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Population regulation in sexual and asexual rotifers: an eco-evolutionary feedback to population size?

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Summary

1. Population size is often regulated by density dependence, that is negative feedbacks between growth and population density. Several density-dependent mechanisms may operate simultaneously in a population.
2. In this study, I focus on two different mechanisms of density-dependent population regulation, resource exploitation (RE) and density-dependent sexual reproduction (DDS).
3. I analyse both mechanisms in clonal populations of the rotifer *Brachionus calyciflorus*, which differ in the investment in sex because of a polymorphism at a single Mendelian locus. Some clones were cyclical parthenogens (CP) and possessed both mechanisms of population regulation (RE + DDS), while other clones were obligate parthenogens (OP) and thus lacking the DDS mechanism.
4. Equilibrium population size was considerably lower in CP clones, compared with OP clones, regardless of the exact measurement variable for population size (numbers of individuals or total biovolume/biomass). Interestingly, the decrease in population size was most pronounced in CP clones that heavily invested in sexual reproduction.
5. This suggests that the DDS mechanism can significantly contribute to population regulation and that genotypes lacking this mechanism (because of a mutation in genes affecting this trait) reach substantially higher population sizes. Apparently, the DDS mechanism operates already at much lower population densities than the RE, causing CP populations to stop growing before they are limited by resources.
6. As these differences in population regulation were caused by genetic variation within a single species and as rapid selective sweeps by OP clones are common in *B. calyciflorus*, this study provides an example for an eco-evolutionary feedback on an important ecological variable – equilibrium population size.

Keywords

carrying capacity; cost of males; density dependence; evolution of sex; population size; rapid evolution

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Introduction

Equilibrium population size in animals is often the result of an endogenous, density-dependent regulation, mediated by negative feedbacks between population growth and density (Murdoch 1994; Turchin 1999). Many different types of mechanisms might provide the basis for such a feedback. For instance, overexploitation of resources can result in reduced per capita birth rates or increased mortality and thereby provides an upper limit to population size (Hairston, Smith & Slobodkin 1960; Arcese & Smith 1988). If there are time-lags involved, this mechanism might also result in periodic fluctuations around a 'carrying capacity' (McCauley et al. 1999). Other density-dependent mechanisms include competition for territories or breeding sites (Krebs 1971; Rodenhouse, Sherry & Holmes 1997), autotoxins (Kirk 1998) or direct interference among individuals (Post, Johannes & McQueen 1997). Elucidating these different mechanisms, and their interactions, is a major prerequisite for understanding and predicting how populations are regulated and how they will respond to exogeneous changes, such as climate change or other human-driven alterations in the environment. Moreover, microevolutionary changes in traits affecting these mechanisms will likely create eco-evolutionary feedbacks to population size. Such feedbacks are considered essential to a more profound understanding of ecological dynamics (Hairston et al. 2005; Fussmann, Loreau & Abrams 2007; Pelletier, Garant & Hendry 2009; Schoener 2011).

Several density-dependent mechanisms may operate simultaneously in a population. It is important to study how such mechanisms act independently or together. Nevertheless, past studies have almost invariably focussed on single mechanisms (but see Rodenhouse et al. 2003). In this study, I focus on two different mechanisms of density-dependent population regulation: resource exploitation (RE) and density-dependent sexual reproduction (DDS). These two mechanisms apply to a number of facultatively sexual animals with density-dependent induction of sexual stages (Kleiven, Larsson & Hobæk 1992; Stelzer & Snell 2003; Schröder & Gilbert 2004; Timmer-meyer & Stelzer 2006): at low population densities, such populations reproduce clonally, while at higher population densities, an increasing proportion of sexual stages is produced. In such cases, sexual reproduction can provide a negative feedback to population growth because of the 'costs of sexual reproduction' (Snell 1987; Serra & King 1999; Fussmann, Ellner & Hairston 2003; Stelzer 2011). In this study, I analyse both mechanisms (RE and DDS) in populations that differ in their investment in sex. I make use of a recently discovered polymorphism in the monogonont rotifer *Brachionus calyciflorus* (Fig. 1), in which a single Mendelian locus can determine the reproductive mode of a population (Stelzer et al. 2010). This can result in entirely asexual populations, that is populations completely lacking the DDS component of population regulation, as opposed to facultatively sexual populations of the same species, which possess the DDS component of population regulation.

