsatellite genotype analyses indicated moderate to high levels of population isolation in both *T. cristinae* (average pairwise *F*<sub>st</sub> values: 0.075, range 0.032–0.153; electronic supplementary material, table S1) and *T. poppensis* (average pairwise *F*<sub>st</sub> values: 0.207, range 0–0.389; electronic supplementary material, table S2). Of the 36 pairs of populations, 28 (10 in *T. cristinae* and 18 in *T. poppensis*) displayed significant differentiation (electronic supplementary material, tables S1 and S2), even though many populations were geographically close (less than 10 km apart). In contrast to *F*<sub>st</sub> values, none of the (within-population) *F*<sub>is</sub> values was significant after correcting for multiple testing. However, populations with more female-biased sex ratios were characterized by relatively larger *F*<sub>is</sub> values ( $\rho = 0.69$ ,  $t_{11} = 3.14$ ,  $p = 0.01$ ), consistent with the idea that female bias largely results from tycho-parthenogenetic offspring production in these populations.

#### 4. DISCUSSION

There are several mechanisms that may generate new parthenogenetic lineages from a sexual ancestor, including infection by micro-organisms, interspecific hybridization and spontaneous mutations in genes involved in meiosis and gamete production. The smallest step from sexual to parthenogenetic reproduction is presumed to involve the preservation of meiotic oocyte production (White 1964, 1973; Bell 1982), whereby diploidy of the zygote is restored secondarily by either fusion or duplication of haploid meiotic products (automixis). Because automictic tycho-parthenogenesis is widespread, it has been suggested that this mechanism may provide a first step towards the transition from sexual reproduction to parthenogenesis (Bell 1982; Suomalainen *et al.* 1987). However, it has not been clear how an incipient parthenogen could persist in a sexual population, given the low egg-hatching success associated with tycho-parthenogenesis (Templeton 1982; Kramer & Templeton 2001). In this paper, we provide a combination of evidence from theory and data that the transition to parthenogenesis via tycho-parthenogenesis may be driven by a positive feedback mechanism that surmounts these constraints, given that tycho-parthenogenesis is more likely to occur if females are mate-limited and if the level of mate limitation depends on the population sex ratio.

Our analytical model revealed that an interaction between sex ratio and female mating probability could indeed result in the loss of males from a sexual population with an initially balanced sex ratio, even if the rate of tycho-parthenogenesis could not increase via evolutionary changes. The model could also explain the highly female-biased sex ratios in some *Timema* populations characterized by moderate tycho-parthenogenesis rates (figure 2), if females in these populations frequently fail to find mates. These results should be fairly robust because our model is conservative: we did not evaluate the effect of three factors likely to increase the parameter space in which males can be lost. First, we assumed a linear relationship between female mating probability and the proportion of males in the population, although this probability probably decreases more rapidly with the frequency of males in the population. Second, we did not consider that population size and density decrease as the proportion of tycho-parthenogenetic offspring in the population increases, owing to unmated females producing fewer offspring than mated females. Thus, tycho-parthenogenesis should amplify mate limitation not only by generating female-biased sex ratios, but also through an additional reduction in population density. Finally, we did not include stochastic effects on demography, which, especially if only a small proportion of males persist in the population, may contribute to the complete loss of males. Although demographic stochasticity should increase the parameter space in which males can be lost from the population, it may also reduce the number of transitions to parthenogenesis that persist, given that tycho-parthenogenesis reduces population size, and demographic stochasticity is an important factor leading to local extinction of very small populations (Lande 1988).

The transition from tycho-parthenogenesis to obligate parthenogenesis may also be hampered by selection for male production in populations with female-biased sex ratios (Hamilton 1967), which could generate dynamics where population sex ratios fluctuate from female bias to more even sex ratios and back. For example, in species with haplo-diploid sex determination (where males develop from unfertilized haploid eggs), female-biased sex ratios may select for mutations causing mating reluctance and/or increased production of male eggs (Huigens & Stouthamer 2003). In species with chromosomal sex determination, mechanisms increasing the production of male offspring are somewhat more complex (Werren & Beukeboom 1998), so that the likelihood of mated females producing male-biased sex ratios depends on how quickly the transition to an all-female population

occurs. Our proposed feedback mechanism can result in the loss of males very quickly (typically within 10–40 generations, depending on the rate of tycho-parthenogenesis and the level of mate limitation). In combination to the small census sizes in the female-biased populations, this may render the evolution of novel sex ratio biasing mechanisms before the loss of males relatively unlikely.

