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Authors: Funk, David H., Sweeney, Bernard W., and Jackson, John K.

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ROSEMARY MACKAY FUND ARTICLE

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In this 7th article of the series, D. H. Funk, B. W. Sweeney, and J. K. Jackson argue that facultative parthenogenesis occurs as a widespread adaptation in mayflies, giving unmated or incompletely fertilized females the ability to reproduce at the price of reduced genetic variability in their offspring. Authors are all at the Stroud Water Research Center: David H. Funk is Entomologist and Director of Facilities, Bernard W. Sweeney is Director, President, and Senior Research Scientist, and John K. Jackson is Senior Research Scientist. The 3 share a common research interest in the ecology, genetics, and taxonomy of temperate and tropical aquatic insects and their role in various aspects of water-quality management.

Why stream mayflies can reproduce without males but remain bisexual: a case of lost genetic variation

David H. Funk[1], Bernard W. Sweeney[2], AND John K. Jackson[3]

Stroud Water Research Center, 970 Spencer Road, Avondale, Pennsylvania 19311 USA

Abstract. Mayflies (Ephemeroptera) are important to the food web of most stream, river, and lake ecosystems and are critical to water-quality monitoring programs. They are widespread and ancient (predate dinosaurs) and have primitive reproductive systems and the shortest adult life spans of all insects. Here we formulate and test the hypothesis that facultative parthenogenesis occurs as a widespread adaptation in most, if not all, mayflies. A rare form of reproduction, facultative parthenogenesis combines the short-term advantages of parthenogenesis, which doubles reproductive output, with the long-term advantages of genetic variation associated with sex. For 7 species of bisexual mayflies, we show that their eggs hatched whether fertilized or not and that unfertilized eggs took longer to develop and hatch. However, once hatched, larvae produced via parthenogenesis grew and developed to the adult in a manner similar to that of larvae produced sexually. In addition, for all study species, female adults produced parthenogenetically were diploid and could reproduce either sexually (producing males and females equally) or parthenogenetically (producing mostly females and some males). Males produced parthenogenetically were viable and occurred either as 1st generation (F1) offspring or as offspring of F1 gynandromorphs (intersex individuals). Last, we show that parthenogenetic descendants lose ~10 to 22% of their genetic variation as a result of each parthenogenetic generation. We use these findings and observations and other published data to support the hypothesis that most, if not all, mayflies are facultatively parthenogenetic. We propose that this ability increases mayfly reproductive success by giving eggs from unmated or incompletely fertilized females a 2nd chance. We then formulate 3 additional hypotheses: 1) parthenogenetic development of mayfly eggs proceeds automatically soon after emergence but can be and normally is preempted by fertilization, and that this mechanism both helps to compensate for and is enabled by the peculiarities of life history and primitive reproductive biology in this ancient group; 2) that facultative parthenogenesis enhances mayfly dispersal and gene flow because it enables virgin females to transfer genes within and among catchments and to form bisexual populations in new habitat; and 3) that mayflies remain bisexual despite being able to reproduce without males because of lost genetic variation associated with the parthenogenetic process.

Key words: facultative parthenogenesis, mayflies, genetic variation, streams, sexual reproduction, allozyme.

[1] E-mail addresses: dhfunk@stroudcenter.org
[2] sweeney@stroudcenter.org
[3] jkjackson@stroudcenter.org

Mayflies (Ephemeroptera) are important aquatic insects in the food webs of most streams, rivers, and lakes (Edmunds et al. 1976, Brittain 1982) and are a mainstay of water-quality monitoring programs

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(Bauernfeind and Moog 2000) and fly fishermen (Knopp and Cormier 1997) throughout the world. As one of the earliest and most primitive winged insect groups (Grimaldi 2001, Kjer et al. 2006), mayflies are widespread and have persisted for >300 million years (my) (Kukalová-Peck 1985) despite having the most primitive reproductive systems and the shortest adult life spans (Needham et al. 1935) of all Insecta, as well as having other anomalies, such as high sensitivity of larvae to environmental change, nonfeeding adults, and the need to molt as an adult. Here we formulate and test the hypothesis that many, if not all, mayflies are facultatively parthenogenetic and that this widespread adaptation helps explain this paradox. Facultative parthenogenesis is a rare form of reproduction (Templeton 1983, Corley et al. 2001, Matsuura and Kobayashi 2007) that combines the short-term advantage (doubling of reproductive output) of parthenogenesis (Cuellar 1977) with the long-term advantages of genetic variation associated with sex (Maynard Smith 1978).

