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Variation in allocation to sexual and asexual reproduction among clones of cyclically parthenogenetic *Daphnia pulex* (Crustacea: Cladocera)

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Organisms reproducing by cyclical parthenogenesis combine the benefits of both sexual and asexual reproduction within the same life cycle. Few studies have examined the evolution of variation in the pattern of investment in parthenogenetic compared to sexual reproduction. Seven clones of *Daphnia pulex* (Crustacea: Cladocera) varying in allocation to sexual reproduction, as measured by the production of males, were raised in isolation and together in a microcosm to study the pattern of sexual reproduction and the effect of this variation on clone fitness. Sex allocation for clones raised together a microcosm was similar to their allocation when raised in isolation, suggesting a genetic basis to the variation. Three clones showed a cost of producing males that lead to their extinction after about 30 days due to the lack of females required for the clones to persist by parthenogenetic reproduction. The remaining four clones persisted until the end of the 72-day experiment. Clones with little or no allocation to males showed no increased allocation to sexual females. The seven clones showed a greater variation in estimated fitness through male and female function than in total estimated fitness. The clone with the greatest total fitness gained most of its fitness through male function but also had a relatively high fitness through female function. Although one clone produced only females it had the next highest fitness. The three clones that went extinct because of a high investment in males had estimated fitness as high as some clones that persisted in the microcosm because of a higher investment in parthenogenetic reproduction. The similarity in total fitness among clones suggests that *Daphnia pulex* populations in temporary habitats maintain a sex polymorphism where different genotypes vary in functional gender ranging from female to primarily male.

ADDITIONAL KEY WORDS:—sex and evolution – sex allocation – cost of males – microcosm.

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INTRODUCTION

Asexual reproduction is often considered a simpler, more efficient mode of reproduction than sexual reproduction. This assumption generates an apparent paradox because sexual reproduction is the dominant mode of reproduction for most plant and animal species (Williams, 1975; Maynard Smith, 1978). Cyclically parthenogenetic species (monogonont rotifers, aphids and cladocerans) have attracted much interest because they maintain both sexual and asexual reproduction within the same life cycle. Williams (1975) proposed that cyclically parthenogenetic organisms provide evidence for the short-term benefit of sex or else sexual reproduction in these organisms would be replaced by strictly asexual reproduction. Pearse, Pearse & Newberry (1989) dismiss the paradox of sex as argued by Williams (1975) and Maynard Smith (1978), and consider asexual (clonal) proliferation a form of growth prior to investment in sexual reproduction, an idea Janzen (1977) used to explain the life history of dandelions and aphids. Whether the clonal descendants of a sexually produced individual should be considered equivalent to somatic growth or the alternative that parthenogenetic eggs are equivalent to sexual eggs except for the absence of genetic recombination is a matter of debate (Charlesworth, 1980; Bulmer, 1982). However, if parthenogenetic reproduction is treated as a form of somatic investment then life-history evolution of cyclically parthenogenetic species can be studied as a problem in resource allocation between parthenogenetic and sexual reproduction including allocation to male and female sex function (Charnov, 1982; Hughes & Cancino, 1985; Hughes, 1987).

The life history of cyclically parthenogenetic organisms involves a trade-off between parthenogenetic and sexual reproduction. Early investment in sexual reproduction is expected to reduce clonal growth while delaying sexual reproduction allows parthenogenetic reproduction to increase the size of a clone and hence make a greater contribution to the pool of sexual propagules (Hughes & Cancino, 1985; Hughes, 1987). Postponing sexual reproduction until the population density is increased by parthenogenetic reproduction is also expected to increase mating success in species that are initially established as sparse populations (Muenchow, 1978; Gerritsen, 1980). For cyclical parthenogens in a seasonal environment the optimal investment in parthenogenetic and sexual reproduction will be a function of the length of time the environment is favourable for parthenogenetic reproduction and mating success which will be a function of population density and sex ratio. Although detailed observations on allocation to sexual and parthenogenetic reproduction in cyclical parthenogens are lacking, demographic models suggest that

an intermediate level of investment in sexual reproduction maximizes sexual egg production for a variety of environments (Snell, 1987; Aparici, Carmona & Serra, 1996, 1998; Serra & King, 1999).

In most species of the cyclically parthenogenetic cladoceran *Daphnia*, parthenogenetic reproduction occurs when conditions are favourable for growth while sexual reproduction is stimulated by unfavourable conditions such as high population density or changes in photoperiod that are correlated with the onset of unfavourable conditions (Hebert, 1978). Parthenogenetic eggs develop immediately, generating a clone of genetically identical descendants from a single female. Parthenogenetic reproduction in *Daphnia* may be viewed as a form of somatic growth prior to investment in sexual reproduction (Janzen, 1977; Hughes & Cancino, 1985). The environment determines sex in *Daphnia* and the transition to sexual reproduction is initiated when parthenogenetic females begin to produce broods of diploid males. Females then switch from parthenogenetic to sexual egg production. Following mating and fertilization of meiotically produced eggs, sexual females release these eggs into their ephippium, a protective structure modified from the carapace. The sexual eggs are diapausing, surviving freezing and desiccation to hatch when favourable conditions return. Diapausing eggs are also the main dispersal stage of the *Daphnia* life cycle.

