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Avoiding the cost of males in obligately asexual *Daphnia pulex* (Leydig)

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Asexual organisms are thought to gain an advantage by avoiding the cost of producing males. In the cladoceran *Daphnia pulex* (Leydig), male production is determined by the environment and is independent of the origin of the asexual obligate parthenogens from the sexual cyclical parthenogens. If there is a cost to producing males, successful obligate parthenogens should have reduced or eliminated male production. Field and laboratory observations showed that obligate parthenogens have much-reduced male production compared to cyclical parthenogens. Although the reduction or elimination of males in the obligate parthenogens suggests that the cost of males is avoided, the coexistence of sexual and asexual forms of *D. pulex* may be partially explained by cyclical parthenogens compensating for the cost of males by having greater fecundity. In addition, the absence of a mating constraint for the obligate parthenogens may favour an increased allocation to asexual diapausing eggs earlier in the season compared to the cyclical parthenogens which require mating with males to produce sexual diapausing eggs. No difference in the production of diapausing eggs was observed, probably because males were abundant in populations of cyclical parthenogens and do not appear to limit the production of sexual diapausing eggs. *D. pulex* is a useful system for determining the ecological consequences of abandoning sexual reproduction and explaining the coexistence of sexual and asexual forms of a species.

Keywords: obligate parthenogenesis; cyclical parthenogenesis; sex and evolution; cost of males; evolution

1. INTRODUCTION

Explaining the ubiquity of sex among plants and animals continues to be a major preoccupation in evolutionary biology (Williams 1975; Maynard Smith 1978; Bell 1982; Stearns 1987; Michod & Levin 1988; Hurst & Peck 1996; Hines & Culotta 1998). The evolution and dominance of sexual compared to asexual reproduction has been described as a paradox because, while the costs of sex are explicit, the benefits are vague (Williams 1975; Maynard Smith 1978). For example, a female which produces all female offspring asexually avoids the cost of producing males and has twice the reproductive output compared to a female which produces the same number of sexual eggs, half of which become male. All else being equal, asexual females should easily out-compete sexual females in the short term and become the dominant mode of reproduction.

Many asexual taxa appear to be recently derived from closely related sexual species and there has been much interest in documenting the ecological differences which may explain their coexistence (Vrijenhoek 1979, 1993; Lynch 1984). The theoretical twofold advantage of asexual reproduction has rarely been tested in order to determine whether the coexistence is truly a paradox or whether asexual taxa suffer ecological or genetic disadvantages which may offset the apparent advantage due to avoiding the cost of males (Michaels & Bazzaz 1986; Dunbrack *et al.* 1995; Jokela *et al.* 1997). For example, comparisons between some closely related parthenogenetic and sexual insect species have revealed that parthenogenetic forms often exhibit reduced fecundity, hatching success and offspring survival (Lamb & Willey 1979; Lynch 1984; Corley & Moore 1999). Therefore, avoiding the cost of males may provide no real advantage for some parthenogenetic species.

Direct competition experiments between closely related sexual and asexual forms have also been used to provide information on the relative fitness of each mode of reproduction. Within the *Artemia* species complex, Old World sexual species were consistently out-competed by parthenogenetic species (Browne & Halanych 1989). However, the outcome appeared to be related to the greater brood size, rate of reproduction and life span of parthenogenetic females rather than a fitness advantage of not producing males (Browne *et al.* 1984; Browne 1992). In contrast, a study comparing coexisting diploid sexual and triploid parthenogenetic freshwater snails (*Potamopyrgus antipodarum*) found very little in the way of life-history differences and a parthenogenetic clone showed greater population growth when in competition with a sexual population (Jokela *et al.* 1997). The outcome suggested that factors compensating for the cost of males must be involved in the maintenance of sex in this species. In addition, Dunbrack *et al.* (1995) provided experimental evidence that a greater evolutionary potential for sexual reproduction may easily compensate for the cost of producing males.

