What Ecological Factors Favor Parthenogenesis over Sexual Reproduction? A Study on the Facultatively Parthenogenetic Mayfly *Alainites muticus* in Natural Populations

Maud Liegeois,[1,2] **Michel Sartori,**[1,2] **and Tanja Schwander**[1,*]

1. Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland;   2. Cantonal Museum of Zoology, Palais de Rumine, Place de la Riponne 6, CH-1014 Lausanne, Switzerland

ABSTRACT: Different reproductive modes are characterized by costs and benefits that depend on ecological contexts. For example, sex can provide benefits under complex biotic interactions, while its costs increase under mate limitation. Furthermore, ecological contexts often vary along abiotic gradients. Here, we study how these factors simultaneously influence the frequency of sex in the facultatively parthenogenetic mayfly *Alainites muticus*. We first verified that parthenogenesis translates into female-biased population sex ratios. We then measured the density of individuals (a proxy for mate limitation) and community diversity (biotic interaction complexity) for 159 *A. muticus* populations covering a broad altitudinal gradient and used structural equation modeling to investigate their direct and indirect influences on sex ratios. We found no effect of community diversity or altitude on sex ratios. Furthermore, even when females can reproduce parthenogenetically, they generally reproduce sexually, indicating that the benefits of sex exceed its costs in most situations. Sex ratios become female-biased only under low population densities, as expected if mate limitation was the main factor selecting for parthenogenesis. Mate limitation might be widespread in mayflies because of their short adult life span and limited dispersal, which can generate strong selection for reproductive assurance and may provide a stepping stone toward obligate parthenogenesis.

Keywords: sex ratios, facultative parthenogenesis, mate limitation, natural populations, ecological factors.

Introduction

Sexual reproduction is by far the most abundant reproductive mode in the animal kingdom, a pattern that is difficult to explain given the many costs associated with sex (reviewed in Lehtonen et al. 2012). A number of theories have been developed that can help explain the advantage of sex, but empirical tests in natural populations remain scarce (Neiman et al. 2018). An ideal approach to identifying conditions under which sex provides benefits is to study variations in the frequency of sex in species capable of facultative parthenogenesis. Such species avoid problems inherent to comparisons between sexual and parthenogenetic species, as there may be species-specific traits that are confounded with reproductive mode. Facultative parthenogenesis is rare among animals overall (Normark 2003; Burke and Bond) but is widespread among mayflies (Ephemeroptera), with at least 49 species reproducing by facultative parthenogenesis (Liegeois et al. 2021). Here, we used the facultatively parthenogenetic mayfly species *Alainites muticus* (Baetidae) to identify ecological correlates of the frequency of sex versus parthenogenesis in natural populations.

Specifically, we evaluated three ecological contexts hypothesized to affect the costs and benefits of sex versus parthenogenesis. First, population densities can affect the level of mate availability, and parthenogenesis can provide benefits through reproductive assurance, especially when males are scarce (Gerritsen 1980; Schwander et al. 2010). Population densities, by affecting the frequency of harassment by males, can also favor parthenogenesis at low densities because in this case the cost of resisting male harassment is low (Gerber and Kokko 2016; Burke and Bonduriansky 2018). Second, it has been suggested that sex, because it has the potential to generate genetic diversity, provides important benefits in complex and saturated communities (Bell 1982; Gaggiotti 1994; Scheu and

* Corresponding author; email: tanja.schwander@unil.ch.
  ORCIDs: Liegeois, https://orcid.org/0000-0001-5657-807X; Sartori, https://orcid.org/0000-0003-3397-3397; Schwander, https://orcid.org/0000-0003-1945-5374.

Drossel 2007; Song et al. 2011). Parthenogenetic species are often genetically uniform, which might result in a narrower niche breadth than in sexual species. Such narrow niches may limit the success of parthenogenesis in complex and saturated communities where biotic interactions, including parasitism, predation, and competition, might be particularly intense and where resources may be more limiting (Bell 1982; Gaggiotti 1994; Scheu and Drossel 2007; Song et al. 2011). Increasing benefits of sex in complex communities is notably believed to explain why parthenogenetic species are rare in the tropics (Bell 1982). Third, a frequently observed pattern in geographical parthenogenesis is that obligately asexual species are more likely to be found at higher altitudes than their sexual counterparts, but hypotheses that could explain the distribution differences remain largely untested (reviewed in Tilquin and Kokko 2016). For example, high altitudes may represent range edges, where reproductive assurance is important and where sexual species are negatively affected by bottleneck consequences or by outbreeding depression because of gene influx from range cores (Peck et al. 1998; Haag and Ebert 2004). Alternatively, parthenogenetic species might be more prevalent in marginal habitats because these habitats harbor communities of reduced complexity and because resources may be less diverse or in shorter supply relative to core habitats (Gaggiotti 1994; Song et al. 2011). Finally, different factors may interact with each other in facultative parthenogens. Indeed, recent theory showed that steep resource gradients can lead to low population densities and less mating at population edges, which results in female-biased populations (Burke and Bonduriansky 2018). However, there are currently no data available to test whether geographical parthenogenesis with more parthenogenesis at higher altitudes also holds for facultative parthenogens. This would indicate that it is direct or indirect consequences of the reproductive mode per se that favor parthenogenesis at high altitudes rather than correlates of parthenogenesis, such as polyploidy and hybrid ancestry, or particular life history or ecological traits enriched in obligately parthenogenetic species.

To elucidate how biotic and abiotic ecological variables, such as the density of individuals, community complexity, and altitude, can affect reproductive strategies, we used structural equation modeling (SEM), a multivariate statistical approach that allows considering interactions between factors and hidden indirect effects (e.g., Grace et al. 2010; Eisenhauer et al. 2015; Fan et al. 2016). Using 159 populations of *A. muticus*, we thus tested whether low population densities favor parthenogenesis, complex communities favor sex, the frequency of parthenogenesis increases with altitude, and how these variables are interconnected with each other.

