



Symposium Article

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

Maud Liegeois, Michel Sartori, and Tanja Schwander

From the Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland (Liegeois, Sartori, and Schwander); and Cantonal Museum of Zoology, Palais de Rumine, Place de la Riponne 6, CH-1014 Lausanne, Switzerland (Liegeois and Sartori).

Address correspondence to M. Liegeois at the address above, or e-mail: maud.liegeois@outlook.com.

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

Corresponding Editor: Maria Orive

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 catalog 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 find 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.

Subject area: Genotype to phenotype

Keywords: mayflies, parthenogenesis, natural populations, evolution of sex

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. The proportion of parthenogenetic species remains however unknown as there are only 2 quantitative estimates (based on species lists) of the frequency of

parthenogenesis (i.e., in vertebrates: White 1973; Vrijenhoek 1998; and in haplodiploids: van der Kooij et al. 2017). This is unfortunate as such species lists are invaluable for addressing when and how parthenogenetic reproduction is favored over sex in natural populations (e.g., Ross et al. 2013; van der Kooij et al. 2017) and thus for helping to solve the paradox of sex. In this review, we provide such a quantitative estimate by summarizing 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, unpublished), 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” (Box 1). Furthermore, a single species can feature mixed reproduction (some females reproduce sexually, others parthenogenetically), either sympatrically or in allopatry (i.e., geographical parthenogenesis).

We conducted a detailed literature review to establish a catalog of all (to the best of our knowledge) mayfly species capable of reproducing parthenogenetically (Supplementary Appendix). We then used this catalog 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 fertilized and unfertilized 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 unfertilized eggs develop through parthenogenesis). Given the low hatching success of unfertilized eggs, population sex ratios remain equal. (B) Facultative parthenogenesis, means that eggs may either be fertilized or develop parthenogenetically. The hatching success of unfertilized 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 fertilized, 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.

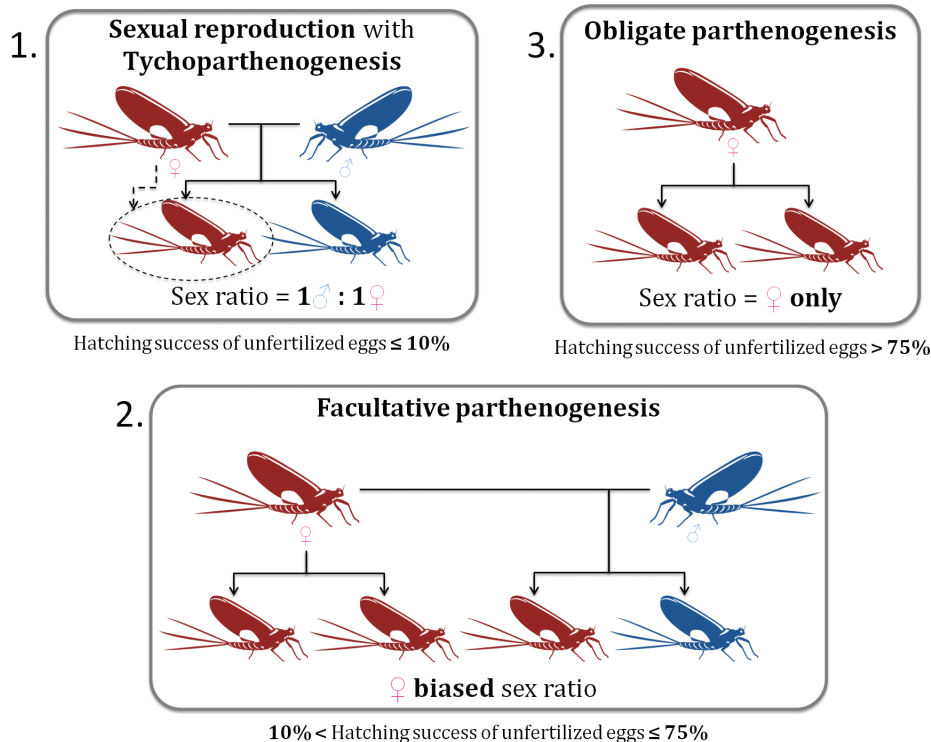


Table 1. Frequency of parthenogenesis and sex determination in Ephemeroptera taxa

Family	Parthenogenetic species	Sexual species	Total number of described species	% of parthenogenesis		Sex determination (2n+♂:♂)	References
				Among described species	Among studied species		
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; Ueno 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; Sivaramkrishnan et al. 1991; Lowen and Flannagan 1992; Sweeney et al. 1993; Jackson and Sweeney 1995; Harker 1997; Salas and Dudgeon 1999; Gattoliat 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, personal observation
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, personal communication
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; Sekimé 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, personal observation
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

Table 1. Continued

Family	% of parthenogenesis					References
	Parthenogenetic species	Sexual species	Total number of described species	Among described species	Among studied species	
Palingeniidae	1	-	33	3	100	Landolt et al. 1997
Polymitarcyidae	2	2	104	1.9	50	Degrange 1960; Britt 1962; Tojo et al. 2006; Sekin� and Tojo 2010b, 2010a; Sekin� et al. 2013, 2015
Potamanthidae	-	1	25	0	0	Degrange 1960; Soldan and Putz 2000
Prosopistomatidae	1	1	29	3.4	50	Degrange 1960; Campbell and Hubbard 1998
Siphonuridae	2	1	48	4.2	66.7	Degrange 1954, 1955, 1960; Gibbs and Siebenmann 1996; Soldan and Putz 2000
Total	65	71 ^a	3,666	1.8%	47.8%	

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, unpublished). NA: unknown. Note that all studied sexual and parthenogenetic species are diploid (2n).

^aAmong the 71 sexual species, 38 can reproduce by tytoparthenogenesis (53.5%).

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. The Tree of Sex database (Ashman et al. 2014) comprised only 8 sexual and one parthenogenetic mayfly species and was therefore not useful for our survey. Our survey combined with personal communications and observations generated a list of 136 species, as described in our database (Table 1, Supplementary Appendix). When available for a given species, information on its geographical distribution, cytological mechanism of parthenogenesis, and sex determination was included in the database (Supplementary Appendix).

Classification of Parthenogenesis

To classify mayfly species by forms of parthenogenesis (Box 1), we focused on the hatching rate of unfertilized eggs. We defined 2 main categories according to this rate: “sexual species” (including species with tytoparthenogenesis), when less than 10% of unfertilized eggs hatch (typically 0–5.3% for population means), and “parthenogenetic species,” when hatching success of unfertilized 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 unfertilized 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 parthenogens 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 the section on geographical parthenogenesis).

