

# The Cost of Sex and Competition between Cyclical and Obligate Parthenogenetic Rotifers

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**ABSTRACT:** The ubiquity of sexual reproduction is an evolutionary puzzle because asexuality should have major reproductive advantages. Theoretically, transitions to asexuality should confer substantial benefits in population growth and lead to rapid displacement of all sexual ancestors. So far, there have been few rigorous tests of one of the most basic assumptions of the paradox of sex: that asexuals are competitively superior to sexuals immediately after their origin. Here I examine the fitness consequences of very recent transitions to obligate parthenogenesis in the cyclical parthenogenetic rotifer *Brachionus calyciflorus*. This experimental system differs from previous animal models, since obligate parthenogens were derived from the same maternal genotype as cyclical parthenogens. Obligate parthenogens had similar fitness compared with cyclical parthenogens in terms of the intrinsic rate of increase (calculated from life tables). However, population growth of cyclical parthenogens was predicted to be much lower: sexual female offspring do not contribute to immediate population growth in *Brachionus*, since they produce either males or diapausing eggs. Hence, if cyclical parthenogens constantly produce a high proportion of sexual offspring, there is a cost of sex, and obligate parthenogens can invade. This prediction was confirmed in laboratory competition experiments.

**Keywords:** maintenance of sex, fitness, life-history evolution, cost of males, *Brachionus calyciflorus*, parthenogenesis.

## Introduction

Asexual lineages have emerged in almost all multicellular taxa, yet most of them seem to be prone to early extinction (Maynard Smith 1978; Bell 1982). Theoretically, transitions to asexuality in sexual species should confer substantial reproductive benefits and lead to rapid displacement of all sexual ancestors (Lively 1996). Likewise, transitions to obligate parthenogenesis in organisms with mixed reproductive modes (e.g., cyclical parthenogens) should give rise to obligatory parthenogenetic lineages that are competitively superior to their ancestors with mixed

reproductive mode. In real natural systems, however, such competitive replacements seem to be rare, and many theories have been proposed explaining why sexual reproduction is stabilized in almost any animal and plant life cycle (e.g., Kondrashov 1993; Barton and Charlesworth 1998; Agrawal 2006; Otto 2009).

There have been few rigorous tests of one of the most basic assumptions in the paradox of sex: the competitive superiority of asexuals directly after they originated from their sexual ancestors. Previous comparisons involving sexual and asexual reproductive types have yielded mixed results. In several studies, asexual lines actually had lower fitness than sexuals. For instance, it was often found that obligate parthenogens had higher mortality (Roth 1974; Corley and Moore 1999; Kramer and Templeton 2001) or reduced fecundity (Wetherington et al. 1987; Kearney and Shine 2005). Such observations have led to criticisms that there may be no paradox of sex from an empirical point of view (Lamb and Willey 1979). Yet in other studies, it was found that asexuals and sexuals had similar fecundity and survival schedules (Jokela et al. 1997; Wolinska and Lively 2008), implying that sexual females will produce fewer female offspring and thus pay a cost of sex. In these cases, sex is paradoxical, and alternative explanations for the maintenance of sex need to be sought.

Most of these previous studies compared fitness and competitive abilities of asexuals versus sexuals sampled from field populations (but see Wetherington et al. 1987). A potential problem with this approach is that the closest sexual relative of an asexual line is usually unknown, and this may have implications on the validity of the fitness comparisons. Inferences about sexual relatives are usually drawn indirectly by phylogenetic reconstruction (e.g., Crease et al. 1989; Schön and Butlin 1998; Delmotte et al. 2001; Neiman et al. 2005; Paland et al. 2005). Thus, the exact age of an asexual line is difficult to determine, since a sample may have missed the closest sexual relatives (for a discussion of this problem, see Neiman et al. 2005). Fitness estimates will therefore be confounded, because the evolutionary histories of such lines might have been

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separated for a long time, and asexuals may already suffer from secondary effects of long-term asexuality, such as mutation accumulation. In addition, the phylogenetic approach likely underestimates the rate at which asexual lineages arise, since it can recognize only moderately successful transitions during evolution of a clade, which may lead to an upward bias of fitness estimates of asexual lines. Less successful asexual lineages that gave rise to only small and transient populations are likely missing in such analyses. In summary, fitness comparisons between asexuals and sexuals sampled from field populations are influenced by a variety of factors, which prevents measurements of the immediate fitness effects of transitions to asexuality.

Here I test the fitness effects of transitions to obligate parthenogenesis in an experimental model system, the monogonont rotifer *Brachionus calyciflorus*. This model system avoids the previously mentioned pitfalls because obligate parthenogens can be generated experimentally. Monogonont rotifers normally reproduce by cyclical parthenogenesis, an alternation between ameiotic parthenogenesis and sexual episodes (Nogrady et al. 1993). In cyclical parthenogenetic rotifers, sex is initiated with the production of sexual females, whose oocytes undergo meiosis and develop into haploid males (if not fertilized) or diploid diapausing eggs (if fertilized). In *Brachionus* and in several other rotifers, the production of sexual females is induced at high population densities by a chemical that is produced by the rotifers themselves (Stelzer and Snell 2003; Snell et al. 2006; Timmermeyer and Stelzer 2006). But there are also several documented cases of *Brachionus* strains that have permanently lost the ability to reproduce sexually (Boraas 1983; Buchner 1987; Bennett and Boraas 1988; Fussmann et al. 2003; Stelzer 2008). Obligate parthenogens are not able to produce sexual females, and therefore they also lack males and diapausing eggs. Thus, obligate parthenogens in *Brachionus* avoid two types of costs associated with sexual reproduction: (1) the production of male-producing females and (2) the production of diapausing-egg-producing females. The production of diapausing eggs presents a reproductive cost during the growing season or in very stable habitats, because they do not contribute to immediate population growth (Serra and Snell 2009).

