

## CHAPTER 12

# The timing of sex in cyclical parthenogenetic rotifers

Manuel Serra, Terry W. Snell, and Charles E. King

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**Cyclical parthenogenesis** is a mode of reproduction that combines **parthenogenesis** with episodic sexual recombination. It has evolved independently several times, but is largely restricted to parasitic trematodes, gall wasps, gall midges, aphids, and two groups of **zooplankters**: cladocerans and monogonont rotifers (Bell 1982; see also De Meester *et al.*, Chapter 11). Cyclical parthenogenesis can be regarded as an optimal combination of a demographic phase during which there is rapid clonal propagation, and a sexual phase during which meiosis and recombination create new genotypic variation. Each of these phases has its own evolutionary and ecological advantages and costs.

Cyclical parthenogens provide a group of model organisms for examining several important aspects of the evolution of sexual reproduction. First, the consequences of sexual and asexual reproduction on the evolutionary dynamics of **adaptation** can be analyzed in a single species (De Meester *et al.*, Chapter 11). Second, because sex is facultative in cyclical parthenogens, the level of sexual reproduction can be correlated with environmental factors and habitat characteristics. The costs of sex can be easily measured and incorporated into models accounting for different patterns of sexual reproduction. The optimal allocation of resources into sexual and asexual offspring can be analyzed with similar methods to those used in the study of other life-history trade-offs. Third, the effects of “bad choices” regarding when to switch from asexual to sexual reproduction are readily investigated in this group, as well as the effects of environmental

changes, including those from anthropogenic sources, on sexual and asexual cycles.

Rotifers make up a phylum composed of taxa that differ greatly in their mode of reproduction: Seasonids are obligate sexuals, bdelloids are obligate parthenogens, and monogononts are cyclical parthenogens. Monogonont rotifers live in seasonal and ephemerally favorable aquatic habitats and differ in their seasonal distribution and their residence time in the plankton. In rotifers, asexual reproduction by **apomixis** produces **clonal** females (Fig. 11.1(b) in De Meester *et al.*, Chapter 11). Multiple generations of asexual reproduction are punctuated with occasional bouts of male production and sexual recombination. The product of sexual reproduction in monogononts is a thick shelled **resting egg** that typically has an extended period of **dormancy** before hatching. Monogonont rotifer populations in temperate climates are re-established each year by hatching from a **resting egg bank** in the sediments (Pourriot and Snell 1983). After a phase of clonal propagation in the plankton, sex is triggered by environmental factors such as population density (e.g. Snell and Boyer 1988; Carmona *et al.* 1995). Sexual reproduction starts when asexual females parthenogenetically produce both sexual and asexual daughters. Sexual females then produce haploid eggs that develop into either haploid males or, if fertilized, into resting eggs. Haploid egg fertilization is only possible if the sexual female is inseminated within a few hours of birth (Snell and Childress 1987). If a sexual female is not inseminated while young, she will only produce males. Sexual, resting eggs are able to survive adverse

conditions and may remain viable in the sediment for decades (Marcus *et al.* 1994; see also Lampert, Chapter 10). Resting eggs are likely to be the major mode of geographic dispersal.

In this paper, we review a considerable amount of information from modeling, laboratory, and fieldwork on the rotifer life cycle. Intensive research has been performed on a rotifer genus (*Brachionus*), and we will focus mainly on this research. Our aim is to get insights into the evolutionary forces shaping the timing of the asexual and sexual phases of reproduction in monogonont rotifers. We address the causes of the maintenance of sex in rotifers and its optimal timing. Finally, we consider the factors operating on the reproductive process in a variety of habitats and relate the effects of environmental change to the probability of population extinction.

### Evolutionary hypotheses for the origin and maintenance of sex in rotifers

The field of population biology has been deeply concerned—some might say obsessed—with the question “why sex?” (Bell 1982). It is easy to understand the reason for this concern. Assuming that a typical asexual female is able to produce the same number of offspring as a typical sexual female, and further assuming that offspring **fitness** is independent of mode of reproduction, an asexual female will have twice as many daughters as a sexual female. That is, since only females can produce eggs, asexual reproduction will have a twofold advantage over sexual reproduction. With this huge fitness advantage, any mutation that leads to a substitution of parthenogenesis for sexual reproduction would be expected to spread rapidly through the population. The twofold cost of sex can be stated as the cost of producing males or, if we focus on the parent–offspring relationship, as the 50 percent loss in the proportion of genes shared by a female and her sexual offspring. Given that parthenogenesis has such a massive fitness advantage over sexual reproduction, why is it that almost all species reproduce sexually?