There is also a study bias for certain taxa, with 70 species in our database belonging to only 2 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 Supplementary 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 2 families (using solely the species of the 15 remaining families; Table 1). Excluding the 2 families from the analyses did not change any of our main conclusions (see below).

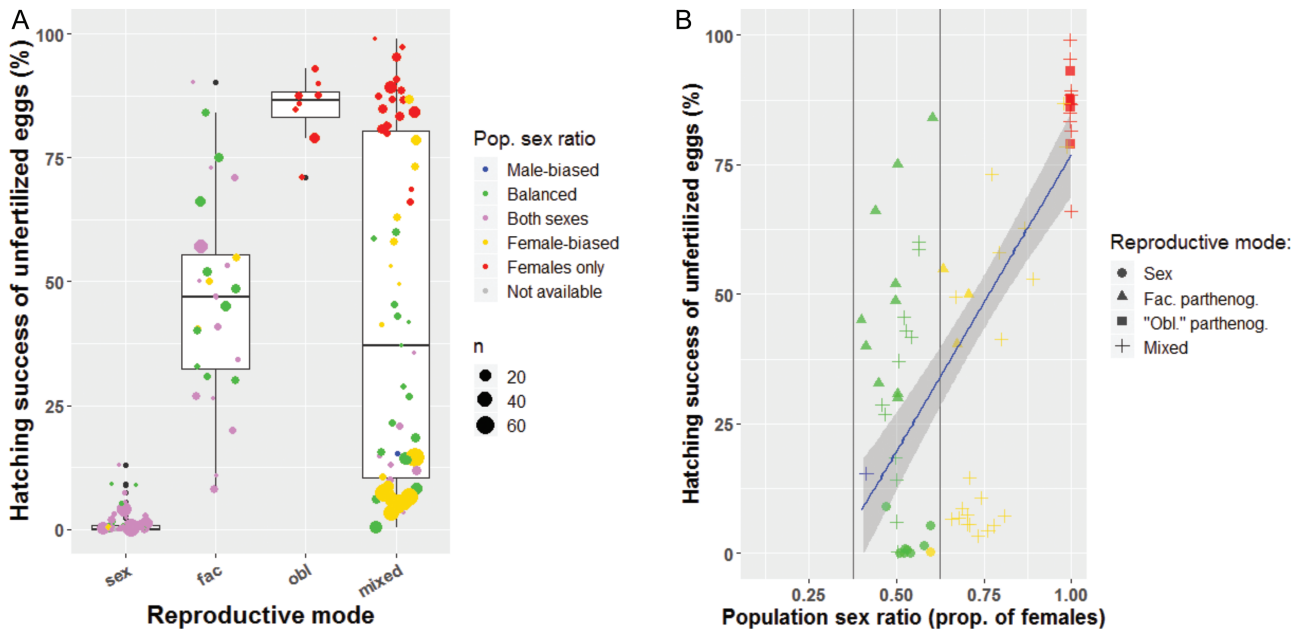
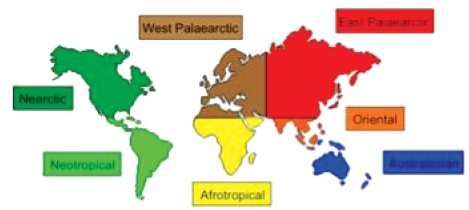


Figure 1. (A) Population-average hatching success of unfertilized eggs and reproductive modes. x axis: sex: sexual reproduction, fac: facultative parthenogenesis, obl: "obligate" parthenogenesis, mixed: mixed reproduction (in sympatry and/or in allopatry). Pop. sex ratio: population sex ratio. Both sexes (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 Baetidae (32.3%) and Heptageniidae (27.4%), see also Supplementary Figures 1 and 2). **(B)** Population-level correlation between unfertilized 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 Baetidae (23.8%) and Heptageniidae (20.2%), see also Supplementary Figures 3 and 4).

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)	% of parthenogenesis among studied species (right)
NEA	26	26	611	4.3	50.0
PAL (west + east)	23	44	839	2.7	34.3
NEO	7	0	902	0.8	100
AFR	5	2	642	0.8	71.4
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, unpublished).

Statistical Analyses

We first verified that our classification into sexual (with or without tytoparthenogenesis) and parthenogenetic species (facultative, mixed, and obligate) was biologically meaningful, by comparing the distribution of hatching successes of unfertilized eggs for different groups (Figure 1A). We further tested whether species with high egg-hatching successes were often characterized by female-biased population sex ratios, by using a quasibinomial Generalized 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 Generalized 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 6 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 rate of fertilized and unfertilized 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 2 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 2 frequency estimates, one using the total number of taxonomically described mayfly species, and one using only species where the reproductive mode was studied.

Results

Unfertilized Egg-Hatching Successes and Population Sex Ratios

Analyzing the information we collected in our database revealed that the parthenogenetic capacity of females varied widely between and within populations (see Supplementary Appendix for details). Nevertheless, our classification into sexual (with or without tytoparthenogenesis) and parthenogenetic species (facultative or obligate) is biologically meaningful, given the largely non-overlapping values for population-average hatching success of unfertilized eggs (Figure 1). Note that some species with mixed reproduction show a low population-average hatching success of unfertilized 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 unfertilized egg-hatching successes were studied in the same populations. In these species, the

parthenogenetic capacity and population sex ratios were significantly positively correlated (Figure 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).

Frequency of Parthenogenesis Among Mayflies

Parthenogenesis occurs in all well-studied mayfly families (Table 1, Figure 3). We were able to classify the reproductive mode of 136 species from 17 families (Table 1, see Supplementary Appendix for details). Seventy-one of these species are sexual (from 16 families), and 38 of these are able to perform tytoparthenogenesis, 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% (Figure 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 tytoparthenogenesis (75.7% of the studied species).

Parthenogenesis occurs in an array of families and genera without any evidence for phylogenetic clustering (Figure 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;

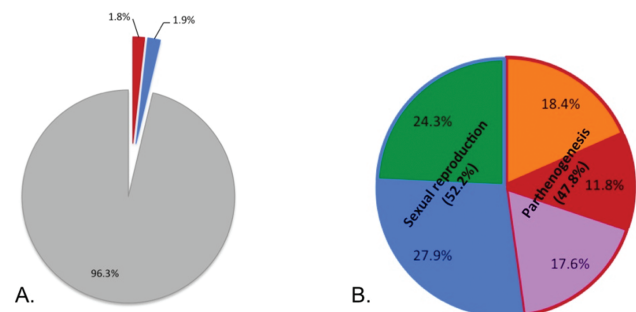


Figure 2. Frequency of parthenogenesis among described mayfly species (A) and among species studied for their reproductive mode (B). Less than 4% of the mayfly species (from 17 out of 42 families) have been studied for their reproductive mode. Parthenogenesis (warm colors): facultative (orange), “obligate” (red) and mixed reproduction (purple). Sexual reproduction (cold colors): “obligate” sexual reproduction (green) and sexual reproduction with tytoparthenogenesis (blue). At least 47.8% of the 136 studied species are able to reproduce parthenogenetically.