Although it is not possible to directly test whether a positive feedback loop mechanism acts in natural populations, our analysis of sexual *Timema* populations has shown that the three key conditions were present for the feedback loop to contribute to high tycho-parthenogenesis rates and female-biased sex ratios. First, females are mate-limited, which was revealed by the significantly larger proportion of non-inseminated adult females in populations characterized by a low density of individuals as compared with high-density populations. Second, tycho-parthenogenesis rates (figure 3b) and the proportion of females (figure 3a) increased exponentially as the density of individuals decreased, consistent with the idea that mate limitation selects for tycho-parthenogenesis. However, because we did not directly estimate female mating probability but used density as a surrogate, it is not possible to distinguish whether the nonlinear effect of density is due to accelerating selection for tycho-parthenogenesis as the level of mate limitation increases or the level of mate limitation is nonlinearly related to our density estimate.

Finally, the rate of tycho-parthenogenesis was significantly positively correlated with the proportion of females across 41 *Timema* populations, suggesting that female-biased sex ratios are due to a significant proportion of offspring being produced by tycho-parthenogenesis. An additional line of evidence for the idea that female-biased sex ratios are due to tycho-parthenogenetic reproduction stems from our microsatellite analyses, which indicated that the level of inbreeding (as measured by the statistic  $F_{is}$ ) was positively correlated with the population sex ratio. Alternatively, significant  $F_{is}$  values in the female-biased populations could result from differences in the geographical area sampled for high- versus low-density populations. Because we needed a minimum number of individuals per population to estimate tycho-parthenogenesis rates and sex ratios, we often had to sample across larger areas when the density of individuals was low. Given that female bias only occurred in low-density populations, local population structure could also explain the high  $F_{is}$  values in the female-biased populations.

The most probable explanation for the positive correlation between density and tycho-parthenogenesis is that relatively high tycho-parthenogenesis rates only persist if females are mate-limited. An alternative explanation would be that populations vary randomly in their rate of tycho-parthenogenesis and that density decreases in populations with large tycho-parthenogenesis rates because tycho-parthenogenesis strongly reduces the number of offspring produced as compared with sexual reproduction. Two lines of evidence are incompatible with this idea. First, tycho-parthenogenesis in *Timema* is probably costly to females in populations where females are not mate-limited. Inseminated females produced significantly fewer tycho-parthenogenetic offspring than virgin females of the same population, suggesting that tycho-parthenogenetic eggs produced by inseminated females largely fail to

develop, possibly because fertilization of the (diploid) tycho-parthenogenetic eggs results in inviable, triploid zygotes. Tycho-parthenogenesis may be even more costly if tycho-parthenogenetic offspring have low fitness as compared with sexual offspring as a consequence of inbreeding depression. Second, the level of variation in tycho-parthenogenesis rates among populations was extremely large, ranging from 0 to almost 23 per cent, which seems unlikely to be explained by random variation, especially if tycho-parthenogenesis is costly.

Selection for tycho-parthenogenesis as a consequence of mate limitation mirrors selection for reproductive insurance via self-compatibility in plants. The evolution of self-incompatibility in plants has largely been explained by inbreeding avoidance (Lloyd 1979; Charlesworth 1988; Byers & Meagher 1992). Self-incompatibility in plants depends on a gene cluster referred to as the S-locus; individuals sharing recognition alleles at this locus cannot produce offspring. When populations are small and genetic diversity at the S-locus is lost as a consequence of drift, individuals may become mate-limited (see Busch & Schoen 2008 for a review). Thus, while outcrossing (and self-incompatibility) may be favoured in large populations because of inbreeding depression, pollen limitation in small populations may select for self-compatibility and self-fertilization (Lloyd 1979; Byers & Meagher 1992; Busch & Schoen 2008). The apparent hatching failure of tycho-parthenogenetic eggs laid by inseminated *Timema* females may impose costs similar to inbreeding depression in a self-compatible plant. Thus, such a cost could explain why tycho-parthenogenesis may only persist in populations where finding a mate is difficult or impossible.

Selection for reproductive insurance and positive feedback loop dynamics is more likely to contribute to female-biased sex ratios if there is relatively little migration between populations, because locally adapted tycho-parthenogenesis rates are less likely to be swamped by gene flow, and dispersal would not tend to homogenize sex ratios across populations. Indeed, given that populations with selection for high tycho-parthenogenesis rates harbour only a small proportion of all individuals, a flux of individuals from high- to low-density populations may completely prevent locally increased tycho-parthenogenesis rates if the migration of individuals among populations is important. Our microsatellite-marker-based estimates of population isolation in *Timema* revealed relatively high levels of isolation even between geographically close populations (average pairwise  $F_{st}$  values: *T. cristinae*, 0.07; *T. poppensis*, 0.21), consistent with a previous study that showed, using mark-recapture techniques, that individuals only disperse over approximately 12 m per generation (Sandoval 1994). High levels of population isolation in *Timema* could be explained by the patchy distribution of suitable host plants and winglessness in both sexes in this genus.

