

Evolutionary Ecology of Rotifers

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Preface

Ecology has been traditionally defined at the species level. That is, most patterns and processes were explained by interactions at the species level or higher taxonomic levels. Populations within a species have been considered more or less homogeneous, with respect to the properties of their individuals. Hence genetic variation, variation in fitness among individuals, or evolution was not explicitly taken into account. Sometimes even representatives of very different taxonomic backgrounds have been clustered into guilds, if they fulfilled the same “role” in an ecosystem. Even though ecologists have acknowledged that species can ultimately evolve and change, the time scales at which these changes happen have been considered much longer than the time scales of most ecological processes (Slobodkin 1961). Consequently, intrapopulation variation has been traditionally neglected, as well as the fact that the genetic composition of populations may change over short time scales, due to selection or drift (Hairston et al. 2005; Schoener 2011).

However, within the last two decades the mainstream of ecological research has shifted from a purely guild- or species-oriented view towards a finer resolution, both temporally and taxonomically. This change has been accompanied by the inclusion of several concepts from evolutionary biology, for example genetic variation, selection, or (local) adaptation. Several discoveries have paved the way towards this new viewpoint:

- 1) The use of highly resolving molecular markers showed that a considerable number of morphological species were actually comprised of several evolutionary distant lineages, often separated by millions of years (Gomez et al. 2002; Hebert et al. 2004; Knowlton 1993). In some cases, these cryptic species complexes also showed a biogeographic structure, which challenges the claim of cosmopolitan species (e.g., Mills et al. 2007). Upon closer examination many of these cryptic species were also ecologically differentiated (e.g., Ortells et al. 2003).
- 2) There has been ever increasing evidence that populations can evolve rapidly, sometimes within a few generations (Hairston et al. 2005; Reznick et al. 2004). Thus most populations contain substantial amounts of genetic variation, which allows them to quickly adapt to new environmental conditions. Such adaptations can sometimes even feedback to ecological processes (Fussmann et al. 2007; Schoener 2011).

With this shift in viewpoint, many traditional views have been challenged, or re-evaluated (e.g. Yoshida et al. 2007). Also, many new aspects and questions have come into the focus of ecological research. For example, ecologists have become more and more concerned with the influence of variation below the species level on ecological processes (Becks et al. 2010; De Meester et al. 2007). Similarly, species definitions themselves have come into the focus of ecologists, as well as related problems (e.g., ecological diversification of closely related species).

In the publications compiled for this “Habilitation-thesis” I have studied questions that are located at the interface between ecology and evolution. Thus, most of the problems addressed are treated with a mindset that ecology and evolution are tightly linked. Specifically I am addressing the following problems and questions:

- Linking demography to fitness: how do individual life history patterns translate into competition (between species) and selection (among individuals, within a species)?
I am addressing these questions in part 1 and 2 of this thesis.
- What are the causes and consequences (ecological and evolutionary) of transitions to obligate asexuality?
These problems are addressed in Part 2 of this thesis.
- How similar/different are closely related (morphologically similar) species?
My publications related to this question are summarized in Part 3 of this thesis.

This “Habilitation-thesis” is mainly intended to summarize my own research conducted after my PhD thesis. I will also pay reference to the general developments in the field “Evolutionary Ecology of Rotifers”, although, as a whole, this thesis will be strongly biased towards my own work.

Publications covered by this thesis

Publications are listed in thematic order according to the three parts of this thesis.

Publications in which I was not the sole author are followed by a ***brief statement of my contribution.***

Part 1: Life history strategies and population ecology

- 1) Stelzer, C. P. (2002). Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: mechanisms and adaptive significance. *Functional Ecology* 16: 835-841.
- 2) Stelzer, C. P. (2005). Evolution of rotifer life histories. (Review) *Hydrobiologia* 546: 335-346.
- 3) Stelzer, C.P. (2006). Competition between two planktonic rotifer species at different temperatures: an experimental test. *Freshwater Biology* 51: 2187-2199.
- 4) Stelzer, C.P. (2009) Automated system for sampling, counting, and biological analysis of rotifer populations. *Limnol. Oceanogr. Methods* 7: 856-864.

Part 2: Evolution of cyclical and obligate parthenogenesis

- 5) Stelzer, C.P. (2008). Obligate asex in a rotifer and the role of sexual signals. *Journal of Evolutionary Biology* 21: 287-293.
- 6) Stelzer, C.P., J. Schmidt, A. Wiedroither, S. Riss (2010) Loss of sexual reproduction in a small metazoan. *PLoS ONE* 5(9). E12854.

I designed the experiments, contributed to data collection, analyzed most of the data and wrote the manuscript.

- 7) Stelzer, C.P. (2011) The cost of sex and competition between cyclical and obligate parthenogenetic rotifers. *American Naturalist* 177(2): E43-E53.
- 8) Scheuerl, T., S. Riss, C.P. Stelzer (2011) Phenotypic effects of an allele causing obligate parthenogenesis in a rotifer. *Journal of Heredity* 102(4): 409-415.

I designed the experiments, contributed to data analysis, and wrote most of the manuscript.

- 9) Stelzer, C.P. (in press) Population regulation in sexual and asexual rotifers: an eco-evolutionary feedback to population size? *Functional Ecology*.

Part 3: Sexual signals and speciation

10) Stelzer, C. P. and T. W. Snell (2003). Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. *Limnology and Oceanography* 48: 939-943.

I designed the experiments (together with TWS), collected and analysed the data, and wrote most of the manuscript.

11) Snell, T. W. and C. P. Stelzer (2005). Removal of surface glycoproteins and transfer among *Brachionus* species. *Hydrobiologia* 546: 267-274.

TWS and I designed the experiments. I contributed to data collection and was involved in writing the manuscript, which was mainly written by TWS.

12) Stelzer, C. P. and T. W. Snell (2006). Specificity of the crowding response in the *Brachionus plicatilis* species complex. *Limnology and Oceanography* 51: 125-130.

I designed the experiments (together with TWS), collected and analysed the data, and wrote most of the manuscript.

13) Snell, T. W., J. Kubanek, W. Carter, A. B. Payne, J. Kim, M. Hicks and C. P. Stelzer (2006). A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). *Marine Biology* 149: 763-773.

TWS, JK and I designed the experiments. Data collection was done mainly by WC, ABP, JK, and MH. I was involved in writing the manuscript, which was mainly written by the TWS.

14) Timmermeyer, N. and C. P. Stelzer (2006). Induction of sexual reproduction in *Synchaeta tremula* (Rotifera, Monogononta). *Journal of Plankton Research* 28: 1233-1239.

