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PARTHENOGENESIS IN MAYFLIES (EPHEMEROPTERA)

Liegeois Maud

Liegeois Maud, 2020, PARTHENOGENESIS IN MAYFLIES (EPHEMEROPTERA)

Originally published at : Thesis, University of Lausanne

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Document URN : urn:nbn:ch:serval-BIB_884B595C1DDC3

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Faculté de biologie
et de médecine

Département d'Écologie et Évolution

PARTHENOGENESIS IN MAYFLIES (EPHEMEROPTERA)

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

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Lausanne 2020

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale
Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président·e	Monsieur	Prof.	Christian	**Fankhauser**
Directeur·trice de thèse	Madame	Prof.	Tanja	**Schwander**
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le Conseil de Faculté autorise l'impression de la thèse de

Madame Maud Cécile Clara Liégeois

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intitulée

Parthenogenesis in Mayflies (*Ephemeroptera*)

Lausanne, le 20 mai 2020

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Niko GELDNER
Directeur de l'Ecole Doctorale

À mon papa...

REMERCIEMENTS

Avant toute chose, je souhaite remercier mes deux superviseurs, **Tanja Schwander** et **Michel Sartori**, pour m'avoir permis de réaliser cette thèse. Merci de m'avoir fait confiance et de m'avoir aussi bien encadrée, tout en me laissant beaucoup d'autonomie. Qui aurait cru que ce projet puisse être mené à bien, alors que je n'avais aucune connaissance ni sur les éphémères, ni sur l'évolution des modes de reproduction ? J'ai dû tout apprendre depuis le début, mais entourée des deux meilleurs spécialistes, je me suis régalée, et je vous en suis très reconnaissante.

Merci également aux autres membres de mon jury de thèse, à savoir mon président **Christian Fankhauser**, et mes experts **Patrice David** et **Olivier Glaizot**, pour avoir pris le temps d'évaluer et commenter, de manière constructive, mes travaux de recherche.

Un grand merci à tous les 'Sexperts' du groupe Schwander, passés ou présents. Que vous soyez post-doctorant(e), doctorant(e), technicienne ou bioinformaticien, vous avez tous contribué à ce projet un peu fou, tant scientifiquement que socialement. Alors, merci **Silvia Paolucci**, **Kirsten Jalvingh**, **Jens Bast**, **Darren Parker**, **Susana Freitas**, **Chloé Larose**, **Casper van der Kooi**, **Karim Ghali**, **Jeli Djordjevic**, **Guillaume Lavanchy**, **Eléonore Genzoni**, **Zoé Dumas**, **Marjorie Labédan** et **Patrick Tran Van** pour votre présence au quotidien, vos conseils avisés et votre aide précieuse.

Merci également à **Luca Sciuchetti**, **Aline Cardinaux**, **Nathalie Caloz**, **Manon Pribille** et **Victor Othenin-Girard**, mes aides estudiantines, pour toutes les tâches ingrates que vous avez effectuées avec brio. Merci **Franck Chalard**, notamment pour tes dons de bricoleur, ta gentillesse et ta patience. Merci **Léna Kolececk** et **Marlène Petit**, les reines de l'administration, pour m'avoir porté secours aux moments opportuns.

Je teins bien sûr à remercier tous mes ami.e.s et collègues, que je ne pourrais nommer un par un. Que vous soyez du Musée, de l'UniL ou d'ailleurs, comment aurais-je pu survivre sans ces apéros, pokers, soirées dansantes, défis sportifs, barbecues au bord du lac, et j'en passe… Mais tout de même, un merci particulier à **Marie Sgandurra**, **Marion Podolak**, **Clotilde Berling**, **Aline Dépraz**, **Chloé Larose**, **Eléonore Genzoni** et **Susana Freitas**, pour aussi avoir été mes 'psychothérapeutes' dans les moments difficiles. Et merci à **Stéphanie Mermet** et **Jérôme Gippet** pour ce coup de foudre amical !

Thank you to **Sean McGregor** and **Katja Hoedjes**, my amazing office mates. Thanks for all kinds of moment we shared together. With a special thank you to **Sean** for being my close friend, and who improved my English a lot (as much as your French?).

Un grand merci à ma famille sans qui je ne serais pas celle que je suis aujourd'hui, curieuse et passionnée. Merci donc à mes parents **Florence** et **Jean-Marc**, à mes sœurs et mon frère **Fanny**, **Magali**, **Vincent** et **Anaïs**, pour m'avoir transmis, entre autres, partage et ouverture d'esprit.

Et, le meilleur pour la fin, MERCI **Yannick** pour m'avoir soutenue depuis le début (et surtout à la fin), pour avoir pris soin de moi, et m'avoir supportée au quotidien malgré le stress et le confinement.

Alors **merci** à tous, sans vous, je n'aurais jamais été capable d'accomplir cette thèse…

RÉSUMÉ VULGARISÉ

Sexe et reproduction ne sont pas synonymes, bien qu'ils soient très souvent liés puisque la plupart des animaux se reproduisent de façon sexuée. Le sexe allie méiose et fécondation pour donner un nouvel individu possédant 50% des gènes de chacun de ses deux parents. Mais plusieurs organismes ont évolués vers une reproduction sans sexe. Par exemple, la reproduction végétative chez les plantes ne fait ni appel à la méiose, ni à la fécondation. Les descendants sont produits par mitose, ce sont des clones de leur « parent ». La fragmentation est aussi une reproduction clonale, notamment connue chez les éponges et les étoiles de mer, où un individu entier peut être régénéré à partir d'un fragment de son « parent ». La parthénogenèse est un autre mode de reproduction asexuée, où il peut y avoir méiose mais pas fécondation. Le nouvel individu n'hérite alors que des gènes de sa mère mais n'est pas toujours son clone.

Bien que moins fréquente, la reproduction asexuée semble plus simple, plus rapide et moins coûteuse que la reproduction sexuée. En effet, il n'y a pas besoin de perdre du temps et de l'énergie pour trouver le bon partenaire sexuel, pas de risques de transmission de maladies ou de parasites, pas de rivalité entre femelles ou de concurrence entre mâles, pas de conflits… Alors, pourquoi ne pas se cloner comme les étoiles de mer, ou faire de la parthénogenèse comme les phasmes ? Se passer de mâles et se passer de sexe !? D'autant plus que les mâles semblent inutiles puisqu'ils ne sont pas capables de donner la vie à proprement parler. Les raisons du maintien du sexe, ou d'une évolution vers une reproduction asexuée, sont aujourd'hui encore mal connues. Le sexe offrirait des avantages considérables qui permettraient de contrebalancer ses coûts. Par exemple, la diversité génétique s'avère être très importante quand il s'agit de s'adapter pour survivre (lorsque l'environnement change ou lorsqu'il faut faire face à des prédateurs ou des parasites). Le sexe serait donc la source de la diversité nécessaire à la sélection naturelle, le moteur de l'évolution. Mais alors, comment font les populations asexuées pour évoluer, s'adapter et persister, sans brassage génétique ?

En étudiant les éphémères, des insectes dont la vie adulte très courte ne laisse pas beaucoup de temps pour se reproduire, nous comptons éclaircir ces points encore obscurs : Quelle est la réelle fréquence de la parthénogenèse utilisée dans la nature ? A quel point la capacité parthénogénétique affecte les sex-ratios en population naturelle ? Y-a-t-il des différences de comportement notables en fonction du mode de reproduction utilisé ? Quels sont les facteurs écologiques qui favorisent la parthénogenèse par rapport à la reproduction sexuée en condition naturelle ? Si elles existent, les différences observées sont-elles dues à une plasticité phénotypique ou à une adaptation génétique ? Et plus globalement, pourquoi le sexe est-il si omniprésent alors que la parthénogenèse présente des avantages considérables ? Cette thèse permet d'éclairer certains mystères, d'apporter des éléments de réponse quant au « paradoxe du sexe »…

RÉSUMÉ SCIENTIFIQUE

L’évolution et le maintien de la reproduction sexuée font partie des questions majeures en écologie évolutive depuis quelques décennies. Sexe et reproduction ne sont pas synonymes, bien qu’ils soient souvent liés puisque la plupart des animaux se reproduisent de façon sexuée. Cependant, la reproduction sexuée (qui allie méiose et fécondation) est très coûteuse, elle doit donc pouvoir fournir des avantages considérables qui compensent ses coûts. De plus, les coûts et les avantages du sexe peuvent varier en fonction de différents facteurs, comme les cycles biologiques ou l’écologie des espèces. Plusieurs modèles théoriques ont étudié cette variabilité entre coûts et bénéfices du sexe, mais les études empiriques restent rares.

Plusieurs organismes ont évolué vers une reproduction sans sexe, comme la parthénogenèse. L’étude des modes de reproduction alternatifs est fondamentale pour identifier l’équilibre entre les coûts et les bénéfices du sexe par rapport à la parthénogenèse. Cela pourrait aider à comprendre comment la parthénogenèse a évolué dans la nature, et pourquoi elle est si peu abondante.

Les éphémères (insectes de l’ordre des Ephéméroptères) font partie des rares organismes ayant recours à différentes formes de reproduction, comme le sexe et la parthénogenèse. Cette dernière peut également être facultative, c'est-à-dire qu’une femelle peut « choisir » de s’accoupler ou non. De plus, le faible taux de dispersion et la durée de vie très courte à l’état adulte des éphémères, créent souvent des situations où les femelles ne trouvent pas de mâles. Ces situations devraient engendrer une forte sélection pour la parthénogenèse, afin d’assurer une descendance. Ainsi, les éphémères sont les organismes idéaux à étudier pour mieux comprendre ce qui maintient la reproduction sexuée dans la nature, ou encore ce qui engendre une évolution vers la parthénogenèse.

Cette thèse vise à évaluer la fréquence de la parthénogenèse chez l’ensemble des éphémères, et ce, afin de mieux comprendre les avantages de la parthénogenèse par rapport à la reproduction sexuée en population naturelle. Ensuite, cette thèse se concentrera sur l’espèce *Alainites muticus*, afin de mesurer le polymorphisme reproducteur de cette espèce et d’identifier les facteurs, notamment écologiques, qui sélectionnent pour l’un ou l’autre mode de reproduction.

Nous avons constaté que les avantages présumés de la reproduction sexuée ne compensent pas toujours ses coûts, ce qui conduit à une transition vers la parthénogenèse. D’ailleurs, ce phénomène se produit bien plus souvent qu’on ne le pensait, au vu du grand nombre d’espèces d’éphémères capables de se reproduire sans mâles. Ces transitions sont influencées par des traits d’histoire de vie, tels que la taille du corps ou la capacité de se disperser. Ces transitions sont aussi influencées par des facteurs démographiques et environnementaux, tels que la densité de population et la complexité des communautés.

Dans son ensemble, cette thèse améliore nos connaissances sur les éphémères, et donne de nouveaux éléments qui éclairent le mystère de l’abondance de la reproduction sexuée alors que d’autres modes de reproduction existent. Les éphémères sont clairement sous-estimés quant à leur potentiel pour aider à résoudre le « paradoxe du sexe ».

ABSTRACT

The evolution and maintenance of sexual reproduction has been one of the major questions in evolutionary ecology for the last decades. Sex and reproduction are not synonymous, although they are often linked since the vast majority of animals reproduce sexually. Sexual reproduction, which combines meiosis and fertilisation, is associated with profound costs. This means that sex must provide significant benefits that can compensate for its costs. In addition, the balance between relative costs and benefits of sex may vary according to different factors, such as life-histories or ecology of species. A collection of theoretical models has studied variables costs and benefits of sex, but empirical studies are scarce.

Although less frequent than sex in animals, several organisms have evolved towards a reproduction without sex, such as parthenogenesis. Studying such alternative forms of reproduction is of fundamental importance for identifying the balance between costs and benefits of sex that could tip towards a selection for parthenogenesis, and thus help understanding the evolution of reproductive polymorphisms in the wild.

Ephemeroptera (mayflies) are one of the few animal groups where sexual reproduction co-occurs with different types of parthenogenesis at the inter- and intra-species levels, including facultative parthenogenesis. In addition, their typically low dispersal ability and short and fragile adult life may frequently generate situations of mate limitation in females, which may strongly select for reproductive assurance. Mayflies thus provide ideal conditions to progress in understanding the maintenance of sexual reproduction in natural populations, and the transition towards parthenogenesis.

This thesis aims to investigate the frequency of parthenogenesis in mayfly species overall, as a first step towards developing this group as a model system for investigating the balance between the benefits of sexual reproduction compared to parthenogenesis in natural populations. Then, this work focuses on the mayfly species *Alainites muticus* to better understand the evolution of reproductive polymorphism in the wild, and to identify ecological correlates of different reproductive strategies in natural populations.

We find that the indirect benefits of sex do not always compensate for its costs, which leads to a transition from sexual reproduction to parthenogenesis, more often than previously thought given the high frequency of parthenogenesis in mayflies. We find that life-history traits, such as body sizes, population growth rates and dispersal abilities are contributing to the transition rate from sexual to parthenogenetic reproduction. Such transition is also influenced by demographic and environmental factors, such as population density and community diversity.

Overall, this thesis improves our knowledge on life-histories and ecologies of mayflies, and gives novel insights into why sexual reproduction is the dominant reproductive mode in the wild. Mayflies are currently clearly underappreciated for their value as an outstanding model system to help resolving the “paradox of sex”.

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– GENERAL INTRODUCTION –

GENERAL INTRODUCTION

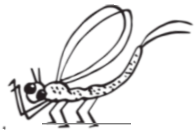
Sex and reproduction are not synonymous, although they are often linked since the vast majority of animals reproduce sexually. Sex combines meiosis and fertilisation to produce new individuals with 50% of the genes of each of their two parents. However, several organisms have evolved towards a sexless reproduction, and various forms of asexual reproduction exist. For example, **vegetative** reproduction in plants does not involve meiosis or fertilisation. The offspring are produced by mitosis, also called clonal reproduction (Asker & Jerling, 1992). **Binary fission** is another clonal reproductive mode. Bacteria and amoeba reproduce this way, by splitting themselves into two identical individuals (Clark & Pazdernick, 2013). **Fragmentation** is also an asexual reproductive mode, when individuals can produce a new individual from a piece of themselves, such as coral, sponges or starfishes (Fisher, 1925; Maldonado & Riesgo, 2008). **Parthenogenesis** can involve meiosis (but does not have to) but not fertilisation. In this case, offspring also only inherit genes from their mother, but are sometimes not clones of their mother (Suomalainen *et al.*, 1987). Even though all these different reproductive modes exist, sex is the most frequent form of reproduction among animals.

Why bother having sex?

Although less frequent than sex in animals, asexual reproduction (hereafter parthenogenesis) is simpler, faster and less costly than sexual reproduction. In order to better understand “the paradox of sex”, and why the prevalence of sex is considered the “Queen of problems” in evolutionary biology (Bell, 1982), it is important to understand the costs and benefits of this reproductive mode.

The costs of sex

Sexual reproduction can be considered as a **waste of time and energy**. For example, sex implies finding a good mating partner (Landolt, 1997), sex increases the risk of disease transmission (*e.g.*, Lockhart *et al.*, 1996; Thrall *et al.*, 1997) and the risk of predation during copulation (*e.g.*, Sakaluk 1990), sex creates rivalries between females or competitions between males (*e.g.*, Andersson, 1994), and sex is the source of many sexual conflicts with costly outcomes (Chapman *et al.*, 2003; Rankin & Kokko, 2007). One typical example of sexual conflict is sexual harassment. In many species, males harass females to achieve mating (Rowe *et al.*, 1994), resulting in fitness costs (fecundity or survival) in females resisting such harassment (*e.g.*, Shine *et al.*, 2000; Rönn *et al.*, 2006; Gosden & Svensson, 2009).



In addition, producing males is a **waste of resources**, as they do not give birth themselves, and typically do not take care of the offspring (Lehtonen *et al.*, 2012). Indeed, the main evolutionary disadvantage of sex compared to parthenogenesis, is the **cost of males** (Fig. 1).

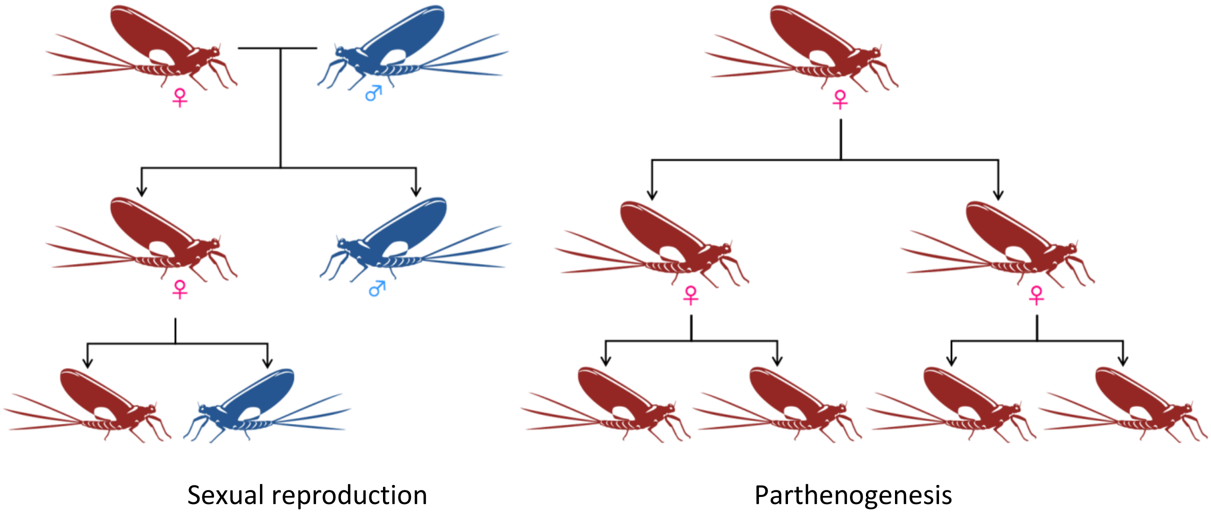


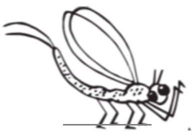
Figure 1: **A schematic representation of the cost of males**, which is two-fold when males only contribute their genes to the offspring.

In species where males contribute no resources to offspring production (except their genetic material), after *n* generations, parthenogenetic females will have 2^n times more offspring than sexual females, which confer an immediate two-fold demographic advantage to parthenogenesis (Maynard Smith, 1971, 1978). Furthermore, parthenogenesis provides reproductive assurance by avoiding the risk of not finding a mating partner, which would lead to **reproductive failure** in sexually reproducing females (*e.g.*, Lively, 1992; Johnson, 1994; Hörandl, 2006; Schwander *et al.*, 2010).

Despite these many costs, sexual reproduction remains the most abundant reproductive mode among animals. Even though a number of theories have been developed to help explain the advantages of sexual reproduction (Otto & Lenormand 2002), its maintenance in natural populations remains poorly understood (Schön & Martens, 2018; Neiman *et al.*, 2018).

The benefits of sex

The many costs generated by sexual reproduction (non-exhaustively presented above) are believed to be compensated by indirect benefits. The indirect benefits conferred by sexual reproduction are recombination, segregation and genetic exchange between individuals. One of the most common

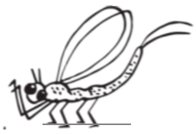


hypotheses is that sexual reproduction alleviates selective interference between loci (reviewed in Otto & Lenormand, 2002), which improve the efficiency of natural selection.

The **Muller's Ratchet** hypothesis (Muller, 1964) is based on the fact that deleterious mutations are accumulating over time in all species. Yet, only recombination can eliminate those deleterious mutations. This argument is largely supported by empirical evidence showing an increased accumulation of deleterious mutations in parthenogenetic as compared to sexual species (*e.g.*, Normark & Moran 2000; Paland & Lynch 2006; Neiman *et al.*, 2010; Henry *et al.*, 2012). Therefore, parthenogenetic reproduction in animals is viewed as an evolutionary dead end, a relict that is observed only rarely, because the lack of recombination, among other things, hampers the elimination of deleterious mutations and may be unfavourable during adaptation (Muller, 1964; Hartfield & Keightley, 2012).

The **Red Queen** hypothesis proposes that sexual reproduction can be favoured in complex communities with fast co-evolutionary dynamics and various types of biotic interactions (*e.g.*, Van Valen, 1973; Jaenike, 1977; Hamilton, 1980; Lively, 2010). Indeed, during co-evolutionary interactions between hosts and parasites, the increased efficiency of selection conferred by sex and recombination favour rare and different host genotypes, thus maintaining genetic polymorphism. If parasites track the most common host genotype (due to local adaptation to these hosts), populations with sexual reproduction, which are genetically diverse, would be less vulnerable to parasitism over time and remain relatively constant in size, while populations with parthenogenetic reproduction, which are genetically similar, would be more likely to be infected and decrease in size over time (*e.g.*, Jokela *et al.*, 2009; Neiman & Koskella, 2009).

Similarly, the **Tangled Bank** and **Sib Competition** hypotheses propose explanations for the prevalence of sex in relatively saturated communities, where competition for resources is likely to be continuous and intense (*e.g.*, Bell, 1982; Willson *et al.*, 1987; Scheu & Drossel, 2007; Otto, 2009). Genetically different individuals can extract more food from their environment than genetically similar individuals, and thus exploit more niches to find the less crowded one. Plus, sex allows for the production of genetically diverse offspring, which avoid direct competitions between siblings. These hypotheses overlap with the **Lottery** hypothesis that supposes environments to be various in space and often changing in time (Williams, 1975). Therefore, it might be advantageous to produce heterogeneous offspring, adapted to different habitats, to increase the probability of generating at least one offspring adapted to the novel future conditions. Assuming that genetic diversity leads to ecological diversity (which is not necessary the case), sexually produced offspring could exploit the environment to a larger extent than parthenogenetically produced offspring, which are genetically uniform.



Despite this collection of theories, progress in understanding the benefits of sex in natural populations has thus far been hampered by a lack of empirical studies supporting the hypotheses mentioned above. Questions, such as what may have allowed asexual populations to evolve, persist and diversify without sex thus far, remain outstanding (Neiman *et al.*, 2018).

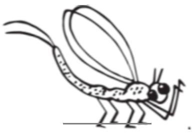
Geographical distribution of sex and parthenogenesis

Ecological conditions favouring the maintenance of sexual reproduction can lead to a different geographical distribution of sexual and parthenogenetic species. In nature, several patterns show that sexual and their parthenogenetic relative species often inhabit different ranges (*i.e.*, geographical parthenogenesis, reviewed in Tilquin & Kokko, 2016). These patterns suggest that sexual species occur mostly in stable habitats with high selective pressures and stressful conditions (Ram & Hadany, 2016), while parthenogenetic species occur mostly in recently colonised and unpredictable habitats with relaxed biotic interactions. The geographical parthenogenesis patterns can thus provide some insight to help resolving the paradox of sex in natural populations.

What about facultative parthenogenesis?

Most studies on the evolution and maintenance of sexual reproduction consider organisms with obligate sex *versus* obligate parthenogenesis (*e.g.*, Pongratz *et al.*, 2003; Wolinska & Lively, 2008; Neiman *et al.*, 2010), whereas organisms with facultative parthenogenesis received little attention. Species that use sex or parthenogenesis facultatively potentially gain the advantages of both reproductive strategies, as they reduce costs of sexual reproduction, while maintaining access to the benefits of genetic recombination (Green & Noakes, 1995; Dacks & Roger, 1999; Hadany & Otto, 2009), at least theoretically. For example, parthenogenesis can allow for fast population growth (as explained above with the two-fold demographic advantage), while sexual reproduction, even in small amounts, allows for sufficient recombination to avoid the genetic costs of parthenogenesis (Charlesworth *et al.*, 1993; D'Souza & Michiels, 2010). Furthermore, studying organisms with facultative parthenogenesis might help to better understand the evolution of reproductive modes, while avoiding problems inherent to comparisons between sexual and parthenogenetic species. Indeed, there may be species-specific traits that are confounded with reproductive mode. However, facultative parthenogenesis is rare among animals, for reasons that are not well understood.

To address when and how parthenogenesis is favoured over sex in natural populations, and thus for helping to solve the paradox of sex, it is important to study one of the few animal groups where sexual reproduction co-occurs with different forms of parthenogenesis, including facultative parthenogenesis, such as **mayflies** (Ephemeroptera).



Mayflies, a good model system

Mayflies constitute one of the most basal (early diverging) lineages of insects (Edmunds and McCafferty, 1988), their origin dating back to ~300 Mya (Brittain and Sartori, 2009). Widespread around the world, they are unique among the winged insects by having two adult stages, the subimago and the imago (Fig. 2).

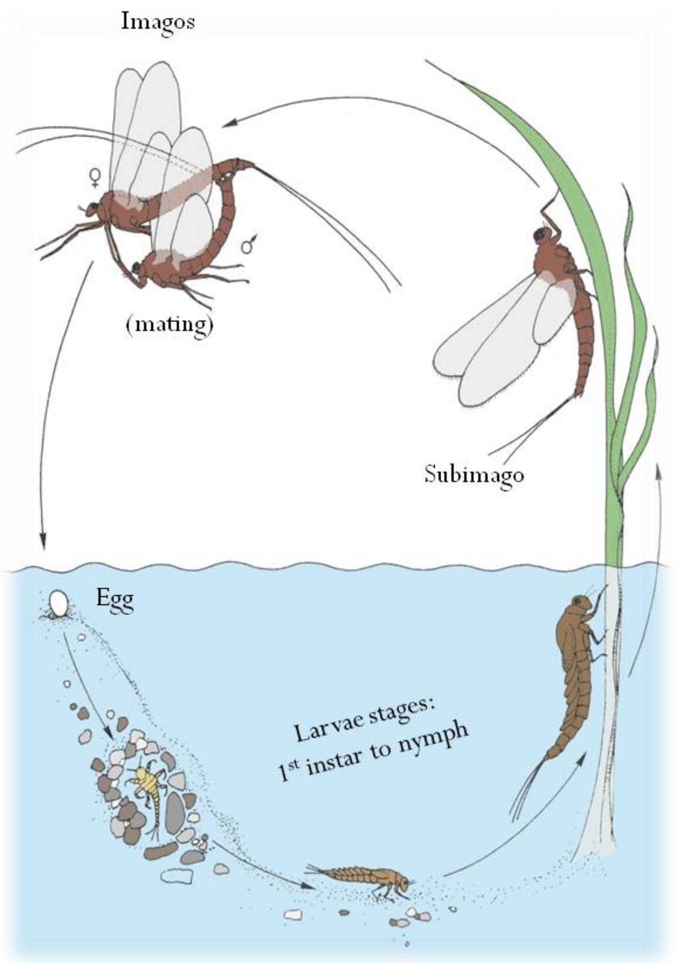


Figure 2: **Life cycle of mayflies.**
Adapted from Studemann *et al.*, 1992.

Mayfly nymphal aquatic life spans vary from several weeks to a couple of years, while the adult stages range from few hours to days, depending on the species. Once adult, mayflies do not feed at all, this stage being only dedicated to reproduction. Because of their typically low dispersal ability and their short and fragile adult life, mayflies have a short window in time and space for reproduction, which we argue is one of the factors that may have selected for the evolution of parthenogenesis in this group.

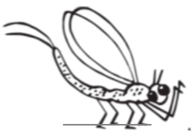
Goals and outlines of my thesis

The first step of my PhD thesis was to conduct a detailed literature review of the different reproductive modes in mayflies. Such species lists are rare but needed to quantitatively estimate the frequency of parthenogenesis overall in natural populations, and to test global hypotheses for the maintenance of sex, or differences between parthenogenetic and sexual species *via* comparative studies. **Chapter I** thus presents a summary of the current knowledge on sexual and parthenogenetic reproduction in Ephemeroptera (mayflies), as a first step towards developing this group for the study of benefits of sex in natural populations. We found a surprisingly high amount of parthenogenetic species compared to other groups surveyed thus far (White, 1973; Vrijenhoek, 1998; van der Kooi *et al.*, 2017). We also discovered that the variation in reproductive mode between mayfly species also holds within species.

Considering that the balance between relative costs and benefits of sex is expected to vary according to species-specific factors, such as life-history traits, or ecological conditions (Meirmans *et al.*, 2012), we decided to extend the species database generated in the first chapter by collecting information on life-history traits and specific microhabitats, in order to elucidate what can influence reproductive strategies in natural populations of mayflies. **Chapter II** shows how life-history traits and ecological factors interact in complex ways, and affect the relative costs of sex and parthenogenesis in natural populations.

The completion of the two first chapters of this thesis raised some interesting questions, such as how does the parthenogenetic capacity affect population sex ratios in the wild? Are there any differences in behaviour according to the reproductive mode? These were therefore the focus of **Chapter III**, in order to elucidate how frequently parthenogenesis is used under natural conditions and how it affects population sex ratios. We specifically studied one mayfly species, widespread in Switzerland, and able to reproduce by facultative parthenogenesis: *Alainites muticus*. We surprisingly found three reproductive modes within the species, highlighting reproductive polymorphism with behavioural differences and geographical parthenogenesis. Plus, we underline that population sex ratios are good estimates of parthenogenesis frequencies in natural populations of mayflies, and thus that this group is clearly underappreciated for its value as an outstanding model system for testing benefits of sex in natural populations.

Finally, in **Chapter IV**, we aimed at exploring ecological correlates of parthenogenesis *versus* sexual reproduction in the mayfly species *Alainites muticus*. We tested some theoretically predicted hypotheses, such as reproductive assurance or Tangled Bank. We found that biotic and abiotic ecological factors interact in complex ways, and affect the reproductive strategies in natural populations.



– CHAPTER I –

Extremely widespread parthenogenesis and a trade-off between alternative forms of reproduction in mayflies (Ephemeroptera)

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(Published in Journal of Heredity)

American Genetic Association

Journal of Heredity, 2021, 45–57
doi:10.1093/jhered/esaa027
Symposium Article
Advance Access publication September 12, 2020

OXFORD

Symposium Article

Extremely Widespread Parthenogenesis and a Trade-Off Between Alternative Forms of Reproduction in Mayflies (Ephemeroptera)

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Received January 16, 2020; First decision April 22, 2020; Accepted July 28, 2020.

Corresponding Editor: Maria Orive

Chapter I

Extremely widespread parthenogenesis and a trade-off between alternative forms of reproduction in mayflies (Ephemeroptera)

Abstract

Studying alternative forms of reproduction in natural populations is of fundamental importance for understanding the costs and benefits of sex. Mayflies are one of the few animal groups where sexual reproduction co-occurs with different types of parthenogenesis, providing ideal conditions for identifying benefits of sex in natural populations. Here, we establish a catalogue of all known mayfly species capable of reproducing by parthenogenesis, as well as species unable to do so. Overall, 1.8% of the described species reproduce parthenogenetically, which is an order of magnitude higher than reported in other animal groups. This frequency even reaches 47.8% if estimates are based on the number of studied rather than described mayfly species, as reproductive modes have thus far been studied in only 17 out of 42 families. We found that sex is a more successful strategy than parthenogenesis (associated with a higher hatching success of eggs), with a trade-off between the hatching success of parthenogenetic and sexual eggs. This means that improving the capacity for parthenogenesis may come at a cost for sexual reproduction. Such a trade-off can help explain why facultative parthenogenesis is extremely rare among animals despite its potential to combine the benefits of sexual and parthenogenetic reproduction. We argue that parthenogenesis is frequently selected in mayflies in spite of this probable trade-off because their typically low dispersal ability and short and fragile adult life may frequently generate situations of mate limitation in females. Mayflies are currently clearly underappreciated for understanding the benefits of sex under natural conditions.

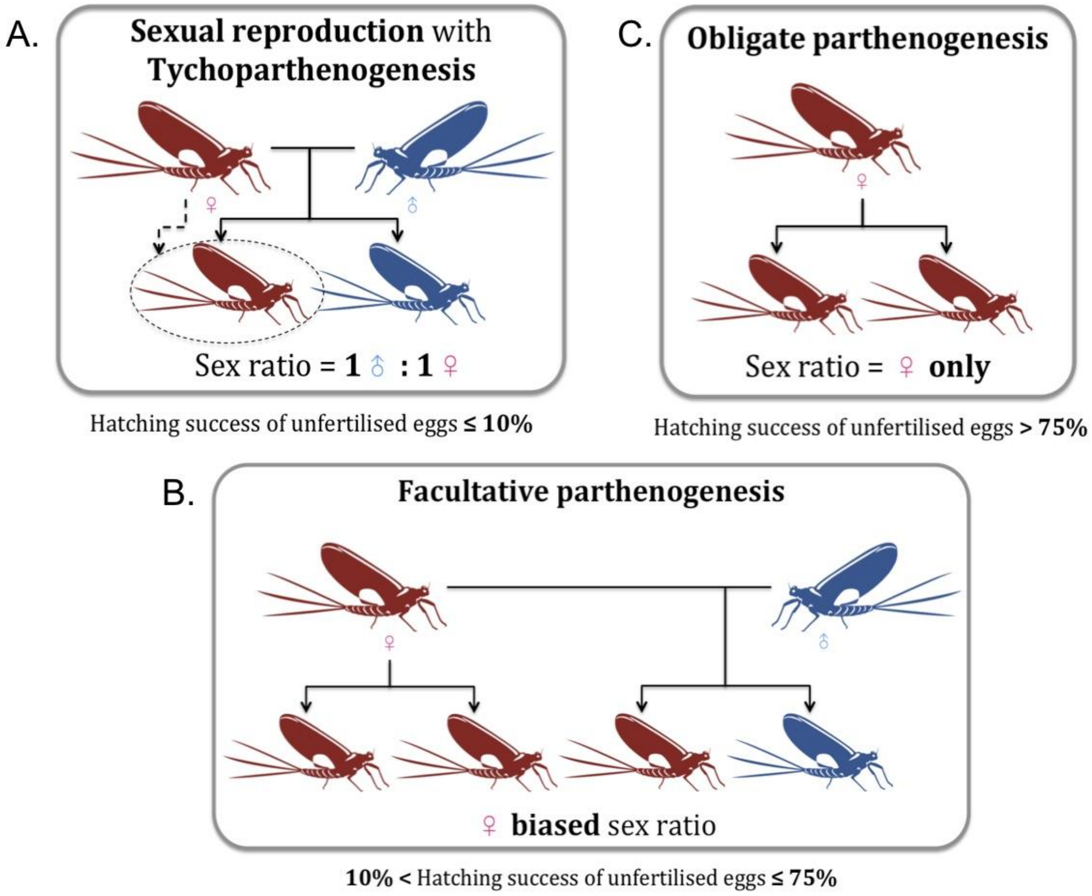
Key Words: mayflies, parthenogenesis, natural populations, evolution of sex.

INTRODUCTION

The evolution and maintenance of sexual reproduction has been one of the major questions in evolutionary ecology for the last decades (*e.g.*, Agrawal, 2006; Otto, 2009; Jalvingh *et al.*, 2016). Sex is associated with profound costs (reviewed in Lehtonen *et al.*, 2012), yet it is the most widespread reproductive mode among animals. Female-producing parthenogenesis (thelytoky) would largely avoid the costs associated with sex, yet only a minority of animals are known to reproduce parthenogenetically. How small this minority is remains however unknown as there are only two quantitative estimates (based on species lists) of the frequency of parthenogenesis (*i.e.*, in vertebrates: White, 1973; Vrijenhoek, 1998; and in haplodiploids: van der Kooi *et al.*, 2017). This is unfortunate as such species lists are invaluable for addressing when and how parthenogenetic reproduction is favoured over sex in natural populations (*e.g.*, Ross *et al.*, 2013; van der Kooi *et al.*, 2017) and thus for helping to solve the paradox of sex. In this review, we provide such a quantitative estimate by summarising the current knowledge on sexual and parthenogenetic reproduction in Ephemeroptera (mayflies), as a first step towards developing this group for the study of benefits of sex in natural populations.

Mayflies constitute one of the most basal (early diverging) lineages of insects (Edmunds and McCafferty, 1988), their origin dating back to ~300 Mya (Brittain and Sartori, 2009). Widespread around the world with 3,666 described species (42 families, 472 genera; adapted from Sartori and Brittain, 2015; MS unpubl.), they are mostly studied for fly fishing purposes (Knopp and Cormier, 1997), and for their potential as bioindicators of water quality (Bauernfeind and Moog, 2000). Mayflies do not feed as adults, relying solely on the energy reserves accumulated during their aquatic larval stages. Adult life span is extremely short, lasting from few hours to some days depending on the species. Because of their typically low dispersal ability and their short and fragile adult life, mayflies have restricted opportunities for reproduction, which we argue is one of the factors that may have selected for the evolution of parthenogenesis in this group. Parthenogenesis in mayflies can be largely accidental (*i.e.*, tychoparthenogenesis), facultative or ‘obligate’ (see **Box 1**). Furthermore, a single species can feature mixed reproduction (some females reproduce sexually, others parthenogenetially), either sympatrically or in allopatry (*i.e.*, geographical parthenogenesis).

We conducted a detailed literature review to establish a catalogue of all (to the best of our knowledge) mayfly species capable of reproducing parthenogenetically (see Appendix). We then used this catalogue to study whether the frequency of parthenogenesis varies among mayfly clades, in order to evaluate how parthenogenesis evolves in this group, and whether it tends to be phylogenetically clustered. We conducted cross-species comparisons with respect to the cellular mechanisms of parthenogenesis and the hatching success of fertilised and unfertilised eggs, to test whether the abilities of reproducing parthenogenetically or sexually are negatively correlated. We also looked at how the capacity for parthenogenesis affects population sex ratios, as female-producing parthenogenesis should bias the population towards females (Box 1). Finally, we compared the geographical distribution of sexual and parthenogenetic populations, to detect large- and small-scale patterns of geographical parthenogenesis.



Box 1. **Three forms of female-producing (thelytokous) parthenogenesis in mayflies.**

(**A**) Tychoparthenogenesis or "spontaneous parthenogenesis", occurs in sexual species (typically less than 10% of unfertilised eggs develop through parthenogenesis). Given the low hatching success of unfertilised eggs, population sex ratios remain equal. (**B**) Facultative parthenogenesis, means that eggs may either be fertilised or develop parthenogenetically. The hatching success of unfertilised eggs in this case is intermediate (typically 10-75%), leading to female-biased population sex ratios. (**C**) Under 'obligate' parthenogenesis, eggs always develop parthenogenetically and likely cannot be fertilised, with a hatching success typically higher than 75%. Only females are present in these populations. Note that an individual species can feature multiple forms of parthenogenesis, in the same or different populations.

MATERIAL AND METHODS

Data collection

The species list was compiled by collecting information from a thorough search of the literature, using Google Scholar, and Web of Science (publications until August 2019), as well as Ephemeroptera Galactica (www.ephemeroptera-galactica.com) and Ephemeroptera of the world (www.insecta.bio.spbu.ru/z/Eph-spp/Contents.htm). Four previous reviews (Degrange, 1960; Humpesch, 1980; Sweeney and Vannote, 1987; Funk *et al.*, 2010) comprised 78 mayflies species studied for their reproductive mode. Our survey combined with personal communications and observations generated a list of 136 species, as described in our database (Table 1, Appendix). The Tree of Sex database (Ashmann *et al.*, 2014) comprised only eight sexual and one parthenogenetic mayfly species, and was therefore not useful for our survey. When available for a given species, information on its geographical distribution, cytological mechanism of parthenogenesis and sex determination was included in the database (Appendix).

