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Population variation and ecological correlates of tychoparthenogenesis in the mayfly, *Stenonema femoratum*

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Species in which both sex and parthenogenesis co-occur are extremely valuable for investigating ecological conditions favouring sex. Tychoparthenogenesis is a breeding system characterized by hatching of a small proportion of unfertilized eggs (typically < 10%) from females of sexually reproducing species. With tychoparthenogenesis, both sexual and parthenogenetic reproduction co-occur within the same population. To identify ecological conditions that may favour this breeding system, I quantified population variation in females' capacity for tychoparthenogenesis and investigated biotic and abiotic correlates of tychoparthenogenesis. I estimated tychoparthenognetic capacity (proportion of unfertilized eggs hatching) for females from 12 Missouri populations of the mayfly, *Stenonema femoratum* (Ephemeroptera: Heptageniidae), across three different habitat types – temporary streams, permanent streams and lakes. Tychoparthenogenetic capacity, measured as the population mean hatch success of unfertilized eggs, ranged from 3.8 to 10.7%. Tychoparthenogenetic capacity varied among habitats in 1996, but not in 1997. In 1996, temporary streams showed hatch success of unfertilized eggs twice that of permanent streams and lakes. Tychoparthenogenetic capacity also varied among sampling dates within years. Temporary streams also showed extremely low nymph densities compared to the other two habitats. However, habitats did not differ in adult density. Furthermore, in all populations nymphs showed significantly female-biased sex ratios. In contrast, adult sex ratios were equal or slightly male biased. Tychoparthenogenetic capacity was negatively correlated with nymph density in 1996, but not in 1997, suggesting possible reproductive assurance in some years. Adult densities also suggested that there may be certain times of year when tychoparthenogenesis may provide benefits of reproductive assurance. Although habitats differed significantly in their abiotic characteristics, tychoparthenogenetic capacity was correlated significantly with water temperature only. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2002, **75**, 101–123.

ADDITIONAL KEYWORDS: abiotic factors – breeding system – dispersal – evolution of sex – parthenogenesis – population density – sex ratio.

INTRODUCTION

Since the discovery of parthenogenesis nearly 300 years ago, biologists have sought to explain why sexual reproduction is the predominant reproductive mode in eukaryotes when asexual reproduction offers a more efficient way of reproducing. Despite the predicted twofold advantage of asexual reproduction due to the production of all-female offspring, paradoxically relatively few organisms reproduce asexually and asexual species appear to be evolutionarily short-lived (Ghiselin 1974; Williams 1975; Maynard Smith 1978; Bell 1982; Michod & Levin 1988; Judson & Normark 1996). All else being equal, asexuals have twice the reproductive rate as sexuals, producing twice as many daughters as sexual females. Sexual females are said to pay a 'twofold cost of sex' due to production of sons. Despite this cost, the predomi-

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nance of sex indicates that it must confer large advantages over the long-term. The primary advantage of sex lies in its ability to produce genetically variable offspring. However, the exact way in which this advantage is realized is unknown and has been the subject of numerous hypotheses to explain the advantages of sex (Kondrashov 1993). To understand how these advantages of sex are manifested, evolutionary biologists have focused their attention on the ecological conditions favouring asexual reproduction. Asexual reproduction is generally found in early successional or marginal habitats that are biotically 'simple', containing few predators, parasites, or competitors (Bell 1982; Bierzychudek 1985).

Species in which both sex and parthenogenesis co-occur are extremely valuable for investigating ecological conditions favouring sex. Tychoparthenogenesis is a little studied, yet relatively common type of parthenogenesis in invertebrates in which females from sexually reproducing species exhibit a small capacity for parthenogenesis (Bell 1982; Templeton 1982; Suomalainen *et al*. 1987). The proportion of unfertilized eggs hatching is typically less than 10% although enormous variation in hatch success among females has been shown (McCafferty & Huff 1974; Templeton 1982; Ball 2000). With tychoparthenogenesis, both sex and parthenogenesis occur within the same population. Population variation in tychoparthenogenetic capacity (i.e. population mean proportion of unfertilized eggs hatching) offers an ideal opportunity to investigate ecological factors associated with different amounts of sexual reproduction. Tychoparthenogenesis offers additional advantages for investigating short-term ecological advantages of sex. Because the hatching success of unfertilized eggs is low, relative to that of fertilized eggs, the predicted twofold advantage of parthenogenesis does not exist with tychoparthenogenesis. This suggests that some other advantage must be responsible for its maintenance. Tychoparthenogenesis also offers several other advantages for testing hypotheses for the maintenance of sex. Both sex and parthenogenesis occur not only within the same species, but also within the same population. Thus, sexuals and parthenogens can be compared without confounding factors related to differences in habitat or phenology. Direct comparisons of sexuals and parthenogens in the same habitat are rare (Joekela *et al*. 1997). Moreover, tychoparthenogenesis is not confounded by hybridity. While all known unisexual vertebrates are of hybrid origin (Cuellar 1974; Vrijenhoek 1989) tychoparthenogens are not (Templeton 1982). Tychoparthenogenesis also offers advantages as a system for studying the evolution and maintenance of sex because of the diversity of genotypic consequences. All known tychoparthenogens are automictic, reproducing via meiotic parthenogenesis (Bell 1982; Suomalainen *et al*. 1987). Different automictic mechanisms can result in different genotypic consequences (White 1973). Therefore, automixis provides an opportunity to examine the consequences of parthenogenesis in different genetic backgrounds.

Despite these advantages, tychoparthenogenesis has been largely ignored by evolutionary biologists. Due to the low hatching success of unfertilized eggs, tychoparthenogenesis has often been referred to as 'accidental' or 'occasional' parthenogenesis (White 1973) and has been dismissed as a type of artificial parthenogenesis that is unimportant to evolution in natural populations (Maynard Smith 1978; Mingo 1978; Brittain 1982). There have been no detailed ecological studies of tychoparthenogens while detailed genetic studies exist for a few tychoparthenogenetic insects (*Drosophila*, Stalker 1951, 1952, 1954; Carson 1961, 1967, 1973; Templeton *et al*. 1976; Templeton 1982; *Bacillus* stick insects, Bullini 1994; *Lonchoptera* flies, Ochman *et al*. 1980; *Locusta migratoria*, Pardo *et al*. 1995). However, these studies have been confined primarily to laboratory investigations.

The purpose of this study was to quantify population variation in tychoparthenogenetic capacity of natural populations of the mayfly, *Stenonema femoratum* (Ephemeroptera: Heptageniidae) and to investigate ecological conditions correlated with this breeding system. Specifically, I examined whether natural populations showed evidence of tychoparthenogenetic reproduction and the extent to which tychoparthenogenetic capacity varied spatially (among habitats and populations) and temporally (between and within years). I also examined biotic and abiotic correlates of tychoparthenogenesis to elucidate the biological and physical conditions under which tychoparthenogenesis occurs and therefore, to identify potential advantages of tychoparthenogenesis.

Species and Study Sites

Parthenogenesis has been documented in 24 North American and 35 European mayfly species (DeGrange 1960; Huff & McCafferty 1974; Friesen & Flannagan 1976; Gibbs 1977; Bergman & Hilsenhoff 1978; Mingo 1978; Humpesch 1980; Sweeney & Vannote 1987; Gillies & Knowles 1990; Harker 1997). In North America, six species are known from obligately parthenogenetic populations, while remaining species have been classified as facultative parthenogens or tychoparthenogens. *Stenonema femoratum* is broadly distributed in North America, ranging from the northeastern United States and Canada to the foothills of the Rocky Mountains and south to Texas (Bednarik & McCafferty 1979). It occupies a wide range of habi-

tats from rocky shoreline of lakes to temporary and permanent streams. In Missouri, *S. femoratum* has at least two generations per year (S. L. Ball, personal observation). Overwintering adult nymphs emerge as early as March. In central Missouri, adults can be found throughout the summer and have been observed as late as November (S. L. Ball, pers. observ.). Unlike most insects, mayflies have retained a subadult (subimago) stage. Subimagoes emerge from their nymphal exuvia and spend up to 24 h resting on vegetation near water before they molt into the final adult (imago) stage. Imago lifespan is brief, lasting ~24–48 h although occasionally female imagoes have been kept alive in the laboratory for up to 7 days (S. L. Ball, pers. observ.). Subimagoes carry mature gametes but, in most species, are unable to mate because the external genitalia are not fully developed (Edmunds & McCafferty 1988). Subimagoes are easily distinguished from imagoes by their dull, grey appearance and translucent wings. Unlike many mayfly species, *S. femoratum* has asynchronous emergence which results in relatively small mating swarms of ~5–100 males. Male imagoes form mating swarms in late afternoon or early evening, flying 10–50 cm above the water surface. Female imagoes join these mating swarms 5–15 min later and copulation, which lasts ~30 s, takes place in flight. After copulating, females immediately lay eggs by flying a few cm above the water surface and dipping the tip of their abdomen into the water repeatedly, each time releasing a small numbers of eggs.

This study was conducted from 1996 to 1999 at 12 sites across central and southern Missouri (Table 1). Sites (hereafter referred to as populations) were classified into one of three habitat types: lakes, permanent streams which flow all year, and temporary streams which dry in late summer forming small, isolated pools. Streams were classified as temporary if the entire width of the stream bed was completely dry by late August, at a minimum of two locations along the streambed, separated by at least 50 m. A total of three temporary streams, four permanent streams, and five lakes were sampled. Each lake or stream was divided into a series of transect points 50 m apart, with up to ten transect points per lake or stream. The number of transect points depended on the amount of rocky shoreline or stream reach that could be waded safely, with three to eight transect points sampled in lakes and 7–10 transect points in streams. The same transect points were sampled each season and year. However, for three sites fewer transect points were sampled on one occasion.

METHODS

Evidence of tychoparthenogenetic reproduction

To determine whether natural populations showed evidence of tychoparthenogenetic reproduction, I measured sex ratios of nymphs, imagoes (adults) and subimagoes (subadults). With tychoparthenogenetic reproduction, populations are expected to exhibit female biased sex ratios. To estimate nymph sex ratios, I collected nymphs from each of the 12 populations in 1997 and 1998. Collections were made twice per year, in late May to July and again in late August to early November to assess seasonal variation. For each population, I collected nymphs from two randomly chosen locations at each transect point. I used Schrader's '10-rock' method (cited in Hynes 1972) to estimate nymph density. At two randomly chosen

Table 1. Locations of *S. femoratum* populations sampled in 1996 and 1997

Population	Abbreviation	Habitat*	County	Latitude	Longitude
Beaver Creek	BC	T	Phelps	37° 52' N	91° 48' W
Honey Creek	HNC	T	Cole	38° 29' N	92° 14' W
Rock Bridge Gans Creek	RBG	T	Boone	38° 52' N	92° 20' W
Cedar Creek	CC	P	Callaway	38° 45' N	92° 11' W
Grindstone Creek	GC	P	Boone	38° 56' N	92° 19' W
Jacks Fork River	JF	P	Shannon	37° 09' N	91° 27' W
Meramec River	MR	P	Crawford	37° 48' N	91° 26' W
Little Prairie Lake	LPL	L	Phelps	37° 60' N	91° 42' W
Little Dixie Lake	LD	L	Callaway	38° 55' N	92° 06' W
Rocky Forks Lake	RF	L	Boone	39° 03' N	92° 18' W
Winegar Lake	WL	L	Cole	38° 28' N	92° 24' W
Whetstone Lake	WS	L	Callaway	38° 57' N	91° 42' W

*T = temporary stream, P = permanent streams, L = lakes.

locations at each transect point, without looking, I picked up ten unimbedded rocks and collected all the *S. femoratum* nymphs on that rock. By collecting all nymphs, I eliminated any size biases, which can bias nymph sex ratios as males and females may grow and develop at different rates. The 10-rock method works exceptionally well with heptageniid mayflies because nymphs cling tenaciously to rocks; preliminary studies showed that very few nymphs washed off as rocks were lifted out of the water. Rocks ranged from ~3 to 40 cm in diameter. A similar range of rock sizes was obtained from each population. Nymphs from each collection were placed in plastic Whirl Pack bags containing water and transported back to the laboratory where the total number of nymphs per ten rocks and the number of males and females was counted. Males were easily identified by the presence of developing genital forceps, which are visible in very early instars. In the few instances where presence of forcep could not be confidently ascertained, nymphs were excluded from sex ratio calculations. Nymph sex ratios were calculated as percentage female by pooling nymphs from the two 10-rock samples at each transect point.