I designed the experiments together with NT, contributed to data analysis, and wrote most of the manuscript.

15) Stelzer, C.P. (2011) A first assessment of genome size diversity in Monogonont rotifers. *Hydrobiologia* 662: 70-82.

16) Stelzer, C.P., S. Riss, P. Stadler (2011) Genome size evolution at the speciation level: The *Brachionus plicatilis* species complex (Rotifera). *BMC Evol. Biol.* 11: 90.

I designed the experiments, contributed to data collection and analysis, and wrote most of the manuscript.

A brief Natural History of Monogonont rotifers

Monogonont rotifers are an important component of aquatic ecosystems, forming a link between the microbial loop and higher trophic levels (Nogrady et al. 1993; Wallace et al. 2006). They are widely distributed in inland water bodies, such as in lakes, rivers and ponds, but also in extreme environments, like sewage ponds, acidified water bodies, alkaline lakes, pitcher plants, or in the thin water film covering mosses, lichens, and liverworts. Owing to their fast reproductive rates and dispersal capabilities they can quickly colonize new habitats and often numerically dominate these communities. Thus, there has been a long tradition of aquatic ecological research on freshwater rotifers.

When compared to most other multicellular animals, rotifers are relatively small, short-lived and fast-reproducing organisms. Lifespans of individuals are typically in the range of few days. Reproductive rates of individual females can reach about 20 asexual offspring. These offspring are produced strictly sequentially (Stelzer 2005), which differs from other planktonic animals that produce “clutches” (e.g. most cladocerans or copepods). This sequential offspring production is probably a constraint of large relative offspring size in most Monogonont rotifers (Relative egg sizes are as high as 72% of body volume, e.g. Stelzer 2011b). Nevertheless, Monogonont rotifers are among the fastest reproducing organisms. This is mostly due to their short generation times. For example, *Brachionus calycifloris* completes egg development in only eight hours at 25°C, matures at the age of 22 hours, and can reach egg laying intervals of one egg in every 3-4 hours (Bennett and Boraas 1989). This results in population growth rates of 1.92 d⁻¹, which is equivalent to a doubling of population density every 8.7 hours.

The life cycle in Monogonont rotifers typically involves cyclical parthenogenesis (Fig. 1), an alternation between ameiotic parthenogenesis and sexual episodes (Wallace et al. 2006). In this life cycle, 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). The triggers for sex induction can be quite diverse in Monogonont rotifers. A causal role of tocopherol has been established for several *Asplanchna* species (Gilbert 1980), while some *Trichocerca* and *Notomata* use day length as sex-inducing factor (reviewed by Gilbert 1992). By contrast, high population density appears to be responsible for sex induction

in many other genera, for example, *Brachionus*, *Rhinoglena*, *Epiphanes*, and *Synchaeta* (Carmona et al. 1993; Gilbert 1963; Schröder and Gilbert 2004; Stelzer and Snell 2003; Timmermeyer and Stelzer 2006). The actual trigger for density-dependent sex induction is a chemical substance produced by the rotifers themselves, which is analogous to *quorum sensing* in bacteria (Kubanek and Snell 2008). The sex inducing chemical can be “harvested” from high density cultures (in the form of conditioned medium) and applied to individually cultured females, which will produce sexual daughters after this treatment (Stelzer and Snell 2003; Stelzer and Snell 2006; Timmermeyer and Stelzer 2006). In one *Brachionus* species, the sex inducing chemical has been recently characterized as a protein (Snell et al. 2006). I will summarize the current knowledge in this field in Part 2 of this thesis.

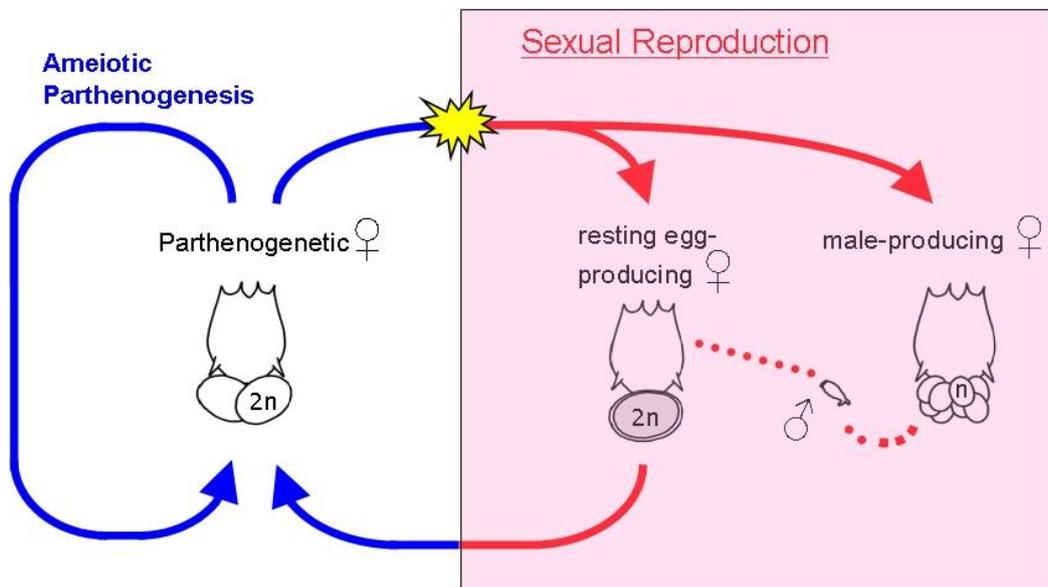


Fig. 1: A simplified scheme of the cyclical parthenogenetic life cycle of Monogonont rotifers. Blue arrows correspond to the asexual part in the life cycle; red arrows correspond to the sexual part. The entry into the sexual part of the life cycle is marked in yellow (“sex induction”). For further details, see main text.

Part 1: Life history strategies and population ecology

There are strong mechanistic links between the life histories of individuals and population growth, and a rich theoretical population biology theory formalizes these connections. For example, matrix population models are defined on the age-specific (or stage-specific) schedules of survival and reproduction (Caswell 2001). Such models can be powerful tools to ecologists since they allow prediction of population growth based on properties of individuals, but they also capture other features, such as the sensitivity of the population growth to minor changes at specific age-specific survival and fecundity. In addition, matrix models can capture non-equilibrium aspects of population growth, in particular if populations deviate from their stable age distribution. Matrix population models are commonly based on the so called “Leslie Matrix”, a system of linear equations describing the age structure of a population by specifying the survival probabilities during transitions through the different age classes, and recruitment of newborn offspring (i.e., the first age class) from each of these age classes. The Leslie matrix can be an important tool for population biologists and conservation ecologists. For instance, sensitivity analysis can help to optimize conservation efforts by identifying the age class that contributes most strongly to population recruitment (Caswell 2001). Matrix population models can be easily implemented for rotifers, since life tables - the raw data for a Leslie matrix - can be established within a few days (Stelzer 2002; Stelzer 2005). In the first half of this section, I will give a few examples of my work in which Matrix models were used. In the second half, I will focus on experimental studies at the population level, in particular on new methodological approaches for studying rotifer population dynamics.