Most *Brachionus* species are capable of sexual and asexual reproduction (cyclical parthenogenesis, hereafter: CP). At low population densities, clonal reproduction dominates: females produce genetically identical daughters by ameiotic parthenogenesis. At higher population densities, sexual daughters are produced, whose oocytes undergo meiosis and

develop into haploid males (if not fertilized) or diploid diapausing eggs (if fertilized). The actual trigger for sex induction at high population densities is a chemical substance produced by the rotifers themselves, which is analogous to *quorum sensing* in bacteria (Kubaneck & Snell 2008). For example, the sex-inducing chemical can be 'harvested' from high-density cultures (in the form of conditioned medium) and applied to individually cultured females, which produce sexual daughters after this treatment (Stelzer & Snell 2003, 2006). In one *Brachionus* species, the sex-inducing chemical has been recently characterized as a protein (Snell et al. 2006). However, this life cycle of *Brachionus* is often unstable as there are several examples of strains that have completely lost their ability to reproduce sexually (Boraas 1983; Fussmann, Ellner & Hairston 2003; Stelzer 2008; Serra & Snell 2009). Recently, it has been shown that the loss of sexual reproduction (obligate parthenogenesis, hereafter: OP) in some *B. calyciflorus* strains is caused by a single recessive allele *op*, for obligate parthenogenesis (Stelzer et al. 2010). *Brachionus* clones homozygous for this allele (genotype: *op/op*) have completely and permanently lost the ability of sexual reproduction and are unable to respond to the chemical cue that induces sex (Stelzer 2008). By contrast, heterozygous clones (+/*op*) and wild-type clones (+/+) are cyclical parthenogens (CP) and undergo sexual reproduction at high population densities. The *op* allele appears to be completely silent in heterozygotes, because +/*op* clones are virtually indistinguishable from +/+ clones with respect to a large number of life-history traits related to growth and reproduction (Scheuerl, Riss & Stelzer 2011). Unexpectedly, Stelzer et al. (2010) found a notable size difference between the two reproductive types, OP clones being approximately half the size of CP clones. This indicated that genes conferring small body size hitch-hiked along with the *op* allele. This body size difference slightly complicates the measurements of population size in terms of numbers of individuals, but it can be accounted for by considering total biovolume or biomass.

Density-dependent sex provides a cost to population growth in rotifers, and it can result in substantial competitive advantages for obligate parthenogenesis (Serra & Snell 2009; Stelzer 2011). This is because sexual females in *Brachionus* do not contribute to current population growth. They produce either males, which do not produce offspring, or diapausing eggs, which undergo a substantially longer development compared with asexual eggs, sometimes several weeks to months (e.g. Hagiwara & Hino 1989). Thus, as a rough approximation, the fitness of such sexual females can be regarded as 'zero' when compared with asexual females in an actively growing population (Fussmann, Ellner & Hairston 2003). Note that this simplification puts emphasis on the short-term advantage of a sexuality and that it also includes a 'cost of dia-pause' (which cannot be separated from sex in *Brachionus*). Overall, this conceptual model has been verified in a recent study with *B. calyciflorus*, which showed that selective sweeps of OP clones invading into CP populations in chemostats could be quantitatively predicted based on such costs of sex and diapause (Stelzer 2011). In natural systems, however, there may be strong adaptive benefits of producing diapausing eggs, most notably bet-hedging strategies in unpredictable environments (Schroder 2005; Stelzer 2005). *Brachionus* populations often inhabit small ponds, which may dry up in summer or completely freeze in winter. In such situations, diapausing eggs are probably the only way to ensure the long-term survival of a population. In more permanent habitats, this situation may be relaxed, because individuals can also persist during harsh periods in their asexual

phase. This strategy has been described for some populations of *Daphnia*, another cyclical parthenogen that normally produces dormant eggs (Rellstab & Spaak 2009; Slusarczyk 2009; Lampert, Lampert & Larsson 2010). Finally, in natural environments, there may be benefits related to sexual recombination itself (Becks & Agrawal 2010). Nevertheless, in favourable environments, or during benign time periods within a growing season, there is usually selection against sexual reproduction in *Brachionus* (Carmona et al. 2009).