The literature is replete with suggestions on how the twofold cost of sex is paid. More than 20 theoretical explanations attempt to account for the

short-term advantage of sex (see West *et al.* 1999). An in-depth review of these explanations is beyond the scope of the present chapter. Instead we will describe them briefly, focusing on aspects that have particular relevance to understanding the life history of rotifers.

A number of hypotheses on the maintenance of sexual reproduction are based on fitness advantages of behaviors involved with either sexual selection or male parental care. For example, **sexual selection**—that is, female choice of mates or male–male competition for mates—may lead to selective incorporation of high fitness paternal genes. Alternatively, the cost of sex may be reduced if male participation in parental care permits females to enlarge their number of progeny. Both of these hypotheses have greater relevance to organisms with a more sophisticated behavioral repertoire than rotifers. Despite the fact that rotifer mating behavior has been reasonably well studied, no evidence of either male rejection by females or parental care by males has been reported. A third category of hypotheses is based on the fact that sexual recombination produces genetically variable offspring. It is presumed that parthenogenetic species have limited evolutionary potential to adapt to environmental heterogeneity, or that they are less able than sexual forms to take advantage of rare, independent mutations that can recombine sexually to produce high-fitness individuals. Perhaps sex functions to purge deleterious mutations (as claimed by **Müller’s ratchet** and the **mutational deterministic hypothesis**; see García-Dorado *et al.*, Chapter 3), or to facilitate rapid evolution in a host–parasite “arms race” (the **“Red Queen” hypothesis**). These, as well as other mechanisms, might interact in a synergistic fashion to maintain sex (West *et al.* 1999).

Müller’s ratchet envisions an obligate asexual organism in which there exists a class of individuals that has the smallest number of deleterious mutations in the population. Assuming that fitness is inversely related to the number of mutations, this class represents the group with the highest fitness in the population. Note that groups of any size, including the class with the highest fitness, are subject to loss from the population because of

stochastic processes. Under Müller's ratchet, if the class with the highest fitness is lost, the group with the next smaller number of mutations then becomes the class with the highest fitness. In other words, under this model, fitness must always deteriorate.

At first glance it would seem that the rotifers would provide perfect material to test the ideas incorporated in Müller's ratchet. Bdelloid rotifers lack sexual reproduction and might be expected to undergo a stochastic deterioration, whereas the periodical sexual reproduction of monogononts should allow them to purge part of their **mutational load**. The huge size of many rotifer populations makes it unlikely that whole fitness classes will be lost by chance. In fact, even though beneficial mutations tend to have very low probabilities (approximately  $10^3$  times lower than rates of harmful mutations), their occurrence is much more likely in most populations than is the loss of an entire fitness class due to stochastic events. It is not surprising that Mark Welch and Meselson (2001) found that nucleotide **substitution rates** in monogononts are similar to those of the asexual bdelloids. The authors, therefore, concluded that mutational load is not a selective pressure for the maintenance of sexual reproduction in monogononts. While exclusion during recombination is one means by which deleterious mutations can be eliminated, monogonont rotifers have a life-history stage that may also contribute to the same purgative effect. The haplodiploid mechanism of sex determination in monogononts suggests that male haploidy may function to reduce mutational load by exposing deleterious recessive mutations to selection. Males are typically smaller and morphologically and physiologically less complex than females of the same species. For instance, males lack a functional digestive system. It is however reasonable to speculate that they still express an important part of their genome (e.g. constitutive genes for energy metabolism). Therefore, the estimation of the mutational load is expected to reflect, at least in part, whether or not the analyzed gene is expressed in males.

The search for evolutionary advantages of sex has not been accompanied by a corresponding effort to look for fitness benefits of asexual reproduction

(Brookfield 1999). Several features of parthenogenesis besides the twofold argument would facilitate its evolution from obligate sexual ancestors. A very powerful advantage of parthenogenesis is that it enables clonal propagation without breaking up maternal gene combinations. Clonal genotypes with the highest state of adaptation to an environment will be passed on without dilution by recombinational load since the entire genome is the unit of selection. In cyclical parthenogens a slippage in directional evolution may be associated with sexual reproduction (Lynch and Deng 1994). Ultimately, therefore, parthenogenesis leads to the production of a large number of high-quality females that are adapted to their environment.