The phenomenon of parthenogenesis (females produced solely from maternal germ cells), although rare, occurs in all animal groups except mammals and has been documented in most insect orders (White 1973, Suomalainen et al. 1987). In most known cases, parthenogenesis is an either/or condition, i.e., eggs that are able to develop parthenogenetically cannot develop sexually and vice versa. Individual germ cells with the potential to develop either sexually or parthenogenetically into diploid progeny (facultative parthenogenesis) are particularly rare (Templeton 1983). Parthenogenetic production of fertile males from germ cells of noncyclical or nonarrhenotokous parthenogens (White 1973) also is rare.

For mayflies, unisexual (obligately parthenogenetic) populations occur (Sweeney and Vannote 1987) and can represent as high as 14% of the mayfly species in streams of a given small catchment (e.g., 7 of 50 in White Clay Creek, Chester County, Pennsylvania; Funk et al. 2006). In contrast, previous reports have considered the occasional hatching of unfertilized eggs (tychoparthenogenesis) in bisexual mayflies to be anomalous and ''unlikely to be of importance in population dynamics'' (Brittain 1982, p. 122). However, a review of the literature (Appendix) combined with recent unpublished data from our laboratory shows that the level of unfertilized egg hatch is higher for bisexual mayflies (median = 13.5% for 50 species) than for any other insect group (e.g., <0.1% for species of Diptera:*Drosophila*; Templeton 1983). We have observed unfertilized egg hatch in nearly every bisexual mayfly species tested so far in our laboratory (23 of 25 to date). These data and observations form the basis for our hypothesis of facultative parthenogenesis for mayflies.

In this paper, we explore in detail the propensity for individuals of 7 species of bisexual mayflies to produce viable eggs both parthenogenetically and through fertilization. We quantified the viability of those eggs (i.e., rate of embryonic development, hatch success, larval growth and survivorship, adult emergence, adult size and reproductive viability) and sex ratio and level of genetic variation of the offspring. We then repeated the analysis for 2nd and sometimes 3rd generation offspring to evaluate fully whether parthenogenesis is occasional, generational, or facultative; to infer the underlying cytological mechanism for parthenogenesis; and to evaluate its short- and long-term implications for genetic variation at the individual level.

Methods

All 7 test species belong to the mayfly family Baetidae (*Acerpenna macdunnoughi* [Ide 1937], *Acerpenna pygmaea* [Hagen 1861], *Centroptilum alamance* [Traver 1932], *Centroptilum semirufum* McDunnough 1926, *Procloeon fragile* [McDunnough 1923], *Procloeon rivulare* [Traver 1935], and *Pseudocloeon frondale* [McDunnough 1925]). We chose these species because they have short life cycles (4–12 wk) and, thus, were more amenable to laboratory studies spanning multiple generations than univoltine species. We obtained all initial test specimens included here from natural bisexual populations with ~1:1 sex ratios (Table 1). We collected *A. macdunnoughi* from the Neversink River, New York (lat 41.90175°N, long 74.58072°W), *A. pygmaea* from Cheaha Creek, Alabama (lat 32.45649°N, long 85.86919°W), and *C. alamance* from Big Alamance Creek, North Carolina (lat 35.9450°N, long 79.07389°W). We collected the remaining 4 species from White Clay Creek, Pennsylvania (lat 39.86061°N, long 75.78383°W). In the laboratory, we induced mating artificially for each species by aligning male and female genitalia. After mating, females oviposited freely in water-filled jars. Unmated females of these species also oviposited freely, except for *Ps. frondale* whose eggs we dissected (Table 1). Methods for studying egg development and egg hatch success and for rearing larvae to the adult stage are described in detail elsewhere (Sweeney et al. 1993, Funk et al. 2006). For this study, we allowed females to oviposit in 30-mL glass museum jars (Wheaton number 225543; Wheaton, Millville, New Jersey) filled part way with filtered (0.45 μm), sterilized stream water from White Clay Creek (WCC) and incubated at 20°C. Eggs attached themselves to the bottom of the jars, enabling us to decant and enumerate hatched larvae daily. After the cessation of

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TABLE 1. Summary of key sexual, developmental, behavioral, and genetic characteristics for 7 bisexual mayfly species. n = number of individuals tested (for proportion of females in wild populations and heterozygosity loss) or number of clutches (for hatch rates and survivorship).