Recently, it was found that obligate parthenogenesis (OP) in the rotifer *B. calyciflorus* is controlled by a single Mendelian locus (Stelzer et al. 2010). Obligatory parthenogenetic *Brachionus* were homozygous for a recessive allele, *op* (for obligate parthenogenesis), which caused an inability to respond to a chemical signal that normally triggers sexual reproduction in this species (Stelzer 2008). Heterozygotes (+/*op*) or homozygotes for the wild-type allele (+/+) were normal cyclical parthenogens (CPs) and underwent sexual reproduction at high population den-

sities. Interestingly, obligate parthenogens (OPs) were also dwarfs (i.e., body size was reduced by 50%, on average), indicating pleiotropy or linkage with genes that strongly affect body size. There were no differences in ploidy between CPs and OPs (Stelzer et al. 2010).

Obligatory parthenogenetic *B. calyciflorus* have several unique features, which make them an attractive experimental system to investigate the fitness consequences of transitions to asexuality. First, they can be created experimentally by crosses or self-fertilization of heterozygotes; hence, they can be studied directly after their origin. Second, cyclical and obligate parthenogens are genetically highly similar to each other. In this study, all comparisons between CPs and OPs involved clones that were siblings and derived from self-fertilization of the same parental heterozygote clone (+/*op*). Thus, even though inbreeding depression may affect clones that were generated in this way, it would do so equally in CPs and OPs. Third, the smaller body size of obligate parthenogens provides a morphological marker for tracking competitive interactions between CPs and OPs. In this study, I used an automated image analysis system to track competition between cyclical and obligate parthenogens by measuring changes in the body size distributions of populations that were a mixture of both reproductive types.

The overall aim of this study was to provide a comprehensive analysis of the immediate fitness consequences of transitions to obligate parthenogenesis in *B. calyciflorus*. This encompassed direct measurements of fitness based on lifetime reproductive success in cyclical versus obligate parthenogenetic clones, model calculations that included the specific costs of sex in this organism (i.e., cost of males and diapause), and competition experiments, which were addressed to answer the following questions: Can obligate parthenogens invade populations of cyclical parthenogens? Are they themselves resistant against invasion of cyclical parthenogens?

## Material and Methods

### *Strains, Clones, and General Culture Conditions*

Two geographic strains of the rotifer *Brachionus calyciflorus*, a Florida and a Georgia strain, were used in this study (kindly provided by J. J. Gilbert, Dartmouth College). Each of these two strains was split up into several clones (i.e., asexual descendants of single females), which were subsequently cultured in isolation. These clones were propagated either asexually or sexually (by self-fertilization). Note that self-fertilization results in highly similar yet new and unique genetically recombined clones. In this way, large pedigrees of clones with known ancestry could be generated (for more details, see Stelzer et al. 2010). Rotifers

were cultured in COMBO medium (Kilham et al. 1998) with the unicellular algae *Chlamydomonas reinhardtii* as food source (strain SAG11-32b, Sammlung fuer Algenkulturen, Goettingen). Food algae were supplied at ad lib. concentrations ( $\sim 400,000$  cells  $\text{mL}^{-1}$ ). Cultures were kept at  $24^\circ\text{C}$ , and continuous illumination was provided with daylight fluorescent bulbs ( $30\text{--}40 \mu\text{Einstein m}^{-2} \text{s}^{-1}$  for rotifers;  $200 \mu\text{Einstein m}^{-2} \text{s}^{-1}$  for algae). Clonal stock cultures of rotifers were reinoculated once or twice per week by transferring a few asexual females to fresh culture medium.

### Cyclical and Obligate Parthenogenetic Clones

In this study, cyclical and obligate parthenogenetic clones were generated by self-fertilization of parental clones, which were heterozygous for a loss-of-function allele *op* causing obligate parthenogenesis (*+/op*). Such self-fertilizations resulted in approximately 25% obligate parthenogenetic offspring clones. A detailed description of the experimental procedures can be found in the study by Stelzer et al. (2010). Briefly, *+/op* clones were grown in mass culture in 1-L flasks to high population densities until they produced diapausing eggs. This usually took about 7–10 days. Diapausing eggs were harvested and concentrated by sedimentation, and aliquots of the concentrated suspension were distributed among several microcentrifuge tubes, dried overnight in a rotation evaporator at  $30^\circ\text{C}$ , and subsequently stored in the dark at  $4^\circ\text{--}7^\circ\text{C}$ . This procedure kills all live individuals and eggs, except for diapausing eggs, which are resistant to desiccation. After a storage period of at least 2–3 weeks, hatching was induced by flooding the diapausing eggs with food suspension and incubation at  $25^\circ\text{C}$  and high light intensities. Clonal cultures were initiated from individual hatchlings of these diapausing eggs. Subsequently, experimental screens of the reproductive mode were used to detect obligate parthenogens. In these screens, each clone was propagated by transferring five to six asexual females into fresh algal suspension every 3–4 days. After each transfer, the old clonal cultures were examined for sexual stages (females with male eggs, males, diapausing eggs). Clones that did not show any sexual stages during 3 weeks were considered obligate parthenogens. Genetic variation among these clones, as estimated by amplified fragment length polymorphism markers, was very small. For instance, within the Florida strain, 93.2% of a total of 118 marker alleles were identical among 11 tested clones (Stelzer et al. 2010). This high similarity is likely the result of self-fertilization.