Classification of parthenogenesis

To classify mayfly species by forms of parthenogenesis (Box 1), we focused on the hatching rate of unfertilised eggs. We defined two main categories according to this rate: "sexual species" (including species with tychoparthenogenesis), when less than 10% of unfertilised eggs hatch (typically 0-5.3% for population means), and "parthenogenetic species", when hatching success of unfertilised eggs is higher than 10% (typically 18.5-97.3% for population means). Parthenogenetic species include all species with facultative parthenogenesis, 'obligate' parthenogenesis and mixed reproduction. Note that species with mixed reproduction have low population-average hatching success of unfertilised eggs when sexual and parthenogenetic females occur in sympatry (see results). We also considered species as parthenogenetic if female-only populations were reported in the literature, even if these species were not directly tested for their parthenogenetic capacity (this was the case for 18 of the 136 species in our database).

Within parthenogenetic species, we further distinguished ‘obligate’ from facultative parthenogens. Excluding rare events of sex in putatively obligate pathenogens is difficult (reviewed in Schurko *et al.*, 2009). We used the term obligate parthenogens for species where no males are known, or where rare males (typically <0.1% of all individuals) are most likely vestiges of sexual reproduction (van der Kooi and Schwander, 2014), indicating that parthenogenesis is the main form of reproduction. We also found very rare mentioning of deuterotoky (where both males and females are produced parthenogenetically). Specifically, the baetid species *Centroptilum luteolum*, *Acerpenna pygmaea*, *Acerpenna macdunnoughi* and *Anafroptilum semirufum* were inferred to be deuterotokous in breeding studies (Degrange, 1956; Funk *et al.*, 2010) because parthenogenetic broods contained high frequencies (2-17%) of males. Occasional deuterotoky is also the most likely explanation for the occurrence of rare males in ‘obligately’ parthenogenetic species as mentioned above. It is currently unclear how such males are produced (*e.g.*, via environmental influences on sex determination or X-chromosome losses in species with XX/X0 sex determination). The few known deuterotokous species are counted as parthenogenetic species in our classification.

Quality of the data and limitations of the database

Our database includes the species for which information concerning their reproductive mode is available. Of course, research efforts are not homogeneously distributed among the different taxa or different geographic ranges, which constraints the conclusions one can draw from the available information. For example, there is a strong study bias for species in the Northern Hemisphere which precludes broad geographic comparisons for the distribution of sexual and parthenogenetic species (see section on geographical parthenogenesis).

There is also a study bias for certain taxa, with 70 species in our database belonging to only two families (Baetidae and Heptageniidae; Table 1). In the Baetidae family, 45 species have been studied for their reproductive mode, which represent one third of the species in our database (Table 1, see Appendix for details). However, this family is also the most species rich one (with 1076=29.4% of the taxonomically described species; Table 1). The Heptageniidae family contributes 25 species to our database, which, again, largely reflects its taxonomic richness (610=16.6% of the taxonomically described species).

Nevertheless, in order to account for these taxonomic biases, we repeated our statistical analyses based on our database after excluding these two families (using solely the species of the 15 remaining families; Table 1). Excluding the two families from the analyses did not change any of our main conclusions (see below).

Statistical analyses

We first verified that our classification into sexual (with or without tychoparthenogenesis) and parthenogenetic species (facultative, mixed and obligate) was biologically meaningful, by comparing the distribution of hatching successes of unfertilised eggs for different groups (Fig. 1A). We further tested whether species with high egg-hatching successes were often characterised by female-biased population sex ratios, by using a quasibinomial Generalised Linear Mixed Model (GLMM) with the R v.3.3.3 (R Development Core Team, 2017) 'MASS' (Venables and Ripley, 2002) and 'car' (Fox and Weisberg, 2011) packages. To account for population means based on low numbers of individuals, we weighted our analyses with the number of females tested for each population.

We compared the prevalence of parthenogenetic species among mayfly families, using the most recent phylogeny available (Ogden *et al.*, 2019). Lack of phylogenetic information at lower taxonomic levels precluded further analyses. We thus tested whether variations in the frequency of parthenogenesis among families were explained by their phylogenetic relatedness by using binomial Generalised Linear Models (GLMs) and Tukey tests with the ‘multcomp’ (Hothorn *et al.*, 2008) package. In addition, we tested whether the frequency of parthenogenesis in mayflies varies among the six broad geographical regions: Nearctic, Palearctic, Neotropical, Afrotropical, Oriental and Australasian (see map in Table 2). Finally, we tested for potential trade-offs between parthenogenetic and sexual reproduction by studying hatching success of fertilised and unfertilised eggs at the population level of a given species (Figure 5).

A key aim of our study was to estimate the frequency of parthenogenesis among mayfly species. Estimating the frequency of parthenogenetic species in a taxon is difficult because the reproductive mode for the vast majority of species has not been studied. The two available previous estimates of the frequency of parthenogenesis among animals (in vertebrates: White, 1973; Vrijenhoek, 1998; and haplodiploid insects: van der Kooi *et al.*, 2017) assumed that all described species without evidence for parthenogenesis were sexual. However, this assumption severely underestimates the frequency of parthenogenesis. To account for this underestimation, we generated two frequency estimates, one using the total number of taxonomically described mayfly species, and one using only species where the reproductive mode was studied.

Table 1. **Frequency of parthenogenesis and sex determination in Ephemeroptera taxa**. Only families with at least one species studied for its reproductive mode are shown (17 families out of 42 (40.5%) have been studied). The numbers of described species have been adapted from Sartori and Brittain (2015) and updated (MS, unpubl.). NA: unknown. Note that all studied sexual and parthenogenetic species are diploid (2n).

				% of parthenogenesis			
Family	Parthenogenetic species	Sexual species	Total number of described species	among described species	among studied species	Sex determination (2n+♀:♂).	References
Ameletidae	4	1	60	6.7	80.0	16+XX:XY	Morgan, 1911; Clemens, 1922; Needham, 1924; Traver, 1932; Katayama, 1939; Wolf, 1946; Burks, 1953; Sweeney and Vannote, 1982, 1987; Jazdzewska and Wojcieszek, 1997; Funk *et al.*, 2006; Reding, 2006
Baetidae	35	10	1076	3.3	77.8	8+XX:XY	Bengtsson, 1913; Dodds, 1923; McDunnough, 1925, 1936; Ide, 1930; Degrange, 1954, 1955, 1956, 1960; Tjønneland, 1960; Wolf, 1960; Hirvenoja, 1964; Uéno, 1966; Bohle, 1969; Gibbs, 1977; Kiauta and Mol, 1977; Bergman and Hilsenhoff, 1978; McCafferty and Morihara, 1979; Kluge, 1980; Harper and Harper, 1984; Sweeney and Vannote, 1984, 1987; Sivaramakrishnan *et al.*, 1991; Lowen and Flannagan, 1992; Sweeney *et al.*, 1993; Jackson and Sweeney, 1995; Harker, 1997; Salas and Dudgeon, 1999; Gattolliat and Sartori, 2000; Soldan and Putz, 2000; Funk *et al.*, 2006, 2008, 2010; Encalada and Peckarsky, 2007; Giberson *et al.*, 2007; Webb *et al.*, 2012; Martynov, 2013; ML pers. obs.
Baetiscidae	-	1	12	0	0	NA	Pescador, 1973; Pescador and Peters, 1974
Behningiidae	-	1	7	0	0	NA	Peters and Peters, 1977; Harvey *et al.*, 1980; Sweeney and Vannote, 1982
Caenidae	5	4	258	1.9	55.6	6+XX:XO	Degrange, 1960; Froehlich, 1969; Mol, 1978; Gillies and Knowles, 1990; Da-Silva, 1993, 1998; Hofmann *et al.*, 1999; Soldan and Putz, 2000; Malzacher and Staniczek, 2007; FFS pers. com.
Dipteromimidae	-	1	2	0	0	NA	Takenaka *et al.*, 2019
Ephemerellidae	7	4	154	4.5	63.6	6, 14+XX:XY	Needham, 1924; Degrange, 1960; Kazlauskas, 1963; Fiance, 1978; Mol, 1978; Harper and Harper, 1982; Sweeney and Vannote, 1987; Soldan and Putz, 2000; Glazaczow, 2001; Funk *et al.*, 2008, 2010; Webb *et al.*, 2012
Ephemeridae	1	10	85	1.2	9.1	10, 12+XX:XO	Neave, 1932; Wolf, 1946; Hunt, 1951; Degrange, 1960; Britt, 1962; Thomforde and Fremling, 1968; Friesen and Flannagan, 1976; Mol, 1978; Sweeney and Vannote, 1987; Soldan and Putz, 2000; Funk *et al.*, 2010; Sekiné and Tojo, 2010a
Heptageniidae	3	22	610	0.5	12	18+XX:XY	Degrange, 1955, 1960; Huff and McCafferty, 1974; McCafferty and Huff, 1974; Mingo, 1978; Mol, 1978; Humpesch, 1980; Salas and Dudgeon, 1999; Soldan and Putz, 2000; Ball, 2001, 2002; Funk *et al.*, 2010; ML pers. obs.
Isonychiidae	-	1	35	0	0	NA	Sweeney and Vannote, 1987
Leptophlebiidae	4	10	726	0.6	28.6	12-14+XX:XY	Degrange, 1960; Clifford *et al.*, 1979; Savage, 1986; Sweeney and Vannote, 1987; Jackson and Sweeney, 1995; Salas and Dudgeon, 1999; Soldan and Putz, 2000; Funk *et al.*, 2010
Oligoneuriidae	-	1	66	0	0	14+XX:XY	Degrange, 1960; Soldan and Putz, 2000
Palingeniidae	1	-	33	3	100	NA	Landolt *et al.*, 1997
Polymitarcyidae	2	2	104	1.9	50	10, 14+XX:XO	Degrange, 1960; Britt, 1962; Tojo *et al.*, 2006; Sekiné and Tojo, 2010b, 2010a; Sekiné *et al.*, 2013, 2015
Potamanthidae	-	1	25	0	0	6+XX:XO	Degrange, 1960; Soldan and Putz, 2000
Prosopistomatidae	1	1	29	3.4	50	NA	Degrange, 1960; Campbell and Hubbard, 1998
Siphlonuridae	2	1	48	4.2	66.7	16+XX:XY	Degrange, 1954, 1955, 1960; Gibbs and Siebenmann, 1996; Soldan and Putz, 2000
Total	**65**	**71***	**3,666**	**1.8%**	**47.8%**		

* Among the 71 sexual species, 38 can reproduce by tychoparthenogenesis (53.5%).

RESULTS

Unfertilised egg-hatching successes and population sex ratios

Analysing the information we collected in our database revealed that the parthenogenetic capacity of females varied widely between and within populations (see Appendix for details). Nevertheless, our classification into sexual (with or without tychoparthenogenesis) and parthenogenetic species (facultative or obligate) is biologically meaningful, given the largely non-overlapping values for population-average hatching success of unfertilised eggs (Figure 1). Note that some species with mixed reproduction show a low population-average hatching success of unfertilised eggs when sexual and parthenogenetic females occur in sympatry (*e.g.*, average of 5.7% for one population of *Stenonema femoratum*, with egg-hatching successes varying among females from 0 to 77.9%). In order to determine whether a higher capacity for parthenogenesis translates into female-biased population sex ratios, we used species where both sex ratios and unfertilised egg-hatching successes were studied in the same populations. In these species, the parthenogenetic capacity and population sex ratios were significantly positively correlated (Fig. 1B, *GLMM, r=0.72, p-value <0.001*). The parthenogenetic capacity of females in strongly biased populations (>60% of females) was always very high (median: 83.4%, range: 40.4-97.3%), except for the species with mixed reproduction in sympatry as mentioned above (median: 7.8%, range: 3.3-15.5%). Conversely, unbiased population sex ratios were not indicative of species with obligate sexual reproduction, as they frequently comprised females with a high parthenogenetic capacity (Figure 1).

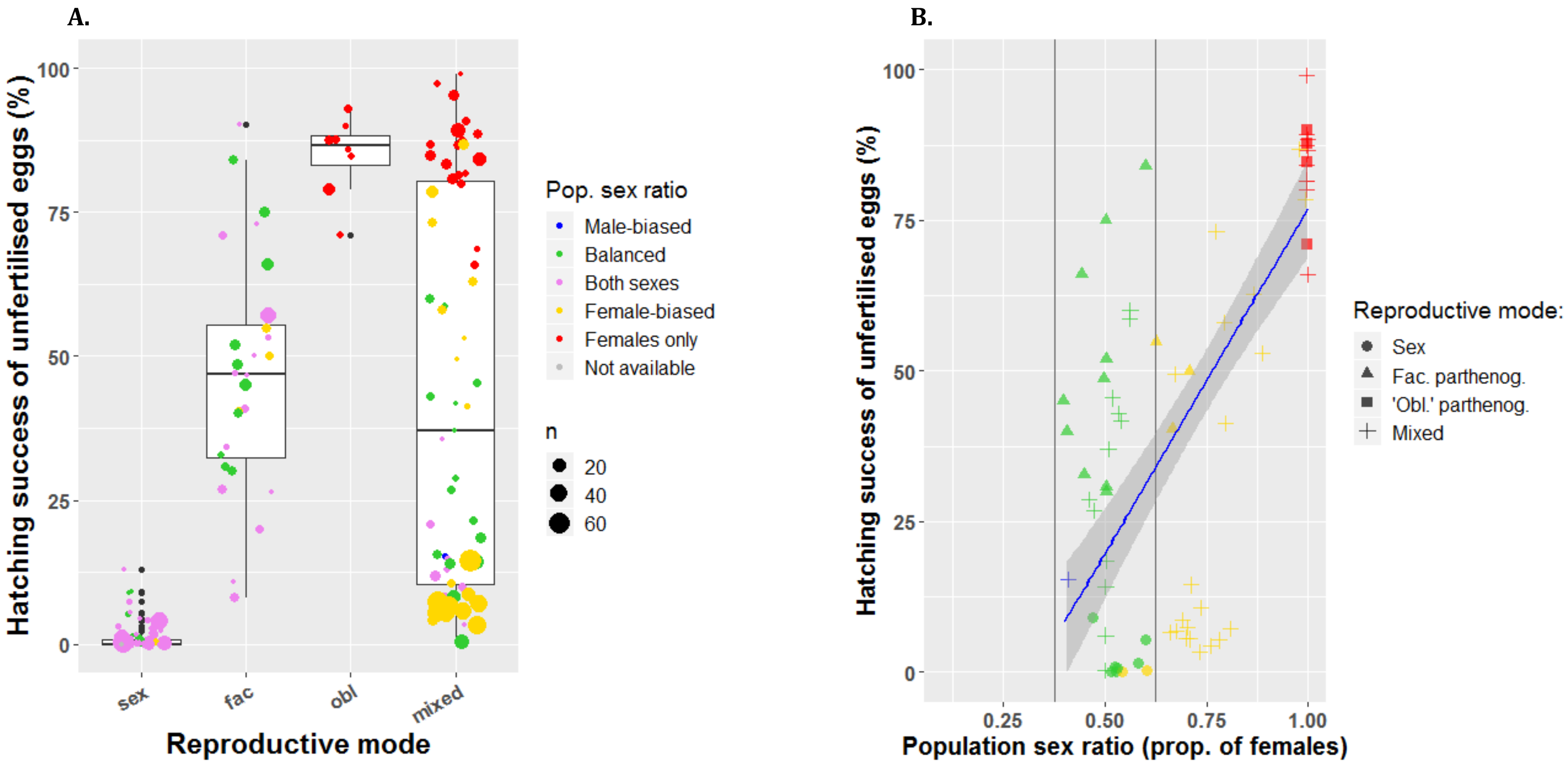


Figure 1. (A) **Population-average hatching success of unfertilised eggs and reproductive modes**. X-axis: sex: sexual reproduction, fac: facultative parthenogenesis, obl: 'obligate' parthenogenesis, mixed: mixed reproduction (in sympatry and/or in allopatry). Pop. sex ratio: population sex ratio; Both sexes (purple dots) means that males are present in these populations but exact sex ratios were not recorded; n: number of females used for determining the egg-hatching success in a given population. In total, 186 populations from 108 different species are represented (with species from 17 families but mostly the Baetidae [32.3%] and Heptageniidae [27.4%], see also supplementary Figures 1 and 2). (**B**) **Population-level correlation between unfertilised egg-hatching success and sex ratio** (*GLMM, r=0.72, p-value<0.001*). Sex: sexual reproduction, Fac. parthenog.: facultative parthenogenesis; 'Obl.' parthenog.: 'obligate' parthenogenesis, Mixed: mixed reproduction (in sympatry and/or in allopatry). Data are available for 84 populations from 31 species (with species from 11 families but mostly the Baetidae [23.8%] and Heptageniidae [20.2%], see also Supplementary Figures 3 and 4).

Frequency of parthenogenesis among mayflies

Parthenogenesis occurs in all well-studied mayfly families (Table 1, Fig. 3). We were able to classify the reproductive mode of 136 species from 17 families (Table 1, see Appendix for details). Seventy-one of these species are sexual (from 16 families), and 38 of these are able to perform tychoparthenogenesis, while 65 species are parthenogenetic (from 11 families). Assuming the 3,666 described mayfly species without information concerning their reproductive mode are sexual, 1.8% of all mayfly species are able to reproduce parthenogenetically, which is at least an order of magnitude higher than the available estimate for vertebrates (0.1%, White, 1973; Vrijenhoek, 1998), and comparable to the frequencies in other arthropod orders. For example, the frequency of parthenogenesis in orders with haplodiploid sex determination varies from 0 to 1.5% (van der Kooi *et al.*, 2017). However, if one uses the frequency estimates based on the number of mayfly species studied for their reproductive mode (n=136), the estimated frequency of parthenogenesis reaches 47.8% (Fig. 2), being about 25 times higher. These findings suggest that half of the mayfly species might be able to reproduce parthenogenetically, or even, that most mayflies are able to reproduce at least by tychoparthenogenesis (75.7% of the studied species).

Parthenogenesis occurs in an array of families and genera without any evidence for phylogenetic clustering (Fig. 3). A similar pattern is observed in haplodiploid taxa (van der Kooi *et al.*, 2017). These findings suggest that putative predispositions for the evolution of parthenogenesis do not have a strong phylogenetic inertia within the studied groups. However, clustering occurs at lower taxonomic levels as the proportion of parthenogenetic species varies significantly among mayfly families (G-tests of independence; studied species, $p<0.001$; described species, $p<0.001$). Indeed, parthenogenetic mayfly species are rarer in the families Heptageniidae, Leptophlebiidae and Ephemeridae (<1.5% among the taxonomically described mayfly species, or <30% among the one studied for their reproductive mode), than in the families Baetidae, Ameletidae and Ephemerellidae (>3.0% or >60%), with not enough data in the remaining families to detect potential differences.

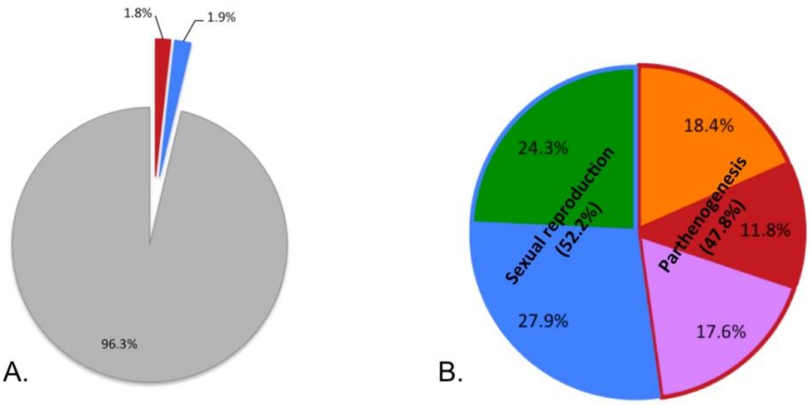


Figure 2. **Frequency of parthenogenesis among described mayfly species (A) and among species studied for their reproductive mode (B).** Less than four percent of the mayfly species (from 17 out of 42 families) have been studied for their reproductive mode. Parthenogenesis (warm colours): facultative (orange), 'obligate' (red) and mixed reproduction (purple). Sexual reproduction (cold colours): 'obligate' sexual reproduction (green) and sexual reproduction with tychoparthenogenesis (blue). At least 47.8% of the 136 studied species are able to reproduce parthenogenetically.

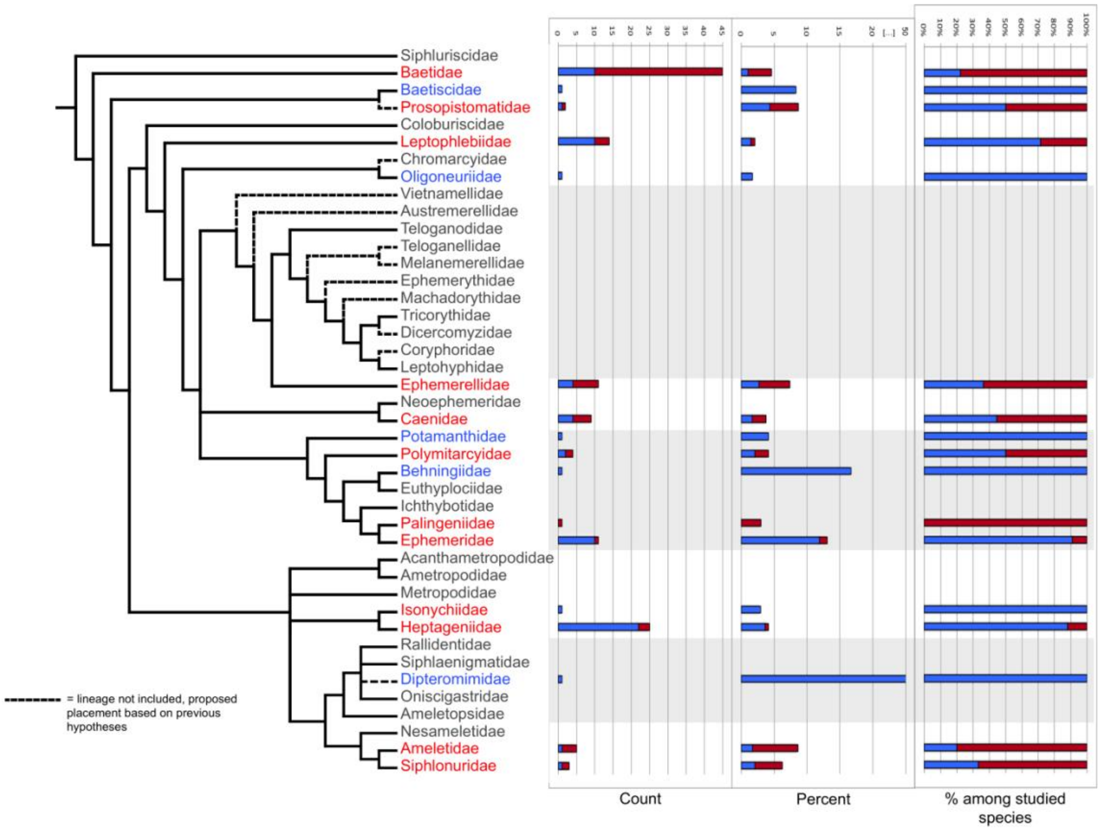


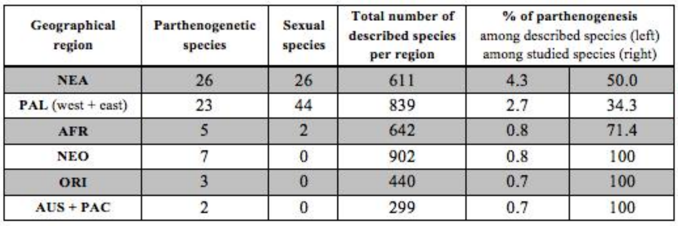
Figure 3. **Phylogenetic distribution of parthenogenesis among mayfly families.** Parthenogenetic species (facultative, obligate and mixed), Sexual species (with or whithout tychoparthenogenesis), Families without information regarding species' reproduction (n=25). The families Heptageniidae, Leptophlebiidae and Ephemeridae show a low propensity for parthenogenesis, whereas the families Baetidae, Ameletidae and Ephemerellidae show a high propensity for parthenogenesis (see main text for details). Count: number of mayfly species studied for their reproductive mode so far; Percent: percentage among the taxonomically described mayfly species in a given family; % among studied species: percentage among the mayfly species studied for their reproductive mode in a given family. Phylogeny adapted from Ogden *et al.* (2009, 2019). Note that we consider sexual reproduction (with tychoparthenogenesis) to be the ancestral state in mayflies.

Geographical parthenogenesis

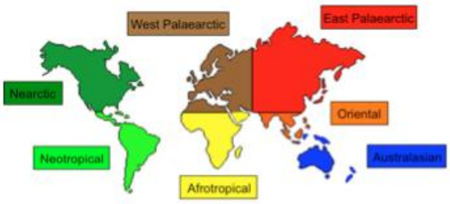
The term geographical parthenogenesis is used when parthenogenetic populations are found at higher altitudes or latitudes than their sexual counterparts, in harsher environmental conditions or have wider distributions and/or ecological niches (Vandel, 1928). Such different distributions of sexual and asexual species could provide insights into ecological conditions that favour sex or parthenogenesis in natural populations, but quantitative comparisons are scarce (reviewed in Tilquin and Kokko, 2016).

Considering very broad geographical scales and among-species comparisons, we found no evidence for geographic clustering of parthenogenetic species. The six regions compared comprised approximately equal proportions of studied parthenogenetic species (Table 2, see Appendix for details). However, even if such differences existed, we would not be able to uncover them with the currently available data. Indeed, among the 136 studied species, 117 come from Nearctic and Palearctic regions (86%), with very little data available for the remaining regions of the world.

Table 2. **World distribution of parthenogenesis in Ephemeroptera.**



Geographical region	Parthenogenetic species	Sexual species	Total number of described species per region	% of parthenogenesis among described species (left) among studied species (right)	
NEA	26	26	611	4.3	50.0
PAL (west + east)	23	44	839	2.7	34.3
AFR	5	2	642	0.8	71.4
NEO	7	0	902	0.8	100
ORI	3	0	440	0.7	100
AUS + PAC	2	0	299	0.7	100



NEA= Nearctic, **NEO**=Neotropical, **PAL**=Palearctic, **AFR**=Afrotropical, **AUS**=Australasian, **PAC**=Pacific islands. Adapted from Barber-James *et al.* (2008) and updated (MS unpubl.).

Considering within-species comparisons at smaller geographical scales, there are at least 18 mayfly species (27.7% of the parthenogenetic species) with both sexual and parthenogenetic populations (see Appendix for details). Two of them feature geographical parthenogenesis, but with distinct distribution differences between parthenogens and sexuals. Parthenogenetic populations of *Eurylophella funeralis* (Ephemerellidae) mostly occur at the periphery of the species ranges in North America (Sweeney and Vannote, 1987), while parthenogenetic populations of *Ephemerella notata* (Ephemerellidae) occur at lower latitudes than sexual ones in Poland (Glazaczow, 2001). One of the 18 species does not feature geographical parthenogenesis. Indeed, no geographical pattern is observed for *Ephoron shigae* (Polymitarcyidae), a species where sexual and parthenogenetic populations broadly overlap in Japan (Watanabe and Ishiwata, 1997). Finally, for 15 of the 18 species where sexual and parthenogenetic populations are known to occur in separate geographical areas, the number of described populations is too small to identify a systematic distribution difference between sexual and parthenogenetic populations (Appendix). Thus, the lack of data for these species does not allow us to conclude anything regarding potential geographical patterns.

More than the two discussed cases of geographical parthenogenesis likely exist in mayflies but are not detected because of a lack of studies, especially in the southern hemisphere. However, it seems that geographical parthenogenesis is not necessarily associated with particular ecological factors, as is the case in most other taxonomic groups studied thus far (Tilquin and Kokko, 2016).

Cytological mechanisms of parthenogenesis in mayflies

In animals, different cytological mechanisms can underlie thelytokous parthenogenesis, which vary with respect to their consequences on heterozygosity in offspring (reviewed in Suomalainen *et al.*, 1987). In mayflies, some of these mechanisms have been identified or suggested, but studies remain scarce. Nevertheless, the available information suggests that obligate parthenogens use cytological mechanisms that potentially allow for maintenance of heterozygosity across generation (but see Jaron *et al.*, 2018), while facultative parthenogens are invariably automictic and produce parthenogenetic offspring that are highly homozygous relative to sexual offspring. Specifically, nine out of the 10 studied 'obligately' parthenogenetic mayflies are functionally clonal without a detected loss of heterozygosity between generations (Sweeney and Vannote, 1987; Sweeney *et al.*, 1993; Funk *et al.*, 2006, 2008, 2010). Three mechanisms can be responsible of the complete maintenance of heterozygosity between generations: apomixis (parthenogenesis is functionally mitotic), endoduplication, or automixis with central fusion (without recombination). Which one(s) of these mechanisms occur in mayflies is currently unknown. The cytological mechanism of the remaining species, *E. shigae* in Japan, was suggested to be automixis with terminal fusion (Sekiné and Tojo, 2010b), indicating that some 'obligate' parthenogens might not be clonal.

All seven studied facultatively parthenogenetic mayflies are automictic (Appendix). Indeed, for the populations with both males and females of seven Baetidae species (*Acerpenna macdunnoughi*, *A. pygmaea*, *Anafroptlilum semirufum*, *Labiobaetis frondalis*, *Neocloeon alamance*, *Procloeon fragile*, *P. rivulare*) parthenogenesis appears to be automictic with terminal or central fusion (with recombination), given the partial loss of heterozygosity between generations, but further details are not known (Funk *et al.*, 2010). Finally, the cytological mechanism in *Ephoron eophilum* (Polymitarcyidae), a mostly sexual species with some facultatively parthenogenetic females (*i.e.*, mixed reproduction in sympatry) is either automixis with terminal fusion (without recombination) or gamete duplication, where complete homozygosity is achieved in one generation (Sekiné *et al.*, 2015). Cytological mechanisms of parthenogenesis in mayflies clearly require additional studies, but the major mechanisms identified to date are summarised in Figure 4.

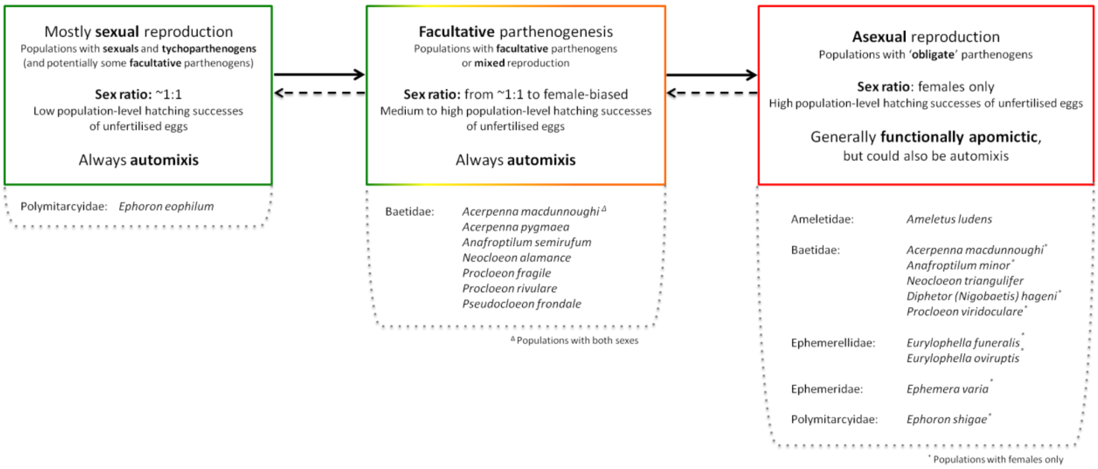


Figure 4. **Cytological mechanisms identified to date in mayflies and possible transitions between parthenogenesis forms**.

Trade-off between reproductive modes

Overall, mayfly species are better at reproducing sexually than asexually (measured as egg-hatching success, Fig. 5A, *p-value <0.001*). Only in obligate parthenogens is egg-hatching success decreased upon mating, presumably because (even partial) fertilisation interferes with the normal development of asexual eggs. Furthermore, there is a significant negative correlation between hatching success of fertilised and unfertilised eggs at the population level of a given species (Fig. 5B, *GLMM*, *r=-0.50*, *p-value=0.013*). This negative correlation suggests that there are trade-offs between parthenogenetic and sexual reproduction, meaning that improving the capacity for parthenogenesis may come at a cost for sexual reproduction, even in facultative parthenogens. If such a trade-off indeed exists, it could help explain why facultative parthenogenesis is extremely rare among animals in spite of its potential to combine the benefits of sexual and parthenogetetic reproduction.

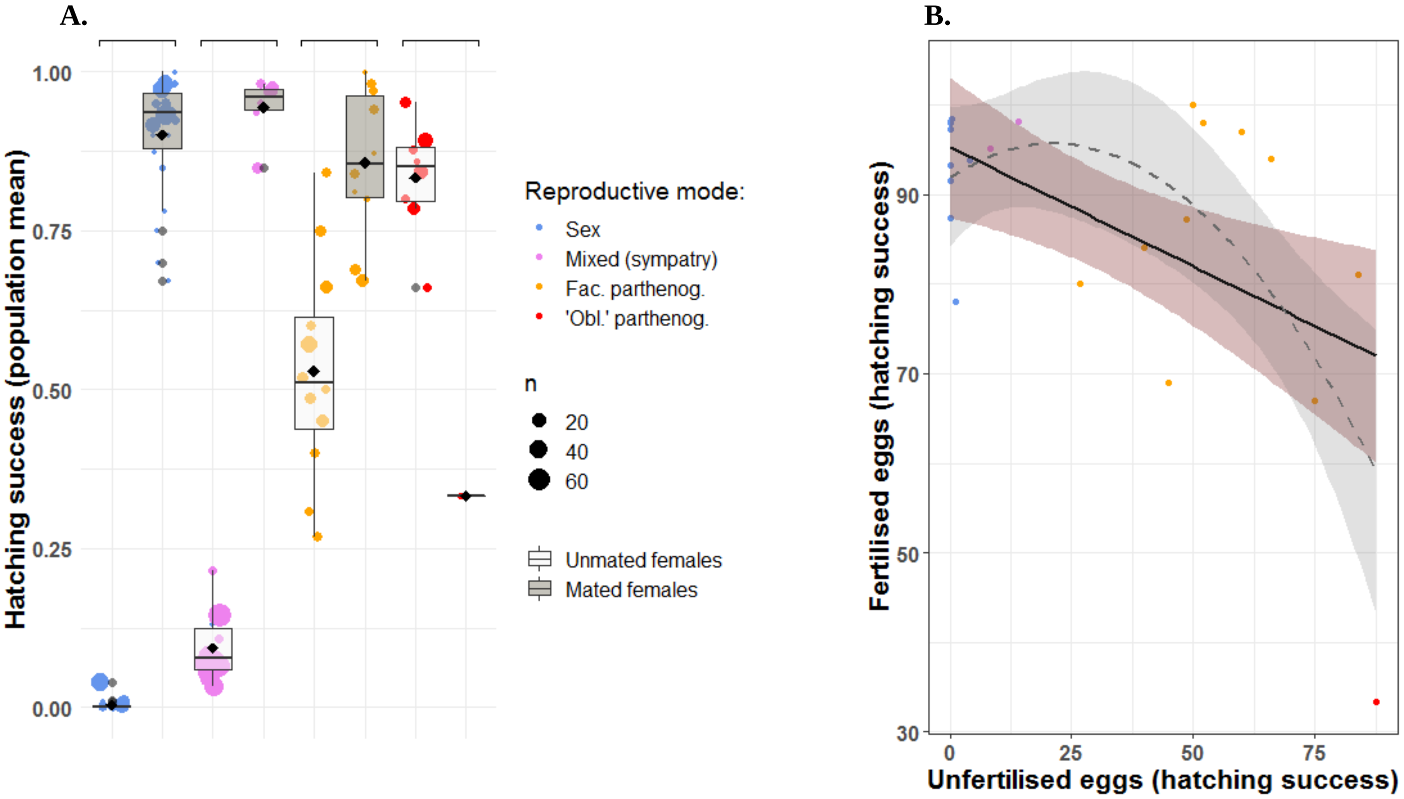


Figure 5. (A) **Hatching success of fertilised and unfertilised eggs for species with different reproductive modes and mating status.** Data are available for 75 populations from 32 different species, among height families (but mostly Baetidae [29.3%] and Heptageniidae [28.0%], see also Supplementary Figures 5 and 6). n: number of females tested for a given population. Sex: sexual reproduction (*$p<0.001$*), Mixed: mixed reproduction in sympatry (*$p<0.001$*), Fac. parthenog.: facultative parthenogenesis (*$p<0.001$*), 'Obl.' parthenog.: 'obligate' parthenogenesis (*$p<0.1$*); (B) **Trade-off between parthenogenesis and sexual reproduction** of a given population (*GLMM, r=-0.50, p-value=0.013*). Each point represents one population for which hatching success of fertilised and unfertilised eggs were measured. Data are available for 22 populations from 19 species, among seven families (but mostly Baetidae [45.4%] and Heptageniidae [9.1%]).

Origins of 'obligate' parthenogenesis in mayflies

There are at least four ways in which parthenogenetic lineages could arise from sexual species in animals (reviewed in Simon *et al.*, 2003): (1) Hybridisation between two sexual species, which is the major route to parthenogenesis in vertebrates (Avise *et al.*, 1992); (2) Contagious origin from pre-existing parthenogenetic lineages, where males produced by parthenogenetic species generate new lineages by mating with sexual females (*e.g.*, in aphids Jaquiéry *et al.*, 2014; and water fleas Xu *et al.*, 2015); (3) Infection by microorganisms (*e.g.*, *Wolbachia*, *Cardinium*, *Rickettsia*), mostly in species with haplodiploid sex determination (reviewed in Ma and Schwander, 2017) and (4) Spontaneous transitions through mutations, for example with tychoparthenogenesis as a first step (Carson *et al.*, 1957; Kramer and Templeton, 2001; Schwander and Crespi, 2009; Schwander *et al.*, 2010).

In mayflies, there is no evidence of parthenogenesis induced by hybridisation or endosymbiont infection, but there is very little data informing on the origins of parthenogenesis. A hybrid origin seems unlikely because it usually results in high levels of heterozygosity (recently reviewed in Jaron *et al.*, 2018), which is not the case for unisexual populations of the mayfly species studied so far (Sweeney and Vannote, 1987; Funk *et al.*, 2006; Sekiné and Tojo, 2010b). Endosymbiont induced parthenogenesis is also unlikely in mayflies because parthenogenesis in this group is often facultative, while endosymbiont infection normally causes obligate parthenogenesis (reviewed in Ma and Schwander, 2017). In addition, sex determination is male heterogamety (not haplodiploïdy, Table 1, see Appendix for details), further reducing the probability for endosymbiont-induced parthenogenesis.

In mayflies, it has been speculated that facultative and obligate parthenogenesis originates from tychoparthenogenesis (Sweeney and Vannote, 1987; Tojo *et al.*, 2006). Although this is a plausible hypothesis given how widespread tychoparthenogenesis is among mayflies (27.9% of the studied species, Fig. 2, see Appendix for details), there is no actual evidence for this suggestion. Indeed, there is currently very little information available that allows inferring how (facultative or obligate) parthenogenesis evolved in any of the known mayfly species. Nevertheless, because of their low dispersal ability and their short and fragile adult life, mayflies have restricted opportunities for reproduction, which may frequently generate situations of mate limitation in females. Mate limitation has been shown to favour parthenogenesis in other insect species (Schwander *et al.*, 2010), and is very likely to also select for parthenogenesis in mayflies, in spite of the probable trade-off with sexual reproduction we highlighted above.