Winglessness may contribute to the transition to parthenogenesis not only via higher levels of population isolation, but also because limited movement of individuals may result more often in mate limitation. Wings have been lost many times independently in insects, and the prevalence of wingless species varies widely among insect orders (Roff 1990, 1994). Interestingly, several insect orders characterized by a high proportion of

wingless species, such as Orthoptera, Thysanoptera, Embiidina and Phasmida (Roff 1994), also tend to have many described cases of thelytokous parthenogenesis (Bell 1982). Conversely, parthenogenesis appears to be rare in orders including no or only a small proportion of wingless species, such as Odonata, Plecoptera, Coleoptera and Lepidoptera. Flightlessness and parthenogenesis also tend to co-occur within insect orders. For example, among Coleopterans and Lepidopterans, both winglessness (Roff 1990, 1994) and parthenogenesis are rare, but there are many parthenogenetic species in weevils (Lanteri & Normark 1995) and in bagworm moths (Rhainds *et al.* 2009), two families that also have a comparatively large proportion of wingless species. Although the required phylogenetic data are currently unavailable to test directly whether parthenogenetic insect lineages indeed derive more frequently from wingless sexual ancestors than from good dispersers, the apparent co-occurrence of parthenogenesis and winglessness across and within insect orders is at least suggestive that it might be the case.

If mate limitation and population isolation generally favour the transition to parthenogenesis in metazoans, these processes may also help to explain the poorly understood pattern of geographical parthenogenesis. Geographical parthenogenesis refers to the higher-latitude (in the Northern hemisphere) and higher-altitude distributions in asexual as compared with related sexual taxa (Vandel 1928; Glesener & Tilman 1978; Kearney 2005). Most higher-latitude and higher-altitude habitats have been colonized relatively recently, at the end of the Pleistocene glacial periods. Species living in these habitats are often characterized by large fluctuations in population sizes and/or low densities of individuals (Kearney 2005). Together, these conditions may favour transitions to parthenogenesis through mate limitation and population isolation in a wide range of organisms, and contribute to the abundance of parthenogenetic organisms in high-latitude and high-altitude habitats.

We investigated the rate of tytoparthenogenesis in populations of sexually reproducing *Timema* species. The genus *Timema* also comprises five obligate parthenogens, raising the question of whether one or several of them may have evolved from their sexual ancestors via increasingly high rates of tytoparthenogenesis. Because diploidy in tytoparthenogenetic eggs is restored by automixis, even a relatively small number of tytoparthenogenetic generations would greatly reduce the level of heterozygosity, similar to high levels of inbreeding (Percy *et al.* 2006). In contrast to the sexual species, the *Timema* obligate parthenogens reproduce by apomixis, where offspring are clones of their mother and heterozygosity can increase across generations as a consequence of new mutations (Schwander & Crespi 2009). If the obligate parthenogens evolved from tytoparthenogenetic ancestors, heterozygosity at most loci should stem from such new mutations. However, a large proportion of heterozygosity at microsatellite loci in the *Timema* parthenogens involves two alleles that are both present in the sexual ancestor, suggesting that the alleles have been inherited from the ancestor rather than being generated from new mutations. Unless mutations at these microsatellite loci generate high levels of homoplasy, these patterns are more consistent with a direct transition

from sexual reproduction to apomictic parthenogenesis than with a transition to apomixis via automixis (Schwander & Crespi 2009).

A hypothesis that tytoparthenogenesis is more likely to generate automictic parthenogens (obligate or facultative), whereas apomixis usually evolves directly from sexual reproduction, remains to be investigated. Independent of the detailed origin of the obligate *Timema* asexuals, our results suggest that mate limitation may select for tytoparthenogenesis and that tytoparthenogenesis generates female-biased sex ratios, possibly further enhancing selection for parthenogenesis. Initially, low rates of tytoparthenogenesis may result in the loss of males through a positive feedback interaction between the population sex ratio, female mating probability and the selection for tytoparthenogenesis rates, and provide a route from sexual reproduction to facultative or obligate parthenogenesis. More generally, positive feedback mechanisms may be especially likely to drive major transitions in evolution, given the strength and rapidity with which they can generate large-scale change compared with other mechanisms (Crespi 2004).

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## APPENDIX A

The system defined by equation (2.1) has two positive solutions, one at  $p_F^* = 1$  (a population without males) and one at

$$p_F^* = \frac{\beta(3v - 1) - 2v}{2\beta(2v - 1)}. \quad (\text{A1})$$

When females produce a relatively large proportion of tytoparthenogenetic eggs,  $v > 1/2$ , males are always lost from the population ( $p_F^* = 1$ ). If  $v \leq 1/2$ , the population sex ratio depends on the value of  $\beta$ , the relationship between the mating probability and the proportion of males in the population. If  $\beta > 2v/(1 - v)$ , then the system will converge to the equilibrium  $p_F^* = 1$ , a population without males. If  $\beta < 2v/(1 - v)$ , some proportion of males persist in the population.

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