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 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%). In the remaining families, the data are too scarce to detect potential differences.

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 favor 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 the geographic clustering

of parthenogenetic species. The 6 regions compared comprised approximately equal proportions of studied parthenogenetic species (Table 2, see Supplementary 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.

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 Supplementary 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

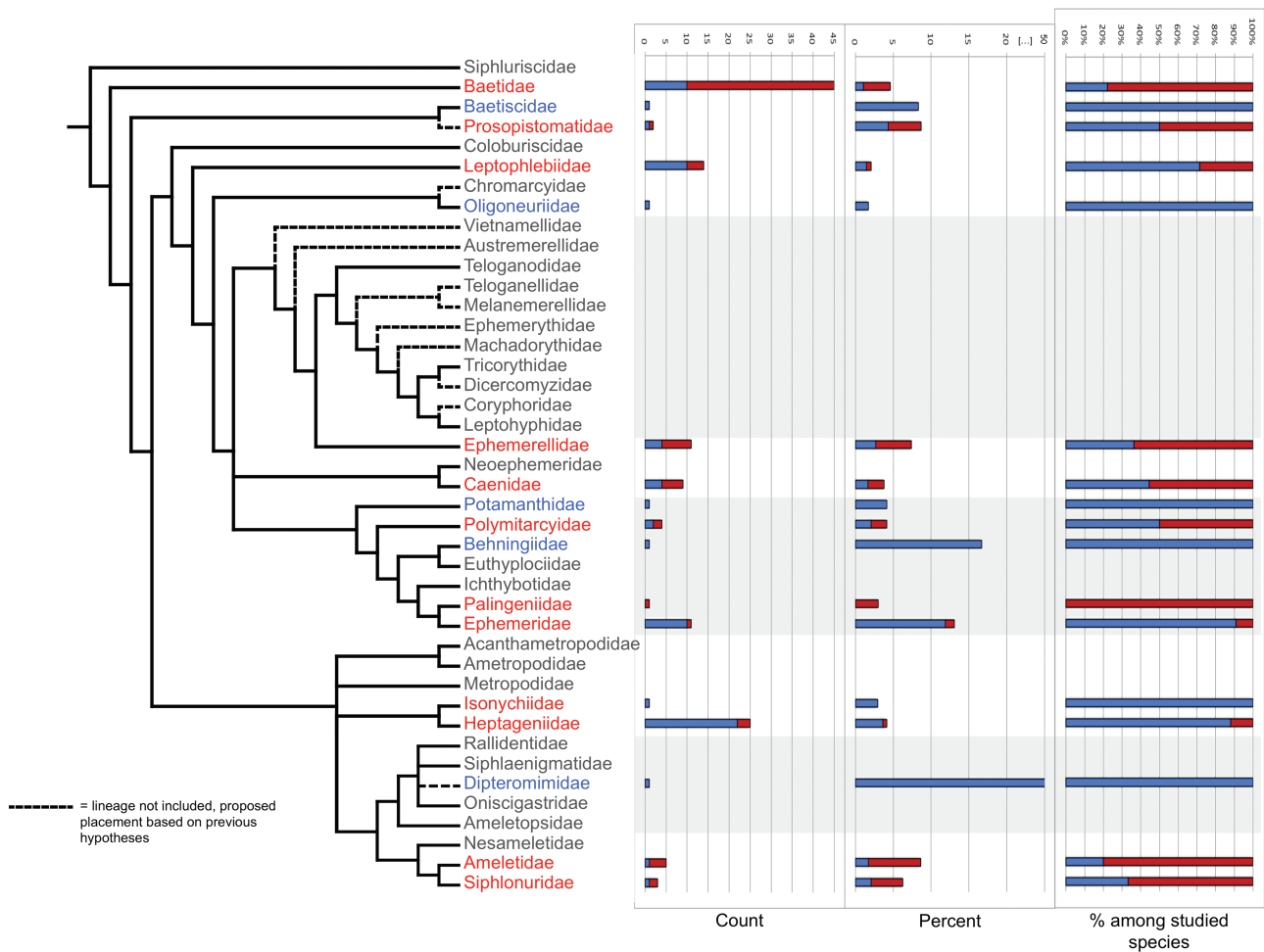


Figure 3. Phylogenetic distribution of parthenogenesis among mayfly families. red color: Parthenogenetic species (facultative, obligate, and mixed), blue color: Sexual species (with or without tycho-parthenogenesis), gray color: Families without information regarding species' reproduction ($n = 25$). 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 tycho-parthenogenesis) to be the ancestral state in mayflies.

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 (Supplementary Appendix). Thus, the lack of data for these species does not allow us to conclude anything regarding potential geographical patterns.

More than the 2 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 generations (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, 9 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 for 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 7 studied facultatively parthenogenetic mayflies are automictic (Supplementary Appendix). Indeed, for the populations with both males and females of 7 Baetidae species (*Acerpenna macdunnoughi*, *A. pygmaea*, *Anafroptilum 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 summarized in Figure 4.

Trade-Off Between Reproductive Modes

Overall, mayfly species are better at reproducing sexually than asexually (measured as egg-hatching success, Figure 5A, P -value < 0.001). Only in obligate parthenogens is egg-hatching success decreased upon mating, presumably because (even partial) fertilization interferes with the normal development of asexual eggs. Furthermore, there is a significant negative correlation between hatching rate of fertilized and unfertilized eggs at the population level of a given species (Figure 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 parthenogenetic reproduction.