Applications of demographic theory

Purely demographic processes can have a measurable impact on population dynamics. Most previous studies on rotifers have used population models without any demographic structure, while a few studies incorporated rudimentary demographic structures, such as mortality rates (Fussmann et al. 2000), or a discrimination between eggs and a (size-structured) rotifer population (McNair et al. 1998). In both studies, introducing demographic structure made the models more realistic by better accounting for some features of population dynamics. But there are some aspects about population dynamics that are purely caused by demographic processes. For example, dampened oscillations in the egg ratio are predicted if reproduction is concentrated in the young adult age (Stelzer 2006). Such concentration might be due to

shorter egg laying intervals in young adults, which is a quite common in rotifers (see Fig. 2). These demographic oscillations are independent of other environmental conditions, but they will eventually decline as the population converges to its stable age distribution. Nevertheless these oscillations will affect population growth, by causing deviations from equilibrium growth rates.

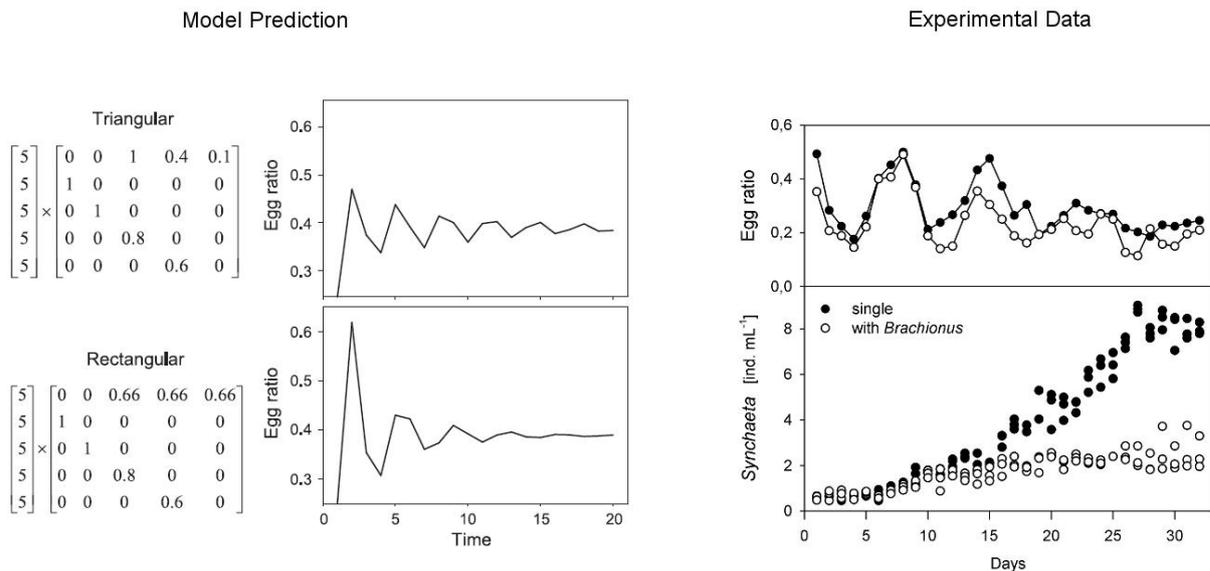


Fig. 2: Demographic egg ratio oscillations in rotifers. Left (Model prediction): Two hypothetical Leslie matrices and their predicted oscillations in the egg ratio in a growing rotifer population (Note that this population deviates from its stable age distribution in the beginning). Triangular fecundity schedule: reproduction is highest at early adult age; rectangular fecundity schedule: reproduction is equal across age classes. Graphs on the left show population data from *Synchaeta pectinata* - with and without a competitor (*Brachionus calyciflorus*). This figure was compiled from Stelzer (2006).

Another interesting application of matrix population models is the calculation of sensitivities. Sensitivity is defined as the contribution of individual elements of the Leslie matrix to its dominant eigenvalue λ , in other words, the contribution of age-specific fecundity or survival values to the overall population growth rate. Knowing such contributions is important because the population growth rate is often used as a measure of fitness or adaptation, in particular in clonally reproducing organisms. Sensitivity analysis can be used to identify precisely those life history changes that most strongly improve the fitness of a clone.

An example may illustrate the application of sensitivities: In a study on phenotypic plasticity, Stelzer (2002) examined “Bergmann’s rule” in the rotifer *Synchaeta pectinata*. If

exposed to low temperatures, these rotifers produced larger eggs than at high temperatures (e.g., 35% larger at 4°C vs. 12°C). The smaller egg size at 12°C appeared to be adaptive, since offspring from small eggs achieved higher population growth rates at this temperature. Sensitivity analysis showed that the largest contribution to the difference in growth rates was caused by an increased fecundity in young adults, which was (in this case) equivalent due to a faster juvenile development.

Methodological developments

The study of population dynamics usually involves tracking populations for several generations. This is time consuming both in terms of the total study duration and the amount of time spent on individual population censuses. Generally, the duration of a study can be reduced by selecting model organisms with short generation times. Accordingly there has been a long tradition of using zooplankton for such tasks, in particular rotifers. Their small body size, fast population growth and ease of keeping large populations in a relatively small space make them ideal for experimental approaches at the population level. Various general questions have been addressed using laboratory populations of rotifers, for example, assimilation efficiency and productivity of populations (Boraas 1983; Rothhaupt 1985; Walz 1993), food chains (Van der Stap et al. 2007; Verschoor et al. 2004), resource competition (Boraas et al. 1990; Ciroso-Perez et al. 2001a; Rothhaupt 1988; Stelzer 2006), population dynamics (Fussmann et al. 2000; Kirk 1998; Stelzer in press; Yoshinaga et al. 2001), nutrient limitation (Rothhaupt 1995), and evolutionary change (Becks and Agrawal 2010; Fussmann et al. 2003; Yoshida et al. 2007; Yoshida et al. 2003). In such experiments, rotifers have to be sampled at least once per day to achieve an adequate temporal resolution. After fixation, samples are concentrated in sedimentation chambers and analysed by microscopic examination under an inverted microscope. If adequate replication is used and/or if populations are studied for several weeks, the work effort in such experiments quickly reaches levels that are barely manageable. Moreover, the sampling interval is usually constrained to 24 hours, which may be too long to resolve fine patterns of population dynamics.