We focused on the mayfly species *A. muticus* (Baetidae) because of its widespread distribution across altitudinal ranges and its ability to reproduce by facultative, female-producing parthenogenesis (thelytoky; Degrange 1960). To estimate the frequency of sex and parthenogenesis in each population, we used population sex ratios as a proxy. With facultative parthenogenesis, population sex ratios are expected to be female biased if females reproduce at least partly by parthenogenesis, whereas population sex ratios are expected to be approximately equal if females reproduce sexually. To corroborate that population sex ratios indeed reflect the capacity for parthenogenesis, we measured the hatching success of unfertilized eggs laid by females and tested whether hatching successes were correlated with population sex ratios.

Finally, we also assessed the stability of population sex ratios over time by integrating information from two surveys per population, separated by 5 years. Our analyses allow us to test whether community complexity, the density of individuals, factors varying along an altitudinal gradient, or interactions between these different components are key drivers of the frequency of sex in natural populations of a facultative parthenogen.

Material and Methods

Study Sites, Sex Ratios, and Ecological Data

We used samples of *Alainites muticus* from 159 sites (i.e., populations). These samples stem from a biodiversity-monitoring survey performed at 500 sites across Switzerland (fig. S1), following the IBCH (*Indice Biologique Global Normalisé* [IBGN] adapted for Switzerland [CH]) method (described in Stucki 2010). This standardized sampling technique was originally designed to monitor the water quality of streams and rivers on the basis of aquatic macroinvertebrate communities. In brief, each site is sampled from early March to mid-June, covering the ecologically relevant altitudinal gradient in Switzerland (from 193 to 2,635 m; sampling at higher altitudes is done later in the season). To cover all substrates of the riverbed and the range of current velocity, eight square areas (25 cm × 25 cm) are sampled by scraping the riverbed for 30 s and by capturing the macroinvertebrates using a standard-sized net. The corresponding samples are then stored in 80% ethanol. Individuals are sorted to the family level and used for the calculation of a water quality index. For each sampling site, standard data are systematically collected (e.g., altitudes, GPS coordinates). Nymphs of three insect orders (Ephemeroptera, Plecoptera, and Trichoptera) were further identified to the species level and stored at the Museum of Zoology in Lausanne, where we obtained the *A. muticus* samples. The biodiversity monitoring started in 2010, and it took 5 years

to cover all 500 sites across Switzerland. Since 2015, each site has been sampled a second time to evaluate how water quality changes over time.

Alainites muticus occurred at 214 of the 500 sites (fig. S1) across an altitudinal range of 195–1,931 m. For further analysis, we selected all of the sites where more than 15 *A. muticus* individuals could be sexed according to external morphology ($n = 159$ of 214; fig. 1). Nymphs smaller than 2 mm were excluded from sex ratio calculations, as they cannot be sexed reliably. The straight-line distance between these 159 sites ranges from 5 to 300 km (mean = 99.7, median = 96.4). We then assessed biotic and abiotic variables of each site: population densities, community diversities, and altitudes. We used two different density estimates—the total number of nymphs found in each population and the sexed individuals only—but results were not affected by the estimate used. Results included in this article are based on the number of sexed individuals. Community diversities were estimated using the Shannon's index ($H = -\sum_{i=1}^{S} p_i \times \ln(p_i)$, where p_i is the proportion of individuals belonging to the *i*th species in the data set of interest). Given the different level of identification for different taxa, (species level for Ephemeroptera, Plecoptera, and Trichoptera; family level for all other macroinvertebrates), the index was based on abundances

at the family level. Note that we also used other biodiversity indexes, including Margalef's index, Simpson's index, and Hill's numbers (Simpson 1949; Margalef 1956; Heip et al. 1988; Magurran 2004; Keylock 2005; Jost 2006; Daly et al. 2018), to ascertain that our main conclusions were consistent according to different diversity measures.

To test whether *A. muticus* sex ratios, densities, and macroinvertebrate community diversities were relatively stable over time, we used the 92 of the 159 sites that were surveyed twice (with a time gap of 5 years). For the remaining 67 populations, we have data from only a single sampling event.

Parthenogenetic Capacity

Parthenogenetic reproduction is expected to generate female-biased sex ratios, with a more extreme female bias in populations with a higher frequency of parthenogenesis. To determine whether the parthenogenetic capacity of females is positively correlated with population sex ratios, we aimed to obtain virgin females from 20 of the 159 *A. muticus* populations by rearing female nymphs in the absence of males. Populations were chosen to cover the range of population sex ratios found in *A. muticus* (fig. 1). Up to 20 late-instar female nymphs (range = 4–20,
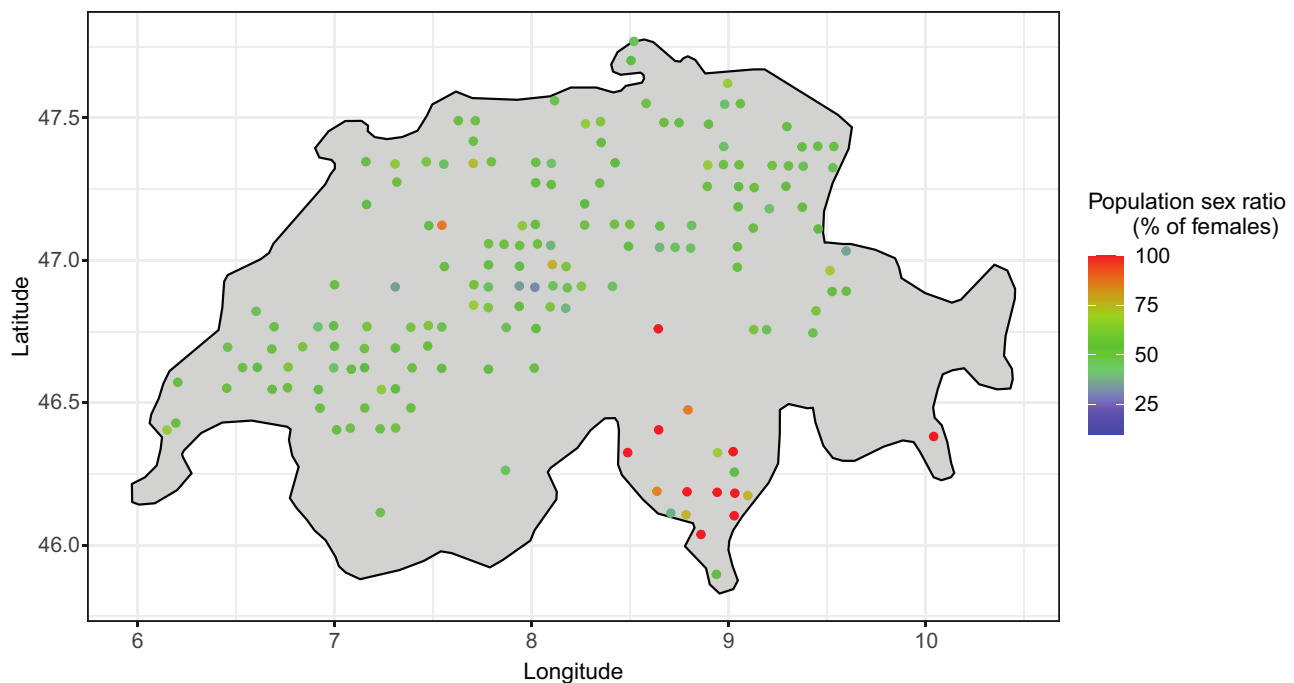
Figure 1: Distribution across Switzerland of the 159 sampling sites used in our study. *Alainites muticus* sex ratios significantly vary between populations (generalized linear model, $P < .001$). Populations are colored according to population sex ratio, given as the proportion of nymphs large enough to be sexed that are female.