Fate of sexual traits in 'obligate' parthenogenetic mayflies

Sexual traits in asexual species decay more or less rapidly depending on whether they become costly or neutral upon transitions to parthenogenesis (reviewed in van der Kooi and Schwander, 2014). Selection favours the reduction of costly traits, contrary to neutral traits which decay via drift. For example, sexual traits which could decay in parthenogenetic females are: (1) pheromones, (2) the capacity to produce males, (3) the ability to fertilise their eggs, and (4) the synchrony of emergences with males.

Sex pheromones are chemical signals involved in mate choice (reviewed in Johansson and Jones, 2007) that can disappear in asexual lineages (*e.g.*, Schwander *et al.*, 2013; Tabata Jun *et al.*, 2017). However, there are apparently no volatile pheromones in mayflies, with mate choice and species recognition based on visual signals and tactile recognition (Landolt *et al.*, 1997; MS pers. obs.).

Occasional production of males has been reported in a range of 'obligately' parthenogenetic mayfly species (*e.g.*, in *Ameletus ludens* Clemens, 1922; Needham, 1924; and in *Neocloeon triangulifer* Funk *et al.*, 2006, 2010) similar to most parthenogenetic species in other animal groups (van der Kooi and Schwander, 2014). In species with male heterogamety, male development in parthenogenetic lineages likely follows the accidental loss of an X chromosome during oogenesis (Schwander *et al.*, 2013). Accidental males produced by parthenogenetic females are often still able to fertilise eggs of females from sexual populations (van der Kooi and Schwander, 2014), but there is currently little information on the fertility of accidental males in mayflies. If fertile, as shown for two baetid species (Funk *et al.*, 2010), such males could potentially generate new lineages by matings with sexual females (*i.e.*, contagious parthenogenesis as explained above), which could help explain the high frequency of parthenogenesis in mayflies.

The ability of parthenogenetic females to fertilise their eggs is unknown in mayflies overall, as only one species, the baetid *Neocloeon triangulifer*, has been studied thus far (Funk *et al.*, 2006). In this species, the ability to fertilise eggs is maintained at least at low levels. Viable progeny could be obtained by crossing *Neocloeon alamance* males (a sexual species with XY male heterogamety) with parthenogenetic *N. triangulifer* females. In such crosses, 66.6% of offspring were normal, clonal *N. tringulifer* females with high fertility, suggesting they were produced parthenogenetically from unfertilised eggs. However, the remaining 33.3% were genetically intermediate between the two species (as indicated by allozyme genotypes), suggesting they were hybrids produced from fertilised eggs. Approximately half of this hybrid progeny were females, perhaps triploid, with low fertility, the second half consisted of sterile gynandromorphs (with both male and female morphological characteristics). These findings suggest that even ‘obligately’ parthenogenetic mayfly species still produce haploid eggs, which could explain why there is always a small proportion of unfertilised eggs that never hatch (typically 3-22%), although more data are clearly needed.

Depending on how synchronous the emergences of both sexes are, the temporal windows to find a mate can be affected. Accordingly, obligate parthenogenesis might lead to a decay of the synchronised activity patterns in mayfly species. Tropical species of Trichoptera and Ephemeroptera appear to follow this theory (Tjønneland, 1970). However, this does not hold for several other mayfly species from temperate regions (*e.g.*, Ameletidae: *Ameletus ludens*; Baetidae: *Neocloeon triangulifer*; Ephemerellidae: *Ephemerella notata* and *Eurylophella funeralis*), where emergence patterns of parthenogenetic mayflies are at least as synchronous as for sexual species (Sweeney and Vannote, 1982; Glazaczow, 2001). These findings might indicate that synchronisation of emergence is not costly in temperate regions, that there are other factors such as predation that select for the maintenance of emergence synchrony (Sweeney and Vannote, 1982), or perhaps that there are still rare events of sexual reproduction even in species believed to be obligately parthenogenetic.

CONCLUSION

We found evidence for parthenogenesis in at least 65 mayfly species, which represent 1.8% of the 3,666 described species. However, this frequency is likely underestimated given that among the 136 species whose reproductive mode was studied, this value reaches 47.8%, currently the highest estimate known in non-cyclical parthenogenetic organisms. Parthenogenesis in mayflies thus appears to be widespread and is certainly an order of magnitude more frequent than in animal groups surveyed thus far. Among the 71 mayfly species found to reproduce sexually, 38 (53.5%) can produce offspring by accidental parthenogenesis (*i.e.*, tychoparthenogenesis). Such accidental parthenogenesis could function as a pre-adaptation for facultative parthenogenesis, which may often be selected in mayflies because their short adult life frequently generates situations of mate limitation.

We found that sex is a more successful strategy (associated with a higher hatching success of eggs) than parthenogenesis. Indeed, we found a trade-off between the hatching successes of parthenogenetic and sexual eggs across mayfly species, meaning that improving the capacity for parthenogenesis may come at a cost for sexual reproduction, even in facultative parthenogens. Such a trade-off can help explain why facultative parthenogenesis is extremely rare among animals in spite of its potential to combine the benefits of sexual and parthenogenetic reproduction.

Additional studies focused on species from areas other than North America and Europe would be necessary to obtain a fully representative overview of the frequency of parthenogenesis in mayflies and for uncovering potential lineage-level or geographical-ecological correlates of parthenogenesis in this group. Additional studies are also needed regarding the cytological mechanisms and the origin of parthenogenesis in mayflies. In spite of these constraints, mayflies are currently clearly underappreciated for their value as outstanding model systems for testing benefits of sex in natural populations.

Funding

This work was supported by the Swiss National Science Foundation (grant numbers PP00P3_139013 and PP00P3_170627 to T.S.).

Acknowledgements

We would like to thank Luca Sciuchetti for his contribution to review the literature and help for building the database. We are also grateful to researchers who provided unpublished information that allowed us to improve our database. We thank the two anonymous reviewers and the editor for constructive comments.

Supplementary material

Supplementary material (Supplementary Figures 1 to 6) is available at *Journal of Heredity* online.

All data (Supplementary Appendix) are deposited in Dryad under https://doi.org/10.5061/dryad.fttdz08qf.

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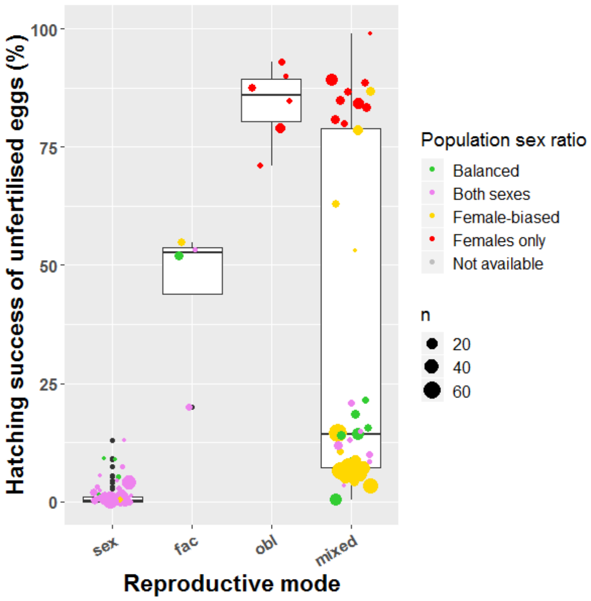
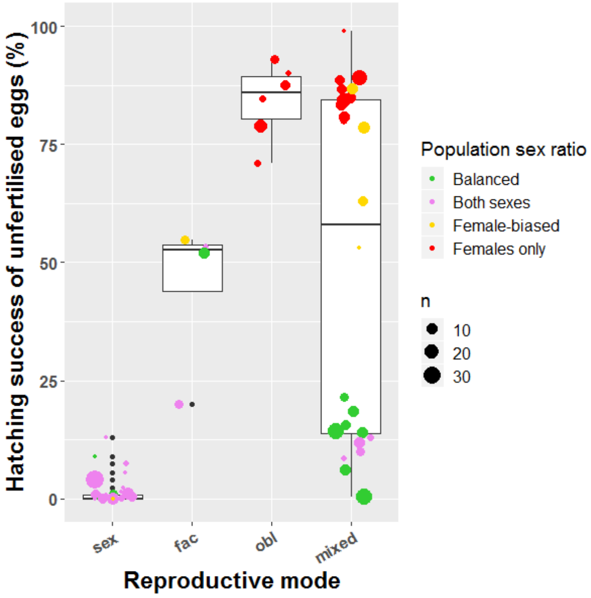
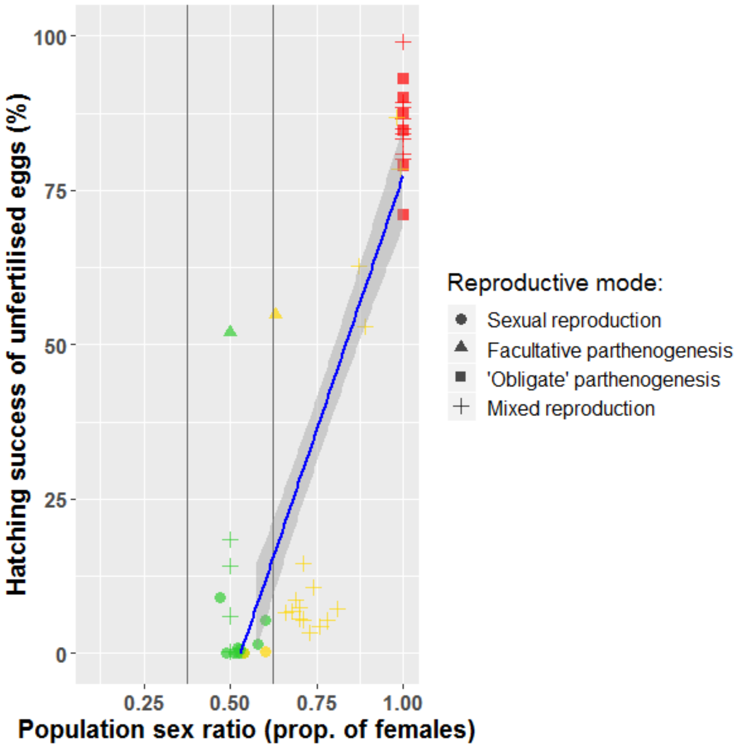
Supplementary Materials



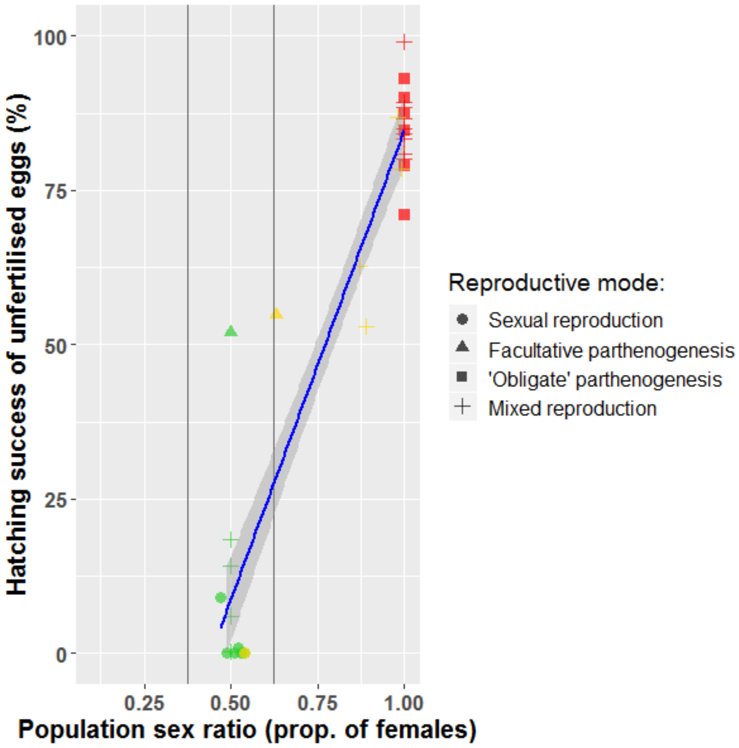
Supplementary Figure 1. Population-average hatching success of unfertilised eggs and reproductive modes (**without the Baetidae family**). X-axis: sex: sexual reproduction, fac: facultative parthenogenesis, obl: 'obligate' parthenogenesis, mixed: mixed reproduction (in sympatry and/or in allopatry). Population sex ratio with both sexes (purple dots) means that males are present in these populations but exact sex ratios were not recorded; n: number of females used for determining the egg hatching success in a given population. In total, 126 populations from 75 different species are represented (with species from 16 families but mostly Heptageniidae [40.5%], see also Supplementary Figure 2).



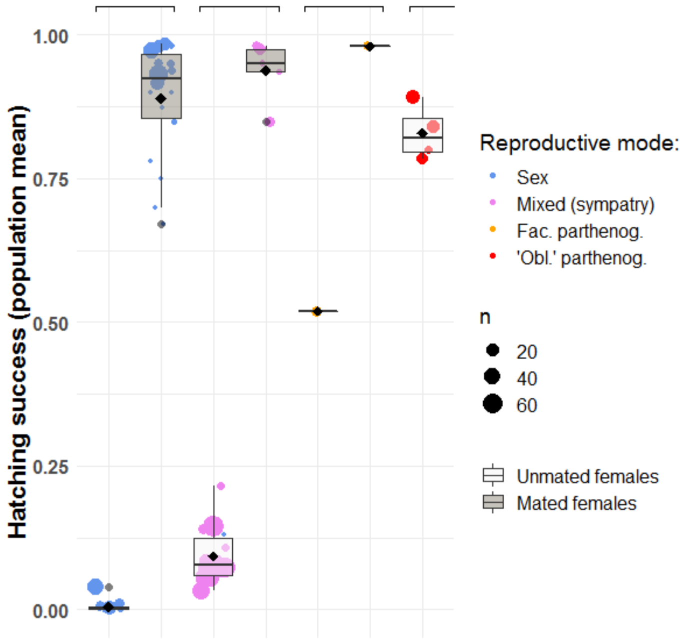
Supplementary Figure 2. Population-average hatching success of unfertilised eggs and reproductive modes (**without the Baetidae and Heptageniidae families**). X-axis: sex: sexual reproduction, fac: facultative parthenogenesis, obl: 'obligate' parthenogenesis, mixed: mixed reproduction (in sympatry and/or in allopatry). Population sex ratio with both sexes (purple dots) means that males are present in these populations but exact sex ratios were not recorded; n: number of females used for determining the egg hatching success in a given population. In total, 75 populations from 52 different species are represented (with species from 15 families).



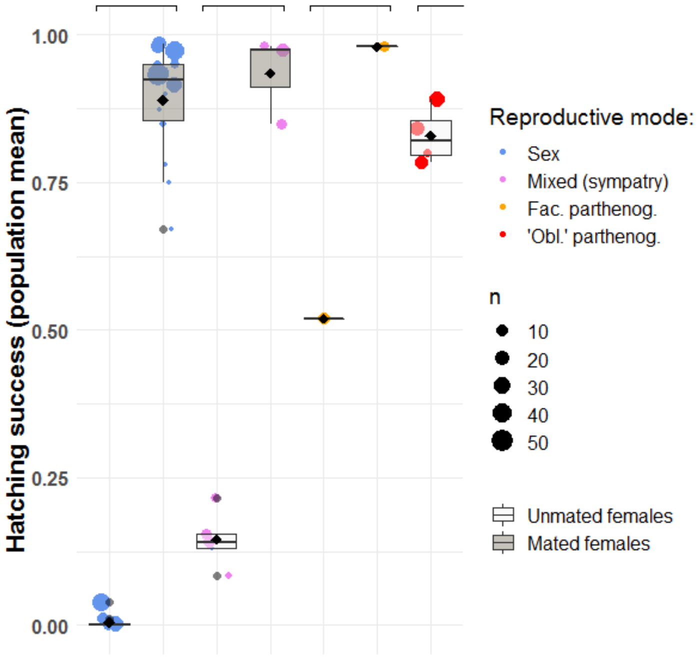
Supplementary Figure 3. Population-level correlation between unfertilised egg-hatching success and sex ratio (**without the Baetidae family**; *GLMM, r=0.88, p-value <0.001*). Data are available for 49 populations from 18 species, among 10 families (but mostly Heptageniidae [36.7.2%], see also Supplementary Figure 4).



Supplementary Figure 4. Population-level correlation between unfertilised egg-hatching successes and sex ratios (**without the Baetidae and Heptageniidae families**; *GLMM, r=0.97, p-value <0.001*). Data are available for 32 populations from 14 species, among 9 families.



Supplementary Figure 5. Hatching success of fertilised and unfertilised eggs for species with different reproductive modes and mating status (**without the Baetidae family**). Data are available for 65 populations from 19 different species, among 7 families (but mostly Heptageniidae [35.4%], see also Supplementary Figure 6). n: number of females tested for a given population. Sex: sexual reproduction (*p<0.001*), Mixed: mixed reproduction in sympatry (*p<0.001*), Fac. parthenog.: facultative parthenogenesis (*p=0.053*), 'Obl.' parthenog.: 'obligate' parthenogenesis.



Supplementary Figure 6. Hatching success of fertilised and unfertilised eggs for species with different reproductive modes and mating status (**without the Baetidae and Heptageniidae families**). Data are available for 42 populations from 14 different species, among 6 families. n: number of females tested for a given population. Sex: sexual reproduction (*p<0.001*), Mixed: mixed reproduction in sympatry (*p<0.001*), Fac. parthenog.: facultative parthenogenesis (*p=0.003*), 'Obl.' parthenog.: 'obligate' parthenogenesis.

– CHAPTER II –

Fast life-cycles and active dispersal favour parthenogenetic reproduction in mayflies

Maud Liegeois, Michel Sartori and Tanja Schwander

(in preparation)

Chapter II

Fast life-cycles and active dispersal favour parthenogenetic reproduction in mayflies

Abstract

Sexual reproduction is extremely costly yet ubiquitous in natural populations, meaning it must provide significant benefits that can compensate for the costs. The balance between relative costs and benefits of sex is expected to vary according to species-specific factors, such as certain life-history traits or ecological conditions, but empirical tests of such predictions are scarce. Studying organisms that use sex or parthenogenesis facultatively is an ideal approach to identifying factors acting to tip the balance towards sex or parthenogenesis in natural populations. Such organisms avoid problems inherent to comparisons between sexual and parthenogenetic species, as there may be species-specific traits that are confounded with reproductive mode. In this study, we used mayflies (Ephemeroptera), an insect order where sex co-occurs with facultative and ‘obligate’ parthenogenesis at the inter- and intra-species levels, in order to elucidate what life-history traits and/or ecological variables can influence reproductive strategies in natural populations. Using structural equation modelling, a statistical approach that allows taking into account direct and indirect interactions between factors, we found that fast life-cycles and active dispersal directly favour parthenogenesis, while habitats with fast currents and coarse river-bed substrates indirectly favour sexual reproduction by selecting for large body sizes. These findings support theories predicting that sex is favoured under *K*-selection, while parthenogenesis is favoured under *r*-selection.

Key Words: mayflies, reproductive strategies, natural populations, evolution of sex, structural equation modelling (SEM).

INTRODUCTION

Sexual reproduction is extremely costly yet ubiquitous in natural populations, meaning it must provide significant benefits that can compensate for the costs. The most significant cost is the cost of males which is two-fold in species where males contribute no resources (except their genetic material) to offspring production (Maynard Smith, 1971, 1978). This cost is believed to be compensated by indirect benefits conferred by sex, which are generated by reducing selective interference between loci (reviewed in Otto & Lenormand, 2002). However, whether such benefits are able to fully compensate for the cost of males is unclear. Furthermore, the relative costs and benefits of sex are expected to vary according to species-specific factors, such as life-history traits, or ecological conditions (Meirmans *et al.*, 2012), but empirical tests of such predictions are scarce.

For example, theory predicts that the cost of males can increase in species with a fast generation time, high fecundity, and high mortality (*e.g.*, MacArthur & Wilson, 1967; Pianka, 1970), which may tip the balance to generate a net benefit for parthenogenesis. In addition, when population growth rates are high, the extra time required for sexual reproduction as compared to parthenogenesis may further increase the costs of sex (Burt, 2000). More generally, sex is less likely to generate a net benefit under *r*-selection than under *K*-selection. This can lead to a different geographical distribution of sexual and parthenogenetic species, with sexual species occurring mostly in stable habitats (characterised by *K*-selection), and parthenogenetic species occurring mostly in recently colonised and unpredictable habitats (characterised by *r*-selection) (*i.e.*, geographical parthenogenesis, see Tilkin & Kokko, 2016 for a recent review).

In addition to life-history traits, specific ecological conditions can also affect the relative costs and benefits of sex. For example, biotic interactions and intense competition at the inter- or intra-specific levels can favour sex over parthenogenesis by different mechanisms (reviewed in Lively & Morran, 2014). The Red Queen hypothesis proposes that sexual reproduction can be favoured when co-evolution with parasites causes strong selection (*e.g.*, Hamilton, 1980;

Bell, 1982), while both the Tangled Bank and Sib Competition hypotheses propose explanations for the prevalence of sex in relatively saturated communities, where competition for resources is likely to be continual and intense (*e.g.*, Williams, 1975; Willson *et al.*, 1987; Scheu & Drossel, 2007). Thus, a general prediction is that sexual species should occur under ecological conditions where biotic interactions are intense, while parthenogenetic species should be prevalent under conditions with relaxed biotic interactions.

An ideal approach to identifying ecological conditions and species' life-history traits that favour sex in natural populations is to identify factors selecting for high or low frequencies of sex in facultative parthenogens. 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. They might help identifying factors acting to tip the balance towards one or the other reproductive mode in natural populations.

In this study, we used mayflies (Ephemeroptera) as a model system because parthenogenesis in this group is more frequent than in other animal taxa (Liegeois *et al.*, 2021). Furthermore, many mayfly species reproduce by facultative parthenogenesis at different frequencies, providing a continuous gradient in reproductive strategies, from 'obligate' sexual to 'obligate' parthenogenetic reproduction. We used the mayfly species list compiled in Liegeois *et al.*, (2021), in order to elucidate whether particular life-history traits (specifically, generation time, body size, and dispersal abilities) can influence reproductive strategies in natural populations. Because we had no information on the intensity of biotic interactions in specific ecological niches of mayflies, we instead probed whether there were differences in parthenogenesis abilities among specific microhabitats (characterised by water current velocity and river-bed substrate size). Given that different life-history and ecological traits are likely linked to each other, for example because certain microhabitats favour particular life-history traits as explained above, we used Structural Equation Modelling (SEM), a statistical approach that allows taking into account interactions between factors and their hidden indirect effects on reproductive modes.

MATERIAL and METHODS

Data collection

Our analyses are based on the database compiled in Liegeois *et al.* (2021) which comprises 136 mayfly species studied for their reproductive mode. This list includes 71 sexual species (among which 38 can reproduce by tychoparthenogenesis), 49 'facultatively' parthenogenetic species (among which 24 feature mixed reproduction, with some females reproducing sexually, and others by facultative or 'obligate' parthenogenesis), and 16 'obligately' parthenogenetic species (see Liegeois *et al.*, 2021 for further details). We complemented this database with information on life-history traits and ecological variables likely to alter the relative costs and benefits of sexual reproduction *versus* parthenogenesis (see Supplementary Table 1). Specifically, we evaluated whether parthenogenetic mayfly species tend to have faster generation times, higher dispersal abilities and smaller body sizes than sexual species. As a proxy for generation time, we used 'voltinism', namely the number of generations per year for a given species. As a proxy for dispersal abilities, we used 'dispersal phenotype', namely the ability of a given species to actively disperse by swimming, which is strongly correlated to the morphology of the nymphs in mayflies (for example, body size, body shape).

Finally, we tested for variations in the incidence of parthenogenesis across different microhabitats, which we characterised as 'substrate size' (the granulometry of the sediment in a species' habitat) and 'current velocity' (the rate of water flow in a species' habitat). We considered probing additional ecological and life-history variables, including diet, saprobity and temperature tolerance, for their correlation with parthenogenesis abilities (see Supplementary Table 1). However, information on these variables were not available for the majority of species in our database, so we only included variables with information on 87% or more of the species, given that our statistical approach, based on structural equation modelling, is sensitive to missing data (*e.g.*, Enders & Bandalos, 2001; Wolf *et al.*, 2013).

Statistical analyses

Reproductive modes, life-history traits and ecological variables were analysed at the species level in order to identify correlates of parthenogenesis in natural populations. Specifically, we tested whether life-history traits and/or specific microhabitats significantly explained variations in the parthenogenesis ability across mayfly species. In order to disentangle the direct and indirect influences of each variable on reproductive mode, we used structural equation modelling (SEM) (*e.g.*, Grace *et al.*, 2010; Eisenhauer *et al.*, 2015; Fan *et al.*, 2016). This multivariate technique tests for the nature and the magnitude of effects of multiple, interacting factors, based on hypothesised causal relationships and correlations among the variables in the SEM. Our initial meta-model (Fig. 1) permitted us to develop a structural equation model (Fig. 2, see results) that was analysed using the R v.3.3.3 (R Development Core Team, 2017) 'lavaan' package (Rosseel, 2012). For the three variables with missing data (*i.e.*, voltinism, current velocity and substrate size, 7-13% of missing values per variable), we used the random imputation methods within the SEM (Gelman & Hill, 2007). On the basis of model fit indices, calculated for the overall goodness of fit (*i.e.*, χ^2, cfi) 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 meta-model, as well as to determine the support for tested links. The χ^2 tests the hypothesis that there is a discrepancy between the model-implied covariance matrix and the original covariance matrix. Therefore, a non-significant discrepancy ($p>0.05$) indicates an acceptable model fit. The comparative fit index (cfi) represents the amount of variance that has been accounted for in the covariance matrix. A higher value indicates a better model fit (best is >0.95). We used these two indices as well as AIC from the 'AICcmodavg' package (Mazerolle, 2019) for model selection.

RESULTS and DISCUSSION

Variation in life-history traits and ecology among species is predicted to change the relative costs and benefits of parthenogenesis *versus* sexual reproduction (*e.g.*, Lively, 2010; Meirmans *et al.*, 2012; Gerber *et al.*, 2018), but empirical tests of such predictions are scarce. We used structural equation modelling to analyse co-variation between reproductive modes, life-history traits and ecology across mayfly species (Fig. 1).

We found clear evidence that high population growth rates favour parthenogenetic reproduction in mayflies. Indeed, parthenogenesis abilities were approximately 30% higher in mayfly species with multiple (two or more) generations per year than in species with one (or less than one) generation per year (Fig. 3a), with voltinism having a strong direct and positive effect on parthenogenesis abilities (Fig. 2, $r = 0.34, p<0.001$). This finding supports the idea that the costs of sex increase in *r*-selected species (MacArthur & Wilson, 1967; Pianka, 1970), resulting in higher frequencies of parthenogenesis. Indeed, theory has shown that the cost of males in short-lived species with high population growth rates is high compared to species with low growth rates (Lively, 2010).

We also found that active dispersal abilities favour parthenogenesis both directly (Figures 2 and 3b, $r = 0.27, p=0.002$) and indirectly *via* a positive effect on population growth rates (Figures 2 and 3d, $r = 0.53, p<0.001$). In mayflies, dispersal abilities are correlated with body shape and size, meaning that small mayfly species with active dispersal are more likely to reproduce parthenogenetically than large species with passive dispersal ($r = 0.45$). In addition, small body sizes are typically correlated with fast generation times in animals (*e.g.*, Gillooly, 2000; Denney *et al.*, 2002; Savage *et al.*, 2004), further supporting the idea and evidence described above that parthenogenesis is favoured in species with high population growth rates. In other words, small mayfly species with active dispersal and multiple generations per year are more likely to reproduce parthenogenetically than large species with passive dispersal and one, or less than one, generation per year.

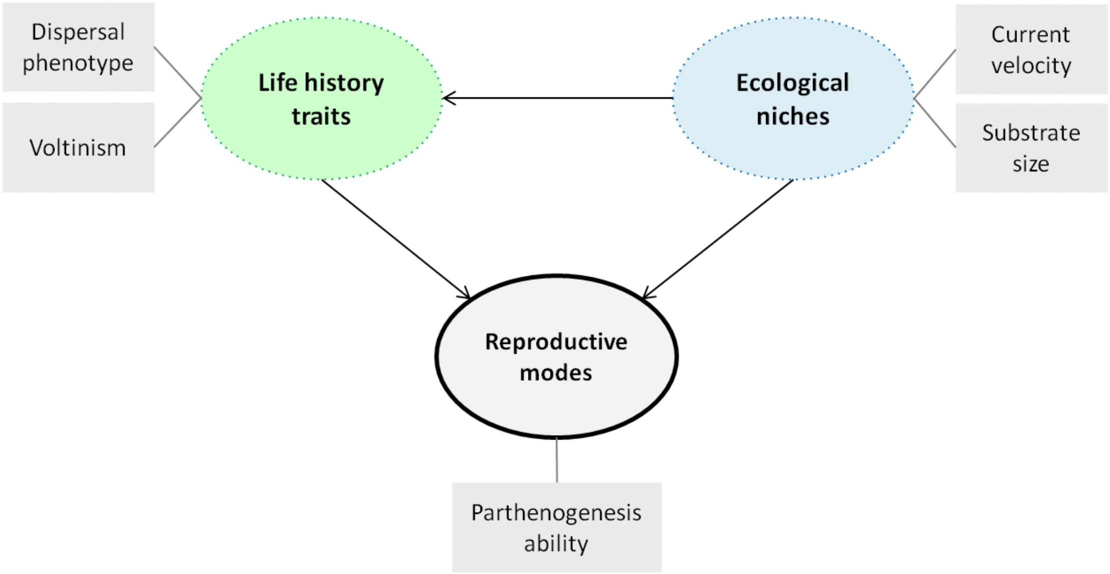


Figure 1. **Structural equation meta-model showing interconnections between life-history traits, ecological niches and reproductive modes.** Black arrows represent expected direct effects between two variables. Rectangles represent empirical variables that were used to run the model.

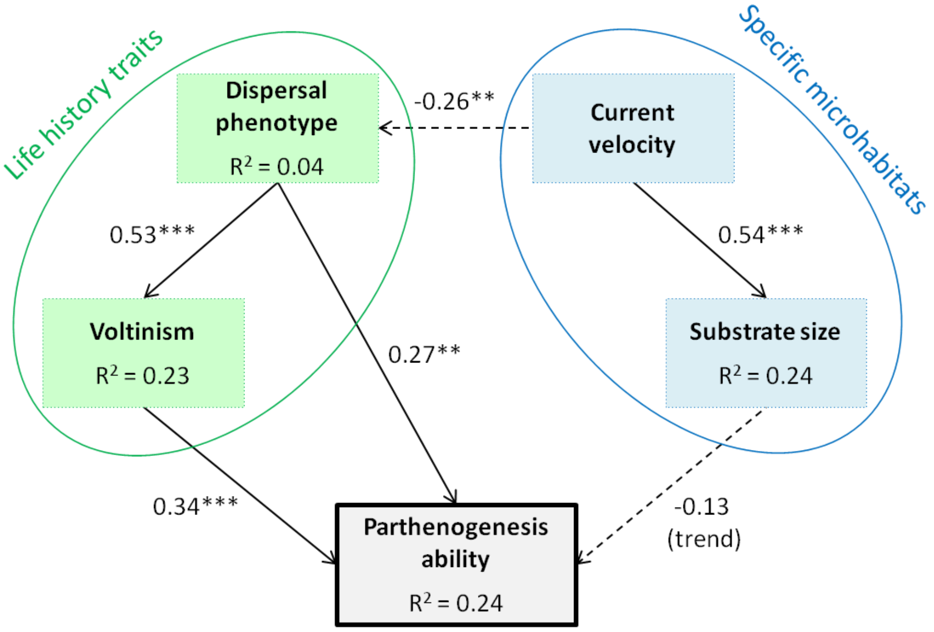


Figure 2. **Final Structural Equation Model fitted to our data.** Arrows represent significant paths with positive (solid) and negative (dashed) effects. Path coefficients correspond to standardised effects. R^2 values are displayed on response variables, representing the proportion of variance explained. Test statistic = 1.38, with 3 degrees of freedom, p-value = 0.71 and cfi = 1 (indicating close model-data fit, see Material and Methods for details).

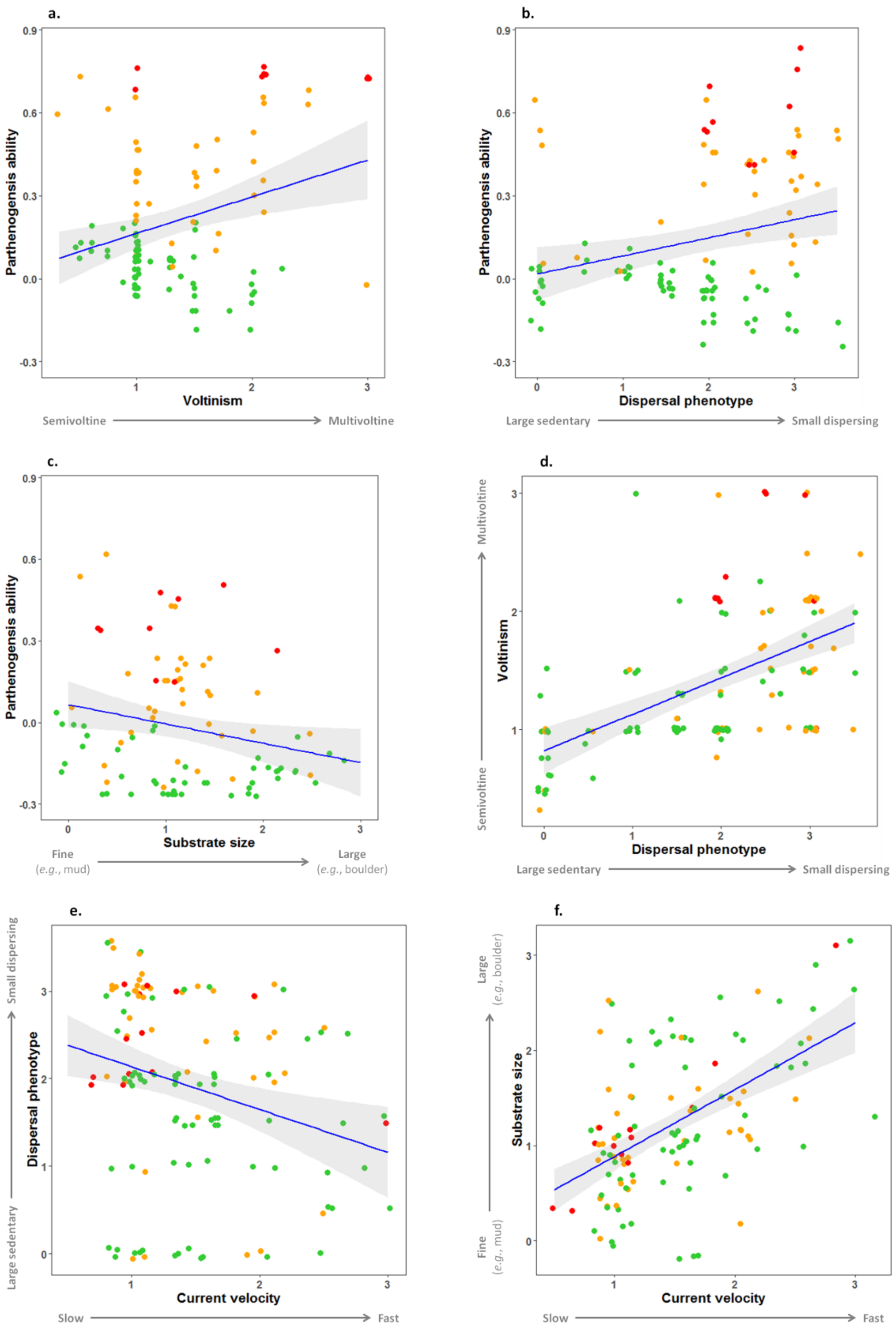


Figure 3. **Multivariate partial plots** (n=136 species) **for the SEM paths in Figure 2**. Different reproductive modes are represented in the graphs by different colours: green=sexual species (with or without tychoparthenogenesis); orange=facultative parthenogenesis/mixed reproduction; and red='obligate' parthenogenesis; note that these categories are used solely for visualisation as statistics are based on quantitative estimates of parthenogenesis abilities. Plots **a to c** visualise partial **direct** effects of variables on parthenogenesis abilities. Plots **d to f** visualise partial relationships between variables with **indirect** effects on parthenogenesis abilities.

Differences in body size and/or generation time between related sexual and parthenogenetic species are commonly found in nature (*e.g.*, in the lichen *Lasallia pustulata*: Hestmark, 1992; in *Schmidtea* flatworms: Weinzierl *et al*., 1999; in *Heteronotia* lizards: Kearney *et al*., 2004; in *Brachionus* rotifers: Beck & Agrawal, 2013), but not always in the same direction we found it in mayflies. Notably, in cases where parthenogenetic species are larger than sexual species, this is typically due to polyploidy in the parthenogens.

High dispersal rates may also facilitate the evolution of parthenogenesis directly by constraining the possibility of parasites to adapt to their local host genotype. For example, a study in bdelloid rotifers suggested that these asexual animals suffer little from parasites because they are able to form resting stages. These resting stages result in extensive dispersal of different genotypes over space and time, limiting the scope of co-evolution in rotifer parasites (Wilson, 2011). Interactions between local adaptation and dispersal rates have been well studied (*e.g.,* Blanquart & Gandon, 2014; Berdahl *et al.*, 2015), but only one case discussed it in relation with sexual reproduction (Lagator *et al.*, 2014), suggesting trade-offs between benefits of sex and migration according to the timing of adaptation. Furthermore, a recent theoretical model showed that co-variation between reproductive modes, dispersal and dormancy act and evolves differently within or between species (Gerber & Kokko, 2018).

We also expected different reproductive strategies in mayflies to be associated with different microhabitats, as microhabitats often differ between closely related sexual and parthenogenetic species (*e.g.,* in *Potamopyrgus* fresh water snails: Jokela *et al.*, 1997; in *Taraxacum* dandelion plants: Verduijn *et al.*, 2004; in *Daphnia* aquatic crustaceans: Lehto & Haag, 2010). We found no strong evidence that different microhabitats directly favour or disfavour parthenogenesis in mayflies. Indeed, the partial effect of substrate size on reproductive mode was rather weak (Figures 2 and 3c, $r = -0.13$, $p=0.06$), and we did not detect a direct effect of current velocity on reproductive mode (resulting in the removal of this non-significant path from the model). However, fast currents lead to a decrease in active dispersal (Figures 2 and 3e, $r = -0.26$, $p=0.001$), which in turn decreases parthenogenesis abilities ($r = -0.12$). Plus, fast currents generate coarse substrates, which could explain the weak effect of substrate size on parthenogenesis abilities in our model (Figures 2 and 3f, $r = 0.54$, $p<0.001$). In other words, mayfly species living mostly in stagnant water are rather small, actively disperse by swimming and are more likely to reproduce parthenogenetically, whereas species living in fast currents are rather large, passively disperse by drift and are more likely to reproduce sexually.