To improve this situation, I have developed an automated system for sampling and analyzing experimental rotifer populations (Stelzer 2009). A schematic drawing is presented in Fig. 3. The system relies on image analysis of digital photographs taken from subsamples of the culture. The system works completely autonomously for several weeks and can sample

up to 40 rotifer cultures at time intervals down to a few hours. It allows quantitative analysis of female population density at a precision equivalent to that of conventional methods (i.e., manual counts of samples fixed in Lugol's solution), and it can also recognize males, which allows detecting temporal variation of sexual reproduction in such cultures. Another parameter that can be automatically measured with the image analysis system is female body size. So far, I have used this system in several studies, e.g., to estimate the selection coefficients for obligate parthenogenesis (Stelzer 2011a), to compare population parameters of different rotifer genotypes (Scheuerl et al. 2011), or to quantify carrying capacities of cyclical vs. obligate parthenogenetic rotifers (Stelzer in press).

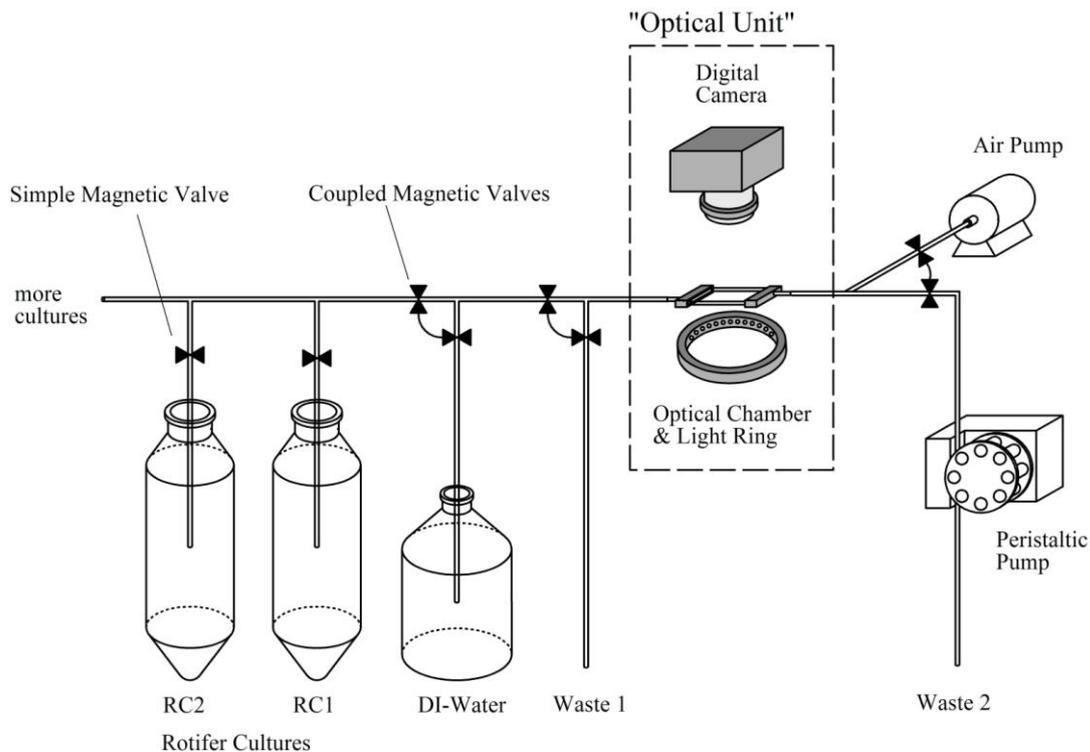


Fig. 3: Schematic drawing of the sampling and image analysis system. For simplicity, only two rotifer cultures are displayed (the most recent version of this system can handle up to 40 cultures). More details on the design and implementation can be found in Stelzer (2009).

Part 2: Evolution of cyclical and obligate parthenogenesis

The evolution of the cyclic parthenogenetic life cycle in rotifers is interesting for several reasons: First, it is interesting in its own right since rotifers exhibit one unique type of the “heterogonic life cycle”. Similar life cycles exist in aphids or crustaceans, two evolutionary quite distant animal taxa. However there are also notable differences to the cyclical parthenogenesis in rotifers, e.g. concerning the ploidy of males, or sex determination. The second reason why cyclical parthenogenesis is an interesting topic is a theoretical one that relates to the “paradox of sexual reproduction” (Bell 1982; Maynard Smith 1978; Williams 1975). Cyclical parthenogens are organisms that regularly engage in sex, but obligate parthenogenesis (OP) should evolve readily in such organisms, because this transition involves merely a loss of the sexual function. In fact, there are several independent studies reporting such a loss of sexual reproduction in *Brachionus* (Bennett and Boraas 1988; Boraas 1983; Buchner 1987; Fussmann et al. 2003; Stelzer 2008). Theoretically, OP mutants should be able to invade populations of cyclical parthenogens. Thus it is interesting to know which factors ultimately stabilize the sexual part in this life cycle.

Mechanisms of cyclical parthenogenesis

The mechanisms of the cyclical parthenogenesis have a long tradition in rotifer research. Early works in this area have been summarized by Birky & Gilbert (1971) and Gilbert (1992). This has led to the current understanding of the Monogonont life cycle, which was presented in Fig. 1. However fundamental research on cyclical parthenogenesis is still one of the most active areas in rotifer research, and many new insights have been gained in the last two decades. Recent additions to this knowledge include:

- (1) Details on the mechanisms of cyclical parthenogenesis. In particular, more details on density-dependent sex induction have been elucidated. Several studies have proven that density dependent sex is mediated by water soluble chemicals (Carmona et al. 1993; Stelzer and Snell 2003; Timmermeyer and Stelzer 2006). So far these efforts have culminated in the isolation of the MIP (=misis inducing protein) in *B. manjavacas* (Snell et al. 2006). Furthermore, a better understanding of the downstream signalling cascade has been gained, which may involve a progesterone signalling system (Stout et al. 2010). Other important findings concern developmental details

about the timing of sex-induction (Gilbert 2007), or a preliminary characterization of genes expressed during mixis induction (Suga et al. 2011) and diapausing egg production (Denekamp et al. 2011).