The aim of this study was to use CP and OP clones of *B. calyciflorus* as a model system for two types of density-dependent population regulation: density-dependent sex vs. resource depletion. I used two-stage chemostats to study the equilibrium population size in the clones of the two reproductive types (CP and OP). Two-stage chemostats are flow-through systems in which the culture is continuously diluted by fresh algal suspension at a constant dilution rate. As rotifers are washed out together with food algae or excretion products, populations tend to grow to an equilibrium size, at which rotifer population growth rate equals the dilution rate (Boraas 1983). I will define this as the ‘equilibrium population size’ under the experimental conditions. A two-stage chemostat can be viewed as an idealization of a very simple ecosystem consisting of only one predator and its prey, while the dilution rate of the chemostat may resemble mortality imposed by a top-predator or ‘fixed proportion harvesting’ in a managed population (Fryxell, Smith & Lynn 2005). Theoretically, an extremely high sex induction rate (e.g. close to 100%) can lead to ‘washout’, that is a collapse of the population beyond recovery. In this study, stationary population size was operationally defined in two ways: based on abundances (ind. mL⁻¹) – stationary population density (SPD) – and based on biomass concentration (rotifer biovolume per millilitre) – stationary biovolume (SBV). The latter was used to account for body size differences among clones. Biovolume can be used as a surrogate for biomass, as I will also show in this article. I used the above-described experimental system to test the following hypotheses: (i) populations of CP clones reach a lower stationary population sizes than OP clones, because of the additional density-dependent regulation mechanism, and (ii) populations of CP clones with high levels of sexual reproduction have an even lower stationary population size, compared with CP clones with low levels of sexual reproduction.

Materials and methods

Rotifer Clones and General Culture Conditions

Clonal cultures (i.e. asexual descendants of a single female) of the rotifer *B. calyciflorus* were used in this study. All clones were derived from two conspecific strains of the rotifer *B. calyciflorus*, a Florida strain and a Georgia strain (kindly provided by J.J. Gilbert, Dartmouth College, USA). The geographical origin of these two strains has been described in detail in an earlier study (Gilbert & Walsh 2005). Both strains exhibited a polymorphism regarding their mode of reproduction: some clones were obligate parthenogens (OP) and were thus unable to produce sexual offspring, while other clones were CP and produced sexual offspring at high population density (Stelzer 2008). This polymorphism is controlled by a single Mendelian locus (Stelzer et al. 2010): OP clones are homozygous for a recessive allele, *op* (for *obligate parthenogenesis*), which causes 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 (+/ +) are regular CP. Thus, CP and OP clones could be generated by self-fertilization from heterozygote clones (+ /*op*) – such self-fertilizations resulted in a Mendelian segregation pattern with 25% OP clones and 75% CP clones (Stelzer et al. 2010). Interestingly, OP clones were also dwarfs (i.e. body size was reduced by approximately 50%, compared with CP clones), which indicated pleiotropy or linkage of the *op* allele with genes that strongly affect body size (Stelzer et al. 2010). Despite the differences in body size, there was no overall difference in the survival or fecundity of these two reproductive types. Each experiment of this study used a set of OP and CP clones that were derived from the same parental clone through selfing. Hence, genetic variation among the experimental clones was generally very low.

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). The nitrogen concentration of the medium was set to 350 $\mu\text{mol L}^{-1}$. Food algae in stock cultures were supplied at *ad libitum* concentrations (approximately 400 000 cells mL^{-1}). Cultures were kept at 24 °C, and continuous illumination was provided with daylight fluorescent bulbs (30–40 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ for rotifers and 200 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ for algae). Clonal stock cultures of rotifers were re-inoculated once or twice per week by transferring 20 asexual females to a 20-mL fresh culture medium.

Chemostat Cultures

Population dynamics of OP and CP clones were investigated in two-stage chemostats, that is flow-through cultures of rotifers (the second stage) that were continuously supplied with food suspension from a separate algal culture (the first stage). Details of this method can be found in the study by Stelzer (2008). Briefly, the culture system consisted of the first stage – one large algal chemostat (volume: 2 L) together with a smaller mixing reactor (volume: 380 mL), where algal suspension was mixed 1 : 1 with fresh COMBO medium, and a second stage – 8–12 rotifer chemostats (each with 380 mL volume). The equilibrium concentration in the algal chemostat was approximately 750 000 *Chlamydomonas* cells per millilitre. Dilution rates of the rotifer chemostats, that is the fraction of volume replaced per time, were 0.3 per day for the very first 4–5 days of an experiment (to facilitate fast establishment of the rotifer populations) and 0.55 per day thereafter.