In order for reproduction by clonal propagation to be advantageous, maternal and offspring environments must be similar so that the fitness value of particular gene combinations remains rather constant. In rotifers, sex is associated with dormancy, and thus with dispersal in time and space. This is a quite general pattern in the living world, from plants to animals: if sexual and asexual reproduction are combined in a single life cycle, then sex tends to occur when there is a low correlation between parental and offspring environments. It is under these conditions that high genetic diversity may play a major role in facilitating adaptation. However, in rotifers it is not obvious that environmental correlations between parents and their sexual offspring must be particularly low, as we have little information on the environmental cues that trigger hatching of resting eggs and few measures of the intensity of clonal selection that must occur among the post-hatching offspring.

Because of space limitations, we have not dealt with the question of how rotifers adapt to changes in their environment through the generation of new genetic variation. This process must involve, at least in part, the recombinant resting eggs produced by sexual reproduction. However, theoretical models also emphasize that recombination is not needed to produce new favorable combinations of alleles. Instead, given the huge population sizes of rotifers in even moderate-sized ponds, mutation is fully capable of generating high levels of new variation (King 1980). Moreover, clonal selection

can rapidly amplify favorable novel mutations and combinations of mutations rendering them far less likely to be lost by random **genetic drift** than are newly formed genotypes produced by sexual recombination (King and Schonfeld 2001). Results from these models suggest that sex in rotifers may have relatively little value as a means of generating novel genotypes.

Historical and developmental constraints may be relevant for the maintenance of sex in rotifers. Estimations of population densities in the field suggest that most rotifers lack the capacity to survive the adverse periods that occur during parts of a typical annual cycle in temperate zone lakes. This implies that long-term fitness in these cyclical parthenogens depends upon the size and quality of the resting egg pool. Since resting eggs are produced sexually, the critical functional role of dormancy may by itself be a strong enough selective factor to maintain sex in monogononts. A similar argument has been developed for the maintenance of sex in cyclically parthenogenetic aphids (see De Meester *et al.*, Chapter 11). In contrast to monogononts, bdelloid rotifers lack both sex and resting eggs. Instead, **anhydrobiosis** is used to survive periods in which the environment is unfavorable. Asexual resting eggs are produced by some cladocerans and in a monogonont rotifer species. These cases underscore the function of the resting egg as a life-history stage promoting survival through adverse periods. However, they are clearly exceptional even though they do indicate that it is possible to decouple sex and resting egg production. The fact that most species have not done so may reflect historical constraints, or provide clues to the function of sex in cyclical parthenogenetic rotifers.

### Empirical studies on the timing of sex in rotifers

Theoretical studies have demonstrated the importance of habitat predictability, variation in resource availability, and demographic parameters in determining the timing of rotifer sex (Serra and King 1999). Two parameters have been useful for characterizing the timing of sex: the **sexual reproduction threshold** (i.e. the population density at which

sexual reproduction is initiated, this determining when sex occurs in a population growth cycle), and the **sexual reproduction ratio**, which indicates the proportion of females that are sexual.

One of the most consistent observations about sexual reproduction is its association with population density. Over the past 70 years researchers studying a variety of rotifer species in laboratory and natural environments have reported a positive relationship between sexual reproduction and population density (e.g. Snell and Boyer 1988; Carmona *et al.* 1995). In an intensively studied genus (*Brachionus*), sexual reproduction appears to be triggered when population density exceeds a species-specific or population-specific threshold and is switched off when population density falls below that threshold. This density response can be modified by a variety of environmental factors such as food quantity (Snell and Boyer 1988). Environmental factors such as temperature, salinity (see Pourriot and Snell 1983), and free ammonia (Snell and Boyer 1988) can suppress sexual reproduction even when population density thresholds are exceeded. Vitamin E and long photoperiods are primary environmental triggers in other genera (*Asplanchna* and *Notomata*, respectively; see Gilbert 1993). Several authors have estimated the sexual reproduction threshold for natural and laboratory rotifer populations. For natural populations of *Asplanchna*, sexual reproduction thresholds were estimated to range from 2.3 to 100 females/l (Snell *et al.* 1999). Carmona *et al.* (1995) estimated sexual reproduction thresholds of 6.6 and 23 females/l for congeneric populations of *Brachionus*. Snell and Boyer (1988) recorded a sexual reproduction threshold of 147 females/l in laboratory populations of *B. plicatilis*.