- (2) Exceptions of the “textbook cyclical parthenogenesis”. There have been several recent discoveries that complement the basic mode of cyclical parthenogenesis. One of them is transgenerational plasticity for sex induction: in several studies it has been shown that females are more or less unresponsive to sex-inducing stimuli during the first few generations after hatching from diapausing eggs, yet after after 10-12 generations the responsiveness to sex-induction cues is back to normal (Gilbert 2002; Gilbert 2003a; Schröder and Gilbert 2004). The mechanism behind this phenomenon is unknown, but it may involve epigenetic inheritance. Similar mechanisms may be involved in the effects of starvation treatments on sex-induction of subsequent generations (Hagiwara et al. 2005). An extreme case of suppression of sexual reproduction is obligate parthenogenesis, which has been found in several lines of *Brachionus calyciflorus*. More details on this exception of the life cycle will be presented below (see section “Obligate parthenogenesis in Monogonont rotifers”). Finally there are also cases of “obligate sexuality” in Monogonont rotifers. For example, populations of the rotifer *Hexarthra* sp. in the Chihuahuan Desert are composed of genotypes that hatch as sexual females *directly from* diapausing eggs, thus bypassing the asexual phase of the life cycle (Schroder et al. 2007).
- (3) Quantitative aspects of cyclical parthenogenesis. Much more information has been gathered on quantitative variation of sex-related traits. It is now well established that rotifer populations can harbour substantial amounts of genetic variation in terms of the percentage of sexual offspring, or in terms of the threshold for sexual induction (Campillo et al. 2009; Carmona et al. 2009). Even intraclonal variation for sex induction has been described in *Brachionus calyciflorus* (Gilbert and Schroder 2007). Moreover there are some nice examples of local adaptation for such sex-related traits. For instance, populations typically show higher rates of sex in less predictable habitats than populations that occupy constant habitats (Campillo et al. 2010).
- (4) Modelling studies have led to a much better understanding of the adaptive significance of the cyclical parthenogenetic life cycle. This involves various aspects, e.g. the frequency of sexual offspring, sexual thresholds, sex allocation, and diapause (Serra et al. 2008; Serra and King 1999; Serra et al. 2004). Further, some of these modelling

efforts also covered the “exceptions” of the standard life cycle, such as obligate parthenogenesis (Serra and Snell 2009; Stelzer 2011a), or transgenerational plasticity for sexual reproduction (Serra et al. 2005).

Obligate parthenogenesis (OP) in Monogonont rotifers

The basic mechanism of loss of sexual reproduction is reasonably well understood in the rotifer *Brachionus calyciflorus*. It has been demonstrated that this inability is caused by a loss of responsiveness to the chemical signal that induces sex (Stelzer 2008). As a consequence, populations of obligate parthenogens can grow to extremely high population densities (without ever inducing sex), whereas cyclical parthenogens readily induce sexual reproduction as soon as population densities exceed one female per ml (Stelzer in press; Stelzer et al. 2010). 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. By contrast, heterozygous clones (*+/op*) and wild-type clones (*+/+*) are cyclical parthenogens and undergo sexual reproduction at high population densities. If heterozygote clones undergo selfing, their offspring display the expected 3:1 segregation ratio into CP and OP clones (Fig. 4). The *op*- allele appears to be silent in heterozygotes, since *+/op* clones are virtually indistinguishable from *+/+* clones with respect to a large number of life-history traits related to growth and reproduction (Scheuerl et al. 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 pleiotropic effects, or that genes conferring small body size have hitch-hiked along with the *op*-allele.

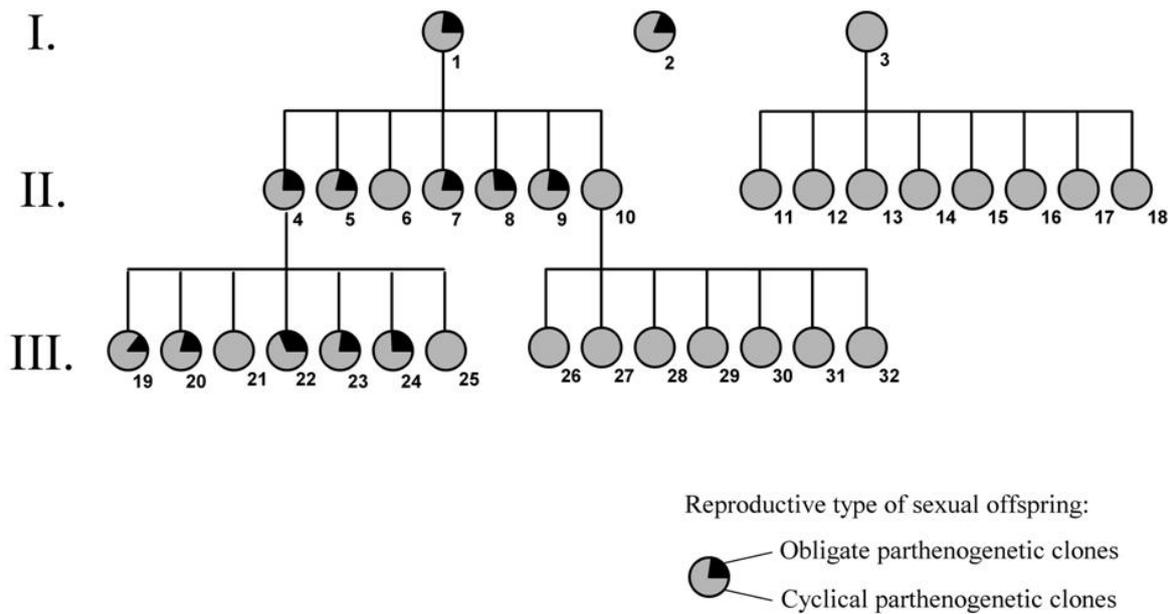


Fig. 4: Mendelian inheritance of obligate parthenogenesis in *Brachionus calyciflorus*. The figure shows an overview of different rotifer clones, represented by numbered pie charts, which were propagated by self-fertilization. Roman numbers indicate successive sexual generations; Arabic numbers indicate individual clones. Pie charts display the proportion of obligate vs. cyclical parthenogens among the sexually produced offspring clones of each clone (Stelzer et al. 2010).

Evolutionary and ecological consequences of OP

All else equal, an asexually reproducing female should produce much more offspring than a sexually reproducing female (often twice as many). This suggests a potential for population invasions by obligate parthenogens. The theoretical fitness consequences of such asexual transitions have been analyzed recently by Serra and Snell (2009) and by Stelzer (2011a). Over short time scales (i.e., few generations) a transition to obligate asexuality can result in a substantial competitive advantage. This is because OP clones completely lack the induction of sexual females, males, and diapausing eggs, which are all components that can severely slow down immediate (asexual) population growth. Such competitive substitutions have been modelled and quantitatively confirmed using laboratory experiments (Stelzer 2011a). Selection coefficients for OP can be as high as $0.39-0.65 \text{ d}^{-1}$, under some circumstances, which means that OP invaders can sweep through CP populations within a few days.