Origins of “Obligate” Parthenogenesis in Mayflies

There are at least 4 ways in which parthenogenetic lineages could arise from sexual species in animals (Simon et al. 2003): 1) Hybridization between 2 sexual species, which is the major route

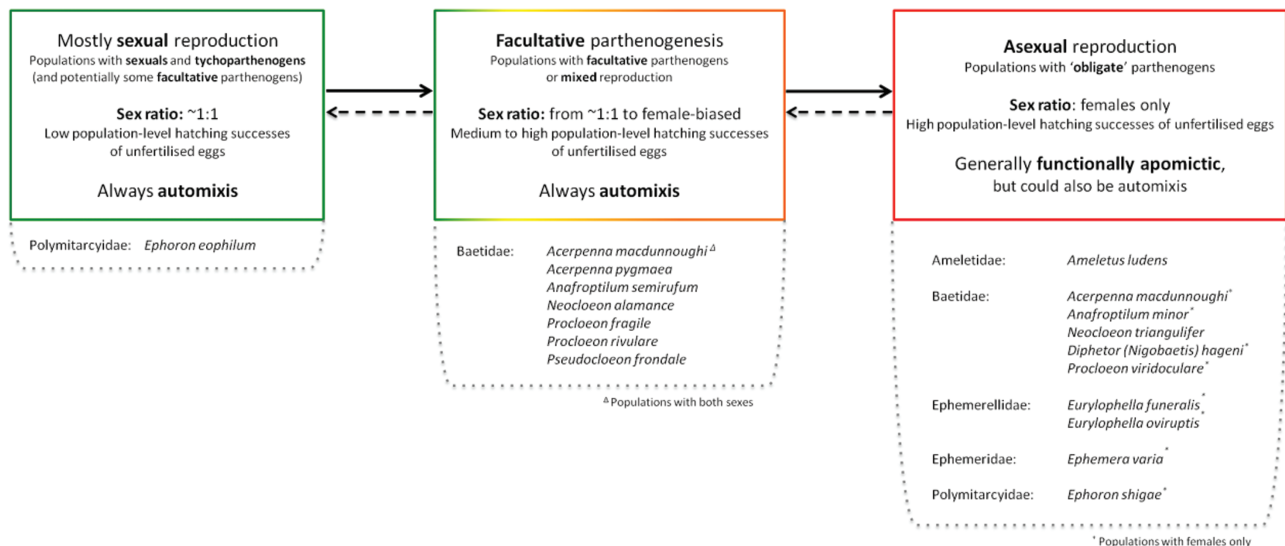


Figure 4. Cytological mechanisms identified to date in mayflies and possible transitions between parthenogenesis forms.

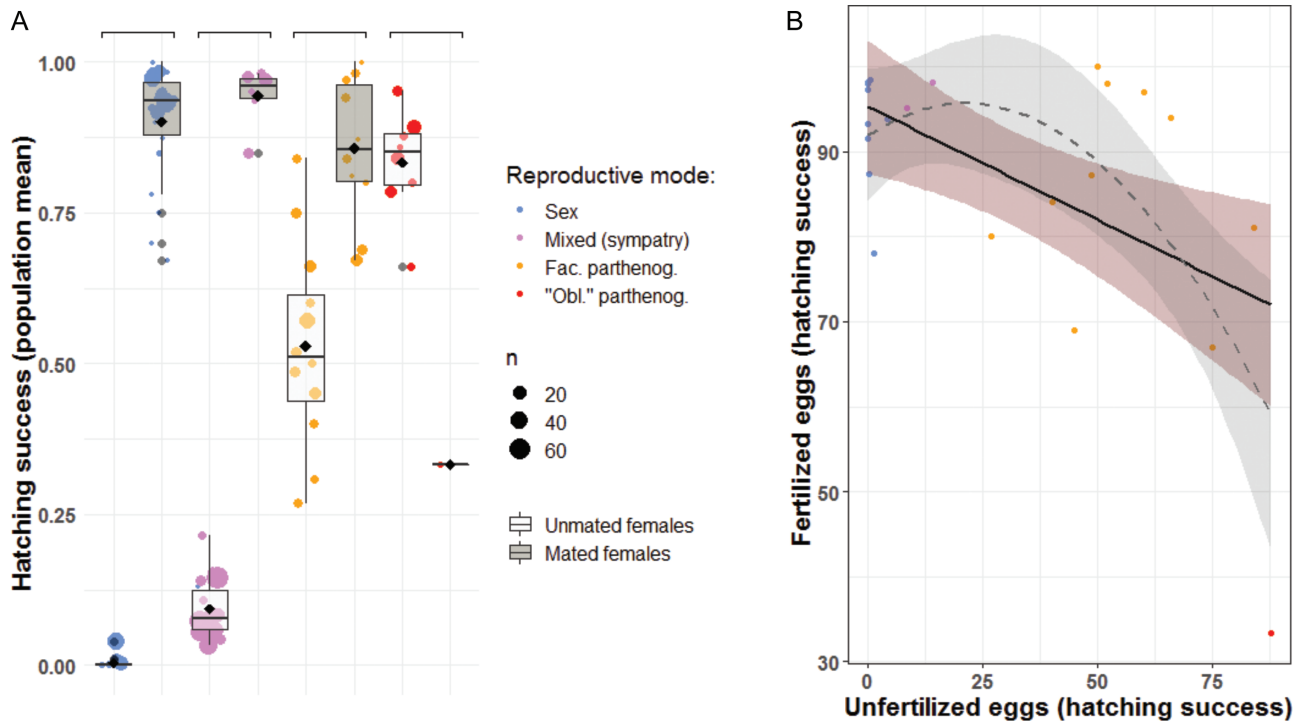


Figure 5. (A) Hatching success of fertilized and unfertilized eggs for species with different reproductive modes and mating status. Data are available for 75 populations from 32 different species, among 8 families (but mostly Baetidae (29.3%) and Heptageniidae (28.0%); see also [Supplementary Figures 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 fertilized and unfertilized eggs were measured. Data are available for 22 populations from 19 species, among 7 families (but mostly Baetidae [45.4%] and Heptageniidae [9.1%]).

to parthenogenesis in vertebrates (Avisé et al. 1992); 2) Contagious origin from preexisting 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 tytoparthenogenesis 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 hybridization 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 haplodiploidy, Table 1, see [Supplementary Appendix](#) for details), further reducing the probability for endosymbiont-induced parthenogenesis.

In mayflies, it has been speculated that facultative and obligate parthenogenesis originates from tytoparthenogenesis (Sweeney and Vannote 1987; Tojo et al. 2006). Although this is a plausible hypothesis given how widespread tytoparthenogenesis is among

mayflies (27.9% of the studied species, Figure 2, see [Supplementary 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 favor 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 Kooij and Schwander 2014). Selection favors the reduction of costly traits, contrary to neutral traits that decay via drift. For example, sexual traits that could decay in parthenogenetic females are 1) pheromones, 2) the capacity to produce males, 3) the ability to fertilize 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 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 personal observation). 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 fertilize 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 2 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 fertilize 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 fertilize eggs is maintained at least at low levels. Viable progeny could be obtained by crossing *Neocloeon alamanca* males (a sexual species with XY male heterogamety) with parthenogenetic *N. triangulifer* females. In such crosses, 66.6% of offspring were normal, clonal *N. triangulifer* females with high fertility, suggesting they were produced parthenogenetically from unfertilized eggs. However, the remaining 33.3% were genetically intermediate between the 2 species (as indicated by allozyme genotypes), suggesting they were hybrids produced from fertilized 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 unfertilized 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 synchronized 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; Glazacow 2001). These findings might indicate that synchronization 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., tycho parthenogenesis). 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 success 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 the benefits of sex in natural populations.