mean = 14) were collected from 16 of the 20 populations (during May to July 2016, May to June 2017, and April to May 2018); no late-instar nymphs could be obtained from the four remaining populations. Female nymphs were taken to the laboratory and reared to adulthood in aquariums in a climate chamber (12°C ± 1°C water temperature, 22°C ± 2°C room temperature, 50% relative humidity, 12L:12D photoperiod). Aquariums were supplied with lake water to provide nutrients for nymphal growth until the final molt. The water temperature was maintained at 12°C (±1°C) using a thermoregulator and continuous water flow. Oxygenation was supplied through the water flow as well as from bubblers placed in each aquarium. Nymphs were reared in partially immersed floating cages (fig. S2). Submerged parts were surrounded by thin mesh that allowed water flow while preventing mayflies from escaping, and the aerial parts allowed individuals to emerge. Emergences were checked daily, and subimagoes were transferred to individual cages. Once they completed their final molt (from subimago to imago), we allowed them to lay (unfertilized) eggs in a Petri dish (55 mm in diameter) filled to three-fourths with filtered (0.2 μm) lake water. Using these conditions, we obtained 56 virgin *A. muticus* females, of which 11 did not lay any eggs spontaneously. For these females, we extracted the eggs by dissecting their abdomen after they died (within 0–12 h). We counted the number of eggs per clutch, divided them into two or three new Petri dishes (35 mm in diameter) to reduce egg densities per dish and facilitate observation of hatchlings, and maintained them at 10°C with a 12L:12D photoperiod. After about 3 weeks of incubation, the eggs began to hatch. Hatchlings were counted and removed from each Petri dish every day, and the cumulative number of hatched eggs was recorded for each virgin female. After the cessation of hatching, we calculated the hatching success (proportion of unfertilized eggs that hatched) for each female. The population-level parthenogenetic capacity was estimated as the mean hatching success across all females collected from that population. In total, we obtained one to seven egg clutches (i.e., eggs from one to seven females, mean = 3.5) for each of the 16 populations.

Statistical Analyses

We first determined whether there was significant variation in sex ratios among sites using a binomial generalized linear model (GLM) in R (ver. 3.6.2; R Development Core Team 2019). We then tested whether the parthenogenetic capacity in each population (measured as the mean hatching success of unfertilized eggs) was related to the population sex ratio. To do so, we ran a quasibinomial GLM (GLM with binomial error distribu-

tion, corrected for overdispersion) followed by an *F*-test from the car package (ver. 3.0-6; Fox and Weisberg 2019). Data points were weighted in the analysis according to sample size (the number of females tested per population).

Finally, to identify ecological correlates of female-biased sex ratios (i.e., frequency of parthenogenesis), we used SEM, which tests for the nature and the magnitude of direct and indirect effects of each explanatory variable on the response variable (e.g., Grace 2006; Fan et al. 2016). Specifically, we tested whether *A. muticus* population density, macroinvertebrate community diversity, and altitude significantly explained sex ratio variation across populations. On the basis of hypothesized causal relationships and correlations among the variables in the SEM, we built our initial metamodel and developed an SEM (fig. 2; see "Results") that was analyzed using the piecewiseSEM package (ver. 2.1.0; Lefcheck 2016). An SEM is built using a list of structured equations, which can be specified using common linear modeling functions in R and thus can accommodate nonnormal distributions, nonindependence of observations, and so on. Then on the basis of model fit indexes calculated for the overall goodness of fit (i.e., Fisher's *C*, comparative fit index) and for each path (i.e., *P* value and standard error), we evaluated model-data consistency to determine whether there were missing links in the initial metamodel, as well as to determine the support for tested links. The Fisher's *C* statistic tests the hypothesis that there is a discrepancy between the model-implied covariance matrix and the original covariance matrix. Therefore, a nonsignificant discrepancy (*P* > .05) indicates an acceptable model fit. The comparative fit index represents the amount of variance that has been accounted for in the covariance matrix. A higher value indicates a better model fit (best is comparative fit index > 0.95). We used these two indexes as well as the Akaike information criterion from the AICcmodavg package (ver. 2.2-2; Mazerolle 2019) for model selection. In the final model, we corrected the model outputs for spatial autocorrelation using Moran's *I* (Moran 1950) within the ape package (ver. 5.3; Paradis and Schliep 2018). We also performed Grubbs's tests from the outliers package (ver. 0.14; Komsta 2011) to identify outliers in our data, and we reran all of the analyses without outliers to assess the robustness of our results. Removing outliers did not qualitatively affect our results, and thus outliers have no impact on our conclusions.

