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Parthenogenesis and Experimental Reproductive Biology in Four Species of the Mayfly Genus *Stenonema*[1]

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INTRODUCTION

The phenomenon of parthenogenesis in mayflies in North America has received little attention in the past. The present state of knowledge was reviewed by McCafferty and Huff (1974) at which time they presented preliminary data indicating the presence of parthenogenesis in a single species of *Stenonema*. The paucity of information on parthenogenesis has obviously been due in part to the inability to manipulate and maintain mayflies in the laboratory. Since parthenogenetic reproduction may have important ramifications in regards to population biology, and since improved experimental techniques would have a broad application for studying many aspects of mayfly biology, we undertook the development of adequate experimental techniques. These techniques have led to the discovery of parthenogenesis in 4 species of *Stenonema*, which are *S. femoratum* (Say), *S. interpunctatum* (Say), *S. pulchellum* (Walsh), and *S. vicarium* (Walker).

METHODS

All experimental organisms were collected in the larval stage from streams in Indiana from March through May, 1973. In the labora-

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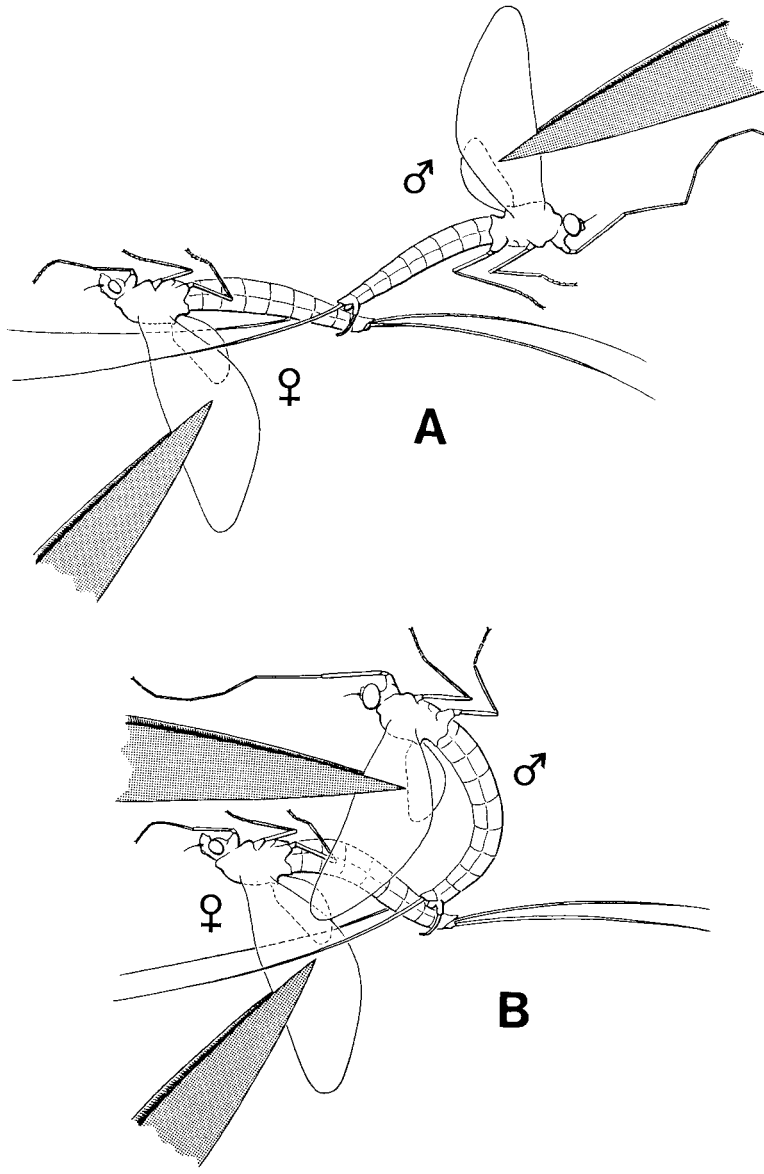

FIGURE 1. Forced copulation of male and female mayflies. A, initiation of coupling and positioning genital forceps; B, bending of male abdomen and final copulatory posture.

tory, female larvae were sorted out and placed into aerated rearing aquaria apart from male larvae. These individuals were acclimatized and maintained at a temperature of between 22 and 24°C. Newly emerged subimagos were immediately removed from the rearing aquaria and placed into individual chambers for final rearing to the adult stage. Last instar larval exuviae were retained with the respective male and female adults for stage association.