### Life-History Experiments

Fitness, in terms of the intrinsic rate of increase, was estimated from life tables of various cyclical and obligate parthenogenetic clones. For each clone, cohorts of individuals were established from hatchlings of asexual eggs (age  $<6$  h). Twelve hatchlings per clone were individually kept in 1 mL food suspension in 24-well tissue culture plates and were transferred to fresh food suspension every 12 h until death. Survival was checked at each transfer, and fecundity was measured by collecting the offspring produced by each female within the preceding 12-h interval. For logistic reasons, the life tables had to be analyzed in three consecutive experiments, leading to a total of four separate trials (because experiment 1 involved clones of two different strains): trial 1 (eight Florida clones in experiment 1), trial 2 (eight Georgia clones in experiment 1), trial 3 (experiment 2, 17 Georgia clones), Trial 4 (experiment 3, 10 Florida clones). Each of these trials contained CP and OP clones that were siblings, derived from self-fertilization of an individual heterozygous clone (*+/op*). In the statistical analysis, these trials were considered as four separate “studies,” each testing for an effect of the reproductive type on the intrinsic rate of increase, and were therefore analyzed separately.

The intrinsic rate of increase  $r$  was calculated by solving the Euler-Lotka equation iteratively:

$$\sum e^{-rx} \times l_x \times m_x = 1,$$

where  $l_x$  is the survivorship until age  $x$  and  $m_x$  is the mean number of offspring of a female at age  $x$ . Confidence intervals (95%) for the intrinsic rate of increase were estimated for 1,000 bootstrapped replicates according to Meyer et al. (1986).

Statistical analysis of the life table data was conducted in two steps and was done separately for each trial. First, randomization tests were used to test for significant variation in the population growth rate  $r$  among clones. In these tests, individuals were permuted randomly, each individual carrying its own record of survival and fecundity, among the different clones. After each permutation, a test statistic  $\theta$  (i.e., the variance of  $r$  among clones) was calculated and compared with  $\theta_{\text{obs}}$ , the observed variance. Variation of  $r$  among clones was considered significant, if the probability of  $\theta > \theta_{\text{obs}}$  was smaller than 0.05 (Caswell 2001). The randomization test was implemented in the MATLAB programming language, and 10,000 permutations were used for each experimental trial. Second,  $t$ -tests were used for each trial to test for an effect of the reproductive mode (OP vs. CP) on  $r$ . Finally, the probabilities of these  $t$ -tests were combined, analogous to meta-analysis (Sokal and Rohlf 1995).

*Theoretical Costs of Sex*

To provide theoretical estimates for the cost of sex in *B. calyciflorus*, life tables of all cyclical parthenogenetic clones were combined into one life table ( $n = 209$ ) in order to obtain an average lifetime fecundity schedule. This combined life table was then used to recalculate the intrinsic rates of increase for different proportions of sexual offspring (from 0% to 95% sexual daughters). It was assumed that all sexual offspring have zero fitness contributions in terms of immediate population growth (e.g., Fussmann et al. 2003). Note that this puts emphasis on the short-term advantage of asexuality and that it also includes a “cost of diapause” (which cannot be separated from sex in *Brachionus*). Each of these “cost-of-sex-adjusted” growth rates was then compared with the theoretical maximum growth rate (under the assumption that all offspring are asexual). The difference between the two values was the growth rate reduction due to sex, which resembles the cost of sex in *Brachionus*. These growth rate reductions were also used to estimate the “ $N$ -fold cost of sex” (per generation) to provide a comparison to costs of sex in other organisms. The  $N$ -fold cost of sex was estimated with respect to the average generation time, which is the average time  $T$  from birth to the peak of offspring production:

$$T = \frac{\sum x \times l_x \times m_x}{\sum l_x \times m_x}.$$

The  $N$ -fold increase of an obligately parthenogenetic clone is then

$$N = e^{\Delta r \times T},$$

where  $\Delta r$  is the growth rate reduction due to the induction of sexual daughters.

*Competition Experiments*

Competition between obligate and cyclical parthenogenetic clones was investigated in two-stage chemostats, that is, flow-through cultures that were continuously supplied with food suspension from a separate algal culture. Details on this method can be found in the study by Stelzer (2008). Briefly, the culture system consisted of one large algal chemostat (volume, 2 L); a smaller mixing reactor (volume, 380 mL), where algal suspension was mixed 1 : 1 with fresh COMBO medium; and eight to 10 rotifer chemostats (each with a volume of 380 mL). The equilibrium concentration in the algal chemostat was approximately 750,000 *Chlamydomonas* cells mL<sup>-1</sup>. Dilution rates of the rotifer chemostats—that is, the fraction of volume replaced per time—were 0.3 day<sup>-1</sup> for the very first 4–5 days of an experiment (to facilitate fast establishment of the rotifer populations) and 0.55 day<sup>-1</sup> thereafter.

For the competition experiments, pairs of OP and CP clones were used, which were derived by self-fertilization from the same parental clone and had similar survival and fecundity schedules. Chemostats were inoculated with either a 1 : 1 mixture of both competitor clones or just one clone in isolation (control treatments). I also used “invasion” treatments, in which individual clones were first allowed to establish a population, followed by inoculation of a small proportion of invaders of the opposite clone (approximately 10% of residents were replaced by invaders). Invasions were started in both directions, obligate parthenogens invading cyclical parthenogens and vice versa. In all treatments, two replicates (= chemostats) were used. Two separate competition experiments were conducted: the first experiment contained two clones of the Georgia strain, while the second contained two clones of the Florida strain.

