Selection of low investment in sex in a cyclically parthenogenetic rotifer

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sex maintenance.

Abstract

Cyclical parthenogens, which combine asexual and sexual reproduction, are good models for research into the ecological and population processes affecting the evolutionary maintenance of sex. Sex in cyclically parthenogenetic rotifers is necessary for diapausing egg production, which is essential to survive adverse conditions between planktonic growing seasons. However, within a planktonic season sexual reproduction prevents clonal proliferation. Hence, clones with a low propensity for sex should be selected, becoming dominant in the population as the growing season progresses. In this context, we studied the dynamics of the heritable variation in propensity for sexual reproduction among clones of a *Brachionus plicatilis* rotifer population in a temporary Mediterranean pond during the period the species occurred in plankton. Clonal isolates displayed high heritable variation in their propensity for sex. Moreover, the frequency of clones with low propensity for sex increased during the growing season, which supports the hypothesized short-term selection for low investment in sex within a growing season. These results demonstrate (1) the inherent instability of the cyclical parthenogenetic life cycle, (2) the cost of sexual reproduction in cyclical parthenogens where sex produces diapausing eggs and (3) the role of the association between sexual reproduction and diapause in maintaining sex in these cyclical parthenogens.

Introduction

Sexual reproduction plays a critical role in the genetic structure, adaptation rates and, ultimately, organic evolution of a population. Despite this, there is no widely accepted explanation for the evolutionary processes maintaining sexual reproduction (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Stearns, 1987; West *et al.*, 1999; Peck & Waxman, 2000; Butlin, 2002). Compared with asexual reproduction, sexual reproduction is costly because of resource allocation to males, since males only contribute to population growth by fertilizing females. In diploid anisogamic organisms, where half the reproductive effort is allocated to males, this implies a two-fold cost of sex (Maynard Smith, 1971). Several theories have been proposed to explain the short- and long-term benefits that counterbalance the cost of sex, mostly stating that sexual progeny are more diverse than asexual ones (West *et al.*, 1999; Butlin, 2002; Simon *et al.*, 2002, 2003). Some theories are based on selection at the species or population level, assuming that asexual species are evolutionary dead ends, prone to extinction because of their slow rates of adaptation (Maynard Smith, 1998; Brookfield, 1999). If asexuals arise at low rates, their high extinction rate would imply a steady-state with a low frequency of asexual species (Brookfield, 1999).

The paradox of sex is particularly intriguing in cyclical parthenogens, which primarily propagate asexually but display occasional bouts of sexual reproduction. Cyclical parthenogenesis would seem to take advantage of the best of both reproductive modes (Lynch & Gabriel, 1983; Simon *et al.*, 2002), fully retaining the evolutionary advantage of recombination while minimizing the cost of producing males. However, there are only ~15 000 animal species spread over six taxonomic groups, that are cyclical parthenogens (Hebert, 1987). Cyclical parthenogenesis might also have physiological and cytogenetic evolutionary constraints (Hebert, 1987; Rispe *et al.*, 1998;

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Simon *et al.*, 2002) and be inherently unstable, since the loss of sex does not require the acquisition of a new reproductive mode (Burt, 2000; Peck & Waxman, 2000). After an obligate asexual appears in a population of cyclical parthenogens, its frequency will often increase up to fixation because of the short-term cost of sex (Bulmer, 1982). The evolutionary constraints on the origin of cyclical parthenogenesis, together with the trend for sex loss could explain why this reproductive mode is rare. However, sex may have been maintained in cyclical parthenogenetic populations because of indirect selection pressures. In aphids, cladocerans and rotifers – the best known representatives of cyclical parthenogenesis – sexual reproduction is associated with the production of dormant, resistant stages, which are essential to survive recurring adverse periods. In this way, selection maintaining resistant stages would indirectly maintain sexual reproduction (Simon *et al.*, 2002).