The transition from parthenogenetic to sexual reproduction in *Daphnia* has received much attention. Bell (1982: 244–249) reviews the older literature and concludes that the transition to sexual reproduction occurs at or soon after a peak in population density for both field and laboratory populations. Although the appearance of sexual stages often precedes conditions unfavourable for growth, the association between sex and peak population density suggests that the timing of sex may be related to the increased probability of finding a mate (Muenchow, 1978; Gerritsen, 1980). *Daphnia* often initiate the growing season as sparse populations in which parthenogenetic reproduction would be favoured over sexual reproduction. Sexual reproduction would then be favoured once population density reaches a level that ensures a high mating success. However, *Daphnia* in small temporary ponds may not have the luxury of a period of parthenogenetic reproduction prior to sexual reproduction if the time required for both is longer than the average time the habitat is in existence. Furthermore, in the low volumes of these shallow temporary ponds, the problem of finding mates may be less acute. Dense populations of *Daphnia pulex* Leydig are very common in small, shallow ponds in southern Ontario that may only be suitable for a few weeks before they dry up leaving only diapausing eggs. These populations invest in sexual reproduction through the production of males soon after the population is re-established by females hatching from resting eggs in the spring (Innes, 1997). The brief period of parthenogenetic reproduction and emphasis on sexual reproduction makes these populations ideal for studying patterns of allocation to sexual and asexual reproduction.

Understand life-history evolution in cyclical parthenogens requires information on the genetic component of reproductive variation and the relationship between this variation and fitness. Fitness can be estimated as the total number of diapausing eggs that each clone has contributed to by the end of the season either as a male or female parent. Genotypes can vary in allocation to parthenogenetic reproduction as opposed to sexual reproduction and individual genotypes can also vary in relative allocation to male and female sexual function. A previous study showed that some genotypes of *Daphnia pulex* in temporary ponds invest in both male and female sexual

function while other genotypes invest in only female sexual function (Innes & Dunbrack, 1993). Other studies have also provided evidence for variation among genotypes in the relative allocation to male and female sexual function (Ferrari & Hebert, 1982; Larsson, 1991; Yampolsky, 1992; Innes & Singleton, 1994; Deng, 1996). Nevertheless, a genotype should adopt a strategy that balances investment in male and female sexual function with the production of parthenogenetic offspring such that it maximizes its contribution of genes to the next generation (Hughes, 1987; Hughes & Cancino, 1985; Serra & King, 1999).

Few studies have examined allocation to sexual and parthenogenetic reproduction in any cyclically parthenogenetic species (Rispe, Bonhomme & Simon, 1999). Because it is difficult to obtain information on sex allocation for individual genotypes of *Daphnia* under natural conditions (Innes, 1997), most studies have been conducted in the laboratory, often using only a single clone and hence there is a lack of information on variation in sex allocation (Stross, 1969, 1971; Hobaek & Larsson, 1990, Kleiven *et al.*, 1992). Given the difficulty of following the reproductive pattern of individual clones in nature, we have attempted to mimic this in a simple laboratory microcosm. A microcosm has the advantage of allowing an interaction among different clones that occurs in nature but is missing from most laboratory experiments on *Daphnia*. Interactions among individuals may influence the sex allocation pattern of different clones. Furthermore, microcosm experiments can be replicated and have proven useful for exploring a number of ecological questions (Beyers & Odum, 1993, Weider, 1992, Fraser & Keddy, 1997, Sarnelle, 1997). The present experiment was designed to follow variation in allocation to parthenogenetic and sexual reproduction, and determine the relationship between sex allocation pattern and estimated fitness for seven clones of *Daphnia pulex*. The following specific questions were addressed: (1) Do clones that exhibit a particular sex allocation pattern when raised in isolation exhibit the same pattern when raised together with other clones? (2) Is there a cost to producing the sexual stages that compromises the ability of a clone to persist in the population? (3) Do clones differ in the relative investment in sexual females and is there an inverse relationship between the relative investments in males compared with sexual females? (4) How do different allocation patterns influence the estimated fitness of a clone?

METHODS

Clonal isolates

Clonal genotypes for the experiments were established from individual females collected from two similar temporary pond populations (Long Point 8A and 8B) separated by about 3 kilometres and described in previous studies (Innes, 1991, 1997). Both are shallow woodland ponds, typically inhabited by *Daphnia* only during April and May of each spring. Although populations of obligately parthenogenetic *Daphnia pulex*, in which there is no sexual reproduction, commonly occur in southern Ontario (Hebert, Ward & Weider, 1988; Hebert *et al.*, 1989), these two ponds contain individuals reproducing by cyclical parthenogenesis (Innes, 1991). Based on preliminary observations, clones were chosen to represent a range of investment in males and could also be distinguished using two enzyme markers (*Pgm, Pgi*). Methods

for cellulose acetate electrophoresis used to determine enzyme genotypes are outlined in Herbert & Beaton (1989). The origin of the seven clones used in the experiments and their *Pgm*/*Pgi* genotype are: clone 1 (8B, SF/MM), clone 2 (8A, MF/SF), clone 3 (8B, SM/MM), clone 4 (8B, FF/MM), clone 5 (8A, MM/SM), clone 6 (8A, MF/SM), clone 7 (8A, MM/MF) where S (slow), M (medium), and F (fast) are the alleles at each locus designated by relative electrophoretic mobility. Females from these clones were set up to produce broods of female young (neonates) to start the experiments. Neonates were collected from the fourth brood of these females so that they were all about the same age. In the Cup experiment, single females were followed to determine the sex allocation pattern of each clone under low-density conditions. The Microcosm experiment was used to follow the allocation pattern of the microcosm population as a whole as well as the pattern for individual clones distinguished using the genetic markers. Both Cup and Microcosm experiments were set up at the same time under the same temperature and photoperiod (20 ± 1°C.; 18L: 6D photoperiod).