Sampling and analysis of the chemostat populations were done with a fully automated image analysis system (Stelzer 2009). The image analysis system allowed quantitative analysis of female population density, could recognize males, and also provided an estimate of the body size of each female in the sample. The competition experiments were mainly analyzed with respect to the body size measurements. Since obligate parthenogenetic females were consistently smaller in body size than cyclical parthenogens (Stelzer 2009; Stelzer et al. 2010), changes in mean body size of a population were indicative of shifts in the relative fraction of the reproductive types. In other words, if one competitor replaced the other, the mean body size of the population should shift toward that of the superior competitor in pure culture. As the output variable in the competition experiments, I calculated the proportion of obligate parthenogens on the basis of the mean body size of the population. Note that values above 100% or below 0% are possible because of stochasticity in the sampling process. To provide an additional direct measure of the clonal composition, 48–96 females were sampled at the end of each experiment from each chemostat population. Bioassays were used to determine the proportion of obligate parthenogens in the sample. These bioassays were identical to those described in “Cyclical and Obligate Parthenogenetic Clones,” except that females could also be sexual (i.e., male or diapausing egg producing). Such sexual females were scored as cyclical parthenogens.

For statistical analysis of the 1 : 1 competition treatment, a runs test above and below the median was used (Sokal and Rohlf 1995). By contrast, multiple before-after control-impact (MBACI) analysis was used for all invasion treatments (Downes et al. 2002). For MBACI, the time courses of mean body size of the rotifer populations were grouped into two time periods representing the before and after periods, respectively. The before period included the

4 days immediately preceding the invasion treatment, while the after period included days 5–8 after the invasion treatment. Note that days 1–4, directly after invasion, were not included in the analysis. This was because successful invasion requires population growth, and 4 days (i.e., approximately two *Brachionus* generations) were considered the minimum time period for such a growth response. Successful invasion was detected by significant  $C \times B$  interactions, that is, interactions between the two main factors of the MBACI analysis: impact-control and before-after (Downes et al. 2002).

## Results

### Life-History Experiments

There was significant variation in the intrinsic rates of increase among clones in each trial (table 1; fig. 1). The overall highest growth rate was achieved by an obligate parthenogen with a value of  $1.9 \text{ day}^{-1}$ . This is equivalent to a doubling in population size every 8.7 h, an extremely high value for metazoans, however, consistent with earlier reports for fast-growing *Brachionus calyciflorus* strains (Bennett and Boraas 1989). The slowest growth rate of  $0.31 \text{ day}^{-1}$  was calculated for a cyclical parthenogen (fig. 1, top right).

Do obligate parthenogens grow faster, on average, than cyclical parthenogens? In each trial there was a slight trend, but it was not significant (table 1). Even after combining the probabilities of these independent tests of significance into a single test (Sokal and Rohlf 1995), the overall  $P$  value was still only marginally significant ( $\chi^2 = 13.81$ ,  $df = 8$ ,  $P = .087$ ). Thus, there was no overall effect of reproductive mode on the intrinsic rate of increase. However, there was a conspicuous pattern in the rank order of the clones. Each panel in figure 1 comprises groups of clones that were siblings (offspring resulting from self-

fertilization of the same parental clone), and in all four cases, the fastest-growing clone was an OP clone (see fig. 1). In other words, OPs provided significantly more often the fastest-growing clone in a group of siblings than CPs (Fisher’s exact test, two-tailed,  $P = .028$ ). Overall, OPs and CPs had similar mean life spans of  $4.7 \pm 0.17$  (SEM) and  $4.3 \pm 0.11$  days, respectively (Students  $t$ -test,  $t = 1.65$ ,  $df = 42$ ,  $P = .106$ ). In terms of fecundity, OPs even performed significantly better than CPs, having on average  $13.1 \pm 0.93$  (SEM) versus  $9.3 \pm 0.95$  offspring per female (Student’s  $t$ -test,  $t = 2.88$ ,  $df = 42$ ,  $P = .006$ ). However, as shown above, this difference in fecundity did not translate into a significant difference in the mean intrinsic rate of increase. This is probably caused by the fecundity pattern of rotifers: eggs are produced sequentially rather than in clutches; hence, the age at first reproduction and the few very first offspring contribute disproportionately strongly to the intrinsic rate of increase (Stelzer 2005).