In 1998 and 1999, I estimated imago and subimago sex ratios from light trap collections to determine if adult stages showed biased sex ratios. Light trapping was carried out repeatedly at six of the 12 populations: two temporary streams (Honey Creek and Rock Bridge Gans Creek), two permanent streams (Cedar Creek and Grindstone Creek) and two lakes (Rocky Forks Lake and Little Dixie Lake). At each site, the trap was placed in the same location each time. I used a BioQuip Light Trap (22 V Circline UV light bulb) powered by a 12-V battery. The trap was filled with 50 mL of 70% ethanol and placed on the ground within 2 m of the water. The light was turned on automatically, ~10 min after sunset, using a BioQuip light sensor. The trap was run for 60 min in 1998 and 90 min in 1999. Captured insects were taken to the laboratory and preserved in 70% ethanol. All *S. femoratum* adults were picked from these samples and stored separately in 20 mL glass vials. Ten minutes after trapping began and 10 min before it ended, air temperature, relative humidity, and wind speed were measured. Temperature and relative humidity were measured using a handheld digital thermometer-hygrometer, hung ~20 cm from the trap. Wind speed was measured, over 2 min, by holding an MJP Student anemometer ~2 m above the ground, near the light trap.

In 1998, light trap samples were collected every 21–28 days except for sample 2, which was collected ~14 days after sample 1. Trapping began on July 6th and ended on September 23rd. A total of four samples per population were collected. In 1999, trapping was carried out every 12–16 days, beginning on April 2nd. Frequent rainfall in late April precluded trapping. Sampling resumed on May 23rd and continued until September 17th, for a total of nine samples per population.

Spatial and temporal variation in tychoparthenogenetic capacity

To test for spatial variation in tychoparthenogenetic capacity, I measured habitat and population variation in the mean proportion of unfertilized eggs that hatched. I did this for a sample of females in each population and calculated population mean tychoparthenogenetic capacity. To test for temporal variation in tychoparthenogenetic capacity, I obtained estimates of the population mean hatching success of unfertilized eggs for 1996 and 1997. Within each year, I also compared the proportion of unfertilized eggs hatching on different sampling dates. Five populations were sampled in 1996 and seven additional populations were sampled in 1997. Late instar nymphs were collected from each population and brought to the laboratory where they were kept in aerated glass aquaria containing water from the lake or stream in which they were collected. I also added several algae covered rocks (8×10 cm) as a food source for nymphs. Water temperature was maintained at 24 °C. Only female nymphs were placed in aquaria to ensure that all were virgins. Mesh lids were placed on aquaria and subimagoes collected daily as they emerged. The majority of females emerged after 1–3 days in the lab but a few spent up to 10 days in aquaria before emerging. Subimagoes were placed in small plastic vials with mesh windows in the caps. As soon as the final molt occurred, female imagoes were dissected and their eggs removed. Females were killed by decapitation. Each female was dissected onto a microscope slide containing a drop of carbon-filtered tap water. Care was taken to remove all eggs. Eggs were then transferred to 35-mm diameter plastic Petri dishes containing ~10 mL of carbon-filtered tap water. All pieces of exoskeleton, reproductive tissue were removed from Petri dishes to discourage bacterial and fungal growth. Petri dishes were placed in random locations on shelves in the laboratory. Room temperature was maintained at 23–25 °C. Every 1–5 days, Petri dishes were examined for the presence of hatchlings which appeared 11–13 days after eggs were removed from females. All hatchlings were counted and removed from the Petri dishes and the cumulative number of hatchlings per Petri dish was recorded. I ceased checking Petri dishes when no hatchlings had appeared in a particular dish for 3 weeks. Most eggs hatched within a week of each other, but some eggs hatched 10–12 days after the first eggs hatched. Once hatching had ceased, the Petri dishes were stored at 4 °C in

sealed, plastic containers. Inviable and hatched eggs can be maintained indefinitely under these conditions without disintegration or loss.

To determine the proportion of unfertilized eggs hatching for each female, I photographed each Petri dish using a video camera attached to a dissecting microscope. A clear, Plexiglas grid was placed under each Petri dish to aid in counting. Eggs were photographed at 20 × magnification and digital images of each Petri dish were taken using NIH IMAGE software. Images were printed on paper and I counted eggs by marking them with a felt tipped pen to avoid inadvertent recounting. A female's tychoparthenogenetic capacity was estimated as the proportion of her unfertilized eggs hatched and was calculated by dividing the number of hatchlings per Petri dish by the total number of eggs (hatched and unhatched) per Petri dish. In 1996, eggs from each female were placed into a Petri dish and the proportion of unfertilized eggs hatching was calculated for each female. In 1997, I divided a female's clutch into two Petri dishes to reduce losses due to bacterial or fungal infection. Proportion of unfertilized eggs hatching was calculated for each Petri dish separately and a females' tychoparthenogenetic capacity was calculated as the mean of the two Petri dishes. Population mean tychoparthenogenetic capacity was calculated as the mean hatch success of all females in that population. Mean tychoparthenogenetic capacity in each habitat was calculated as the mean of all populations in that habitat type.

To test for temporal variation in tychoparthenogenetic capacity, in 1996, hatch success was measured on two sampling dates ~4 weeks apart for three of the five populations tested. In 1997, six of the 12 populations were tested two, three or four times. Sampling dates for each population differed as did the time elapsed between repeated samples. On average, sampling intervals were ~3–4 weeks.

Biotic correlates of tychoparthenogenesis

I estimated nymph, imago, and subimago densities for each habitat and population. Density can affect both mate availability and the level of intraspecific competition for resources. Because *S. femoratum* spend > 95% of its lifecycle in the nymph stage and because adults do not posses functional mouthparts, competition for resources is primarily a function of nymph densities rather than imago or subimago densities. In 1997 and 1998, I estimated nymph density for each population from '10-rock' samples collected for the sex ratio analyses described above. Nymph density per transect point was estimated by taking the mean of the two '10-rock' samples. In 1998 and 1999, I estimated imago density and combined imago and subimago density (hereafter referred to as total density) from light trap samples collected for the sex ratio analyses described above. Imago and subimago densities were calculated as the number of individuals per hour of light trapping.

Abiotic correlates of tychoparthenogenesis

I collected data on abiotic aspects of each habitat during 1997 and 1998. For each of the 12 populations, I measured stream or shoreline water depth, percent of stream bed or shoreline that was dry (habitat dryness), water current velocity, and water temperature. Measurements were taken in summer and fall to test for seasonal differences in abiotic factors because temporary streams usually cease flowing and form small pools by late summer. For each population, measurements were taken at each transect point. For streams, water depth was measured at 2-m intervals across the streambed, except for Beaver Creek, which was measured at 1-m intervals due to the small size of the stream. Water depth at lakeshores was measured, beginning at the edge of permanent vegetation, at two points, 1 m apart. Only two measurements were made because water quickly became too deep to measure. For lakes, these measurements were repeated 2 m to the left and right of the first measurement for a total of six measurements per transect point. For each stream, habitat dryness (% of the stream bed that was dry) was calculated for each transect point by dividing the number of locations across the stream bed that were dry, by the total number of locations measured. For lakes, measurements at the three locations per transect point were pooled so that the total number of locations without water was divided by six, the total number of locations at that transect point. In 1997, current velocity was measured once at each transect point using a neutrally buoyant weighted, Styrofoam fishing line float. The amount of time taken for the float to travel 1 m was recorded. For lakes, current velocity was scored as zero as the float usually did not move unless conditions were extremely windy. In 1998, current velocity was measured using an MJP Student Stream Flowmeter electronic velocity meter. Water temperature was measured once at each transect point by holding a thermometer for 1 min at a known depth.

Statistical Analyses

Categorical data modelling (Proc CATMOD, SAS Institute 1990) was used to test effects of year, season, habitat, and population nested within habitat on nymph, imago, and pooled imago and subimago sex ratios (% female). This was carried out for the 12 pop-

ulations sampled for nymphs in 1997 and 1998 and for six populations sampled for imagoes and subimagoes in 1998 and 1999. Separate analyses were carried out for nymphs and imagoes. For these analyses, year, season, and habitat were considered fixed effects, while population was considered a random effect and was nested within habitat. The response variable in these analyses was the number of males and females. Log-likelihood analysis was used to test whether population sex ratios (F:M) deviated significantly from 1:1. In cases where the sample size was <25, I used a binomial exact test (Zar 1984) to test for deviations from 1:1. For each population, I pooled data for all transect points and performed *G*-tests on frequency of female nymphs separately for each year. For clarity, data are plotted as percent of females in each population although the analysis was performed on frequencies. Similar analyses were used to test for significant deviations from 1:1 sex ratios of imagoes as well as imagoes and subimagoes combined.

I used separate nested ANOVA to test effects of habitat and population on tychoparthenogenetic capacity (% hatch success of unfertilized eggs) and nymph density. In all nested ANOVA tests, year and habitat were considered fixed effects. Population was considered a random effect nested within habitat. The habitat main effect was tested using population mean squares as the error term. The population effect was tested using the error mean squares as the error term (Zar 1984). I performed analyses for each year separately and then tested for a year effect by including only those populations sampled in both years. For 1996 only, I used separate one-way ANOVAS to test the effects of habitat and population on ranked hatch success because I had only one permanent stream population; therefore nested ANOVA could not be used. Instead, I examined the effect of habitat and used residuals to test the effect of population. Analysis of nymph densities also included season as a fixed main effect and transect point, which was considered a random effect nested within population. Fixed main effects (year, habitat, season and interactions between them) were tested using population mean squares as the error term. Population was tested using transect mean squares as the error term and transect point was tested using the error mean squares as the error term (Zar 1984). Data were tested for normality and homogeneity of variance assumptions. If these assumptions were violated, I used ranked dependent variables in the nested ANOVA. When main effects or interactions were significant, I used Tukey's HSD for posthoc multiple comparisons.

