Phenotypic Effects of an Allele Causing Obligate Parthenogenesis in a Rotifer

THOMAS SCHEUERL, SIMONE RISS, AND CLAUS-PETER STELZER

From the Institute for Limnology, Austrian Academy of Sciences, 5310 Mondsee, Austria.

Address correspondence to C.-P. Stelzer at the address above, or e-mail: claus-peter.stelzer@oeaw.ac.at.

Abstract

Transitions to obligate asexuality have been documented in almost all metazoan taxa, yet the conditions favoring such transitions remained largely unexplored. We address this problem in the rotifer Brachionus calyciflorus. In this species, a polymorphism at a single locus, \( op \), can result in transitions to obligate parthenogenesis. Homozygotes for the \( op \) allele reproduce strictly by asexual reproduction, whereas heterozygous clones (+/\( op \)) and wild-type clones (+/+ ) are cyclical parthenogens that undergo sexual reproduction at high population densities. Here, we examine dosage effects of the \( op \) allele by analyzing various life-history characteristics and population traits in 10 clones for each of the 3 possible genotypes (\( op / \( op \), +/\( op \), and +/+ ). For most traits, we found that \( op / \( op \) clones differed significantly \( (P < 0.05) \) from the 2 cyclical parthenogenetic genotypes (+/+ and +/+ \( op \)). By contrast, the 2 cyclical parthenogenetic genotypes were almost indistinguishable, except that heterozygote individuals were slightly but significantly smaller in body size compared with wild-type individuals. Overall, this indicates that the \( op \) allele is selectively neutral in the heterozygous state. Thus, selective sweeps of this allele in natural populations would first require conditions favoring the generation of homozygotes. This may be given by inbreeding in very small populations or by double mutants in very large populations.

Key words: asexuality, cyclical parthenogenesis, evolution of sex, gene dosage effect, mendelian inheritance

Transitions to asexuality have occurred in almost all higher metazoan taxa (reviewed by Bell 1982). The mechanisms responsible for such transitions are highly diverse: They include interspecific hybridization, polyploidization, dominant meiosis suppressors, or infections with microorganisms that cause their hosts to reproduce parthenogenetically (reviewed by Simon et al. 2003). In addition, a recent study has demonstrated a new mechanism of transition to asexuality, showing that obligate parthenogenesis (OP) in the monogonont rotifer Brachionus calyciflorus is controlled by a single Mendelian locus (Stelzer et al. 2010).

Normally, monogonont rotifers reproduce by cyclical parthenogenesis (CP), an alternation between ameiotic parthenogenesis and sporadic sexual episodes (Nogrady et al. 1993). 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 monogonont 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 and Stelzer 2005; Snell et al. 2006; Timmermeyer and Stelzer 2006).

However, there are also several documented cases of Brachionus strains that have permanently lost the ability to reproduce sexually (Buchner 1987; Bennett and Boraas 1988; Fussmann et al. 2003; Stelzer 2008; Serra and Snell 2009). These obligate parthenogens are not able to produce sexual females, and thus, they lack males and diapausing eggs. It has been demonstrated that this inability is caused by a loss of responsiveness to the chemical signal that induces sex (Stelzer 2008). More recently, this result was extended by the finding that obligatory parthenogenetic Brachionus were homozygous for an allele, \( op \) (for obligate parthenogenesis). Heterozygotes (+/\( op \)) or homozygotes for the wild-type allele (+/+ ) were regular cyclical parthenogens that underwent sexual reproduction at high population densities. Interestingly, obligate parthenogens were also dwarfs (i.e., body size was reduced by 50%, on average), which indicated pleiotropy or linkage with other genes that strongly affect body size (Stelzer et al. 2010).

An open question that remains is whether the \( op \) allele exhibits dosage effects. For instance, Stelzer et al. (2010) hypothesized that heterozygote clones might show higher population density thresholds for sex, if compared with wild-type clones. This might be the case, for instance, if the \( op \) mutation would directly affect the receptor molecule for the sex induction chemical, thus causing a lower density of intact receptors in heterozygote clones. Likewise, heterozygous clones might show an overall lower propensity for sexual reproduction, for example, they might only produce...
30% sexual offspring on receiving a full dose of the sexual signal rather than 50%. Our previous study (Stelzer et al. 2010) could not resolve such subtle dosage effects; rather, we used population densities that were far above normal density thresholds for sex induction, in order to exclude the possibility of erroneously assigning obligate parthenogenesis to clones that just did not receive a sufficiently strong cue. Moreover, we did not quantify the propensity for sexual reproduction in cyclical parthenogenetic clones.