Cup experiment

Single neonate females from each of clones 1–7 were placed in individual plastic cups with about 80 ml of zooplankton media (Lynch, Weider & Lampert, 1986). Four replicate females were set up from each clone except three replicate females from clone 4. Cups were fed daily 2 ml of an algal slurry (predominantly *Chlorella* sp.) from an aquarium culture system. Brood release was synchronous and all females released four broods over an 11-day period. As each female released neonates, their number and sex were noted for the first four broods as they were removed from each cup. Average proportion of male broods and standard errors were calculated among the replicates. The total number of offspring produced by each female was also compared among the clones.

Microcosm experiment

For each of clones 1–7, three female neonates were placed into each of four separate jars with about 390 ml of zooplankton media. Females were fed daily 8 ml of algal slurry and monitored for survival and initiation of reproduction. After 4 days, the contents of seven jars (one for each clone) were combined into a 4 litre jar and zooplankton media added up to the 3 litre mark. This process was repeated three more times to produce four replicate jars containing three young, pre-reproductive females from each of the seven clones. The four experimental jars were maintained at 20°C and 18L: 6D photoperiod with frequent rotation within an incubator. Each jar was fed the same amount of 50–150 ml of aquarium-cultured algae every other day.

Each jar was sampled 10 times (days 11, 15, 19, 23, 27, 31, 38, 45, 52, 72) during the experiment. Replicate 150 ml samples were taken by mixing the contents of each jar and sampling a column using a glass tube (with a silicon ring at one end) pressed to the bottom of the jar. The contents of the water column were removed and individuals classified into males (3 stages of maturity) and females (ephippial, carrying a brood, no brood, immature) prior to determining the *Pgm* and *Pgi*

genotype to assign clone membership of each individual. Total density of females, males and ephippial females was estimated on each sampling date. The volume of each jar was maintained at 3.01 by replacing the media removed during sampling with zooplankton media.

A dilution was imposed on each jar on days 15, 23, 38, 45, 52 by removing an additional 450–500 ml and replacing with zooplankton media. Dilution was imposed to prevent the *Daphnia* population of each jar from drastically overshooting carrying capacity and crashing to very low levels. Dilution was also used to reduce the possibility of one or a few clones dominating the population. Weider (1992) found that such a disturbance maintained clonal diversity in a laboratory microcosm of *D. pulex*.

Sex allocation and clone fitness

The density of females, males and ephippial females was determined for each clone identified in the samples using the two genetic markers. Total reproductive investment for each clone during the 72 days of the experiment was calculated as the sum of females, males and ephippial females in the samples and expressed as a proportion of the three life history stages.

For each clone, fitness through male function was estimated indirectly as the product of the proportion of mature males for each clone and the total number of ephippial females in the 300 ml samples (two 150 ml samples combined) collected from each jar during each sample. Fitness through female function for each clone was estimated as the number of ephippial females for each clone in 300 ml samples as long as at least one mature male from any clone was present in the sample to fertilize diapausing eggs. Fitness through male and female function for each clone during the 72 days of the experiment was calculated by summing fitness estimates for all samples in each jar. Total fitness for each clone for the duration of the experiment was estimated as the sum of the fitness estimates through male and female function. Therefore, total fitness for each clone was defined as the total number of haploid genomes from male or female gametes estimated to contribute to the next generation through diapausing eggs.

Statistical analysis

The statistical analysis used SAS v. 6.12. Variables were tested for normality using PROC UNIVARIATE. Non-normal variables were transformed or anlaysed using PROC NPAR1WAY if the transformation failed to normalise the data. Scheffe's multiple-comparison procedure was used to test for significant differences among individual mean values and the Spearman rank correlation (r_s) was used to measure association among estimated fitness and allocation variables.

RESULTS

Cup experiment

No mixed-sex broods were observed for any of the 107 broods produced by the individual females. The seven clones showed significant (Kruskal–Wallis test, $\chi^2 =$

TABLE 1. Offspring production and sex allocation for *Daphnia pulex* clones in the Cup Experiment based on four single females per clone (three for clone 4) and the first four broods per female. Standard errors in parentheses. Means with the same letter are not significantly different

Clone	Total number of broods	Mean proportion of male broods per female	Mean total number of offspring per female	Mean total number of females per female	Mean total number of males per female
1	16	0.63 (0.07)^{A}	105.3 (1.31)^{A,B}	31.8 (11.4)^{A}	73.5 (12.0)^{A}
2	15	0.46 (0.04)^{A}	86.5 (7.42)^{A,B}	48.0 (4.1)^{A,C}	38.5 (4.5)^{A,C}
3	16	0.75 (0.00)^{A}	120.5 (9.42)^{B}	28.5 (6.7)^{A}	92.0 (5.7)^{A}
4	12	0.08 (0.08)^{A}	96.7 (14.19)^{A,B}	83.7 (1.5)^{B,C}	13.0 (13.0)^{B,C}
5	16	0.00^{B}	72.5 (5.52)^{A}	72.5 (5.5)^{B,C}	0.0^{B}
6	16	0.00^{B}	97.5 (4.99)^{A,B}	97.5 (5.0)^{B,C}	0.0^{B}
7	16	0.00^{B}	97.5 (4.84)^{A,B}	97.5 (4.8)^{B,C}	0.0^{B}