I used a nested ANOVA to test the effect of sampling date, nested within population, on ranked hatch success for three populations that were sampled twice in 1996 and for six populations that were sampled repeatedly in 1997. Sampling date was considered a nested effect, instead of a repeated measure, because populations were sampled a different number of times and at slightly different time intervals. The population main effect was tested using sampling date mean squares as the error term while sampling date was tested using the error mean squares as the error term (Zar 1984).

To determine if populations with higher hatch success also tended to have a higher frequency of parthenogenetic females, I tested effects of habitat, population nested within habitat and sampling date nested within population on frequency of parthenogenetic females in each population. Frequency of parthenogenetic females is the number of females exhibiting nonzero hatch success, divided by the total number of females tested for that population. I used categorical data modelling to perform log linear tests using a nested ANOVA model. Analysis was performed separately for 1996 and 1997. To test for differences between years, I used only the five populations sampled in both years.

A repeated measures ANOVA was used to test effects of year, habitat, population (nested within habitat) and sampling date (the repeated measure) on density of imagoes. Separate analysis was performed for imagoes and subimagoes combined (hereafter referred to as total density). I performed separate analyses for 1999 data only, to test effects of habitat and sampling date on mean density of imagoes and total density. Because emergence and adult flight may be affected by temperature, relative humidity and wind speed, I used linear regression to test for relationships between these variables and imago and total density. Both imago ($r^2 = 0.20$, $N = 53$, $P < 0.001$) and total density ($r^2 = 0.27$, $N = 53$, $P < 0.001$) were significantly related to temperature. Therefore, I used residuals of these regressions in the repeated measures ANOVA. Neither relative humidity nor wind speed was significantly related to imago or total density.

Pearson's correlation was used to test whether hatch success in a population was related to the frequency of females exhibiting parthenogenesis in 1996 and 1997. In each year, I also used Pearson's correlation to test for relationships between population mean hatch success and population sex ratios, nymph density and imago and total density. Separate correlations were performed for 1996 and 1997 hatching success. Correlations between 1996 hatch success and imago and combined imago and subimago sex ratios were not calculated due to extremely small sample sizes ($N = 3$). If correlations were large, but nonsignificant, I calculated the power (1-β) of the correlation (Zar 1984), which is the probability of rejecting the null hypothesis when the null hypothesis is false.

I used Pearson's correlation to test for relationships between population mean hatching success and nymph, imago and total densities. Because nymph densities did not vary between years, I used densities averaged across the two years. Imago and total densities from 1999 were used because sampling in 1999 covered a greater proportion of the mayfly's emergence season (March through to September) compared to 1998. Population mean imago and total densities were obtained by combining all samples collected over the entire emergence season.

To determine whether the three habitats differed significantly in their abiotic characteristics, I performed two MANOVA tests, one including depth and habitat dryness (% dry) as dependent variables and the other including current velocity and temperature. I performed separate MANOVA tests because, where percentage dry was 100%, there were no measurements for current velocity or temperature. Because current velocity was measured in different units in different years, I used standardized values (Z scores) in the MANOVA. I tested main effects of year, season, habitat and population within habitat on the linear combination of depth and dryness and on the linear combination of current velocity and temperature. Significant main effects were followed by univariate analysis of those effects on each of the dependent variables. Following significant univariate main effects and interactions, Tukey's HSD was used to test for differences among levels of the factor.

I compared habitat variability by estimating the population mean coefficient of variation for each of the four abiotic variables, corrected for sample size (Sokal & Rohlf 1981). Using coefficients of variation of each of the abiotic variables, I performed a MANOVA, testing effects of year and habitat. If the multivariate effect was significant, I used Hotelling's T^2 to perform pairwise comparisons for the linear combination of all four abiotic variables. I also performed univariate ANOVA tests on each of the dependent variables separately to determine which of the dependent variables contributed to the multivariate significance. I used Tukey's HSD to perform multiple comparisons on significant main effects for each univariate analysis.

I used stepwise regression to test for a relationship between tychoparthenogenetic capacity and water depth, habitat dryness, and current velocity. I also used stepwise regression to investigate relationships between tychoparthenogenetic capacity and coefficients of variation for each of the four abiotic variables. Water temperature was not included in the analysis because it did not contribute to the multivariate differences in habitat variability. I calculated partial correlation coefficients for each abiotic variable and hatching success for 1996 and 1997, separately.

RESULTS

Field Evidence of tychoparthenogenetic reproduction

If females utilize their parthenogenetic capacity, then populations should show female-biased sex ratios. In 1997 and 1998, all populations were significantly

Table 2. Tests of deviation from 1:1 sex ratios for nymphs collected from each population in 1997 and 1998. Values are ratios of Female : Male. Sample sizes are in parentheses. Significance levels are shown with an asterisks

		Year	
Population	Habitat†	1997	1998
Beaver Cree	T	9.00:1 (20)*	2.67:1 (22)^{NS}
Honey Cree	T	9.00:1 (50)***	2.27:1 (98)***
Rock Bridge	T	3.33:1 (26)**	2.09:1 (68)**
Cedar Creek	P	2.05:1 (119)***	1.87:1 (198)***
Grindstone Creek	P	–	2.26:1 (140)***
Jacks Fork River	P	2.89:1 (35)**	5.17:1 (37)***
Meramec River	P	2.80:1 (38)**	2.41:1 (58)**
Little Dixie Lake	L	2.45:1 (494)***	2.46:1 (384)***
Little Prairie Lake	L	2.38:1 (71)***	2.08:1 (157)***
Rocky Forks Lake	L	2.55:1 (213)***	2.93:1 (299)***
Winegar Lake	L	3.41:1 (207)***	2.34:1 (204)***
Whetstone Lake	L	2.69:1 (381)***	2.01:1 (268)***

*Significant at $0.005 < p < 0.05$; **significant at $0.001 < p < 0.005$; ***significant at $p < 0.001$; ^{NS}non-significant. †T = temporary stream, P = permanent streams, L = lakes.

female-biased (Table 2). Nymph sex ratio differed among years (χ^2 = 17.77, d.f. = 1, P < 0.0001) and populations (χ^2 = 46.76, d.f. = 10, P < 0.0001). The percentage of females per population ranged from 67% in Cedar Creek to 92% in Beaver Creek in 1997 and from 62% in Cedar Creek to 84% in the Jacks Fork River and in 1998. There were significant habitat by year (χ^2 = 10.59, d.f. = 2, P = 0.005) and habitat by season interactions (χ^2 = 11.03, d.f. = 2, P = 0.004), but no significant main effect of habitat (χ^2 = 0.63, d.f. = 2, P = 0.73), season (χ^2 = 0.001, d.f. = 1, P = 0.99) or the three–way interaction (χ^2 = 4.99, d.f. = 2, P = 0.082). In 1996, temporary streams had a greater female bias compared to permanent streams and lakes while in 1997, all three habitats had similar sex ratios (Fig. 1A). Lakes showed a greater female bias in fall than in summer while both temporary and permanent streams showed no difference in sex ratio across seasons (Fig. 1B).

Unlike nymph sex ratios, there were no consistent sex biases among different habitats, populations, or years for imagoes and for imagoes and subimagoes combined. Results were similar when imagoes and subimagoes were combined; therefore results are reported only for imagoes. In 1998, only three populations showed significant deviations from 1:1 female : male sex ratios for imagoes (Table 3). In both cases, sex ratios were male biased. In 1999, only three populations showed significantly male biased imago sex ratios. There were significant effects of year (χ^2 = 8.51, d.f. = 1, P = 0.004), habitat (χ^2 = 7.92, d.f. = 2, P = 0.019), year by habitat interaction (χ^2 = 26.82, d.f. = 2, P < 0.0001) and population nested within habitat (χ^2 = 42.45, d.f. = 6, P < 0.0001) on sex ratio of imagoes. Similar results were obtained when imagoes and subimagoes were combined. Temporary streams showed a significant male bias in 1998 but not in 1999 (Fig. 2). In contrast, lakes showed a significant female bias in 1998 but not in 1999. Permanent streams showed equal sex ratios in 1998, but in 1999 ratios were significantly male biased.

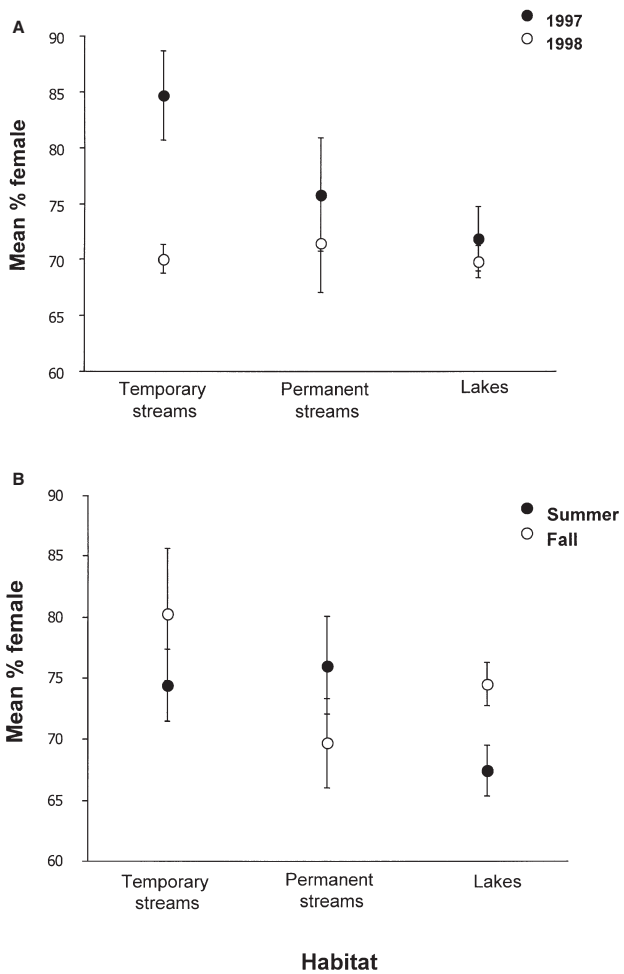

Figure 1. Effects of (A) habitat by year and (B) habitat by season interactions on nymph sex ratio (mean percent female). Values are means ± SE.

Spatial and temporal variation in tychoparthenogenesis

In 1996, tychoparthenogenetic capacity differed significantly among habitats ($F_{2,141}$ = 4.53, P = 0.012). Females from temporary streams had twice the hatch success of unfertilized eggs than those from lakes or permanent streams (Fig. 3). However, there was no significant effect of population within habitat ($F_{4,139}$ = 0.30, P = 0.88). Within populations, females showed large variation in tychoparthenogenetic capac-

Table 3. Log-likelihood tests of deviation from 1:1 sex ratios for imagoes for samples collected from each population in 1998 and 1999. Values are ratios of Female:Male; sample sizes are in parentheses. Bonferroni corrected probabilities are shown with an asterisks

Population	1998	1999
Honey Creek	0.63:1 (112)	1.25:1 (117)
Rock Bridge	0.71:1 (758)***	0.78:1 (673)
Cedar Creek	0.93:1 (268)	0.89:1 (285)
Grindstone Creek	0.52:1 (260)***	0.51:1 (557)***
Little Dixie Lake	0.82:1 (482)	0.52:1 (285)**
Rocky Forks Lake	0.75:1 (1288)***	0.52:1 (894)***

*Significant at 0.005 < P < 0.05; **significant at 0.001 < P < 0.005; ***significant at P < 0.001.