The possible existence of dosage effects would have high relevance for the evolutionary dynamics of the $op$ allele because this would allow for gradual transitions to asexuality. Recent theoretical studies suggest that higher sexual thresholds may be adaptive under certain environmental conditions (Serra and King 1999; Serra et al. 2004), and there is also empirical evidence for selection on this kind of variation in natural populations (Carmona et al. 2009). Likewise, there are studies documenting genetic variation in the propensity for sexual reproduction in Brachionus rotifers (e.g., Gilbert 2003, 2004). On the other hand, complete dominance of the wild-type allele over the $op$ allele would also have important implications as this should strongly constrain the evolution of obligate parthenogenesis in natural populations.

The aim of this study was to rigorously test for gene dosage effects of the $op$ allele. In total, we tested 30 clones, with 10 clones for each of the 3 different genotypes ($op/op$, $op/+$, and $++/+$). In particular, we were interested whether heterozygotes ($+/op$) are in some way intermediate between homozygote clones ($op/op$) and wild-type clones ($++/+$). We tested the following hypotheses:

1. Heterozygote clones exhibit a higher threshold density for induction of sexual reproduction than wild-type clones (i.e., they should grow to a higher population density before they undergo sexual reproduction).
2. Heterozygote clones show a lower total investment into sexual reproduction, if compared with wild-type clones.
3. Body size and/or egg size of heterozygote clones ranges intermediate between obligate parthenogens and wild-type clones.
4. Parameters of population growth (e.g., growth rate, maximum population density, and biomass production) of heterozygote clones range intermediate between obligate parthenogens and wild-type clones.

Materials and Methods

General Culture Conditions

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, Germany). Algae were supplied at ad libitum concentration (in general, more than 400 000 cells ml$^{-1}$). Experimental cultures were kept at 24°C and continuous illumination was provided with daylight fluorescent bulbs (30–40 $\mu$Einstein m$^{-2}$ s$^{-1}$ for rotifers; 200 $\mu$Einstein m$^{-2}$ s$^{-1}$ for algae). Stock cultures of rotifers were maintained at 20°C throughout the experiments by transferring 20 asexually reproducing females every 3–4 days to 30 ml of fresh culture medium provided in polystyrene petri dishes. This rather high transfer frequency ensured that diapausing eggs, which were occasionally produced in the cyclic parthenogens, could not hatch between transfers. In other words, this guaranteed that the genotypes of our experimental clones were faithfully transmitted during the whole experimental period, except for mitotic recombination or rare mutation.

Generation of Experimental Clones

For the experiments of this study, we used 30 clones, 10 for each genotype ($++/+$, $op/+$, and $op/op$). These were all derived by self-fertilization of one heterozygous clone (Clone “Florida 23,” shown in Figure 1 of reference Stelzer et al. 2010), a procedure which is possible in monogonont rotifers because CP clones have both male and female function (Stelzer 2005). The self-fertilization protocol is described in detail in Stelzer et al. (2010). Briefly, a clonal culture was initiated by inoculating a few asexual females of one clone into a bottle with algal food suspension, aerated with sterile air through a glass tube. A few days after inoculation, the rotifer population increased exponentially and males and diapausing eggs were produced. Each diapausing egg in such a culture is genetically unique because it was sexually produced. However, genetic variation among these diapausing eggs is low because they were generated by self-fertilization. Because the self-fertilized clone was heterozygous for the $op$ allele, the diapausing eggs thus produced contained obligate parthenogens ($op/op$) and cyclical parthenogens, which included both heterozygote ($op/+$) and homozygote ($++/+$) genotypes. Diapausing eggs in the cultures were concentrated by sedimentation and stored for 2 weeks in the dark at 4°C. Aliquots of the concentrated suspension were then distributed among several 1.5-ml microcentrifuge tubes, dried in a rotation evaporator at 30°C, and stored at $-20^\circ$C.