Finally, to test whether population variables were relatively stable over time, we developed a new SEM (fig. 3; see "Results") using the data available for both surveys (i.e., 92 populations). All plots were generated using the ggplot2 package (ver. 3.3.5; Wickham 2016), in addition to the rworldmap package (ver. 1.3-6; South 2011) for the map in figure 1.
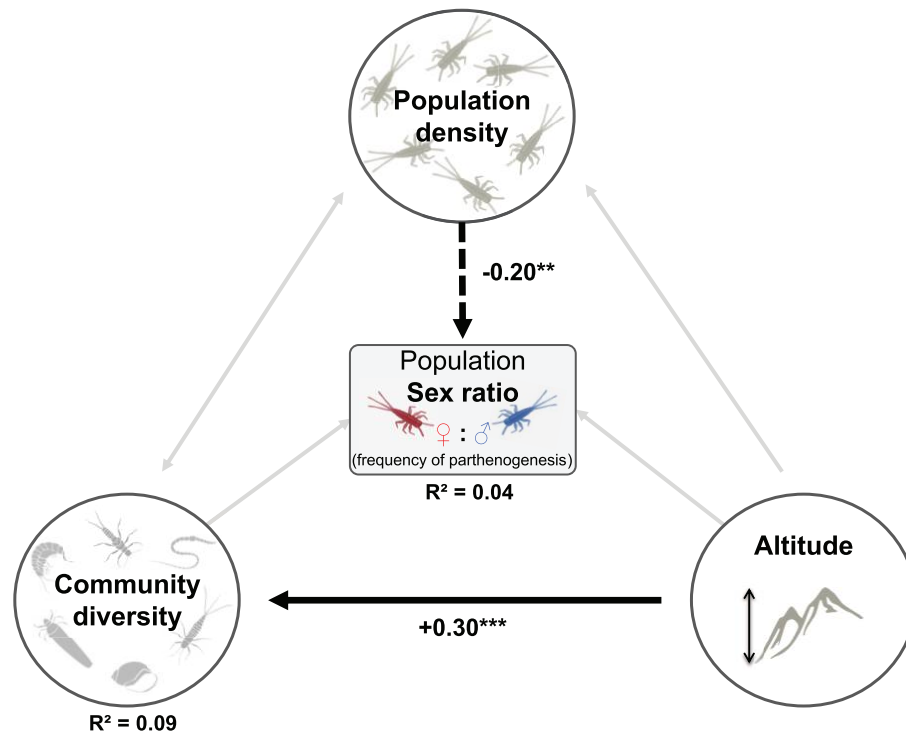
Figure 2: Structural equation metamodel showing expected interconnections between ecological variables and the pathways fitted to collected data. Black arrows represent significant paths with positive (solid line) or negative (dashed line) effects ($P < .05$). Gray arrows represent nonsignificant paths that were evaluated in the metamodel but removed from the final model ($P > .30$). Path coefficients correspond to standardized effects (strength and direction of the linear relationship between the two variables) and their probability. The R^2 values are displayed on response variables, representing the proportion of variance explained. Test statistic $F = 5.61$, df $= 6$, $P = .47$, comparative fit index $= 1$ (indicating close model-data fit; see "Material and Methods" for details). $**P \leq .01$; $***P \leq .001$.
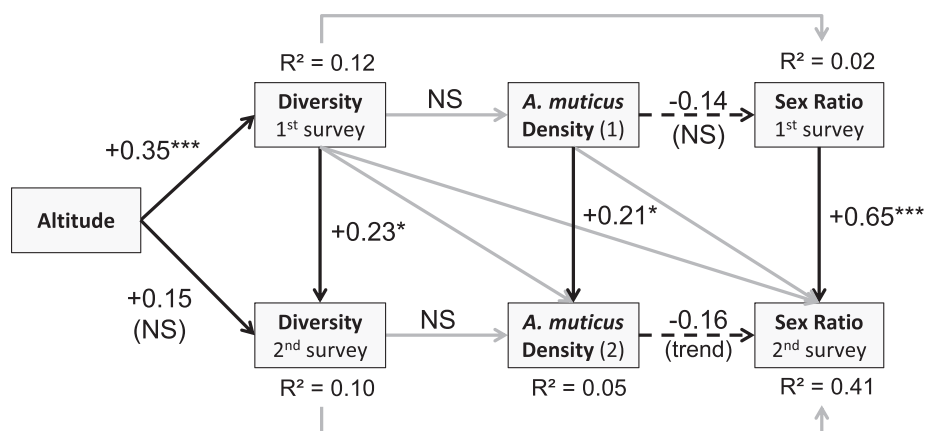
Figure 3: Structural equation model including two surveys per site ($n = 92$) to consider potential temporal fluctuation of population variables. Gray arrows represent nonsignificant paths that were removed from the initial model. Black arrows represent paths with positive (solid line) or negative (dashed line) effects. Path coefficients correspond to standardized effects (strength and direction of the linear relationship between two variables) and their probability. The R^2 values are displayed on response variables, representing the proportion of variance explained. Test statistic $F = 31.37$, df $= 26$, $P = .22$ (indicating close model-data fit; see "Material and Methods" for details). $*P \leq .05$; $***P \leq .001$.