However, even though OP clones may grow faster than cyclical parthenogens they should be at a strong disadvantage in long term, because they are not able to produce diapausing eggs. Thus obligate parthenogens might increase in frequency during a growing season, but they should completely die out once the habitat deteriorates, whereas cyclical parthenogens could recolonize from diapausing eggs (Serra and Snell 2009). Nevertheless, during the growing season there is high clonal competition in populations of cyclical parthenogens (De Meester et al. 2006), hence shifts towards obligate parthenogenesis might occur towards the end of a season. In addition, competition with OP clones might have implications for the clonal composition of CP clones and their resting egg banks. Interestingly, there are some species of monogonont rotifers that can produce diapausing eggs without sex. For instance some clones of the rotifer *Synchaeta pectinata* can asexually produce diapausing egg that hatch after about 14 days, or up to several months (Gilbert 1995). If such species were able to give rise to obligate parthenogens, they might also be successful in longer terms.

Transitions to obligate parthenogenesis should also have strong ecological consequences. Similar to cyclical parthenogens, one OP female should be sufficient to colonize a habitat. However, OP populations might avoid several costs related to sex induction that are present in CP populations. First, the initial population expansion should be faster due to higher realized population growth rates (by avoiding the costs of males and diapause). Many CP populations constantly face these costs since sexual reproduction is *not* always concentrated to the “end of the growing season” (Carmona et al. 1995). Second, stationary populations of OP clones should reach a higher carrying capacity, because asexuals might transfer more of their assimilated energy into immediately reproducing offspring. This prediction has recently been confirmed for experimental populations in laboratory (Fig. 5 and Stelzer in press). Consequently, all else equal, a transition to asexuality should result in higher grazing pressure on the food algae and thus a greater impact on the environment. However it remains to be investigated whether these observations also apply to natural populations.

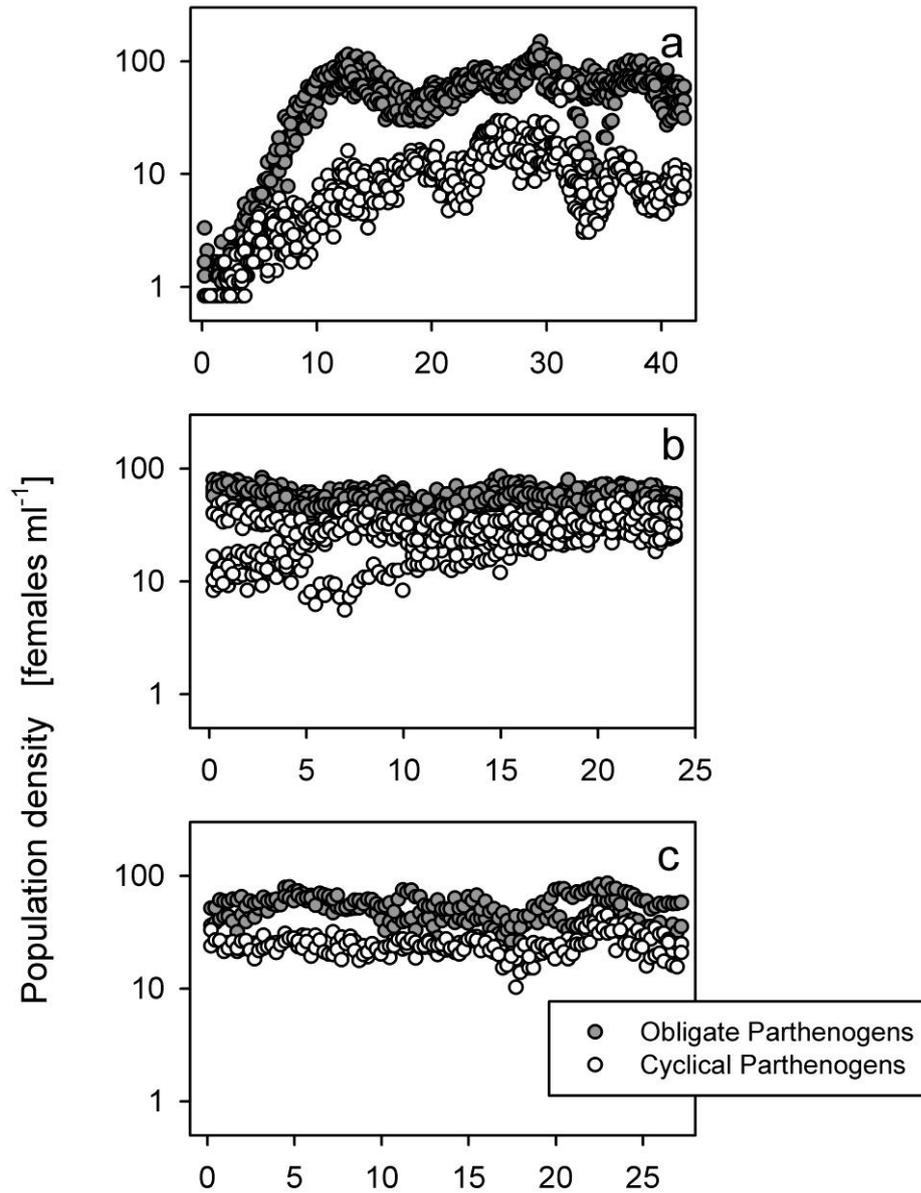


Fig.5: Population dynamics of experimental CP and OP populations. Each panel shows the time courses of population density of several chemostat populations. Each of the chemostats was inoculated with either a CP clone or OP clone, respectively. Individual symbols represent a measurement of population density (6h intervals) with the image analysis system described in Part 1 of this thesis (see “Methodological developments”). Note that population density of OP populations is almost always higher than that of CP populations (Stelzer in press).