Hatching of diapausing eggs was induced by flooding the dried eggs with food suspension and incubation at room temperature at high light intensities. Usually after 24 h, the first hatchlings emerged. Clonal cultures were initiated by transferring these individuals separately into 5 ml of food suspension provided in 6-well tissue culture plates. The reproductive mode of these clones (OP or CP) was determined using experimental screens (Stelzer et al. 2010). In such screens, clonal cultures were propagated by transferring 5–6 asexual females every 3–4 days into fresh algal suspension. Between these transfers, the population size of CP clones typically increased to about 40–60 individuals. Populations of OP clones usually reached more than 100 individuals per well after 3–4 days. After each transfer, the old culture was examined for sexual stages (females with male eggs, males, and diapausing eggs). These screens were run for 3 weeks (i.e., ca. 10 asexual generations). Clones that did not show any sexual stages during this time were considered as obligate parthenogens.

To determine the exact genotype of cyclical parthenogenetic clones ($+/op$ vs. $++/+$), we followed the CP clones for an
additional round of self-fertilization. The procedure was the same as described above. Again, we screened the hatchlings of the diapausing eggs: if hatchlings of one clone contained obligate parthenogens, the parental clone was heterozygous; if they contained only cyclical parthenogens, the parental clone was homozygous. Finally, 10 clones of each of the 3 genotypes (+/+, op/+, and op/op) were randomly selected and were used for the following experiments.

**Experiment 1: Threshold Population Density for Sex**

The threshold population density for sex was defined as the population density (in a growing population) at which the first male was observed. This parameter serves an indirect measure for the sensitivity of a clone to the density-dependent chemical mixis signal (Carmona et al. 2009). To provide asexually reproducing females for this experiment, the 20 CP clones were precultured at low population densities (0.02–0.05 females ml⁻¹) for 7–10 days. Newborn offspring of such low-density cultures were used to initiate experimental populations. Briefly, one juvenile was inoculated into a petri dish with 10 ml of algal food suspension. We used 10–12 replicates (≈petri dishes) for each clone. All experimental populations were examined every 24 h. Once the first male was observed in a population, population size was determined by picking out all females under a dissecting microscope. Population density (in females per milliliter) was calculated based on the remaining volume of medium in each dish. Losses in volume due to evaporation were usually not higher than 5% because the petri dishes were kept in closed boxes.

**Experiment 2: Body Size and Egg Size Measurements**

Newborn females (age < 4 h) were isolated from low-density cultures and cultured individually in 1 ml food suspension, with daily transfers to fresh food suspension. We used 12–18 females for each of the 30 experimental clones. After 3 days, when females were adult, they were fixed in Lugol’s solution and transferred to plankton sedimentation chambers. Body size was measured using inverted microscopy at 200-fold magnification. Body volume was estimated from 3 distance measurements on each individual (total length, widest breadth, and breadth at the anterior end) according to Ruttner-Kolisko (1977). Asexual eggs were measured in the same way, and egg volume was calculated based on the remaining volume of medium in each dish. Losses in volume due to evaporation were usually not higher than 5% because the petri dishes were kept in closed boxes.

**Experiment 3: Population Traits**

Our third experiment addressed differences in the population dynamics among the 3 genotypes. We followed populations of our 30 rotifer clones through a growth cycle in batch culture, that is, rotifers were inoculated into 1-L aerated algal cultures (750 000–1000 000 Chlamydomonas cells ml⁻¹) at low population densities (12 asexual females L⁻¹). The resulting population dynamics in such cultures were 1) exponential growth to a maximum population density, 2) male and diapausing egg production at high population densities (only CP clones), and 3) a crash of the population due to depletion of the algal food resource. Figure 1 shows example data for a cyclical parthenogenetic clone. Sampling and counting in this experiment were accomplished using an automated image analysis system, which was developed by one of us (Stelzer 2009). Image analysis samples were taken automatically from the fourth day after inoculation, in 6-h intervals, usually for a time period of 7–10 days (this was the typical time when the population crashed). The accuracy of the image analyzing system was checked using manual counts of samples, which were sporadically taken during the experiment. Manual samples were fixed in Lugol’s solution and counted using inverted microscopy at 100-fold magnification.