Eggs were obtained from both fertilized and unfertilized female adults by 1 of 3 methods. A female would occasionally achieve complete evacuation of the eggs when placed on the surface of the liquid culture medium. More commonly, oviposition was induced by gently bending the last 3 abdominal segments dorsally at nearly a right angle. This would be continued in a rhythmic manner until oviposition began. If these methods failed the abdomen was dissected between the seventh and eighth segments and the eggs were forced from the oviducts by gently squeezing the abdomen from anterior to posterior with a pair of blunt larval forceps.

Fertilized eggs were obtained through forced copulation of males and females reared in our laboratory. This procedure involved grasping the female by the wings with a pair of forceps and placing her in a fixed inverted position. Coupling was then achieved by placing the male against the female so that their external genitalia were directly opposite (fig. 1A). Once the male genital forceps were positioned in the normal clasping position around the abdomen of the female, a slight bending of the male abdomen was required before copulation and insemination would proceed (fig. 1B). Thus, mating was initiated with the individuals facing in opposite directions in an unnatural copulatory posture (Cooke, 1940; Brinck, 1957). To insure maximum fertilization forced copulation was repeated at intervals throughout the oviposition period. It had been observed that females, field captured while mating, commonly had only a small percentage of eggs in the oviducts. Presumably, these females had mated and oviposited previously.

Eggs from individual females were incubated at 18°C. in covered petri dishes containing 15 ml. of sterile 10 percent Steinberg's solution (Carnegie Institute Washington, 1956) buffered with Tris (hydroxymethyl) aminomethane (Sigma Chemical Company) at pH 7.4. Steinberg's solution simulates the ionic balance of surface water by incorporating the chloride, nitrate, and sulphate salts of sodium,

TABLE 1. *Eclosion in* S. femoratum *eggs.*

	Number of eggs incubated	Number of neonate larvae	Eclosion %
Unfertilized 17 females	65,673	5,512	8.39
Fertilized 3 females	10,541	10,029	95.14

potassium, calcium, and magnesium. The problems of salt precipitation and pH change sometimes associated with phosphate and bicarbonate buffers were avoided by using the Tris buffer. Bacterial growth was suppressed by using 5.0, 3.0, and 1.0 microgram per milliliter of streptomycin, penicillin, and garamycin respectively.

Direct egg counts were made under a variable-power swing-arm dissecting microscope with the aid of a standard colony counter grid placed beneath the petri dish.

RESULTS

The results from unfertilized and fertilized eggs of *S. femoratum* are summarized in table 1. Of the unfertilized eggs from 17 virgins, 8.39 percent underwent eclosion. Three fertilized females yielded 95.14 percent hatch (probably reflecting the very small proportion of eggs which were not fertilized in the oviducts). The mean number of eggs from the unfertilized females was 3,863 while the mean for the 3 fertilized females was 3,514.

Figure 2 illustrates the distribution of percent hatch of eggs for the 17 unfertilized females of this species. Average percent hatch in each class interval is given above the bars. Nine of the 17 females possessed less than 5.25 percent parthenogenetic eggs. However, the remaining 8 females yielded higher percentages up to a maximum of 36.50 percent for 1 individual. A correlation coefficient less than

TABLE 2. *Post-oviposition embryonic development in* S. femoratum.

	Minimum	Maximum
Unfertilized eggs	18 days	29 days
Fertilized eggs	12 days	15 days

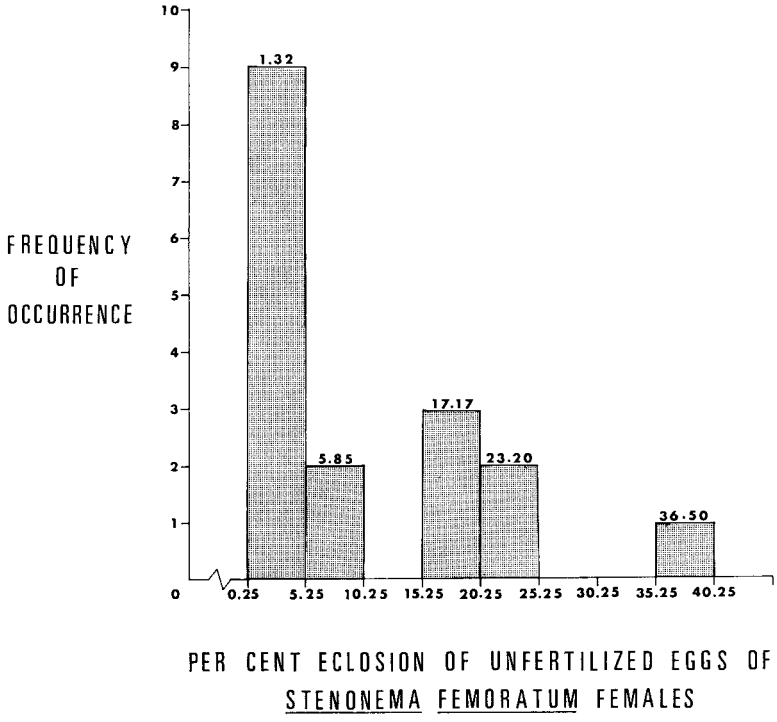

FIGURE 2. Distribution of percent eclosion in unfertilized eggs of *Stenonema femoratum*.