Monogonont rotifers (with over 1500 species) are small-sized (50–2000 μm) invertebrates inhabiting aquatic and moist habitats (Wallace *et al.*, 2006; Wallace & Smith, 2009). As a result of their high reproductive rates, they often reach large population sizes (up to about 1000 individuals L^{-1}) constituting a substantial part of continental zooplankton, and playing a critical role in aquatic food webs (Armengol *et al.*, 2001; Wallace & Smith, 2009). At temperate latitudes, rotifer populations are typically temporary, in many cases annually colonizing the water column (planktonic growing season) by hatching of diapausing eggs from lake sediments (Gilbert, 1974; Pourriot & Snell, 1983). The hatchlings are diploid asexual females (amictic females) that produce genetically identical daughters by ameiotic parthenogenesis (Wallace *et al.*, 2006). Typically, there is a period of exclusively asexual reproduction, followed by a period in which both sexual and asexual reproduction co-occur. The sexual phase is induced by environmental factors and asexual females start to parthenogenetically produce sexual (mictic) females among their offspring. Sexual females produce haploid eggs, which if not fertilized, develop into haploid, dwarf males. If sexual females are inseminated while young, their fertilized, sexual eggs will develop into encysted diploid embryos, called diapausing eggs. Diapausing eggs, which settle in the sediment, are able to resist desiccation and other adverse conditions, remaining viable for decades (Marcus *et al.*, 1994; Kotani *et al.*, 2001; García-Roger *et al.*, 2006a). After an obligatory dormant period (Hagiwara & Hino, 1989), and when suitable conditions resume in the water column, a proportion of the diapausing eggs hatch and a new growing season begins.

In the extensively studied genus *Brachionus*, sexual reproduction (mixis) is density-dependent, and induced by a chemical signal produced and released into the environment by the rotifers themselves (Gilbert, 1963, 2003a; Snell & Boyer, 1988; Carmona *et al.*, 1993, 1994; Stelzer & Snell, 2003, 2006). A recent study (Snell *et al.*, 2006) has shown that this signal is a protein, called the mixis inducing protein (MIP). As population density increases, this protein concentration increases in the medium until reaching a threshold that triggers the initiation of sexual reproduction, commonly at rotifer densities of around 0.1 individuals per millilitre (Timmermeyer & Stelzer, 2006).

Since diapausing eggs are produced by sexual reproduction in *Brachionus*, and this type of egg is needed to survive adverse periods, the cost of sex operating within an annual growing season is, in fact, the cost of dormancy (Serra & Snell, 2009). For instance, a *Brachionus* clone could have a high investment in sexual reproduction by initiating sex at low population density – i.e. low MIP concentration. This would lead to an extended period of sexual reproduction within the annual planktonic growth period, resulting in a slow rate of clonal growth. Note that, because of the association between sexual reproduction and dormancy, none of the sexual females contribute to the current growth of the clone, as they only produce males or diapausing eggs. Thus, the cost of sex on this time scale is not the cost of producing males, but the fraction of sexual females to total females produced (i.e. the mixis ratio). Therefore, another clone having a low investment in sexual reproduction by initiating sex a high density would be selected for during the growing season, even if this feature implies lower diapausing egg production compared with the alternative strategy. In contrast, since survival to the next planktonic growing season is only possible through diapausing eggs, between-year cycle selection would select against the clone with low investment in sex.

The current study addresses the hypothesis that there is heritable variation for investment in sexual reproduction in natural populations of cyclical parthenogenetic rotifers, which leads to short-term selection of clones with low investment in sex during the annual growing period (i.e. the period when the species occurred in plankton). With this aim, the *Brachionus plicatilis* rotifer population inhabiting the Mediterranean Pond Poza Sur (Spain) was studied. Genetic variation in the rate of diapausing egg production (S. Campillo, E. García-Roger, M. J. Carmona & M. Serra, unpublished) and in the timing of sexual reproduction (Aparici *et al.*, 2001) have been reported in this species. A large foundation of ecological data exists for the population under study (e.g. Gómez *et al.*, 1995) including annual planktonic growth periods (Gómez *et al.*, 1995; Ortells *et al.*, 2003) and reportedly long periods of sexual reproduction (Carmona *et al.*, 1995; Aparici *et al.*, 2002).