Supplementary Material

Supplementary material is available at *Journal of Heredity* online.

Funding

This work was supported by the Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Swiss National Science Foundation: grant numbers PP00P3_139013 and PP00P3_170627 to T.S.).

Acknowledgments

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 2 anonymous reviewers and the editor for constructive comments.

Data Availability

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

References

- Agrawal AF. 2006. Evolution of sex: why do organisms shuffle their genotypes? *Curr Biol*. 16:R696–R704.
- Avisé JC, Quattro JM, Vrijenhoek RC. 1992. Molecular clones within organismal clones. In: Hecht MK, Wallace B, Macintyre RJ, editors. *Evolutionary biology*, Vol. 26. Boston: Springer. p. 225–246.

- Ashman TL, Bachtrog D, Blackhom H, Goldberg EE, Hahn MW, Kirkpatrick M, Kitano J, Mank JE, Mayrose I, Ming R, et al. 2014. Tree of sex: a database of sexual systems. *Sci Data*. 1:140015.
- Ball SL. 2001. Tychoparthenogenesis and mixed mating in natural populations of the mayfly *Stenonema femoratum*. *Heredity (Edinb)*. 87:373–380.
- Ball SL. 2002. Population variation and ecological correlates of tycho-parthenogenesis in the mayfly, *Stenonema femoratum*. *Biol J Linn Soc*. 75:101–123.
- Barber-James HM, Gattoliat JL, Sartori M, Hubbard MD. 2008. Global diversity of mayflies (Ephemeroptera: Insecta) in freshwater. *Hydrobiologia*. 595:339–350.
- Bauernfeind E, Moog O. 2000. Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity: a methodological approach. *Hydrobiologia*. 422:71–83.
- Bengtsson S. 1913. Undersökningar öfver äggen hos Ephemeriderna. *Entomol Tidskr*. 34:271–320 (+pl. 1–3).
- Bergman E, Hilsenhoff W. 1978. Parthenogenesis in mayfly genus *Baetis* (Ephemeroptera: Baetidae). *Ann Entomol Soc Am*. 71:167–168.
- Bohle VHW. 1969. Untersuchungen über die Embryonalentwicklung und die embryonale Diapause bei *Baetis vernus* (Curtis) und *Baetis rhodani* (Pictet) (Baetidae, Ephemeroptera). *Zool Jahrb Abt Für Anat Ontog Tiere*. 86:493–575.
- Britt NW. 1962. Biology of two species of Lake Erie mayflies, *Ephoron album* (Say) and *Ephemera simulans* Walker. (Ephemeroptera). *Bull Ohio Biol Surv*. 1:1–72.
- Brittain JE, Sartori M. 2009. Chapter 91 - Ephemeroptera: (Mayflies). In: Resh VH, Cardé RT, editors. *Encyclopedia of insects*. 2nd ed. San Diego, CA: Elsevier Academic Press. p. 328–334.
- Burks BD. 1953. The mayflies, or Ephemeroptera, of Illinois. *Bull Ill Natl Hist Surv*. 26:1–216.
- Campbell IC, Hubbard MD. 1998. A new species of *Prosopistoma* (Ephemeroptera: Prosopistomatidae) from Australia. *Aquat Insects*. 20:141–148.
- Carson HL, Wheeler MR, Heed WB. 1957. A parthenogenetic strain of *Drosophila mangabeirai* Malogolowkin. *Genet Drosoph*. 5721:115–122.
- Clemens WA. 1922. A parthenogenetic mayfly (*Ameletus ludens* Needham). *Can Entomol*. 54:77–78.
- Clifford HF, Hamilton H, Killins BA. 1979. Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera: Leptophlebiidae). *Can J Zool*. 57:1026–1045.
- Da-Silva ER. 1993. Descrição do imago macho de *Caenis cuniana* Froehlich, com notas biológicas (Ephemeroptera, Caenidae). *Rev Bras Zool*. 10:413–416.
- Da-Silva ER. 1998. Estratégias de adaptação das espécies de Ephemeroptera às condições ambientais da Restinga de Maricá, Estado do Rio de Janeiro. In: Nessimian JL, Carvalho AL, editors. *Ecologia de insetos aquáticos, Series Oecologia Brasiliensis*, Vol. 5. Rio de Janeiro, Brasil: PPG-UF RJ. p. 29–40.
- Degrange C. 1954. Deux cas de parthénogenèse chez les Ephéméroptères - *Siphonurus aestivalis* Eat. et *Centroptilum luteolum* Müll. *Comptes Rendus Séances Académie Sci*. 239:1082–1083.
- Degrange C. 1955. Nouveaux cas de parthénogenèse chez les Ephéméroptères. *Comptes Rendus Séances Académie Sci*. 241:1860–1861.
- Degrange C. 1956. La parthénogenèse facultative deutérotoque de *Centroptilum luteolum* (Müll.) (Ephéméroptères). *Comptes Rendus Séances Académie Sci*. 243:201–203.
- Degrange C. 1960. Recherches sur la reproduction des Ephéméroptères. *Trav Lab Hydrobiol Piscic Univ Grenoble*. 50–51:7–193.
- Dodds GS. 1923. Mayflies from Colorado. Descriptions of certain species and notes on others. *Trans Am Entomol Soc*. 49:93–114.
- Edmunds GF Jr, McCafferty WP. 1988. The mayfly subimago. *Annu Rev Entomol*. 33:509–529.