Results

Field Evidence of Parthenogenetic Reproduction

To determine whether natural populations with a higher proportion of females also feature a higher parthenogenetic capacity, we used the average level of hatching success of unfertilized eggs of all 16 surveyed populations and checked whether it was related to the population sex ratio. In total, we were able to obtain unfertilized eggs from 56 females (one to seven females per population, mean = 3.5), and 51,771 eggs were observed for their parthenogenetic development (~1,000 eggs per female; range = 372–2,764). Hatching successes of unfertilized eggs (averaged across females) were significantly related to population sex ratios (fig. 4; GLM, $r = 0.90$, $P < .001$), meaning that a high parthenogenetic capacity of females in the field likely translates into female-biased population sex ratios. Given this result, we used sex ratios as a proxy of parthenogenetic capacity in natural populations.

Densities and Mate Limitation

Population densities of the 159 *Alainites muticus* sites ranged from 15 to 450 sexed individuals per sampled sur-

face (mean = 77.4, median = 52). Our SEM revealed that population densities have a significant direct and negative effect on population sex ratios (the proportion of females; figs. 2, 5*A*; $r = -0.20$, $P = .002$), suggesting that mate limitation (or reduced harassment from males) selects for increased parthenogenetic capacity in natural *A. muticus* populations (see also fig. S3).

Community Diversity and Competition Hypotheses

As explained above, different hypotheses predict that sexual reproduction may be more prevalent in complex and diverse communities. Using Shannon's index as an indicator of community diversity within the SEM (see "Material and Methods" for details), we did not find any direct effect of community diversity on *A. muticus* sex ratios (i.e., parthenogenesis frequencies), resulting in the removal of this nonsignificant path from the model (figs. 2, S5*A*). In addition, we did not find any indirect effect of community diversity on sex ratios (figs. 2, S5). Thus, our analyses do not detect an effect of community diversity on selection for sex in natural populations, either directly or indirectly. Note that similar results are obtained using
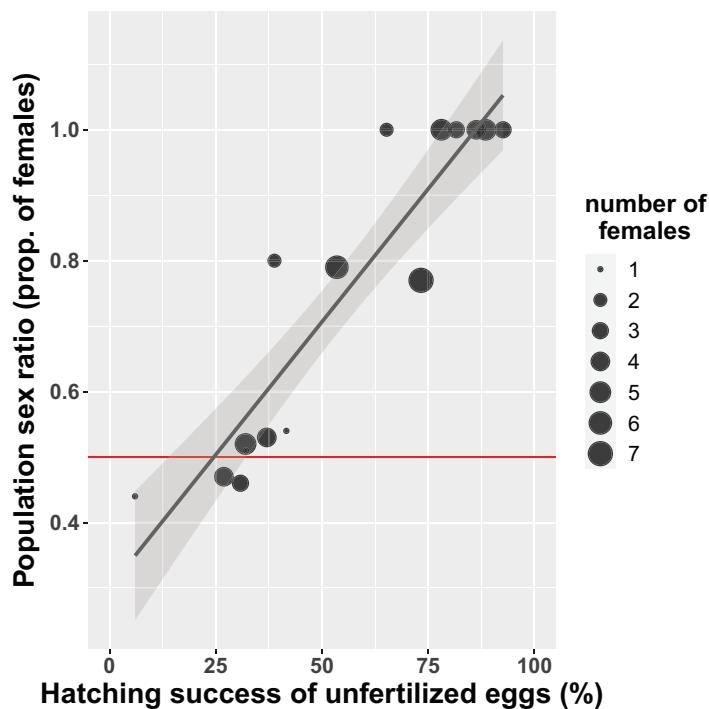
Figure 4: Parthenogenesis correlates strongly with female-biased sex ratios in the field. Circle sizes are proportional to the number of females used to measure the parthenogenetic capacity for each population (i.e., one to seven females). Population sex ratios were estimated on the basis of 15–322 sexed individuals per population (mean = 90.4, median = 54). The black line represents the fitted linear model (generalized linear model, $r = 0.90$, $P < .001$), and the gray shading represents the 95% confidence interval on the fitted values. The horizontal line represents a balanced sex ratio with equal numbers of males and females.
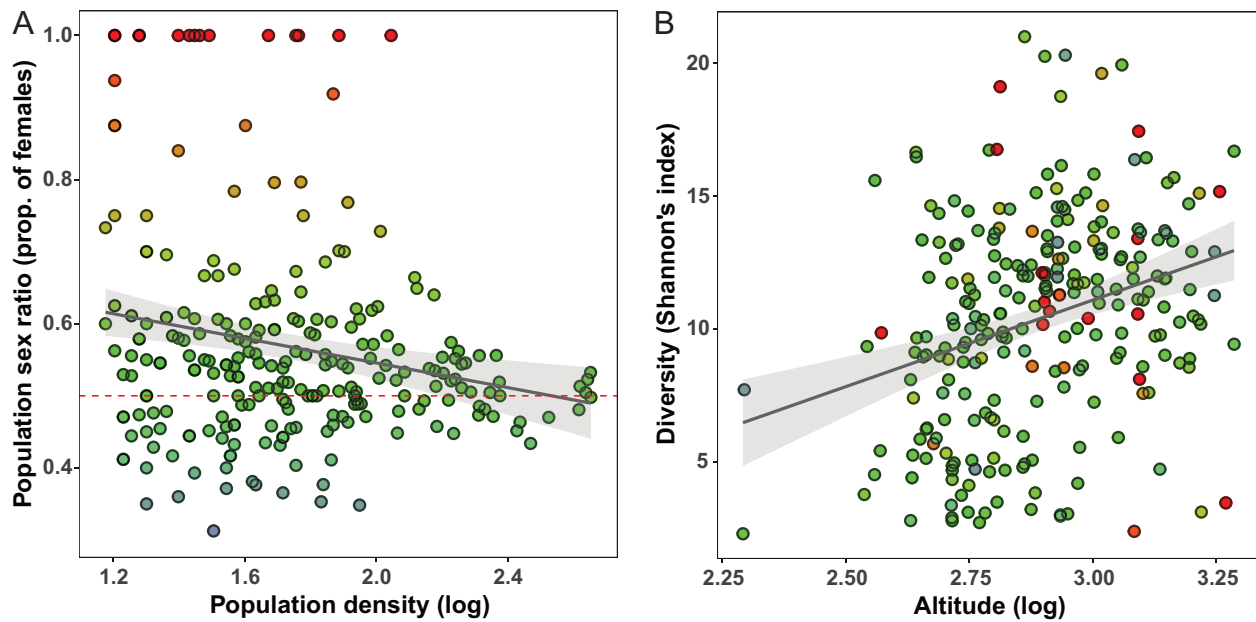
Figure 5: Multivariate partial plots for the significant paths in figure 2. Note that for the 92 of the 159 populations that were surveyed twice, both values are plotted (site ID was included as a random factor in the model). The partial plots for the nonsignificant paths are in figure S5. The color gradient shows the population sex ratios (proportion of females), as in figure 1. The black line represents the fitted linear model, and the gray shading represents the 95% confidence interval on the fitted values. *A*, Partial relationship of the direct significant effect of population densities on population sex ratios. The horizontal dashed line represents a balanced sex ratio (see also fig. S3). *B*, Relationship between altitude and community diversity (see also fig. S4). Note that the positive effect of altitude on community diversity was for the altitudinal range of *Alainites muticus* populations. Across the full altitudinal range of all surveyed sites (including those without *A. muticus*), diversity peaks at midelevation (see fig. S6).