The planktonic cladoceran *Daphnia pulex* (Leydig) consists of cyclical parthenogens with sexual reproduction and obligate parthenogens with no sexual reproduction (Hebert *et al.* 1988). Both forms occupy small temporary ponds in the same area but rarely coexist in the same pond (Hebert *et al.* 1988, 1989). Evidence from mitochondrial DNA and genetic studies has confirmed that the genotypically diverse obligate parthenogens are of recent origin and new clones are likely to be continually produced (Innes & Hebert 1988; Crease *et al.* 1989). Successful and persistent obligately parthenogenetic

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clones are expected to be a subset of all the clones produced. Therefore, studies on *D. pulex* provide an opportunity for determining whether the transition to obligate parthenogenesis results in clones with specific characteristics which differ from the sexual forms and explains their coexistence.

Cyclical and obligate parthenogens both produce broods of parthenogenetic eggs which develop immediately and separate diapausing eggs enclosed in ephippia (modified carapace) which survive freezing and desiccation and represent the primary dispersal stage. Populations are re-established from diapausing eggs in the spring. Environmental conditions such as a short-day photoperiod and increased density stimulate the production of males and sexual females in the cyclical parthenogens. Sexual females produce haploid, diapausing eggs which require fertilization in order for eggs to be released into the developing ephippium. Unmated females will not release eggs and moult an empty ephippium. Thus, the cost in time and energy required for diapausing egg and ephippia production suggests that the appearance of sexual females should be tied to male production resulting in a mating constraint on the timing of ephippia production in the cyclical parthenogens.

Obligate parthenogens suppress meiosis and release diploid (unreduced) diapausing eggs into the ephippium (Hebert & Crease 1983; Innes & Hebert 1988). Because there is no mating requirement, the production of diapausing eggs is not as constrained as it is for the cyclical parthenogens. Furthermore, males are no longer required, but the loss or reduction of males is independent of the suppression of meiosis during the formation of diapausing eggs. In fact, some obligate parthenogens have retained the ability to produce males and mating of these males with sexual females from the cyclical parthenogens is thought to be the mechanism which generates new obligately parthenogenetic clones (Innes & Hebert 1988; Hebert *et al.* 1989). Genetic variation for diapausing egg and male production, including genotypes producing no males, occurs within the cyclical parthenogens (Larsson 1991; Innes & Dunbrack 1993; Innes & Singleton 1994; Deng 1996; Innes 1997). Newly produced obligately parthenogenetic clones are expected to show a range of investment in males and variation in the pattern of diapausing egg production for natural selection to act on. Given the cost of males, clones with a reduced investment in males should have a selective advantage.

In the present study, a combination of field and laboratory observations compared male and diapausing egg production in cyclical and obligately parthenogenetic *D. pulex*. Specifically, it was predicted that investment in males should be eliminated or significantly reduced in the obligate parthenogens compared with the cyclical parthenogens. In addition, because of the absence of a mating constraint, obligate parthenogens may also show a higher investment in diapausing eggs earlier in the season compared with cyclical parthenogens.