Experiment With Direct Population Counts

One experiment, lasting 25 days, focused on the initial phase of population establishment, assessing differences in resource depletion between CP and OP clones and estimating sexual reproduction using conventional methods (direct observations and microscopic counts). Chemostat cultures were inoculated at densities of one female per millilitre with clones of the Florida and Georgia strain, respectively, and were sampled daily (for rotifer and algae concentrations) or every 2–3 days (for the estimates of sexual reproduction). Two replicate chemostats were used for each clone. For the concentration estimates, rotifers and algae were fixed with Lugol's solution and enumerated using inverted microscopy (rotifers) or a CASY® electronic particle counter (Schaefer Systems, Reutlingen, Germany) for the algae.

Image Analysis Experiments

Four long-term experiments (duration: 16–42 days, or 64–168 sampling events, spaced at 6-h intervals, respectively) addressed the hypothesis that CP clones should reach lower carrying capacities than OP clones. Only the Florida strain was used in these experiments. Each chemostat was inoculated with only one clone (either a CP clone or an OP clone). Each experiment utilized 4–12 clones that were siblings, derived from self-fertilization of an individual heterozygous clone (genotype: + /*op*). Hence, the clones within one experiment were highly related to each other but differed in their reproductive modes. On the other hand, the parental clone (the one that was selfed) was a different one in each experiment, so there was some genetic variation contributing to variation among the four experiments. Sampling and analysis of the rotifer populations was carried out using a fully automated image analysis system (for a detailed description, see Stelzer 2009). Briefly, this system allowed quantitative analysis of female and male population density. It automatically sampled all chemostats in 6-h intervals using a peristaltic pump and several magnetic valves, which allowed switching among different rotifer cultures. Samples were drawn into an observation chamber, and a digital camera automatically took pictures of each culture and sent them to a PC for further analysis using image recognition software. As the image analysis system could discriminate between males and females, it was possible to calculate male ratios (males per female), which served as a rough estimate for the current degree of sexual reproduction.

I used two variables for determining the equilibrium population size of a rotifer population: SPD and SBV. Both variables were calculated as the mean values during the stationary phase of a population. The stationary phase was defined using the following heuristic rule: (i) each population time series was split into two halves of equal duration; (ii) the minimum population density during the second half was looked up; and (iii) this population density served to define the first data point to be included from the first half of the population time course. This procedure allowed excluding the phase of exponential population increase in the beginning while providing an objective selection criterion for the inclusion of data points. SBV was calculated by multiplying SPD with average female body volume for each clone. Average female body volume was determined from the size measurements of at least 30 adult females for each clone. These females were grown from birth to the age of 72 h at *ad libitum* food concentrations with daily transfers to fresh food suspension. The females were then fixed with 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 three distance measurements on each individual (total length, widest breadth and breadth at the anterior end) according to Ruttner-Kolisko (1977). Statistical analyses on SPD and SBV were carried out using two-way analysis of variance, with reproductive type (CP and OP) as fixed factor and experiment as random factor.

Calibration of Rotifer Biovolume Vs. Dry Weight

As biovolume (rather than biomass) was used as a measure for the standing population in a chemostat, it was necessary to ensure that the two reproductive types (CP and OP) did not differ in the density of their body tissues (i.e. biomass/biovolume ratio). To test this aspect, I grew mass cultures of different clones of both reproductive types and estimated

their biovolumes *and* biomasses (=dry weight of the rotifer populations). Briefly, 1–2 L batch cultures were inoculated with a few individuals of each clone and grown for 7–10 days until they reached population densities of 50–200 ind. mL⁻¹. The animals were then transferred from the algal suspension into sterile culture medium using 30-µm mesh nylon sieves, starved for 5–6 h and filtered onto pre-weighed GFC filters (Whatman, Kent, UK). A small aliquot of each culture was fixed in Lugol's solution for microscopic estimation of the total biovolume (females, males and eggs). GFC filters were dried for 2 h at 100 °C and weighed again. Microscopic measurements of biovolume were taken similar to the procedure described previously, except that various stages of the rotifer were classified: adult females, juvenile females, males, asexual eggs, diapausing eggs and male eggs. Males and all egg types were measured in two dimensions (length and breadth), and their shape was approximated as ellipsoids of rotation. From microscopic counts of the various life cycle stages, I calculated the total biovolume for each clonal culture. Statistical analysis was carried out using a *t*-test on arcsine-transformed values of the biomass/biovolume ratio.