other diversity indexes, such as Margalef's index, Simpson's index, or Hill's numbers (fig. S7).

Altitude and Geographical Parthenogenesis Patterns

Various abiotic characteristics vary along altitudinal gradients, and frequencies of parthenogenesis could be affected by those abiotic factors, directly or indirectly. However, we found no direct effect of altitude on population sex ratios (figs. 2, S5*B*), which does not corroborate the pattern observed in among-species comparisons (Tilquin and Kokko 2016.). We also did not find any indirect effect of altitude on sex ratios (figs. 2, S5). Nevertheless, there was a significant direct and positive effect of altitude on community diversity (figs. 2, 5*B*; $r = 0.30$, $P < .001$; see also fig. S4), which means that at higher altitudes, aquatic communities are more diverse. Note that similar results are obtained using other diversity indexes, such as Margalef's index, Simpson's index, or Hill's numbers (fig. S7). This is typical for aquatic communities within the observed altitudinal range in Switzerland; streams at higher altitudes are closer to the source

and less affected by pollution from human activities, which results in more diverse but less abundant communities (Lang and Reymond 1993).

Temporal Variability of Populations

To assess the stability of population variables over time, we ran a new SEM with the 92 (of 159) populations that were surveyed twice (fig. 3). We found that sex ratios remained relatively stable between surveys, as shown by the significant positive correlation between sex ratios of the two surveys (figs. 3, 6*A*; $r = 0.65$, $P < .001$). This result suggests that sex ratios and thus the frequency of parthenogenesis within populations are relatively stable (41% of the variance explained). Most variation in sex ratios between years was for populations with low densities, which is most likely due to sampling effects, since sex ratio estimates are less exact for small sample sizes. Population densities also remained relatively stable between surveys, as shown by the significant positive correlation between densities of the two surveys (figs. 3, 6*B*; $r = 0.21$, $P = .04$).
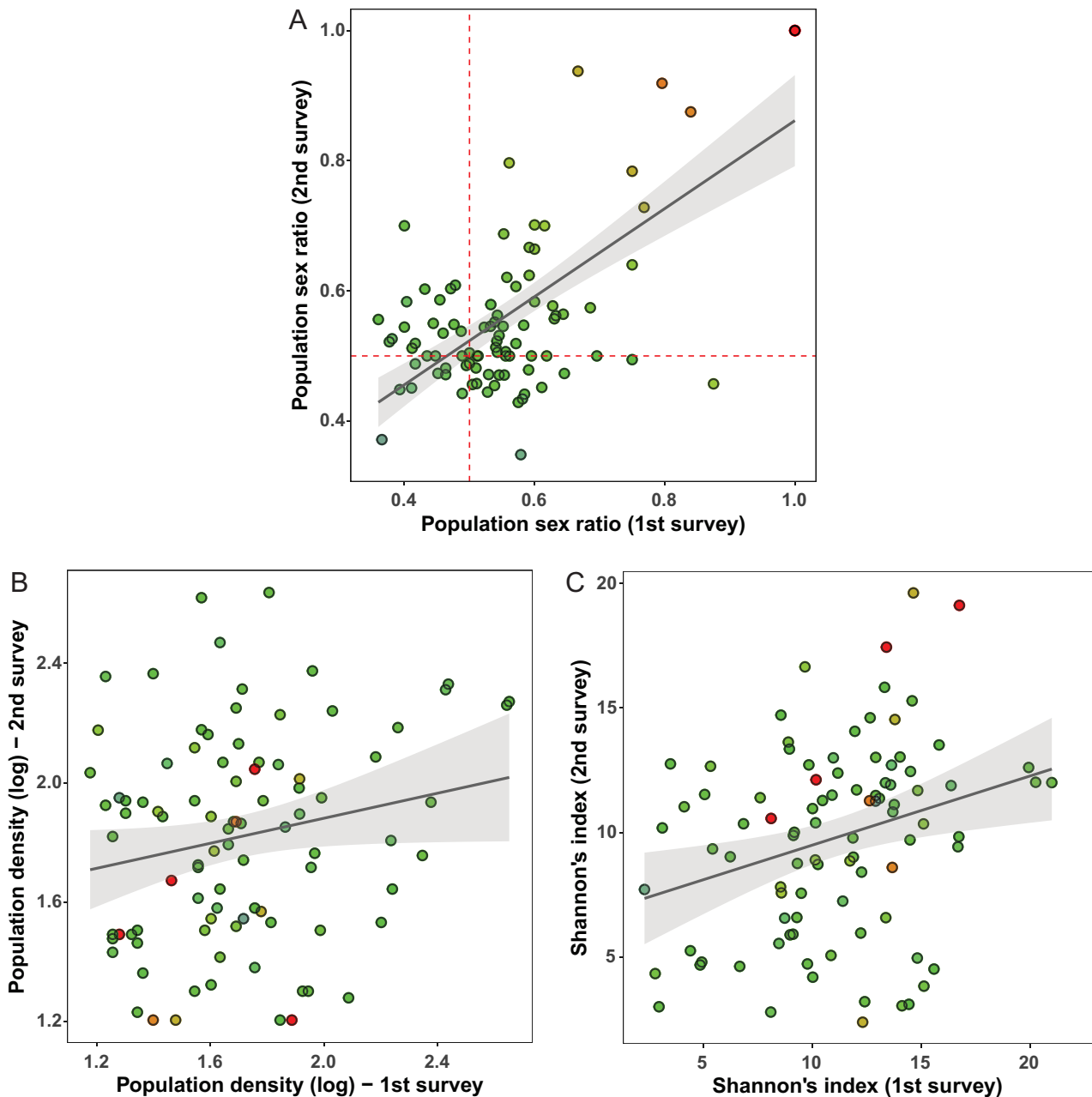
Figure 6: Multivariate partial plots over time. Significant positive effect of the first survey on the second one for population variables. The black line represents the fitted linear model, and the gray shading represents the 95% confidence interval on the fitted values. *A,* Population sex ratios (red dashed lines represent the balanced sex ratios; $r = 0.65, P < .001$). *B, Alainites muticus* population densities (estimated from the number of sexed individuals; $r < 0.21, P = .04$). *C,* Macroinvertebrate community diversities ($r = 0.23, P = .04$). The color gradient shows the population sex ratios (proportion of females), as in figure 1.