2. MATERIAL AND METHODS

(a) *Samples from natural populations*

During early spring of 1997 (between 29 April and 13 May), seven ponds containing obligate parthenogens (Lz, Bl, Mor, War, Rus, W1 and W2) and six ponds containing cyclical parthenogens (Beth, Mar, Tex, LP8A, LP8B and LP9A) were sampled in southern Ontario and eastern Michigan soon after the ponds became re-established from diapausing eggs. The mode of reproduction of the individuals in the ponds was determined from previous surveys based on the genotypic structure of allozyme variation. The pond identification numbers previously sampled by Hebert *et al.* (1989) are ponds 17 (Lz), 21 (Bl), 69 (Mor), 73 (War), 27 (Rus), 76 (Beth), 74 (Mar) and 72 (Tex). Ponds W1 and W2 were described by Hebert & Crease (1983) and ponds LP8A, LP8B and LP9A by Innes (1991). The samples were preserved in 95% ethanol for later enumeration of females, males and ephippial females. Frequencies were analysed using a *G*-test (Sokal & Rohlf 1981). Samples of live, brood-carrying females from each pond were returned to the laboratory and the sex of the released brood determined. Individual females were also set up in separate 150 ml plastic beakers with zooplankton media (Lynch *et al.* 1986), and fed a slurry of aquarium-cultured algae (primarily *Chlorella* sp.). Parthenogenetic reproduction of the isolated females established clones of genetically identical offspring from both the obligate and cyclical parthenogens. The clones were examined periodically for the presence of males under uncontrolled crowding in the laboratory.

(b) *Clonal variation in ephippia production*

A total of 13 obligately parthenogenetic clones (Lz (O1), Mor (O2 and O3), War (O4–O6), W1 (O7–O9) and W2 (O10–O13)) and 17 cyclically parthenogenetic clones (Beth (C1–C3), Mar (C6), Tex (C7), 8A (C9–C12 and C14), 8B (C16–C18) and 9A (C19–C21)) were tested for their rates of ephippia production in four experiments (A1–A4). Each clone was set up in duplicate 150 ml plastic beakers with 40 ml of zooplankton media in each experiment and ten young, non-ephippial females maintained in an incubator at 15 °C under a 16 L:8 D photoperiod. All beakers received 2–3 ml of aquarium-cultured algae daily. The beakers were examined daily and any released ephippia counted and removed. Any neonates and excess media which accumulated due to feeding were removed to maintain a constant volume and density. Experiment A1 was run for 15 days, A2 for 28 days, A3 for 34 days and A4 for 20 days.

SAS PROC GLM was used to perform a nested ANOVA for each experiment with clone nested within reproductive type (obligate or cyclical parthenogen) on the number of ephippia produced per day per female following a square-root transformation (Sokal & Rohlf 1981). The α-level was set at $0.05/4 = 0.0125$ to correct for multiple tests.

(c) *Clonal variation in offspring and male production*

Four male-producing obligately parthenogenetic clones (from Lz, Mor and War), a fifth from Spru (pond 1 described by Hebert *et al.* (1989)) and five male-producing cyclically parthenogenetic clones (from Tex, 8A and 8B) were tested for their total offspring and male production under two densities (one female per 40 ml and ten females per 40 ml) and two photoperiods (long day 16 L:8 D and short day 8 L:16 D) with two replicates per treatment. Except for Tex-27 (C8) and War-15 (O6), different clones were used because most of the clones previously described in the ephippia experiment were no longer in culture. The high-density beakers received 2 ml of algae per day and the low-density beakers received 1 ml of algae per day. Each beaker was examined daily and newly released neonates were counted, sexed and removed during the 15 day experiment. Statistical

Table 1. *Proportion of females, males and ephippial females in natural populations of obligately and cyclically parthenogenetic* D. pulex

(The column of male broods shows the number of male broods observed among brood-carrying females sampled from each pond (few brood-carrying females were present in the obligate parthenogen ponds). The last column shows the number of laboratory clones which produced males. *n*, sample size.)

pond	*n*	female	male	ephippial	*n*	male broods	*n*	clones producing males
obligate parthenogens								
Lz	275	1.000	0.000	0.000	—	—	30	9
W1	266	0.936	0.008	0.056	6	0	38	0
W2	242	0.983	0.000	0.017	6	1	21	0
BL	118	0.975	0.025	0.000	—	—	17	0
Mor	110	1.000	0.000	0.000	—	—	28	10
War	107	0.935	0.000	0.065	—	—	10	6
Rus	51	0.941	0.000	0.059	—	—	18	0
cyclical parthenogens								
Tex	222	0.613	0.374	0.014	21	11	26	18
Mar	52	0.827	0.173	0.000	19	10	16	9
Beth	104	0.856	0.134	0.010	16	9	29	24
LP8A	169	0.822	0.089	0.089	21	11	99	56
LP8B	101	0.970	0.030	0.030	20	11	83	56
LP9A	153	0.876	0.124	0.000	21	12	61	28

analysis used the SAS GLM procedure following an arcsine, square-root transformation for the proportion of males and a square-root transformation for counts of the total numbers of offspring (Sokal & Rohlf 1981).