In combination, our results suggest a “parthenogenesis syndrome” in mayflies: high parthenogenesis abilities are favoured in species with small body sizes that disperse actively, and these species tend to live in stagnant water with muddy to sandy substrates. We are not able to formally distinguish whether this “syndrome” is driven directly by certain life-history traits (fast population growth rates, small body sizes, high dispersal abilities) favouring parthenogenesis as discussed above or whether these life-history traits are adaptations to certain habitat types that favour parthenogenesis for other reasons. For example, in streams and rivers, slow currents are known to feature increased water temperatures and accumulate pollution, which typically causes eutrophication (*e.g.*, Yang *et al.*, 2008). Such disturbed habitats, often associated with human activities, may be more suitable for parthenogens (Vrijenhoek, 1984; Vrijenhoek & Parker, 2009). By contrast, fast current habitats are sometimes characterised by stronger parasite pressure (*e.g.*, in salmons: Samsing *et al.*, 2015). In such habitats, sexual species would likely be more successful than parthenogens (*i.e.*, Red Queen hypothesis).

CONCLUSION

Our results showed that specific habitats, such as slow current and fine substrates, tend to select for small individuals with active dispersal and high growth rates, which in turn select for parthenogenesis. This "parthenogenesis syndrome" we uncovered with our structural equation model accounts for the variation in parthenogenesis abilities among mayfly species surprisingly well, with 24% of variation explained. However, we were not able to correct for phylogenetic non-independence of species, given the relevant phylogenies are currently not available for mayflies. Among the 65 mayfly species that are able to reproduce parthenogenetically, about half belong to the Baetidae family, and among the 71 sexually reproducing species, about one third belongs to the Heptageniidae family. Since species within families may share phenotypic similarities through shared ancestry, our results have to be interpreted with care.

Independently of the detailed mechanisms that drive the "parthenogenesis syndrome" in mayflies, our findings show how life-history traits and ecological factors interact in complex ways, and affect the relative costs of sex and parthenogenesis in natural populations.

Acknowledgements

We would like to thank Manon Pribille for her contribution to review the literature and help for collecting information on life-history traits and ecology of mayfly species. We also thank Dr. James B. Grace for his helpful explanations on Structural Equation Modelling. This work was supported by funding from the University of Lausanne and the Swiss National Science Foundation (grant numbers PP00P3_139013, PP00P3_170627).

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Supplementary Materials

Supplementary Table 1. Life-history traits and ecological variables likely to alter the relative costs and benefits of sexual reproduction *versus* parthenogenesis in mayflies (based on the species list compiled in Liegeois *et al.*, 2021).

SYSTEMATIC		REPRODUCTIVE STRATEGY		LIFE-HISTORY TRAIT		MICROHABITAT		ADDITIONAL INFORMATION			
Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Ameletidae	*Ameletus.cryptostimulus*	**0**	0,001	1	3	1	2	1.7	NA	0	0.5
Ameletidae	*Ameletus.lineatus*	**2**	0,800	1	3	1	1	NA	NA	0.5	1.5
Ameletidae	*Ameletus.ludens*	**2**	0,850	1	3	1.5	1.5	NA	NA	0.5	1
Ameletidae	*Ameletus.tertius*	**1**	0,200	1	3	1	1	NA	NA	1	1
Ameletidae	*Metreletus.balcanicus*	**1**	0,500	1	2,7	1	0.5	1.5	0,9	0.5	NA
Baetidae	*Acentrella.sinaica*	**1**	0,263	1	2,5	2	2.5	1.5	0,8	1	1.5
Baetidae	*Acentrella.turbida*	**1**	0,500	1.5	3	2	1	1.7	1,3	0.5	1
Baetidae	*Acerpenna.macdunnoughi*	**1**	0,718	2.5	3	2	1.5	1.7	NA	1	1
Baetidae	*Acerpenna.pygmaea*	**1**	0,840	2.5	3,5	1	1	NA	1,8	1	1.5
Baetidae	*Alainites.muticus*	**1**	0,577	2	2,5	2	1.5	1.5	1,4	1	1.5
Baetidae	*Americabaetis.sp*	**1**	0,472	2.1	3	NA	NA	1	2,0	2	0
Baetidae	*Anafroptilum.minor*	**1**	0,500	1	3	1	1	NA	NA	1	NA
Baetidae	*Anafroptilum.semirufum*	**1**	0,500	1.5	3	1	NA	NA	NA	1	NA
Baetidae	*Baetis.alpinus*	**0**	0,000	1.4	2,5	2.5	2.5	1.5	1,0	0.5	1
Baetidae	*Baetis.bicaudatus*	**1**	0,500	1.5	2,5	2.5	2	1.5	1,3	0.5	0.5
Baetidae	*Baetis.brunneicolor*	**0**	0,000	1.8	3	1.5	1	1	1,8	1	1
Baetidae	*Baetis.bundyae*	**1**	0,500	1	3	1	1	NA	0,8	0.5	0.5
Baetidae	*Baetis.foemina*	**2**	0,800	NA	3	1	1	NA	NA	0	0
Baetidae	*Baetis.gemellus*	**0**	0,000	2.25	2,5	2.5	3	1.5	NA	0.5	1
Baetidae	*Baetis.hudsonicus*	**1**	0,500	NA	3	1	2	NA	NA	0	0
Baetidae	*Baetis.noshaqensis*	**2**	0,800	NA	3	NA	NA	NA	NA	0	0

Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Baetidae	*Baetis.rhodani*	**0**	0,000	2	2,5	2	2	1.5	2,0	1	2
Baetidae	*Baetis.scambus*	**1**	0,467	1.7	2,5	2	1	1.5	1,8	0.5	0
Baetidae	*Baetis.tricaudatus*	**0**	0,000	2	3	2	1.5	2	1,3	0.5	2
Baetidae	*Baetis.vernus*	**0**	0,000	2	2,5	1	1.5	1.4	2,1	0.5	1.5
Baetidae	*Bugilliesia.notabilis*	**1**	0,500	2.1	3	NA	NA	NA	NA	2	0
Baetidae	*Bungona.sp*	**1**	0,710	3	3	NA	NA	NA	NA	2	1
Baetidae	*Callibaetis.ferrugineus*	**0**	0,000	1.5	3,5	1	0.5	1.5	1,8	0.5	1
Baetidae	*Callibaetis.fluctuans*	**0**	0,000	1.5	3	1	1	1.5	1,8	0.5	2
Baetidae	*Centroptilum.lituratum*	**1**	0,903	NA	3	1.5	1.5	NA	NA	1	NA
Baetidae	*Centroptilum.luteolum*	**1**	0,529	2	3,2	1	1.5	1.6	1,9	1.5	2
Baetidae	*Centroptilum.sp*	**1**	0,410	2.1	3	NA	NA	1.6	NA	2	1
Baetidae	*Cloeon.dipterum*	**0**	0,000	2	3,5	1	0.5	1.2	2,1	1	2
Baetidae	*Cloeon.praetextum*	**1**	0,500	NA	3,5	1	NA	1.2	1,7	1	NA
Baetidae	*Cloeon.simile*	**1**	0,247	1.7	3	1	0.5	1.2	2,0	1	2
Baetidae	*Cloeon.sp*	**1**	0,268	1	3	1	NA	NA	NA	0.5	NA
Baetidae	*Diphetor.hageni*	**1**	0,745	2.1	3	1.5	1.5	1.4	NA	1	1
Baetidae	*Guloptiloides.gargantua*	**2**	0,800	2.1	3	2	2	3	NA	2	0.5
Baetidae	*Labiobaetis.frondalis*	**1**	0,484	1.5	3	1	1	NA	1,3	1	2
Baetidae	*Labiobaetis.geminatus*	**1**	0,328	2.1	3	1	1.5	NA	NA	2	0
Baetidae	*Labiobaetis.propinquus*	**1**	0,404	2	2,5	1	0.5	1.5	1,5	1	1
Baetidae	*Neocloeon.alamance*	**1**	0,750	2.1	3	1	1	NA	NA	1	1
Baetidae	*Neocloeon.triangulifer*	**2**	0,846	3	3	1	1	1.5	1,3	1	1.5
Baetidae	*Nigrobaetis.niger*	**1**	0,578	1.7	2,5	1.5	1	1.4	1,8	0.5	0
Baetidae	*Procloeon.fragile*	**1**	0,400	NA	3	1	1.5	2	NA	1	1
Baetidae	*Procloeon.pennulatum*	**1**	0,324	1.7	3,2	1	0.5	1.6	1,5	1.5	2
Baetidae	*Procloeon.rivulare*	**1**	0,450	1.5	3	1.5	1	NA	NA	1	1
Baetidae	*Procloeon.rufostrigatum*	**0**	0,001	1.5	3	1.5	1	1.4	NA	1	1

Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Baetidae	*Procloeon.viridoculare*	**1**	0,500	2.1	3	1	0.5	1.8	NA	1	1
Baetidae	*Procloeon.sp*	**1**	0,730	2.1	3	NA	NA	NA	NA	2	1
Baetiscidae	*Baetisca.rogersi*	**0**	0,010	1	1,5	1.5	1	1.5	NA	1	1
Behningiidae	*Dolania.americana*	**0**	0,000	0.6	0	2.5	1	3	1,0	1	NA
Caenidae	*Caenis.catherinae*	**2**	0,800	2.1	2	1	1	NA	NA	2	0
Caenidae	*Caenis.cuniana*	**2**	0,792	2.1	2	0.5	0.5	1.3	NA	2	0.5
Caenidae	*Caenis.femina*	**2**	0,800	2.1	2	0.5	0.5	NA	NA	2	0
Caenidae	*Caenis.horaria*	**0**	0,005	2	2	1	0.5	1	2,2	1	1.5
Caenidae	*Caenis.knowlesi*	**2**	0,930	2.1	2	1	1	1.3	NA	2	0
Caenidae	*Caenis.luctuosa*	**0**	0,059	2	2	1.5	1	1.2	1,6	1	2
Caenidae	*Caenis.macrura*	**0**	0,000	1.5	2	1.5	1	1.2	1,5	1	NA
Caenidae	*Caenis.robusta*	**0**	0,000	1.5	2	1	0.5	1	2,2	1	2
Caenidae	*Caenis.vanuatensis*	**2**	0,800	2.1	2	1	NA	NA	NA	2	NA
Dipteromimidae	*Dipteromimus.tipuliformis*	**0**	0,000	1	3	1	NA	NA	NA	1.5	NA
Ephemerellidae	*Ephemerella.aurivillii*	**1**	0,500	1	2	2	1.5	1.6	NA	0	1
Ephemerellidae	*Ephemerella.dorothea*	**0**	0,001	1	2	1	1	1.4	NA	1	2
Ephemerellidae	*Ephemerella.invaria*	**1**	0,500	1	2	1	1	NA	0,3	1	1
Ephemerellidae	*Ephemerella.mucronata*	**1**	0,384	1	2	2	1	1.6	1,0	0.5	1
Ephemerellidae	*Ephemerella.notata*	**1**	0,500	1	2	2	1	1.6	1,9	1	1
Ephemerellidae	*Eurylophella.doris*	**0**	0,100	1	2	1.5	1	2	NA	1	1.5
Ephemerellidae	*Eurylophella.enoensis*	**0**	0,100	1	2	1	NA	NA	NA	1	NA
Ephemerellidae	*Eurylophella.funeralis*	**1**	0,647	0.75	2	1	1	1.6	NA	1	2
Ephemerellidae	*Eurylophella.oviruptis*	**2**	0,795	NA	2	1	NA	NA	NA	1	NA
Ephemerellidae	*Serratella.ignita*	**1**	0,135	1.3	2	1.5	1.5	1.5	1,9	1	1.5
Ephemerellidae	*Torleya.major*	**0**	0,011	1	2	1.5	1	1.5	1,5	1	1
Ephemeridae	*Ephemera.danica*	**0**	0,000	0.5	0	1.5	1	0.5	1,5	1	2
Ephemeridae	*Ephemera.glaucops*	**0**	0,000	0.5	0	1.5	NA	0.5	NA	1	NA

Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Ephemeridae	*Ephemera.japonica*	**0**	0,011	1	0	1.5	0	NA	NA	1.5	NA
Ephemeridae	*Ephemera.orientalis*	**0**	0,004	1.5	0	1	0	NA	NA	1	NA
Ephemeridae	*Ephemera.simulans*	**0**	0,000	0.75	0	1	0.5	0.5	NA	1	NA
Ephemeridae	*Ephemera.strigata*	**0**	0,007	0.75	0	1	0	NA	NA	1	NA
Ephemeridae	*Ephemera.varia*	**1**	0,630	0.5	0	1	0.5	0.5	NA	1	NA
Ephemeridae	*Ephemera.vulgata*	**0**	0,000	0.5	0	1	0	0.5	2,1	1	NA
Ephemeridae	*Hexagenia.bilineata*	**0**	0,000	1.3	0	1	0	0.5	1,1	1	1
Ephemeridae	*Hexagenia.limbata*	**0**	0,025	0.6	0	1.5	0	0.5	1,0	1	1.5
Ephemeridae	*Hexagenia.rigida*	**0**	0,040	0.45	0	1	0	0.5	NA	1	1
Heptageniidae	*Afronurus.sp*	**0**	0,000	2.1	1,5	NA	NA	NA	NA	2	NA
Heptageniidae	*Dactogenia.coerulans*	**0**	0,000	1	1,5	2	1.5	1.6	2,0	1.5	NA
Heptageniidae	*Ecdyonurus.dispar*	**0**	0,003	1.3	1,5	1.5	2	1.5	1,9	1	2
Heptageniidae	*Ecdyonurus.forcipula*	**0**	0,002	NA	1	1.5	2	NA	1,6	0.5	0.5
Heptageniidae	*Ecdyonurus.heleticus*	**0**	0,000	1.1	1,5	1.5	2	NA	0,9	0.5	0
Heptageniidae	*Ecdyonurus.insignis*	**0**	0,016	1	1,5	1.5	2	1.6	1,9	1	NA
Heptageniidae	*Ecdyonurus.picteti*	**0**	0,006	1	1,5	1.5	NA	NA	0,9	0.5	0
Heptageniidae	*Ecdyonurus.torrentis*	**1**	0,209	1.1	1,5	1.5	2	1.6	1,2	1	NA
Heptageniidae	*Ecdyonurus.venosus*	**0**	0,012	1.3	1,5	1.5	2	1.7	1,5	1	2
Heptageniidae	*Electrogena.lateralis*	**0**	0,045	1	1,5	1.5	2	1.7	1,5	1	1.5
Heptageniidae	*Electrogena.sp*	**0**	0,000	3	1	NA	NA	NA	NA	2	NA
Heptageniidae	*Epeorus.alpicola*	**0**	0,041	1	1	3	3	NA	0,8	0	0
Heptageniidae	*Epeorus.assimilis*	**0**	0,009	0.9	0,5	3	2.5	2	0,8	0.5	0
Heptageniidae	*Heptagenia.sulphurea*	**0**	0,005	1.3	1,5	1.5	1.5	1.6	2,0	0.5	0
Heptageniidae	*Maccaffertium.mediopunctatum*	**0**	0,000	1	1	2	2.5	NA	0,9	1	NA
Heptageniidae	*Maccafertium.mexicanum*	**0**	0,100	1.5	1	2	2	1	1,8	1	NA
Heptageniidae	*Maccafertium.modestum*	**0**	0,100	1.5	1	1.5	2	1.5	0,6	1	NA
Heptageniidae	*Maccafertium.pulchellum*	**0**	0,003	1	1	1.5	2.5	1.7	2,1	1	2

Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Heptageniidae	*Maccaffertium.vicarium*	**0**	0,016	1	1	1	2	2.1	0,6	1	2
Heptageniidae	*Rhithrogena.hybrida*	**0**	0,001	1	1	2.5	2	NA	0,3	0.5	0
Heptageniidae	*Rhithrogena.loyolaea*	**0**	0,018	0.6	0,5	2.5	2.5	2	0,2	0	0
Heptageniidae	*Rhithrogena.picteti*	**0**	0,011	1	0,5	2.5	2	2	0,9	0.5	1
Heptageniidae	*Rhithrogena.semicolorata*	**1**	0,092	1	0,5	2.5	1.5	2	1,1	0.5	1
Heptageniidae	*Stenacron.interpunctatum*	**0**	0,046	1.5	1	1	2.5	1.8	1,0	0.5	1
Heptageniidae	*Stenonema.femoratum*	**1**	0,073	1.5	1	1	2.5	1.8	1,6	1	1
Isonychiidae	*Isonychia.bicolor*	**0**	0,001	1.5	2,5	2.5	2	1.2	NA	1	NA
Leptophlebiidae	*Choroterpes.picteti*	**0**	0,000	1	2	2	1	1.4	2,1	1	2
Leptophlebiidae	*Choroterpes.sp*	**1**	0,013	3	2	NA	1	1.4	NA	2	NA
Leptophlebiidae	*Fittkaulus.cuiabae*	**2**	0,800	3	2,5	1	1	1.8	NA	2	NA
Leptophlebiidae	*Fittkaulus.cururuensis*	**2**	0,800	3	2,5	1	1	1.8	NA	2	NA
Leptophlebiidae	*Habroleptoides.auberti*	**0**	0,000	1	2	1	0.5	NA	NA	0	0
Leptophlebiidae	*Habroleptoides.modesta*	**0**	0,001	1	2	1.5	0.5	1.1	1,3	1	0
Leptophlebiidae	*Habrophlebia.lauta*	**0**	0,000	1	2	1	0.5	1	1,5	1	2
Leptophlebiidae	*Leptophlebia.cupida*	**0**	0,065	1	2	1.5	0.5	1.5	NA	1	2
Leptophlebiidae	*Leptophlebia.marginata*	**0**	0,000	1	2	1	1	1	1,9	0.5	0
Leptophlebiidae	*Leptophlebia.vespertina*	**0**	0,022	0.9	2	1	1	1	1,8	1	1
Leptophlebiidae	*Paraleptophlebia.debilis*	**0**	0,100	1.3	2	1.5	1	1.5	NA	1	NA
Leptophlebiidae	*Paraleptophlebia.submarginata*	**0**	0,000	1	2	1.5	1	1	1,6	1	1
Leptophlebiidae	*Thraulodes.sp*	**2**	0,847	2.3	2	NA	NA	2	1,5	2	0
Leptophlebiidae	*Thraulus.bellus*	**0**	0,000	1	2	1	1	NA	NA	1	1.5
Oligoneuriidae	*Oligoneuriella.rhenana*	**0**	0,000	1	1,5	2.5	2	0.5	1,8	1	1.5
Palingeniidae	*Palingenia.longicauda*	**1**	0,520	0.33	0	2	0	0.5	2,0	1	2
Polymitarcyidae	*Ephoron.album*	**0**	0,090	1	0	1.5	0	0.5	NA	0.5	1.5
Polymitarcyidae	*Ephoron.eophilum*	**1**	0,155	1	0	1	0	0.5	NA	1.5	NA
Polymitarcyidae	*Ephoron.shigae*	**1**	0,525	1	0	2	1	0.5	NA	1	NA

Family	***Species***	**Reprod**	**Parth**	**voltinism**	**dispersal**	**current**	**substrate**	**diet**	**saprobity**	**Climate**	**rangeTemp**
Polymitarcyidae	*Ephoron.virgo*	**0**	0,000	1	0	2	0.5	0.5	2,3	1	2
Potamanthidae	*Potamanthus.luteus*	**0**	0,000	1	1,5	1.5	1	0.8	2,2	1	2
Prosopistomatidae	*Prosopistoma.foliaceum*	**0**	0,000	1	1,5	3	1.5	1.7	NA	1.5	2
Prosopistomatidae	*Prosopistoma.pearsonorum*	**2**	0,800	NA	1,5	3	3	NA	NA	2	NA
Siphlonuridae	*Siphlonisca.aerodromia*	**1**	0,548	1	3,5	1	1	2.5	NA	0.5	1.5
Siphlonuridae	*Siphlonurus.aestivalis*	**0**	0,054	1.3	2,7	1	1	1.1	2,0	0.5	2
Siphlonuridae	*Siphlonurus.lacustris*	**1**	0,119	1.3	2,5	1	1	1.1	1,4	1	2

Reprod: reproductive mode; 0=sexual reproduction (including tychoparthenogenesis); 1=facultative parthenogenesis (including mixed reproduction in sympatry and/or in allopatry); 2=’obligate’ parthenogenesis (unisexual populations with only females).

Parth: parthenogenesis ability, the mean proportion of hatching success of unfertilised eggs at the species level (see the database in Liegeois *et al.*, 2021).

Voltinism: number of generation(s) per year for a given species; ≤0.75=mostly semivoltine; >0.75-≤1.5= mostly univoltine; >1.5-≤2.5=mostly bivoltine; >2.5=mostly multivoltine. The diversity and plasticity of mayfly life cycles are species-specific with no consistent patterns at the family or genus level (Clifford, 1982).

Dispersal phenotype: larval dispersal ability, correlated to body shape and size; 0=burrowers (almost never swim and live hidden into the bottom substrate, quite large nymphs generally >2.4cm); 1=clingers (able to actively swim but usually stay under a rock in fast to very fast current, typically flat nymphs with size ~2cm); 2=walkers and crawlers (awkward swimmers that avoid fast current, typical size ~1-2cm); 3=swimmers (can easily swim by fish-like movements, typical size <1.5cm).

Current: current velocity, the rate of water flow in a species’ habitat; 0=null; 1=slow; 2=moderate; 3=fast.

Substrate: substrate size, the granulometry of the sediment in a species’ habitat; 0=very small, <2.5mm (mud, silt, fine sand); 1=small, from 2.5mm to 2.5cm (gravel, coarse sand, leaf-litter, algae, moss); 2=medium, from 2.5cm to 25cm (stone, pebble, rubble, branch, root); 3=large, >25cm (block, rock, boulder, cobble, log).

Diet: feeding regime, the size of the particle ingested; 0=filters (of fine particulate organic matter); 1=collector-gatherers (of diatoms and small decomposing particulate organic matter); 2=grazer-scrapers (of algae, plants and coarse particulate organic matter); 3=predators (carnivorous).

A combination of stream order, substrate size, current velocity preference and diet define the microhabitat of mayfly species (Vannote *et al.*, 1980).

Saprobity: degree of tolerance to water pollution, based on the species saprobic value (Sladecek, 1973; Moog *et al.*, 1997; Zahradkova *et al.*, 2009; Barinova, 2017); 0=very sensitive; 1=sensitive; 2=tolerant; 3=resistant.

Climate: the species geographic range; 0=boreal and/or polar (cold); 1=temperate; 2=tropical (warm).

rangeTemp: range of temperature tolerance; 0=stenothermal (narrow range); 1=metathermal; 2=eurythermal (wide range).

NA: not available.

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– CHAPTER III –

Reproduction and sex ratios in natural populations of the mayfly *Alainites muticus* (Baetidae)

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(in preparation)

Chapter III

Reproduction and sex ratios in natural populations of the mayfly *Alainites muticus* (Baetidae)

Abstract

The ability to reproduce parthenogenetically has been reported for a number of sexual species under laboratory conditions. However, it remains typically unknown whether or how frequently species capable of parthenogenesis use this reproductive mode under natural conditions. A possible approach to estimating the frequency of parthenogenesis in natural populations is to study population sex ratios. Indeed, with thelytokous (female producing) parthenogenetic reproduction, natural populations are expected to exhibit female-biased sex ratios. However, other factors such as sex-biased mortality can generate deviations from 1:1 sex ratios. Before using population sex ratios as a proxy for parthenogenesis in natural populations, it is therefore important to verify that the parthenogenetic capacity is correlated to sex ratios. We used the facultatively parthenogenetic mayfly *Alainites muticus* (Baetidae) to test whether the ability to produce parthenogenetic brood generates a distortion of the 1:1 sex ratios normally found in sexual species, and whether the parthenogenetic capacity varies between and/or within populations. To address these two questions, we measured the hatching success of unfertilised eggs of 57 females from 17 Swiss populations. We found a significantly positive relationship between the parthenogenetic capacity and the proportion of females in natural populations, suggesting that *Alainites muticus* females do use their parthenogenetic potential in the wild and that sex ratios can be used as a proxy of parthenogenesis in natural populations. We also found a reproductive polymorphism between females within populations, highlighting three different reproductive modes in *A. muticus*: obligate sexual, obligate parthenogenetic and facultative parthenogenetic reproduction.

Keywords: mayflies, parthenogenesis, sex ratios, natural populations, behaviour.

Introduction

The maintenance of sexual reproduction in natural populations, despite its many costs, is an evolutionary paradox (Schön & Martens, 2018). Studying alternative forms of reproduction, such as parthenogenesis, can help understand the evolution of reproductive polymorphism, but experimental tests in natural populations remain scarce (Jalvingh *et al.*, 2016; Neiman *et al.*, 2018).

In mayflies, sexual reproduction co-occurs with three forms of parthenogenesis (tycho-, facultative and obligate parthenogenesis) at different levels (see Liegeois *et al.*, 2021 for a recent review). However, this ability to reproduce parthenogenetically has been reported mainly under laboratory conditions, and it remains unknown whether or how frequently parthenogenesis is used under natural conditions. A possible approach to estimating the frequency of parthenogenesis in the wild is to study population sex ratios. Indeed, with thelytokous (female producing) parthenogenetic reproduction, natural populations are expected to exhibit female-biased sex ratios. However, other factors such as sex-biased mortality can generate deviations from 1:1 sex ratios. Before using population sex ratios as a proxy of parthenogenesis in natural populations, it is therefore important to verify that the parthenogenetic capacity affects population sex ratios in the wild.

To address this question, we used the mayfly species *Alainites muticus* (Baetidae) because of its widespread distribution across Switzerland, and its ability to reproduce *via* facultative parthenogenesis (Degrange, 1960). We first tested whether the ability to produce parthenogenetic broods generates a distortion of the 1:1 sex ratios normally found in sexual species. Second, we tested whether the parthenogenetic capacity varies among females from the same or different populations. Finally, we compared the egg-laying propensity of virgin females, and the development time of unfertilised eggs, between females with high *versus* low capacities of parthenogenesis, to uncover potential traits associated with parthenogenesis in *A. muticus*.

Material & Methods

Population sex ratios

To determine whether sex ratios (proportion of females) reflect reproductive strategies of a given population, we sampled 36 stream sites (hereafter referred to populations) in Switzerland across a broad altitudinal range (200-2000m). The populations were sampled between March and June (according to altitude) following the IBCH method (described in Stucki, 2010), a standardised sampling technique used for biomonitoring of Swiss watercourses. In short, each station was sampled over a transect ten times longer than the width of the stream. Within that transect, 8 parcels of 25cm^2 were sampled by scraping the riverbed during 30 seconds in front of a Surber net (fine mesh of 500µm). The macrozoobenthos living on and in the substrate was so freed and collected in the Surber net. The eight samples of each station were then pooled and stored in 80% ethanol until later examination and identification. In the laboratory, samples were passed successively through several sieves, from 5 mm to 650 µm. Retained material was then examined under a binocular microscope, and macrozoobenthos separated from debris. We identified mayflies of the Baetidae family to the species level following Studemann *et al.* (1992). Individuals of the species *Alainites muticus* were then sexed and counted for each population. This allowed us to calculate population sex ratios and identify those significantly deviating from 1:1 (binomial test).

Parthenogenetic capacity

To determine whether sex ratios (proportion of females) are correlated to parthenogenetic potentials in natural populations of *A. muticus*, we re-sampled 20 of the 36 sites mentioned above (during May-June 2017 and April-May 2018). These populations were chosen to cover the range of population sex ratios found in *A. muticus* identified during the first sampling (Fig. 1). To measure the parthenogenetic capacity, up to 20 late instar female nymphs were collected from 17 of the 20 populations, no late instar nymphs could be obtained from the three remaining populations. Female nymphs were taken to the laboratory and reared to adulthood in aquariums in a climate chamber (water temperature: 12±1°C, room temperature: 22±2°C, relative humidity: 50%, 12L:12D photoperiod). Aquariums were supplied with lake water, in order to provide nutrients for nymphal growth until the final moult. 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 bubblers in each aquarium. Nymphs were reared in partially immersed floating cages (Fig. 2). Submerged parts were surrounded by thin mesh allowing 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 moult (from subimago to imago), we allowed them to lay their (unfertilised) eggs into a Petri dish (Ø 55 mm) filled to ¾ with filtered (0.2 µm) lake water. Some females (28/91=30.8%, see results) did not lay spontaneously under these conditions. For these females, we extracted the eggs by dissecting their abdomen after they died (within 0-12 hours). We counted the number of eggs per clutch, divided them into two to three new Petri dishes (Ø 35 mm) to reduce egg densities per dish and facilitate observation of hatchlings, and maintained them at 10°C with a 12L:12D photoperiod. After about three weeks of incubation, hatchings started. 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 unfertilised eggs that hatched) for each female. The population-level parthenogenetic capacity was estimated as the mean hatching success of all females in that population. In total, we obtained one to seven egg clutches (*i.e.*, eggs from 1-7 females) per population (Table 1). For each of these clutches, we also determined the mean development time by averaging the number of days, from oviposition to hatching, for all eggs that hatched.

Statistical analyses

We tested whether the mean parthenogenetic capacity of each population was correlated to the sex ratio measured in the field. To do so, we ran a quasibinomial GLM (Generalised Linear Model, corrected for overdispersion) followed by an *F*-test from the ‘car’ package (Fox & Weisberg, 2011). Data points were weighted in the analysis according to sample size (the number of females per population). We also analysed the mean development time of unfertilised eggs to see whether there was variation among females according to their parthenogenetic capacity, using a binomial GLMM (Generalised Linear Mixed Model) from the ‘lme4’ package (Bates *et al.*, 2015). Finally, we checked whether non-spontaneous ovipositions were enriched in populations with 1:1 sex ratios using a Fisher’s exact test within the ‘rcompanion’ package (Mangiafico, 2017). All analyses were performed with R v.3.3.3 (R Development Core Team, 2017), and all graphs using the ‘ggplot2’ package (Wickham, 2016).

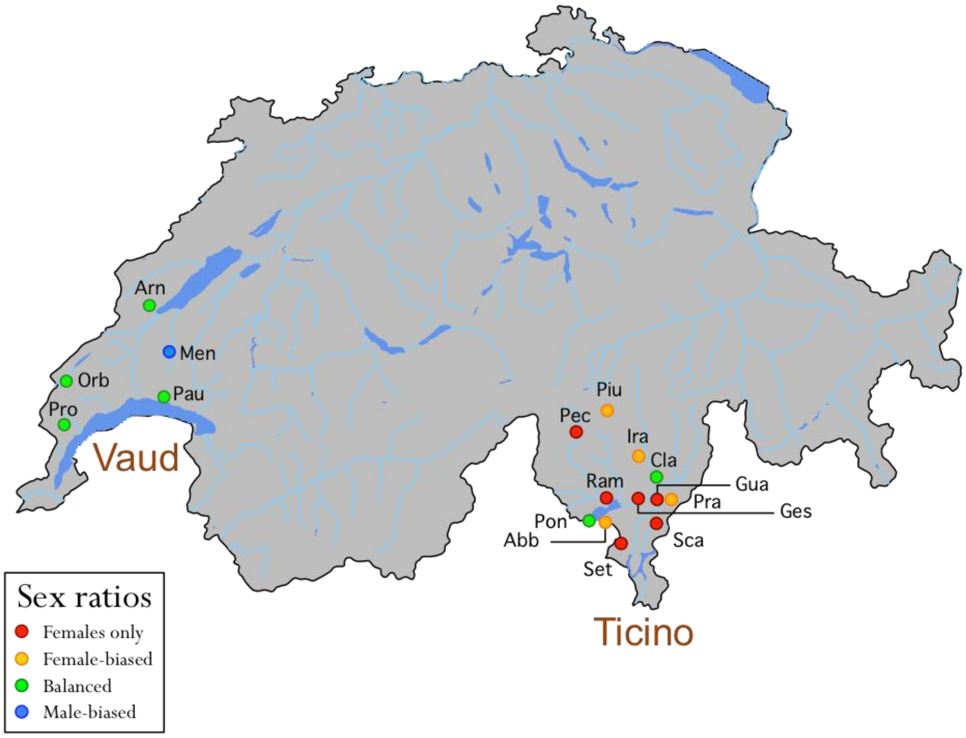

Figure 1. **The 17 sampling sites from Vaud and Ticino cantons (Switzerland).** Each colour represents the population sex ratio: significantly male-biased (blue), approximately equal (green), significantly female-biased (orange), and female-only (red).

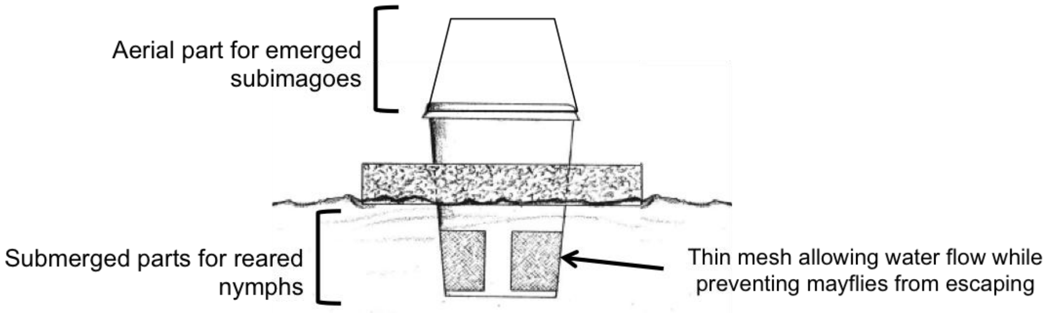

Figure 2. **Schema of a partially immersed floating cage.**

Results

Variations in the parthenogenetic capacity between and within populations

Laboratory experiments to determine the hatching success of unfertilised eggs showed that the parthenogenetic capacity of females varied widely between and within populations (Table 1, Fig. 3A). In total, we were able to obtain unfertilised eggs from 57 females (one to seven females per population) and 52’010 eggs were observed for their parthenogenetic development (~1’000 eggs per females, range 239-2765). The average level of parthenogenetic hatching per population ranged from 6.0% to 92.4%, with large variations among females within populations (*e.g.*, from 2.1 to 98.0% for the “Pra” population, n=6 females).

Field evidence of parthenogenetic reproduction

In order 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 unfertilised eggs of each population, and correlated it with the population sex ratio. Hatching successes of unfertilised eggs were significantly positively correlated with the population sex ratios (Fig. 3B), meaning that a high parthenogenetic potential of females in the field translates into female-biased population sex ratios (*GLM*, $r = 0.91$, $p<0.001$). Therefore, we can use sex ratios as a proxy of parthenogenetic capacity in natural populations of *A. muticus*. Furthermore, the variations observed between females within populations (Table 1, Figure 3), suggest that there are three types of *A. muticus* females (sexual females, facultatively parthenogenetic, and ‘obligately’ parthenogenetic ones) with different frequencies in different populations (see also Fig. 6B). Populations with 1:1 sex ratios consist largely of sexual individuals (capable of tychoparthenogenesis) with few facultatively parthenogenetic females at most, while populations with female-biased sex ratios consist of either facultatively parthenogentic females or a relatively even mixture of sexual and parthenogenetic ones. Finally, female-only populations are most likely ‘obligate’ parthenogens (Figure 3).

Table 1. **Population sex ratios and hatching successes** (%) **of unfertilised eggs for 17 *A. muticus* populations across Switzerland**.

Population	**n**	**Hatching success of unfertilised eggs – population mean** (%, range)	**Development time – population mean** (days, range)	**Total number of eggs observed**	**Sex ratio**
Men	1	6.0	70.7	1055	Male-biased
Pro	5	29.5 (10.3-49.7)	75.0 (66.9-83.5)	4611	Balanced
Arn	4	29.6 (2.7-55.3)	69.5 (68.2-70.7)	4100	Balanced
Orb	4	35.8 (5.4-86.4)	71.8 (60.3-82.0)	3331	Balanced
Pau	1	41.6	72.1	2764	Balanced
Cla	1	32.1	68.0	1124	Balanced
Pon	3	38.8 (6.2-93.6)	48.5 (30.4-59.5)	1333	Balanced
Ira	1	49.8	43.3	239	Female-biased
Abb	7	66.7 (28.9-89.4)	43.3 (34.1-63.4)	4433	Female-biased
Pra	6	50.4 (2.1-98.0)	39.9 (26.9-60.9)	4844	Female-biased
Piu	2	38.9 (7.0-70.9)	50.1 (45.5-54.8)	973	Female-biased
Pec	5	67.7 (12.9-98.5)	36.7 (25.1-53.7)	4214	Females only
Set	3	81.1 (63.7-97.6)	32.6 (30.7-35.0)	4022	Females only
Ram	5	85.3 (67.4-94.1)	35.5 (31.7-40.5)	8144	Females only
Gua	2	65.5 (36.2-94.9)	36.4 (30.2-42.6)	1346	Females only
Ges	4	87.1 (74.8-93.7)	34.8 (31.8-39.4)	3702	Females only
Sca	3	92.4 (89.0-93.9)	33.7 (29.8-37.5)	1775	Females only

n: number of virgin females tested. See also Figure 1 for geographical information.

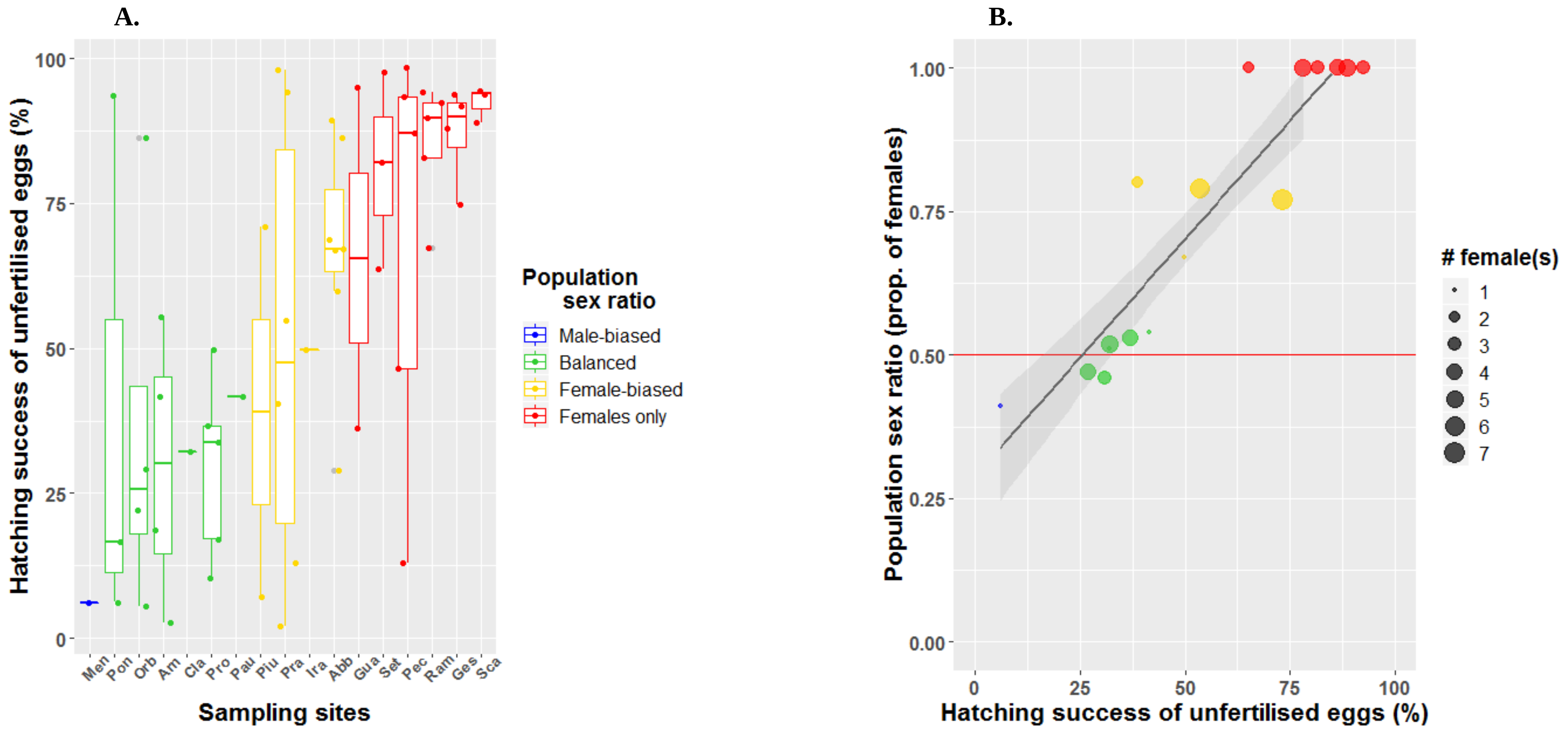

Figure 3. (**A**) **Large variation in hatching success of unfertilised eggs** between and within populations of *A. muticus*. (**B**) **Parthenogenesis generates female-biased sex ratios** in the field (*GLM*, *p<0.001*, *r = 0.91*). The size of the dots represents the number of virgin females tested for each population.