- Encalada AC, Peckarsky BL. 2007. A comparative study of the costs of alternative mayfly oviposition behaviors. *Behav Ecol Sociobiol*. 61:1437–1448.
- Fiance S. 1978. Effects of pH on the biology and distribution of *Ephemerella funeralis* (Ephemeroptera). *Oikos*. 31:332–339.
- Fox J, Weisberg S. 2011. *An R companion to applied regression*. 2nd ed. Thousand Oaks, CA: Sage.
- Friesen MK, Flannagan JF. 1976. Parthenogenesis in the burrowing mayfly *Hexagenia rigida* (Ephemeroptera). *Can Entomol*. 108:1295–1295.
- Froehlich CG. 1969. *Caenis cuniana* sp.n., a parthenogenetic mayfly. *Beitr Zur Neotropischen Fauna*. 6:103–108.
- Funk DH, Jackson JK, Sweeney BW. 2006. Taxonomy and genetics of the parthenogenetic mayfly *Centroptilum triangulifer* and its sexual sister *Centroptilum alamanca* (Ephemeroptera: Baetidae). *J North Am Benthol Soc*. 25:417–429.
- Funk DH, Jackson JK, Sweeney BW. 2008. A new parthenogenetic mayfly (Ephemeroptera: Ephemerellidae: *Eurylophella* Tiensuu) oviposits by abdominal bursting in the subimago. *J North Am Benthol Soc*. 27:269–279.
- Funk DH, Sweeney BW, Jackson JK. 2010. Why stream mayflies can reproduce without males but remain bisexual: a case of lost genetic variation. *J North Am Benthol Soc*. 29:1258–1266.
- Gattoliat JL, Sartori M. 2000. *Guloptiloides*: an extraordinary new carnivorous genus of Baetidae (Ephemeroptera). *Aquat Insects*. 22:148–159.
- Gibbs KE. 1977. Evidence for obligatory parthenogenesis and its possible effect on emergence period of *Cloeon triangulifer* (Ephemeroptera: Baetidae). *Can Entomol*. 109:337–340.
- Gibbs KE, Siebenmann M. 1996. Life history attributes of the rare mayfly *Siphonisca aerodromia* Needham (Ephemeroptera: Siphonuridae). *J North Am Benthol Soc*. 15:95–105.
- Giberson DJ, Burian SK, Shouldice M. 2007. Life history of the northern mayfly *Baetis bundyae* in Rankin Inlet, Nunavut, Canada, with updates to the list of mayflies of Nunavut. *Can Entomol*. 139:628–642.
- Gillies MT, Knowles RJ. 1990. Colonization of a parthenogenetic mayfly (Caenidae: Ephemeroptera) from Central Africa. In: Campbell IC, editor. *Mayflies and stoneflies: life histories and biology, Series Entomologica*, Vol. 44. Dordrecht (the Netherlands): Springer. p. 341–345.
- Glazaczow A. 2001. Parthenogenetic and bisexual populations of *Ephemerella notata* Eat. in Poland. In: Domínguez E, editor. *Trends in research in ephemeroptera and plecoptera*. Boston: Springer. p. 227–231.
- Harker JE. 1997. The role of parthenogenesis in the biology of two species of mayfly (Ephemeroptera). *Freshw Biol*. 37:287–297.
- Harper F, Harper PP. 1984. Phenology and distribution of mayflies in a southern Ontario lowland stream. In: Landa V, Soldán T, Tonner M, editors. *Proceedings of the fourth international conference on ephemeroptera*. České Budějovice (Czech Republic): Institute of Entomology, Czechoslovak Academy of Sciences. p. 243–251.
- Harper PP, Harper F. 1982. Mayfly communities in a Laurentian watershed (Insecta; Ephemeroptera). *Can J Zool*. 60:2828–2840.
- Harvey RS, Vannote RL, Sweeney BW. 1980. Life history, developmental processes, and energetics of the burrowing mayfly *Dolania americana*. In: Flannagan JF, Marshall KE, editors. *Advances in Ephemeroptera biology*. New York: Plenum Press. p. 211–230.
- Hirvenoja M. 1964. Studien über die Wasserinsekten in Riihimäki (Südfinland). IV: Ephemeroptera, Odonata, Hemiptera, Lepidoptera und Coleoptera. *Ann Entomol Fenn*. 30:65–93.
- Hofmann C, Sartori M, Thomas A. 1999. Les Ephéméroptères (Ephemeroptera) de la Guadeloupe (petites Antilles françaises). *Mém Soc Vaudoise Sci Nat*. 20:1–96.
- Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biom J*. 50:346–363.
- Huff BL, McCafferty WP. 1974. Parthenogenesis and experimental reproductive biology in four species of the mayfly genus *Stenonema*. *Wasmann J Biol*. 32:247–254.
- Humpesch UH. 1980. Effect of temperature on the hatching time of parthenogenetic eggs of five *Ecdyonurus* spp. and two *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and English rivers and lakes. *J Anim Ecol*. 49:927–937.
- Hunt BP. 1951. Reproduction of the burrowing mayfly, *Hexagenia limbata* (Serville), in Michigan. *Fla Entomol*. 34:59–70.
- Ide FP. 1930. Contribution to the biology of Ontario mayflies with descriptions of new species. *Can Entomol*. 62:204–213, 218–231.