Results

Experiment With Direct Population Counts

Rotifers of both reproductive types (CP and OP) showed the typical exponential increase (i.e. linear increase on a log scale) until they reached population densities of 30–120 ind. mL⁻¹ (Fig. 2a, d). At these densities, further population growth diminished, likely due to resource depletion and/or high induction of sexual offspring. Resource depletion appeared to be much more pronounced in OP clones than in CP clones, as is indicated by lower concentrations of residual algae in OP cultures (Fig. 2c, f). For instance, in the Georgia strain, this difference was as high as one order of magnitude (Fig. 2c). The CP clones showed considerable variation in their rates of sexual reproduction during the experiment (Fig. 2b, e). Occasionally, these rates were close to 100% (e.g. in the Georgia strain; Fig. 2b).

Mage Analysis Experiments

These experiments consisted of four independent chemostat experiments and involved different clones, a larger number of replicates and higher temporal resolution in terms of the sampling intervals than the exploratory experiment described previously (see *Experiment with direct population counts*). Figure 3 summarizes the population densities of CP and OP clones in all four experiments. This graph shows that the population densities of OP clones were almost always considerably higher than those of CP clones. In agreement with this observation, stationary population densities of OP clones were always considerably higher than those of CP clones (Fig. 4a). This impression was corroborated by statistical analysis, which showed that OP reached significantly higher SPDs than CP (Table 1). However, there were also significant interactions between the factors 'reproductive type' and 'experiment', indicating that the magnitude of the 'reproductive type' effect was variable among the experiments.

The picture changes slightly if SBVs are used as a measure for the carrying capacity (Fig. 4b). The SBVs of CP were increased to some extent because of the larger body size of

CP clones. In the experiments 2 and 3, this led to the situation that CPs reached similar SBVs as OPs. Nevertheless, in experiments 1 and 4, the SBVs of OPs were 1.8- to 2.8-fold higher than the SBVs of CPs. Overall, the SBV in OP was significantly higher than in CP (Table 1). However, there was again a significant interaction between reproductive type and experiment, indicating that the magnitude of the ‘reproductive type’ effect was variable among the experiments.

I suspected that the significant interaction in the two-way ANOVAS was caused by variation in sexual rates among the four experiments. In other words, the effect of a lowered equilibrium population size is expected to be most pronounced in the populations of CP with high rates of sex. To test this hypothesis, I calculated the average male ratio (an indicator of the rate of sex) for each CP population of all four experiments. Male ratios ranged from 0.001 to 0.149 among the 15 CP populations. I also determined the ‘relative SBV’ for each CP population, defined as the ratio of SBV of CP relative to the mean SBV of OP in the same experiment. In these calculations, the biovolume of males was added in CP populations, even though male biovolumes amounted to <2% in the most extreme cases (i.e. in the populations with the highest male ratios). This ‘relative SBV’ reflects the degree to which the equilibrium population size of a CP population is lowered relative to an OP population. For instance, a relative SBV of 100% would indicate that the cyclical parthenogen reached a SBV equal to the mean of all OP in the same experiment. As can be seen in Fig. 5, such high values were never reached. Relative SBVs ranged between 31% and 84% across experiments and chemostat populations within experiments. Indeed, there was a significant negative correlation between male ratio and the relative SBV, which indicates that higher rates of sexual reproduction in CP populations were accompanied by lowered SBVs (Spearman’s rank correlation: $n=15$, $\rho=-0.954$, $P<0.001$).

There was no significant difference in the body tissue densities of the two reproductive types (Student’s t -test: $P=0.599$). Tissue density was $0.13 \pm 0.03 \mu\text{g per } 10^6 \mu\text{m}^3$ for OP and $0.14 \pm 0.04 \mu\text{g per } 10^6 \mu\text{m}^3$ for CP. These values are highly consistent with literature values for *B. calyciflorus*, which are in the range of 0.12–0.15 $\mu\text{g per } 10^6 \mu\text{m}^3$ (Dumont, Velde Van De & Dumont 1975; Breitig & Tümping Von 1982). Overall, this confirms that biovolume can be used as a surrogate for biomass when estimating the carrying capacity of a population.