From these population experiments, we extracted several parameters (Figure 1): exponential growth rate (unit: day⁻¹), measurements starting at a density of 10 rotifers ml⁻¹ until 70% of the maximum rotifer density (that culture), maximum rotifer density (individuals ml⁻¹), maximum rotifer biovolume (μm³ ml⁻¹), and the rate of biovolume increase (analogous to the population growth rate). The image analysis system also provided estimates of the mean body size of females, which could be deduced from the pixels corresponding to signals of individual females in the digital photograph (Stelzer 2009). This provided an independent estimate for body size, in addition to the

**Figure 1.** Example data of the image analysis system: population dynamics of a batch culture of a cyclical parthenogenetic clone. Filled circles in (a): female abundances. Gray bars in (a): males per female. Open circles in (b): rotifer biovolume. Some of the extracted population traits are indicated by arrows (maximum population density, population density at first male observation, and maximum biovolume). Dashed line indicates part of the growth curve that was used to calculate the population growth rate (10 females ml⁻¹ to 70% of maximum density).
measurements in experiment 2. More specifically, the image analysis system “integrates” body size and egg size because gravid females are photographed with their eggs attached to the body. Finally, the image analysis system can also recognize males (Stelzer 2009), which allowed a classification of the level of sexual reproduction in CP clones. We extracted 2 parameters: density threshold for sex (unit: females ml$^{-1}$; in analogy to experiment 1) and male index (dimensionless; defined as males per females, summed over all samplings in which males were counted). The latter parameter is an estimate of the propensity to which clones engaged in sexual reproduction—it integrates the intensity and duration of male production. At the end of the experiment, we took well-mixed samples (10–30 ml) of each culture (only cyclical parthenogens) and counted the number of diapausing eggs to estimate the total number of diapausing eggs that were produced during the experiments.

For logistic reasons, the population experiments were conducted in 4 independent trials, with each trial containing all 30 experimental clones (the image analysis system could only handle 30 different cultures). For this reason, we used a randomized block model for statistical analysis (see below). Unfortunately, there was an electrical power outage close to the end of the fourth trial, which prevented reliable estimates for some parameters in that trial (maximum rotifer density and biovolume). Thus, for these parameters, we could only include 3 trials in the statistical analysis.

**Statistical Analysis**

Experiments 1–3 were analyzed using hierarchical analyses of variance with “genotype” as a fixed factor and “clone” as a random factor nested within genotype. In experiment 1 (density threshold for sex), only the 2 genotypes of cyclical parthenogens were compared (op/+ vs. +/+). In experiment 2 (body size and egg size determinations), the factor genotype composed 3 levels, op/op, op/+ and +/+ Differences among genotypes were analyzed using 2 a priori contrasts: 1) obligate parthenogens versus cyclical parthenogens (i.e., op/op vs. op/+ and +/+) and 2) heterozygote versus homozygote cyclical parthenogens (i.e., op/+ vs. +/+). Experiment 3 (population traits) was analyzed using a randomized block model with “trial” as a random (blocking) variable, genotype as a fixed factor, and clone as a random variable, nested within genotype. A priori contrasts were defined in the same way as for experiments 2 and 3. Because experiment 3 involved tests of 8 population traits on the same data set, we corrected for a family-wise Type I error rate by using the Dunn–Sidak procedure (Quinn and Keough 2002). In all 3 experiments, dependent variables were log transformed, if necessary, to meet the requirements for parametric testing. All calculations were done using SPSS Statistics 17.0.

**Results**

We generated clones with the 3 different genotypes (op/op, +/op, and +/+ by self-fertilization of one clone, of which we knew that it was heterozygous for the $\phi$ allele, based on the results of an earlier study (Stelzer et al. 2010). We hatched 64 resting eggs, which were produced by selfing of this clone, and obtained 46 cyclical parthenogenetic clones (71.9%) and 18 obligate parthenogenetic clones (28.1%). These percentages are consistent with the expected 3:1 CP:OP ratio. We then randomly selected 10 obligate parthenogenetic and 32 cyclical parthenogenetic clones and established stock cultures. The 32 cyclical parthenogenetic clones were subjected to a second experimental screen to distinguish heterozygotes from wild-type clones (see Material and Methods). This experimental screen showed that 21 of the 32 CP clones were heterozygotes and 11 were wild-type clones. Again, we randomly selected 10 clones of each of the 2 genotypes. Together with the 10 OP clones, these comprised our 30 experimental clones.

Our first experiment was intended to test the hypothesis that +/op clones engage into sexual reproduction later than +/+ clones, when growing from low to high population densities. Our results clearly rejected this hypothesis because the population density thresholds were almost indistinguishable. They were 8.51 (±1.71 standard deviation) versus 7.89 (±1.28) individuals ml$^{-1}$ in heterozygote versus wild-type clones, respectively. Even though there was significant variation among clones in the population density threshold for sex, the factor genotype had no significant effect (Table 1).