### Theoretical Cost of Sex

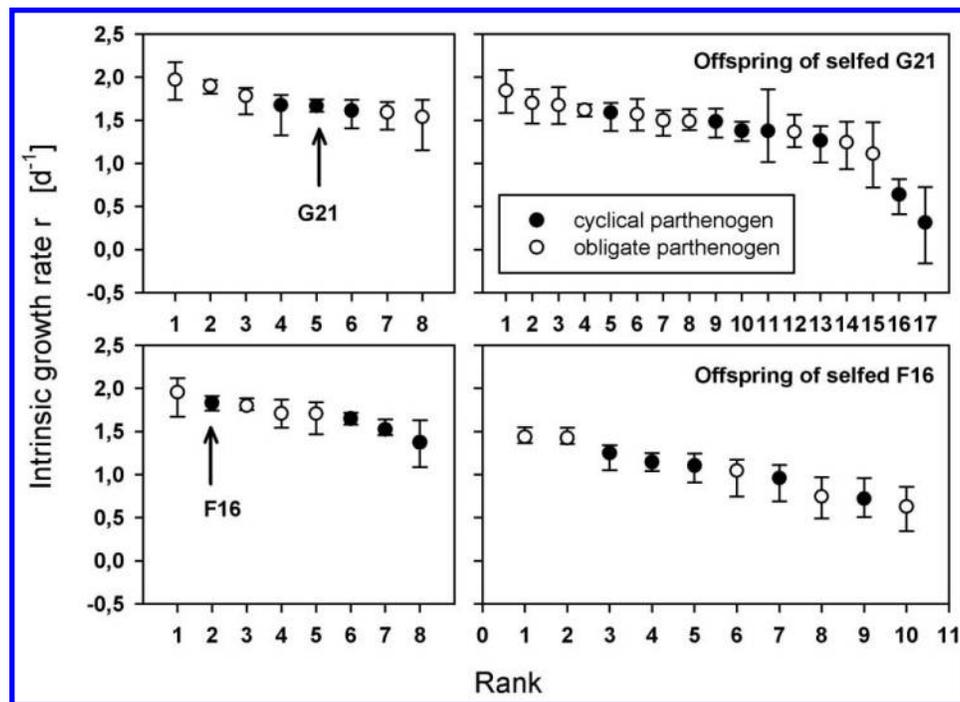
The cost of sex, expressed as the  $N$ -fold increase in population size per generation of a hypothetical obligate parthenogen, increased with the proportion of sexual offspring (fig. 2a). If only a small proportion of sexual offspring were produced (0%–10%), the cost of sex remained well below 2. However, for example, in the case of 60% sexual offspring, the cost of sex increased to 3.8 if these sexual offspring were produced equally across age classes. This picture changed dramatically if sexual offspring were produced preferentially early or late in life, respectively. For simplicity, I considered only the most extreme cases: (1) females produce all sexual offspring early in life and then completely switch to the production of asexual daughters (early sex strategy) and (2) females first produce asexual daughters only and then switch to

**Table 1:** Statistical analysis of intrinsic rates of increase in cyclical versus obligate parthenogenetic clones in *Brachionus calyciflorus*

Trial	Variation in $r$ among all clones		Variation in $r$ between reproductive types					
	Randomization test		Mean (SE)					
	Variance ( $\theta_{\text{obs}}$ )	$P$	Obligate parthenogens	Cyclical parthenogens	$t$	$\chi^2$	df	$P$
1	.0317	.0027	1.79 (.057)	1.59 (.094)	1.77		6	.127
2	.0214	.0124	1.80 (.072)	1.65 (.021)	1.567		6	.168
3	.1474	<.001	1.51 (.069)	1.15 (.18)	2.106		15	.052
4	.0787	<.001	1.06 (.16)	1.03 (.069)	.125		8	.903
Combined <sup>a</sup>						13.81	8	.087

Note: Each trial contained clones that were siblings derived from the one parental clone: trial 1 (eight Florida clones), trial 2 (eight Georgia clones), trial 3 (17 Georgia clones), trial 4 (10 Florida clones).

<sup>a</sup> Combined probabilities of all four trials.



**Figure 1:** Intrinsic rates of increase in clones of *Brachionus calyciflorus*. *Left*, first generation of inbred clones in two rotifer strains (Georgia strain and Florida strain). *Right*, second generation of inbred clones. These clones were established by self-fertilization of the clones G21 and F16 (arrows). Values for  $r$  are based on life tables with 12 individuals per clone on average. Means and 95% confidence intervals estimated for 1,000 bootstrapped replicates.

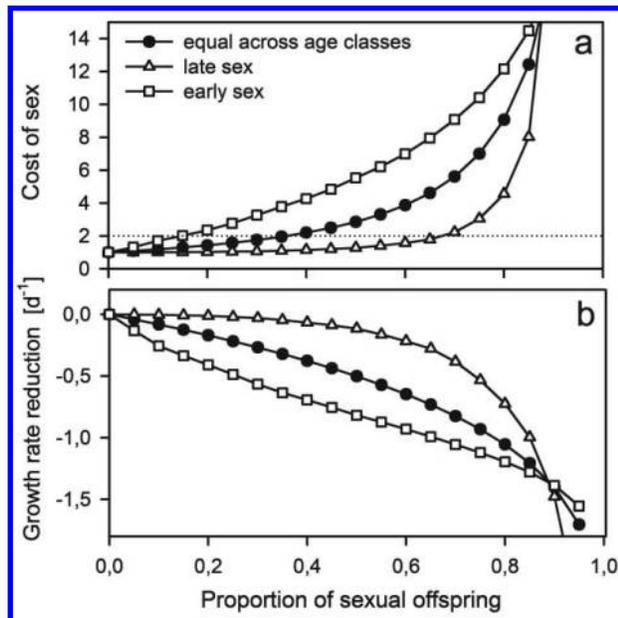
the production of sexual daughters (late sex strategy). In the late sex strategy, the cost of sex is minimized. Up to 67% sexual daughters can be produced until the cost of sex exceeds 2. By contrast, in the early sex strategy, the twofold cost of sex is already exceeded when more than 15% sexual daughters are produced.

If the cost of sex were expressed as the growth rate reduction of a cyclical parthenogen versus an obligate parthenogen (fig. 2*b*), the strongest growth rate reductions were found for a high proportion of sexual daughters produced early in life. For instance, the production of 40% sexual offspring equally across age classes resulted in a growth rate reduction of  $-0.377 \text{ day}^{-1}$ . This would mean that a hypothetical asexual invader should spread through a population of cyclical parthenogens within only 12 days (increase from 1% to 99% of the population).