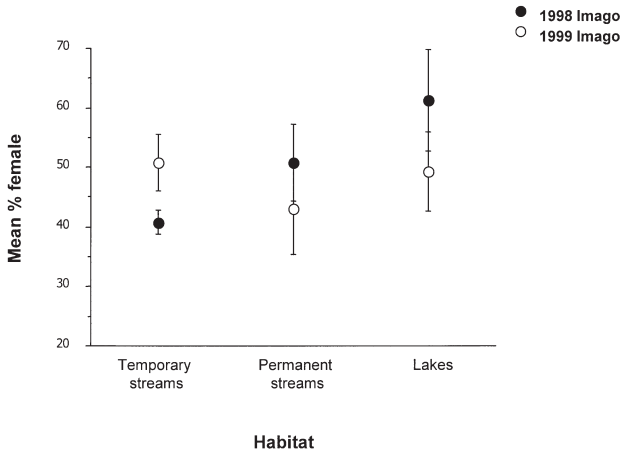

Figure 2. Effects of habitat and year on imago sex ratio (mean percent female). Values are means ± SE.

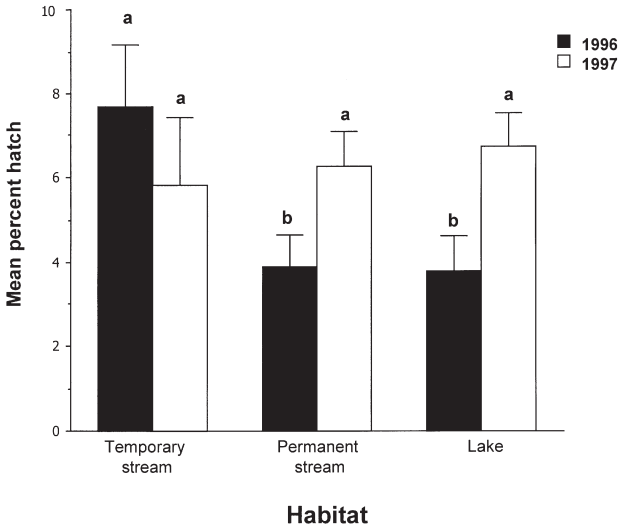

Figure 3. Effects of habitat and year on tychoparthenogenetic capacity (mean % hatching success of unfertilized eggs). Values are means and standard errors. Means with different letters are significantly different (Tukey's HSD, $P < 0.05$).

ity. Patterns of variation in hatch success were highly positively skewed, with most females exhibiting small tychoparthenogenetic capacities (Fig. 4). In all but one population, coefficients of variation in hatch success were greater than one. For populations, which were sampled twice, there was a significant effect of sampling date within populations on ranked hatching success ($F_{3,99} = 3.83$, $P = 0.012$). Hatching success of unfertilized eggs from females collected at Little Dixie Lake on May 29 (mean ± SE = 6.01% ± 1.64%) was significantly higher than that of females collected on June 11th (mean ± SE = 1.27% ± 0.57%).

For 12 populations tested in 1997, there was no significant effect of habitat ($F_{2,9} = 0.39$, $P = 0.61$) or population ($F_{9,377} = 1.24$, $P = 0.27$) on ranked mean hatch success. Females within populations exhibited extremely large variation in hatch success (Table 4). In the most variable population (Rock Bridge Gans Creek) hatch success ranged from 0 to 78%, indicating that within some populations, females showed enormous variation in hatching success. As in 1996, all populations showed significantly positively skewed distributions of female hatch success. For populations sampled repeatedly in 1997, there was a significant effect of sampling date nested within population ($F_{13,566} = 2.16$, $P = 0.01$; Fig. 5). Hatch success for Rock Bridge Gans Creek females was significantly higher on June 29th compared to May 20th or July 14th. Similarly, hatching success of Little Prairie Lake females was significantly higher on July 2nd compared to April 19th or June 2nd. Although Little Dixie females collected on June 10th had higher hatch success compared to females collected during the three other sampling dates, differences were not significant.

For the five populations sampled in both 1996 and 1997, I found significant effects of year ($F_{1,4} = 9.28$, $P = 0.038$) and year by habitat interaction ($F_{2,4} = 8.83$, $P = 0.034$) on ranked hatch success. In 1996, hatching success was greatest in temporary streams compared to permanent streams and lakes while in 1997 hatching success was similar among all habitats (Fig. 3). However, there were no main effects of habitat ($F_{2,4} = 3.28$, $P = 0.14$) or population within habitat ($F_{4,497} = 0.25$, $P = 0.91$) on hatch success.

Hatching success of unfertilized eggs from 1996 was positively correlated with nymph sex ratio ($r = 0.80$, $N = 5$, $P = 0.09$), however, lack of significance may be due to low statistical power ($1 - \beta = 0.65$) resulting from a small sample size. Correlations were not computed for 1996 hatch success and imago and combined imago and subimago sex ratios because data were available from only three populations. Hatch success for 1997 showed no significant correlations with nymph ($r = -0.48$, $N = 12$, $P = 0.40$), imago ($r = 0.67$, $N = 12$, $P = 0.21$) and combined imago plus subimago ($r = 0.59$ $N = 12$, $P = 0.30$) sex ratios.

In 1996, I found no significant variation among populations ($\chi^2 = 0.97$, d.f. = 2, $P = 0.62$) or habitats ($\chi^2 = 2.93$, d.f. = 2, $P = 0.23$) in frequency of parthenogenesis. Similarly, for populations sampled twice, no difference in frequency of parthenogenesis between sampling dates was observed ($\chi^2 = 3.14$, d.f. = 3, $P = 0.37$). Similarly, in 1997 I found no significant difference in frequency of parthenogenesis among habitats ($\chi^2 = 0.12$, d.f. = 2, $P = 0.94$) or among populations within habitat ($\chi^2 = 12.73$, d.f. = 9, $P = 0.18$). However, there was a significant effect of sampling date within

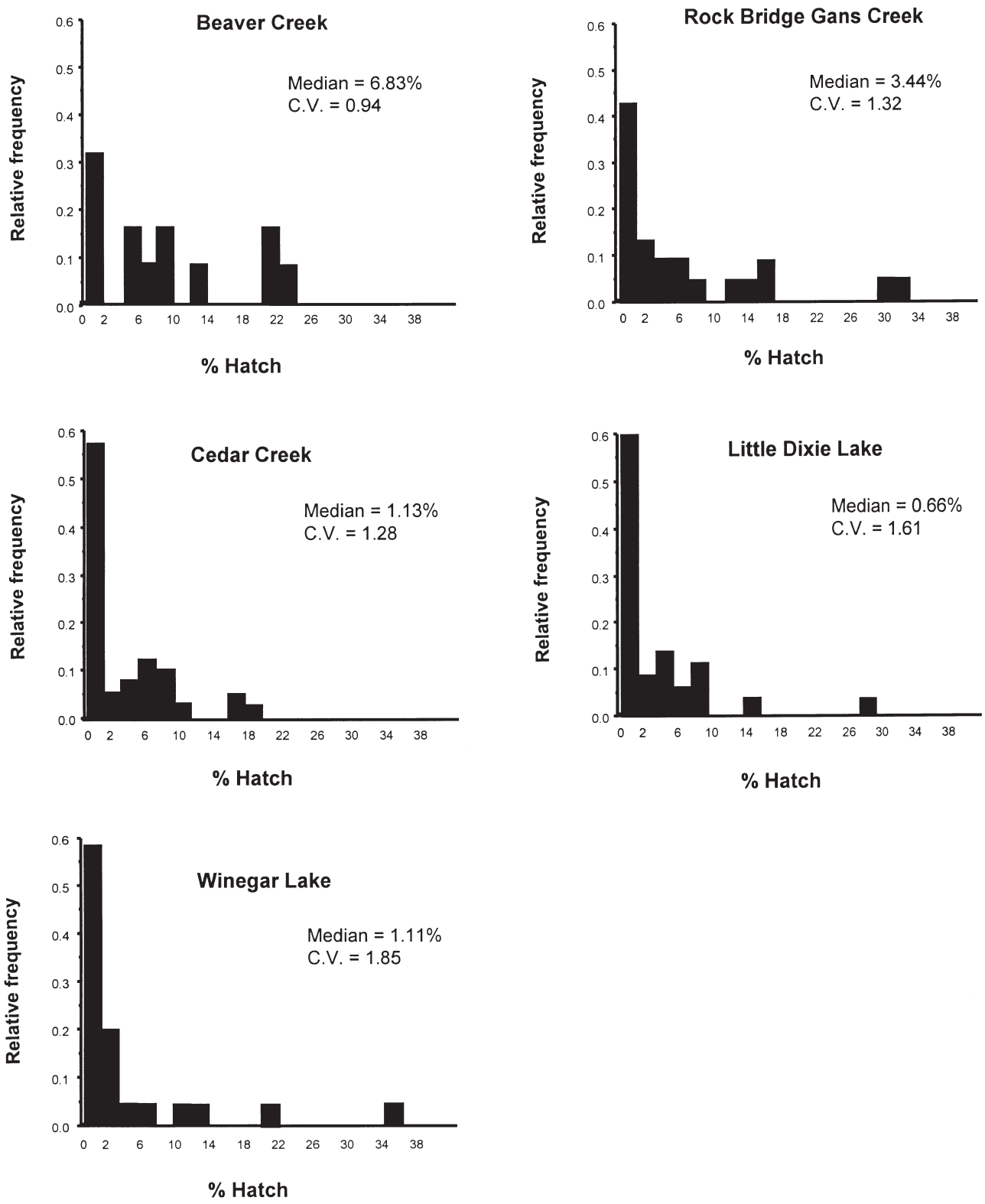

Figure 4. Variation in tychoparthenogenetic capacity (% hatch success of unfertilized eggs) among females in each population sampled in 1996. Bars represent the proportion of females in the population with a given % hatch of unfertilized eggs. Medians and coefficients of variation (C.V.) are also reported for each population.

Table 4. Population variation in hatch success (mean percent) of unfertilized eggs for females from populations sampled in 1997. *N* = number of females tested

Population	Region*	Mean (%)	SD	Range	*N*
Beaver Creek	T	7.31	13.95	0-53.16	15
Honey Creek	T	4.33	7.83	0-25.47	11
Rock Bridge Gans Creek	T	5.66	13.93	0-77.86	38
Cedar Creek	P	6.56	9.64	0-46.52	64
Grindstone Creek	P	8.56	14.77	0-57.32	19
Jacks Fork River	P	5.28	8.28	0-30.96	28
Meramec River	P	5.49	10.25	0-45.76	45
Little Dixie Lake	L	7.96	14.54	0-67.66	71
Little Prairie Lake	L	6.86	7.84	0-32.40	36
Rocky Forks Lake	L	3.31	4.12	0-16.14	43
Winegar Lake	L	10.73	17.58	0-41.64	5
Whetstone Lake	L	7.36	12.93	0-63.38	57

*T = temporary stream, P = permanent streams, L = lakes.