(d) *Microcosm experiment*

Microcosm experiments were conducted in order to determine the total allocation of each clone to males and ephippial females when cultured together. Obligately parthenogenetic and cyclically parthenogenetic clones were placed together in three replicate jars with 2 l of zooplankton media. Each of the clones within a jar was uniquely identified based on three allozyme loci (*Pgm*, *Pgi* and *Amy*). The allozyme genotypes were identified using cellulose acetate electrophoresis (Hebert & Beaton 1989). Each jar was inoculated with four pre-reproductive females from each of the clones and maintained in an incubator at 15 °C under a 16 L:8 D photoperiod. Each jar received 5 ml of aquarium-cultured algae daily and excess media was poured off without losing any animals in order to maintain the volume at 2 l. Experiment B1 contained clones O1, O4, O6, C5, C18 and C21 and was sampled on days 22, 24, 27, 30, 36 and 41. Experiment B2 contained clones O1, O2, O6, C4 and C13 and was sampled on days 25, 32, 39 and 46. Previously described clones were used except two clones (C4, C5) from the DISP pond (D. J. Innes, unpublished results) and C13 was from pond LP8A. During each sample day a selected number of ephippial females, non-reproductive females and males were genotyped to determine clone membership. The total proportion allocated to males and ephippial females for each clone for the duration of each experiment was analysed by a nested ANOVA (clones nested within reproductive type) following an arcsine, square-root transformation.

3. RESULTS

The ponds of cyclical parthenogens contained a much greater frequency of males compared to the ponds of obligate parthenogens (table 1). Out of the 1169 individuals examined from the obligate parthenogen ponds only five males (0.4%) were observed compared with 143 males observed among the 801 (17.9%) individuals from the cyclical parthenogen ponds ($G = 234.0$, d.f. $= 1$ and $p < 0.001$). Ephippial females were rare (table 1) and there was no significant difference ($G = 0.25$, d.f. $= 1$ and $p > 0.05$) in their frequency in the samples from the obligate and cyclical parthenogen ponds (2.5 and 2.4%, respectively). Approximately half of the brood-carrying, cyclically parthenogenetic females were carrying male broods at the time samples were taken from all six ponds. Few brood-carrying females were observed for the obligate parthenogens and only one male brood was observed among the 12 sampled. Out of the 314 cyclical parthenogens established as clones in the laboratory, males were detected in approximately half of the clones from each sample (table 1). In contrast, few of the 162 obligately parthenogenetic clones produced males (table 1). However, males were observed (table 1) in a number of clonal isolates from Lz (nine out of 30), Mor (ten out of 28) and War (six out of ten).

There was significant variation (figure 1) in the rate of ephippia production among clones ($F_{8,10} = 12.8$, 22.7 and 8.17, and $p < 0.002$) within each reproductive type for experiments A1–A3, but no significant difference in the rate of ephippia production between the cyclical and obligate parthenogens within each experiment ($F_{1,8} = 4.88$, 3.07 and 1.25, and $p > 0.05$). The rate of ephippia production was significantly greater for the obligately parthenogenetic clones in experiment A4 (figure 1) ($F_{1,6} = 22.7$ and $p < 0.005$) but there was no significant difference ($F_{6,8} = 3.51$ and $p > 0.05$) between the clones within each reproductive type. Eight clones (O3, O4, O11, O12, C2, C3, C6 and C7) were tested in both experiments A2 and A3 and there was a significantly positive correlation