Development time of parthenogenetic eggs

In facultative parthenogenetic species, fertilised eggs often develop faster than unfertilised ones (*e.g.*, Sweeney & Vannote, 1987; Landolt *et al.*, 1997; Funk *et al.*, 2010), as expected if the onset of unfertilised egg development was delayed to maximise the chance of fertilisation. However, such a delay in development might not be expected for eggs in obligately parthenogenetic females as they will never be fertilised.

The mean development time of eggs from the 57 different clutches varied extensively between and within populations (Table 1, Fig. 4). The higher the egg-hatching success per clutch, the shorter was the mean development time of eggs in that clutch (*GLMM, r = -0.94, p<0.001*). Indeed, for females with the highest hatching successes of parthenogenetic eggs (which are most likely obligately parthenogenetic), hatching occurs on average 36 days after oviposition (typical range 25-40 days), whereas egg development takes on average 64 days (typical range 55-80 days) for females with a low hatching success of parthenogenetic eggs (which are most likely sexual with a capacity for tychoparthenogenesis).

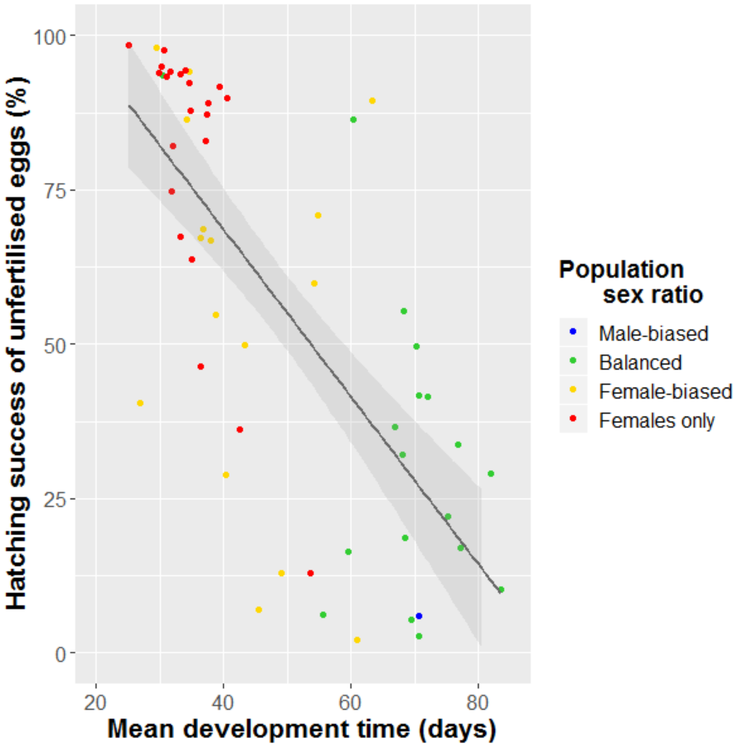

Figure 4. **Relationship between hatching success and development time of unfertilised eggs** of 57 virgin females from the 17 Swiss populations (*GLMM, p<0.001, r = -0.94*).

Oviposition behaviour

Reproductive polymorphism may lead to behavioural changes such as how or when virgin females oviposit. For example, virgin sexual females may delay egg-laying to increase the chances of finding a mate, a behaviour not expected in obligately parthenogenetic females. In order to determine whether a virgin female from a parthenogenetic population is more likely to lay her eggs spontaneously than a virgin female from a sexual population, we classified the 57 females depending on how unfertilised eggs were obtained (*i.e.*, from spontaneous oviposition or from abdomen dissection). We further included information from 34 additional females for which we obtained unfertilised egg clutches but were unable to measure hatching successes (either because eggs were damaged during dissections or because of fungi development). Females from parthenogenetic populations laid their eggs more often spontaneously than females from sexual populations (*i.e.*, 82.8% *vs.* 45.5% of spontaneous oviposition for n=58 and 33 respectively, *p.Fisher<0.001*, Fig. 5A).

We also tested whether unfertilised eggs from spontaneously laid clutches had a higher hatching success than extracted clutches (using the 57 clutches with information on hatching success). For females from parthenogenetic populations, eggs laid spontaneously showed a greater hatching success than extracted ones (Fig. 5B, *p=0.02*), while hatching successes were much lower and showed no difference between spontaneously laid *versus* extracted eggs for females from sexual populations (Fig. 5B, *p=0.42*). In addition, among females who laid spontaneously, unfertilised eggs from parthenogenetic females had a significantly higher hatching success than those from sexual females (Fig. 5B, *p=0.006*). These results suggest that parthenogenetic females are more likely to oviposit spontaneously, and they exhibit a higher hatching success of unfertilised eggs (Figure 5).

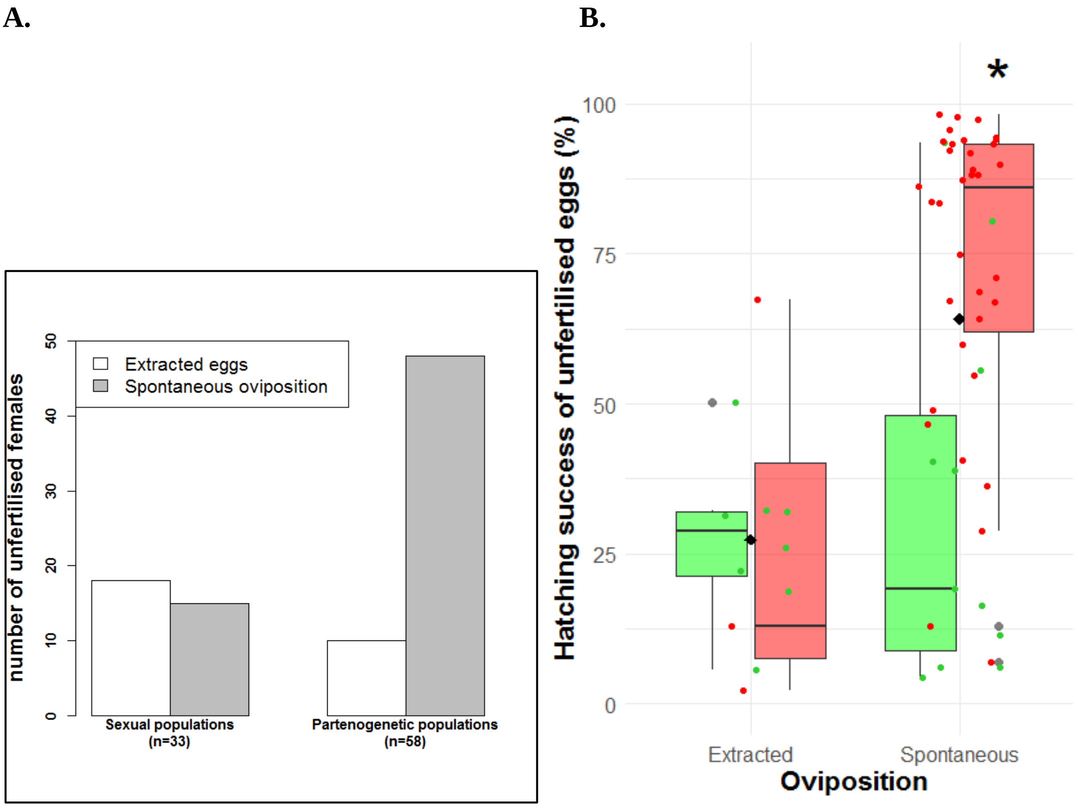

Figure 5. (A) **Variations in oviposition behaviour according to the population reproductive strategy of 91 virgin females.** Thirty-three females were tested from the seven sexual populations (with relatively equal sex ratios), and 58 females were tested from the 10 parthenogenetic populations (with relatively female-biased sex ratios). Fisher's exact test, *$p<0.001$*; see also Supplementary Figure 1. (B) **Variations in hatching success according to the type of oviposition and the population reproductive strategy.** Each dot represents a tested virgin female from either populations with relatively equal sex ratios (green), or populations with relatively female-biased sex ratios (red). Only virgin females from parthenogenetic populations that have laid spontaneously showed a significantly higher hatching success of unfertilised eggs than virgin females from the three remaining categories. See also Supplementary Figure 2.

Discussion

Alainites muticus was thought to be a facultatively parthenogenetic species given the high hatching rate of unfertilised eggs laid by females from a sexual population (*e.g.*, Degrange, 1960). However, our measurements revealed that there are most likely different types of females in *A. muticus* (*i.e.*, mixed reproduction). Indeed, among the 57 studied virgin females, the range of hatching successes was very wide (*i.e.*, from 2.1% to 98.5%, Table 1). In addition, we found unisexual populations in Ticino (Fig. 1, see also Liegeois *et al.*, Chap. IV), where parthenogenetic females exhibit a much higher hatching success of unfertilised eggs than virgin females from sexual populations (Table 1). These results suggest three reproductive modes among *A. muticus* females: obligate sexual, obligate parthenogenetic and facultative parthenogenetic reproduction, a distinction further supported by a K-means cluster analysis (Figure 6). In addition, we found that reproductive polymorphism leads to variation in oviposition behaviour and egg development time among females. Our results showed that parthenogenetic females are more likely to lay unfertilised eggs spontaneously than sexual females, and that their unfertilised eggs develop faster (Figures 4, 5 and 6). Which factors cause these different reproductive strategies remains to be investigated. Unisexual populations of *A. muticus* are also known in eastern Ukraine (Martynov, 2013), corroborating our suggestion of reproductive polymorphism in this mayfly species and suggesting geographical parthenogenesis.

The reproductive polymorphism we found in *A. muticus* likely exists in other mayfly species. For example, the hatching successes of unfertilised eggs can differ extensively between populations (*e.g.*, Sweeney & Vannote, 1987; Watanabe & Ishiwata, 1997; Glazaczow, 2001; Ball, 2002, for *Eurylophella funeralis* (Ephemerellidae), *Ephoron shigae* (Polymitarcyidae), *Ephemerella notata* (Ephemerellidae), and *Stenonema femoratum* (Heptageniidae)), or within populations (*e.g.*, Degrange, 1960; Ball, 2001, for *Siphlonurus lacustris* (Siplonuridae) and *Stenonema femoratum*), highlighting the fact that reproductive polymorphism may be widespread among mayflies (see also Liegeois *et al.*, 2021 for a recent review).

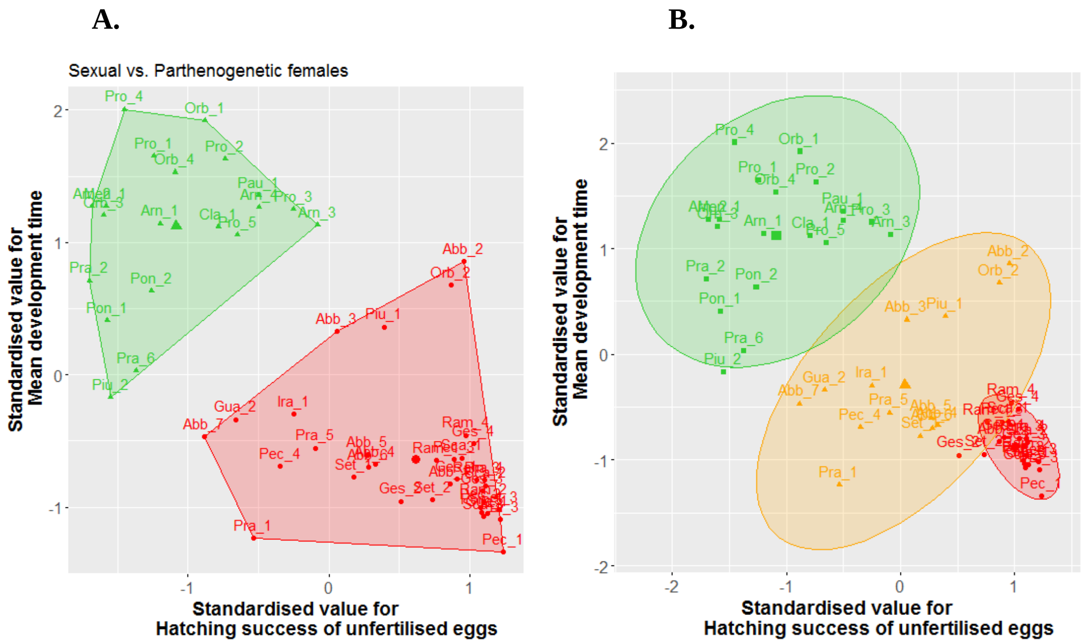

Figure 6. (A) **K-means cluster analysis of *A. muticus* virgin females according to hatching success and development time of their unfertilised eggs.** The two groups represent sexual females (green) *vs.* parthenogenetic females (red). Unfertilised eggs from sexual females show a low hatching success and a prolonged development time, while unfertilised eggs from parthenogenetic females show a high hatching success and a short development time. (**B**) **K-means cluster analysis partitioning females into a set of three groups.** Green: sexual females; Orange: facultatively parthenogenetic females; and Red: 'obligately' parthenogenetic females. Clustering algorithms were used from the 'cluster' package (Maechler *et al.*, 2019), and clustering visualisation from the 'factoextra' package (Kassambara & Mundt, 2019).

For spontaneous oviposition, virgin females in *Eurylophella funeralis* also showed a stronger propensity to oviposit spontaneously if they came from parthenogenetic populations than from sexual populations (Sweeney & Vannote, 1987). As for egg development time, similar patterns to the one we document in the present study have been shown in French populations of *Alainites muticus* and *Siphlonurus lacustris*, where two types of females were found, females with high hatching rates of unfertilised eggs and quite short development times, and females with low hatching rates of unfertilised eggs and prolonged development times (Degrange, 1960). In addition, in mayfly species, fertilised eggs often develop faster than unfertilised ones (*e.g.*, Sweeney & Vannote, 1987; Landolt *et al.*, 1997; Funk *et al.*, 2010).

We suggest that in facultatively parthenogenetic species, fertilised eggs develop faster than unfertilised ones because the onset of unfertilised egg development is delayed to maximise the chance of fertilisation. Such delay in development is however not adaptive for eggs in obligately parthenogenetic females as they will never be fertilised.

Some authors who found parthenogenetic potentials in mayfly species in the laboratory suggested that this potential is never realised in the field (*e.g.*, Huff & McCafferty, 1974; Humpesch, 1980). However, the positive relationship between parthenogenetic capacity and proportion of females in natural populations suggests that *A. muticus* females do use their parthenogenetic potential in the wild (Figure 3B). Field evidence of parthenogenetic reproduction has also been shown for the mayfly species *Stenonema femoratum* (Ball, 2001, 2002), with a mixture of sexual and parthenogenetic females that translate into female-biased population sex ratios. Population sex ratios can therefore serve as a proxy of parthenogenetic capacity within natural populations, at least for the two species *Stenonema femoratum* and *A. muticus*. Nevertheless, there are also cases where high capacities of parthenogenesis do not translate into female-biased sex ratios. Indeed, even though the average hatching success for eggs laid by virgin females from 1:1 populations was rather low (Table 1), eggs from several females of these populations featured quite high hatching successes (above 20%). Thus, potential parthenogenetic reproduction of these females does not affect the population sex ratios. The reasons for this finding remain to be investigated, but could include for example, a very low frequency of such females in 1:1 populations, a lower viability of parthenogenetic than sexual offspring, or a consequence of mate limitation in populations with low densities that would force the female to use her parthenogenetic capacity to overcome the failure to find a mate.

Even though several factors other than parthenogenesis may lead to female-biased sex ratios, it is unlikely that these factors fully explain the female-biased sex ratios in natural populations we observe. For example, differential mortality between sexes could also generate deviations from 1:1 (*e.g.*, if males are less competitive, more sensitive to parasitism or affected by a sex-specific parasite), but further analyses are needed to clarify this possibility. However, differential mortality between sexes in *A. muticus* seems unlikely (or rather weak) according to the strong significant correlation we found between population sex ratios and population-average hatching successes of unfertilised eggs (91% of the variance explained, Fig. 3B).

A distortion of sex ratios in sexually produced broods can also lead to a sex ratio deviating from 1:1 (*e.g.*, Long & Pischedda, 2005; Connallon & Jakubowski, 2009). However, there is currently no evidence for sex ratio distortion within the Baetidae family, since sex ratios of fertilised broods in seven baetid species are relatively balanced (Funk *et al.*, 2010).

Finally, parasitism could affect population sex ratios. Indeed, mayfly nymphs can serve as host for parasites such as nematodes that can affect behaviours in a way that they become more likely to be predated (Benton & Pritchard, 1990; Vance & Peckarsky, 1997; Williams *et al.*, 2001), or by provoking a sex reversal by turning males into quasi-females (Vance, 1996). Investigating these additional potential influences on population sex ratios in *A. muticus* is a challenge for future studies.

Acknowledgements

We would like to thank Luca Sciuchetti for his help in the field, sampling the 36 stream sites, as well as for helping sorting mayflies. We also want to thank Aline Cardinaux for her great job on cleaning and passing all samples through sieves. We are also grateful to Pascale Derleth-Sartori for her explanations and demonstration on IBCH sampling. This study was supported by grants PP00P3_139013 and PP00P3_170627 from Swiss NSF.

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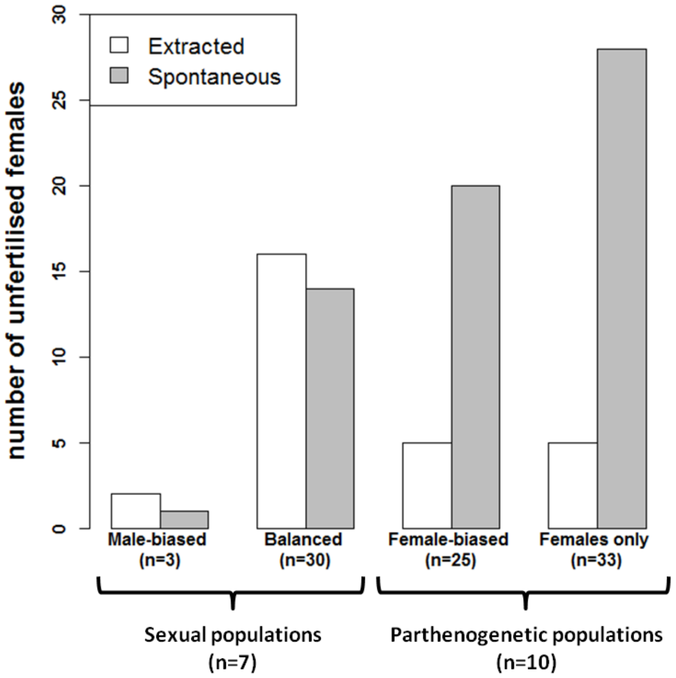
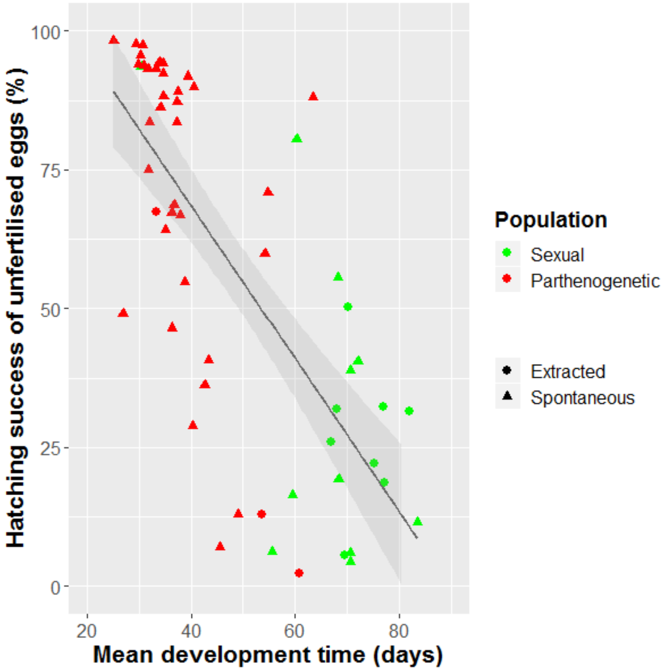
Supplementary Materials



Supplementary Figure 1. **Variations in oviposition behaviour according to the population sex ratio of 91 virgin females.** Eggs were obtained from spontaneous oviposition (gray) or abdomen dissection (white). Three females were tested from the population with male-biased sex ratio, 30 females were tested from the six populations with balanced sex ratios, 25 females were tested from the four populations with female-biased sex ratios, and 33 females were tested from the six populations with female-only.



Supplementary Figure 2. **Correlation between hatching success and development time of unfertilised eggs**, in relation to how a female oviposited (n=57 virgin females). Eggs were obtained from spontaneous oviposition (triangles) or abdomen dissection (circles).

– CHAPTER IV –

What ecological factors favour parthenogenesis over sexual reproduction?

A study on the facultatively parthenogenetic mayfly
Alainites muticus in natural populations.

Maud Liegeois, Michel Sartori and Tanja Schwander

(in preparation)

Chapter IV

What ecological factors favour parthenogenesis over sexual reproduction?

A study on the facultatively parthenogenetic mayfly Alainites muticus in natural populations

Abstract

Different modes of reproduction are characterised by various costs and benefits, which often change depending on ecological contexts. Sexual reproduction is by far the most abundant reproductive mode in the animal kingdom. However, in some taxa, asexual reproduction occurs frequently. For instance, females in many mayfly species are capable of reproducing by facultative parthenogenesis, and produce offspring (females only) if they fail to find a mating partner. Such 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. In addition, complex biotic interactions (*e.g.*, Red Queen hypothesis) and abiotic factors such as altitude (*i.e.*, geographical parthenogenesis patterns) may also play a role in shaping reproductive strategies. We measured the population sex ratio (a proxy of parthenogenesis frequencies), density (a proxy of mate limitation) and community diversity (a proxy of biotic interaction complexity) for 140 populations of the mayfly *Alainites muticus* in Switzerland across an altitude range of 195-1931m, and we used structural equation modelling to investigate both direct and indirect influences of the different variables on reproductive strategies. We found that low population densities generate female-biased sex ratios, as expected if mate limitation selects for parthenogenesis. By contrast, we found no direct effect of community diversity or altitude on the frequency of parthenogenesis. However, community diversity and altitude were indirectly affecting reproductive strategies, by being associated with population densities, highlighting the complexity of interconnections between ecological variables. Together, these results indicate that parthenogenetic reproduction is more common in situations where females are mate-limited, while sexual reproduction is more common in situations presenting suitable habitat conditions for both high community diversities and large population sizes.

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 (recently reviewed in Lehtonen *et al.*, 2012). A number of theories have been developed that can help explain the advantage of sex, but experimental tests in natural populations remain scarce (Jalvingh *et al.*, 2016; 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, but it is well known for several species in the Ephemeroptera order (mayflies). Indeed, among the 3,666 described species, at least 103 have the ability to reproduce by different forms of thelytokous (female producing) parthenogenesis, and at least 49 species are facultatively parthenogenetic (Liegeois *et al.*, 2021). With facultative parthenogenesis, population sex ratios are female-biased if females reproduce at least partly by parthenogenesis, whereas population sex ratios are approximately equal if females reproduce sexually.

The purpose of this study is to identify ecological correlates of the frequency of sex *versus* parthenogenesis in natural populations using sex ratios. Specifically, in order to elucidate ecological conditions selecting for sex or parthenogenesis, we focused on several hypotheses suggested for the maintenance of sex. First, based on the importance of biotic interactions (reviewed in Lively & Morran, 2014), the Red Queen hypothesis proposes that sexual reproduction is generally favoured in more complex communities with fast co-evolutionary dynamics, various types of species interactions, and high levels of competition (*e.g.*, Van Valen, 1973; Jaenike, 1977; Hamilton, 1980). Similarly, the Tangled Bank and Sib Competition hypotheses propose explanations for the prevalence of sex in relatively saturated communities, where competition for resources is likely to be continuous and intense (*e.g.*, Williams, 1975; Bell, 1982; Scheu & Drossel, 2007). Second, population densities can affect the level of mate availability, and parthenogenesis can provide benefits through reproductive assurance, especially when males are scarce (Schwander *et al.*, 2010). Third, a frequently observed pattern in geographical parthenogenesis is that obligate asexual populations are more likely to be found at higher altitudes than their sexual counterparts, for different but often unknown reasons (recently reviewed in Tilquin & Kokko, 2016). However, there are currently no data available to test whether a similar pattern of geographical parthenogenesis holds for facultative parthenogens as well.

In the present study, we focused on the mayfly species *Alainites muticus* (Baetidae) because of its ability to reproduce by facultative parthenogenesis (Degrange, 1960), and because population sex ratios in this species can be used as a proxy of the local parthenogenetic potential (see Liegeois *et al.*, Chap. III). In order to elucidate how biotic and abiotic ecological variables, such as community diversity, the density of individuals, and altitude, can affect reproductive strategies, we used structural equation modelling (SEM), a statistical approach that allows taking into account interactions between factors and hidden indirect effects (*e.g.*, Grace *et al.*, 2010; Eisenhauer *et al.*, 2015; Fan *et al.*, 2016). We thus tested whether intense competition between species favours sex (by studying community diversities), whether mate limitation could select for parthenogenesis (by studying population densities), how sex ratios are correlated with altitude, and how all these ecological variables are interconnected with each other.

Finally, we assessed the stability of parthenogenesis frequencies over time, by testing whether population densities and population sex ratios are relatively constant across years in *A. muticus*.

Material & Methods

Study sites, sex ratios and ecological data

We used samples of *Alainites muticus* from 140 sites (*i.e.*, populations). These samples stem from a biodiversity monitoring survey performed at 500 sites across Switzerland, following the IBCH method (described in Stucki, 2010). This standardised sampling technique is designed to provide an indication of the water quality of streams and rivers on the basis of aquatic macro-invertebrate communities. In brief, each site is sampled from early-March to mid-June, following the relevant altitudinal gradient of Switzerland (from 193 to 2000m, sampling at higher altitudes is done later in the season). In order to cover all substrates of the riverbed and the range of current velocity, eight square areas (25 x 25 cm) are sampled by scraping the riverbed during 30 seconds 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, etc.). Nymphs of three insect orders (Ephemeroptera, Plecoptera and Trichoptera) are further identified to the species level and stored at the Museum of Zoology in Lausanne, from where we obtained the *A. muticus* samples.

Alainites muticus was discovered in 196 of the 500 sites, and we selected all sites where more than 12 *A. muticus* individuals were present (n = 140/196, Fig. 1). For each site, we first quantified sex ratios by sorting male and female nymphs. Males are easily identifiable in early instars by their large eyes and their external genitalia. Nymphs smaller than two millimetres were excluded from sex ratio calculations, as they cannot be sexed reliably. We then assessed biotic and abiotic aspects of each site: population densities, community diversities and altitudes. Two densities were estimated, one on the total number of nymphs found in each population, and one on the sexed individuals, but results were not affected by the density estimate used (see below). Community diversities were estimated using family richness (*i.e.*, total number of macroinvertebrate families) and the Shannon index ($H =$

$-\sum_{n=i}^{R} pi\ x \ln(pi))$. Given the different level of identification for different taxa, (species level for Ephemeroptera, Plecoptera and Trichoptera, while family level for all other macroinvertebrates), the Shannon index was based on abundances at the family level.

Statistical analyses

As a first step, we wanted to determine whether the *A. muticus* sex ratio was significantly unbalanced (*i.e.*, diverged from the equilibrium 1:1) at the whole meta-population level (all sites pooled). To do so, we used a χ^2 test on the total number of males and females over the 140 sites. Subsequently we determined whether there was significant variation among sites in sex ratios. This variation was analysed by using a binomial Generalised Linear Model (GLM).

In a second step, in order to identify ecological correlates of female-biased sex ratios (*i.e.*, parthenogenesis), we used structural equation modelling (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 *et al.*, 2010; Eisenhauer *et al.*, 2015; Fan *et al.*, 2016). Specifically, we tested whether population density, community diversity, and altitude significantly explained sex ratio variations across populations. Based on hypothesised causal relationships and correlations among the variables in the SEM, we built our initial meta-model (Fig. 2) and developed a structural equation model (Fig. 4, see results) that was analysed using the R v.3.3.3 (R Development Core Team, 2017) 'lavaan' package (Rosseel, 2012). On the basis of model fit indices, calculated for the overall goodness of fit (*i.e.*, χ^2, cfi) 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 meta-model, as well as to determine the support for tested links. The χ^2 tests the hypothesis that there is a discrepancy between the model-implied covariance matrix and the original covariance matrix. Therefore, a non-significant discrepancy ($p>0.05$) indicates an acceptable model fit. The comparative fit index (cfi) represents the amount of variance that has been accounted for in the covariance matrix. A higher value indicates a better model fit (best is >0.95). We used these two indices as well as AIC from the 'AICcmodavg' package (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 (Paradis & Schliep, 2018).

Finally, in order to test whether sex ratios were relatively stable or fluctuate over time, we used the 87 of the 140 sites that were sampled twice, with a time gap of five years. Note that for the second sampling, we only had values for population densities and sex ratios but not for community diversities. We chose to use generalised linear models (GLMs) to compare densities and sex ratios between years rather than including both sampling years in the overall SEM, because SEM is sensitive to small sample sizes (Wolf *et al.*, 2013). We performed the GLMs using quasibinomial and quasipoisson approaches to account for overdispersion in our data, followed by *F*-tests from the 'car' package (Fox & Weisberg, 2011). All analyses were performed using log-transformed data to meet the assumptions of statistical tests.

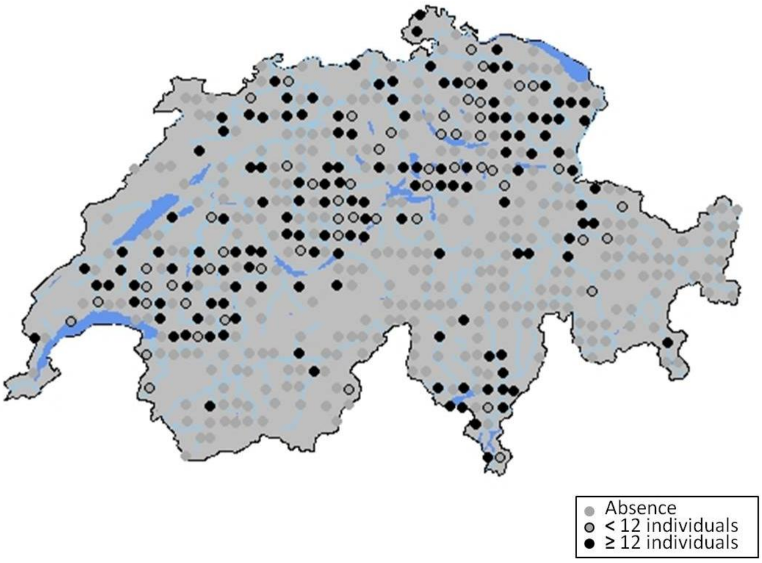


Figure 1. **The 140 sampling sites with at least 12 *Alainites muticus* individuals used in our study** (black dots), among the 500 locations of the biodiversity monitoring survey in Switzerland.

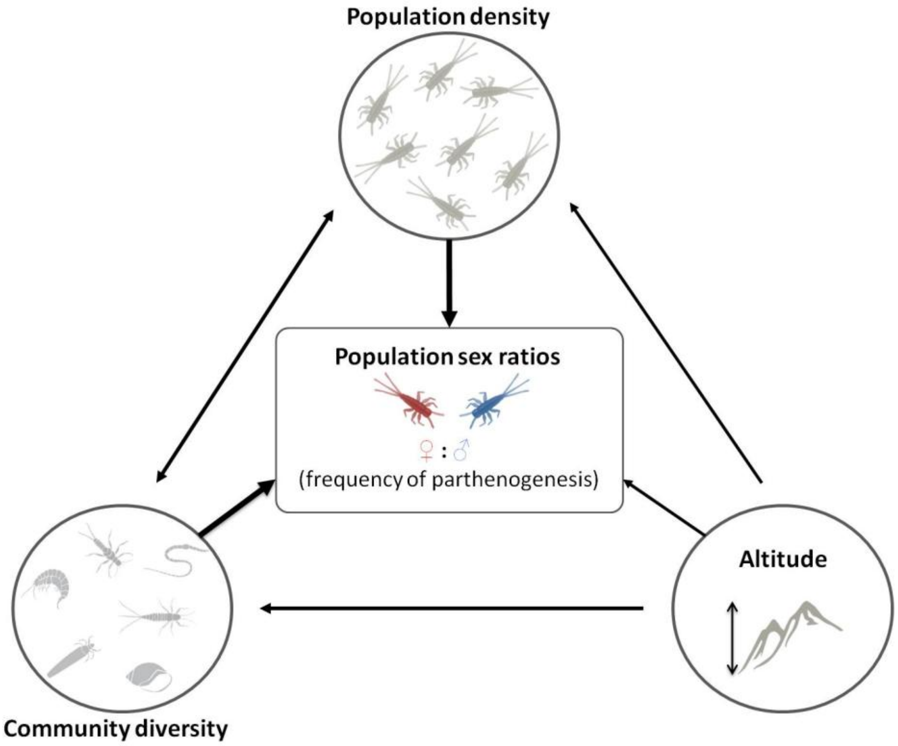


Figure 2. **Structural equation meta-model showing interconnections between ecological variables** (*i.e.*, population density, community diversity and altitude) and frequency of parthenogenesis. Black arrows represent expected direct effects between two variables.

Results

Meta-analysis and population classification

The global *A. muticus* sex ratio was mildly female-biased (54.2% females; χ^2, df=139, *p<0.001*) with significant variation between populations (*GLM, p<0.001*).

For each population, we tested whether the sex ratio differed significantly from equality in order to classify populations into different categories. Because these tests were solely conducted for displaying population categories on a map (Fig. 3), no correction for multiple testing was applied. We defined four categories: females only (unisexual populations), female-biased (significantly different from 1:1, an excess of females), balanced sex ratio (approximately equal), and male-biased (significantly different from 1:1, an excess of males). The percentage of females per population ranged from 33.3% to 100%, with ten unisexual (all-female) populations (7.1%), 15 where sex ratios were significantly female-biased (10.7%), 114 populations with approximately equal numbers of males and females (81.4%) and one significantly male-biased population (0.7%). Seven of the ten unisexual populations are located in the same geographic area (Ticino Canton), while the three others are isolated and located in Uri and Grisons Cantons (Fig. 3).

Densities and mate limitation

There has been little focus to date on the relationship between population densities and reproductive modes in animals, even though density can affect the level of mate availability. Population densities among the 140 *A. muticus* sites ranged from 12 to 450 individuals per sampled surface (average=71.1, median=45). Analysed within the SEM, we found that population densities have a significantly direct and negative effect on population sex ratios (the proportion of females) (Figures 4 and 5A, *r = -0.19*, *p=0.04* after correction for spatial autocorrelation), suggesting that mate limitation selects for increased parthenogenetic ability in natural *A. muticus* populations.

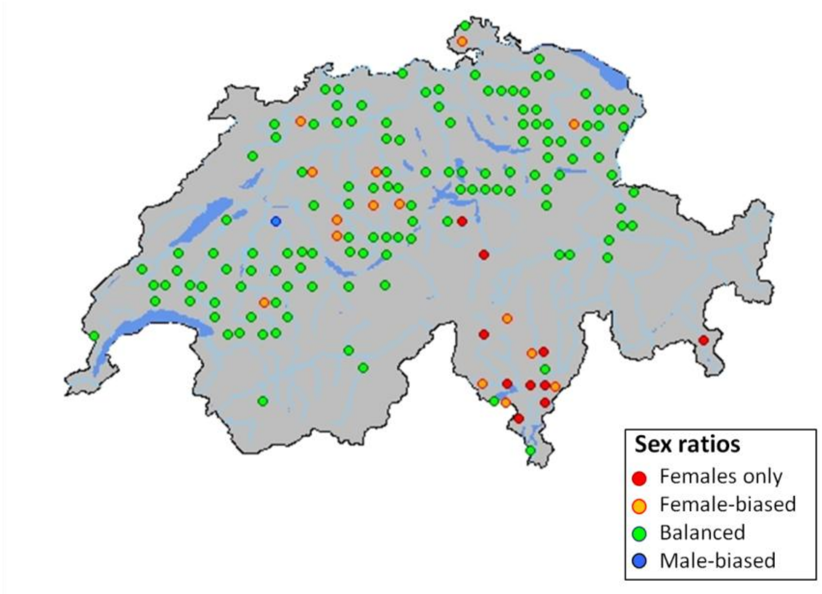


Figure 3. ***A. muticus* sex ratio variations among sites across Switzerland.**

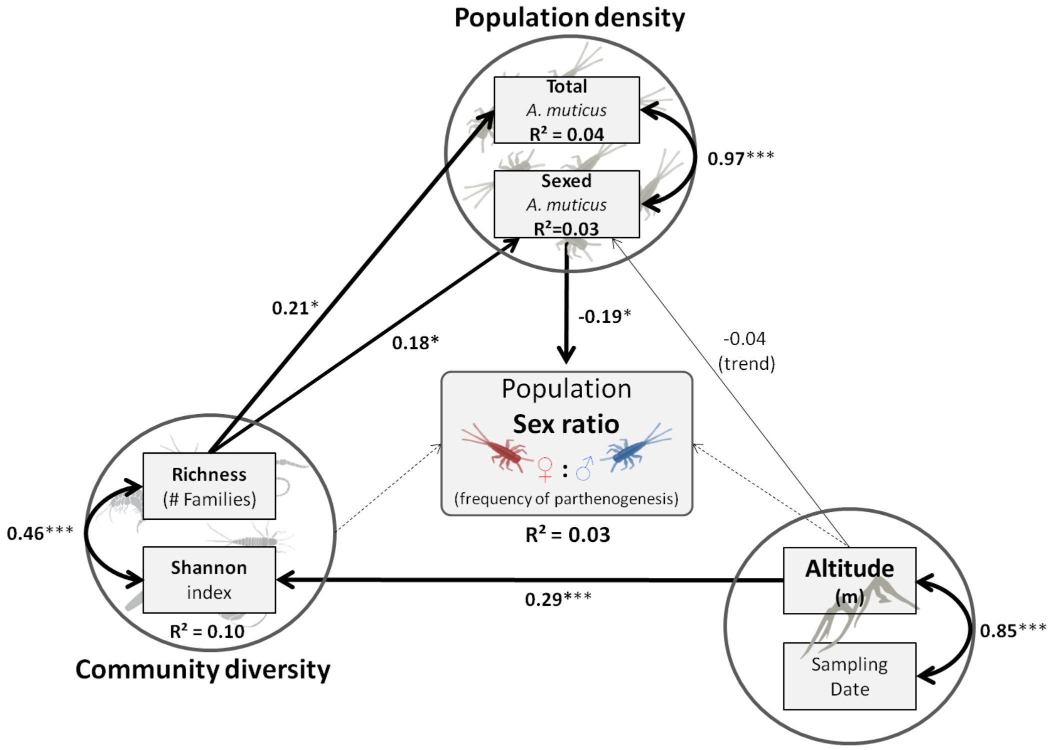


Figure 4. **Finalised Structural Equation Model pathways fitted to our data.** Solid thick arrows represent significant paths with positive or negative effects (*p<0.05*). The solid thin arrow represents a path with a trend (*0.05<p<0.10*). Dashed arrows represent non-significant paths that were removed from the model (*p>0.10*). Double arrows represent error correlations. Path coefficients correspond to standardised effects, and R^2 values are displayed on response variables, representing the proportion of variance explained. Test statistic = 15.28, with 12 degrees of freedom, p-value = 0.23 and cfi = 1 (indicating close model-data fit, see Material & Methods for details).