24.6, df = 6, $P<0.001$) variation in the proportion of male broods produced (Table 1). Clones 1–3 produced a very high proportion of male broods, clone 4 produced one male brood out of the 12 broods examined and clones 5–7 produced no male broods among the 16 broods examined for each clone (Table 1). A nonparametric multiple comparison test (Zar, 1974: 200) showed no difference in the proportion of male broods among clones 1–4, but a significant different between clones 1–4 and clones 5–7.

The total number of offspring produced (square root transformation) in the first four broods varied significantly ($F_{6,20} = 4.70$, $P<0.01$) among the clones (Table 1), but the comparison of means showed that only clones 3 and 5 were significantly different. There was also significant variation among clones in the total number (square root transformation) of males ($F_{6,20} = 40.9$, $P<0.001$) and females ($F_{6,20} = 13.6$, $P<0.001$) produced. Clones 1, 2 and 3 produced significantly more males than clones 4–7 (Table 1). Variation among clones was greater for the number of females (coefficient of variation = 46.7%) and the number of males (121.0%) compared to the total number of offspring produced (19.5%). Although female brood sizes ($22.8 \pm$ SE 1.08) were significantly smaller (Mann–Whitney U test, $\chi^2 = 7.56$, df = 1, $P<0.01$) than male broods (28.5 ± 2.16), there was no significant difference between male and female brood sizes within any of the male-producing clones (1–3).

Microcosm experiment

Figure 1 plots the total density as well as density of the different life history stages during the 72 days of the experiment. Total density, averaged over the four replicate jars, was estimated to be 270/l on day 11 of the experiment. Total density increased to about 700/l before declining due to the dilution imposed on days 15 and 23. Total density increased from about day 27 to day 38 when a dilution produced a decrease in total density. As the populations consisted mostly of females, changes in average density of females closely mirrored the observed changes in total density. Males were almost as common as females in the first sample and reached peak density on day 15. The density of males was also affected by dilution and remained at about 100/l in the samples taken during days 45, 52 and 72. Ephippial females were rare but showed a peak in density for the day-19 sample, slightly after the peak in density of males. No mature males or ephippial females were detected in

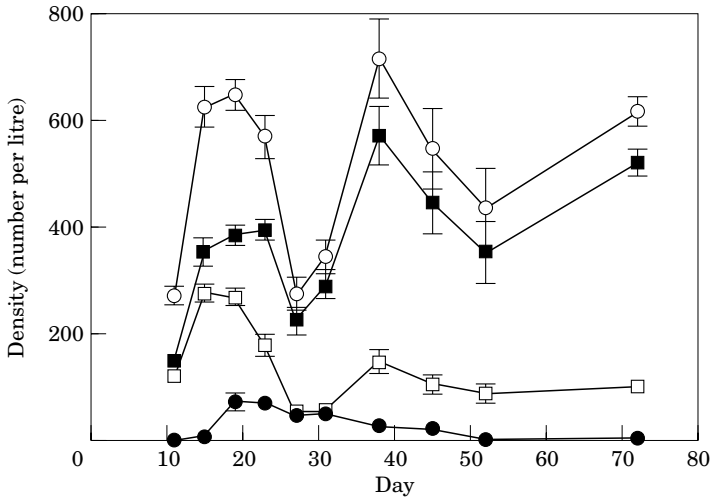

Figure 1. Mean (± SE) density of females (■), males (□), ephippial females (●) and total individuals (○) for samples taken during the 72 days of the Microcosm experiment.

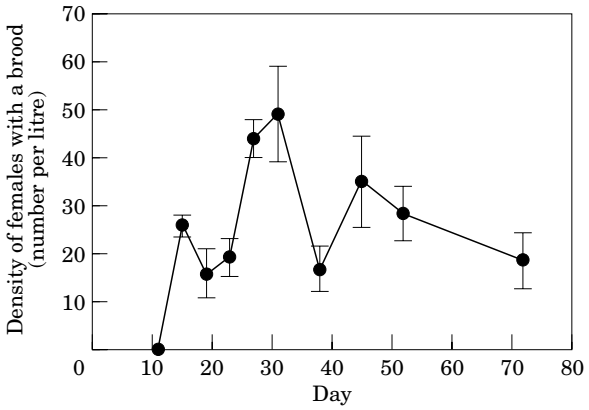

Figure 2. Mean (± SE) density of brood-carrying females during the Microcosm experiment.

the first sample on day 11. Total number of the three life history stages produced over the 72 days (counts based on 300 ml samples) was 85.3 ± SE 9.2 ephippial females, 409.8 ± 33.7 males and 1013.5 ± 41.3 females averaged over the four replicate jars. The four replicate jars showed a remarkably similar pattern of change in density of the life-history stages as indicated by the small standard errors around the mean values.