Material and methods

Sampling

A population of *B. plicatilis* (Müller 1786) was sampled in Poza Sur, a brackish pond located in a marshland in the

Natural Park Prat de Cabanes-Torreblanca (Eastern Spain; 40°08.917′N, 0°10.148′E). This is a small, rectangular (10 × 7 m) and shallow (1 m, average depth) pond, with strong seasonal variation in salinity (Gómez *et al.*, 1995). The biological species *B. plicatilis* belongs to a cryptic species complex. From previous studies it is known that only three species of this complex inhabit Poza Sur (Gómez *et al.*, 1995; Ortells *et al.*, 2003). Individuals of these three species can be distinguished by body size and shape, and care was taken to perform accurate taxonomical identification of *B. plicatilis* (Ciros-Pérez *et al.*, 2001). In previous studies *B. plicatilis* was recorded in the water column of Poza Sur from September to May (Gómez *et al.*, 1995; Ortells *et al.*, 2003).

Poza Sur was sampled every ∼15 days from 29 September 2006 to 11 May 2007, when no rotifers were detected after sampling approx. 20 000 L of water. This gave rise to 15 sampling events. Live zooplankton samples were obtained through horizontal hauls with a 30 μm-mesh net in each sampling event. Samples were re-suspended in approx. 1 L of pond water. Additionally, three preserved quantitative zooplankton samples were obtained from the mid point, east end and west end of the shorter pond axis (i.e. following the dominant wind direction in the pond). Each of these samples was obtained by filtering 3 L of pond water through a 30-μm mesh, and fixing the retained material with formaldehyde (4% final concentration).

Sexual reproduction bioassays

Propensity for sexual reproduction in the isolated females was studied following the procedure described in the literature (Carmona *et al.*, 1993; Aparici *et al.*, 2001; Stelzer & Snell, 2003, 2006) with modifications. For every sampling event, within 4 h of the field collections, 200 *B. plicatilis* females were picked from the live sample under a stereomicroscope and individually isolated in Petri dishes with 60 mL of culture medium. This medium consisted in 12 g L^{-1} salinity artificial seawater (Instant Ocean®; Aquarium Systems, Sarrebourg, Cedex, France), fertilized with modified f/2 medium (Guillard & Ryther, 1962), and contained the microalgae *Tetraselmis suecica* (5×10^5 cells mL^{-1}) as food. Culture conditions were 18 °C and constant illumination (PAR: approx. 35 μmol photons m^{-2} s^{-1}). Microalgae used as food had been cultured in the same medium and conditions, and density was estimated by 750-nm wavelength light extinction using a density vs. absorption calibration curve.

Most isolated rotifer females reproduced asexually, founding a total of at least 108 clones per sampling event (average number of clones per sampling event: 158 ± 18). From the moment the females were isolated in the Petri dish, a pre-experimental culture period started (1) to produce individuals to replicate clones and (2) to control the maternal environmental conditions that might affect the reproductive type of the offspring.

For the latter, the criterion of assaying the third generation cultured under controlled conditions was used (Stelzer & Snell, 2006). Therefore, 2 days after initial isolation of the females, three asexual daughters carrying a single egg of each clone were individually transferred to Petri dishes with 40 mL of fresh culture medium, and allowed to reproduce. After 2 days, a newborn female was isolated from each Petri dish and used for the bioassay. Each newborn was transferred to a well (Nunc™ polystyrene 24-well plate; Nalge Nunc Int., Roskilde, Denmark) containing 0.5 mL of culture medium. These newborns were allowed to grow and reproduce, and were monitored every 12 h until the first male was observed in cultures. Then, both density and time at first male appearance were recorded (Aparici *et al.*, 2001). Genetic variation in the propensity for sexual reproduction was studied by recording population density of a clone when the first male appeared. When the first male appears at low clone density in a laboratory culture, it is indicative of a high propensity to sex (i.e. high investment in sex) during the planktonic growing season. A total of 5474 bioassays were carried out as a result of combining 15 sampling dates, an average of 158 clones per sampling event and an average of ∼2.3 replicates per clone.

Estimation of mixis ratio and population density in the natural population

To estimate the level of sexual reproduction in the natural population for each sampling event, ∼300 *B. plicatilis* females were isolated from the live samples within 4 h of sampling, and individually placed in wells (Nunc™ polystyrene 96-well plate) containing 150 μL of culture medium (culture medium and conditions as above). These females were allowed to reproduce and were then classified as asexual (producing female offspring) or sexual (producing male offspring or diapausing eggs). From these data the mixis ratio (i.e. proportion of sexual females) was calculated for each sampling date.