	Sexual reproduction			Parthenogenetic reproduction			
Species	Proportion of females in wild populations[a] (n)	Hatch rate for fertilized eggs[b] % (n; range)	Survivorship to adult for larvae from fertilized eggs[b] % (n; range)	Oviposition by virgin adult females[b,c]	Hatch rate for unfertilized eggs[b] % (n; range)	Survivorship to adult for larvae from unfertilized eggs[b] % (n; range)	Heterozygosity loss per parthenogenetic generation[b] % (n; no. of loci)
Centroptilum alamance	0.50 (44)	67 (16; 25–90)	49 (15; 20–72)	yes	75 (10; 37–95)	42 (3; 23–62)	22 (98; 6)
Centroptilum semirufum	0.71 (21)	100 (1)	44 (1)	yes	50 (5; 5–75)	30 (3; 20–43)	10 (86; 6)
Procloeon rivulare	0.40 (67)	69 (9; 16–97)	32 (7; 4–79)	yes	45 (14; 11–82)	16 (7; 0–34)	20 (261; 7)
Procloeon fragile	0.41 (12)	84 (6; 70–99)	39 (6; 14–79)	yes	40 (6; 30–57)	32 (4; 14–54)	24 (95; 8)
Acerpenna macdunnoughi	0.56 (34)	97 (4; 95–99)	4 (2; 3–5)	yes	60 (8; 34–83)	6 (6; 3–12)	15 (107; 9)
Acerpenna pygmaea	0.60 (15)	81 (1)	22 (1)	yes	84 (6; 72–95)	7 (6; 1–16)	18 (112; 7)
Pseudocloeon frondale	0.44 (54)	94 (6; 78–99)	58 (2; 51–65)	no	66 (14; 31–92)	38 (6; 29–49)	15 (374; 9)

[a] Field populations
[b] Laboratory populations
[c] When placed on water

hatching, we counted eggs (hatched vs unhatched) under a stereomicroscope. For larval rearing, we transferred 1st-instar larvae to 1.9-L glass canning jars (n = 50 larvae/jar) filled with WCC water and an air stone. We provided fresh algae by adding acrylic plates (70 × 200 mm; 1 mm thick) covered with periphyton (predominantly diatoms) grown in a greenhouse by continuously streaming WCC water over them. We added new plates as needed. We placed rearing jars in a water bath at 20°C with an emergence cage over each jar to collect subimagos. We reared subimagos to the imago in these cages.

All genetics in this paper involved enzyme electrophoresis of adult tissue (see Funk et al. 2006 for methods). We tested the genetic structure of the bisexual field population of *C. alamance*. The population exhibited Hardy–Weinberg equilibrium, insignificant linkage disequilibrium, and heterozygosity levels between 14 and 16%. We did not test any other species for those variables. Loss of heterozygosity (= rate of transition to homozygosity) in parthenogenetically produced progeny was measured as the proportion of individuals homozygous at loci where their mothers were heterozygous, averaged across loci.

Results

The average levels of parthenogenetic hatch among egg clutches of the 7 bisexual species were: *C. alamance* (75%; n = 10 clutches), *C. semirufum* (50%; n = 5), *Pr. fragile* (40%; n = 6), *Pr. rivulare* (45%; n = 14), *A. macdunnoughi* (60%; n = 8), *A. pygmaea* (84%; n = 6), and *Ps. frondale* (66%; n = 14). For all 7 species, eggs from virgin females took about 2× as long to hatch as eggs from mated females (Fig. 1A–G), but overall hatch success in the laboratory was high (40–84%; Table 1). Egg fertilization was confirmed for artificially mated females by showing that offspring reared to the adult stage contained a normal percentage of males and that marker alleles in the offspring were consistent with inheritance from 2 parents. However, eggs hatching after ~15 to 20 d in a given fertilized clutch were interpreted as having developed parthenogenetically because: 1) only females were reared from those hatchlings (e.g., Fig. 1H for *C. alamance*), 2) no paternal alleles were detected, and 3) the time required to hatch matched the time observed for conspecific individuals known to have hatched from unfertilized clutches (Fig. 1A–G).