The second experiment was addressed to determine whether there are differences in body size and egg size among the 3 different genotypes. There were significant differences, such that the $\phi$/op genotype was always significantly smaller than the 2 CP genotypes (Figure 2 and Table 2). Closely examination of the 2 CP genotypes showed that there was no significant difference in egg size between +/op and +/+ clones, yet the difference in body size was significant (Table 2). The mean body volumes of $\phi$/op clones, +/op clones, and +/+ clones were 0.898, 1.391, and $1.527 \times 10^3$ $\mu$m$^3$, respectively. Hence, heterozygote clones (+/op) were roughly 9% smaller than wild-type clones (+/+).

The third experiment addressed differences in population traits among the 3 genotypes. Table 3 lists a summary of these parameters, and significant differences (after Dunn–Sidak procedure correction) are indicated by asterisks. This experiment showed that the 2 CP genotypes (+/op and +/+ did not differ significantly in the 3 sex-related traits: density threshold for sex, male investment, and diapausing egg production (Table 4). Consistent with our second experiment, we found significant differences in body size between all 3

| Table 1 Statistical analysis of the population density threshold for sex (experiment 1) |
|--------------------------------------|---|---|---|---|
| Source of variation | df | Mean square | F | P |
| Log (sex threshold) | Genotype | 1 | 0.424 | 1.148 | 0.297 |
| Clone (genotype) | 19 | 0.369 | 2.789 | <0.001 |
| Residual | 224 | 0.132 |   |   |

df, degrees of freedom.
Statistical analysis, see Table 2.

each genotype (size (egg volume) Log volume Body

Table 2 Statistical analysis of body size and egg size
(experiment 2)

| Source of variation | df | Mean square | F     | P       |
|---------------------|----|-------------|-------|---------|
| Body volume         |    |             |       |         |
| Genotype            | 2  | 12.38       | 103.31| <0.001  |
| Clone (genotype)    | 28 | 0.124       | 2.62  | <0.001  |
| Residual            | 337| 0.047       |       |         |
| A priori contrasts  |    |             |       |         |
| OP versus CP        |    |             |       | <0.001  |
| +/op versus +/+     |    |             |       | <0.001  |
| Log (egg volume)    |    |             |       |         |
| Genotype            | 2  | 16.894      | 294.49| <0.001  |
| Clone (genotype)    | 27 | 0.057       | 6.432 | <0.001  |
| Residual            | 267| 0.009       |       |         |
| A priori contrasts  |    |             |       |         |
| OP versus CP        |    |             |       | <0.001  |
| +/op versus +/+     |    |             |       | 0.578   |

df, degrees of freedom.

Figure 2. Influence of genotype on egg size (a) and body size (b). Bars represent the mean and standard deviation for each genotype (n = 9–11). For this figure, genotype means were calculated from the means for each clone (n = 8–16). For statistical analysis, see Table 2.

population traits related to rotifer biovolume, such as the rate of biovolume increase or the maximum rotifer biovolume.

**Discussion**

This study was intended to fill a gap in our knowledge of the phenotypic effects of the op allele—the question whether it also affects heterozygote carriers in their sexual behavior and other life-history traits or whether heterozygote clones are indistinguishable from wild-type clones. Distinguishing between these 2 possibilities has important implications on the potential of obligate asexuality to spread in natural populations. From our previous studies, we knew that the op allele has profound effects on life history and fitness of homozygote carriers: Obligate parthenogens (op/op clones) were much smaller in body size and egg size (Stelzer et al. 2010), compared with cyclical parthenogens (+/op and +/+ clones), and they could attain higher fitness in terms of the population growth rate because they did not invest into sexual offspring (Stelzer 2011). In competition experiments, it was shown that, if a resident population consisting of cyclical parthenogens invests heavily into sex (say, they produce 40–60% sexual offspring on average), this can result in selection coefficients for obligate parthenogens as high as 0.39–0.65 d⁻¹ (Stelzer 2011). Thus, under such circumstances, obligate parthenogens can virtually displace populations of cyclical parthenogens within 7–10 days (Stelzer 2011). However, if the op allele arises in a natural population by mutation, its first carriers will be heterozygous. Thus, the spread of the op allele will critically depend on whether a clone carrying this mutation would show any phenotypic differences as compared with wild-type clones. Our working hypothesis for this study was that heterozygote clones are intermediate between obligate parthenogens and wild-type clones, showing reduced propensity for sexual reproduction, higher thresholds for sex induction, and reduced body size.