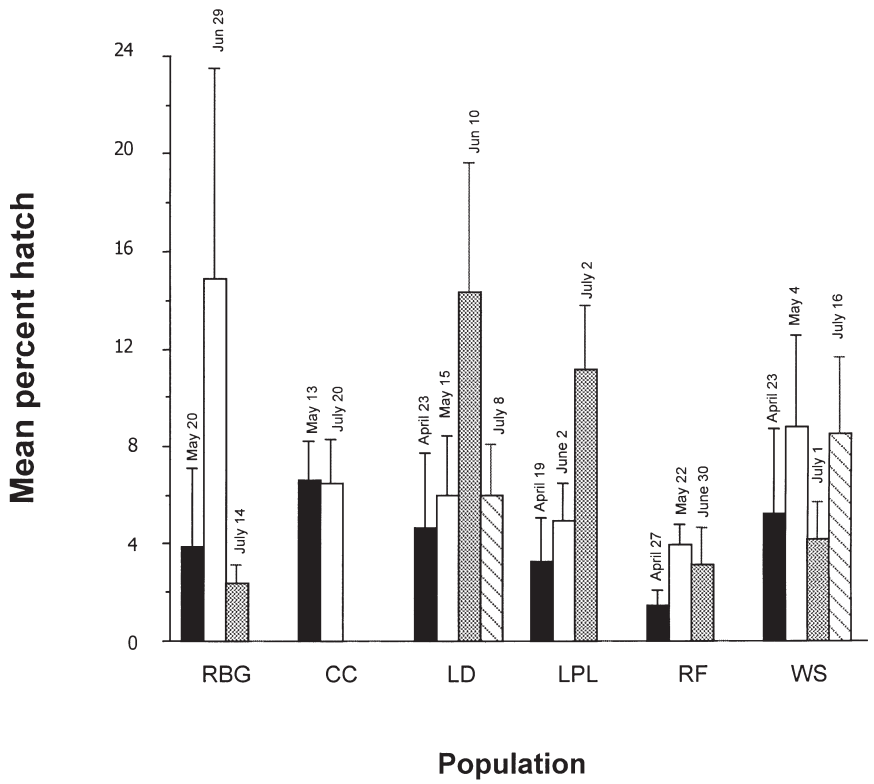

Figure 5. Temporal variation in tychoparthenogenetic capacity among populations sampled in 1997. Values are means ± SE. Sampling dates are given above each bar.

population ($\chi^2 = 26.13$, d.f. = 13, $P = 0.016$; Fig. 6). The greatest variation in frequency of parthenogenesis occurred in Whetstone Lake. On May 4th, 65% of females tested produced offspring parthenogenetically while 100% of females sampled on July 1st produced offspring parthenogenetically. Similarly, both Little Dixie and Rocky Forks also showed large difference in frequency of parthenogenesis among sampling dates. For populations sampled in both 1996 and 1997, I found no significant effect of year ($\chi^2 = 1.15$, d.f. = 1, $P = 0.28$), habitat ($\chi^2 = 3.60$, d.f. = 2, $P = 0.16$) and no significant year by habitat interaction ($\chi^2 = 4.54$,

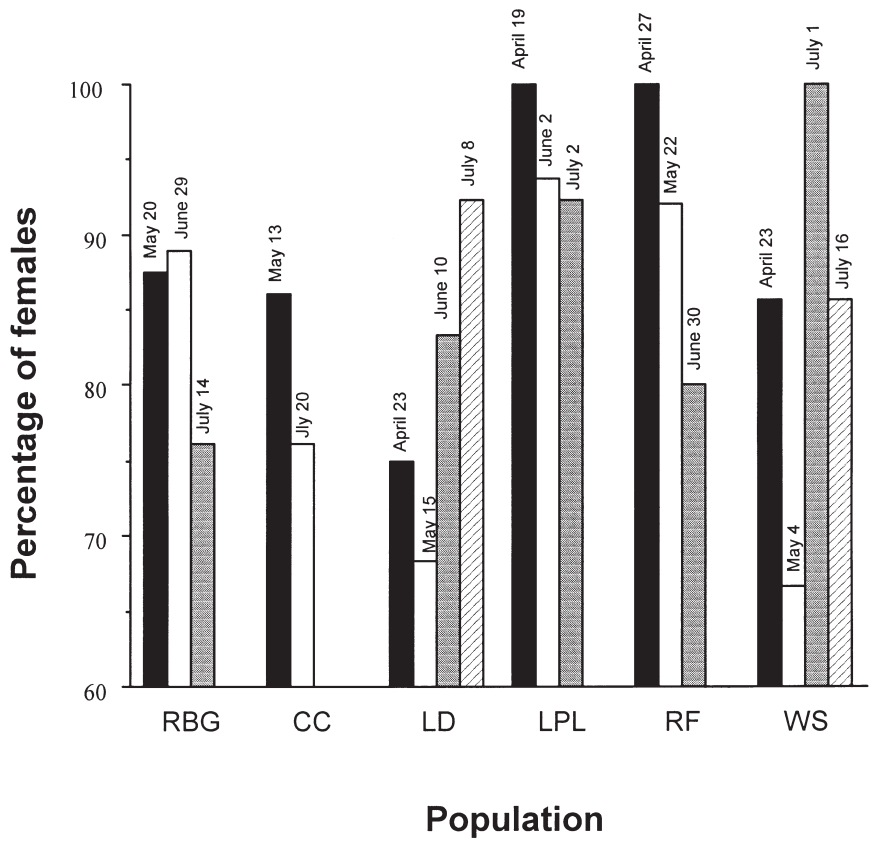

Figure 6. Temporal variation in the percentage of females exhibiting tychoparthenogenetic capacity (non-zero % hatch of unfertilized eggs) for six populations sampled repeatedly in 1997. See Table 1 for population names and habitat designations.

d.f. = 2, $P = 0.10$) on the frequency of parthenogenesis. There was a nearly significant effect of population within habitat ($\chi^2 = 8.04$, d.f. = 3, $P = 0.09$).

Correlation of population mean hatch success in 1996 with mean percent of females producing offspring parthenogenetically was not significant ($r = 0.76$, $N = 5$, $P = 0.13$). However small sample size resulted in low power to detect a statistically significant relationship (1-β = 0.55). Similarly in 1997, variation in hatch success was unrelated to variation in percent of females producing offspring parthenogenetically ($r = -0.09$, $N = 12$, $P = 0.78$).

Biotic correlates of tychoparthenogenesis

Nymph densities did not differ significantly between years, but there were significant differences among habitats (Table 5). Lake densities were an order of magnitude higher than those in either temporary or permanent streams (Fig. 7). Nymph densities also varied significantly among populations. There was a significant effect of transect point, indicating that nymphs were patchily distributed along streambeds and lakeshores. None of the two or three-way interactions between year, habitat, or season were significant.

Repeated measures ANOVA showed a significant effect of population nested within habitat on total density, but no significant effects of year, habitat or any two-way interaction (Table 6). Similar results were obtained for imagoes only. In contrast to nymph densities, Rock Bridge Gans Creek, a temporary stream, showed relatively high total densities while Little Dixie Lake showed low densities that were comparable to those of the permanent streams (Fig. 8A). Total density varied over time, declining significantly in early September. A similar pattern was observed for imago density, although the effect of time was not significant ($P = 0.066$). A separate analysis for 1999 that included all nine sampling dates, from April to September, showed a significant effect of sampling date ($F_{8,24} = 2.67$, $P = 0.029$) on total density, but no effect of habitat ($F_{2,24} = 0.19$, $P = 0.83$) or habitat by sampling date interaction ($F_{16,24} = 1.08$, $P = 0.42$). Similar results were obtained for imago density. Light

Table 5. Nested ANOVA of effects of year, season, habitat, population (nested within habitat) and transect point (nested within population) for mean nymph density (number of nymphs per ten rocks)

Source	df	MS	*F*	*P*
Year	1	52147.48	0.63	0.43
Season	1	25403.17	0.31	0.58
Habitat	2	3566697.98	43.10	0.0001
Year x Season	1	182168.70	2.20	0.15
Year x Habitat	2	159664.69	1.93	0.16
Season x Habitat	2	41265.15	0.50	0.61
Year x Season x Habitat	2	24106.47	0.29	0.75
Population (Habitat)	33	82761.51	2.57	0.0001
Transect point (Population)	288	32213.92	3.54	0.0001
Error	333	3029017.00		

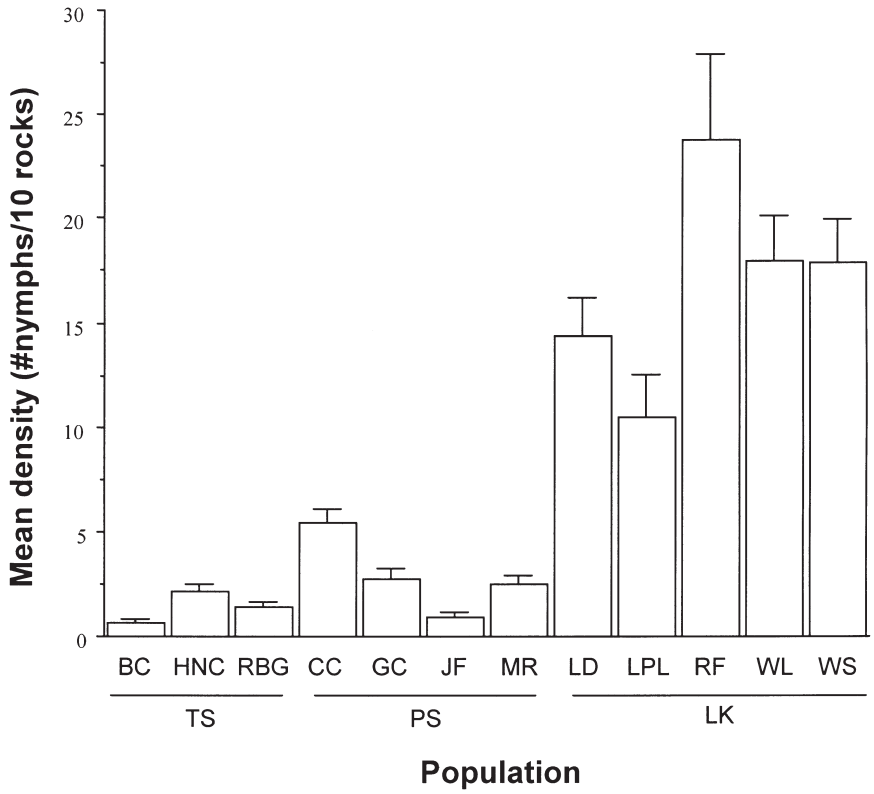

Figure 7. Variation in nymph density among populations and habitats. Populations are grouped by habitats (TS = temporary streams, PS = permanent streams, LK = lakes). Values are means ± SE, averaged across two years (1997 and 1998).

trap samples collected early in April and late May and toward the end of the emergence season in mid September showed extremely low total densities relative to samples collected in June through August (Fig. 8B).