For each of the 7 species, larvae produced from fertilized clutches had higher average survivorship to the adult stage. However, the range of survivorship values between sexually and parthenogenetically

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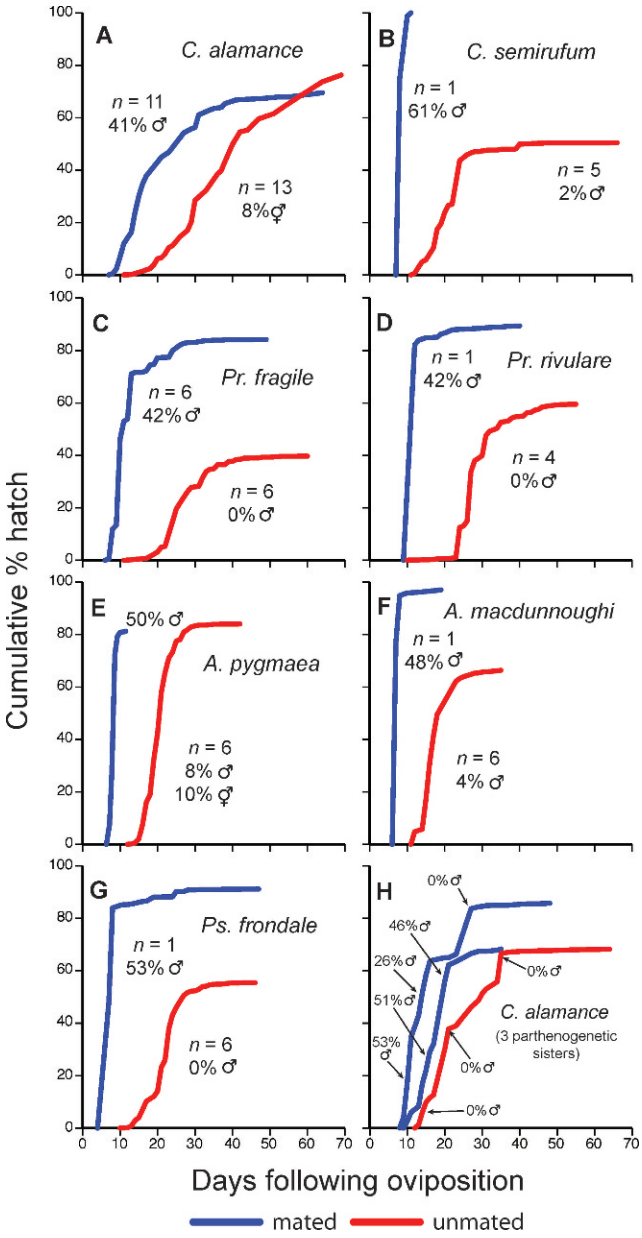


FIG. 1. Timing of hatch and cumulative % hatch success of fertilized and unfertilized eggs obtained from mated and unmated females, respectively, of *Centroptilum alamance* (A), *Centroptilum semirufum* (B), *Procloeon fragile* (C), *Procloeon rivulare* (D), *Acerpenna pygmaea* (E), *Acerpenna macdunnoughi* (F), *Pseudocloeon frondale* (G), and *C. alamance* (H), 7 species of bisexual stream mayflies. Lines in panels A–G represent means for *n* individual egg clutches and in panel H represent individual clutches from 3 parthenogenetic sisters, 2 fertilized and 1 unfertilized (see text), % ♂ = percentage of hatched larvae that were males out of total hatched, % ♂ with long arrow pointing to a curve in panel H = percentage of males for eggs hatching at that point in time in the experiment, % ♀ = percentage of hatched larvae that were gynandromorphs (intersexes).

produced larvae overlapped broadly when *n*-values were >1 clutch (Table 1). Differences among species are probably an artifact of rearing methods used. The 2 *Acerpenna* species typically are found in areas of moderate to high current velocity, and survivorship was relatively low in these species. *Centroptilum*, *Procloeon*, and *Pseudocloeon* species are found in pool and edge habitats where flow is more similar to that experienced in our rearing jars, and survivorship was high in these species. Larval development time (i.e., egg hatch to adult) was the same for fertilized and unfertilized eggs of *Ps. frondale* at 18.5°C (fertilized: 31.1 d, $n = 2$ clutches, range 30.5–31.6 d; unfertilized: 31.2 d, $n = 10$ clutches, range 29.3–32.4 d), but comparable data are not available for the other 6 test species.