The results of this study reject this hypothesis: There was no significant difference between +/op and +/+ clones in terms of sexual induction and in terms of most other life-history traits. The only exception was body size: Heterozygous clones were significantly smaller than wild-type clones, a difference which was significant in 2 completely independent experiments (Tables 2 and 3). However, the magnitude of this difference was small as it amounted to only 9% by volume (experiment 2) and 3% by area (experiment 3). It should kept in mind that these measurements involved cohorts with very narrow age classes (experiment 2) or extremely large sample sizes (experiment 3). Thus, the chances for detecting subtle differences in mean body size were maximized by our experimental design. On the other hand, body size variation within rotifer populations is notoriously high due to developmental variation (adults are typically twice or triple the size of juveniles). In the light of this developmental variation, it is questionable whether the relatively small difference in mean body size has any biological significance. For instance, it is hard to imagine a size-selective predator that prefers one of these 2 genotypes while sparing the other. Overall, we

Figure 2. Influence of genotype on egg size and body (experiment 2).
conclude that the \(qP\) allele is almost completely recessive to the wild-type allele and that it is unlikely that there are selective forces that would favor/disfavor heterozygote clones in a population of wild-type clones.

Our study also provides new insights into potential patterns of resource allocation in CPs versus OPs. These patterns are most evident when examining the population level traits. For instance, we found that OPs reached significantly higher maximum population densities than CPs (212 individuals m\(^{-1}\) vs. 117 and 112 individuals m\(^{-1}\); see Table 3). Similarly, the population growth rate (based on increases in numbers of individuals) was significantly higher in CPs versus OPs. This difference in the population growth rate might have been partially caused by sex induction in CPs during the later phase of exponential growth. We tried to minimize such effects by limiting the population trajectories used for the population growth rate estimates to growth between 10 individuals m\(^{-1}\) and 70% of maximum population density. Nevertheless, a reduction of the population growth rate through sex induction in CPs cannot be completely ruled out. However, it is interesting that all these differences completely disappear, if rotifer biovolume is considered rather than the numbers: Both the rate of biovolume increase as well as the maximum biovolume were indistinguishable in all 3 genotypes. This suggests that OPs are not overall more efficient in converting algal biomass into rotifer biomass but that they simply allocate available resources according to a different scheme.

**Conclusions**

Our study suggests that heterozygotes for the \(qP\) allele are phenotypically more or less indistinguishable from wild-type clones. Does this rule out the spread of occasional mutants for the \(qP\) allele in natural systems? There are actually quite a few scenarios by which the \(qP\) allele might increase in natural systems, even though starting in heterozygous state. The first scenario involves population bottlenecks. Aquatic organisms are often dispersed by waterfowl into new habitats (Bohonak and Jenkins 2003; Green and Figuerola 2005), and this mode of dispersal can result in colonization of new habitats by only a few genotypes (De Meester et al. 2002). Clonal growth during the colonization phase and subsequent mating between males and females derived from \(qP/+\) clones could then give rise to

### Table 3

Summary of population traits of the 3 genotypes (means ± standard error mean)

| Source of variation          | \(qP/qP\) | \(qP/+\) | +/+       |
|------------------------------|-----------|----------|----------|
| Density at first male        | 37.9 (±3.38) | 36.7 (±3.37) |
| Male index*                 | 0.792 (±0.159) | 1.101 (±0.205) |
| Diapausing egg production (# eggs) | 6846 (±1274) | 7885 (±932) |
| Population growth rate (day\(^{-1}\)) | 1.13 (±0.034)** | 0.973 (±0.022) |
| Maximum population density (individuals m\(^{-1}\)) | 212 (±9.8)** | 117 (±7.8) |
| Mean body size (10\(^2\) \(\mu\)m\(^3\)) | 2.45 (±0.202)** | 3.30 (±0.026)* |
| Rate of biovolume increase (day\(^{-1}\)) | 1.09 (±0.038) | 1.10 (±0.027) |
| Maximum biovolume (10\(^3\) \(\mu\)m\(^3\)) | 308.7 (±12.3) | 304.8 (±13.3) |

Sample sizes: 10 clones per genotype, 3–4 blocks (=experimental trials) per clone.