Density of males showed a significant correlation with total density in all four replicate jars ($r_s = 0.65$, 0.81, 0.71, 0.87 all $P<0.05$), but density of ephippial females did not ($r_s = 0.39$, 0.58, −0.40, 0.17 all $P<0.05$) suggesting that male production responded more to the increase in density than ephippial production. The density of females carrying a brood increased during the first 30 days indicating that conditions had not deteriorated sufficiently to prevent a low level of parthenogenetic-brood production (Fig. 2).

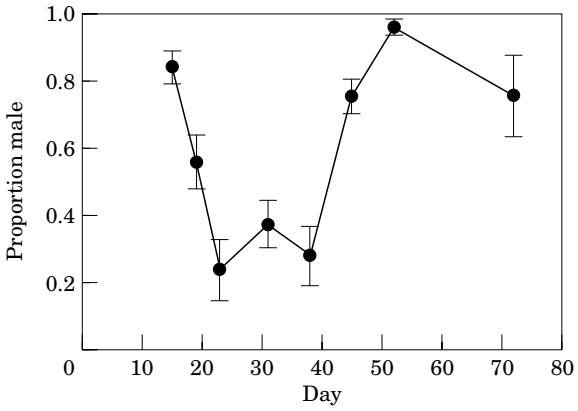

Figure 3. Mean (± SE) sex ratio during the Microcosm experiment. Sex ratio defined as the proportion of mature males relative to the total of mature males plus ephippial females.

Sex ratio (proportion male), based on mature males and ephippial females, showed a wide fluctuation over the course of the experiment (Fig. 3). Mature males were more frequent than ephippial females for all samples except samples taken on days 23, 32 and 38 (Fig. 3). The density of ephippial females declined to near zero during the last half of the experiment while males were still present (Fig. 1).

Density of clones

Clones 1, 2 and 3 showed a similar pattern of change in density over the course of the experiment (Fig. 4). Males were the dominant life-history stage for these clones and showed a peak in density in the first few samples of the experiment. Few females and ephippial females were produced and all three clones were extinct by about day 31. Clones 4 and 5 produced mostly females as well as some males and ephippial females (Fig. 4). Ephippial females were only detected in the early samples while males were detected in most of the samples. No males were detected for clone 6 but ephippial females were observed early in the experiment (Fig. 4). Some ephippial females were detected for clone 7 prior to males being detected in the later samples (Fig. 4).

Sex allocation and clone fitness

The total proportional allocation to males (Kruskal–Wallis Test $\chi^2 = 25.7$, df = 6, $P < 0.001$), females ($\chi^2 = 25.03$, df = 6, $P < 0.001$) and ephippial females ($\chi^2 = 15.11$, df = 6, $P < 0.05$). was significant different among the seven clones in the microcosm (Fig. 5). Clones 1–3 showed the greatest allocation to males while clones 4, 5 and 7 exhibited a much smaller allocation to males. No allocation to males was detected for clone 6, but this clone showed a greater allocation to ephippial females compared with the other six clones. However, no significant difference ($\chi^2 = 11.28$, df = 6, $P > 0.05$) was found among the clones if allocation to ephippial females was calculated relative to all females, excluding males.