The females tested above were collected from wild populations with sex ratios ~1:1, but we could not be certain whether those individuals were progeny of sexual or parthenogenetic reproduction. We were able to conduct experiments to confirm our results with 1 species (*C. alamance*) by using offspring of known origin. Thus, we mated parthenogenetically produced sisters with males and left others to oviposit as virgins. Results confirmed the longer development time for unfertilized eggs (relative to fertilized) and showed conclusively that parthenogenetically produced female mayflies can mate and have their genes re-enter the sexual population pool from which their parents arose (Fig. 1H). In addition, we found that *C. alamance* mothers who were heterozygous at 6 loci produced both heterozygous and homozygous (for each of the maternal alleles) offspring, with ~22% loss in heterozygosity in the F1 generation. We then confirmed and quantified the % lost heterozygosity in the F1 generation with the other 6 species as follows: *C. semirufum* (10%), *Pr. rivulare* (20%), *Pr. fragile* (24%), *A. macdunnoughi* (15%), *A. pygmaea* (18%), and *Ps. frondale* (15%) (Table 1).

Males can be and are produced when a parthenogenetically produced female mates, but we also observed that, occasionally, males can be directly produced parthenogenetically. Thus, males can arise either as F1 offspring or later as parthenogenetic offspring of an F1 gynandromorph (a part male/part female individual produced parthenogenetically; Fig. 2). To date, gynandromorphs, which were reported in the past as anomalous specimens with no known function (Grant and Masteller 1987), have been produced parthenogenetically in our laboratory for 3 of the 7 test species (*C. alamance*, *C. semirufum*, and *A. pygmaea*). Gynandromorphs have low fecundity, but a high proportion of eggs can and did develop parthenogenetically into males (e.g., 78% in *C. alamance*). Moreover, we

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FIG. 2. Photograph of an adult mayfly gynandromorph (intersex) of *Centroptilum alamance* showing the presence of both male and female morphological characters. The left eye (red) is male in size and shape, whereas the right eye is female. A male clasping appendage (forcep) is evident on the right side at the end of the abdomen but absent (as in females) on the left side.

produced bisexual offspring by mating a male produced parthenogenetically from a *C. alamance* gynandromorph with a female produced parthenogenetically (but not from a gynandromorph) and confirmed the biparental inheritance by genetic analysis using several marker loci. In addition, we reared males from eggs taken from parthenogenetically produced gynandromorphs of *C. semirufum* and *A. pygmaea*. For *A. pygmaea*, we also produced bisexual offspring by mating a parthenogenetically produced F1 male with a parthenogenetically produced female. Thus, males produced via parthenogenesis, while infrequent, were viable and fertile.

Discussion

We confirmed that parthenogenesis is widespread in mayflies and showed for 7 species of bisexual mayflies that eggs can and do hatch whether fertilized or not and that parthenogenetically produced offspring are diploid and develop normally into adults. For all bisexual study species, parthenogenetic eggs took longer to hatch, even when they were part of a partially fertilized clutch. In fact, the delayed hatch time for parthenogenetic eggs in a partially fertilized clutch matched the time observed for conspecific individuals known to have hatched from completely unfertilized clutches. This result suggests that the longer egg-development time for unfertilized eggs might be adaptive and probably results because parthenogenetic development is somehow suppressed initially until after the short period when mating and fertilization can occur. Even though egg development of parthenogenetic eggs is delayed, subsequent larval development, adult emergence, and viability appears to be the same as that of fertilized eggs. Thus, our results indicate that female adults produced via parthenogenesis can themselves reproduce parthenogenetically (resulting in mostly female offspring but also an occasional male or gynandromorph) or sexually (producing male and female offspring in about equal numbers).