A second parameter of the sexual reproduction pattern is the sexual reproduction ratio, that is, the proportion of sexual daughters produced by a female when the sexual reproduction threshold is exceeded. A number of studies have estimated sexual reproduction ratios in laboratory and, occasionally, in natural rotifer populations (Snell *et al.* 1999). For laboratory populations of *Asplanchna* a range of 8–69 percent sexual daughters has been reported after sex has been initiated. Estimates for *Brachionus*

range from 1–89 percent, but rarely are higher than 50 percent. Carmona *et al.* (1995) recorded sexual reproduction ratios over 17 months in natural populations of two **sympatric** *Brachionus* species. For *B. plicatilis*, on 67 percent of the tri-weekly sampling dates sexual reproduction ratios were higher than 5 percent and fluctuated around 10 percent. In contrast, *B. rotundiformis* sexual reproduction ratios exceeded 5 percent on only 20 percent of the sampling dates, but then the sexual reproduction ratios were higher than those observed in *B. plicatilis*. These authors describe the pattern of sexual reproduction of *B. plicatilis* as continuous, in contrast to the periodic pattern of *B. rotundiformis*. Although sexual reproduction in both species was correlated with population density, the density threshold in *B. rotundiformis* was approximately three times higher than in *B. plicatilis*, which seems to account for the divergence in sexual reproduction patterns.

### Optimal timing of sexual reproduction and resting egg production

The optimal combination of sexual and asexual reproduction in rotifers can be conceptualized as a trade-off between the short-term advantages of

asexual reproduction and the advantage of sex. Asexual reproduction facilitates fast colonization when habitats become suitable. Sexual reproduction is associated with dormancy and survival during adverse periods. One answer to the question of when sexual reproduction should be initiated is based on the role of resting eggs in allowing survival during adverse periods: sex should occur when the habitat becomes unsuitable. This view will be termed the **habitat deterioration hypothesis** (Table 12.1).

Alternative hypotheses have been proposed. Gilbert (1974) suggested that sexual reproduction is optimally induced at high population density because male–female encounters are more likely. If sexual reproduction occurs at low population density most sexual females would be male producing, but males would still be rare, and resting egg production would be low. In their model simulating the rotifer “male–female encounter hypothesis,” Snell and Garman (1986) showed that the probability of a female being fertilized increases almost linearly when male density increases from 50 to 700 individuals/l. Using branching process analysis, Muenchow (1978) developed a stochastic version of the **male–female encounter hypothesis** (Table 12.1). He suggested that sexual reproduction

**Table 12.1** Hypotheses on the optimal pattern of sexual reproduction in rotifers

Hypothesis	Rationale	Predicted relationship between sexual reproduction and population density
Habitat deterioration	Sex initiation occurs when population growth is no longer possible	<i>Indirect</i> : high density causes habitat deterioration, which in turn induces sex <i>Spurious</i> : habitat deterioration (population decline) produces a peak in the population density time series; habitat deterioration induces sex
Male–female encounter	Sex initiation occurs at high population density because male–female encounters are then more likely	<i>Direct</i> : high density is the selective condition for inducing sex
Resource-demanding	Sex occurs in good environmental conditions because sex (male and resting-egg production) is resource-demanding	<i>Spurious</i> : good conditions result in high density; good conditions induce sex

should occur at high densities as a way to decrease the variance associated with demographic stochasticity, which is highest at low abundances. Note that this conclusion holds whether the fertilization rate is density-dependent or not. High variance in male–female encounter rates would decrease the among-year geometric mean of encounters, the most relevant fitness measure in this case.

Sexual females are sometimes common during the early, exponential phase of population growth in mass cultures, when resources are abundant. Several authors (e.g. Gilbert 1980; Snell and Boyer 1988) noted that sex is resource demanding, because a higher amount of resources seems to be allocated to resting eggs than to asexual eggs. The threshold food concentration for sexual reproduction is 10 times higher than that for asexual reproduction in *B. plicatilis* (Snell and Boyer 1988). Life-table experiments showed that an asexual female has much higher fecundity than a fertilized sexual female (see Serra and Carmona 1993).