However, there was considerably more variation than for sex ratios, with only 5% of the variance explained (fig. 3). Similarly, we also found that community diversities of the two surveys were significantly correlated (figs. 3, 6*C*; $r = 0.23, P = .04$), suggesting that diversities are also relatively stable over time, with 10% of the variance explained (this

correlation is stronger if considering all 500 sites, i.e., including sites without *A. muticus* present; $r = 0.45$).

Note that we did not detect any crossed effect of variables from the first or the second survey. Notably, population density of the first survey does not affect the sex ratio of the second survey, meaning that sex ratios are apparently
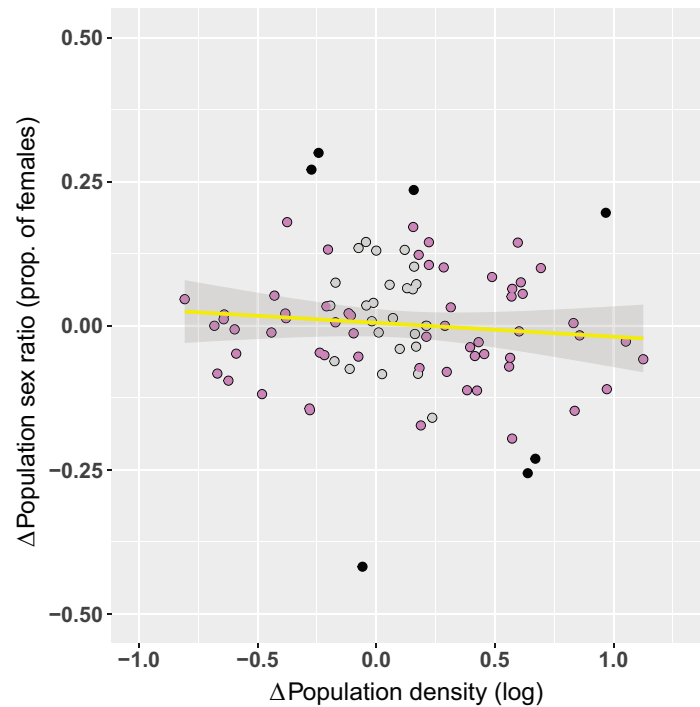
Figure 7: Within-population differences between surveys for sex ratio and density. The yellow line shows the overall relationship ($n = 92$). The black circles show populations that display a significant change in sex ratio over time ($n = 7$). The pink circles show populations that display a significant change in density over time ($n = 65$).

not shifted in the short term according to the past local densities, as would be expected if parthenogenetic capacities were plastic. To further investigate this, we looked at the within-population variation between surveys for sex ratios and densities (fig. 7).

We found no significant correlation between the extent of the within-population density shifts and the within-population sex ratio shifts between surveys (fig. 7; $P = .38$), again suggesting that sex ratios are not shifted in the short term according to the current local densities. However, some populations displayed a significant change in sex ratio ($n = 7$; binomial tests, $P < .05$; fig. 7), and most populations displayed a significant change in population densities ($n = 65$; binomial tests, $P < .05$; fig. 7). For the latter, the change in density was not correlated with the change in population sex ratio (fig. 7, pink circles), as expected if parthenogenetic capacities were not very plastic.

Discussion

The aim of our study was to evaluate how different ecological factors hypothesized to affect the benefits of sex and parthenogenesis affect the frequency of these two reproductive modes in natural populations of the facultatively parthenogenetic mayfly *Alainites muticus*. Specifically, theory predicts that the benefits of sex increase in

complex communities with diversified resources and complex biotic interactions (Bell 1982; Gaggiotti 1994; Scheu and Drossel 2007; Song et al. 2011). On the other hand, benefits of parthenogenesis may increase under mate limitation and if costs from males trying to enforce copulations with parthenogenetic females are low, which is the case in marginal or low-density populations (Gerritsen 1980; Schwander et al. 2010; Gerber and Kokko 2016). Furthermore, community complexity and population densities often vary in accordance with abiotic factors along ecological gradients, such as altitude, whereby interactions between these different components can drive the geographical distribution of sex and parthenogenesis in facultative parthenogenetic species (Burke and Bonduriansky 2018). By simultaneously analyzing the impact of the different components as well as interactions between them, we have shown that population density is the major driver of reproductive mode variation in *A. muticus*. By contrast, we found no evidence for community complexity or biotic or abiotic factors varying along altitudinal gradients to influence reproductive modes.