Females from the preserved quantitative zooplankton samples were counted using an inverted Olympus CK2 microscope (Olympus Corp., Tokyo, Japan). Population density (N_i) of *B. plicatilis* in the pond at each sampling event (*i*) was estimated by computing a weighted average of the counts for the three sampling points (mid point, east end and west end of the shorter pond axis; according to sampled area, weight for middle point count was twice the weight of the other points).

Mixis ratio and population density estimations were combined to compute an integrated investment in sexual reproduction as follows:

$$\frac{\sum_i d_i (m_i N_i + m_{i+1} N_{i+1})/2}{\sum_i d_i (N_i + N_{i+1})/2},$$

where m_i and N_i are respectively the mixis ratio and the population density in the *i*-th sampling event and d_i is the number of days from *i* to *i* + 1 (Carmona *et al.*, 1995).

Stability of propensity for sexual reproduction in the laboratory

From all the clones analysed, those exhibiting first male appearance at the lowest and highest population densities, termed hereafter LDS (for low density sex) and HDS (for high density sex), respectively, were kept in laboratory culture to test short-term stability of their propensity for sexual reproduction. Sexual reproduction bioassays were repeated in these 14 LDS clones and 15 HDS clones 5 months after the sampling program ended. The bioassays followed the procedure described above, except they were performed in 15 mL of medium at 20 °C.

Statistical analyses

Effects on log transformed population density at first male appearance were tested using a nested ANOVA with sampling event as a fixed effect, and clone as a random effect nested to sampling event. Broad-sense heritability (H^2) was estimated from the genetic (i.e. between clones) component of the variance for each sampling event following the procedure for clonal organisms described in Lynch & Walsh (1998). Pearson's correlation and regression analyses were used to study the variation of the mixis ratio in the field, the average log-density at first male appearance and H^2 with the log-density of the natural population, as well as the variation of the average log-density at first male appearance and heritability with the sampling date. Differences between LDS and HDS clones were tested by fixed effect ANOVA on log-density at first male appearance, with clone type and clone nested to clone type as effects. All statistical analyses were performed using SPSS, release 14.0 (SPSS Inc., 2006).

Results

Population density of *B. plicatilis* ranged from 0.17 to 2280 females L^{-1} (Fig. 1) during the period from 29 September 2006 to 11 April 2007, following a downward trend. The highest observed mixis ratio (38%) was recorded on 29 September 2006 (i.e. the first sampling event), and decreased during the time course of the study. No sexual reproduction was observed in five of the six samples after January 2006, the last period of the growing season, where population density was lower than 3 females L^{-1}. The mixis ratio was significantly correlated with population density ($r = 0.64$, $t_{13} = 2.98$, $P = 0.005$). The ratio of integrated number of sexual females to integrated number of total females (i.e. an estimate of the population investment in sexual reproduction) was 0.130.
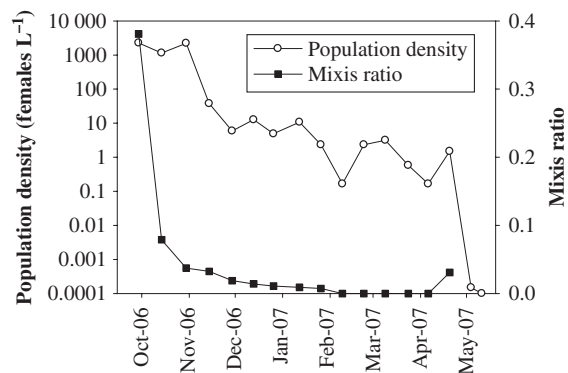

Fig. 1 Population density (log females L^{-1}) and mixis ratio of *Brachionus plicatilis* in Poza Sur during the study period (from 29 September 2006 to 20 April 2007). Note that in the two consecutive samplings after 20 April 2007 population density was lower than 5×10^{-5} females L^{-1}.