There is also evidence for a higher lipid level in resting eggs than in asexual eggs (Gilbert 1980). In addition, sexual reproduction also allocates resources to males. From these observations, it follows that sexual reproduction is more resource demanding than asexual reproduction. Accordingly, Snell and Boyer (1998) proposed that sexual reproduction should occur under good environmental conditions and be suppressed when the physical or chemical environment or food resources are less optimal. This we will term the **resource-demanding hypothesis** (Table 12.1). Other authors have reached similar conclusions from experiments on the effects of environmental factors on resting egg production in rotifer mass cultures (Hagiwara *et al.* 1988). The role of favorable food conditions was also used by Gilbert (1980) to explain the relationship between a vitamin-E rich diet, large *Asplanchna* morphotypes, and sexual reproduction.

Unfortunately, field observations and laboratory experiments have not provided an unambiguous test to discriminate between these hypotheses. The common association of sexual reproduction with high population density is compatible with all of the above hypotheses (Table 12.1). Under the male–female encounter hypothesis, population

density would provide a direct selective advantage for sex initiation. However, if habitat deterioration gives a selective advantage to sex initiation, the correlation with high population density could be explained in two ways: (1) high density is expected to cause habitat deterioration due to resource depletion, and (2) peaks in the population density time series are expected just before habitat deterioration. Associations between sexual reproduction and population density are also predicted by the resource-demanding hypothesis as both are concomitant effects of good environmental conditions. Finally, peaks in a field population density time series could be associated with sex initiation because sexual reproduction would cause a decrease in population density due to the shift from **subitaneous egg** to resting egg production. This is especially true if sexual reproduction occurs at high rates.

### Theoretical analysis of the optimal patterns of sexual reproduction

R. A. Fisher's brilliant insight establishing sex allocation theory demonstrated that the optimal sex ratio in a population is 50 percent males:50 percent females. Since each offspring in a sexual population has one male and one female parent, if there is an excess of males in the population the average female makes a greater reproductive contribution to the next generation than the average male, hence the production of female offspring would be favored. The opposite is true if there is an excess of females. This situation creates a frequency dependent selection that is expected to produce an equal number of male and female progeny. This argument is based on the assumption that it is equally expensive to produce male and female offspring. If this assumption is not true, Fisher's result can be restated in the form: the resources allocated by parents to male and female offspring production must be equal (e.g. Ridley 1996).

Rotifer males are smaller and morphologically simpler and, therefore, energetically less expensive to produce than females. They develop from unfertilized haploid eggs produced by sexual females. Recently, Aparici *et al.* (1998) have extended

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Fisher's theory to rotifers. In many systems it is nearly impossible to accurately quantify the amount of resources devoted to producing each sex under natural conditions. However, sexual females produce either males or resting eggs, but not both. Hence, in rotifers, if there is to be equal investment of resources into male and female offspring, half of the sexual females must produce resting eggs while the other half must produce haploid males. This could be achieved if selection acts to alter the threshold age that marks the latest age at which females can be fertilized. A female that has not been fertilized by the threshold age can only produce male offspring in the future. Thus, if the threshold age is shifted to an earlier point in the life history, male production will be favored. Similarly, if it is shifted later, there will be fewer males produced. The threshold age at which the fertilization probability under a given set of environmental conditions is  $1/2$ , is therefore an **evolutionary stable strategy**. This means that maximization of resting egg production is subject to the constraints imposed by sex allocation as well as the basic physiological and genetic constraints affecting egg formation.

In rotifers, there is an interaction between population density and the threshold age for the loss of fertilization potential. As shown by Snell and Garman (1986), fertilization is more likely at high densities. Sexual reproduction at high densities is expected to be associated with short threshold age of fertilization, so that a compensation maintaining the average fertilization probability at  $1/2$  occurs. In fact, this probability could also be achieved by initiating sexual reproduction at an appropriate density. However, regardless of what trait would adjust the fertilization rate—either the population density at which sexual reproduction is initiated or the threshold age of fertilization is actually selected for—effective fertilization rate is expected to be  $1/2$ , and consequently density-independent when averaged over the realized range of densities. This conclusion seems to militate against the male-female encounter hypothesis in the sense that higher density is not expected to cause higher proportions of resting egg producing sexual females.