Hatch success in 1996 was negatively correlated with nymph density ($r = -0.80$, $P = 0.034$; Fig. 9). Correlations between 1996 hatching success and imago and total density were not performed due to small sample sizes ($N = 3$). Hatch success in 1997 showed no relationship with any of the density measures; in all cases, correlation coefficients were relatively small and nonsignificant ($P > 0.50$ for all correlations).

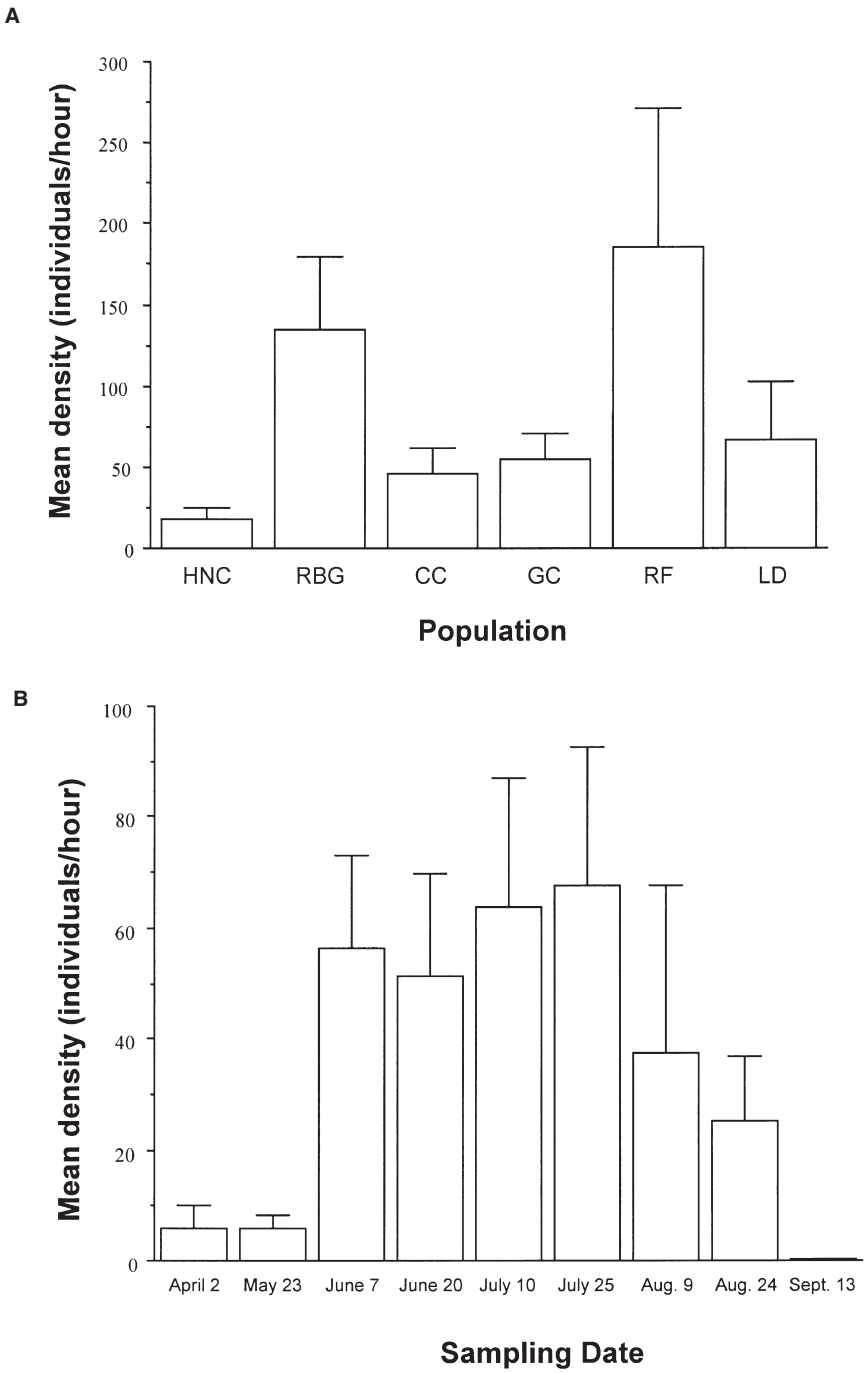

Figure 8. Population variation (A) and temporal variation (B) in mean total density (imagoes plus subimagoes) from light trap samples. Population means in (panel a) are averaged across 2 years (1998 and 1999). Temporal variation (panel b) is shown for 1999 only, across nine different sampling dates. Values are means ± SE.

Table 6. Repeated measures ANOVA for effects of year, habitat, population (nested within habitat) and time (repeated measure) on mean total density . Density is the number of individuals caught per hour of light trapping on four sampling dates: July 10, July 26, August 24, September 9, in 1998 and 1999

Source	df	MS	*F*	*P*
Year	1	96690.40	2.63	0.21
Habitat	2	23649.05	0.64	0.59
Time	3	32854.69	3.78	0.050
Year *x* Habitat	2	33302.06	0.90	0.49
Habitat *x* Time	6	14448.14	1.66	0.24
Population (Habitat)	3	36821.88	4.24	0.039
Time *x* Population (Habitat)	9	8681.92	0.87	0.57
Error	21	9997.92		

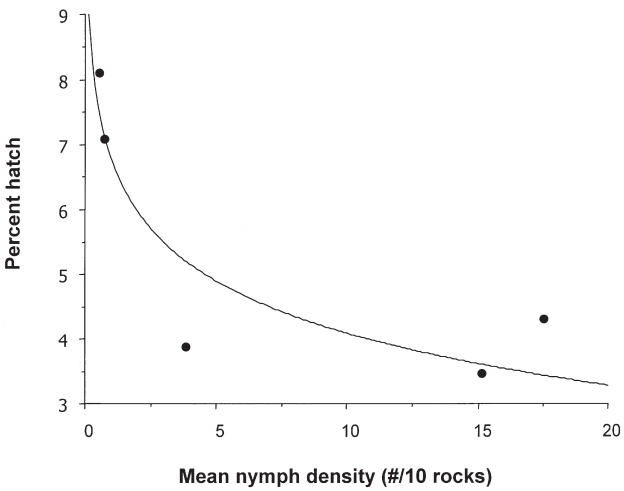

Figure 9. Relationship between tychoparthenogenetic capacity (% hatch of unfertilized eggs) measured in 1996 and nymph density.

ABIOTIC HABITAT CHARACTERISTICS

Multivariate ANOVA revealed that water depth and habitat dryness varied significantly among habitats (Table 7A). Neither year, season, nor any of the two-way or three-way interactions were significant with respect to these two variables. Populations differed significantly, indicating spatial variation for depth and dryness within the different habitats. Univariate analyses showed that significant differences in depth among habitats and among populations within habitats contributed to the multivariate significance (Table 7B). Temporary streams were significantly shallower than either permanent streams or lakes (Fig. 10A). All other main effects were not significant.

Multivariate analysis of current velocity and temperature showed significant differences among habitats and seasons, but no significant differences between years and no significant two- or three-way interactions (Table 8A). Populations within habitats also varied significantly in current velocity and temperature. Univariate analysis of current velocity showed significant variation among habitats and populations within habitats (Table 8B). Permanent streams showed the highest (Fig. 10B) current velocity; temporary streams were intermediate and lakes showed the lowest current velocity. For temperature, univariate tests showed significant differences between seasons and among populations within habitats, but no differences among years, habitats, or with any of the two-way or three-way interactions (Table 8C). Summer water temperatures were significantly higher (mean ± SD = 24.7 ± 4.7 °C) than fall temperatures (mean ± SD = 17.3 ± 6.5 °C).

Univariate analysis showed that habitat dryness also contributed to multivariate significance. There was a significant interaction between year, season, and habitat as well as a significant season by habitat interaction (Table 7C; Figs 10C,D).

Hatch success in 1996 was negatively related to water temperature ($r = -0.95$, $N = 5$, $P = 0.012$) but showed no correlation with percentage dryness ($r = 0.74$, $N = 5$, $P = 0.26$), water depth ($r = -0.41$, $N = 5$, $P = 0.56$) or current velocity ($r = 0.60$, $N = 5$, $P = 0.40$). In 1997, hatch success showed no correlations with water depth ($r = 0.17$, $N = 12$, $P = 0.59$),% dryness ($r = -0.32$, $N = 12$, $P = 0.31$), current velocity ($r = -0.18$, $N = 12$, $P = 0.57$), or water temperature ($r = 0.07$, $N = 12$, $P = 0.84$).

Multivariate ANOVA on coefficients of variation for water depth, dryness, current velocity, and temperature showed significant differences in variability among habitat (Wilk's $\lambda = 0.086$, $F_{8,30} = 9.06$, $P = 0.0001$) and a trend toward differences in variability between years (Wilk's $\lambda = 0.55$, $F_{4,15} = 3.04$, $P = 0.052$) but no multivariate interaction between year and habitat (Wilk's $\lambda = 0.56$, $F_{8,30} = 1.25$, $P = 0.30$). Pairwise multivariate comparisons using Hotelling's T^2 indicated that all three habitats differed significantly in variability ($P < 0.02$ in all cases). Univariate analyses showed that habitats differed significantly in variability of water depth, with temporary streams significantly more variable compared to permanent streams and lakes ($F_{2,18} = 16.81$, $P = 0.0001$; Table 9). Similarly, habitats differed in their variability in dryness, with lakes being significantly more variable compared to temporary or permanent streams ($F_{2,18} = 6.46$, $P = 0.008$; Table 9). There were no significant year or year by habitat interactions for either variability in water depth or dryness (all

P values >0.50). There were also significant habitat differences in variability of current velocity ($F_{2,18}$ = 42.34, P = 0.0001) as well as differences between years ($F_{2,18}$ = 11.63, P = 0.003). Current velocity was most variable in permanent streams and slightly less variable in temporary streams while lakes showed no variation in current velocity (Table 9). The interaction between habitat and year was nearly significant ($F_{2,18}$ = 3.44, P = 0.054). Variability in temperature did not contribute significantly to the multivariate effect ($F_{2,9}$ = 1.20, P = 0.35; Table 9).

Hatch success in 1996 was positively related to variability in water depth (r = 0.98, N = 5, P = 0.02) but showed no correlation with variability in current velocity (r = 0.92, N = 5, P = 0.17) or with variability in dryness (r = –0.43, N = 5, P = 0.98). There were no significant correlations between hatch success in 1997 and variability in depth, dryness or current velocity (P = 0.90 in all cases).