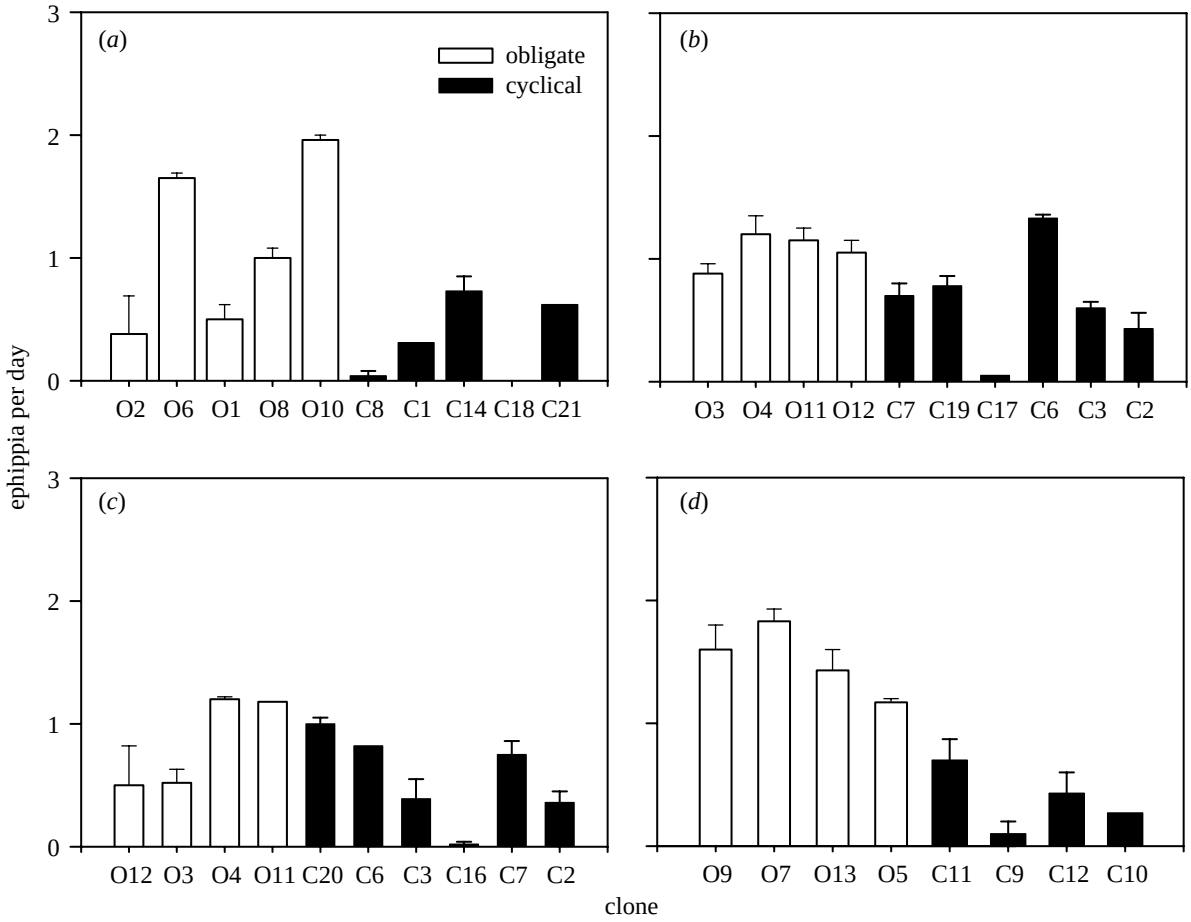

Figure 1. The rate of ephippia production for females from obligate (O) and cyclical (C) parthenogen clones in four experiments; (*a*) A1, (*b*) A2, (*c*) A3, (*d*) A4. Averages of two replicates with standard errors.