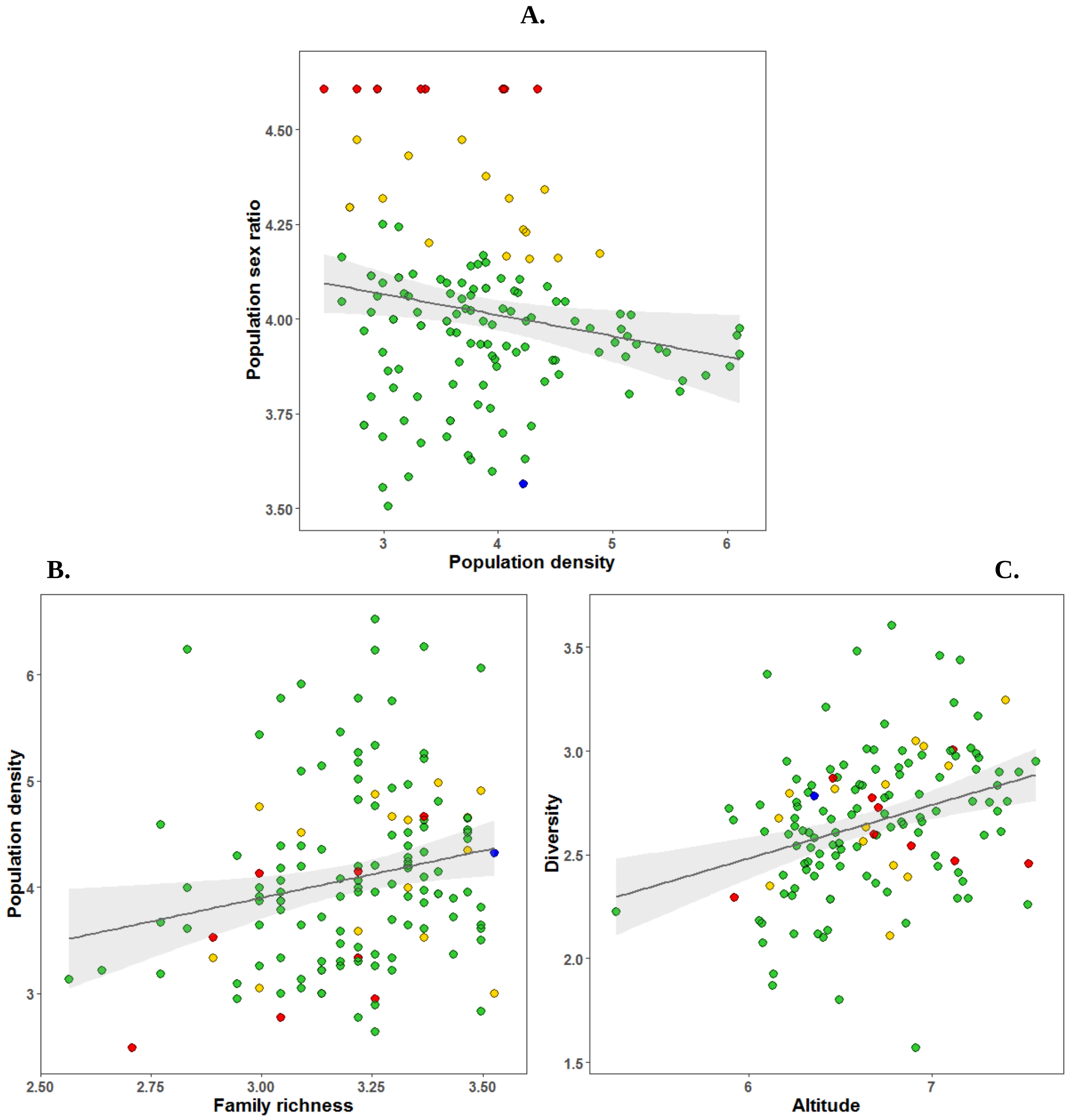


Figure 5. **Multivariate partial plots** (n=140 populations) **for the significant paths in Figure 4.** The different population sex ratio categories are represented in different colours for illustration (but not used in the model): female-only (red), female-biased (orange), balanced (green) and male-biased (blue). Panel **A** visualises the partial relationship of the **direct** significant effect of population densities on population sex ratios. Panels **B** and **C** visualise the partial relationships of the **indirect** significant effects of community diversity and altitude on population sex ratios, respectively.

Community diversity and competition hypotheses

According to the Red Queen, the Tangled Bank and Sib Competition hypotheses, we expected more sexual reproduction in more complex communities because of co-evolutionary dynamics and high levels of competition. Using family richness and the Shannon index as indicators of community diversity within the SEM (see Material & 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 non-significant path from the model (Fig. 4). Thus, complex communities do not seem to favour sex in natural populations, giving no support to the three hypotheses. However, we did find a significant direct and positive effect of family richness on population density (Figures 4 and 5B, $r = 0.21$, $p=0.013$), which in turn favours sexual reproduction. Thereby, complex communities indirectly select for sexual reproduction in natural populations of *A. muticus*.

Altitude and geographical parthenogenesis patterns

Sites can differ in their abiotic characteristics, and frequencies of parthenogenesis could be affected by those abiotic factors. We focused on altitudes within the SEM, because of the pattern frequently observed in among species comparisons, where asexual populations are more likely to be found at higher altitudes than their sexual counterparts (*i.e.*, geographical parthenogenesis). However, we found no direct effect of altitudes on population sex ratios (Fig. 4), which does not corroborate the pattern observed in geographical parthenogenesis. Nevertheless, we detected a significant direct and positive effect of altitude on community diversity, which means that at higher altitudes, aquatic communities are more rich and diverse (Figures 4 and 5C, $r = 0.29$, $p<0.001$). In turn, higher community diversities are associated with larger *A. muticus* population sizes, which eventually decrease the frequency of parthenogenesis. These results show that altitude indirectly affects reproductive strategies, but in a direction opposite to the one typically described for geographical parthenogenesis, with more sexual reproduction at higher altitudes overall. However, altitude also influences population densities directly, with higher densities at lower elevation, but the effect was rather weak (Fig. 4, $r = -0.04$, $p = 0.06$). This slight direct effect of altitude on population densities suggests more parthenogenesis at higher altitude, as described for geographical parthenogenesis, but opposite to the one, much stronger, associated with community diversities.

Parthenogenetic frequencies over time

In order to assess the stability of parthenogenesis frequencies over time, we used a second IBCH sampling (conducted five years after the first one), available for 87 of the 140 populations. We found that the direct influence of mate limitation on parthenogenesis frequencies found during the first sampling was also detected for the second sampling, five years later. Indeed, population densities have a significant effect on population sex ratios for both sampling rounds (Figures 6A and 6B).

We also found that sex ratios of the first sampling were significantly correlated to the sex ratios of the second sampling (Fig. 6C, $r = 0.60$, $p<0.001$), suggesting that sex ratios and thus the frequency of parthenogenesis within populations are relatively stable. Most variation in sex ratios between years was for populations with low densities (Fig. 6C), 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 sampling events, as shown by the significant positive correlation between densities of the two sampling years (Fig. 6D, $r=0.31$, $p=0.03$). However, variations between sampling events was much stronger than for sex ratios, with 69 populations displaying a significant change in density (Fig. 7).

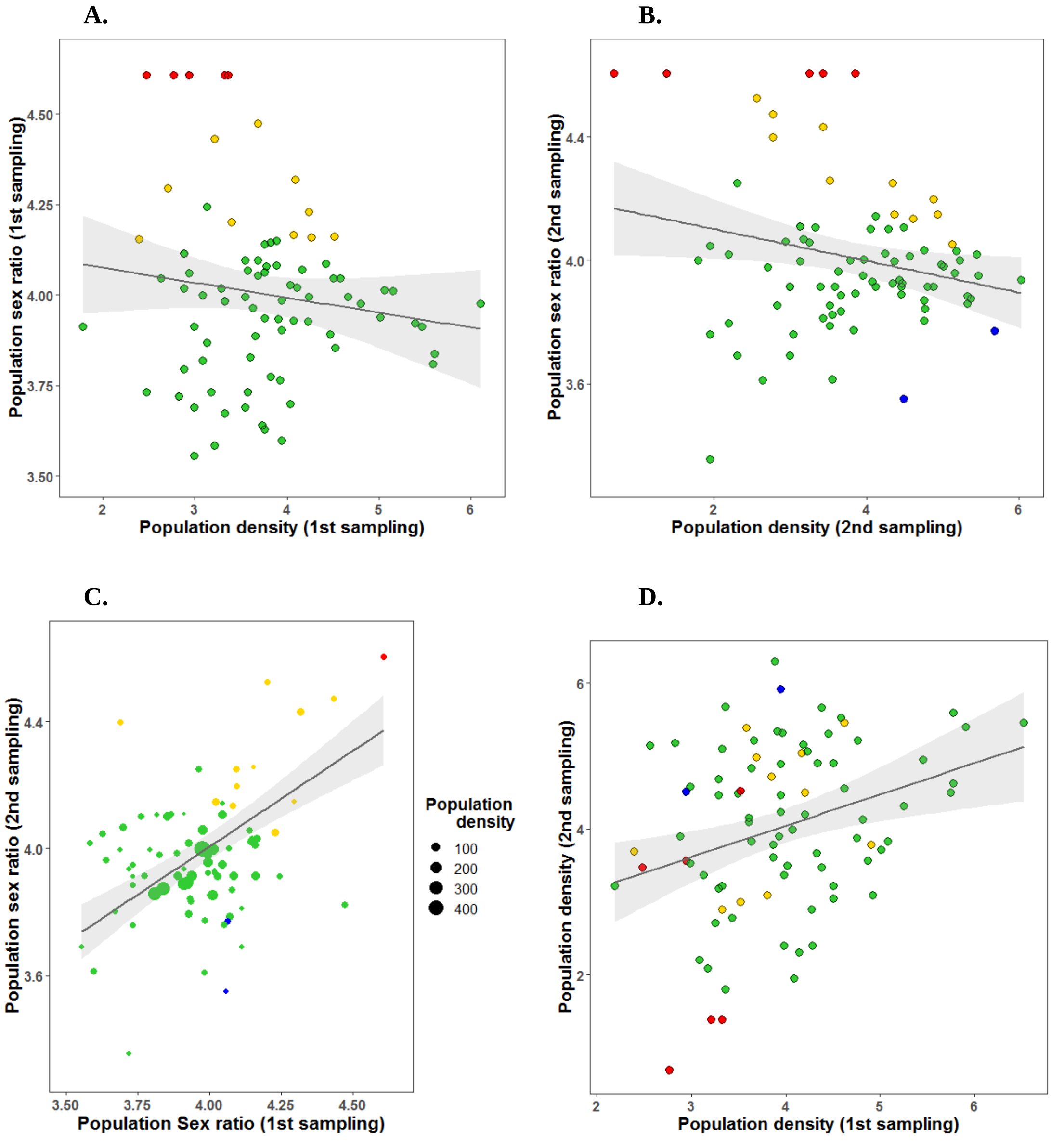


Figure 6. **Panels A and B** show significantly negative correlations between population sex ratios and population densities of the first and second sampling respectively (*GLM*, *r = -0.13* and *-0.23*, corrected *p=0.03* and *p<0.001*, respectively); **Panel C** shows the significantly positive correlation between sex ratios of the first and second sampling (*GLM*, *r = 0.60*, corrected *p<0.001*); **Panel D** shows the significantly positive correlation between population densities of the first and second sampling (*GLM*, *r = 0.31*, *p=0.03*).

With facultative parthenogenesis, population sex ratio differences may be due to phenotypic plasticity. In order to investigate variations within populations, we measured for each of the 87 population the difference in sex ratios and the difference in densities between the two sampling events. Overall, we found no significant correlation between the variation in population sex ratios and the variation in densities (Fig. 7, *p=0.89*), 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=14, Fig. 7A), and most populations displayed a significant change in population densities (n=69, Fig. 7B). For populations where sex ratios changed significantly, the change in population sex ratio was negatively correlated with the change in density (Fig. 7A, *cor = -0.49*), with an increase of parthenogenetic frequencies when densities decreased, further supporting our previous results that mate limitation selects for parthenogenesis. Conversely, for populations where densities changed significantly, the change in density was not correlated with the change in population sex ratio (Fig. 7B, *cor ~ 0*), suggesting that parthenogenetic capacities are not very plastic.

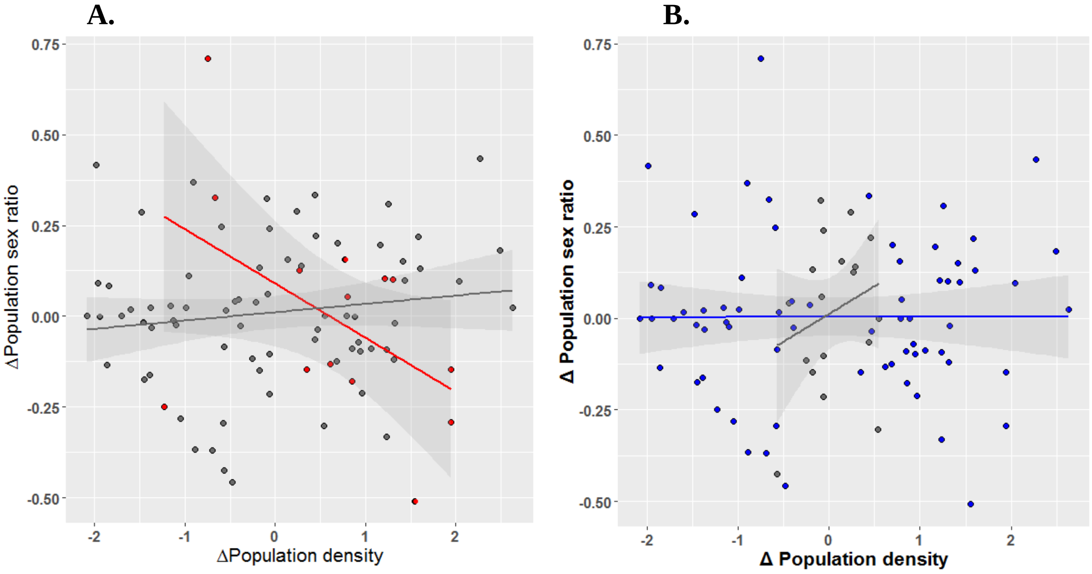


Figure 7. **Population sex ratio and density variation between the two sampling events separated by five years** (n=87, *cor = 0.002*, *p = 0.89*). **A)** Red dots and line show populations that display a significant change in sex ratio over time (n=14, *cor = -0.49*). **B)** Blue dots and line show populations that display a significant change in density over time (n=69, *cor ~ 0*).

Discussion

Liegeois *et al.* (see Chap. III) found a positive relationship between the parthenogenetic capacity of females and population sex ratios (proportion of females), revealing the use of parthenogenesis in natural populations of the mayfly *A. muticus*. However, the high percentage of natural populations with 1:1 sex ratios in our results (81.4% of the 140 sampling sites) suggests that parthenogenesis is only rarely used by this species under natural conditions. Therefore, evaluating what ecological factors could push the species to reproduce parthenogenetically in natural populations was the purpose of this study.

We found a significantly negative effect of *A. muticus* densities on frequencies of parthenogenesis, suggesting that parthenogenesis in this species is mostly used when females have difficulties finding mating partners, even though the explained variation among populations is quite weak (only 3%, Fig. 4). Such mate limitation is expected to be widespread in mayflies as they are characterised by a very short adult life span, which may generate strong selection for reproductive assurance. Sex ratios and/or hatching successes of unfertilised eggs negatively correlated to male densities have also been mentioned for the mayfly species *Eurylophella funeralis* (Sweeney & Vannote, 1987), *Ephemerella notata* (Glazaczow, 2001), *Ephoron shigae* (Tojo *et al.*, 2006) and *Stenonema femoratum* (Ball, 2002). Therefore, the widespread frequency of parthenogenesis among mayflies (see Liegeois *et al.*, 2021 for a recent review) might result from regular situations where females are mate-limited. Indeed, mate limitation, may help the transition from sexual reproduction to parthenogenesis, *via* selection for reproductive assurance through facultative parthenogenesis. The latter generates more strongly female-biased sex ratios, which in turn increases mate limitation for females, suggesting a positive feedback loop. Such a mechanism has also been shown in *Timema* stick insects (Schwander *et al.*, 2010). An alternative explanation to mate limitation selecting for parthenogenesis in natural populations could be that females with facultative parthenogenesis may pay the cost of resisting male harassments, if they try to reproduce parthenogenetically in spite of male presence. Thus, parthenogenesis would be costly to females in populations with high frequencies of males. In this case, females reproducing sexually will be favoured at high densities as harassments would increase with male availability (Gerber *et al.*, 2016). This might also explain why facultative parthenogenesis is not so often used in natural populations of mayfly, as shown by the large amount of populations with relatively equal sex ratios.

Regarding community diversity, the Tangled Bank and Sib Competition hypotheses suggest that sexual reproduction is likely to be favoured by inter-specific respectively intra-specific competition in stable heterogeneous environments, where relatively saturated communities lead to a continuous and intense competition for resources (*e.g.*, Bell, 1982). The Red Queen hypothesis proposes that sexual reproduction is generally favoured by pressure from parasites (*e.g.*, Hamilton, 1980). Regarding these hypotheses, we investigated whether sexual reproduction of *A. muticus* is favoured in more complex communities. However, we found no direct influence of community complexity (*i.e.*, macroinvertebrates family diversities) on the frequency of parthenogenesis (Fig. 4). A potential explanation for why we did not find the expected correlation with community complexity is that our measures of community diversity did not take into account microorganisms or big predators (*e.g.*, parasites, fishes). Indeed, regarding the Red Queen hypothesis, during co-evolutionary interactions between hosts and parasites, parasites are under selection to infect the most common host genotype (*e.g.*, Jokela *et al.*, 2009). Populations with sexual reproduction, which are genetically diverse, would be less vulnerable to parasitism over time and remain relatively constant in size, while populations with parthenogenetic reproduction, which are genetically similar, would be more likely to be infected and decrease in size over time. We might largely miss this effect with our community diversity calculation based on macroinvertebrates. Nevertheless, we found a significantly positive effect of community diversity on *A. muticus* population density, which eventually increased the frequency of sexual reproduction. Indeed, parasites are a more important threat in high-density populations (*e.g.*, Lagrue & Poulin, 2015), which corroborates our findings of an indirect effect of community diversity on reproductive strategies. Similarly, population densities can also affect the level of intra-specific 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 direct effect of community diversity on reproductive strategies. Hence, strong intra-specific competition selecting for sexual reproduction might be concealed by mate limitation selecting for parthenogenesis in *A. muticus*.

As for altitude, we expected a positive relationship with the frequency of parthenogenesis that we did not find in *A. muticus*. Indeed, according to the patterns observed in geographical parthenogenesis, asexual lineages are more likely to be found at higher altitudes then their sexual counterparts (reviewed in Tilquin & Kokko, 2016). Thus, it seems that this abiotic factor favouring sexual over asexual species does not hold for facultative parthenogens (*i.e.*, within species). A probable explanation for this difference is that there may be species-specific traits that are confounded with reproductive modes. Indeed, in geographical parthenogenesis (*i.e.*, between species comparisons), asexuals are often polyploid or hybrids, meaning that the pattern observed with altitude might not be caused by the reproductive mode *per se* (Kearney, 2005). In *A. muticus*, we further detected a positive effect of altitude on community diversity, and a negative trend on population density (Fig. 4). These results suggest that abiotic factors do not directly select for parthenogenesis or sexual reproduction, but instead influence biotic factors of the local populations. Hence, abiotic factors such as altitude would indirectly impact the reproductive strategies, by creating situations presenting suitable habitat conditions for both increased community diversities and population densities. In turn, suitable habitats would remove selection for parthenogenesis by facilitating high community diversities and large population sizes.

Finally, the frequency of parthenogenesis over time does not extensively fluctuate (Fig. 6C). This suggests that variations in parthenogenetic capacities are not fully plastic, but at least partially generated by genetic differences between populations. Similarly, population densities remained relatively stable over time, even though they varied much more than sex ratios between the two sampling events. One explanation, for the population densities varying more than population sex ratios, could be that population densities are more sensitive to random effects such as the person conducting the sampling, weather conditions or characteristics of the river. Another explanation could be that parthenogenetic capacities are relatively stable (not plastic) even in the face of transient changes in population densities, as supported by our results regarding population sex ratio and density variations between the two sampling events (Fig. 7).

To conclude, the extremely short adult life span of mayfly tends to select for females with higher parthenogenetic capacities, especially in cases of mate limitation. In addition, those females seem to transmit their parthenogenetic potential over generations, even though further investigations are needed to fully discriminate phenotypic plasticity. Taken together, these results suggest that parthenogenetic reproduction should be more common in short-lived organisms, which have typically a shorter window in time and space to reproduce. By contrast, sex is expected to be more common in suitable habitats that facilitate high community diversities and large population sizes.

Acknowledgements

We are grateful to the whole team of the BDM survey who provided the *A. muticus* samples, as well as the ecological data for each site. We would like to thank Jean-Luc Gattolliat for his help in the connection between the “BDM team”, the Museum of Zoology and the University of Lausanne. We also thank Victor Othenin-Girard for his help in separating *A. muticus* mayflies for the sex ratio calculations over time. We thank Dr. James B. Grace for his helpful explanations on Structural Equation Modelling. This work was supported by funding from the University of Lausanne and the Swiss National Science Foundation (grant numbers PP00P3_139013 and PP00P3_170627).

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– GENERAL DISCUSSION –

GENERAL DISCUSSION

This thesis aimed to provide empirical studies that test some hypotheses concerning the costs and benefits of sex, in order to progress in understanding the maintenance of sexual reproduction in natural populations. We investigated the frequency of parthenogenesis in mayfly species overall (**Chapter I**), as a first step towards developing this group as a model system for investigating the balance between the benefits of sexual reproduction *versus* parthenogenesis in natural populations (**Chapter II**). We then focused on the mayfly species *Alainites muticus* and population sex ratios to better understand the evolution of reproductive polymorphism in the wild (**Chapter III**), and to identify ecological correlates of different reproductive strategies in natural populations (**Chapter IV**). All together, these results showed how mayflies are currently clearly underappreciated for their value as an outstanding model system for testing benefits of sexual reproduction *versus* parthenogenesis in natural populations, and help resolving the "paradox of sex".

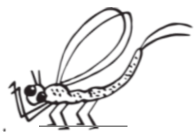
Main conclusions

Frequency of parthenogenesis in mayflies

Mayflies appeared to be an ideal group to study the maintenance of sexual reproduction and the evolution of parthenogenesis in natural populations, given that most species are able to reproduce at least by tychoparthenogenesis, with a lot of reproductive polymorphism between and within species, even within populations between individuals. We consider sexual reproduction (with tychoparthenogenesis) to be the ancestral state in mayflies, with multiple transitions to parthenogenesis. The extensive variation in parthenogenesis frequency among mayfly clades could help identify the genetic and developmental constraints that may reduce the transition from sexual reproduction to parthenogenesis (*see* Engelstädter, 2008). Such quantitative estimates of the frequency of parthenogenesis are helpful determining genetic and ecological effects underlying the evolutionary transitions from sexual reproduction to parthenogenesis.

We also found that sex is a more successful strategy, as we found a trade-off between the capacity of parthenogenesis and sexual reproduction. This means that increasing the capacity of parthenogenesis in a lineage may come at a cost for sexual reproduction, even in facultative parthenogens. Such a trade-off can help explain why facultative parthenogenesis is extremely rare among animals in spite of its potential to combine the benefits of sexual and parthenogenetic reproduction.

These findings raised the following question: what are the factors affecting the balance between the relative costs and benefits of different reproductive modes in mayflies? In other words, what are the potential lineage-level or geographical-ecological correlates of parthenogenesis in this group?

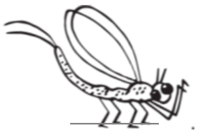


Reproductive polymorphism under natural selection

The balance between relative costs and benefits of sexual reproduction is expected to vary according to evolvable species-specific factors, such as life-history or ecological traits. For example, the costs of sex may increase when population growth is high (Williams 1975; Bell 1982; Burt, 2000), or at low population densities, when encounter rates between individuals are low (Snell & Garman, 1986; Schwander *et al.*, 2010; Sprenger *et al.*, 2011). More generally, sex is less likely to generate a net benefit under *r*-selection than under *K*-selection (MacArthur & Wilson, 1967; Pianka, 1970). Indeed, when the environment is at or near carrying capacity, *K*-selection might favour slow development, high survival rates, low resource thresholds, and strong competitive abilities, leading to constant population sizes, and sexual reproduction, while *r*-selection might favour rapid development, high growth rates, and low survival rates, leading to population sizes below the carrying capacity of the environment, and parthenogenesis.

In mayflies, using cross-species comparisons and a structural equation modelling approach, we found that specific habitats, such as slow currents and fine substrates, tend to select for small individuals with active dispersal and fast generation times, which in turn select for parthenogenesis. These findings are consistent with the idea that *r*-selection favours parthenogenesis, as explained above. However, we were not able to formally distinguish whether this “parthenogenesis syndrome” is driven directly by the life-history traits or whether these life-history traits are adaptations to certain habitat types that favour parthenogenesis for other reasons. Evidences for *r*-selection favouring parthenogenesis in natural populations have also been found in other organisms. For example, agricultural environments such as orchards, vineyards, pastures and crops, favoured parthenogenetic pests with high fitness across multiples generations (Hoffman *et al.*, 2008). Agriculture potentially provides stable and uniform environments with an abundance of resources due to human’s agricultural practices (*e.g.*, monocultures, pesticides, fertilizers), leading to a selective advantage of parthenogenetic lineages over sexual populations. Similarly, *K*-selection favoured sexual reproduction in experimental *Brachionus* rotifer populations (Beck & Agrawal, 2013), a facultatively parthenogenetic genus. The rate of sex evolved to higher levels in the *K*-selected environment, accompanied by selection for lower growth rates.

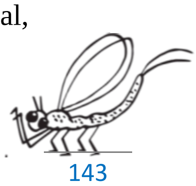
Here, I would argue that in mayflies, habitats are also likely to directly drive the reproductive strategies, even though the effect was rather weak in our SEM. We used water flow and bed substrate as proxies of microhabitat type, but despite their essential contribution into river’s habitat description, they do not take into account the full picture (*e.g.*, food resources such as plants and detritus, water quality). This is a potential explanation for why we did not find a strong direct effect of specific microhabitats on parthenogenesis abilities within the SEM.



Theoretical works have identified several ecological conditions favouring the maintenance of sexual reproduction by different mechanisms (reviewed in Lively & Morran, 2014). For example, biotic interactions with selection over time (*e.g.*, Red Queen) and intense competition with selection in space (*e.g.*, between species: Tangled Bank; within species: Sib Competition) can favour sex over parthenogenesis (*e.g.*, Hamilton, 1980; Bell, 1982; Scheu & Drossel, 2007; Otto, 2009).

In mayflies, using cross-population comparisons (within the facultatively parthenogenetic mayfly species *Alainites muticus*) and a structural equation modelling approach, we found that high diversity levels, measured by the Shannon index and family richness, tend to be associated with high population densities, which in turn select for sexual reproduction. Density-dependant reproductive strategies, correlated with the level of mate availability, is apparently common in mayflies (Sweeney & Vannote, 1987; Glazaczow, 2001; Ball, 2002; Tojo *et al.*, 2006), but is not mayfly specific and is also observed in other organisms (*e.g.*, in rotifers: Stelzer & Snell, 2003; Serra *et al.*, 2008; in stick insects: Schwander *et al.*, 2010). However, we were not able to formally distinguish whether this “sexual syndrome” in mayfly is driven directly by the population density, by the community diversity, or both. Indeed, high population densities are also associated with high competition and low resource availability, and thus with stressful conditions, which are known to induce sexual reproduction, notably in *Daphnia* (reviewed in Ram & Hadany, 2016). In addition, about 95% of a typical mayfly life cycle occurs at the nymphal stage, and adults do not feed, further suggesting that competition for resources is primarily a function of nymph densities. In our SEM model, we used the Shannon index and family richness as proxies of diversity. However, despite them being good estimates of biological diversities (Jost, 2006; Tuomisto, 2010), they do not take into account microorganisms (*e.g.*, parasites), big predators (*e.g.*, fishes), or food resources (*e.g.*, plants, detritus). Yet, the Red Queen, the Tangled Bank and the Sib Competition hypotheses predict that sexual reproduction will be favoured with complex biotic interactions in time and space (further explained above or in “General Introduction”). This is a potential explanation for why we did not find a direct effect of community complexity on reproductive strategies within the SEM. In this context, high population densities are not only associated with sexual reproduction, but also generate general conditions where sex is favoured, further decreasing the costs of sex. Indeed, a recent theoretical model in *Daphnia*, a genus with cyclical facultative parthenogenesis, predicts that sexual reproduction will be favoured with a combination of ecological and demographic factors, when its relative costs are reduced (Gerber *et al.*, 2018). These factors are population density, population growth and habitat stability (*i.e.*, permanent *versus* ephemeral).

Taken together, our results indicate that parthenogenesis is generally more common in species with fast generation times, small body sizes and active dispersal abilities. Small body size and/or fast generation time between related sexual and parthenogenetic species are also found in other organisms (*e.g.*, in the lichen *Lasallia pustulata*: Hestmark, 1992; in *Brachionus* rotifers: Beck & Agrawal,



2013), further supporting our findings. More generally, body sizes, population growth rates and dispersal abilities are likely to contribute to the transition rate from sexual to parthenogenetic reproduction in animals. In addition, parthenogenesis is more common in situations where females are mate-limited, supporting the selection for reproductive assurance, while sexual reproduction is more common in situations presenting habitat conditions that facilitate both large population sizes and high community diversities, thus removing the selection for parthenogenesis. These findings show how life-history traits and ecological factors interact in complex ways, and affect the relative benefits of sexual reproduction in natural populations.

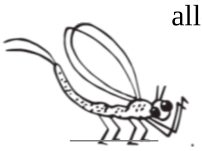
Unanswered questions and perspectives

This thesis has improved our knowledge on mayfly life-histories and ecologies, especially regarding correlates of parthenogenesis. However, there are still a number of gaps that could, once filled, allow us to better understand why facultative parthenogenesis is so rare under natural conditions, and therefore, why sexual reproduction is the dominant reproductive mode in natural populations.

Population stability over time

In order to assess whether populations remain stable or fluctuate over time regarding their reproductive strategies, we investigated the variations in the frequency of parthenogenesis and in population densities within populations across two sampling events. We found that the frequency of parthenogenesis within *A. muticus* populations are relatively stable, remaining equivalent after five years, while population densities were much more fluctuating, suggesting that parthenogenesis frequencies are not shifted in the short term according to the current local densities. These results suggest that reproductive strategies in mayflies are not fully plastic, but at least partially generated by genetic differences between populations. In order to directly test for whether parthenogenesis frequencies are plastic or genetically determined, we could conduct an experiment with different rearing densities. For example, rearing the facultatively parthenogenetic *A. muticus* mayfly in aquariums with low *versus* high nymph densities, and with the same amount of food supply, will potentially tell us whether an individual female can easily switch between sexual and parthenogentic reproduction, after having measured the hatching successes of unfertilised eggs once their emerged. By conducting the same experiment but with different mayfly families, it could further allow us to measure variations across mayfly clades.

We argued multiple times that an asset of mayflies is that the presence of facultative parthenogenesis allows studying the frequency of sex under different ecological conditions without confounding



species-specific effects. However, it remains possible that species with reproductive polymorphism in fact consist of genetically divergent lineages, each characterised by a single reproductive mode. In order to investigate whether *A. muticus* mayflies featuring different forms of parthenogenesis (*i.e.*, tychoparthenogenesis, facultative or obligate parthenogenesis) belong to different genetic lineages, we collected 288 individuals from 75 populations from Switzerland, as well as 307 individuals from 43 populations from 13 additional countries in Europe. We performed PCR amplification of two mitochondrial (*i.e.*, Cytochrome c Oxydase submit I and Cytochrome B) and three nuclear markers (*i.e.*, Elongation Factor 1α, Histone 3 and Ryanodine receptor 44f), and will conduct amplicon sequencing. These genetic markers will allow us to detect the presence of potential cryptic lineages within *A. muticus* as well as investigate genetic polymorphisms in populations characterised by different reproductive modes.

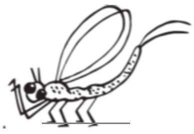
Trade-off between reproductive modes

Overall, mayfly species are better at reproducing sexually than asexually (measured as egg-hatching successes). Only in obligate parthenogens is egg-hatching success decreased upon mating, presumably because (even partial) fertilisation interferes with the normal development of asexual eggs (see Chapter I for details). In order to confirm the trade-off identified between parthenogenetic and sexual reproduction, a comparison between fertilised and unfertilised *A. muticus* females should be conducted. Fertilised eggs can be obtained either by forced copulation between males and females or by obtaining fertilised eggs directly from the field during the mating season. Eggs should then be incubated under standardised laboratory conditions as described in Chapter III. Hatching successes and development times of fertilised and unfertilised eggs can then be measured and compared. If such a trade-off indeed exists, it could help explain why facultative parthenogenesis is extremely rare among animals in spite of its potential to combine the benefits of sexual and parthenogenetic reproduction. This experiment could also help identify whether an ‘obligately’ parthenogenetic female (*i.e.*, from population with females only) can return to sexual reproduction.

In addition, by using genotyping of mothers and their eggs or hatchlings (*e.g.*, with RAD sequencing), it would further be possible to estimate how flexible a facultatively parthenogenetic female can choose between sex and parthenogenesis. This experiment would also give some insight regarding the cytological mechanisms of parthenogenesis in *A. muticus*.

Test of the Red Queen and Tangled Bank hypotheses in mayflies

Regarding that the Red Queen and the Tangled Bank hypotheses are present in most of the chapters of this thesis, it might be good to push a bit our investigations.

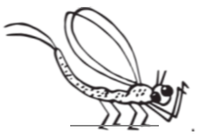


In order to evaluate whether populations with approximately equal sex ratios, and thus mainly sexual reproduction, are also exposed to a crowded habitat with co-evolutionary dynamics, it would be interesting to have a better proxy for habitat and diversity. For example, environmental DNA (eDNA) technique would provide a rapid measurement and monitoring of biodiversity, including parasites, fishes and plants. As we know the main predators of mayflies (*e.g.*, fishes, dragonflies, stoneflies), their main parasites (*e.g.*, endoparasites: mermithid nematods, ectoparasites: *Symbiocladius* and *Epoicocladius* chironomids), and their diet (mostly herbivorous) (reviewed in Sartori & Britain, 2015), it will help identify the selective pressures that are mayfly-specific. Pushing the reasoning even further, EPFL developed a model, using multiple eDNA samples, that can simulate the exact localisation and the frequency of species (Carraro *et al.*, 2018), which would help characterising the realised niches of mayfly species.

General conclusion

All together, the results of this thesis showed that the evolution of reproductive strategies is determined by a trade-off between sex and parthenogenesis. This evolution can be influenced by genetic and developmental constraints that prevent the transition of parthenogenesis from sexual reproduction, as shown for mayflies. This evolution can also be influenced by demographic and environmental factors, as shown for the mayfly *A. muticus*. The indirect benefits of sex do not always compensate for its costs, which lead to a transition from sexual reproduction to parthenogenesis, even more often than we previously thought given the high frequency of parthenogenesis in mayflies.

Mayflies are thus an ideal group for testing benefits of sexual reproduction *versus* parthenogenesis in natural populations, and help resolving the “paradox of sex”, even though they are fragile, often small and not easy to manipulate. In addition, their long aquatic nymphal stages and their ephemeral period of emergence do not help to conduct regular experiment along the year, but they remain good (and cute) insects for answering questions in fundamental as well as applied research, especially for tests in natural populations.



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–APPENDIX–

Sex and Parthenogenesis in Mayflies

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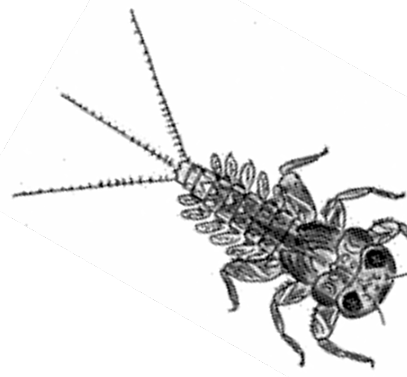
Why having Sex?

The evolution and maintenance of sexual reproduction has been one of the major questions in evolutionary ecology for the last decades – sex is associated with profound costs, yet it is the most widespread reproductive mode among animals. A number of theories have been developed that can help explain the advantage of sex, but experimental tests in natural populations remain extremely scarce.