1.0 indicated no relationship between either the mode of obtaining the eggs or the number of eggs obtained from each female and the percent eclosion.

Duration of post-oviposition embryonic development (table 2) ranged from a minimum of 18 days to a maximum of 29 days for unfertilized eggs and from 12 to 15 days for fertilized eggs under identical conditions.

Parthenogenesis in 3 other species of *Stenonema* has also been demonstrated in our laboratory (table 3) using the procedures described above. A single virgin female of *S. vicarium* yielded 1.64 percent parthenogenetic hatch from 3,172 eggs with a post-oviposition embryonic developmental time ranging from 20 to 31 days. Ten eggs of *S. pulchellum* hatched between 14 and 17 days following ovi-

TABLE 3. *Eclosion of unfertilized eggs in* Stenonema.

Species	Number of eggs incubated	Number of eclosions	Post-oviposition embryonic development	Eclosion %
S. vicarium	3,172	52	20–31 days	1.64
1 female *S. pulchellum*	2,483	10	14–17 days	0.40
1 female *S. interpunctatum*	—	1	32 days	—

position from 3,483 eggs incubated from a single unfertilized female of this species. An undetermined number of unfertilized eggs of *S. pulchellum* from 1 virgin female of *S. interpunctatum* yielded 1 neonate larva after 32 days of incubation following oviposition. In addition, several developing eggs present in this and another egg mass from the same species did not hatch. The results for *S. interpunctatum* came from preliminary tests in which eggs were incubated in stream water and subsequently subjected to a rather high concentration of antifungal agents.

DISCUSSION OF METHODS AND RESULTS

Prior to our use of Steinberg's solution as an incubation medium for the eggs we encountered considerable difficulty in maintaining the eggs in viable condition. Eggs incubated in stream water became infected with bacteria and fungi and gave unreliable results on egg development and eclosion. Infection could be reduced somewhat by changing the water periodically but this resulted in some loss of eggs. Although antibiotics used in the Steinberg's solution prevented bacterial infection of the eggs, some fungal infection occurred in a few cultures during the latter part of the incubation period. We would suggest changing the solution periodically to suppress fungal growth in addition to keeping the containers covered to prevent the entrance of spores. Fungicides would appear feasible as control agents if concentrations necessary for fungal control are not toxic to the developing eggs. Our results with *S. interpunctatum* indicate that some fungicides may have an undesirable effect on normal embryonic development and eclosion, however, no toxicity levels have been established.

Modification of the basic formula of Steinberg's solution may be desirable in certain instances. The experimentation involving *S. femoratum* eggs would indicate that techniques employed for incubation and eclosion are adequate for the laboratory assessment of parthenogenesis in this species and very possibly other mayflies as well.

Experimental control was assured by inducing copulation in the laboratory to obtain fertilized eggs for comparative purposes. Degrange (1960) relied primarily on field captured females taken while either copulating or ovipositing in order to obtain fertilized eggs. He experienced a wide range of results in regards to percent eclosion in the species he studied. The methods we have developed for inducing laboratory mating and maintenance of the eggs are equally applicable to the study of embryology, early larval morphology, developmental time or sequences, and egg diapause. Hopefully these methods will help provide researchers with the means for explaining the discontinuities in our knowledge of the life histories of many of the mayfly species.

The rather broad range of eclosion of unfertilized eggs of *S. femoratum* females (0.50–36.50 percent) could not be explained by an analysis of possible experimental influences. This would possibly indicate an hereditary influence on percent eclosion. Duration of post-oviposition embryonic development was consistently longer for the unfertilized eggs than for the fertilized eggs. Degrange (1960) obtained similar results for many of the European mayfly species he studied and determined that the increase in duration of embryonic development of parthenogenetic eggs was due to a delay occurring early in ontogenetic development.

Although the data for *S. vicarium*, *S. pulchellum*, and *S. interpunctatum* are not as extensive as for *S. femoratum*, they nonetheless support the hypothesis that parthenogenetic potential does exist in these species. There still remains, however, the possibility that this potential is never realized in the field. The rearing of successive parthenogenetic generations in the laboratory in order to determine the sex ratio of parthenogones, along with the study of gametic cytology will be necessary before the type and mode of parthenogenesis can be satisfactorily determined for these species. On the basis of field observations McCafferty and Huff (1974) estimated the parthenogenesis to be facultative and deuterotokous.

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