These data collectively show, for the first time, that parthenogenesis in these 7 bisexual mayflies is facultative. As noted earlier, our 7 test species were chosen for detailed laboratory studies described here because of their short generation times. We acknowledge that they are all from 1 family of mayflies (Baetidae). However, they were chosen from a larger group of bisexual mayflies that we had screened and knew produced offspring parthenogenetically. This larger group included 9 additional species representing 5 other mayfly families (Ameletidae: *Ameletus cryptostimulus* Carle 1978; Heptageniidae: *Maccaffertium modestum* [Banks 1910], *Maccaffertium integrum* [McDunnough 1924]; Ephemeridae: *Hexagenia limbata* [Serville 1829], *Hexagenia rigida* McDunnough 1924; Ephemerellidae: *Ephemerella invaria* [Walker 1853], *Eurylophella enoensis* Funk 1994, *Eurylophella doris* [Traver 1934]; Leptophlebiidae: *Paraleptophlebia debilis* [Walker 1853]). All of these species have significantly longer generation times than our 7 test species (most with at least a 1-y life cycle and, in some cases, a prolonged egg diapause) making full-life-cycle laboratory studies long and difficult. It is important to note that parthenogenetic reproduction has been observed at some level in almost every species of bisexual mayfly where it has been tested for carefully (either in our laboratory or others). Thus, the test data presented here, in combination with observations regarding parthenogenesis on other bisexual mayflies in the literature and our laboratory, provide strong support for our main hypothesis that most, if not all, bisexual mayflies are facultatively parthenogenetic.

From an ecological and evolutionary perspective, these findings are important because they demonstrate that facultative parthenogenesis in mayflies can and does provide a mechanism for the genes of parthenogenetically produced offspring to re-enter the sexual population pool from which their parents arose. These observations lead to 2 additional hypotheses: that the mechanism of facultative parthenogenesis found in mayflies: 1) helps to compensate for and is to some degree enabled by the peculiarities of life history and primitive reproductive biology in this

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ancient group, and 2) enhances mayfly dispersal and gene flow because it enables virgin females to transfer genes within and among catchments and to form bisexual populations in new habitat. In terms of reproduction, mayflies have several unusual characteristics that can compromise reproductive success (Needham et al. 1935). For example, mayflies, unlike all other insects, molt again following metamorphosis, and the adults are generally extremely short-lived (<24 h), cannot feed, and are on the wing only during a brief (few weeks) mating season. Mayfly mating occurs only in association with male swarms that typically last <1 h each day of the seasonal reproductive period and copulation usually occurs once, in flight, and is brief (lasting seconds to <1 min). Mayflies have the most primitive ovaries (panoistic) among insects and lack the spermatheca used by most other insects to store and distribute sperm efficiently. Last, in the species studied here, fertilization occurs en masse within the oviducts, and eggs are laid just after copulation (DHF, unpublished data). Some of these characteristics can be advantageous (e.g., short/synchronous adult emergence period satiates predators; Sweeney and Vannote 1982), but collectively, they seem to increase the possibility of female mayflies laying unfertilized or partially fertilized clutches. Hence, we propose that facultative parthenogenesis is an adaptation that provides a 2nd chance for unfertilized eggs (because of incomplete or absent fertilization) and gives mayflies the known reproductive and dispersal advantages of cyclical parthenogenesis in animals (White 1973), albeit at an intra- rather than intergenerational level. The advantages afforded mayflies by the mechanism described here, whereby parthenogenetic development proceeds automatically unless preempted by fertilization, might depend on the existence of a predictably brief temporal window for fertilization. Animals that have longer-lived adults with less predictable reproductive timing might be at a selective disadvantage should parthenogenetic development proceed in eggs that could still be fertilized. Thus, we predict that facultative parthenogenesis should occur at a lower frequency in the few mayfly species known to delay oviposition. We have some evidence that this might be the case. Two ovoviviparous species we have tested, *Callibaetis fluctuans* (Walsh) and *Cloeon cognatum* Stephens (the latter often treated as a synonym of *Cloeon dipterum* [Linnaeus]), in which oviposition does not occur until 7 to 9 d or 17 to 19 d, respectively, after females molt to the imago, showed no evidence of parthenogenetic development in eggs from unmated females (but see Harker 1997 for *Cloeon dipterum* [Linnaeus]).