Part 3: Sexual signals and speciation

In the past two decades, cryptic species complexes have been described in many animal groups (De Meester et al. 2002; Hebert et al. 2004; Knowlton 1993), mainly due to new molecular markers with improved taxonomic resolution. Small microscopic invertebrates seem to harbour particularly large amounts of genetic diversity, “hidden” within morphotypes that had been traditionally classified as a single species. The rotifer *Brachionus plicatilis* is one of the most striking and well-studied examples of such hidden diversity. It was initially described as a single species, but has subsequently experienced an enormous taxonomic inflation to currently 14-22 postulated species, based on molecular markers (Gomez et al. 2002; Suatoni et al. 2006). This pattern likely holds for other morphological rotifer species as well, for example *Brachionus calyciflorus* (Gilbert and Walsh 2005), *Epiphanes senta* (Schroder and Walsh 2007), or several Bdelloid rotifer species (Fontaneto et al. 2009). Morphological discrimination among some species of the *Brachionus plicatilis* complex is possible, yet difficult, as it involves tight experimental control over environmental and developmental variation (Ciros-Perez et al. 2001b) or sophisticated analysis methods combined with high sample sizes (Fontaneto et al. 2007). Despite the morphological similarity of members of the *B. plicatilis* complex, recent studies have demonstrated extensive ecological diversification in terms of temperature or salinity preferences (Ortells et al. 2003) and prezygotic and postzygotic reproductive isolation among members in this species complex (Gomez and Serra 1995; Rico-Martinez and Snell 1995; Snell and Hawkinson 1983; Snell and Stelzer 2005; Suatoni et al. 2006).

Evolution of pre- and postmating isolation mechanisms

The first studies documenting mating isolation in the *Brachionus plicatilis* complex date back to the early eighties (e.g. Snell and Hawkinson 1983). At this time, modern molecular markers were not available and thus mating variation was interpreted as within-species variation. This has changed since the phylogeny of the *Brachionus plicatilis* complex has become better known. The first molecular evidence that *B. plicatilis* may contain more than one species was provided by allozyme studies (Fu et al. 1991; Gómez et al. 1995). This was confirmed with DNA-based nuclear and mitochondrial markers on *B. plicatilis* sampled from various locations worldwide (Gomez et al. 2002; Suatoni et al. 2006). To date it is well established that the *B. plicatilis* complex consists of at least 14 species and at least 3 major clades (Fig. 6),

each clade containing several species. These major clades have probably evolved separately since several Millions of years (Gomez et al. 2002). Exact dating is not possible in rotifers due to lack of calibration of the molecular clock. Despite the similarity in body shape among members of the *B. plicatilis* species complex, there are notable differences in body size, with mean adult body volumes ranging at least 17-fold (Stelzer et al. 2011). In the following I will briefly summarize the major findings on pre- and postzygotic isolation across the *B. plicatilis* species complex.

Sex induction is the first step in a sequence of biological mechanisms that can result in species barriers. Theoretically such a barrier would exist, if species A would not respond to the sex inducing chemicals of species B (and vice versa). Interestingly evidence so far suggests that this type of isolation mechanism has *not* evolved in the *B. plicatilis* complex. Even very distant lineages such as *Brachionus rotundiformis* and *Brachionus plicatilis* ‘Austria-lineage’ can cross-induce sexual reproduction when presented with conditioned media from the opposite species (Stelzer and Snell 2006). This suggests that the mixis signal did not diversify for several million years during the evolution of the *Brachionus plicatilis* complex. This apparent stasis is remarkable because pre- and postmating isolation is common in this species complex and, due to its cosmopolitan distribution, species often occur in sympatry. Cross-induction between sympatric clones of different lineages has been explicitly studied by Garcia-Roger et al. (2009). Likewise, these authors did not find any evidence for differentiation of the signal between species. This is in contrast to *B. calyciflorus*, a cryptic species complex where divergence in the sex-inducing signal has been demonstrated (Gilbert 2003b).

Mating isolation in rotifers consists mainly of the behaviour of males displayed towards females of a different species. Mating is a multi-step process in *Brachionus*, which includes the following sequence of events and behaviours: encounter, circling behaviour, and copulation (Snell and Morris 1993). Encounters are random in *Brachionus*, since males cannot locate females from a distance (Snell and Garman 1986). Circling behaviour is characterized by the male rapidly circling around the longitudinal axis of the female, for several seconds and up to a few minutes, while maintaining body contact. This behaviour may or may not end in copulation. Finally, copulation is characterized by insemination of the female, typically at the region of the corona or at the base of the food. Copulations can differ

in their intensity and length; both may affect fertilization probability. There are few hints that females may also participate in mate choice. For example, swimming acceleration, food flipping and corona retraction has been interpreted as female resistance, to avoid copulations by unwanted males (Snell et al. 2007). The role of such behaviour in mating isolation between species remains to be investigated.

Behavioural mating isolation has been studied intensively in the *B. plicatilis* complex (e.g., Gomez and Serra 1995; Kotani et al. 1997; Kotani et al. 2001; Rico-Martinez and Snell 1995). In general, mating isolation has been found in many species pairs. However, there also have been reports of heterospecific mating behaviour (Rico-Martinez and Snell 1995; Snell and Stelzer 2005; Suatoni et al. 2006). Often mating isolation is gradual, such that males of species A will just exhibit a reduced probability of displaying mating behaviour towards species B. The mechanistic aspects of mating in *Brachionus* are well studied. Males rely on contact- chemoreception and respond to a glycoprotein on the body surface of females (MRP=mate recognition protein). MRP probably allows species recognition, identification of receptive females, and it possibly directs males to parts of the female body that are most suitable for copulations (located at the head and foot regions, respectively), since MRP is most concentrated in these regions (Snell et al. 1993). MRP can be detached from the female's body surface by a chemical treatment and can be transferred across species (Snell and Stelzer 2005). Recently the structure of this protein has been elucidated for *B. manjavacas*, one species of the *B. plicatilis* species complex (c.f., Fig. 6). The MRP is a 29kD Protein and its gene is called MMR-B3 (=MRP Motif Repeat). Its causal role in male mate recognition has been demonstrated by functional assays using the RNAi method (Snell et al. 2009). Tracking the MMR-B3 gene tree across the *Brachionus plicatilis* complex will likely give fundamental insights into the evolution of mating isolation.

Postzygotic isolation in the *Brachionus plicatilis* complex has also been investigated in several studies (e.g., Fu et al. 1993; Kotani et al. 2006; Suatoni et al. 2006). Postzygotic isolation is the hallmark of the biological species concept and it appears to be common in the *B. plicatilis* complex (Suatoni et al. 2006). One of the most extensive studies on postzygotic isolation in BP has been done by Suatoni et al. (2006), which did not only involve F1-hybrids but also backcrosses. Results for postzygotic isolation were largely concordant with the genealogical species hypothesis based on phylogenetic data from COI and ITS1 genes,

respectively. Cases of interspecific hybridization were confined to a few very closely related lineages and were usually accompanied by very low fertilization success (Suatoni et al. 2006). Nevertheless, it is noteworthy that not all of the putative species of the *B. plicatilis* complex are completely reproductively isolated.