- Jackson JK, Sweeney BW. 1995. Egg and larval development times for 35 species of tropical stream insects from Costa Rica. *J North Am Benthol Soc.* 14:115–130.
- Jalvingh K, Bast J, Schwander T. 2016. Evolution and maintenance of sex. In: Kliman RM, editor. *Encyclopedia of evolutionary biology*. Oxford: Elsevier Academic Press. p. 89–97.
- Jaquiéry J, Stoeckel S, Larose C, Nouhaud P, Rispe C, Mieuze L, Bonhomme J, Mahéo F, Legeai F, Gauthier JP, et al. 2014. Genetic control of contagious asexuality in the pea aphid. *PLoS Genet.* 10:e1004838.
- Jaron KS, Bast J, Ranallo-Benavidez TR, Robinson-Rechavi M, Schwander T. 2018. Genomic features of asexual animals. *bioRxiv.* 497495.
- Jazdzewska T, Wojcieszek A. 1997. *Metreletus balcanicus* (Ulmer, 1920) (Ephemeroptera) in Poland with notes on its ecology and biology. *Pol Pismo Entomol.* 66:9–16.
- Johansson BG, Jones TM. 2007. The role of chemical communication in mate choice. *Biol Rev Camb Philos Soc.* 82:265–289.
- Katayama H. 1939. The sex chromosomes of a may-fly, *Ameletus costalis* Mats. (Ephemera). *Jpn J Genet.* 15:139–144.
- Kazlauskas RS. 1963. New and little-known mayflies (Ephemeroptera) from the fauna of the USSR. *Entomol Obozr.* 42:582–593 (in Russian).
- Kiauta B, Mol AWM. 1977. Behaviour of the spermatocyte chromosomes of the mayfly, *Cloeon dipterum* (Linnaeus, 1761) s.l. (Ephemeroptera: Baetidae) with a note on the cytology of the order. *Genen Phaenen.* 19:31–39.
- Kluge NJ. 1980. To the knowledge of mayflies (Ephemeroptera) of Taimyr National District. *Entomol Obozr.* 59:561–579 (in Russian).
- Knopp M, Cormier R. 1997. *Mayflies: an angler's study of trout water Ephemeroptera*. 1st ed. Helena, MT: Greycliff Publishing Company.
- Kramer MG, Templeton AR. 2001. Life-history changes that accompany the transition from sexual to parthenogenetic reproduction in *Drosophila mercatorum*. *Evolution.* 55:748–761.
- Landolt P, Sartori M, Studemann D. 1997. *Palingenia longicauda* (Ephemeroptera: Palingeniidae): from mating to the larval stage. In: Landolt P, Sartori M, editors. *Ephemeroptera & Plecoptera: biology-ecology-systematics*. Fribourg, Switzerland: MTL. p. 15–20.
- Lehtonen J, Jennions MD, Kokko H. 2012. The many costs of sex. *Trends Ecol Evol.* 27:172–178.
- Lowen RG, Flannagan JF. 1992. Nymphs and imagoes of four North American species of *Proclotron* Bengtsson with description of a new species (Ephemeroptera, Baetidae). *Can Entomol.* 124:97–108.
- Ma WJ, Schwander T. 2017. Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. *J Evol Biol.* 30:868–888.
- Malzacher P, Staniczek AH. 2007. *Caenis vanuatensis*, a new species of mayflies (Ephemeroptera : Caenidae) from Vanuatu. *Aquat Insects.* 29:285–295.
- Martynov AV. 2013. The life cycles of mayflies of the eastern Ukraine. Subfamily *Baetinae* (Ephemeroptera: Baetidae). *Vestn Zool.* 47:35–44.
- McCafferty WP, Huff BL Jr. 1974. Parthenogenesis in the mayfly *Stenonema ferroratum* (Say) (Ephemeroptera: Heptageniidae). *Entomol News.* 85:76–80.
- McCafferty WP, Morihara DK. 1979. The male of *Baetis macdunnoughi* Ide and notes on parthenogenetic populations within *Baetis* (Ephemeroptera: Baetidae). *Entomol News.* 90:26–28.
- McDunnough J. 1925. The Ephemeroptera of Covey Hill, Que. *Trans R Soc Can.* 19:207–223.
- McDunnough J. 1936. A new Arctic baetid (Ephemeroptera). *Can Entomol.* 68:33–34.
- Mingo TM. 1978. Parthenogenesis in the mayfly *Stenacron interpunctatum frontale* (Burks) (Ephemeroptera: Heptageniidae). *Entomol News.* 89:46–50.
- Mol AWM. 1978. Notes on the chromosomes of some West European Ephemeroptera. *Chromosome Inf Serv.* 24:10–12.
- Morgan AH. 1911. May-flies of Fall Creek. *Ann Entomol Soc Am.* 4:93–119.
- Neave F. 1932. A study of the May flies (Hexagenia) of Lake Winnipeg. *Contrib Can Biol Fish.* 7:177–201.
- Needham JG. 1924. The male of the parthenogenetic May-fly, *Ameletus ludens*. *Psyche (Stuttg).* 31:308–310.
- Ogden TH, Breinholt JW, Bybee SM, Miller DB, Sartori M, Shiozawa D, Whiting MF. 2019. Mayfly phylogenomics: Initial evaluation of anchored hybrid enrichment data for the order Ephemeroptera. In: Salles FF, Sobrinho TG, editors. *Proceedings of the joint meeting of the XV International Conference on Ephemeroptera and XIX International Symposium on Plecoptera*. Zoosymposia, Vol. 16. Aracruz, Brazil. p. 167–181.
- Ogden TH, Gattolliat JL, Sartori M, Staniczek AH, Soldán T, Whiting MF. 2009. Towards a new paradigm in mayfly phylogeny (Ephemeroptera): combined analysis of morphological and molecular data. *Syst Entomol.* 34:616–634.
- Otto SP. 2009. The evolutionary enigma of sex. *Am Nat.* 174(Suppl 1):S1–S14.
- Pescador ML. 1973. The ecology and life history of *Baetisca rogersi* Berner (Ephemeroptera: Baetiscidae). In: Peters WL, Peters JG, editors. *Proceedings of the first international conference on Ephemeroptera, Florida, A&M Univ., 17–20 August 1970*. Leiden (the Netherlands): Brill. p. 211–215.
- Pescador ML, Peters WL. 1974. The life history and ecology of *Baetisca rogersi* Berner (Ephemeroptera: Baetiscidae). *Bull Fla State Mus Biol Sci.* 17:151–209.
- Peters WL, Peters JG. 1977. Adult life and emergence of *Dolania americana* in northwestern Florida (Ephemeroptera: Behningiidae). *Int Rev Gesamten Hydrobiol.* 62:409–438.
- R Development Core Team. 2017. *R: a language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing.
- Reding JP. 2006. Notes faunistiques sur *Metreletus balcanicus* (Insecta: Ephemeroptera) et *Ironoquia dubia* (Insecta: Trichoptera), deux espèces d'insectes aquatiques du Jura nouvelles pour la Suisse. *Bull. Soc. Neuchl. Sci. Nat.* 129:73–86.
- Ross L, Hardy NB, Okusu A, Normark BB. 2013. Large population size predicts the distribution of asexuality in scale insects. *Evolution.* 67:196–206.
- Salas M, Dudgeon D. 1999. Parthenogenesis in some Hong Kong mayflies (Ephemeroptera). *Mem Hong Kong Nat Hist Soc.* 22:165–169.
- Sartori M, Brittain JE. 2015. Chapter 34 - Order Ephemeroptera. In: Thorp JH, Rogers DC, editors. *Ecology and general biology: Thorp and Covich's Freshwater Invertebrates*, Vol. 1. Boston: Elsevier Academic Press. p. 873–891.
- Savage HM. 1986. Systematics of the *Terpides* lineage from the Neotropics: Definition of the *Terpides* lineage, methods, and revision of *Fittkaulus* Savage & Peters. *Spixiana.* 9:255–270.
- Schurko AM, Neiman M, Logsdon JM Jr. 2009. Signs of sex: what we know and how we know it. *Trends Ecol Evol.* 24:208–217.
- Schwander T, Crespi BJ. 2009. Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in Timema stick insects. *Evolution.* 63:84–103.
- Schwander T, Crespi BJ, Gries R, Gries G. 2013. Neutral and selection-driven decay of sexual traits in asexual stick insects. *Proc Biol Sci.* 280:20130823.
- Schwander T, Vuilleumier S, Dubman J, Crespi BJ. 2010. Positive feedback in the transition from sexual reproduction to parthenogenesis. *Proc Biol Sci.* 277:1435–1442.
- Sekiné K, Hayashi F, Tojo K. 2013. Phylogeography of the East Asian polymitarcyid mayfly genus *Ephoron* (Ephemeroptera: Polymitarcyidae): a comparative analysis of molecular and ecological characteristics. *Biol J Linn Soc.* 109:181–202.
- Sekiné K, Tojo K. 2010a. Potential for parthenogenesis of virgin females in a bisexual population of the geographically parthenogenetic mayfly *Ephoron shigae* (Insecta: Ephemeroptera, Polymitarcyidae). *Biol J Linn Soc.* 99:326–334.
- Sekiné K, Tojo K. 2010b. Automictic parthenogenesis of a geographically parthenogenetic mayfly, *Ephoron shigae* (Insecta: Ephemeroptera, Polymitarcyidae). *Biol J Linn Soc.* 99:335–343.
- Sekiné K, Tojo K, Bae YJ. 2015. Facultative parthenogenesis in the burrowing mayfly, *Ephoron eophilum* (Ephemeroptera: Polymitarcyidae) with an extremely short alate stage. *Eur J Entomol.* 112:606–612.
- Simon JC, Delmotte F, Rispe C, Crease T. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biol J Linn Soc.* 79: 151–163.