(Spearman's rank correlation = 0.83 and $p < 0.05$) for the rate of ephippia production between the two experiments.

No males were observed for any clone at low density and under a long-day photoperiod (table 2). Only two out of the five obligate parthenogen clones produced a very small number of males under high density and a short-day photoperiod. In contrast, most of the cyclical parthenogens showed an increase in male production with increased density and short-day photoperiod. Four out of the five cyclical parthenogens responded to the high density and short-day photoperiod by producing a significantly ($F_{1,8} = 112.0$ and $p < 0.001$) greater proportion of male offspring than the obligate parthenogens (table 2). There was also significant ($F_{8,10} = 7.5$ and $p < 0.01$) between-clone variation in the proportion of male offspring produced.

The photoperiod did not influence the total number of offspring produced and the photoperiod treatments were pooled. For the lowest density, the average total number of offspring produced per female was not significantly different ($F_{1,8} = 1.05$ and $p > 0.05$) between the obligate and cyclical parthenogens, but there was a significant difference between clones within each reproductive type (table 2). This was particularly obvious for the Spru clone which produced many more offspring than the other obligate parthenogens. Fewer offspring were produced per female at the higher density but the cyclical parthenogens produced significantly more total offspring per female than the obligate parthenogens ($F_{1,8} = 48.9$ and $p < 0.001$). There was also significant variation in the total offspring production between clones within each reproductive type ($F_{8,30} = 23.9$ and $p < 0.001$). Excluding males, there was no significant difference in the total number of female offspring produced between the cyclical and obligate parthenogens either at the higher ($F_{1,8} = 1.50$ and $p > 0.05$) or lower ($F_{1,8} = 0.12$ and $p > 0.05$) densities.

Figure 2 summarizes the total allocation to males and ephippial females for each clone in the microcosm experiments. No males were detected for clone C21 in the stock cultures and no males were detected in the microcosm. The proportion of males produced by the obligate parthenogens was significantly lower ($F_{1,8} = 47.2$ and $p < 0.001$) than the proportion produced by the cyclical parthenogens (figure 2), excluding clone C21. However, the proportion of ephippial females produced by the obligate parthenogens was significantly greater ($F_{1,9} = 11.4$ and $p < 0.01$) than the cyclical parthenogens (figure 2). If males were excluded and the proportion of ephippial females was expressed as a proportion of all females, there was no significant ($F_{1,9} = 0.07$ and $p > 0.05$) difference in allocation to ephippia between the obligate and cyclically parthenogenetic clones.

4. DISCUSSION

Surveying ponds in early spring found dense populations of *D. pulex*, a situation which stimulates the production of males (Innes & Singleton 1994; Innes 1997).

Table 2. *Proportion of male offspring produced and average total number of offspring produced per female after 15 days for obligately and cyclically parthenogenetic clones of* D. pulex *raised under two photoperiods* (*long day and short day*) *and two densities* (*one female per 40 ml and ten females per 40 ml*) *with two replicates per treatment*

(Standard errors in parentheses.)

	proportion of male offspring					
	long day		short day		average total offspring per female	
density	1	10	1	10	1	10
obligate parthenogen clones						
Lz-24	0	0.00	0.00	0.04 (0.04)	15.5 (4.9)	8.8 (1.0)
Mor-28	0	0.00	0.00	0.00	11.3 (2.8)	5.0 (0.7)
War-3	0	0.00	0.00	0.00	11.8 (2.5)	5.8 (1.4)
War-15	0	0.00	0.00	0.00	10.8 (2.9)	3.6 (0.5)
Spru	0	0.00	0.09 (0.09)	0.10 (0.07)	62.8(13.8)	24.9 (4.3)
cyclical parthenogen clones						
Tex-27	0	0.39 (0.053)	0.34 (0.26)	0.59 (0.04)	25.0 (4.3)	18.6 (2.8)
8A-16	0	0.14 (0.022)	0.51 (0.37)	0.73 (0.36)	38.3 (7.6)	29.1 (1.9)
8A-18	0	0.00	0.47 (0.06)	0.74 (0.10)	29.5 (5.3)	21.4 (3.1)
8A-24	0	0.00	0.00	0.08 (0.08)	13.3 (2.2)	7.0 (0.3)
8B-5	0	0.02 (0.020)	0.13 (0.13)	0.30 (0.03)	10.3 (3.4)	8.2 (0.3)