Two different mechanisms could increase the frequencies of parthenogenesis in low-density populations. First, parthenogenesis could be used for reproductive assurance when females are mate limited. Mate limitation is expected to be widespread in mayflies, as they are characterized by a

very short adult life span, which may generate strong selection for reproductive assurance (Liegeois et al. 2021). Consistent with this idea, negative correlation between sex ratios and/or hatching successes of unfertilized eggs and population densities has also been reported for the mayfly species *Eurylophella funeralis* (Sweeney and Vannote 1987), *Ephemerella notata* (Glazaczow 2001), *Ephoron shigae* (Tojo et al. 2006), and *Stenonema femoratum* (Ball 2002). Reproductive assurance through facultative parthenogenesis can generate more strongly female-biased sex ratios, which in turn increases mate limitation for females and selection for parthenogenesis in a positive feedback loop, which can result in the loss of males (Schwander et al. 2010). Widespread facultative parthenogenesis and selection for reproductive assurance could thus also help explain why mayflies have more known parthenogenetic species than any other animal order (Liegeois et al. 2021). A second mechanism that can generate negative correlation between population densities and parthenogenetic frequencies is costly harassment from males (Kawatsu 2013; Gerber and Kokko 2016; Burke and Bonduriansky 2017). Specifically, females with facultative parthenogenesis may pay the cost of resisting male harassment and mating attempts if they try to reproduce parthenogenetically. Thus, parthenogenesis would be more costly to females in populations with high frequencies of males. In this case, females reproducing sexually will be favored at high densities, as harassment would likely increase with male availability (Gerber and Kokko 2016). Notably, male coercion might also explain why parthenogenesis is only rarely used in our surveyed natural populations with facultatively parthenogenetic females. Indeed, populations in our survey have generally balanced sex ratios (79.2% of the 159 sampling sites), even though most females are capable of parthenogenesis. More generally, our survey suggests that females generally prefer to reproduce via sex rather than via parthenogenesis or that if females have mated, they are unable to produce parthenogenetic eggs.

A potential explanation for why we did not find the expected effect of community complexity is that our measures of community diversity did not take into account microorganisms (i.e., the most likely parasite communities) or big predators (e.g., fishes). In addition, parasites can be a more important threat in high-density populations (e.g., Arneberg et al. 1998; Lagrue and Poulin 2015), and population densities can also affect the level of intraspecific competition for resources. Because about 95% of a typical mayfly life cycle occurs at the nymphal stage and because adults do not feed, competition for resources is primarily a function of nymph densities. This might be another explanation for why we did not find the expected effect of community diversity on reproductive strategies but why

females in high-density populations generally reproduce via sex instead of parthenogenesis in *A. muticus*.

As for altitude, we expected a positive relationship with the frequency of parthenogenesis, which we did not find in *A. muticus*. Thus, it seems that altitude may affect the relative success of sexual and asexual species (reviewed in Tilquin and Kokko 2016), but it does not influence the frequency of reproductive modes in facultative parthenogens (i.e., within species). A probable explanation for this difference is that the altitudinal pattern is driven by species-specific traits that are confounded with reproductive modes. Indeed, in geographical parthenogenesis (i.e., in between-species comparisons), asexuals are often polyploid or hybrids, meaning that the pattern observed might not be caused by the reproductive mode per se (Kearney 2005). In our survey, we further detected a positive effect of altitude on community diversity for sites where *A. muticus* was present. This result suggests that abiotic factors, such as altitude, could create situations presenting suitable habitat conditions for increased community diversities and potential effects on population sizes that we did not detect in our data set.

Finally, to investigate whether differences in population sex ratios were more likely due to plasticity or genetic differences between populations, we evaluated whether population densities and sex ratios were relatively stable over time. We found that both population sex ratios and densities were correlated between sampling years. We also found no significant correlation between the extent of the within-population density shifts and the within-population sex ratio shifts. In combination, these results suggest that sex ratios are not modified in the short term according to the current local densities and are thus more likely generated by differences in parthenogenetic capacities between populations rather than plastic changes in the use of parthenogenesis.

Our population surveys also revealed an unexpected clustering of female-only populations of *A. muticus* south of the Alps, which could indicate the presence of obligately parthenogenetic lineages. Unisexual populations of *A. muticus* are also known in eastern Ukraine (Martynov 2013), supporting the idea that this species might be characterized by reproductive polymorphism and geographical parthenogenesis. However, formally testing this idea, as well as investigating potential genetic differentiation between females with different reproductive modes, required different types of data and is a challenge for future studies.

To conclude, we suggest that low population density is the major driver of reproductive mode variation in facultatively parthenogenetic mayflies, with little or no impact from other factors, such as community complexity or biotic and abiotic factors correlated with altitude. Low

population densities may generate situations of mate limitation for females or reduced costs associated with male coercion. The extremely short adult life span and limited dispersal abilities of mayflies may frequently generate locally low population densities and thus provide a stepping stone toward obligate parthenogenesis. Yet even when females have the capacity to reproduce via parthenogenesis, they generally reproduce sexually, indicating that the benefits of sex for females must exceed its costs in most ecological situations.

Statement of Authorship

M.L. and T.S. designed the study. T.S. acquired funding. M.L. collected, analyzed, and visualized the data with input from T.S. M.L. wrote the original draft of the manuscript. M.L., T.S., and M.S. revised and edited the manuscript. M.S. and T.S. provided resources.

Data and Code Availability

Data, codes, and supplemental information are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.g79cnp5s7; Liegeois et al. 2022).

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Associate Editor: Emily H. DuVal
Editor: Jennifer A. Lau

"Under exciting circumstances one cannot take in the whole situation at once. The ground work of the flanks of my ophidian was of a beautiful clear yellow, intermediate between a lemon and orange shade, much brighter than a cream-yellow, and which prevented me from determining him at first as an *Ancistrodon contortrix*." From "The Copperhead" by Richard E. Kunzé (*The American Naturalist*, 1883, 17:1229–1238).