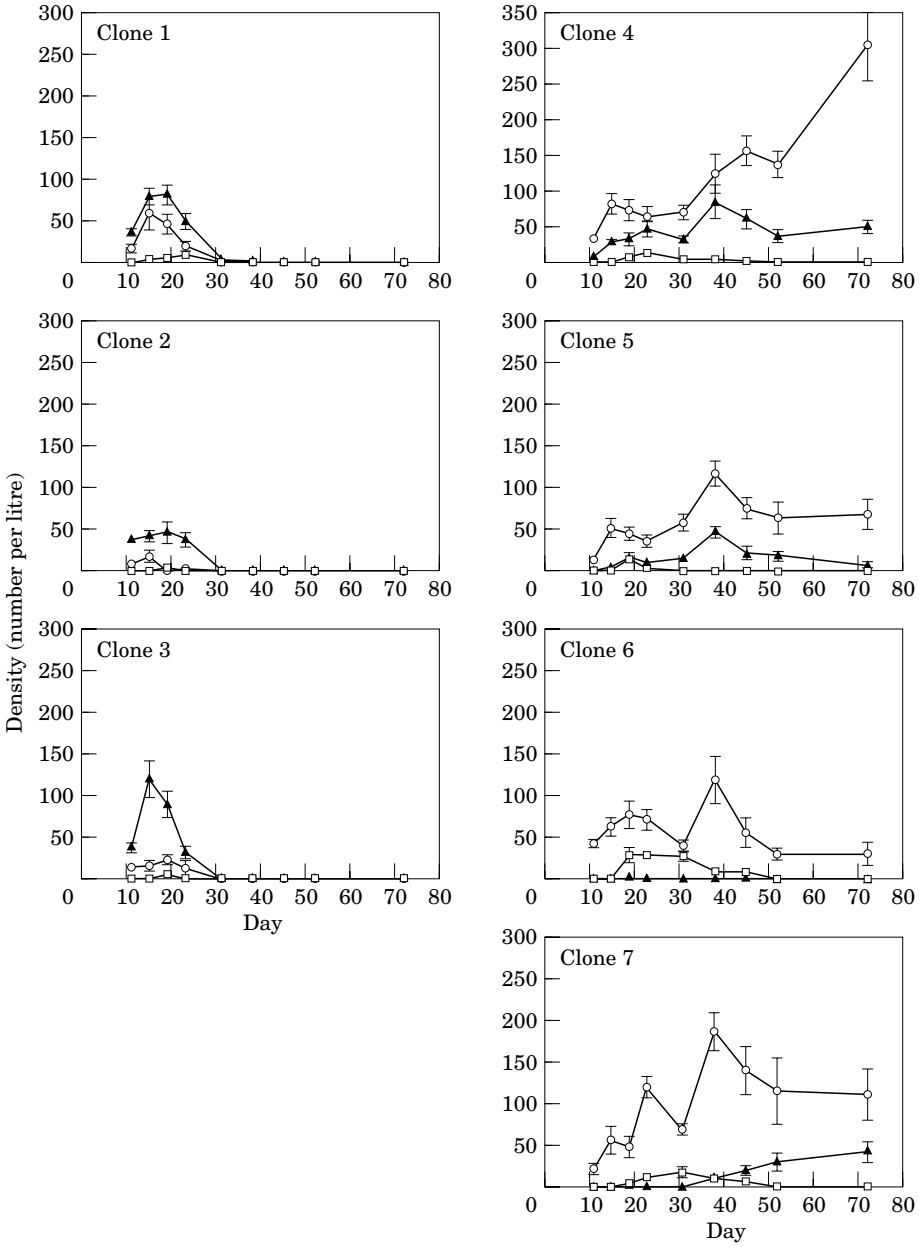

Figure 4. Mean (± SE) density of three life history stages—females (○), males (▲), ephippial females (□)—for clones 1–7 during the Microcosm experiment.

There was a significant difference among the clones in estimated total fitness ($F_{6,21}$ = 4.61, P<0.005) but the comparison of means suggested that only the difference between clones 2 and 4 was significant (Table 2). Greater differences were observed in fitness derived through male ($F_{6,21}$ = 16.8, P<0.001) and female ($F_{6,21}$ = 11.7, P<0.001) function among the clones. The high-male-producing clones (1, 2, 3)

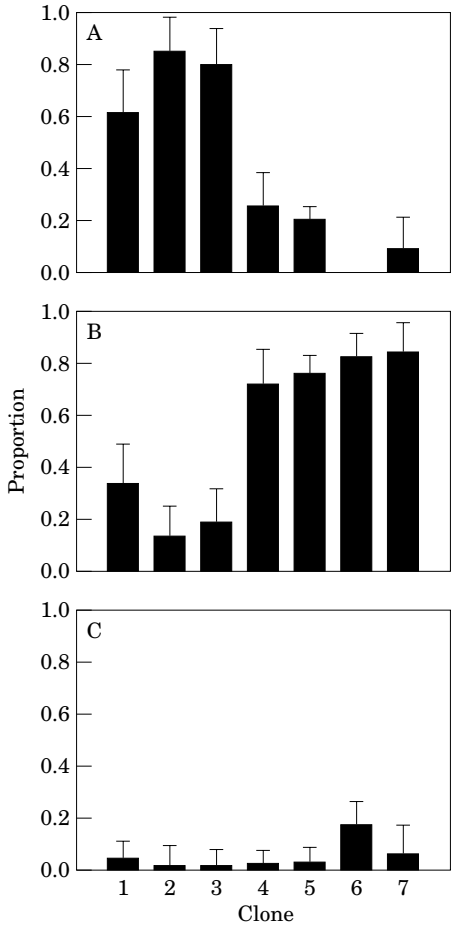

Figure 5. Mean (± SE) relative allocation to (A) males (B) females and (C) ephippial females for clones 1–7 during the 72-day Microcosm experiment.

TABLE 2. Estimated mean fitnesses for *Daphnia pulex* clones over the 72-day Microcosm experiment based on four replicates. Fitness is estimated as the number of haploid genomes contributed to diapausing eggs by each clone in each 300 ml sample summed over the 72-day experiment. Standard errors in parentheses. Fitness estimates are not significantly different for clones with the same letter

	Fitness			
Clone	Relative	Total	Male	Female
1	0.55	17.11 (1.96)^{A,B}	12.86 (1.51)^{A,B}	4.25 (1.60)^{B}
2	0.31	9.58 (1.74)^{B}	8.33 (0.86)^{B,C,D}	1.25 (0.95)^{B}
3	0.45	13.77 (1.54)^{A,B}	12.02 (1.91)^{B,C}	1.75 (0.85)^{B}
4	1.00	30.85 (4.75)^{A}	22.60 (3.36)^{A}	8.25 (1.70)^{B}
5	0.38	11.69 (3.40)^{A,B}	5.94 (2.12)^{B,C,D}	5.75 (1.44)^{B}
6	0.90	27.75 (4.03)^{A,B}	0.00 (–)^{D}	27.75 (4.03)^{A}
7	0.56	17.20 (6.25)^{A,B}	2.20 (1.27)^{C,D}	15.00 (5.29)^{A,B}

derived most of their fitness through male function, as expected (Table 2). Clone 4 also derived most of its fitness through male function due to a combination of producing males early when sexual females were most abundant and the extended period over which it produced males (Fig. 4). Male production in Clone 5 was later than Clone 4 and this clone derived about an equal amount of its fitness through both male and female function over the period of the experiment (Table 2). Clone 6 produced no males and hence all fitness was derived through female function. Clone 7 derived most of its fitness through female function (Table 2) because it produced males later in the experiment, when few sexual females were present (Fig. 4).