Discussion

The results of this study confirmed that DDS can provide a mechanism of population regulation in rotifers: clones with high propensity for sexual reproduction were restricted to significantly lower equilibrium population sizes than clones with low sexual propensity or obligatory asexual clones. This result held for both measures of the stationary population size, SPD and SBV, further confirming that the difference was caused by the reproductive mode rather than by the body size differences between CP and OP clones. DDS affected populations at resource levels that were not yet limiting for population growth. This was suggested by the observation that residual algal concentrations in CP populations were substantially higher than in OP populations (Fig. 2). These elevated algal concentrations may have strong implications on competitive interactions between the two reproductive

modes, because they resemble an ‘Achilles heel’ of CP populations in terms of potential invasions by OP clones.

Two previous studies have addressed the population-level consequences of asexuality in comparable experimental systems. First, Fussmann, Ellner & Hairston (2003) used single-stage chemostats (i.e. rotifers and algae growing in the same vessel) to study eco-evolutionary dynamics of mixed-clonal populations of *B. calyciflorus*. They found that the propensity for sexual reproduction declined during the experiment, which was presumably caused by the selection of clones with low levels of sexual reproduction. To account for such evolution, Fussmann, Ellner & Hairston (2003) constructed a simulation model that included the evolution of a quantitative trait called ‘propensity for sex’ and showed that accounting for evolution improved the fit between model predictions and observed population dynamics. A distinctive feature of Fussmann *et al.*’s experimental system is that they used single-stage chemostats in combination with high nutrient concentrations, which led to the extinction of rotifer populations after only one predator–prey cycle, rather than the sustained populations of the present study.

A second study examining population-level consequences of asexuality was recently published by Scheuerl, Riss & Stelzer (2011), who compared the population growth of the three possible genotypes (*op/op*, *+ / op* and *+ / +*) in batch cultures. They obtained similar ‘boom and crash’ dynamics as in Fussmann, Ellner & Hairston’s (2003), but found that the peak in population density was higher in OP clones (genotype: *op/op*) than in the two CP genotypes. However, this difference became insignificant if total biovolume was considered instead of numbers of individuals. This is in contrast to the present study, where SBV was also lowered in CP genotypes. This discrepancy is likely the result of contrasting dilution regimes in the two studies: In Scheuerl, Riss & Stelzer’s (2011) study, the batch cultures experienced a dilution rate of 0 (by definition), whereas in the present study, the dilution rate was set to 0.55 per day. In the former, populations increased at their maximum possible growth rate (i.e. population doublings up to every 7–8 h). This rate of population increase was probably too fast for the DDS mechanism to significantly affect the reproduction *before* the resources were used up completely. By contrast, the dilution rate of this study prevented excessive population growth and slowed population dynamics, allowing a persistent feedback of the DDS mechanism to population growth.

The present study shows that small differences in the genetic composition of a population (genetic variation with respect to the *op* allele) can have a significant effect on equilibrium population size. The CP and OP clones used in this study were genetically highly similar to each other, because they were generated by selfing of the same parental *op/ +* clone. In a previous study, this genetic similarity was confirmed by AFLP (Amplified Fragment Length Polymorphism) markers: 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). Thus, the CP and OP clones likely differed only in the *op* allele and in few closely linked alleles. Another previous study has shown that populations with a mixed genetic composition with regard to the *op* allele can evolve rapidly (Stelzer 2011). For instance, OP clones are often favoured over CP clones in chemostats and can sweep to fixation within only a few asexual generations. In conclusion, the DDS mechanism (and its underlying genetic variation)

combined with rapid evolution of this trait can give rise to eco-evolutionary feedbacks that can significantly affect an important ecological variable – population size.

The DDS mechanism likely acts independent of the RE mechanism of population regulation. This is because DDS operates already at low-to-medium population densities, when resource depletion is still low. For instance, thresholds for sex induction in rotifers are often in the range of 0.1 females per millilitre (Snell & Boyer 1988; Stelzer & Snell 2003) and sometimes even lower (Carmona, Gomez & Serra 1995; Gilbert & Dieguez 2010). Second, the increase in sexual reproduction with population density is caused by population density *per se*, rather than indirectly by resource depletion. This has been demonstrated by experiments involving conditioned medium, harvested at different rotifer population densities and applied to rotifers at a constant, high food concentration (Stelzer & Snell 2003; Snell & Stelzer 2005; Timmermeyer & Stelzer 2006), and by the isolation of a sexinducing protein in *Brachionus plicatilis* (Snell et al. 2006). This is in contrast to other CP, for example *Daphnia*, where food reduction can also act as a trigger for sexual induction (Kleiven, Larsson & Hobaek 1992).