#### Competition Experiments

In the first competition experiment, an OP clone competed against a CP clone of the Georgia strain. In the 1 : 1 treatment, both reproductive types were inoculated at the same time and ratio. During the first 3 days, both clones appeared to be equally abundant. However, in the following

days, the obligate parthenogens spread, and eventually they completely replaced the cyclical parthenogens (fig. 3*a*). This was confirmed by direct determinations of reproductive mode of females in these chemostats: all females sampled on day 14 were obligate parthenogens. Overall, direct determinations in both competition experiments were highly consistent with measurements of the image analysis system at the respective times ( $r^2 = 0.907$ ,  $df = 12$ ,  $P < .001$ ). The time courses (% OP) for both chemostats of the 1 : 1 treatment showed a significant increase in the proportion of obligate parthenogens (Runs test; each chemostat: only two runs with  $n_1, n_2 = 6, 7$  and  $7, 7$  respectively;  $P < .001$ ). In addition, obligate parthenogens successfully invaded established populations of cyclical parthenogens (fig. 3*b*). Invasions started at the end of day 5 with 10% obligate parthenogens, and it took only 8 days until obligate parthenogens had increased to 70%–90% (fig. 3*b*). Invasion success of the OP clone was statistically significant, since there was a significant  $C \times B$  interaction in the MBACI analysis of this treatment (table 2). By contrast, established populations of obligate parthenogens were resistant against invasion by cyclical parthenogens (fig. 3*c*; table 2). However, there was a significant before-after ( $B$ ) effect, even in the treatment where



**Figure 2:** Theoretical predictions of the cost of sex in *Brachionus calyciflorus*. Calculations are based on a combined life table of all cyclical parthenogenetic clones of this study ( $n = 209$  individuals). This life table was used to recalculate the intrinsic rates of increase for different proportions of sexual offspring (0%–95%) under the assumption that sexual offspring do not contribute to immediate population growth. *a*, Cost of sex. The cost of sex was defined as the  $N$ -fold increase in population size of a hypothetical obligate parthenogen relative to a cyclical parthenogen (within the average generation time of *B. calyciflorus*; i.e., 2.2 days). The dotted line represents the “twofold cost of sex,” which is the null model for most organisms. *b*, Growth rate reduction, that is, the absolute difference of the intrinsic rate of increase of obligate versus cyclical parthenogens.

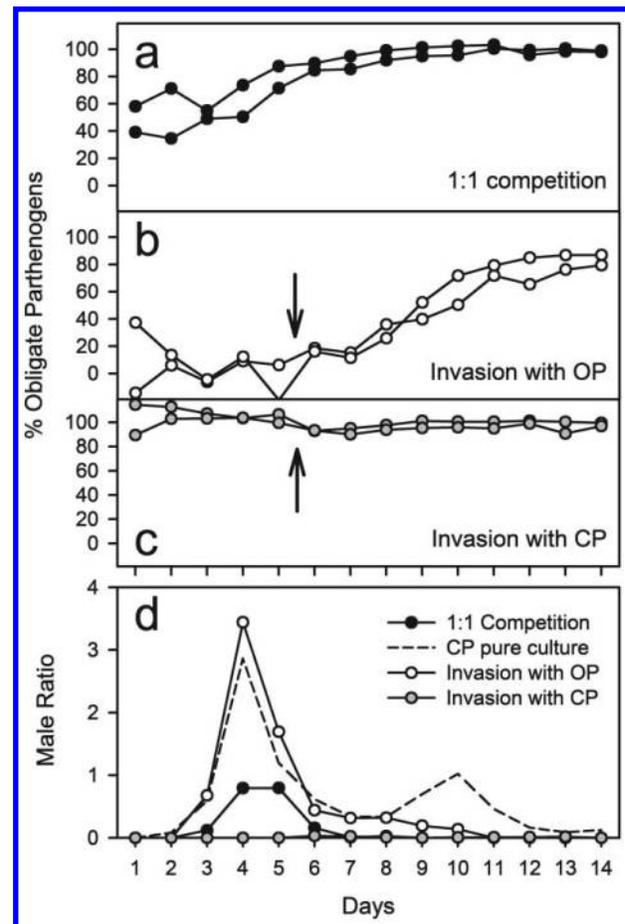
CPs invaded OPs. This was likely caused by a slight overall decline in mean body sizes, which occurred during this experiment. However, this change was unrelated to the invasion treatment.

In the second competition experiment, an OP clone competed against a CP clone of the Florida strain. Unfortunately, for logistic reasons, the 1 : 1 treatment could not be performed in this experiment. Interestingly, the invasion treatments in this experiment did not result in a clear winner. Rather, it seemed that the outcome of competition was undetermined, since there was no indication of a selective sweep in any direction (fig. 4). Each treatment (invasion with CP or OP) contained one replicate in which the proportion of invaders was increasing, while in the other replicate the resident clones appeared to be resistant against invasion (fig. 4). The male ratio (an indicator for the level of sexual reproduction) was much lower in the second competition experiment than in the first experi-

ment (see figs. 3*d*, 4*c*). MBACI analysis did not reveal any significant  $C \times B$  interactions and hence gave no indication of successful invasion. By contrast, the  $L(C) \times B$  interactions were significant, which reflects the highly divergent trajectories among replicates of the same treatment.