Among the seven clones there was a significantly positive correlation ($r_s = 0.96$, $P < 0.001$) between phenotypic gender (proportion male allocation relative to total male and ephippial allocation) and realized gender (proportion of fitness through male function relative to fitness through male and female function). There was also a significantly positive correlation ($r_s = 0.96$, $P < 0.001$) between allocation to females and fitness through female function. However, no significant ($P > 0.05$) correlation was found between fitness through male and female function ($r_s = -0.50$), between allocation to males and fitness through male function ($r_s = 0.64$), or between allocation to ephippial females and allocation to males ($r_s = -0.56$).

DISCUSSION

Cyclical parthenogenesis in *Daphnia* has been characterized as having environmental sex determination, but it is becoming increasingly clear that populations inhabiting ephemeral habitats are polymorphic for investment in sexual reproduction and this variation appears to be expressed as an interaction between genetic variation and environmental conditions (Korpelainen, 1986a, b,1989a,b; Ruvinsky *et al.*, 1986, Larsson, 1991; Yampolsky, 1992; Innes & Dunbrack, 1993; Innes & Singleton, 1994; Deng, 1996). Similar variation in sex allocation has recently been described for cyclically parthenogenetic aphids (Rispe *et al.*, 1999). Explaining the evolution and maintenance of sexual polymorphism in cyclical parthenogenetic species requires information on the pattern and genetic control of the observed reproductive variation. The present study showed that clones of *Daphnia pulex*, under laboratory conditions, exhibited a range of allocation to parthenogenetic compared to sexual reproduction as measured by the relative production of females and males, respectively. Comparable results have been observed in other studies but this was the first to demonstrate reproductive variation in a microcosm where individuals from several clones could interact in a common environment. Clones that produced no males when isolated under low density appeared to be stimulated to produce males by an increase in population density in the microcosm but they may have also been influenced by the presence of males from the high-male-producing clones. Regardless of the mechanism, these clones had a much smaller allocation to males in the microcosm than the three high-male-producing clones. No males were detected for one clone even when exposed to high population density and abundant males in the microcosm. The observed difference in male production among the clones, raised together in a common environment, suggests a significant genetic component to this variation and supports a similar conclusion for *D. pulex* clones raised in isolation (Innes & Dunbrack, 1993; Innes & Singleton, 1994).

Clones with a relatively high investment in males are expected to be at a competitive disadvantage compared to clones that invest primarily in females. Females produce more females and increase the size of a clone in the population. Producing males reduces the growth of a clone and has been referred to as the 'cost of males' (Maynard Smith, 1978). The cost of males has received much attention from a theoretical perspective but has rarely been tested empirically (Ruvinsky *et al.*, 1986; Dunbrack *et al.*, 1995; Jokela *et al.*, 1997; Innes, Fox & Winsor, 2000). The three clones with the greatest investment in males soon went extinct in the microcosm despite the production of some female offspring while the remaining clones with a lower investment in males persisted until the end of the 72-day experiment. The extinction of the high-male-producing clones was not due to smaller brood sizes nor did it appear to be due to greater mortality, confirming that there was a cost to producing males for these clones.

Very high investment in males prevents a clone from persisting in competition with clones with a lower investment in males (Ruvinsky *et al.*, 1986). However, if the growth of a clone was solely due to its relative investment in males, clones with no investment in males should have the greatest advantage. This was clearly not the case in the microcosm experiment. Population density of the clone with no male investment was no greater than for clones with a moderate investment in males. In addition to differences in brood production and survivorship, growth of a clone may also be influenced by differences in the rate females produce ephippia. Environmental conditions stimulate females to interrupt parthenogenetic brood production and produce an ephippium that will contain the sexually produced diapausing eggs if the female has mated or an empty ephippium if not. In either case, a female that invests in an ephippium will delay the opportunity to increase the size of the clone through parthenogenetic reproduction. However, few ephippia were produced in the microcosm and it is unlikely that variation in ephippial production had much of an influence on growth of any of the clones except, perhaps, the clone producing no males. This clone produced the most ephippia and their production may have reduced any advantage the clone gained in growth by not producing males.

A significant component of the total variation in ephippial production in *Daphnia* appears to be due to genotype × environment interaction (Carvalho & Hughes, 1983; Larssen, 1991; Deng, 1996), similar to that found for variation in male production. It was expected that clones with a relatively low allocation to males might exhibit an increased allocation to sexual (ephippial) females to compensate for the loss of fitness through male function. However, ephippial production was low for all clones in the microcosm and the negative association between allocation to males and ephippial females was not significant. Clone 6 showed no allocation to males, but the relative allocation to ephippial females among females (excluding males) was no greater than for any of the male-producing clones. A negative but non-significant relationship was also observed between the estimates of fitness through male and female function. Further studies with a larger number of clones are required to determine if a trade-off exists for the allocation of resources between males and ephippial females in *D. pulex*.