Evolution of genome size in the *B. plicatilis* complex

Genome size, measured as the haploid nuclear DNA content (C-value), is extremely variable among eukaryotes. This variation has long puzzled biologists, because it could not be accounted by organismal complexity or the total number of genes (C-value paradox). In the last decades it has become evident that the observed genome size variation is largely caused by differences in the content of non-coding and/or repetitive DNA, such as introns, pseudogenes, or transposable elements (Gregory 2005b; Lynch 2007). Nevertheless there are still many unanswered questions about genome size diversity, such as the actual causes driving the differences in DNA content, speed and mode of changes in genome size over population genetic and longer evolutionary time scales, or the cellular and organismal consequences of large vs. small genome size (Gregory 2005a). So far, most genome size comparisons in animals have been done at high taxonomic levels, e.g., between classes, families, or genera (e.g., Gregory et al. 2000; Lynch and Conery 2003; Oliver et al. 2007; Tsutsui et al. 2008). In my own work I have focussed on the evolution of genome size in the *Brachionus plicatilis* species complex (but see also Stelzer 2011b).

Stelzer et al. (2011) found an unexpectedly high genome size variation in this species complex, ranging approximately seven-fold (haploid '1C' genome sizes: 0.056-0.416 pg). Most of this variation (67%) could be ascribed to the major clades of the species complex, i.e. clades that are well separated according to most species definitions. However, substantial variation (32%) was also at lower taxonomic levels - within and among genealogical species – and, interestingly, among species pairs that are not completely reproductively isolated. In one genealogical species, called *B. plicatilis* 'Austria', Stelzer et al. (2011) found greatly enlarged genome sizes that could roughly be approximated as multiples of the genomes of its closest relatives, which suggests that whole-genome duplications have occurred early during separation of this lineage. This suggests that substantial genome size variation can build up early during speciation, potentially even among isolated populations.

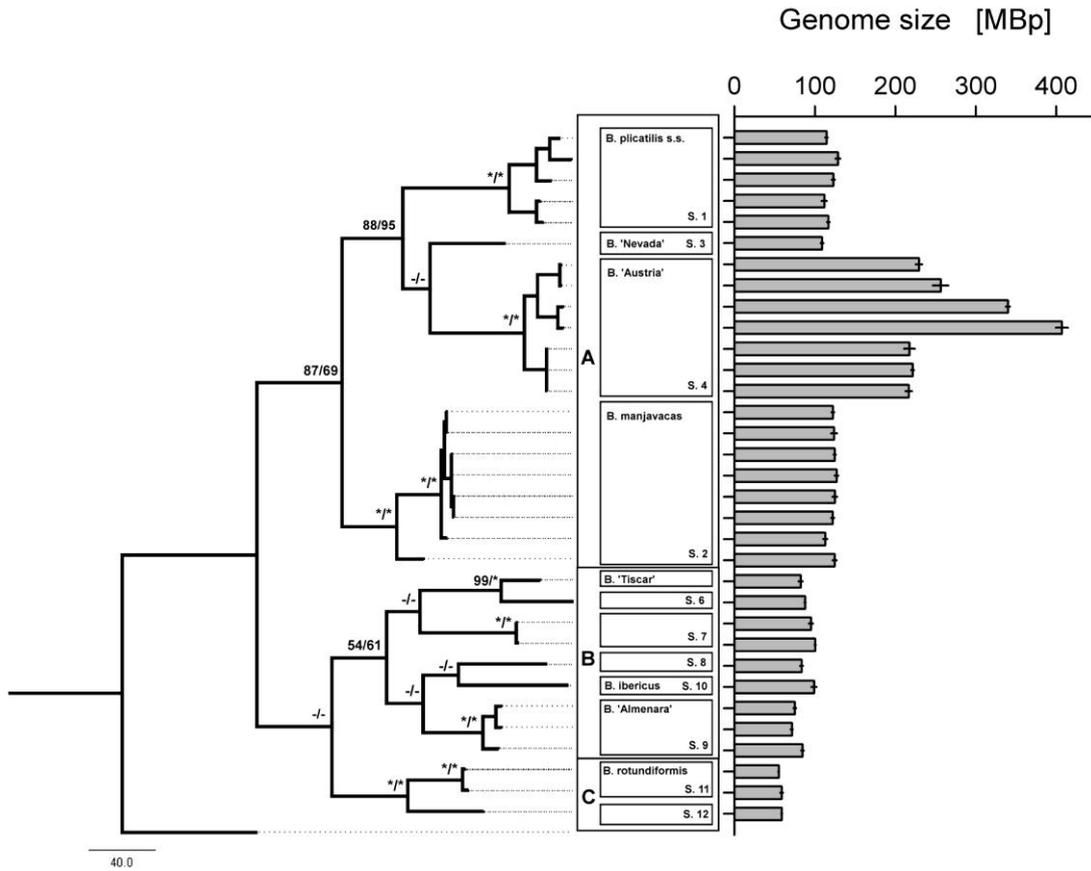


Fig. 6: Genome size diversity in the *Brachionus plicatilis* species complex. Maximum Parsimony tree, based on combined analysis of partial mitochondrial COI and ribosomal ITS1 sequences, with *Brachionus calyciflorus* as outgroup is shown on the left. Boxes indicate the cryptic species identified by Suatoni et al. (2006) and Gomez et al. (2002), as well as the three major clades (A, B, and C). Bars represent the mean haploid genome sizes of the different clones (\pm s.e.m.)

Conclusions and perspectives

Research on rotifers has yielded significant progress in the past two decades, especially with regard to the following two aspects: First, many new insights have been gained in terms of rotifer biology *sensu strictu* (e.g., Snell et al. 2006; Snell et al. 2009; Stelzer et al. 2010) and, second, rotifers have been successfully established as model organisms for general ecological and evolutionary problems (Campillo et al. 2010; Fussmann et al. 2000; Stelzer 2011a; Stelzer in press; Weithoff 2007). Such advances were only possible due to the foundations laid by earlier research efforts, e.g. previously established culturing methods such as chemostat culture (Boraas 1980; Walz 1993), but also because of technological advances in biochemistry, molecular biology or sequencing techniques. To date, it can be fairly stated that rotifers have become fully-fledged model organisms, suitable for a variety of questions. Future research will likely gain new insights several different fields. Possible directions are:

- A better understanding of the phylogenetic relationships among Monogonont genera.
- Elucidation of more details on mechanisms specific to the rotifer life cycle (mate-recognition related genes, sex-induction related genes, signal transduction pathways)
- Increased use of rotifers as model organisms for experimental evolution, population dynamics, evolution of sex, aging, etc.

It is also likely that some of these research avenues will merge. For example, future studies of experimental evolution might be accompanied by a functional and genomic analysis of the evolved traits.

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