### Discussion

The aim of this study was to test whether obligate parthenogens in *Brachionus calyciflorus* can realize higher pop-



**Figure 3:** First competition experiment (with descendants of clone G21). Each line with symbols represents the time course of one replicate population (chemostat). Estimates for the percent obligate parthenogens were based on changes in the mean body size of the population, obtained with the image analysis system. Note that values slightly above 100% or below 0% are possible because of the stochasticity of the sampling process (see “Material and Methods”). *a*, Obligate and cyclical parthenogens were inoculated at equal proportions (1 : 1) at the beginning of the experiment. *b*, *c*, Invasion treatments with 10% of the opposite reproductive type inoculated at the end of day 5 (arrows). *d*, Male ratio (males per female), mean value of two replicates for each treatment.

**Table 2:** Multiple before-after control-impact analysis of the invasion treatments in the competition experiments

		df	First competition experiment						Second competition experiment					
			OP invades CP			CP invades OP			OP invades CP			CP invades OP		
			MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
Impact vs. control	<i>C</i>	1	90,763.7	159.5	.006	43.2	.1	.774	2,717.7	.5	.546	6,620.2	11.8	.075
Chemostat	<i>L(C)</i>	2	569.2			403.0			5,231.2			561.2		
Before vs. after	<i>B</i>	1	11,3928.8	30.7	.031	13,041.9	34.8	.028	10,571.7	7.6	.111	4,841.4	6.2	.131
Times (within <i>B</i> vs. <i>A</i> )	<i>T(B)</i>	6	3,574.2	8.3	.001	219.9	.6	.702	302.9	2.0	.140	103.6	3.1	.044
<i>C</i> × <i>B</i>		1	75,908.8	20.4	.046	502.4	1.3	.366	11,764.1	8.4	.101	6,032.7	7.7	.109
<i>C</i> × <i>T(B)</i>		6	385.8	.9	.528	35.7	.1	.995	172.6	1.2	.390	7.6	.2	.959
<i>L(C)</i> × <i>B</i>		2	3,711.9	8.6	.005	374.7	1.1	.370	1,396.4	9.3	.004	785.8	23.8	.000
<i>L(C)</i> × <i>T(B)</i> /error		12	430.9			347.0			149.5			33.0		

Note: Successful invasions are characterized by significant main effects (*C*, *B*) and, in particular, by a significant *C* × *B* interaction. Significant *L(C)* × *B* interactions indicate that replicate chemostats (i.e., either impact or control) behaved differently between the periods. Before-after comparisons refer to days 2–5 (=before the invasion treatment) and 10–13 (=after).

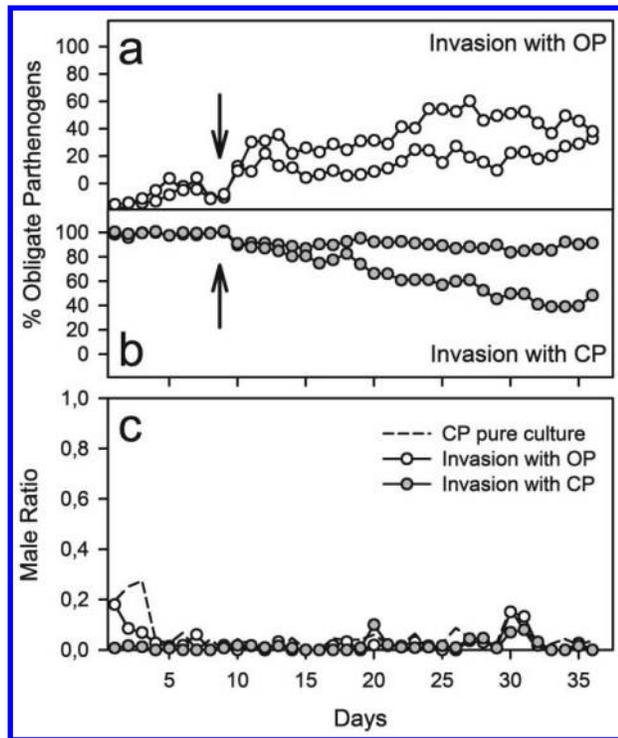
ulation growth rates than cyclical parthenogens and thus invade and displace populations of cyclical parthenogens. On the basis of results from life table experiments, OPs were predicted to grow at similar rates as CPs, provided that all offspring were weighted equally with respect to their contribution to future population growth (i.e., there is no cost of males and diapausing eggs). This supports the “all else equal” assumption in terms of the population growth rate. There were two minor deviations from this general pattern. First, obligate parthenogens appeared to have a slightly higher chance to contain clones with very high population growth rates. Moreover, obligate parthenogens produced on average more offspring than cyclical parthenogens, even though this difference was too small to result in differences in the intrinsic rate of increase.

Theoretical calculations suggested that strong differences in the intrinsic rates of increase can be expected if the sexual offspring in cyclical parthenogens are accounted for (i.e., male-producing and diapausing-egg-producing females). Especially with high rates of sex induction, CPs were predicted to grow much slower than OPs (see fig. 3). Altogether, this suggested that OPs in *B. calyciflorus* have at least equal but often higher fitness in terms of the intrinsic rate of increase and thus should have the potential to competitively displace populations of cyclical parthenogens.