*Defined as the ratio of males per females, summed over all sampling events (further details in main text).

**P < 0.01, *P < 0.05 (significant differences after Dunn–Sidak procedure; further statistical details in Table 4).

### Table 4

Statistical analysis of population traits (experiment 3)

| Source of variation          | df    | F    | P     | df    | F    | P     | df    | F    | P     |
|------------------------------|-------|------|-------|-------|------|-------|-------|------|-------|
| Genotype                     | Density at first male | 1, 18 | 0.04 | 0.842 | 1, 18 | 1.44 | 0.245 | 1, 18 | 0.70 | 0.413 |
| Block                        | Male ratio | 2, 38 | 7.04 | 0.003 | 3, 57 | 10.46 | <0.001 | 3, 57 | 30.27 | <0.001 |
| Clone (genotype)             |       | 18, 38 | 1.82 | 0.061 | 18, 57 | 1.45 | 0.144 | 18, 57 | 1.36 | 0.186 |
| A priori contrasts           | OP versus CP | Population growth rate | 2, 27 | 6.25 | 0.006 | 2, 27 | 51.36 | <0.001 | 2, 27 | 0.39 | 0.676 |
|                            | +/\(qP\) versus +/+ | Maximum population density | 3, 87 | 12.68 | <0.001 | 2, 58 | 15.95 | <0.001 | 3, 87 | 11.39 | <0.001 |
|                            |       | Rate of biovolume increase | 27, 87 | 3.08 | <0.001 | 27, 58 | 1.28 | 0.212 | 27, 87 | 2.21 | 0.003 |
|                            | OP versus CP | Maximum biovolume | 27, 87 | 3.08 | <0.001 | 27, 58 | 1.28 | 0.212 | 27, 87 | 2.21 | 0.003 |

Notice that a priori contrasts were only considered, if the factor “genotype” was statistically significant. df, degrees of freedom.
homozgygotes. The second scenario involves quite the opposite situation: In very large populations, double mutants might occur. Given the huge population sizes that can be reached by rotifers in a lake, this scenario might not be completely unrealistic. Consider a relatively small water body, such as a hypothetical shallow lake with the dimensions $100 \times 100 \times 1 \text{ m} (= 10^7 \text{ L})$. Such a lake may well hold a population of $10^{10}$ rotifers, assuming a population density of 1 individual $\text{ml}^{-1}$. Third, $op$ alleles could spread simply by random drift. Interestingly, the effectiveness of this process would even be enhanced by the recessivity of the $op$ allele. Even though the chances for fixation by drift might be small, if an individual population is considered, the overall probability of asexual transitions through drift can become high if we allow for many populations, longtime scales, and recurrent $op$ mutations. Finally, $op/op$ clones might be generated from heterozygous clones by gene conversion and mitotic crossing over, if such processes occurred at the relevant genomic regions and during asexual propagation (e.g., Omilian et al. 2006).

It remains to be determined if the $op$ allele is widespread in natural populations or whether differences in the frequencies of such an allele. Because several studies by independent authors have reported a loss of sexual reproduction in rotifers that have been introduced from natural habitats into lab culture (Buchner, 1987; Bennett and Boras, 1988; Fussmann et al. 2003; Stelzer, 2008), mutations affecting the sexual reproductive mode might actually be quite common.

**Funding**

FWF (Fonds zur Foerderung der wissenschaftlichen Forschung) Grant (P20735-B17 to C.-P.S.).

**Acknowledgments**

We thank Nicole Laufenstein for technical assistance during some of the particularly work-intensive phases of the experiments.

**References**

Bell G. 1982. The masterpiece of nature. San Francisco (CA): University of California Press.

Bennett WN, Boras ME. 1988. Isolation of a fast-growing strain of the rotifer *Brachionus calyciflorus* Pallas using turbidostat culture. Aquaculture. 73:27–36.

Bohonak AJ, Jenkins DG. 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. Ecol Lett. 6:783–796.

Buchner H. 1987. Untersuchungen über die Bedingungen der heterogenen Fortpflanzungsarten bei den Rädereriten III. Über den Verlust der mitotischen Potenz bei Brachionus ureoclasus Archiv für Hydrobiologie. 109:333–354.

Carmona MJ, Dimas-Flores N, Garcia-Roger EM, Serra M. 2009. Selection of low investment in sex in a cyclically parthenogenetic rotifer. J Evol Biol. 22:1975–1983.