This study suggests that asexual lineages might, in general, exert a stronger impact on their environment (higher population size → higher grazing impact) than their sexual counterparts, because of a higher realized equilibrium population size. In *Brachionus*, this was likely caused by a release from the costs of sexual reproduction and diapause. Theoretically, one should expect that asexuals can transfer more of their assimilated energy into immediately reproducing offspring. Consequently, if asexuals and sexuals are similar with regard to other aspects of their life history, a transition to asexuality should result in higher grazing pressure and thus a greater impact on the environment. As many invasive species are asexual (Sakai et al. 2001), it is tempting to speculate that their negative effects on environments and communities derive, to some extent, from a more efficient energy transfer caused by a release from the cost of sex. Extrapolating such an effect would mean that ecosystems favouring asexuality (e.g. stable habitats) might experience higher biomass turnover than ecosystems favouring sexuality.

The results of this study have been derived from a very simple and well-controlled laboratory system. Under such conditions, OP clones (i) are competitively superior over CP clones (Stelzer 2011) and (ii) exert a measurable impact on their environment owing to their lack of the DDS mechanism of population regulation (in this study). Real ecosystems, on the other hand, likely contain at least some variables that would favour CP clones over OP clones, for example recurrent harsh conditions, which favour diapause, or conditions favouring sexual recombination. Thus, the dominance of OP clones could be only transient under natural conditions. Likewise, the effect of OPs on their environment (higher grazing impact) might be diluted by the sheer complexity of planktonic food webs. Thus, the eco-evolutionary feedback observed in this study might be difficult to observe under field conditions, or only it may be restricted to very simple, constant and species-poor environments, like large inland saline lakes or other ‘extreme’ habitats. In fact, this problem of ‘real-world’ applicability is one of the major challenges in the area of eco-evolutionary dynamics. To conclude, in the future, it will be necessary to study the ecological consequences of asexuality in more complex systems. This could include more

complex experimental systems, such as tri-trophic food chains with a predator on top of *B. calyciflorus* (e.g. Verschoor, Vos & Van Der Stap 2004), or controlled introductions of OP to semi-natural communities, like mesocosms or field enclosures.

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Fig. 1. *Brachionus* female, carrying an asexual egg (Photograph by: Claus-Peter Stelzer).

Fig. 2.

Experiment with direct population counts: population dynamics (a, d), sex induction (b, e) and concentrations of residual algae (c, f) in cyclical parthenogens vs. obligate parthenogens clones of the rotifer *Brachionus calyciflorus*. Panels a–c: Georgia strain; panels d–f: Florida strain. Each line corresponds to a replicate (chemostat). Note the logarithmic scaling on the Y-axis in some panels.

Fig. 3.

Image analysis experiments: population densities of cyclical parthenogens vs. obligate parthenogens clones (Florida strain). Each panel shows the population densities of all chemostats of one experiment. Symbols correspond to individual measurements (sampled at 6-h intervals). Note the logarithmic scaling on the Y-axis.

Fig. 4. Stationary population density (a) and stationary biovolume (b) of cyclical parthenogens vs. obligate parthenogens clones in the image analysis experiments (Florida strain). Each symbol shows the mean $\pm$ standard error for all chemostats of the same reproductive type. For statistical analysis, see Table 1.

Fig. 5. Correlation between male ratio and stationary population density in cyclical parthenogens clones during the image analysis experiment (Florida strain). There was a significant negative correlation between the two variables (Spearman's rank correlation: $n=15$, $\rho = -0.954$, $P < 0.001$).

Table 1

Results of ANOVAs on stationary population density (SPD) and stationary biovolume (SBV). Reproductive type refers to cyclical parthenogens and obligate parthenogens clones, respectively. Experiment refers to experiments 1–4 of the image analysis experiments (see Materials and Methods)

	Source of variation	d.f.	Mean square	<i>F</i>	<i>P</i>
SPD	Reproductive type (R)	1, 3·048	10 001·58	28·712	0·012
	Experiment (E)	3, 3	58·33	0·152	0·922
	R × E	3, 24	384·97	14·4	<0·001
	Residual	24	26·7		
SBV	Reproductive type (R)	1, 3·056	1935·97	294·49	0·045
	Experiment (E)	3, 3	97·51	6·432	0·712
	R × E	3, 24	197·7		<0·001
	Residual	24			