This prediction was confirmed with competition experiments, which were analyzed by tracking changes in the proportion of both reproductive types in chemostat populations. In the first competition experiment, the competitive superiority of obligate parthenogens was evident: OPs completely displaced the CPs if inoculated at equal frequencies (1 : 1 treatment). Likewise, they successfully invaded established populations of CPs, rising from 10%

to >80% in just 8 days (fig. 3). From these time courses, selection coefficients can be calculated as the slope of the linear regression of  $\ln(p/q_t)$  on time *t*, where *p* and *q* are the frequencies of invaders and residents, respectively (Bell 2008). Calculated in this way, the selection coefficients have units of time ( $\text{day}^{-1}$ ) and are equivalent to the difference in the intrinsic rates of increase *r* between invaders and residents. I used the time courses from first day after invasion (or the beginning of the experiment, in the case of the 1 : 1 treatment) until invaders reached 90% (or until the experiment was terminated, whichever came first). Selection coefficients calculated in this way range between 0.39 and 0.65  $\text{days}^{-1}$  for the different chemostat populations of competition experiment 1 (results shown in fig. 3). Such high selection coefficients can be expected if the cyclical parthenogenetic clones produce approximately 40%–60% sexual offspring (equally across age classes; cf. fig. 2). These percentages of sex induction are highly consistent with values reported in the literature for *B. calyciflorus* (Gilbert 2007). Thus, the invasive success of obligate parthenogens in this experiment could be fully explained by the expected costs of sex.

In the second competition experiment, which involved a different pair of clones, cyclical and obligate parthenogens coexisted over the entire duration of the experiment. There were fluctuations in the composition of the populations, yet there was no indication that any reproductive type would completely replace the other. This suggested that obligate and cyclical parthenogens in this experiment essentially did not differ in fitness and, possibly, that the observed changes in the composition of the populations reflected random drift. A possible explanation for equal fitnesses among CP and OP clones could be an extremely low mixis induction of the cyclical parthenogens, which



**Figure 4:** Second competition experiment (with descendants of clone F16). Each line represents the time course of one replicate population (chemostat). Estimates for the percent obligate parthenogens were based on changes in the mean body size of the population, obtained with the image analysis system. Note that values slightly above 100% or below 0% are possible because of the stochasticity of the sampling process (see “Material and Methods”). *a*, *b*, Invasion treatments with 10% of the opposite reproductive type, cyclical or obligate parthenogens, inoculated at the end of day 8 (arrows). *c*, Male ratio (males per female), mean value of two replicates for each treatment.

was suggested by the exceptionally low male counts (fig. 4c). This was clearly not the case in the first experiment, where the male ratios were much higher (fig. 3d). In conclusion, both competition experiments are in agreement with the theoretical expectations: if cyclical parthenogens produce many sexual offspring, there is a high cost of sex and obligate parthenogens can successfully invade populations of cyclical parthenogens, but if cyclical parthenogens invest only minimally into sexual offspring, their fitness should be more or less equal to that of obligate parthenogens.

Can obligate parthenogens invade under natural conditions? The results of this study suggest that this may depend on the level of sexual reproduction in such populations. There have been only a few field studies on sexual reproduction of rotifers. Traditionally, it is assumed that rotifers reproduce asexually during most of the year and switch to sex only once, when the environmental conditions deteri-

orate. This would give only a few chances for obligate parthenogens to invade. However, this view has changed in the past 2 decades. First, field studies have shown that sexual reproduction in rotifer populations can sometimes prevail more or less over the entire season (Carmona et al. 1995; Miracle and Armengol-Diaz 1995). Second, experimental studies have established that sex in rotifers is not induced by deteriorating conditions (e.g., food shortage), as it is in other cyclical parthenogens (e.g., *Daphnia*; Hebert 1978), but rather suppressed by such conditions (Snell 1986; Schroder 2005). Density-dependent induction of sex in *Brachionus* can occur already at moderate population densities (e.g., 0.1 rotifers mL<sup>-1</sup>; Stelzer and Snell 2003), which are often observed in the field. To summarize, under field conditions it is not uncommon that rotifers invest heavily into sexual offspring, even during long periods in the growing season. Hence, there should be a potential for obligate parthenogens to temporally invade such populations.

Even though obligate parthenogens in *B. calyciflorus* may grow faster than cyclical parthenogens, they should be at a strong disadvantage in the long term because they are not able to produce diapausing eggs. Thus, obligate parthenogens might increase in frequency during a growing season but would completely die out once the habitat deteriorates, whereas cyclical parthenogens could recolonize from diapausing eggs (Serra and Snell 2009). Nevertheless, during the growing season, there is high clonal competition in populations of cyclical parthenogens (De Meester et al. 2006); hence, shifts toward obligate parthenogenesis might occur toward the end of a season. In addition, competition with obligate parthenogenetic clones might have implications for the clonal composition of CP clones and their resting egg banks. Interestingly, there are some species of monogonont rotifers that can produce diapausing eggs by parthenogenesis. For instance, some clones of the rotifer *Synchaeta pectinata* can asexually produce diapausing eggs, which hatch after 14 days or up to several months (Gilbert 1995). If such species were able to give rise to obligate parthenogens, they might also be successful in the long term.

Nevertheless, the ultimate factors preventing permanent and widespread establishment of obligate parthenogens in natural systems are probably unrelated to ecological constraints but rather reside in the lack of recombination. Evolutionary theories stress the various benefits of sex: for instance, sex can generate new variation that may be advantageous in coevolutionary arms races of hosts with their parasites (Decaestecker et al. 2007; Lively 1996), or it can aid adaptation by liberating beneficial mutations from poor genetic backgrounds (Goddard et al. 2005). Such mechanisms are likely of importance in natural environments but might not play a role in short-term laboratory experiments, such as those in this study. A promising av-

enue for future research may thus be to add more complexity to this experimental system, step by step, in order to determine the factors that can ultimately stabilize sexual reproduction in the life cycle of cyclical parthenogens.

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Asexual *Brachionus* female carrying an egg. Photograph by Claus-Peter Stelzer.