When tested in the laboratory, clones isolated from the *B. plicatilis* population in Poza Sur showed a wide variation in their propensity for sexual reproduction. Population density of clones at first male appearance ranged from 4 to 477 females mL^{-1} (average over replicates), while the time when the first male appeared ranged from 2.5 to 10 days (average over replicates). Population density at first male appearance differed significantly among clones within sampling events as well as among sampling events (Table 1). Respectively, these results show there is genetic variation for this trait and changes in the genetic component of the density at which males are produced. The frequency of clones with first male appearance at high population densities increased during the planktonic growing season (Fig. 2); for instance, the clone with the highest density at first male appearance was isolated late in the planktonic growing season, on 6 April 2007. Consistently, the average population density at first male appearance was positively correlated to sampling event (Fig. 3), with $r^2 = 0.36$ ($F_{1,13} = 7.18$, $P = 0.019$; significant results were also obtained when all the clones or all replicates, instead of averages, were used) and increased more than twofold over the growing season. The average population density at first male appearance was negatively correlated to population density in the field ($r = -0.57$, $t_{13} = -2.47$, $P = 0.014$).

Table 1 Nested ANOVA on density (log transformed) at first male appearance.

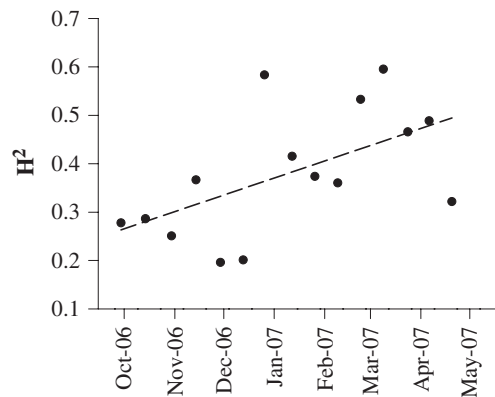| Source of variation	SS	d.f.	MS	*F*-value
Sampling event	145.63	14	10.40	122.01*
Clone (sampling event)	219.49	2359	0.09	2.67*
Error	107.89	3100	0.04	–

*$P < 0.001$.
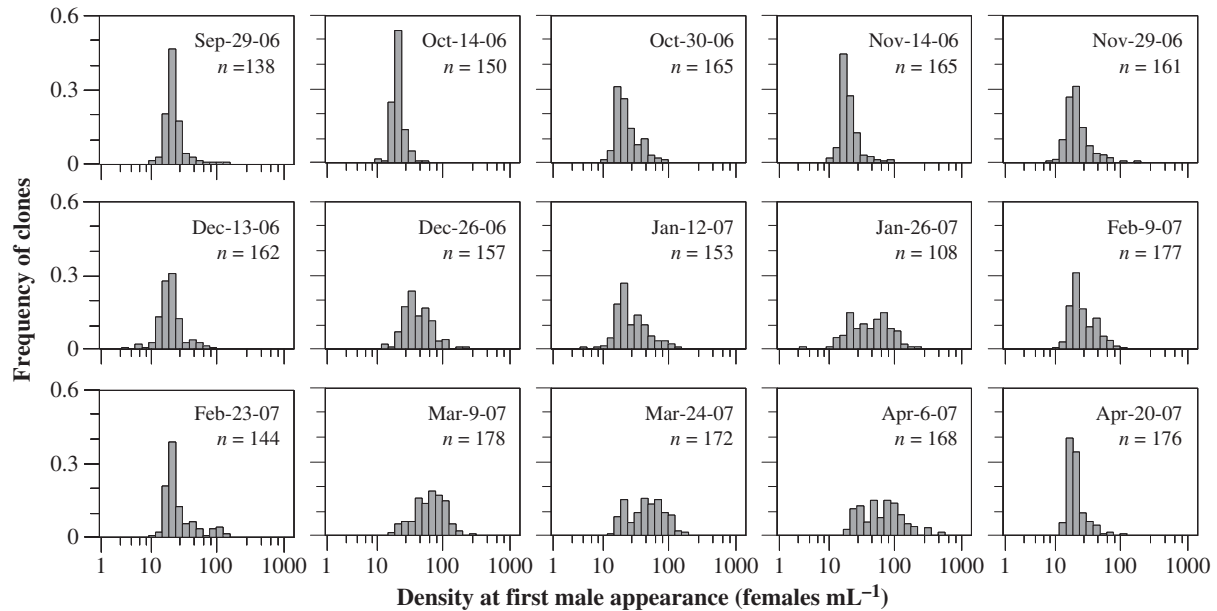
Fig. 2 Frequency distribution for population density at first male appearance in the clones isolated from Poza Sur at each of the 15 sampling events during the study period. Number of clones is indicated.
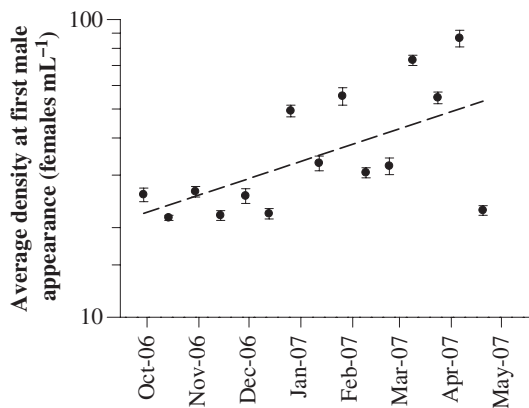
Fig. 3 Population density at first male appearance during the study period (from 29 September 2006 to 20 April 2007). Values are averages over clones; error bars are ± SE.