Delaying sexual reproduction until the end of the season in a cyclically parthenogenetic species will normally result in an increase in fitness due to asexual reproduction increasing the number of individuals of a clone that can participate in sexual reproduction. Conversely, delaying sexual reproduction in a temporary habitat runs the risk of extinction without contributing to the pool of sexual,

diapausing eggs. Thus a trade-off is expected between sexual and asexual reproduction and the pattern of allocation that translates into the maximum contribution to sexual eggs for a particular set of environmental conditions will be favoured (Hughes & Cancino, 1985; Yund, Marcum & Stewart-Savage, 1997). A trade-off in sex allocation does not preclude the possibility of a polymorphism for sex allocation being maintained in a population (Doums, Viard & Jarne, 1998). In addition to the present study, several other studies have demonstrated that populations of *Daphnia* are composed of genotypes that vary in the pattern of allocation to sexual and asexual reproduction (Korpelainen, 1986b, 1989b; Larsson, 1991; Yampolsky, 1992; Spaak, 1995). How variation in different allocation patterns translates into fitness is difficult to determine. The estimates of fitness for each clone in the microcosm experiment are based on the calculation of potential rather than actual mating between males and sexual females for a non-random collection of clones. Nevertheless, the experiment provided an indication of how clonal variation in sex allocation combined into an artificial population might translate into variation in fitness.

Total estimated fitness was divided into a component through male function and a component through female function. Clone 6 obtained fitness only through female function and the remaining clones obtained fitness through both male and female function. Clone 4 had the highest estimated fitness because it had a high allocation to males, but not at the expense of females. Sufficient females were produced to allow the clone to persist and dominate the microcosm unlike the three high-male-producing clones. Clone 4 gained a high fitness because it continued to produce males after clones 1, 2 and 3 had gone extinct and there was little competition for mating with the ephippial females that were still being produced, particularly by clone 6. There was strong competition for mates among males from the three high-male-producing clones early in the experiment possibly reducing their fitness through male function (Yund, 1998). The estimated fitness also showed that although the three clones went extinct due to the cost of producing males, much of this cost was recovered when males from these clones mated with ephippial females. The total fitness for these clones was as high as some clones that persisted in the microcosm because of a higher investment in parthenogenetic reproduction. Clone 6 obtained fitness only through female function, but had the second highest estimated fitness because it produced the greatest number of ephippial females and there was no competition for mates among ephippial females due to the abundance of males in the microcosm. Although the clones varied greatly in fitness obtained through male and female function, total fitness showed much less variation. Similarity of total fitness among clones varying in sex allocation suggests that the observed sex-allocation polymorphism in populations of *D. pulex* is maintained by a trade-off between fitness through male and female function. However, a non-significant negative correlation was observed between fitness through male and female function, but the small number of clones tested probably limited the power to detect a trade-off. Also, the estimates of fitness were based on the relative frequency of males and ephippial females of each clone in the samples rather than direct estimates of the contribution of each clone to diapausing eggs. Recently developed microsatellite genetic markers (J. Colbourne, pers. com.) that can assign paternity from single *D. pulex* diapausing eggs, will undoubtedly be very useful for determining male mating success in future studies and refine the estimates of fitness for individuals varying in sex allocation.

Combining environmental sex determination with parthenogenetic reproduction,

a single genotype of *Daphnia* is capable of producing genetically identical males and sexual females. Although the two sexes are independent units, males and sexual females with the same genotype can be produced over a short period so they can potentially coexist for a period of time. This mode of reproduction bears a resemblance to simultaneous hermaphroditism and models of sex allocation for these organisms may be applicable to species of *Daphnia* (Charnov, 1982; Brunet, 1992; Innes & Dunbrack, 1993). These models attempt to predict the optimum allocation of limited resources to male and female function and can also be used to predict the conditions under which hermaphroditism is favoured over separate sexes (dioecy). Much of the empirical evidence for testing sex allocation theory has come from hermaphroditic plants where there may be some difficulty in deciding what currency should be used to measure sex allocation and how to apportion reproductive resources for structures used to attract pollinators (Brunet, 1992; McKone, Lund & O'Brien, 1998). Empirical measures of sex allocation may be easier in some animals such as *Daphnia* where the separate allocation of resources to male and female function is more straightforward (Johnston, Das & Hoeh, 1998). Clones of *D. pulex* exhibit a wide variation in sex allocation similar to that observed in some hermaphroditic animals (McCartney, 1997; Yund *et al.*, 1997; Yund, 1998). Although the causes of this variation require further study, a sex-allocation model described by Innes & Dunbrack (1993) suggested conditions for maintaining male-producing and non-male-producing clones within a population of *Daphnia pulex*. The model predicted that populations with non-male-producing females should contain females with a biased allocation to males. The current observation of clones with a very high allocation to males provides support for this model.

Cyclical parthenogenesis presents a challenge to sex allocation theory. Several approaches are required to answer basic questions on the evolutionary relationship between sexual and asexual reproduction in *Daphnia* and other cyclically parthenogenetic species. The present study showed that *D. pulex* populations in temporary habitats maintain a sex-allocation polymorphism. In addition, the microcosm experiment was found to be useful for studying the pattern of sex allocation among several interacting *Daphnia pulex* clones and the relationship between parthenogenetic and sexual reproduction. Information from microcosm experiments combined with measurements of sex allocation under precisely controlled laboratory conditions and observations of sexual reproduction in natural populations will provide the information necessary to explain observation life-history variation among species of *Daphnia* inhabiting a variety of pond and lake habitats. *Daphnia* species provide an excellent model for elucidating factors determining the optimal allocation to sexual and asexual reproduction in the same life cycle (Green & Noakes, 1995), particularly since clones varying in sex allocation can easily be reared together in experimental microcosms.

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