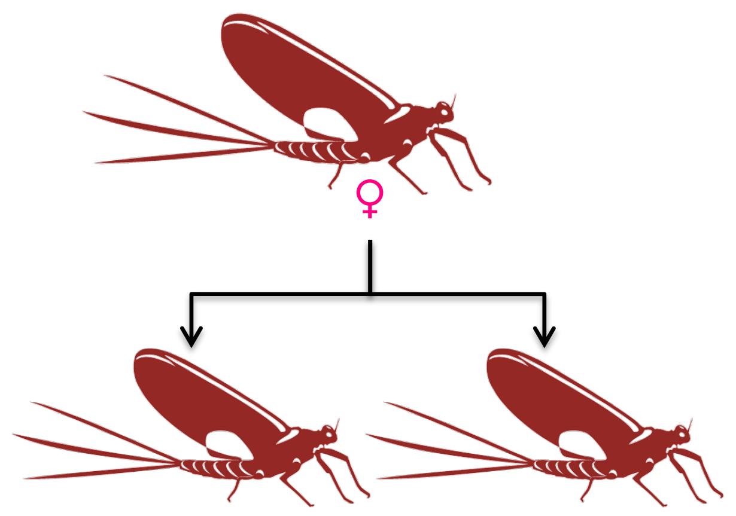


Mayflies

Mayflies are one of the few groups where sexual reproduction co-occurs with different types of parthenogenesis at the intra- and the interspecific level, providing ideal conditions for testing predicted advantages of sex in natural populations.

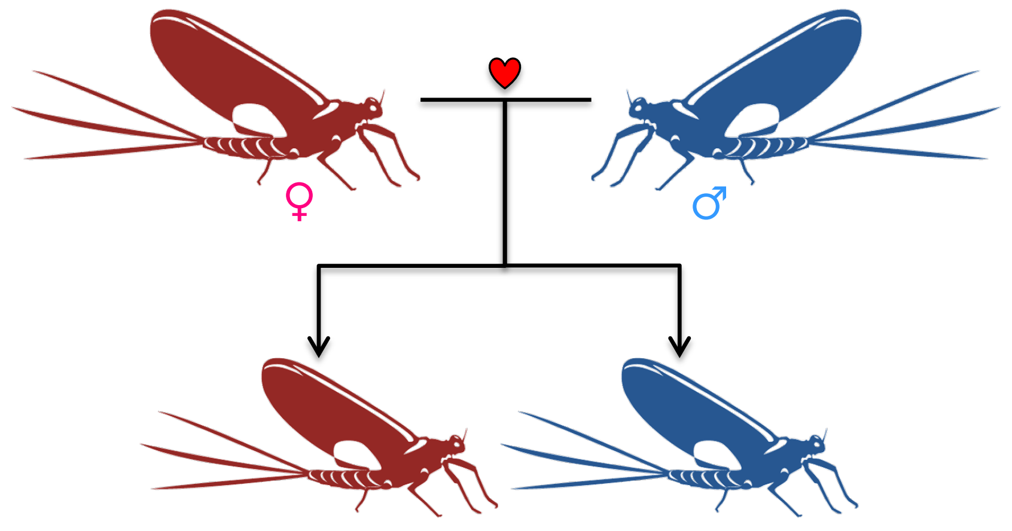
Are there ecological or phylogenetic correlates of high parthenogenesis rates?

Obligate parthenogenesis, when the eggs always develop parthenogenetically

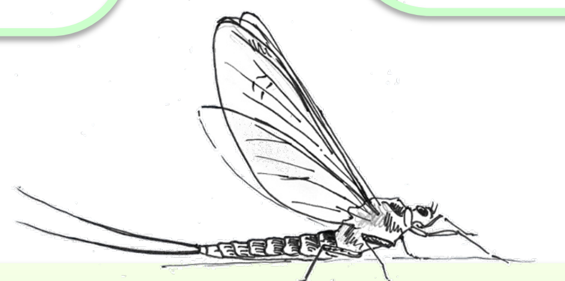


Sex ratio = ♀ **only**

Tychoparthenogenesis, when unfertilized eggs develop accidentally or occasionally through parthenogenesis in a normally sexual species



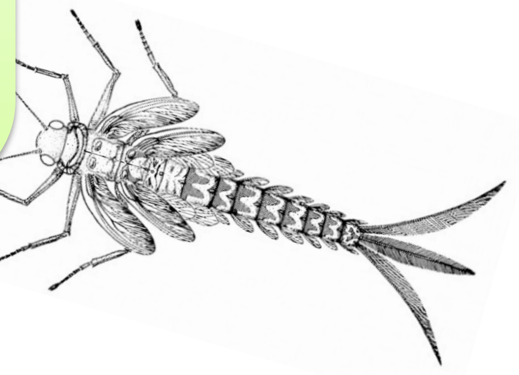
Sex ratio = **1**♂ **: 1**♀



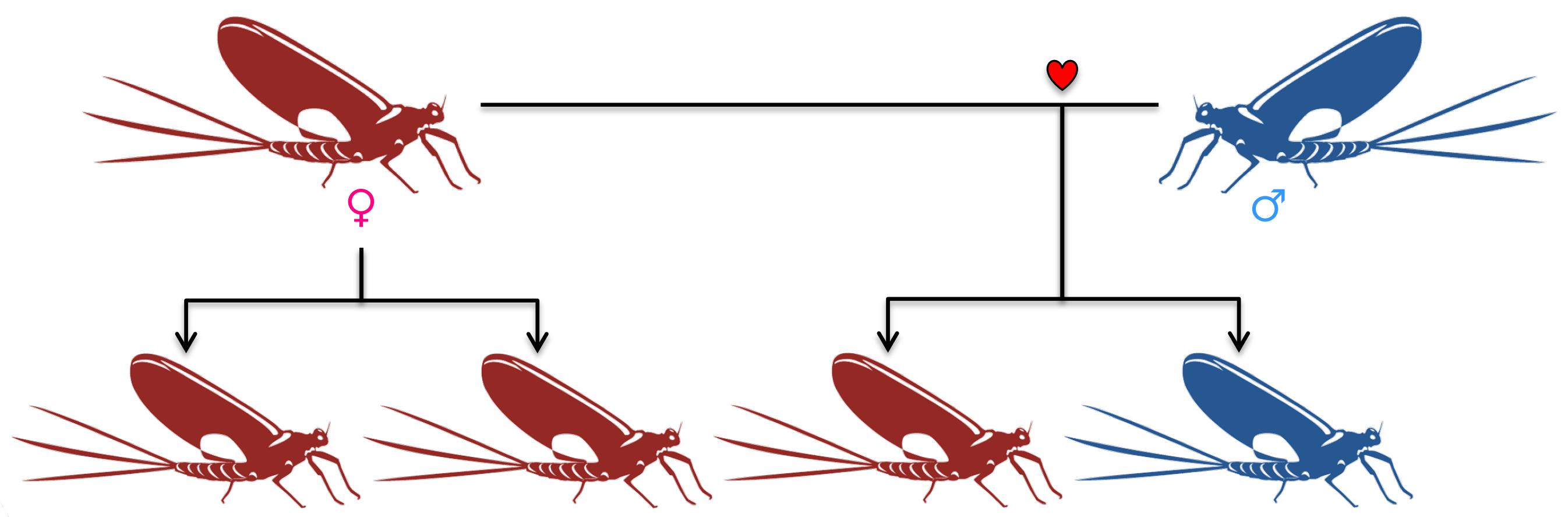
Parthenogenesis

Three different types of parthenogenesis are known.

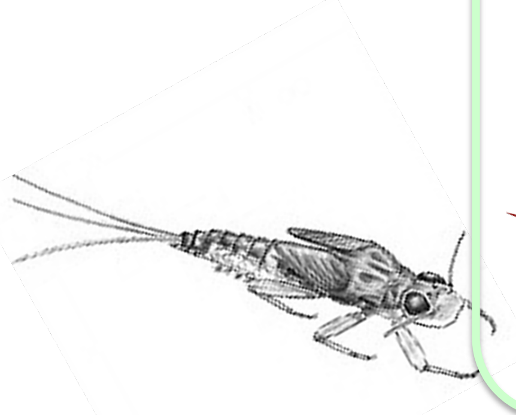
A species can feature different types of parthenogenesis (**mixed reproduction**), whereby obligately parthenogenetic females occur at different locations from their sexual or facultatively parthenogenetic counterparts (**geographic parthenogenesis**). Interestingly, it is rarely known what factors generate such patterns.

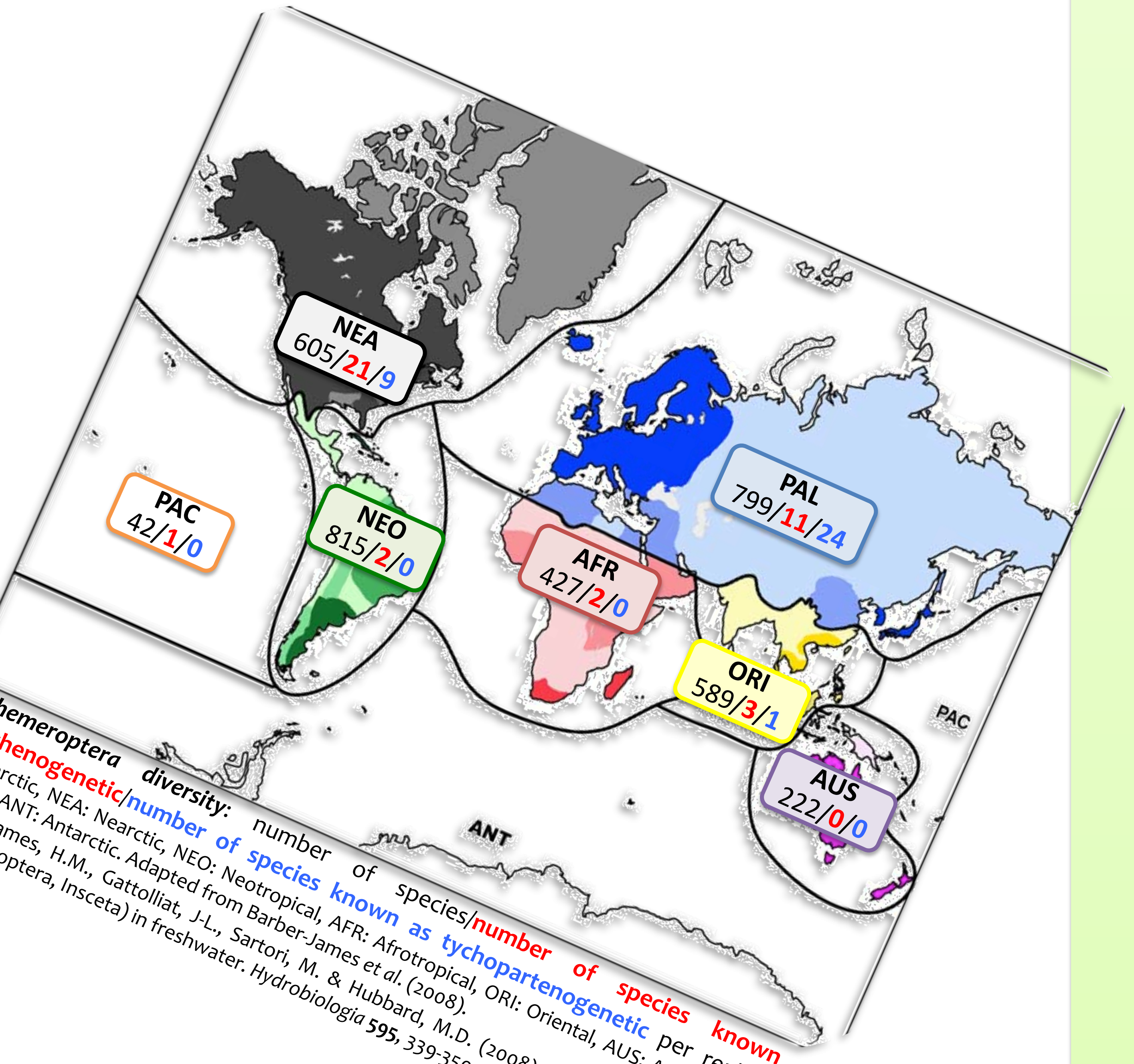


Facultative parthenogenesis, when an egg may either be fertilized or develop parthenogenetically



♀ **biased** sex ratio





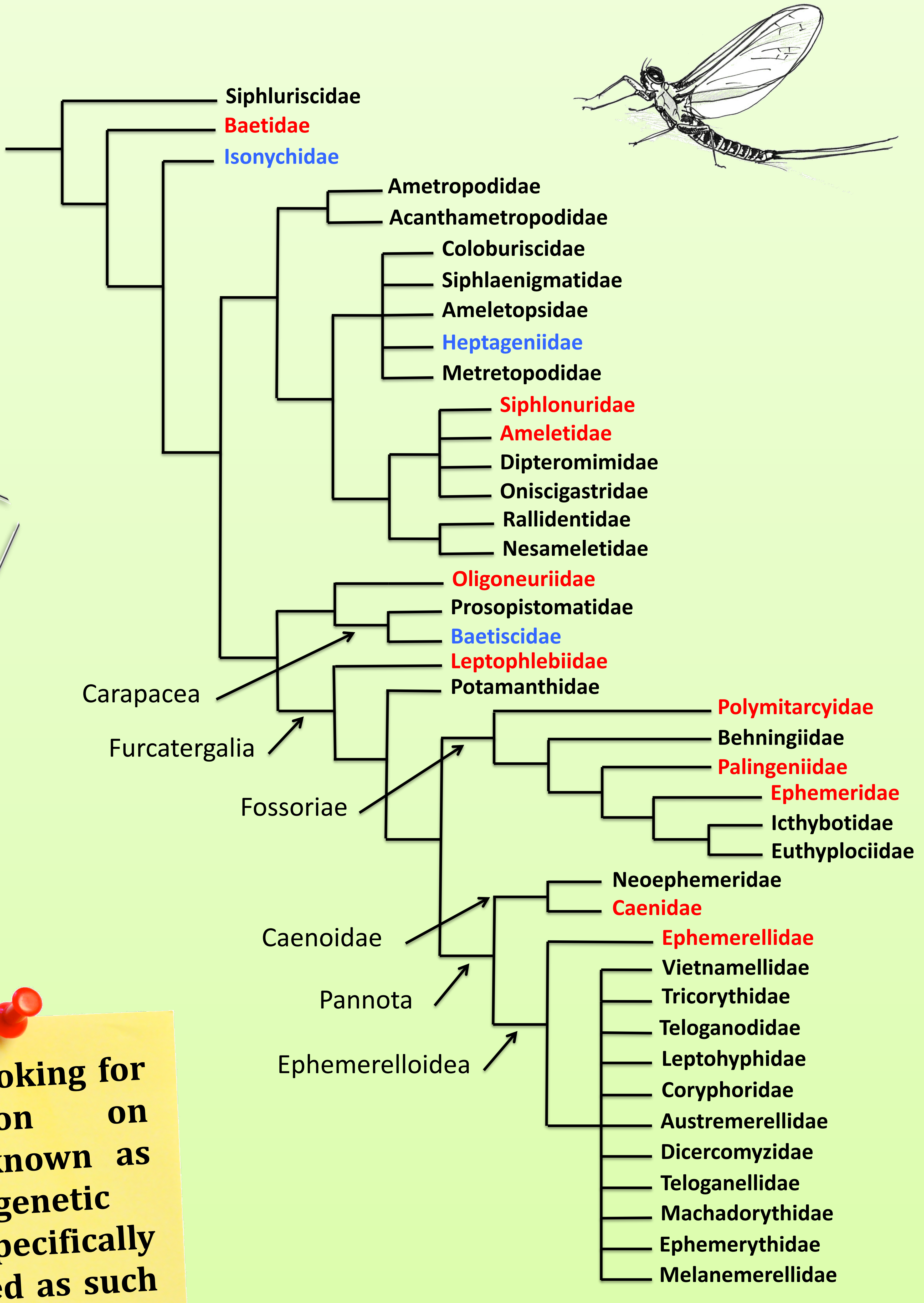
Ephemeroptera diversity: number of species/number of species known as parthenogenetic/number of species known as tychopartenogenetic per realm. PAL: Palaearctic, NEA: Nearctic, NEO: Neotropical, AFR: Afrotropical, ORI: Oriental, AUS: Australasian, PAC: Pacific, ANT: Antarctic. Adapted from Barber-James et *al.* (2008). Barber-James, H.M., Gattolliat, J-L., Sartori, M. & Hubbard, M.D. (2008). Global diversity of mayflies (Ephemeroptera, Insceta) in freshwater. *Hydrobiologia* **595**, 339-350.

Conclusion

All types of parthenogenesis are known in an array of families and genera of mayflies. In some families, **more than 10%** of described species are capable of parthenogenesis, which is an order of magnitude higher than typical in animals. However, this estimate is likely influenced by a publication bias towards species from North America or from Europe.

We are looking for information on species known as parthenogenetic but not specifically mentioned as such in the literature.

Phylogenic distribution of parthenogenesis among mayfly families (from published data)



Siphluriscidae
Baetidae
Isonychidae
Ametropodidae
Acanthametropodidae
Coloburiscidae
Siphlaenigmatidae
Ameletopsidae
Heptageniidae
Metretopodidae
Siphlonuridae
Ameletidae
Dipteromimidae
Oniscigastridae
Rallidentidae
Nesameletidae
Oligoneuriidae
Prosopistomatidae
Baetiscidae
Leptophlebiidae
Potamanthidae
Polymitarcyidae
Behningiidae
Palingeniidae
Ephemeridae
Icthybotidae
Euthyplociidae
Neoephemeridae
Caenidae
Ephemerellidae
Vietnamellidae
Tricorythidae
Teloganodidae
Leptohyphidae
Coryphoridae
Austremerellidae
Dicercomyzidae
Teloganellidae
Machadorythidae
Ephemerythidae
Melanemerellidae

Carapacea
Furcatergalia
Fossoriae
Caenoidae
Pannota
Ephemerelloidea

Tychoparthenogenesis ; Facultative and/or Obligate parthenogenesis ; Unknown data

Adapted from *Ogden et al.* (2009), and updates data.

Ogden, T.H., Gattolliat, J.L., Sartori, M., Staniczek, A.H, Soldan, T. & Whiting M.F. (2009). Towards a new paradigm in mayfly phylogeny (Ephemeroptera): combined analysis of morphological and molecular data. *Systematic Entomology* **34**, 616-634.

Reproduction sexuée ou asexuée ?
Une étude chez les éphémères

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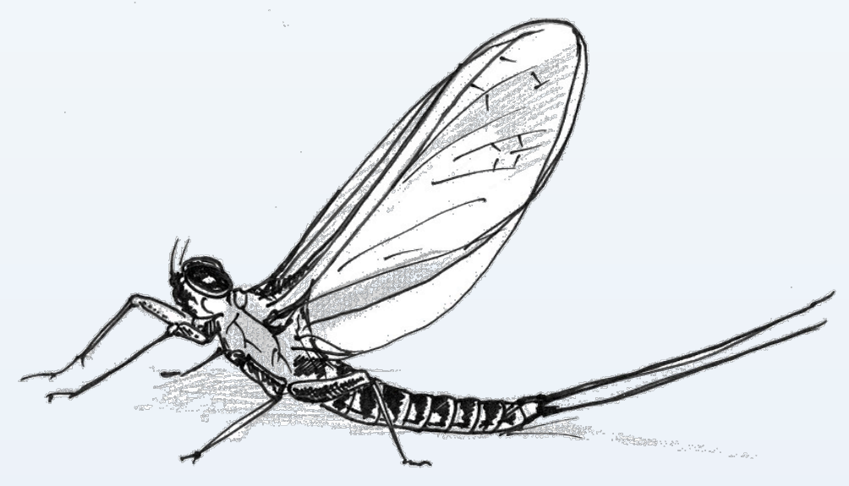
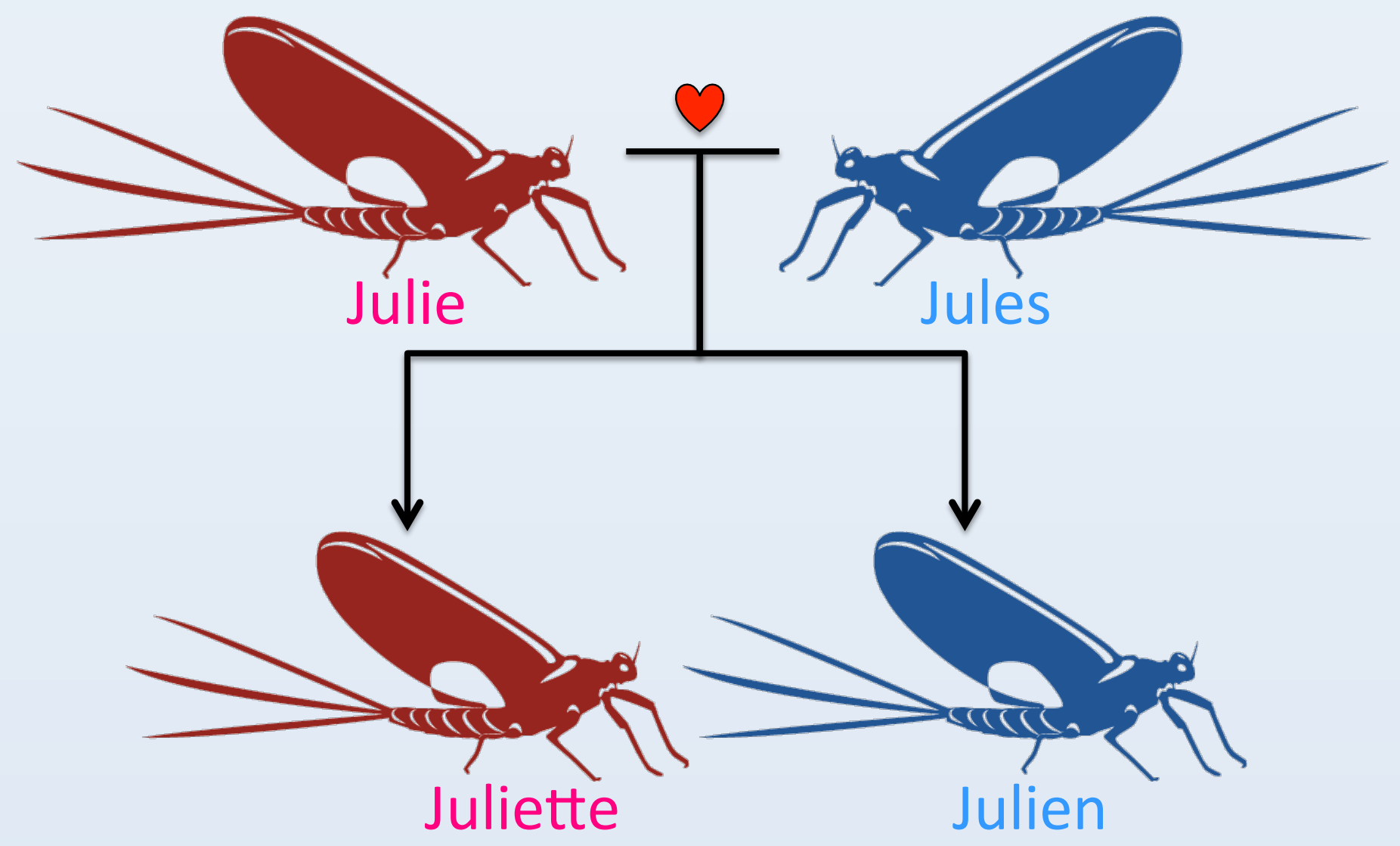


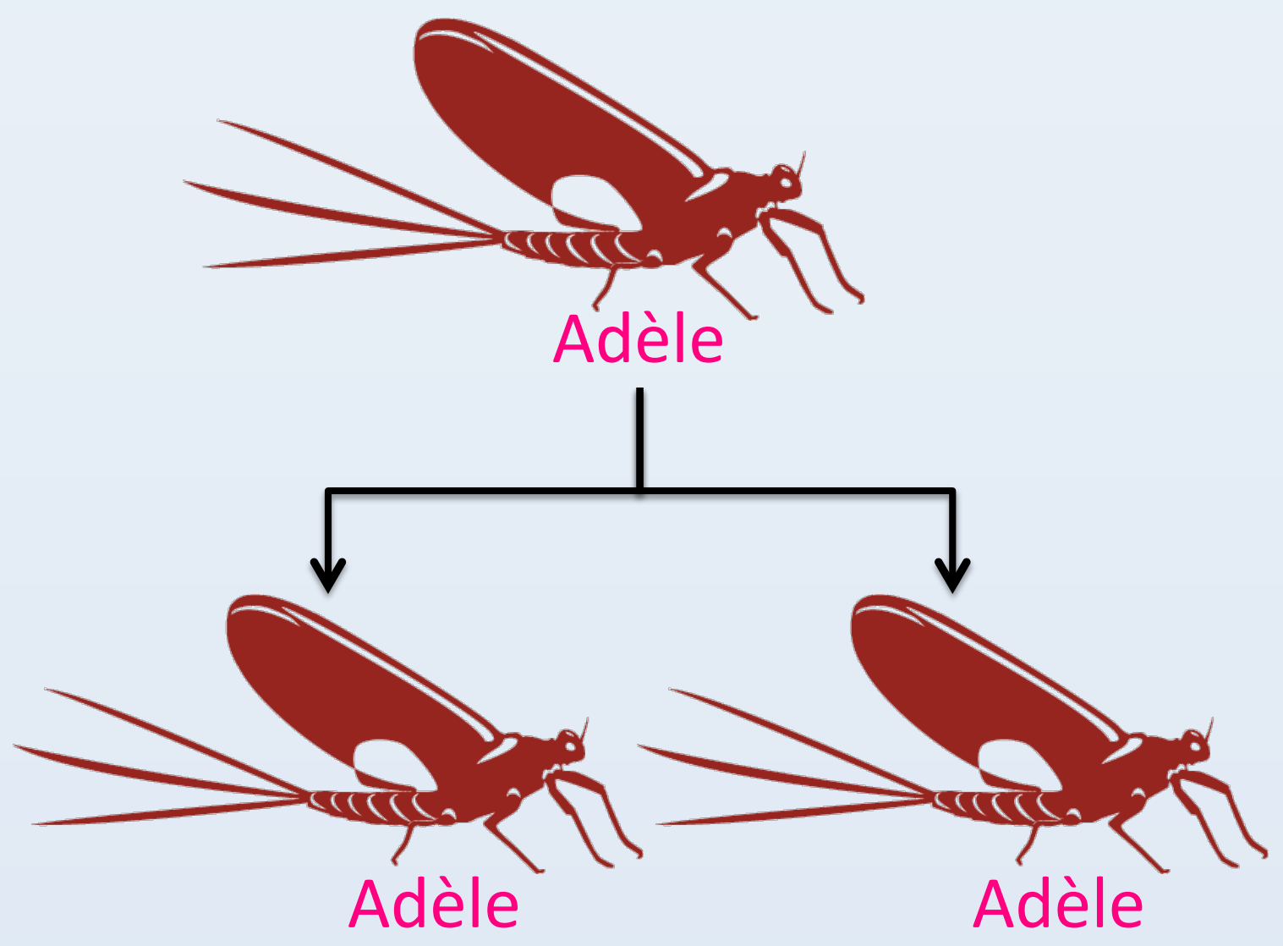
Pourquoi se reproduire de façon sexuée ?

Reproduction sexuée



La majorité des animaux se reproduisent de façon **sexuée**, mais certaines espèces sont capables de se multiplier **sans sexe** : on parle alors de **reproduction asexuée**.

Reproduction asexuée

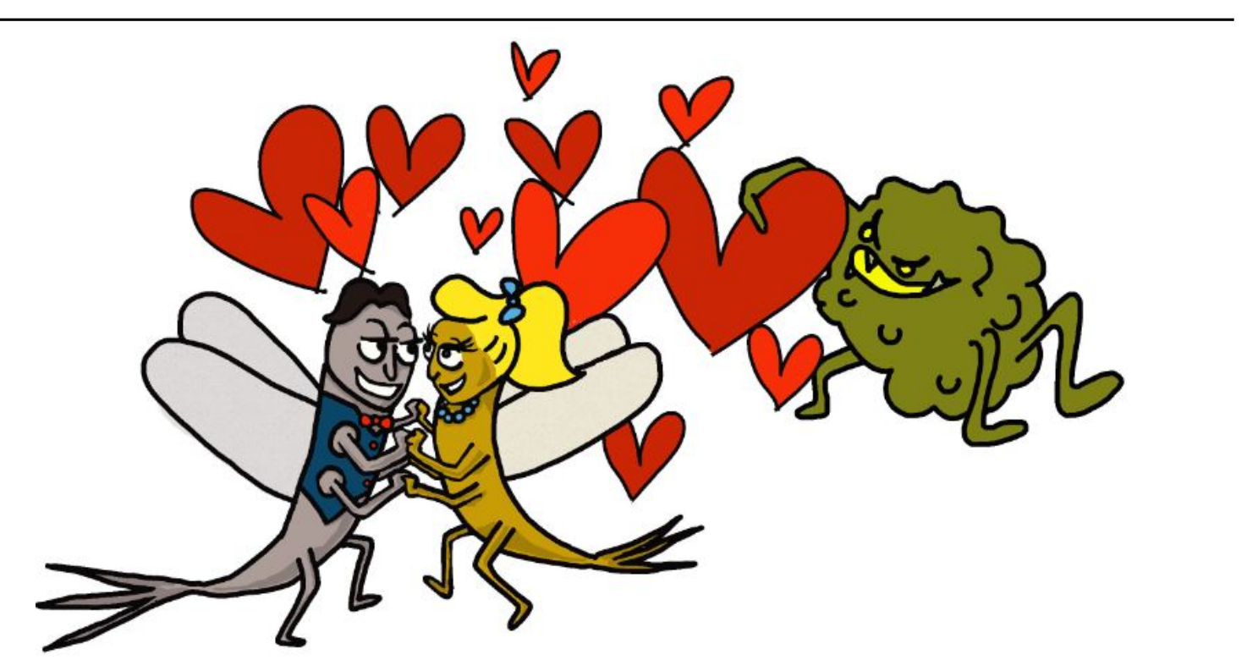


Bien que moins fréquent, se reproduire de façon **asexuée** est plus **simple**, plus **rapide** et **moins coûteux** que de faire du sexe. C'est pourquoi les scientifiques supposent que la reproduction sexuée apporte des avantages qui compensent les inconvénients. Quels sont donc **les coûts et les bénéfices du sexe** ? Plusieurs hypothèses ont été proposées…

Coûts

Nécessité de trouver un partenaire sexuel

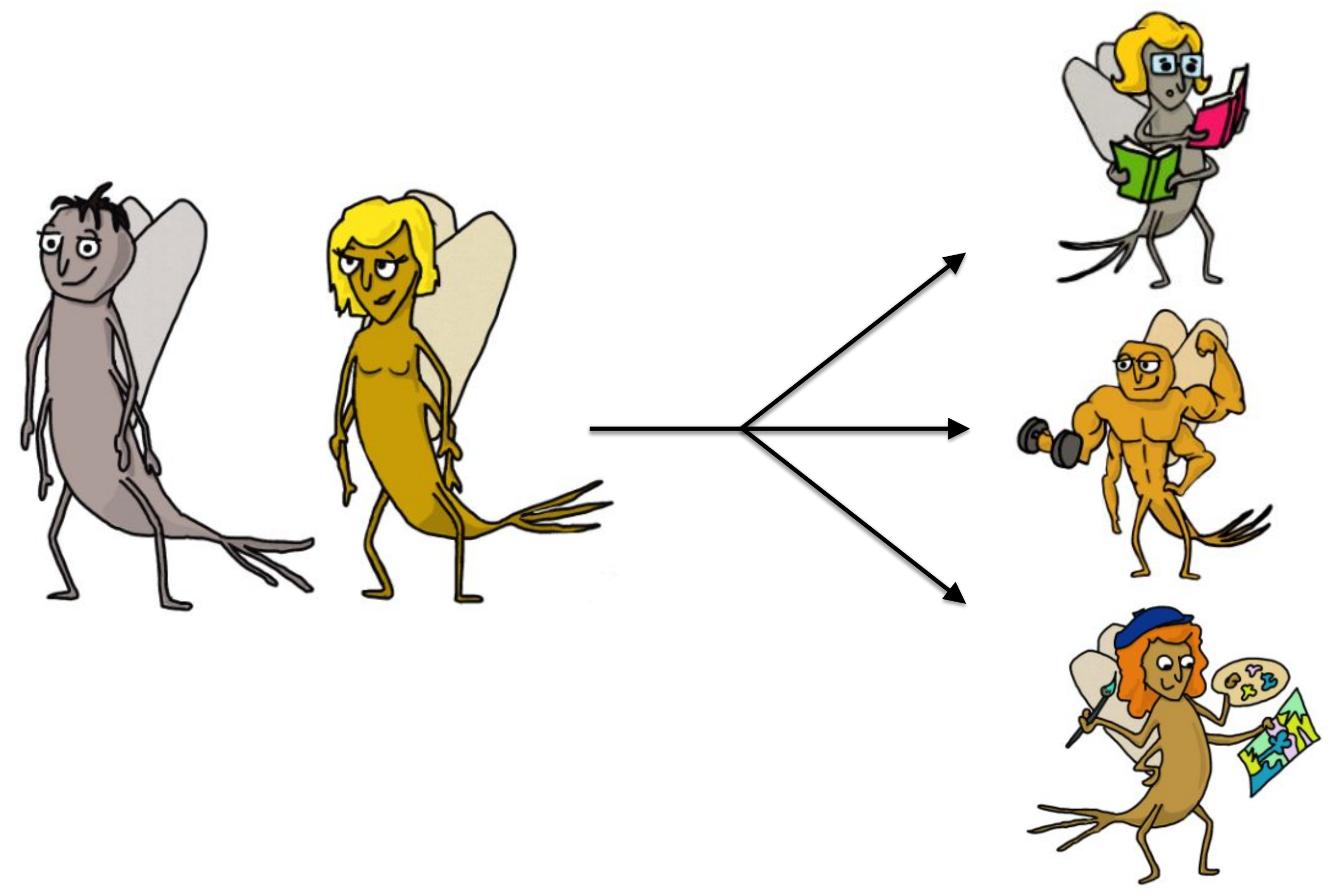
Risque de transmission de maladies



Gaspillage de ressources dans la production de mâles qui ne portent pas d'enfants

Bénéfices

Possibilité de produire des enfants avec de nouvelles performances



Illustrations : Anna Caiata

Mais alors, quels sont les facteurs écologiques et environnementaux qui influencent le type de reproduction ?

Cycle de vie

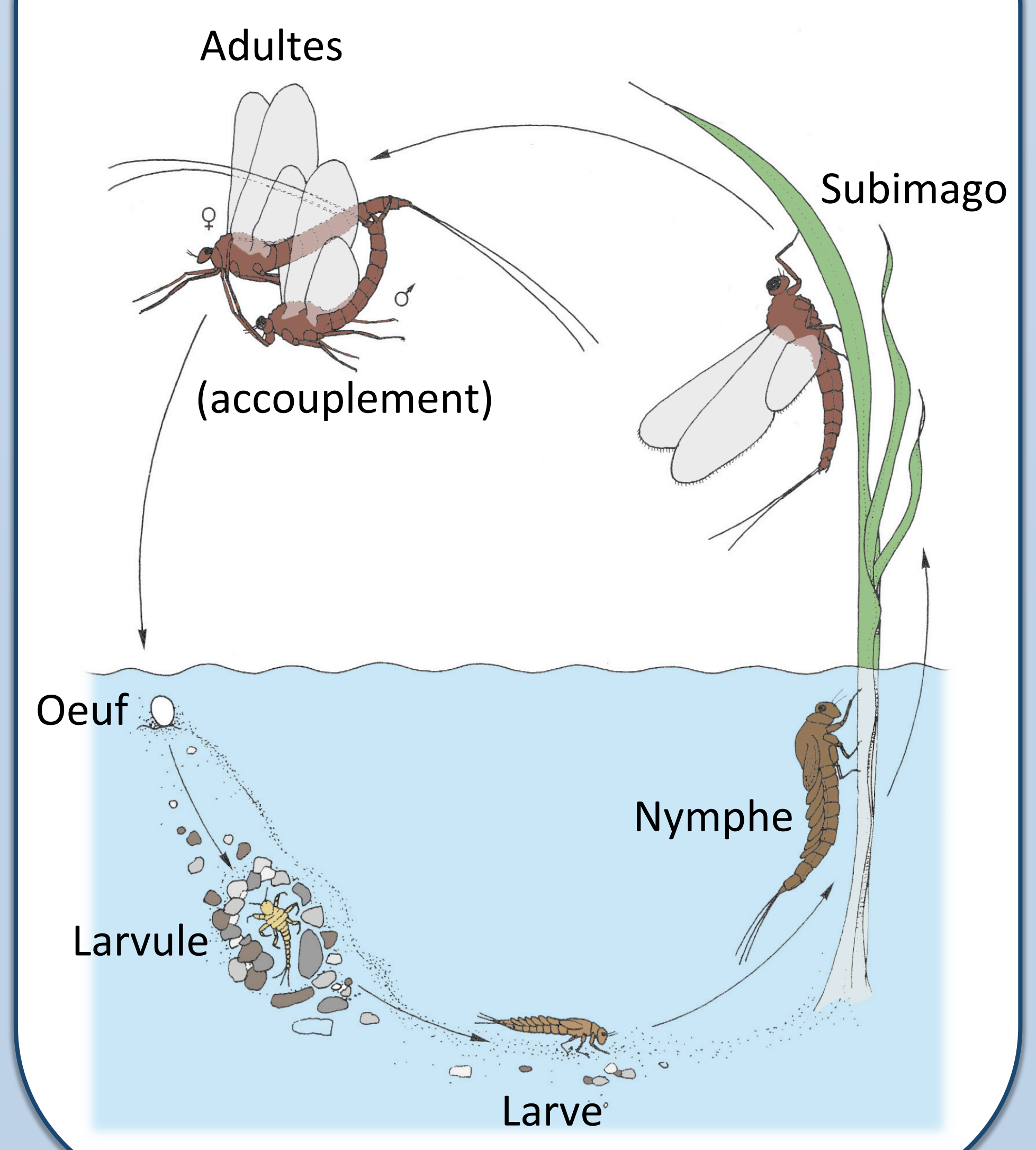
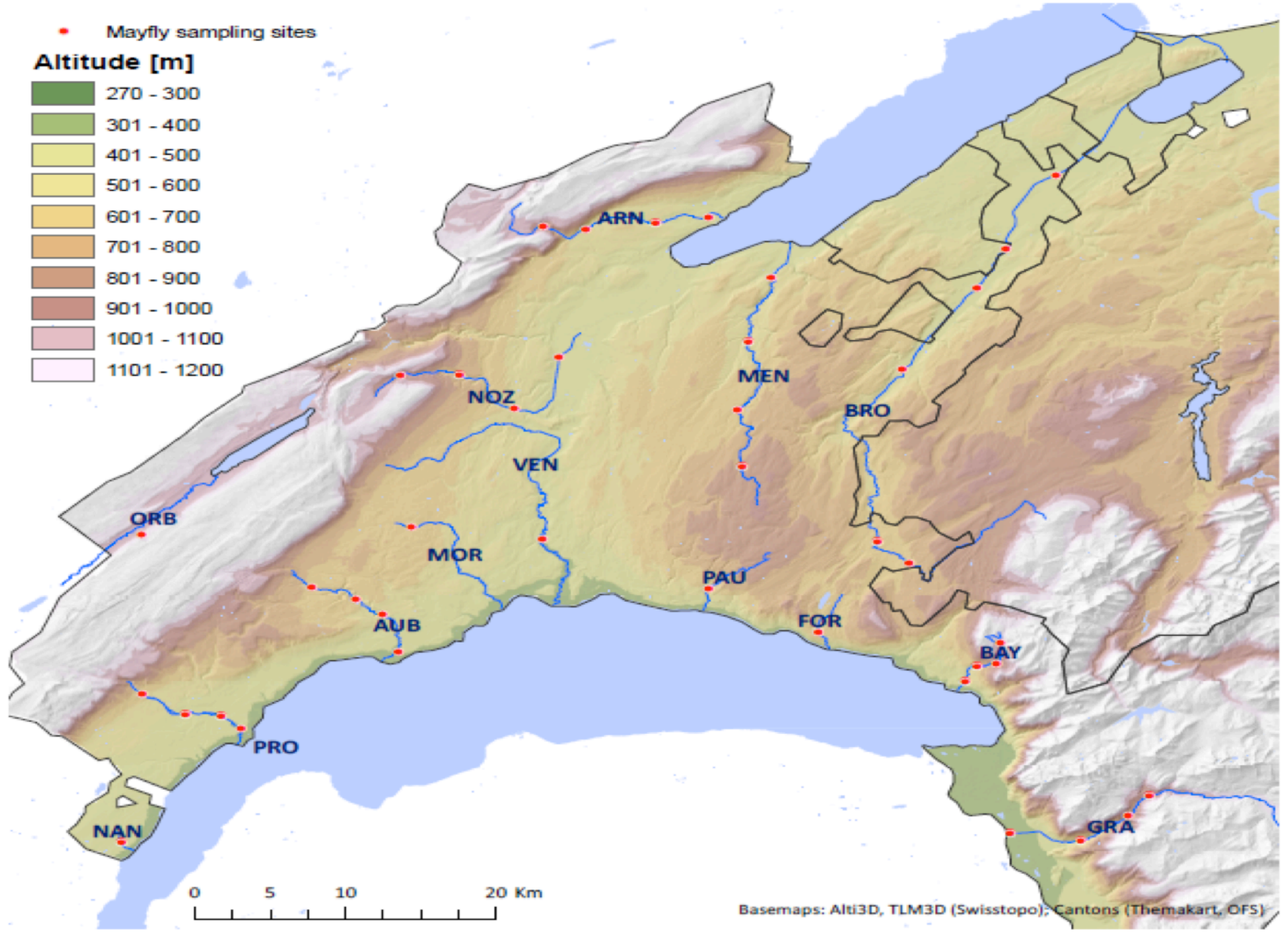


Figure adaptée de : Ephemeroptera, Insecta helvetica, *Fauna* 9, société entomologique Suisse.