DISCUSSION

Field Evidence of tychoparthenogenetic reproduction

In both years sampled, all *S. femoratum* populations showed significantly female biased nymph sex ratios, with populations ranging from moderately (62%) to strongly female biased (92%). These data are consistent with the hypothesis that tychoparthenogenetic reproduction does occur in these natural populations and that hatching of unfertilized eggs was not simply a case of artificial parthenogenesis observed solely in the laboratory. If females reproduce parthenogenetically, then nymphs from the overwintering generation should show female biased sex ratios the following spring. As expected, nymph sex ratios closely reflected tychoparthenogenetic capacity of females tested the previous year. Female biased sex ratios of late instar nymphs has been shown for the tychoparthenogenetic mayfly *Eurylophella funeralis*. Sweeney & Vannote (1987) reported a positive relationship between tychoparthenogenetic capacity and percentage female. One tychoparthenogenetic population they tested in two consecutive years showed female-biased sex ratios of 88% and 89% female. Pescador & Peters (1974) reported slightly female biased nymph sex ratios (52% female) for the tychoparthenogenetic *Baetisca rogersi.* However, hatch success of unfertilized eggs was ~1% (range 0.3–1.6%). With such a low level of parthenogenetic reproduction, only a slight female bias would be expected. Female biases have also been reported for several other parthenogenetic mayfly species (*Baetis spinosus* and *B. frondalis*, Bergman & Hilsenhoff 1978; *Baetis macdunnoughi*, McCafferty & Morihara 1979; *Siphlonisca aerodromia,* Gibbs & Siebenmann 1996; *Cloeon simile*, Harker 1997), including several obligately parthenogenetic species in which males are exceedingly rare or absent (Sweeney & Vannote 1987). In contrast, equal sex ratios were reported for *Dolania americana* nymphs, a species which showed no parthenogenetic capability (Peters & Peters 1977).

Several factors, other than tychoparthenogenesis, may lead to female biased sex ratios. However, it is unlikely that these factors explain entirely the female biased nymph sex ratios in *S. femoratum* populations. Female bias can also result from sampling biases, differential mortality, environmental sex determination, or cytoplasmic symbionts. In addition, mating systems where mating occurs exclusively between sibs are typically characterized by strong female biases (Thornhill & Alcock 1983). It is unlikely that female biases are an artifact of sampling because nymphs were collected randomly without bias toward certain size classes or developmental stages. Like many mayfly species, *S. femoratum* shows protandrous emergence. Collecting nymphs immediately after male emergence begins, but before female emergence begins, could result in female bias. However, female biases were consistent across sampling dates between years and seasons, suggesting that they were not simply a sampling artifact. Sib mating is unlikely to occur frequently enough in *S. femoratum* populations to lead to female biases because of asynchronous adult emergence. The likelihood of sib mating should be less than for synchronously emerging mayfly species. Mating is promiscuous, with swarming males intercepting females as they fly into the swarm, which suggests that assortative mating with sibs in unlikely. Environmental sex determination, which can also lead to biased sex ratios, has never been reported for mayflies, but cannot be ruled out as a possible influence on sex ratio. Preliminary research on these S. *femoratum* populations has also provided no evidence for the involvement of cytoplasmic symbionts in sex ratio distortion (S. L. Ball, unpublished data). Differential mortality may, however, account, in part, for biased nymph sex ratios. However, differential mortality is more likely to lead to a male bias. Female aquatic insects are generally larger and have higher growth rates than males (Butler 1984). If females feed more actively or longer than males, then females are likely to spend more time exposed to predators, compared to males.

In contrast to nymphs, imago and subimago sex ratios were equal or slightly male biased. These differences may reflect differences in nymph and imago sampling methods. Sex differences in attraction to the UV light may result in over-representation of adult males. Peters & Peters (1977) documented, for *D. americana*, male biased subimago sex ratios (75% male) from light trap samples while nymph sex ratios

Table 7. MANOVA and univariate ANOVA of effects of year, season, habitat and population (nested within habitat) on mean depth and mean % dry measured in 1997 and 1998

Source	df	Wilk's *L*	*F*	*P*
(A) MANOVA (depth, % dry)				
Year	2,8	0.97	0.10	0.90
Season	2,8	0.83	0.82	0.47
Habitat	4,16	0.03	19.79	0.0001
Year *x* Season	2,8	0.65	2.11	0.18
Year *x* Habitat	4,16	0.49	1.74	0.19
Season *x* Habitat	4,16	0.46	1.88	0.16
Year *x* Season *x* Habitat	4,16	0.37	2.54	0.081
Population (Habitat)	9,676	0.81	4.29	0.0001
	df	MS	*F*	*P*
(B) Univariate ANOVA (depth)				
Year	1	107.33	0.10	0.76
Season	1	308.69	0.29	0.60
Habitat	2	5764.70	9.51	0.27
Year *x* Season	1	2706.54	2.59	0.14
Year *x* Habitat	2	359.90	0.34	0.72
Season *x* Habitat	2	910.97	0.87	0.45
Year *x* Season *x* Habitat	2	1044.63	1.00	0.41
Population (Habitat)	9	1045.29	6.87	0.0001
Error	339	152.26		
(C) Univariate ANOVA (% dry)				
Year	1	158.20	0.22	0.65
Season	1	1328.30	1.84	0.21
Habitat	2	59700.26	82.87	0.0001
Year *x* Season	1	2998.56	4.16	0.072
Year *x* Habitat	2	2279.16	3.16	0.091
Season *x* Habitat	2	3071.07	4.26	0.049
Year *x* Season x Habitat	2	3648.79	5.06	0.034
Population (Habitat)	9	720.42	1.67	0.094
Error	339	430.52		

were generally equal. However, *D. americana* exhibits protandry and it is not known if light trapping coincided with the predominantly male phase of the emergence period. Males may be more attracted to the UV light because of their attraction to polarized light and to pale objects in the environment, which often act as swarm markers. Attraction to polarized light is thought to facilitate swarming by attracting males to light reflected off the water surface, however, females also exhibit orientation toward polarized light presumably to facilitate oviposition (Kriska *et al*. 1998). Although *S. femoratum* is protandrous, emergence is highly asynchronous, with subimagoes emerging almost daily throughout the season. Light trap samples were collected over nearly the entire emergence period, which occurs from April to November in Missouri. It is unlikely that all nine samples were collected during peaks in male emergence and therefore, timing of sample collection is unlikely to account for male biases. The abundance of imago and subimago males, relative to nymphs, may be due, in part, to greater female mortality. Peters & Peters (1977) documented higher female mortality in *D. americana* imagoes. Females are larger and slower flies than males, which may make them particularly susceptible to aerial predators such as dragonflies and birds.

SPATIAL AND TEMPORAL VARIATION IN TYCHOPARTHENOGENETIC CAPACITY

In 1996, tychoparthenogenetic capacity of females from temporary stream populations was twice that of

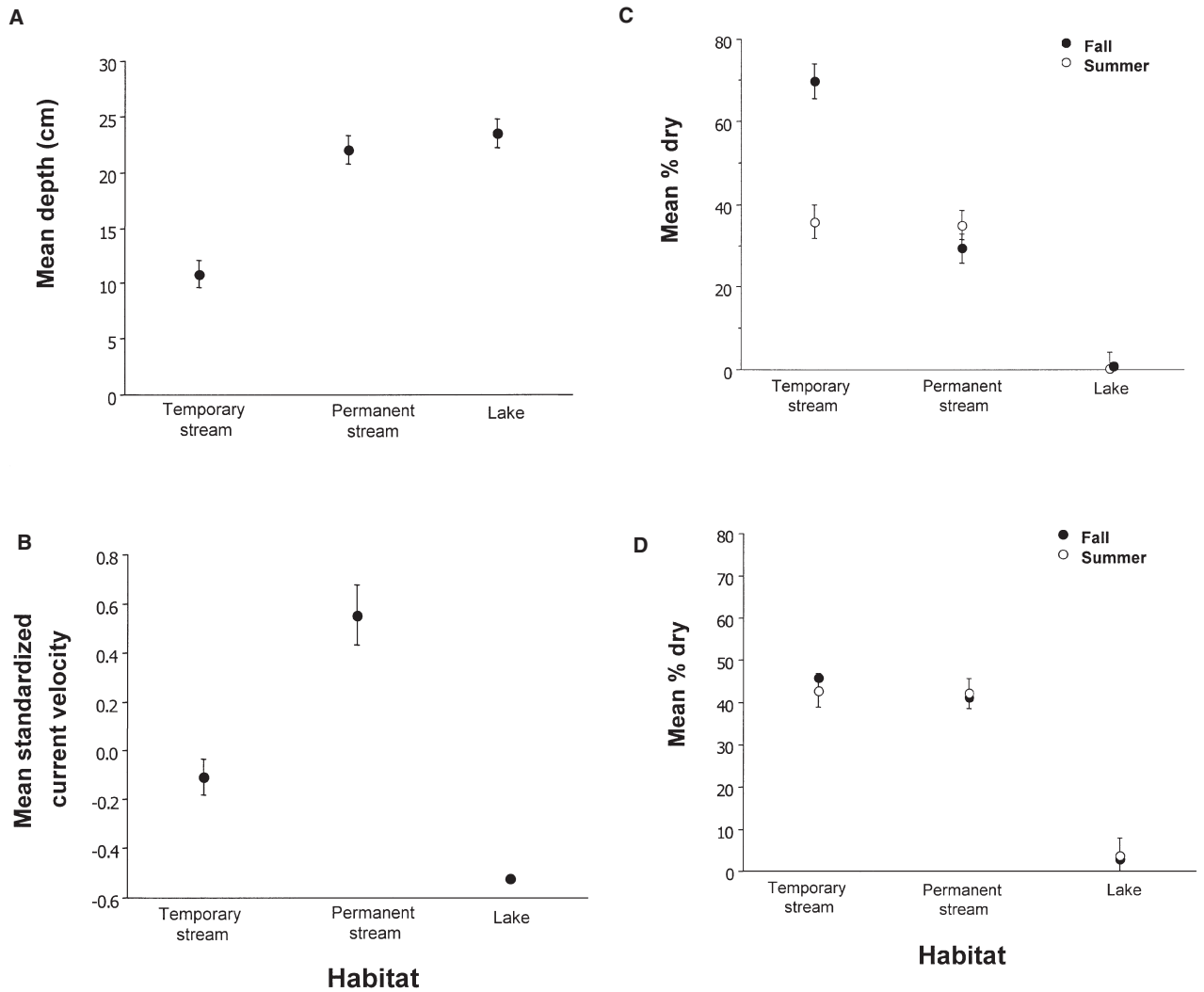

Figure 10. Habitat variation in (A) mean water depth, (B) mean standardized current velocity, (D) habitat dryness, 1997, and (D) habitat dryness, 1998. Values are means ± SE.

females from permanent streams or lakes. This association between tychoparthenogenetic capacity and habitat permanence is consistent with the ecological distribution of parthenogenesis. Parthenogenesis is often found in harsh, disturbed or unpredictable environments, such as ephemeral and man-made habitats, where abiotic factors have a greater influence on population dynamics than biotic factors (Levin 1975; Glesner & Tilman 1978; Bell 1982; Bierzychudek 1985; Martens 1998). Consistent with these patterns, temporary streams exhibited large variability in several abiotic factors, extremely low nymph densities, and greater tychoparthenogenetic capacity (in 1996) relative to permanent streams and lakes. In contrast with the patterns described above, lakes were associated with higher levels of sexual reproduction in 1996, lower abiotic variability and greater nymph densities than temporary streams, suggesting that lakes are relatively stable habitats in which biotic influences predominate.