- Sivaramakrishnan KG, Sridhar S, Rajarajan PA. 1991. Effect of temperature on hatching of parthenogenetic eggs of *Baetis geminatus* Müller-Liebenan & Hubbard, 1985 from south India (Ephemeroptera; Baetidae). *Opusc Zool Flum.* 69:1–8.
- Soldan T, Putz M. 2000. Karyotypes of some Central European mayflies (Ephemeroptera) and their contribution to phylogeny of the order. *Acta Soc Zool Bohem.* 64:437–445.
- Suomalainen E, Saura A, Lokki J. 1987. *Cytology and evolution in parthenogenesis*. Boca Raton (FL): CRC Press.
- Sweeney BW, Funk DH, Standley LJ. 1993. Use of the stream mayfly *Cloeon triangulifer* as a bioassay organism: life-history response and body burden following exposure to technical chlordane. *Environ Toxicol Chem.* 12:115–125.
- Sweeney BW, Vannote RL. 1982. Population synchrony in mayflies: a predator satiation hypothesis. *Evolution.* 36:810–821.
- Sweeney BW, Vannote RL. 1984. Influence of food quality and temperature on life-history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. *Freshw Biol.* 14:621–630.
- Sweeney BW, Vannote RL. 1987. Geographic parthenogenesis in the stream mayfly *Eurylophella funeralis* in eastern North America. *Holarct Ecol.* 10:52–59.
- Tabata J, Ichiki RT, Moromizato C, Mori K. 2017. Sex pheromone of a coccoid insect with sexual and asexual lineages: fate of an ancestrally essential sexual signal in parthenogenetic females. *J R Soc Interface.* 14:20170027.
- Takenaka M, Sekiné K, Tojo K. 2019. The first establishment of “hand-pairing” cross-breeding method for the most ancestral wing acquired insect group. *Zool Sci.* 36:136–140.
- Thomforde LL, Fremling CR. 1968. Synchronous emergence of *Hexagenia bilineata* mayflies in the laboratory. *Ann Entomol Soc Am.* 61:1235–1239.
- Tilquin A, Kokko H. 2016. What does the geography of parthenogenesis teach us about sex? *Philos Trans R Soc B Biol Sci.* 371:20150538.
- Tjønneland A. 1960. The flight activity of mayflies as expressed in some East African species. *Årb Univ Bergen Mat-Naturv Ser.* 1:1–88.
- Tjønneland A. 1970. A possible effect of obligatory parthenogenesis on the flight activity of some tropical larvo-aquatic insects. *Årb Univ Bergen Mat-Naturv Ser.* 3:1–7.
- Tojo K, Sekiné K, Matsumoto A. 2006. Reproductive mode of the geographic parthenogenetic mayfly *Ephoron shigae*, with findings from some new localities (Insecta: Ephemeroptera, Polymitarcyidae). *Limnology.* 7:31–39.
- Traver JR. 1932. Mayflies of North Carolina. *J Elisha Mitchell Sci Soc.* 47:85–161 (+pl. 5–12), 163–236.
- Uéno M. 1966. Mayflies (Ephemeroptera) collected by the Kyoto University Pamir-Hindukush Expedition 1960. *Results Kyoto Univ. Sci. Exped. Karakoram and Hindukush, 1955,* 8:299–326.
- van der Kooij CJ, Matthey-Doret C, Schwander T. 2017. Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. *Evol Lett.* 1:304–316.
- van der Kooij CJ, Schwander T. 2014. On the fate of sexual traits under asexuality. *Biol Rev Camb Philos Soc.* 89:805–819.
- Vandel A. 1928. La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bull Biol Fr Belg.* 62:164–281.
- Venables WN, Ripley BD. 2002. *Modern applied statistics with S*. 4th ed. New York: Springer.
- Vrijenhoek RC. 1998. Animal clones and diversity: are natural clones generalists or specialists? *BioScience.* 48:617–628.
- Watanabe NC, Ishiwata SI. 1997. Geographic distribution of the mayfly, *Ephoron shigae* in Japan, with evidence of geographic parthenogenesis (Insecta: Ephemeroptera: Polymitarcyidae). *Jpn J Limnol.* 58:15–25.
- Webb JM, Jacobus LM, Funk DH, Zhou X, Kondratieff B, Geraci CJ, DeWalt RE, Baird DJ, Richard B, Phillips I, et al. 2012. A DNA barcode library for North American Ephemeroptera: progress and prospects. *PLoS One.* 7:e38063.
- White MJD. 1973. *Animal cytology and evolution*. 3rd ed. London: Cambridge University Press.
- Wolf E. 1946. Chromosomenuntersuchungen an insekten. *Z Für Naturforschung.* 1:108–109.
- Wolf E. 1960. Zur karyologie der eireifung und furchung bei *Cloeon dipterum* L. (Bengtsson) (Ephemerida, Baetidae) *Biol Zbl.* 79:153–198.
- Xu S, Spitze K, Ackerman MS, Ye Z, Bright L, Keith N, Jackson CE, Shaw JR, Lynch M. 2015. Hybridization and the origin of contagious asexuality in *Daphnia pulex*. *Mol Biol Evol.* 32:3215–3225.