Fig. 4 Broad-sense heritability (H^2) during the study period (29 September 2006 to 20 April 2007).

Broad-sense heritability of the population density at first male appearance, as estimated in the laboratory, ranged from 0.18 to 0.57 and showed a twofold increase in value during the sampling period (Fig. 4; $r^2 = 0.32$; $F_{1,13} = 6.16$, $P = 0.027$). Heritability was negatively correlated to population density recorded in the field ($r = -0.50$, $t_{13} = -2.06$, $P = 0.030$).

Our experiment on stability of propensity for sexual reproduction performed on LDS and HDS clones after 5 months of culture showed significant differences in density at first male appearance between these two types of clones (Table 2). However, densities at first male appearance recorded in this analysis were lower than those taken on the same clones just after their isolation from the field.

Discussion

During this study, the *B. plicatilis* population inhabiting Poza Sur was present in the water column in fall, winter and spring, with lowest densities during spring. This pattern is consistent with that observed in previous studies (Gómez *et al.*, 1995; Aparici *et al.*, 2002; Lapesa, 2004). Poza Sur typically dries during the summertime,

Table 2 Nested ANOVA on density (log transformed) at first male appearance, with low density sex (LDS) clones and high density sex (HDS) clones as the main effect.

Source of variation	SS	d.f.	MS	*F*-value
Clone type†	7.62	1	7.62	310.89*
Clone (clone type)	9.41	27	0.35	14.21*
Error	1.96	80	0.03	–

*$P < 0.001$.
†LDS clone average, 8 ± 1.8 females mL^{-1}; range, 2.2–30 females mL^{-1}; HDS clone average, 31.7 ± 9.2 females mL^{-1}; range, 7.8–149.7 females mL^{-1}.

and although the population reached a high density on 29 September 2006 (i.e. the first sampling event), this date should be very close to water column re-colonization (Gómez *et al.*, 1995; Aparici *et al.*, 2002), therefore our sampling program most probably covered the whole planktonic growing season. Mixis ratio was positively correlated with population density, as expected for a density-dependent mechanism where sex is induced by rotifers producing a chemical signal that accumulates in the medium. The integrated investment in sexual reproduction of the *B. plicatilis* population during the growing period (0.130) is strikingly similar to the value (0.124) estimated in a previous study (Carmona *et al.*, 1995). Consistent with previous findings, *B. plicatilis* in Poza Sur displayed an extended period of sexual reproduction (Carmona *et al.*, 1995; Aparici *et al.*, 2002). Such a long sexual reproduction period should afford opportunities for short-term selection of clones with low investment in sex, this being one of the hypotheses we tested in the present study.

We measured the genetic variation of the population density at first male appearance. Density at first male appearance measured in the laboratory is not comparable to the much lower density at which sex is observed in natural populations, because of higher growth rates and maximum densities reached in the laboratory (Carmona *et al.*, 1995; Schröder, 2001). However, both densities are expected to be correlated. A high density at first male appearance is indicative of a low investment in sexual reproduction, as parthenogenetic growth will last longer. Therefore, our results from heritability analysis show that the *B. plicatilis* population inhabiting Poza Sur harbours genetic variation for investment in sexual reproduction. By checking a subset of clones selected because they had shown extreme values for density at sex induction just after field isolation, we found that genetic variation was stable after 5 months since clone isolation. There is additional evidence for genetic variation in sex investment in natural populations of cyclically parthenogenetic rotifers. In a previous study of the *B. plicatilis* population in Poza Sur, Aparici *et al.* (2001) detected heritable variation in the propensity to initiate sex among clones, which was established by hatching diapausing eggs