In 1997, tychoparthenogenetic capacity of females from permanent streams and lakes increased and was not different from that of temporary streams. The lack of habitat or population variation in tychoparthenogenetic capacity complicates interpretation of associations between parthenogenesis and habitat type. The amount of parthenogenesis increased in lakes and permanent streams, relative to those in 1996. This suggests that factors other than those measured in this study may be influencing parthenogenetic reproduction in these populations. Because reproduction is such an important component of individual fitness,

Table 8. MANOVA and univariate ANOVA of effects of year, season, habitat and population (nested within habitat) on mean standardized current velocity and mean water temperature measured in 1997 and 1998

Source	df	Wilk's *L*	*F*	*P*
(A) MANOVA (current,temperature)				
Year	2,8	0.95	0.22	0.81
Season	2,8	0.29	9.71	0.007
Habitat	4,16	0.11	8.00	0.001
Year x Season	2,8	0.50	3.97	0.064
Year x Habitat	4,16	0.92	0.18	0.95
Season x Habitat	4,16	0.44	2.02	0.14
Year x Season x Habitat	4,16	0.66	0.91	0.48
Population (Habitat)	18,620	0.61	9.61	0.0001
	df	MS	*F*	*P*
(B) Univariate ANOVA (current)				
Year	1	0.29	0.14	0.72
Season	1	0.07	0.03	0.86
Habitat	2	35.52	17.12	0.0001
Year x Season	1	1.93	0.93	0.36
Year x Habitat	2	0.64	0.31	0.74
Season x Habitat	2	3.80	1.83	0.22
Year x Season x Habitat	2	0.91	0.44	0.66
Population (Habitat)	9	2.07	2.81	0.004
Error	311	0.74		
(C) Univariate ANOVA (temperature)				
Year	1	132.54	0.49	0.50
Season	1	4538.59	16.62	0.003
Habitat	2	446.71	1.64	0.25
Year x Season	1	993.59	3.64	0.09
Year x Habitat	2	82.87	0.30	0.76
Season x Habitat	2	263.08	0.96	0.42
Year x Season x Habitat	2	216.60	0.79	0.48
Population (Habitat)	9	273.05	18.65	0.0001
Error	311	14.65		

plasticity in timing, frequency and type of reproduction (i.e. sex vs. parthenogenesis) may provide females with the ability to maximize fitness under varying environmental conditions. Although tychoparthenogenesis results in lower offspring production relative to sexual reproduction, it may provide intermittent advantages if females find themselves in situations where biotic (i.e. scarcity of males) or abiotic (i.e. unfavourable weather conditions) factors prevent mating. The importance of these biotic and abiotic influences may be enhanced by the extremely brief (~48–72 h) adult lifespan. Therefore, tychoparthenogenesis may function as a type of bet-hedging strategy, allowing females to salvage some reproduction when mating is not possible.

In addition to annual variation, I found significant variation in tychoparthenogenetic capacity within years. In 1996, the Little Dixie Lake population showed a large decrease in tychoparthenognetic capacity, from 6 to 1%, in samples collected 2 weeks apart. Significant temporal variation was also found in 1997, with three populations (one temporary stream and two lakes) showing significant increases in tychoparthenogenetic capacity in mid summer. If tychoparthenogenesis is a means of reproductive assurance, then these mid-summer increases in tychoparthenogenetic capacity are contrary to what would be predicted based on mate availability. Imago and subimago densities were greater during the summer compared to early spring or fall. However, these mid-summer increases in

Table 9. Multiple comparisons of mean coefficients of variation among habitats. Comparisons were made for each variable separately. Different letters indicate significant differences ($P < 0.05$)

Habitat	Depth	% Dry	Current velocity	Temperature
Temporary stream	120.97[A]	65.09[B]	445.90[A]	27.21[A]
Permanent stream	60.57[B]	60.09[B]	481.90[B]	33.76[A]
Lake	54.46[B]	238.82[A]	0.000[C]	23.91[A]

tychoparthenogenetic capacity may reflect low imago and subimago densities that occurred in between peaks in adult emergence. Although *S. femoratum* emergence is asynchronous, most populations did show distinct emergence peaks in June and July. Both Little Dixie Lake and Rock Bridge Gans Creek populations, which had high midsummer tychoparthenogenetic capacities, also exhibited extremely low mid-summer imago and subimago densities in between peak emergence. Although these patterns suggest that tychoparthenogenetic capacity may be highest when imago and subimago densities are lowest, further research will need to focus on measuring the relationship between tychoparthenogenetic capacity and adult densities in detail.

Biotic correlates of tychoparthenogenesis

Several hypotheses for the maintenance of sex are based on the importance of biotic interactions. The Red Queen Hypothesis proposes that sex provides a frequency-dependent advantage to species involved in a coevolutionary 'arms race' with parasites (Van Valen 1973; Jaenike 1978; Hamilton 1980) while both the Tangled Bank and Sib Competition Hypotheses suggest sex is advantageous because the diversity of genotypes produced will reduce overlap in resource use among sibs (Ghiselin 1974; Williams 1975; Bell 1982). However, little attention has been focused on the relationship between population density and reproductive mode. Templeton (1982) suggested that parthenogenesis simply provides an advantage to females, which find themselves in situations where mates are scarce.

In 1996, greater sexual reproduction was associated with lake and permanent stream populations, two habitats in which biotic factors are likely to predominate over abiotic factors. For example, lake populations contained nymph densities an order of magnitude greater than temporary stream populations; nymphs likely experience much greater intraspecific competition compared to those from sparse, temporary streams. Although nymph densities in permanent streams were more similar to those of temporary streams, preliminary data suggest that the aquatic invertebrate community was more diverse than that of temporary streams (S. L. Ball, unpublished data). Biotic interactions may be much more important to permanent stream than to temporary stream populations. Further studies are being conducted to quantify aquatic invertebrate species diversity to determine the potential importance of biotic interactions to nymphs in each habitat.

Large differences in nymph density among habitats also suggest that tychoparthenogenesis may function in reproductive assurance. In 1996, tychoparthenogenetic capacity was highest in low density temporary streams; there was a negative relationship between tychoparthenogenetic capacity and nymph density. Moreover, large mating swarms, typical of lake populations were never observed in temporary streams. Temporary stream swarms usually consisted of 5–25 males whereas lake swarms often contained over 100 males and two or three different swarms were often observed along lakeshores. In addition, swarming was observed on most evenings in lakes, however, in spring and fall, I frequently did not find any swarming males in temporary streams. On these occasions, I often observed lone females ovipositing. Although tychoparthenogenetic capacity was negatively related to nymph density in 1996, this relationship did not persist in 1997. This suggests that some unmeasured variable may also be influencing tychoparthenogenetic capacity and that this variable may be associated with or interacting with nymph density. The relative influence of different factors may drive the differences in tychoparthenogenetic capacity observed between years.

Temporal variation in imago and subimago density also suggests that tychoparthenogenesis may, at certain times, provide reproductive assurance. Light trap samples showed extremely low imago and subimago densities in early spring and late fall and in midsummer, between peak emergence. Therefore, imago density may be associated with tychoparthenogenetic capacity. However, imago densities were not measured in the same years as tychoparthenogenetic capacity; future studies will need to examine the relationship between these two variables in more detail.

Future studies will also focus on investigating whether tychoparthenogenetic capacity is related to parasite presence. Sexual snail populations are often associated with greater parasite abundance (Lively 1987; Schrag *et al*. 1994a); the genetic diversity generated by sex may provide a frequency dependent advantage over asexual reproduction. Small nematode-like worms and encysted worms have been found in the abdominal cavity of female *S. femoratum* imagoes from Beaver Creek, Cedar Creek, and Little Dixie Lake. Whether these organisms are mermithids, a common mayfly parasite (Vance & Peckarsky 1997), is unknown. Future work will focus on measuring frequency of infection and determining whether these organisms are truly parasitic.

Abiotic correlates of tychoparthenogenesis

Comparison of water depth, habitat dryness, current velocity and water temperature showed that the three habitats differed significantly in their physical characteristics as well as the variability in these characteristics. Some habitat differences also varied seasonally. Temporary streams were characterized by shallow water depths, moderately slow current velocities, and the greatest amount of habitat drying over the season. In contrast, lakes were characterized by deep water with little or no current and very little drying while permanent streams were intermediate in depth and drying, but exhibited the fastest current velocities. In addition, temporary streams showed the greatest variability in water depth, while permanent streams showed the greatest variability in current velocity. Lakes showed the greatest variability in habitat dryness. These three habitats provide very different physical environments and have the potential to exert different selective pressures, particularly for nymphs, which comprise the majority (> 95%) of the mayfly lifecycle in the aquatic environment. Due to drying, temporary stream habitats become fragmented, forming small pools. This, combined with low population densities, may lead to stochastic shifts in sex ratio, which can enhance female biases (Templeton 1982; Chaplin *et al*. 1994). If tychoparthenogenesis is heritable, then these environmental conditions, in addition to the extremely short imago lifespan, may select for females with higher tychoparthenogenetic capacities. Templeton (1982) increased tychoparthenogenetic capacity 1000-fold in *Drosophila mercatorum*, through artificial selection in one generation. If selection does favour tychoparthenogenesis as a means of reproductive assurance, selection would tend to act early in the season (e.g. March to May) and near the end of the season (September to November) when imago densities were low. Further studies are needed to examine in detail, temporal variation in tychoparthenogenetic capacity and mate availability. In addition, studies are needed to determine whether mayfly populations are sperm-limited. Allan & Flecker (1989) observed male mayflies mating with more than one female. If *S. femoratum* males mate with more than one female, then imago densities may not directly reflect sperm availability.

In 1996, tychoparthenogenetic capacity was negatively correlated with water temperature and showed a trend toward a positive correlation with habitat dryness. It is not known why tychoparthenogenesis was negatively correlated with temperature. Temperature may directly affect egg development. However, temperature may also be correlated with some unmeasured variable causing variation in tychoparthenogenetic capacity. A positive relationship between water temperature and outcrossing rates has been shown in the freshwater snail, *Bulinus truncatus* (Schrag & Read 1992; Schrag *et al*. 1994a,b). Woolhouse & Chandiwana (1989) and Schrag (1993) both found that water temperature was a good predictor of future levels of parasitism.

Female biased nymph sex ratios suggest that *S. femoratum* females do use their capacity for tychoparthenogenesis in natural populations and that tychoparthenogenesis is not simply a case of artificial parthenogenesis induced in the laboratory. Results suggest that tychoparthenogenesis may function in reproductive assurance. However, this hypothesis was supported in only one of the two years. Multi-year studies are needed to determined long-term temporal patterns of variation in tychoparthenogenetic capacity. Similarly, tychoparthenogenesis was associated with low water temperature. However, it is not known whether temperature directly affects egg development or whether it was associated with an unmeasured variable such as parasite infection. Further studies are needed to identify possible biotic and abiotic selective forces or environmental cues that may influence tychoparthenogenesis. Similarly, whether tychoparthenogenesis is controlled by genetic or environmental factors, or an interaction between the two, needs to be determined in order to better understand the potential adaptive significance of tychoparthenogenesis.

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