Some males were detected in ponds with obligate parthenogens, but many fewer than in ponds containing cyclical parthenogens. Several of the clonal isolates of the obligate parthenogens established from single females produced males. However, it is possible that some of the isolates were the same clonal genotype since genetic studies have shown that most ponds of obligate parthenogens consist of only one or a few clones (Hebert *et al*. 1988, 1989). Approximately half of the brood-carrying females from ponds of cyclical parthenogens were carrying male broods and males were detected in approximately half of the clonal isolates establishes from single females, consistent with previous studies from this area (Innes & Dunbrack 1993; Innes 1997).

The level of male production for five obligately parthenogenetic clones was found to be very low even under the crowded, short-day photoperiod conditions which stimulated high male production in the cyclically parthenogenetic clones. Although males were observed in all five obligately parthenogenetic clones under uncontrolled crowding in stock cultures, no males were produced by three of the clones during the experiment. Apparently, the level of crowding (or some other factor) in the experiment was not sufficient to stimulate male production in these clones. The much higher male production in the cyclical parthenogens suggests that they have a cost of males which the obligate parthenogens appear to avoid. This cost assumes that the offspring production is the same for both forms. However, the offspring production was greater for the cyclical parthenogens. In fact, the average number of female offspring was similar between the two forms suggesting no cost to males when compared to these particular obligate parthenogens under laboratory conditions. One exception was the Spru clone which produced a much greater number of offspring than the other obligately parthenogenetic clones and most of the cyclically parthenogenetic clones as well. Furthermore, the Spru clone showed a low investment in males suggesting that the cyclical parthenogens have a cost to producing males when compared to this particular clone. Further sampling will be required in order to determine whether obligately parthenogenetic clones with such a high level of offspring production are common.

The lack of males being produced by the obligate parthenogen clones was not an artefact of rearing each clone in isolation. The obligate parthenogen clones tested in the microcosm experiments were exposed to males and females from other clones under crowded conditions, but produced significantly fewer males than the cyclical parthenogens under the same conditions. The reduced number of males produced by the obligate parthenogens did not translate into an automatic advantage because there was no consistent pattern in the relative frequency of the obligate and cyclical parthenogens after around 40 days in the microcosm experiments. Clearly, the cost of producing males by the cyclical parthenogens can be compensated for by increased fecundity and/or survivorship relative to the obligate parthenogens. In addition, the cost of males for the cyclical parthenogens is only relevant when compared to obligate parthenogens which have identical life-history characteristics except for their level of male production.

Hebert *et al*. (1989) identified 36 obligately parthenogenetic clones using 11 polymorphic enzyme loci and males were observed in 13 clones. They noted that rare clones were more likely to produce males than common clones, suggesting that the success of the common clones may be partially due to avoiding the cost of males. The present study showed that obligately parthenogenetic clones which have retained the ability to produce males produce very few males and far fewer than the average production of males by the cyclical parthenogens. This observation is