isolated from the pond sediment. Moreover, within-population heritable variation in sexual reproduction levels has also been detected in a study on the local adaptation of six natural populations of *B. plicatilis*, which included Poza Sur (S. Campillo, E. García-Roger, M.J. Carmona & M. Serra, unpublished). Furthermore, in the co-generic species *B. calyciflorus*, the evolutionary dynamics of populations cultured in continuous flow-through cultures (chemostats) indicated there was genetic variation for the propensity to reproduce sexually in the natural founder population (Fussmann *et al.*, 2003). Regarding other cyclical parthenogens, cladoceran populations inhabiting ephemeral habitats showed variation for investment in sexual reproduction (e.g. Ruvinsky *et al.*, 1986; Larsson, 1991; Innes & Dunbrack, 1993; Innes & Singleton, 1994; Deng, 1996; Innes *et al.*, 2000). Experimental studies suggest this variation has a significant genetic component (Innes & Dunbrack, 1993; Innes & Singleton, 1994; Innes *et al.*, 2000). Similar variation has been described in aphids, where different degrees of investment in sex can be found within the same population (Rispe *et al.*, 1999; Simon *et al.*, 2002).

We detected high levels of heritability for investment in sexual reproduction. Broad-sense heritability measured in the laboratory, where environmental noise should be controlled, is probably much higher than that expressed in the field. Moreover, although minimized by our pre-experimental approach, maternal effects could still increase our heritability estimates. In the rotifer *B. calyciflorus*, Gilbert (2002, 2003b) reported maternal effects for several generations after hatching from the diapausing egg, though stem females hatched from diapausing eggs are regarded as having particular features (Gilbert & Schröder, 2004; García-Roger *et al.*, 2006b). Despite these caveats, it is likely that biologically significant broad-sense heritability in the *B. plicatilis* population is expressed in the wild, hence creating an opportunity for natural selection to act. Note that during the planktonic growing season the genome of a female is inherited as a whole by her daughters, thus selection works on the total genetic variance. Hence, the broad-sense heritability, rather than the narrow-sense heritability, is the measure of genetic variability relevant for selection (Lynch & Walsh, 1998).

In the population of *B. plicatilis* under study, our results suggest that the genetic propensity to reproduce sexually declines with time, thus providing the first time-series evidence for this pattern in a natural population of cyclically parthenogenetic organisms. According to our regression analysis, the average density at first male appearance, measured after controlling environmental factors, increased from 22.4 to 56.7 females L^{-1} in 204 days. This suggests selection of low investment in sex during the planktonic growing season. High population density at first male appearance in a clone might be explained by low production rate of the chemical signal for sex initiation (i.e. MIP). If so, clones with high

population density at first male appearance in the laboratory might show high investment in sex in the wild if co-occurring co-specific clones produce MIP at high rate. In this event, the selective advantage of the former clones could save the cost of MIP production and this advantage would cause their dominance, and low investment in sex in the population as a whole. More likely, a mechanism for high density at first male appearance would involve low responsiveness to MIP, because of alleles affecting MIP receptors, for instance. This mechanism would imply a huge short-term advantage by avoiding the cost of sex. In rotifers, the cost of sex within a planktonic growing season can be quantified as the mixis ratio. For instance, a sexually reproducing clone with a mixis ratio of 10% would proliferate with a growth rate 10% lower than an asexual clone. Alternatively, the increasing frequency of clones showing first male appearance at high density during the planktonic growing season could be reflecting selection for clones adapted to crowding, if sex would be induced when clones cannot grow longer. However, this interpretation is not consistent with our finding of negative correlation between density at first male appearance and population density in the field.