Analyse des populations d'éphémères et de leurs habitats



40 sites

Légende

1. *B. rhodani*
2. *B. alpinus*
3. *A. muticus*
4. *E. helveticus*
5. *E. venosus*
6. *E. assimilis*
7. *R. picteti*
8. *H. confusa*
9. *H. lauta*
10. *S. ignita*

10 espèces

Données recueillies pour chaque site :
1 Nombre d'individus par espèce
2 Proportion ♀ : ♂
3 Variables environnementales
Altitude (m)
Température de l'eau (°C)

La reproduction asexuée, Combien ?

Afin de mesurer la capacité des différentes populations d'éphémères à se reproduire **sans sexe**, il est nécessaire d'obtenir des pontes non fécondées.
Pour ce faire, des larves immatures femelles (nymphes non accouplées) sont prélevées aux 40 sites et sont élevées sans les mâles. Les conditions de leur environnement naturel sont respectées au mieux afin qu'elles émergent en adultes puis pondent leurs oeufs, qui seront donc **non fécondés.**

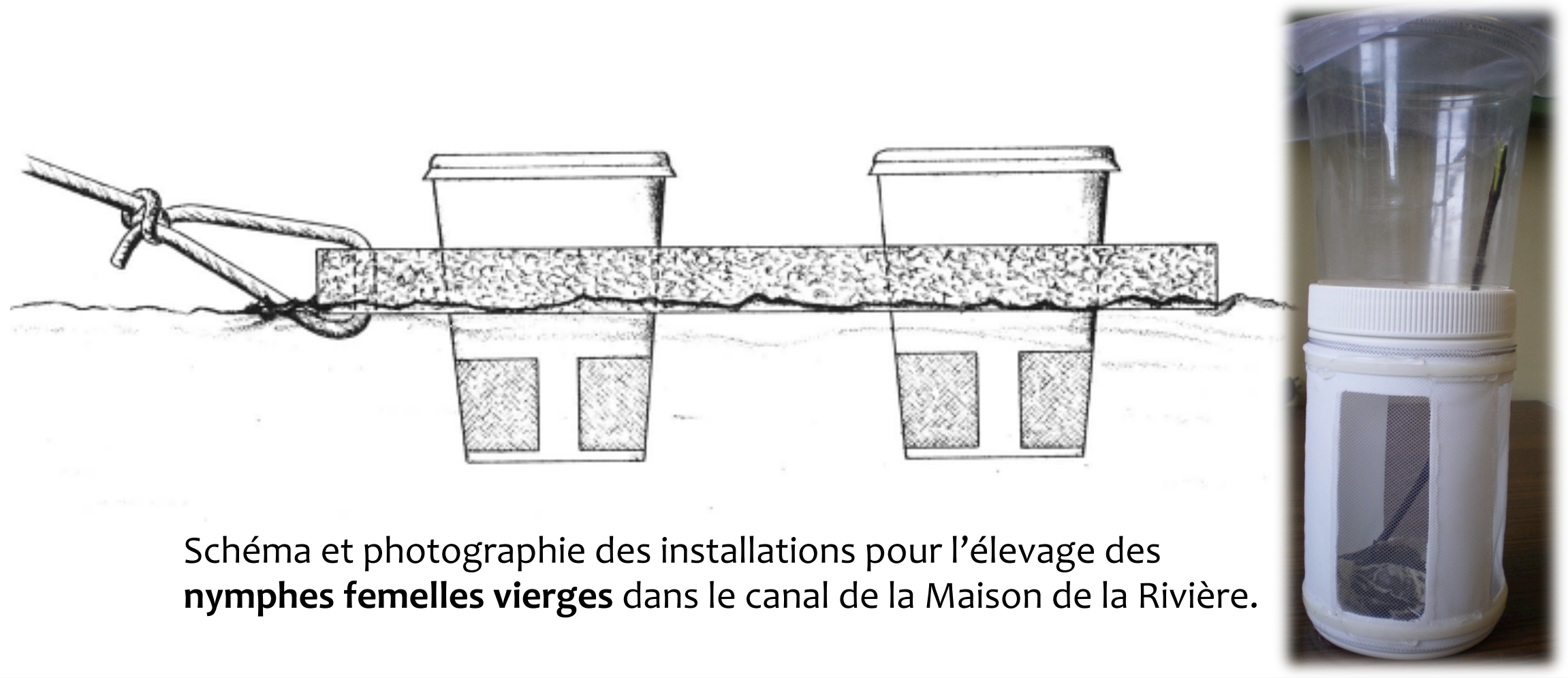


Schéma et photographie des installations pour l'élevage des **nymphes femelles vierges** dans le canal de la Maison de la Rivière.

Le nombre d'oeuf éclos, et donc le nombre de bébés obtenus, définira si les femelles éphémères peuvent faire de la reproduction asexuée et combien.

Plus le nombre de bébés sera grand, plus l'espèce sera apte à la reproduction asexuée !

What ecological factors favour asexual over sexual reproduction?

Maud Liégeois (1,*), Michel Sartori (2, †) and Tanja Schwander (1, †)

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(†) These two authors contributed equally to this work
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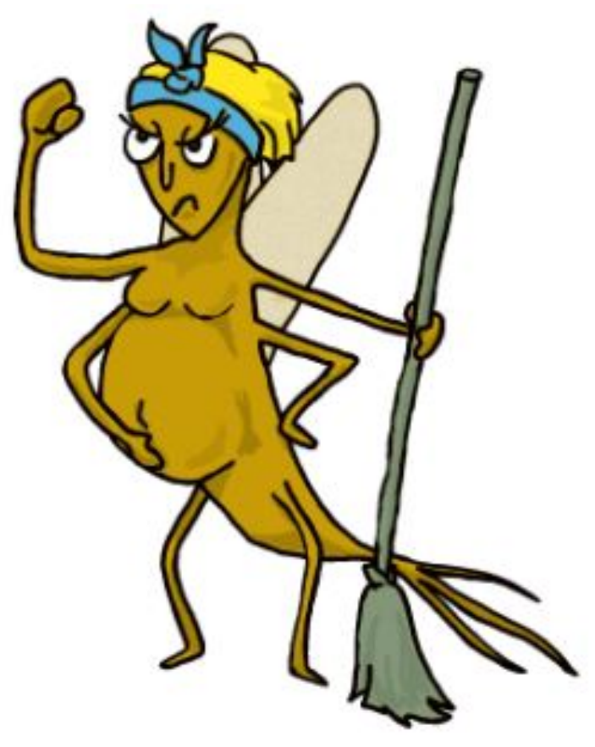
Why bother having sex?

Wasting time and **energy** to find a "good" mating partner

Risk of **disease** transmission
Increase **predation**

Conflicts
Rivalry
Competition

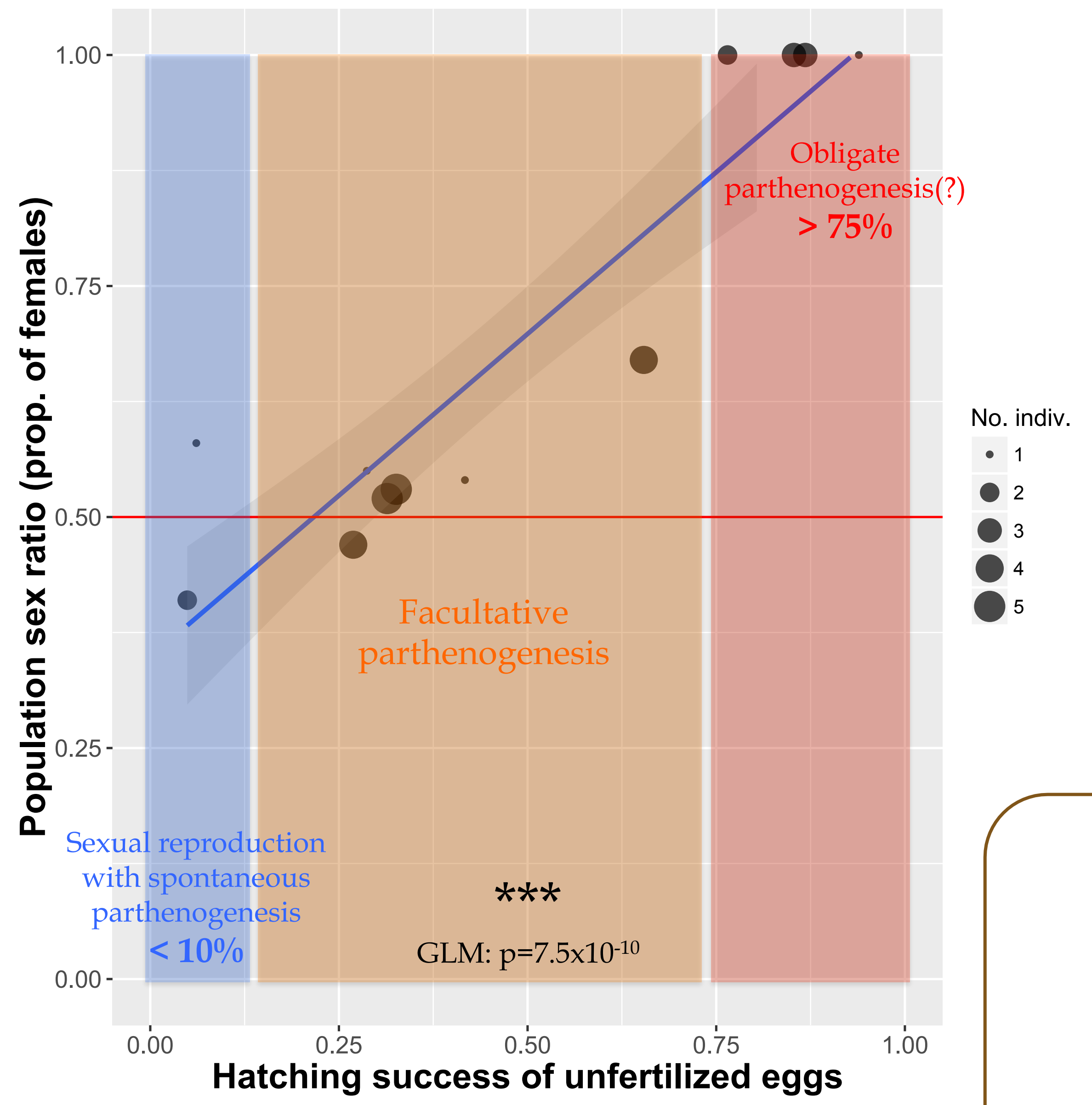


etc...

Changes in the **frequency of sex** across **natural populations** in the **facultatively parthenogenetic** mayfly species: ***Alainites muticus***

1. Parthenogenesis generates female-biased sex ratios in the field

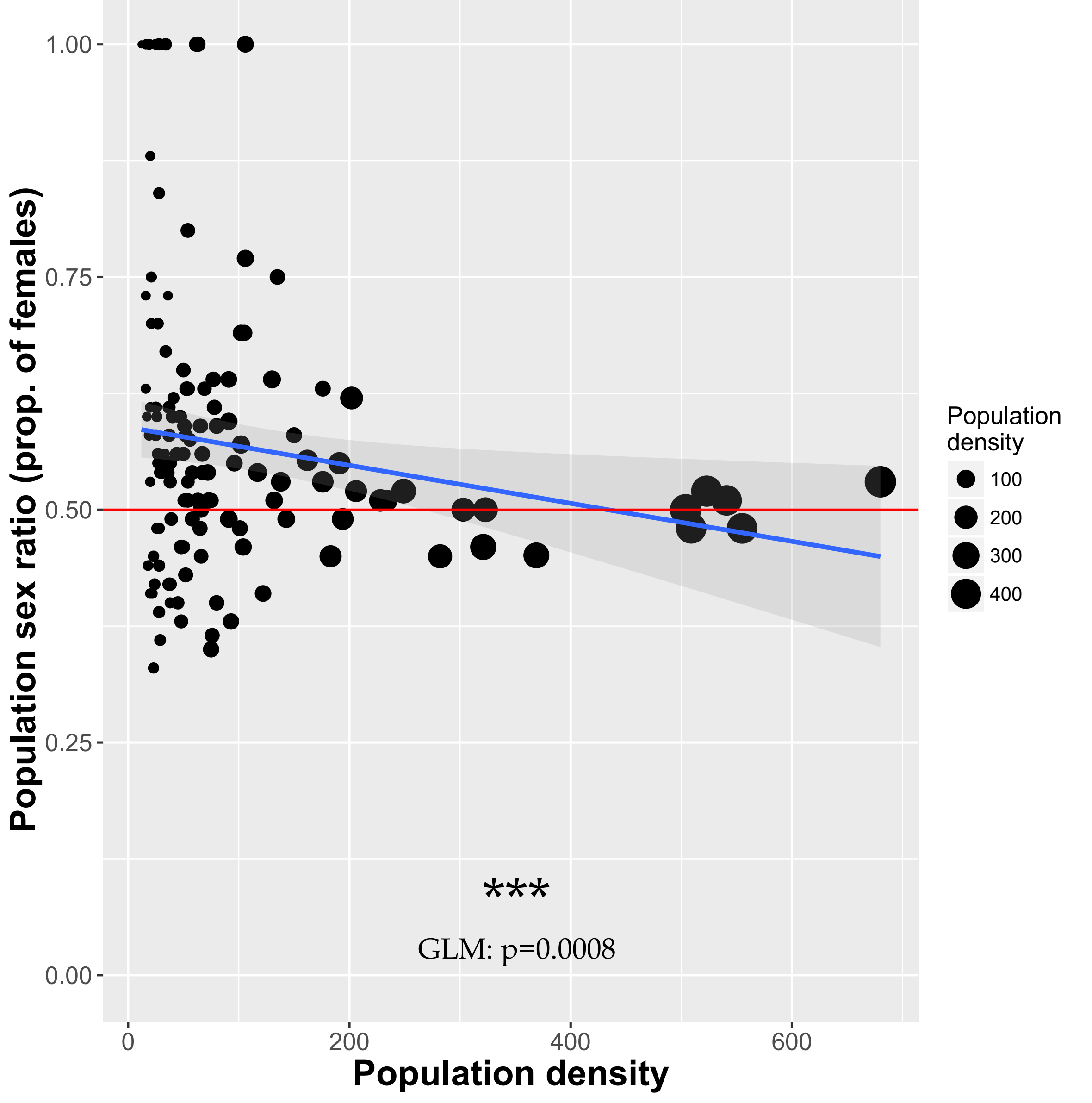
Population sex ratio reflects parthenogenetic potential → we used sex ratio as a proxy for parthenogenesis within a population.

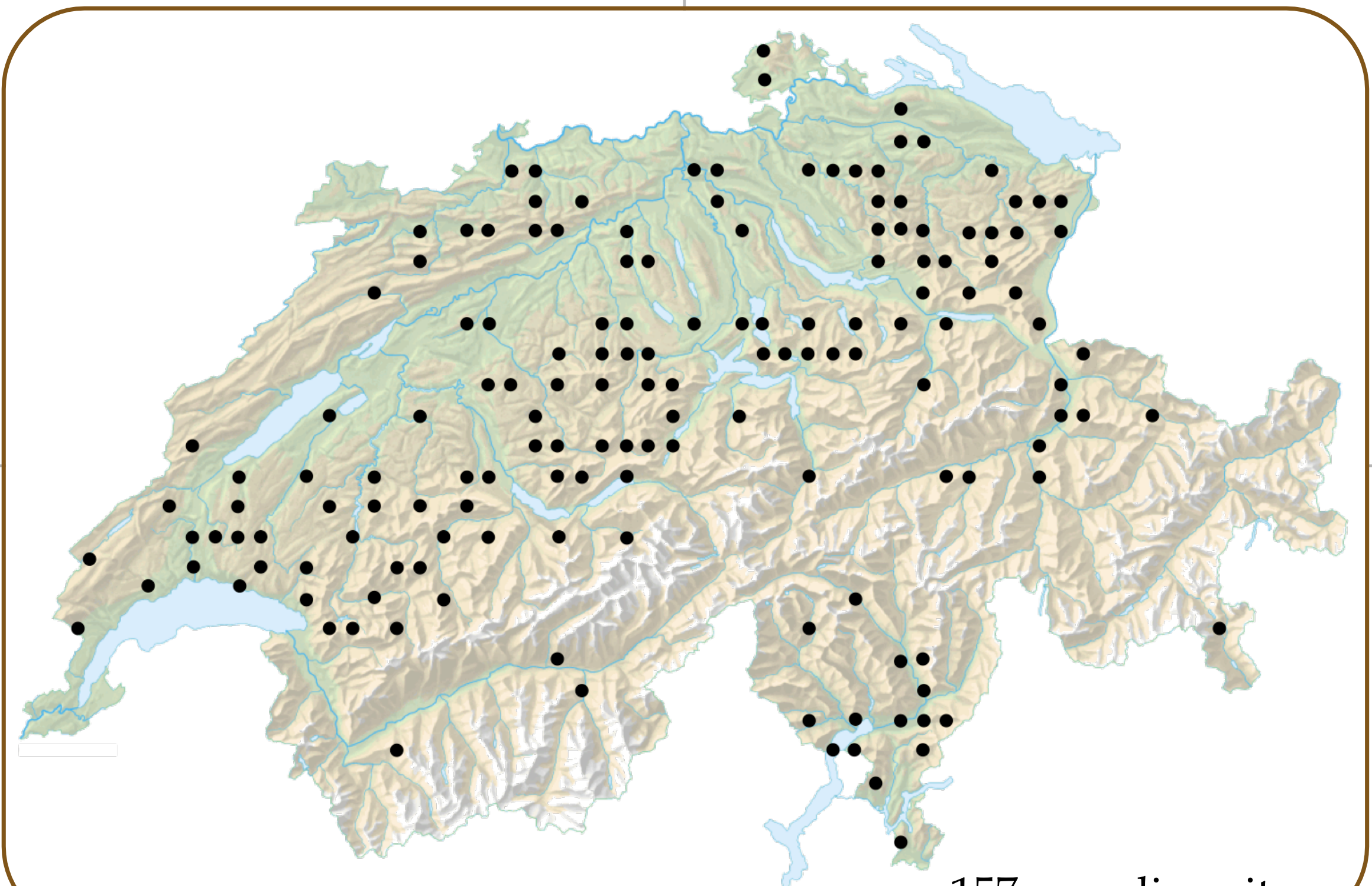


Hatching success of unfertilized eggs
(laid by virgin *A. muticus* females collected from different sampling sites across Switzerland)

2. Mate limitation (low population densities) can select for parthenogenesis

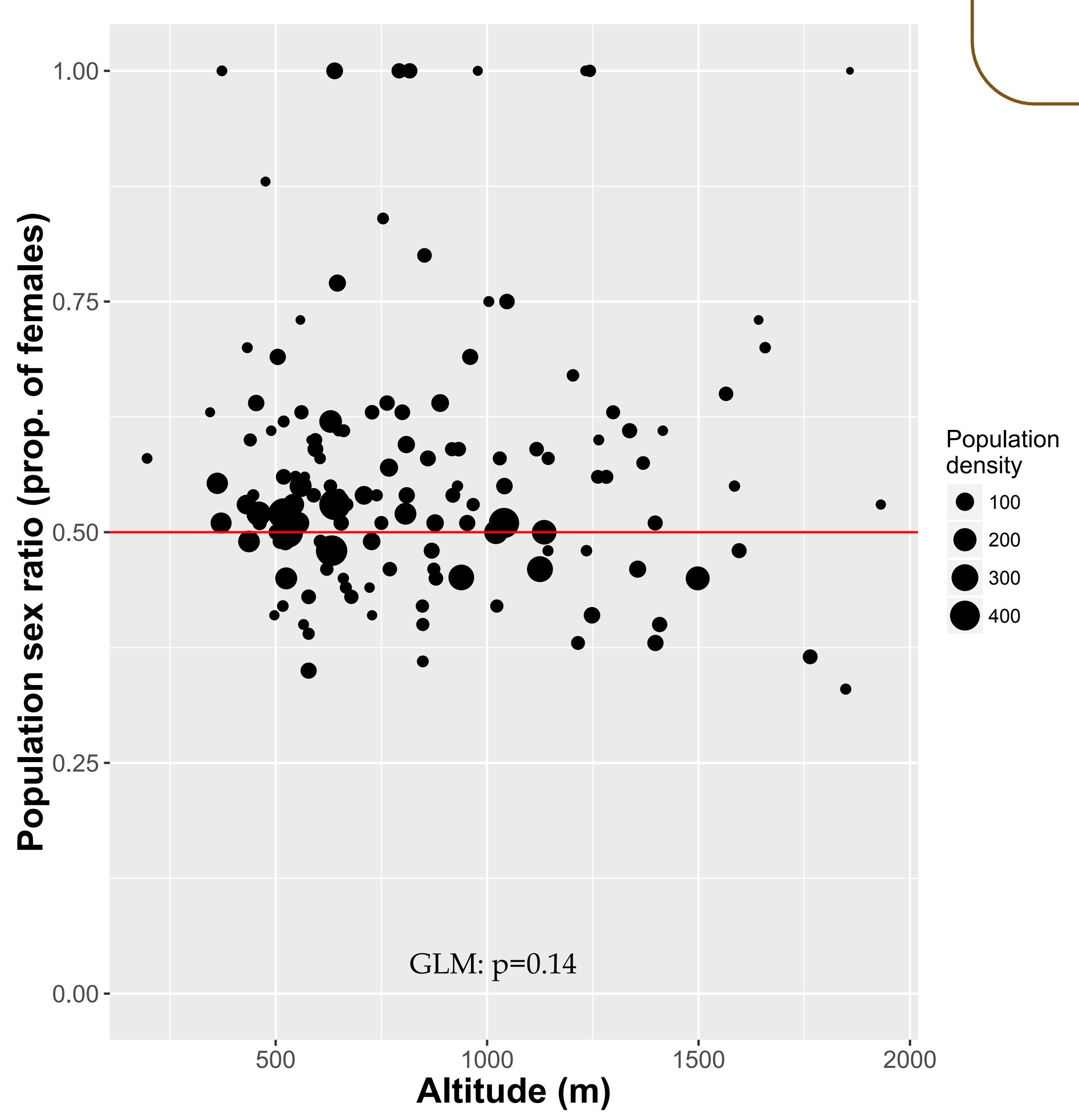
Mate limitation (estimated via population densities) may select for increased parthenogenetic ability as a means to achieve **reproductive assurance**.





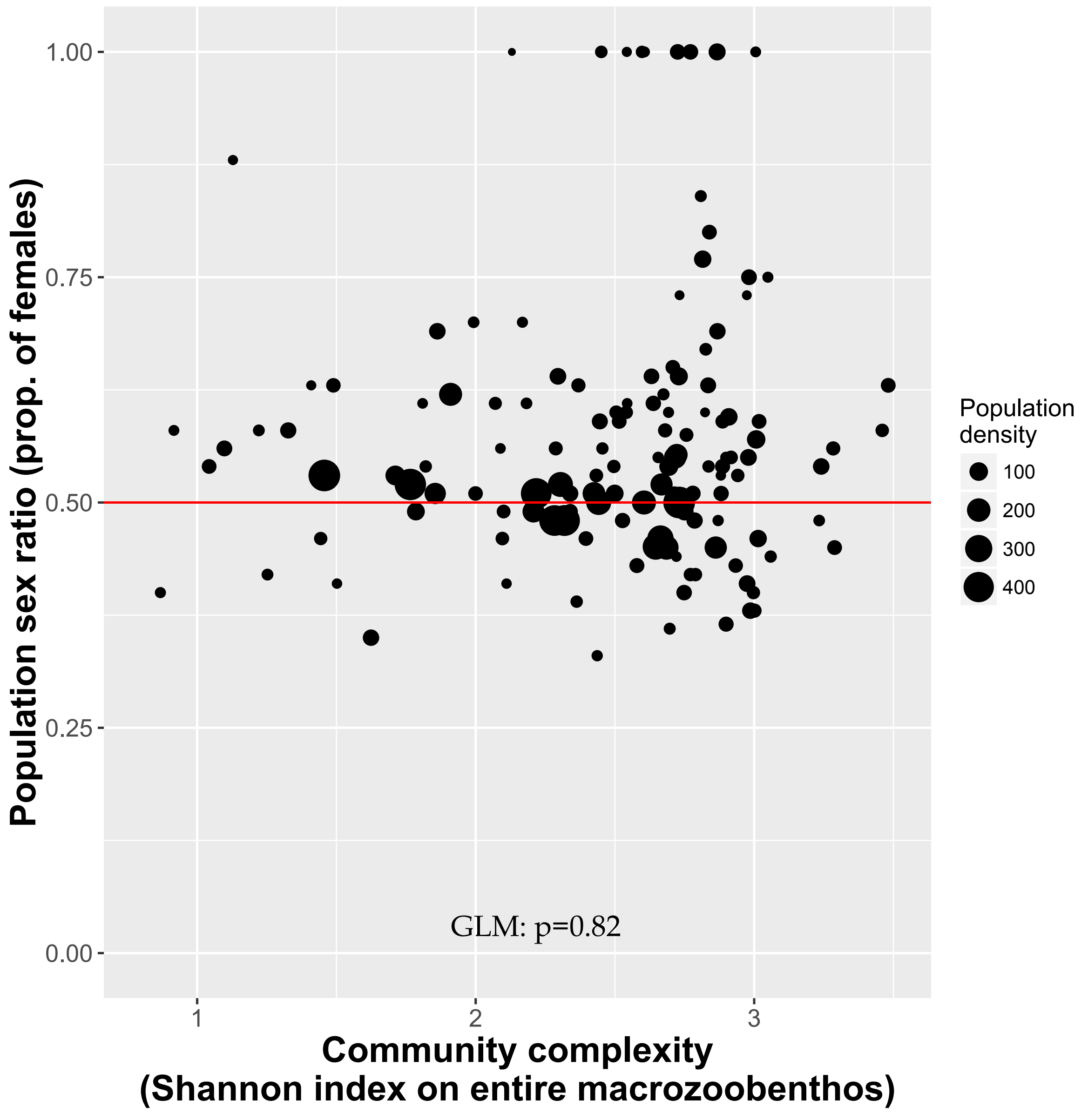
157 sampling sites

4. No correlation between parthenogenesis and altitude



In **geographical parthenogenesis**, obligate asexual populations are more likely to be found in higher altitude than their sexual counterparts. This does not hold for facultative parthenogens.

3. Sex frequency does not increase in complex communities



More complex communities with various types of species interactions, high levels of competition and fast co-evolution dynamics can favor sexual reproduction (e.g. **Red Queen hypothesis**). This does not hold for facultative parthenogens.

Conclusion

Female-biased sex ratios reflect the higher parthenogenetic potential of the population.

Mate limitation selects for parthenogenesis.

Surprising: ecological factors favoring sexual over asexual species may differ from factors favoring sex in species with facultative parthenogenesis.

Drawings : Anna Caiata; Mayfly pictures : Cyril Bennett, Graham Calow, Nick Sidle; Background picture: Maud Liégeois

Award Certificate

The best oral presentation

XV International Conference on
Ephemeroptera
XIX International Symposium on
Plecoptera
Brazil, Aracruz
03-08/June/2018

It is hereby certified that *Maud Liégeois* was awarded in the category of **Best oral presentation – Ephemeroptera** at the XV International Conference on Ephemeroptera and XIX International Symposium on Plecoptera.

Title: Reproduction and sex ratios in natural populations of the mayfly Alainites muticus Linneaus, 1758 (Ephemeroptera: Baetidae)

Frederico Falcão Salles
Universidade Federal do Espírito Santo

Rodolfo Mariano
Universidade Estadual de Santa Cruz

Maud LIEGEOIS

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1004 Lausanne
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PhD en Ecologie et Evolution & Ingénieure Agronome

Née le 24 Juillet 1987

Permis de conduire

Langues : **français** (langue maternelle), **anglais** (courant), allemand (notions)

Expériences Professionnelles

nov. **2020** – oct. **2021**	**Post-Doctorante**, *Université de Lausanne*, Suisse Département d'Ecologie et Evolution (DEE) Evolution de la parthénogenèse chez les éphémères Recherche et publications scientifiques
2020 (fév.-juil.)	**Chargée de Recherche**, *Musée Cantonal de Zoologie*, Lausanne, Suisse Collaboratrice scientifique et conservatrice en **entomologie** Analyses génétiques et collections
fév. **2015** – jan. **2020**	**Doctorante**, *Université de Lausanne*, Suisse Département d'Ecologie et Evolution (DEE) Travail de terrain, en laboratoire et enseignement Gestion de projets et élaboration de protocoles Analyses statistiques et communication des résultats (congrès, publications)
2014 (avr.-oct.)	**Ingénieure de Recherche**, *CIRAD – Centre de coopération Internationale en Recherche Agronomique pour le Développement*, Montpellier, France Unité Propre de Recherche : Bioagresseurs, analyse et maitrise du risque Département : Systèmes Biologiques (Bios) Etude par radio-télémétrie du papillon palmivore *Paysandisia archon*
2013 (mars-sept.)	**Stage de fin d'études**, *EBCL – European Biological Control Labaratory*, Montpellier, France **&** *Chambre d'Agriculture de l'Ain*, Bourg-en-Bresse, France Compréhension des facteurs d'invasion de l'euphorbe ésule (*Euphorbia esula* L.) et des interactions avec ses régulateurs naturels (insectes), en vue d'une régulation par lutte intégrée dans les prairies humides du Val-de-Saône
2012 (fév.-juil.)	**Stage en laboratoire**, *Université de Nottingham*, Royaume-Uni Département : Plants and Crop Sciences Gestion intégrée des maladies fongiques sur céréales (notamment *Fusarium* et *Rhizoctonia*)
2011 (août-déc.)	**Stages de Terrain**, *Madagascar National Parks*, Madagascar Réserve Spéciale Manongarivo : Projet visant à améliorer les pratiques culturales du riz, et élaboration d'unités de compostage pour les potagers Parc National Tsimanampetsotse : Biologie et écologie pour les suivis des espèces et habitats (état de santé de la biodiversité floristique et faunistique du site)
2010 (avr. et juil.)	**Stage en exploitation agricole**, *GAEC au fil des saisons*, Saint-Affrique, France Polycultures-élevage : **ovins lait** pour la fabrication de **Roquefort bio** **Maraîchage bio** : travail sous serre, au champ et vente directe Diagnostic technico-économique de l'exploitation

Formation

2015-2020	**Doctorat en biologie et écologie évolutive**, *Université de Lausanne* (Faculté de Biologie et Médecine), Lausanne, Suisse Thèse : Evolution de la parthénogenèse chez les éphémères Superviseurs : Prof. Tanja Schwander et Dr. Michel Sartori
2009-2013	**Ingénieure Agronome**, *ENSAIA – Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires* (Institut National Polytechnique de Lorraine), Nancy, France Spécialisation : **Protection des cultures**
double diplôme	**Master** **F**orêt **Ag**ronomie et **E**nvironnement, *Université de Lorraine* (Faculté des Sciences et Technologies), Nancy, France Spécialisation : **B**iologie des **I**nteractions **P**lantes-**E**nvironnement
2011-2012	Année de **Césure** à l'étranger

Autres Compétences

Informatique	Bureautique (**World**, **Excel**, **Power Point**)	● ● ● ● ●
	Statistiques (**R**)	● ● ● ● ●
	Géomatique (**QGIS**)	● ● ● ◐ ○
Laboratoire	Biologie moléculaire : extraction d'ADN, PCR, analyses des séquences et phylogénies	
Terrain	Echantillonnages et expérimentations, notamment en Suisse, en France et à Madagascar	
Entomologie	Formation d'étudiants en **entomologie** en tant que responsable des travaux pratiques de 1ère et 3ème années de bachelor Membre de la Société **V**audoise d'**E**ntomologie depuis 2016	
Certiphyto CSPP	Certificat individuel pour les Produits Phytopharmaceutiques. Il atteste de connaissances suffisantes pour sécuriser l'utilisation de pesticides et en réduire l'usage	
Communication	Représentante des doctorants au conseil du département d'écologie et évolution (Université de Lausanne) pendant 3 ans	

Enseignements

2019	**Analyse de données en biologie** Statistiques avec le logiciel R (Travaux Pratiques, 2ème année de bachelor)
2017	**Biodiversité et habitats** : **Faunistique des invertébrés** Caractéristiques des ordres parmi les hexapodes (Cours Magistral, 3ème année de bachelor)
2016-2021	**Biodiversité et habitats** : **Entomologie** Echantillonnage et identification des macro-invertébrés aquatiques selon la méthode IBCH (Travaux Pratiques, 3ème année de bachelor)
2016-2019	**Diversité du vivant** : **Botanique** Origine évolutive et diversification des plantes (Travaux Pratiques, 1ère année de bachelor) Caractéristiques des grandes familles et détermination d'espèces
2015-2018	**Biologie, Ecologie et Conservation** Supervision d'étudiants en master
2015-2019	**Diversité du vivant** : **Zoologie** Responsable des travaux pratiques en **entomologie** (Travaux Pratiques, 1ère année de bachelor) Classification et détermination des espèces d'invertébrés terrestres et aquatiques

Congrès

18-22 August **2018**	**Evolution – II Joint Congress on Evolutionary Biology**, Montpellier, France Talk: What ecological factors favour asexual over sexual reproduction? A study on the facultatively parthenogenetic mayfly *Alainites muticus* in natural populations
03-08 June **2018**	**International Conference on Ephemeroptera and Plecoptera**, Aracruz, Brazil Talk: Parthenogenesis and sex ratios in mayflies (Ephemeroptera) ***Awarded for the best talk***
20-25 August **2017**	**ESEB – Congress of the European Society for Evolutionary Biology**, Groningen, The Netherlands Poster: What ecological factors favour asexual over sexual reproduction? A study in mayflies
11-12 February **2016**	**Biology'16 – The annual Swiss Conference on Ecology, Evolution, Systematic, Biogeography and Conservation**, Lausanne, Switzerland Talk: Can mate limitation select for parthenogenesis? A test in mayflies
31 May-05 June **2015**	**International Conference on Ephemeroptera and Plecoptera**, Aberdeen, Scotland Poster: Sex and Parthenogenesis in Mayflies
07-09 Octobre **2013**	**CBI – 17ème Colloque de Biologie de l'Insecte**, Montpellier, France Poster : « Jeunes longicornes et vieilles euphorbes » : pas de conflit de génération

Publications

Liegeois M., Sartori M. & Schwander T. (**2021**). Extremely widespread parthenogenesis and a trade-off between alternative forms of reproduction in mayflies (Ephemeroptera). *Journal of Heredity*, **112**(1): 45-57. doi:10.1093/jhered/esaa027

Liegeois M., Sartori M. & Schwander T. Fast life-cycles and active dispersal favour parthenogenetic reproduction in mayflies. *Preprint available upon request.*

Liegeois M., Sartori M. & Schwander T. Reproduction and sex ratios in natural populations of the mayfly *Alainites muticus* (Baetidae). *Preprint available upon request.*

Liegeois M., Sartori M. & Schwander T. What ecological factors favour parthenogenesis over sexual reproduction? A study on the facultatively parthenogenetic mayfly *Alainites muticus* in natural populations. *Preprint available upon request.*

Liegeois M., Vuataz L., Wagner A. & Sartori M. (**in prep.**). *Rhithrogena fonticola* (Sowa & Degrange 1987) dans les pré-Alpes vaudoises : première mention pour la Suisse (Ephemeroptera : Heptageniidae). *Entomo Helvetica*.

Liégeois M., Tixier P. & Beaudoin-Ollivier L. (**2016**). Use of radio telemetry for studying flight movements of *Paysandisia archon* (Lepidoptera: Castniidae). *Journal of Insect Behavior*, **29**(2): 199-213. doi:10.1007/s10905-016-9554-4.

Augé M., Le Bourgeois T., **Liégeois M.** & Sforza R.F.H. (**2014**). Cows and insects helping out to control the weediness of leafy spurge in France. *Proceedings of the XIV International Symposium on Biological Control of Weeds*, pp. 156-166. F.A.C. Impson, C.A. Kleinjan and J.H. Hoffmann (eds). 2-7 March 2014, Kruger National Park, SouthAfrica.

Vulgarisation scientifique

2018 **Festival de la Salamandre**
Atelier de vulgarisation scientifique en lien avec les invertébrés aquatiques
Classes de 7^{e} et 8^{e} (10-12 ans)

2015-2019 **Mystères de l'UNIL**
Ateliers de vulgarisation scientifique en lien avec les insectes et autres arthropodes terrestres
Ecoles vaudoises (5^{e} à 8^{e}) et grand public

Subventions/Bourses

2018
FONDATION
POUR L'UNIVERSITÉ
DE LAUSANNE

FUNIL – *Fondation pour l'Université de Lausanne*
Bourse permettant la participation à un congrès scientifique international afin de présenter ses résultats de recherche
2300 CHF

2016
SOCIETE VAUDOISE
D'ENTOMOLOGIE

SVE – *Société Vaudoise d'Entomologie*
Bourse destinée à soutenir des travaux entomologiques comportant une part de terrain en terre Vaudoise
500 CHF

Hobbies

Sports : Randonnée, Trekking à vélo, Equitation, Squash

Musique : Saxophone

Photographie : Macros et paysages

Références scientifiques

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