De Meester L, Gomez A, Okamura B, Schweng K. 2002. The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. Acta Oecol. 23:121–135.

Fussmann GF, Ellner SP, Hairston NG. 2003. Evolution as a critical component of plankton dynamics. Proc R Soc Lond Ser B Biol Sci. 270:1015–1022.

Gilbert JJ. 2003. Environmental and endogenous control of sexuality in a rotifer life cycle: developmental and population biology. Evol Dev. 5:19–24.

Gilbert JJ. 2004. Population density, sexual reproduction and diapause in monogonont rotifers: new data for *Brachionus* and a review. J Limnol. 63:32–36.

Green AJ, Figueroa J. 2005. Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. Divers Distrib. 11:149–156.

Kilham SS, Keeger DA, Lynn SG, Goulden CE, Herrera L. 1998. COMBO: a defined freshwater medium for algae and zooplankton. Hydrobiologia. 377:147–159.

Nogrady T, Wallace RL, Snell TW. 1993. Rotifera: biology, ecology and systematics. Guides to the identification of the microinvertebrates of the continental waters of the world. The Hague (The Netherlands): SPB Academic Publishing.

Omilian AR, Cristescu MEA, Dudycha JL, Lynch M. 2006. Ameiotic recombination in asexual lineages of *Daphnia*. Proc Natl Acad Sci U S A. 103:18638–18643.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Ruttnner-Kolisko A. 1977. Suggestions for biomass calculations of plankton rotifers. Arch Hydrobiol Beih. 87:1–76.

Serra M, King CE. 1999. Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. J Evol Biol. 12:263–271.

Serra M, Snell TW. 2009. Sex loss in monogonont rotifers. In: Schön I, Martens K, Van Dijk P, editors. Lost sex. Berlin (Germany): Springer.

Serra M, Snell TW, King CE. 2004. The timing and proportion of sex in monogonont rotifers. In: Moya A, Font E, editors. Evolution: from molecules to ecosystems. Oxford University Press. p. 135–146.

Simon JC, Delmotte F, Rispe C, Crease TJ. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. Biol J Linn Soc. 79:151–163.

Snell TW, Kubanek J, Carter W, Payne AB, Kim J, Hicks M, Stelzer CP. 2006. A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). Mar Biol. 149:763–773.

Snell TW, Stelzer CP. 2005. Removal of surface glycoproteins and transfer among *Brachionus* species. Hydrobiologia. 546:335–346.

Stelzer CP. 2005. Evolution of rotifer life histories. Hydrobiologia. 546:335–346.

Stelzer CP. 2008. Obligate asex in a rotifer and the role of sexual signals. J Evol Biol. 21:287–293.

Stelzer CP. 2009. Automated system for sampling, counting, and biological analysis of rotifer populations. Limnol Oceanogr Methods. 7:856–864.

Stelzer CP. 2011. The cost of sex and competition between cyclical and obligate parthenogenetic rotifers. Am Nat. 177:E43–E53.

Stelzer CP, Schmidt J, Wiedlroither A, Riss S. 2010. Loss of sexual reproduction and dwarfing in a small metazoan. PloS One. 5:e12854.

Snell TW, Stelzer CP. 2005. Removal of surface glycoproteins and transfer among *Brachionus* species. Hydrobiologia. 546:335–346.

Stelzer CP. 2008. Obligate asex in a rotifer and the role of sexual signals. J Evol Biol. 21:287–293.

Stelzer CP. 2009. Automated system for sampling, counting, and biological analysis of rotifer populations. Limnol Oceanogr Methods. 7:856–864.

Stelzer CP. 2011. The cost of sex and competition between cyclical and obligate parthenogenetic rotifers. Am Nat. 177:E43–E53.

Stelzer CP, Schmidt J, Wiedlroither A, Riss S. 2010. Loss of sexual reproduction and dwarfing in a small metazoan. PloS One. 5:e12854.

Stelzer CP, Snell TW. 2005. Removal of surface glycoproteins and transfer among *Brachionus* species. Hydrobiologia. 546:335–346.

Stelzer CP. 2005. Evolution of rotifer life histories. Hydrobiologia. 546:335–346.

Stelzer CP. 2008. Obligate asex in a rotifer and the role of sexual signals. J Evol Biol. 21:287–293.

Corresponding Editor: Tomoko Steen