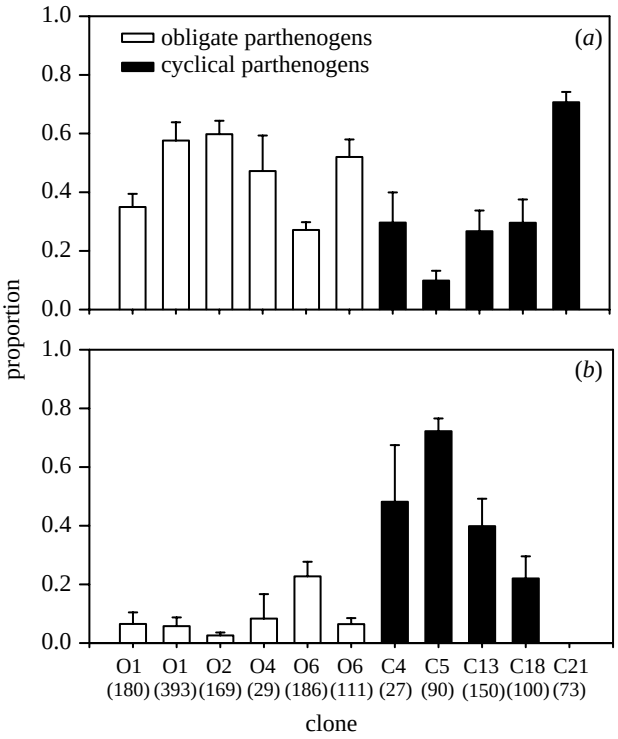

Figure 2. Total proportion allocated (average of three replicates with standard error) to (*a*) ephippia and (*b*) males for obligate (O) and cyclical (C) parthenogen clones raised together in a microcosm. Sample sizes are given in parentheses.

consistent with the origin of new obligately parthenogenetic clones which inherit variation in allocation to males from the cyclical parthenogens followed by selection favouring clones with a reduced or no allocation to males.

The observed pattern of diapausing egg production did not fit the prediction of a greater production by the obligate compared to the cyclical parthenogens. A very low occurrence of ephippial females was observed in natural populations of both the cyclical and obligate parthenogens. In addition, no large differences were observed in the rate of ephippia formation for the obligate and cyclical parthenogen clones in the laboratory experiment or the microcosm. Thus, the obligate parthenogens did not appear to differ from the cyclical parthenogens in ephippia formation, despite the lack of a mating constraint. It is likely that ephippia production for both forms is at a level which allows parthenogenetic reproduction to increase a genotype's representation in the population and, hence, make a greater contribution to the pool of diapausing eggs (Serra & King 1999). Furthermore, males appear to be abundant in cyclically parthenogenetic populations very early in the season suggesting that ephippia formation is rarely constrained by the lack of males (Innes 1997).

The theoretical cost of males assumes that the sexual and asexual forms are identical except for the production of males by the sexual forms (Jokela *et al.* 1997; Corley & Moore 1999). There is no absolute cost of males and the cost can only be relative to a particular sample of sexual and asexual individuals and this must be evaluated in each case. Lynch *et al.* (1989) compared life-history characteristics among 12 obligately parthenogenetic clones with cyclically parthenogenetic *D. pulex*. They concluded that the obligate parthenogens have a fitness disadvantage due in part to the production of smaller clutches. However, male production was not measured and the obligate parthenogens may gain a fitness advantage by avoiding the cost of males. The present study also found a lower fecundity for most of the obligate parthenogens. A lower fecundity combined with an avoidance of the cost of males suggests that the two forms have a similar reproductive output and this may partially explain their coexistence in the same area.

Why do some obligately parthenogenetic *D. pulex* retain the ability to produce males? Periodic contact between obligate and cyclical parthenogens may select for the retention of males by the obligate parthenogens because mating between these males and sexual females from the cyclical parthenogens results in some obligately parthenogenetic progeny (Innes & Hebert 1988). Therefore, avoiding the cost of males in obligately parthenogenetic *D. pulex* has to be examined within the context of the potential benefits of retaining the production of males. A theoretical model has shown that obligate parthenogens with some investment in males may have the greatest fitness advantage (Joshi & Moody 1998). The model also suggested that geographical substructuring such as isolated ponds of *D. pulex* may explain the coexistence of sexual and asexual populations in the same geographical area.

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