Therefore, evidence of a decrease in the genetic propensity for sex in a natural population during the planktonic growing season supports the hypothesis that selection of clones with low investment in sex should be strong within a growing season. If genetic variation exists for sex investment, the trade-off between asexual and sexual reproduction (Snell, 1987; Serra & King, 1999) would imply an increased frequency of clones with low investment in sex during the part of the growing season in which sexual reproduction occurs. Note that clonal propagation depends upon asexual reproduction. For populations in which the period of sexual reproduction is long enough, selection for exclusive asexual reproduction may be possible. Consistent with this prediction, irreversible loss of sexual reproduction has been observed in laboratory cultures of rotifers (Boraas, 1983; Bennet & Boraas, 1989; Fussmann et al., 2003; Stelzer, 2008). Sex loss has also been reported in aphids and cladocerans (e.g. Hebert, 1987; Moran, 1992; Colbourne & Hebert, 1996; Simon et al., 2002). However, most rotifer populations only inhabit water bodies during the period of the year in which conditions are suitable. In these temporary populations, as frequently happens in cladocerans and aphids, sexually-produced diapausing eggs are necessary to survive between growing seasons. The need for diapause acts in opposition to the selective regime acting during the growing season, thus providing an important short-term advantage to the maintenance of sex, as stated by Simon et al. (2002).

In our study, the average increase in density at first male appearance during the planktonic growing season seemed to be related to the increase in the variance of this parameter. Genotypes with high density at first male

appearance are undetectable early in the planktonic growing season but occur more frequently later on (Fig. 2). Accordingly, broad-sense heritability increased during the study period, as expected if selection is favouring genotypes that are initially very rare. However, it is surprising that, given the important cost of sex in these populations (mixis ratio up to 38%) and the high heritability for variation in sex investment detected in the laboratory, we did not observe a more dramatic decrease in the genetic propensity for sexual reproduction. There could be several reasons for this. First, as mentioned above, we probably overestimated the broad-sense heritability expressed in the field. Secondly, the selection period for low investment in sex was when the *B. plicatilis* population was reproducing sexually, this being shorter than the growing season. Thirdly, a low MIP threshold for sex initiation might be associated to a low mixis ratio, which would reduce the associated cost of sex. If so, selection against low responsiveness to MIP might be weaker than expected. Finally, a previous study of a *B. plicatilis* population in Poza Sur using microsatellites as genetic markers found that clonal diversity decreases throughout the planktonic growing period (Gómez & Carvalho, 2000). Moreover, low genetic diversity has been associated with long planktonic growing seasons in other *Brachionus* populations (Ortells et al., 2006). Therefore, genotypes initiating sex at low population densities – i.e. early in the planktonic growth period – would be expected to produce more genetically diverse diapausing eggs than those delaying sex. This enables the former to more effectively retain the advantages that recombination affords fitness (e.g. Bell, 1982; Hurst & Peck, 1996; West et al., 1999; Tagg et al., 2005) and to partially compensate for the higher cost of sex.

A question arising from our results is how the genetic variation in the propensity for sex is maintained in a rotifer population long term. A temporary population of cyclical parthenogens can be conceived of as a collection of clones, where clones cross sexually to produce diapausing eggs, some of which will hatch in the next growing season (i.e. the clones have discrete sexual generations). In this context the total number of diapausing eggs produced by a clone during the growing season can be regarded as a between-year fitness measure (Serra & King, 1999; Serra et al., 2005). Given that production is dependent on both the MIP threshold for sex initiation and the mixis ratio after sex initiation, an optimal pattern is expected to evolve in a given ecological scenario (Serra et al., 2004). However, theoretical analysis of diapausing egg production has shown that a substantial diversity of patterns can be maintained in habitats with uncertain season length (Spencer et al., 2001). We conjecture that among-year variation in the length of the planktonic growing period may be the cause of the underlying variation in the genetic propensity for sex found in *B. plicatilis* in Poza Sur. Note that this pond is located in the Mediterranean area, which is characterized

by a high temporal variation in ecological conditions (Blondel & Aronson, 1999) and its small size confers low inertia to its physical conditions. Such environmental uncertainty must affect the time during which both biotic (competitors and predators) and abiotic conditions (salinity, temperature, etc.) would allow planktonic growth to take place. In this scenario, a high propensity for sex would be advantageous if the length of the growing season was unexpectedly short in a given year, and, vice versa, a low propensity for sex would have increased benefits in years with a long growing season.

Sex in cyclical parthenogens is linked to an ecological function; i.e. diapause and resistance to adverse conditions. This fact modifies the selective regime in sex maintenance. Sex loss is favoured when it is unnecessary to enter diapause, while adverse periods promote the maintenance of sex. The need to endure adverse conditions provides a constraint on the maintenance of sex, regardless of genetic diversity or any other advantages sex may afford.

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