Facultative parthenogenesis also should enhance mayfly dispersal because it can lead to gene flow without the dispersal of either males or inseminated females. This supposition is based, of course, on the assumption that the parthenogenetically produced offspring of a virgin colonist are able to synchronize the timing of their reproduction with male conspecifics of the newly colonized habitat. Such synchronization seems probable, even for long-distance dispersal, based on previous experiments (Sweeney et al. 1992) that showed that eggs collected from the northern or southern limits of a mayfly's range and reared under conditions (light regime, temperature, food) associated with the middle of the range produced adults fully synchronized with the local population after 1 generation. Thus, our observations showing that facultative parthenogenesis allows unmated females to reproduce and can and does involve the production of occasional fertile males and gynandromorphs provides a good basis for our hypothesis that facultative parthenogenesis enhances mayfly dispersal and gene flow.

Although facultative parthenogenesis combines the short-term advantages of parthenogenesis, which doubles reproductive output, with the long-term advantages of genetic variation associated with sex, our study shows that there appears to be a significant cost, i.e., a consistent loss of genetic variation with each parthenogenetic generation. The observed rates of lost genetic variation for 7 bisexual species (mean: 17.7%, range: 10–24%) strongly suggest that the underlying cytological mechanism for facultative parthenogenesis in mayflies is automixis (via central fusion) with recombination (caused by crossing over), which is expected to result in a loss of 0–33% depending on the proximity of a given locus to the centromere (Pearcy et al. 2006). In contrast, mayflies known to be obligately parthenogenetic, such as *Centroptilum triangulifer* (Funk et al. 2006) and certain populations of *Centroptilum minor*, *Acerpenna macdunnoughi*, *Procloeon viridoculare*, *Ameletus ludens*, and *Ephemera varia* (DHF, unpublished data), are clonal with the genetic variation of a given clone being stable over many generations. This pattern indicates that a different mechanism of parthenogenesis occurs in these species (either apomixis [i.e., ameiotic] or some form of automixis without recombination; Funk et al. 2006). Our data suggest that individuals produced via facultative parthenogenesis need to mate within a few generations to remain viable because an average loss of genetic variation of 17.7% per generation compounded over only 3 generations results in a total loss of ~56%. For habitat with established bisexual populations where males are abundant, the prospect

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of mating is good. For small populations or new populations founded by a virgin female, the prospects might not be good because the arrival of males from local bisexual populations might be slow and uncertain given the limitations imposed by a short (<24 h) adult life span on adult dispersal and swarming. Given that lost heterozygosity has been equated with reduced fitness (Ricklefs and Miller 1999, Matsuura et al. 2004), the reproductive advantage of facultative parthenogenesis appears to be of limited duration in mayflies. These observations lead to our last hypothesis that the relatively significant and rapid loss of genetic variation is the principal reason that most mayflies remain bisexual despite being able to reproduce without males (i.e., strong selection pressure exists for facultatively parthenogenetic females to seek mates). This hypothesis is supported by the fact that 7 of 7 bisexual mayfly species tested lost heterozygosity with each parthenogenetic generation, whereas the levels of heterozygosity remained constant from 1 generation to the next in 5 of 5 unisexual mayfly species.

We propose that these novel data and observations provide a theoretical framework that helps explain: 1) the lack of skewed sex ratios for mayflies in nature despite the widespread occurrence of facultative parthenogenesis (viz., parthenogenetic offspring either mate and quickly rejoin the bisexual gene pool or go extinct), 2) how facultatively parthenogenetic mayflies might evolve to become obligately parthenogenetic (viz., eliminate recombination), 3) why unisexual mayfly species are not more common (viz., eliminating recombination is nontrivial), 4) why gynandromorphs persist at low frequencies in natural mayfly populations (viz., a symptom of automictic heterozygosity loss at sex-determining loci), and 5) why some mayfly studies (Hughes et al. 2000) indicate widespread dispersal and gene flow across catchments yet fine-scale population structure within a catchment (viz., local population structure is altered by ongoing unrecognized parthenogenesis). Moreover, the results suggest that genetic recombination plays a dual role in mayflies, both enhancing genetic diversity at the population level but reducing genetic diversity in offspring produced via facultative parthenogenesis.

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APPENDIX. Literature values for the occasional hatching of unfertilized eggs (tychoparthenogenesis) in 44 mayfly species. Average percentage refers to the average proportion of eggs that hatched unfertilized for each species.

Family	Species	Average percentage	Reference
Ameletidae	*Ameletus cryptostimulus* Carle 1978	0.1	Sweeney and Vannote 1987
	Ameletus tertius McDunnough 1938	20.0	Sweeney and Vannote 1987
Baetidae	*Acentrella sinaica* Bogoescu 1931	26.3	Degrange 1960
	Baetis niger (Linnaeus 1761)	35.4	Degrange 1960
	Baetis pumilus (Burmeister)	58.7	Degrange 1960
	Baetis scambus Eaton 1870	46.7	Degrange 1960
	Centroptilum litura (Pictet 1843)	90.3	Degrange 1960
	Centroptilum luteolum (Müller 1776)	48.7	Degrange 1960
	Cloeon dipterum (Linnaeus 1761)	39.0	Harker 1977
	Cloeon simile Eaton 1870	28.0	Harker 1977
	Procloeon pennulatum (Eaton 1870)	32.4	Degrange 1960
	Procloeon rivulare (Traver 1935)	0.1	Sweeney and Vannote 1987
	Pseudocloeon frondale (McDunnough 1925)	23.0	Bergman and Hilsenhoff 1978
	Pseudocloeon propinquum (Walsh 1863)	39.5	Bergman and Hilsenhoff 1978
Baetiscidae	*Baetisca rogersi* Berner 1940	1.0	Pescador and Peters 1974
Caenidae	*Caenis luctuosa* (Burmeister 1839)	5.9	Degrange 1960
Ephemerellidae	*Ephemerella dorothea* (Needham 1908)	0.1	Sweeney and Vannote 1987
	Ephemerella mucronata (Bengtsson 1909)	38.4	Degrange 1960
	Eurylophella funeralis (McDunnough 1925)	14.0	Sweeney and Vannote 1987
	Serratella ignita (Poda 1761)	13.5	Degrange 1960
	Torleya major (Klapalek 1905)	1.1	Degrange 1960
Ephemeridae	*Ephemera varia* Eaton 1883	10.0	Sweeney and Vannote 1987
	Hexagenia rigida McDunnough 1924	9.0	Friesen and Flannagan 1976
Heptageniidae	*Ecdyonurus aurantiacus* (Burmeister 1839)	0.3	Degrange 1960
	Ecdyonurus forcipula (Pictet 1843)	0.2	Degrange 1960
	Ecdyonurus insignis (Eaton 1870)	0.3	Degrange 1960
	Electrogena lateralis (Curtis 1834)	4.5	Degrange 1960
	Epeorus alpicola (Eaton 1871)	4.1	Degrange 1960
	Epeorus assimilis Eaton 1885	0.9	Degrange 1960
	Heptagenia sulphurea (Müller 1776)	0.5	Degrange 1960
	Maccaffertium pulchellum (Walsh 1862)	0.4	Huff and McCafferty 1974
	Maccaffertium vicarium (Walker 1853)	1.6	Huff and McCafferty 1974
	Rhithrogena semicolorata (Curtis 1834)	20	Degrange 1960
	Rhithrogena sp.	0.5	Degrange 1960
	Stenacron interpunctatum (Say 1839)	9.1	Mingo 1978
	Stenonema femoratum (Say 1823)	8.4	Huff and McCafferty 1974
Leptophlebiidae	*Habroleptoides modesta* (Hagen 1864)	0.0	Degrange 1960
	Leptophlebia cupida (Say 1823)	13.0	Sweeney and Vannote 1987
	Leptophlebia vespertina (Linnaeus 1758)	2.2	Degrange 1960
Palingeniidae	*Palingenia longicauda* (Olivier 1791)	50.0	Landolt et al. 1977
Polymitarcyidae	*Ephoron album* (Say 1824)	8.0	Britt 1962
Siphlonuridae	*Siphlonisca aerodromia* Needham 1909	90	Gibbs and Siebenmann 1996
	Siphlonurus aestivalis (Eaton 1903)	5.4	Degrange 1960
	Siphlonurus lacustris (Eaton 1870)	18.4	Degrange 1960

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