Snell (1987) viewed the problem of optimization of the sexual reproduction pattern as a trade-off

between current sexual reproduction and current parthenogenetic growth, and consequently between current and future production of sexual females within a population growth cycle. When the sexual reproduction ratio is low, most females are producing asexual daughters and contributing simultaneously to both present population growth and the future number of sexual females. These trade-offs in the population growth cycle are similar to (1) the trade-off between reproduction (analogous to sexual reproduction) and somatic growth (analogous to parthenogenetic growth) and (2) the trade-off between present and future reproduction. Both of these trade-offs are well known in life-history theory. This view stresses that rotifers have discrete sexual generations whose fitness is the net recruitment rate of resting eggs, that is, the number of resting eggs produced during the growing season.

As a synthesis of the previous and their own theoretical work, Serra and King (1999) proposed a relationship between habitat features and optimal patterns of sexual reproduction (those maximizing resting egg production), which we summarize in Table 12.2. Habitats can be classified following two criteria: their predictability regarding the end of the growth period and the importance of competition (negligible or not). Table 12.2 implicitly assumes that, if competition is important, population density will achieve a quasi-stationary phase, and that the yearly periods of colonization and extinction of the water column population are relatively short and can be neglected. Hence, the optimal pattern in the stationary phase would be the optimal pattern for the whole growth period, and it does not make a difference whether its end is predictable or not. Consequently, three optimal sexual reproduction patterns are expected for four habitat types.

Optimal sexual reproduction patterns in predictable, noncompetitive habitats were studied by Serra and Carmona (1993), who assumed time-dependent, density-independent growth rates. Potential—without sex—population growth rate was assumed to vary from zero at the onset of colonization, to a maximum, then to zero (end of habitat suitability), and then to negative values. Such shifts might occur in habitats with an ephemeral

**Table 12.2** A demographic habitat classification for optimal patterns of sexual reproduction in rotifers (after Serra and King 1999)

Population growth	Sexual reproduction feature	Length of population growth period	
		Predictable	Unpredictable
Density independent	Threshold	Just before the habitat becomes unsuitable; population density relatively high	Habitat deterioration often occurs too soon; population density relatively low
	Ratio	High (close to 1); sexual reproduction is ephemeral	Intermediate; larger for long habitat durations sexual reproduction is extended
Density dependent <sup>a</sup>	Threshold	When the population density approaches the carrying capacity (high density)	
	Ratio	Intermediate; the better the habitat, the higher the sexual reproduction ratio; sexual reproduction is extended	

<sup>a</sup> Density-dependent growth is associated with a long stationary population growth phase. Consequently, it does not make a difference whether the end of the growth period is predictable or not, since the sexual reproduction pattern occurring in the stationary phase dominates over the patterns in the declining phase.

seasonal growth, or with poor conditions so that population densities are low and competition can be neglected. Serra and Carmona (1993) found that a **big-bang strategy** (all reproduction being first parthenogenetic, then sexual) should evolve, a result analogous to that commonly found using life-history models for the allocation of resources into somatic growth and reproduction (all resources first being allocated into growth, then into reproduction). Under these conditions sex should be initiated a short time before the habitat becomes unsuitable, and simultaneously the sexual reproduction ratio should become one. In this way, the maximum possible population density is achieved, providing the maximum number of mothers. Therefore, sexual reproduction is expected to be punctuated, with an ephemeral period of sex just before the habitat becomes unsuitable. This theoretical finding is in agreement with the habitat deterioration hypothesis.

Optimal patterns of sexual reproduction in a competitive habitat were studied by Snell (1987) who conducted simulations using a **discrete**

**growth model** with competition to describe population dynamics. A periodic pattern consisting of several parthenogenetic generations followed by a mixture of sexual and asexual females was assumed, and the effects of both the frequency of the sexual phase and the proportions of sexual females were explored. He found that resting egg production was maximized by higher allocations to sexual daughters at higher intrinsic growth rates. Serra and King (1999) reached similar conclusions using a **continuous growth model** with two state variables (asexual and sexual female densities). Intraspecific competition was assumed to decrease linearly the birth rate. These authors analytically found that the optimal sexual reproduction ratio, if constant, was  $1 - (b/q)^{1/2}$ , where  $b$  is the intrinsic birth rate (without competition) and  $q$  is the mortality rate. This result was interpreted as being due to lower relative costs of sex in good conditions, and can be related to the resource-demand hypothesis, since it provides an optimizing argument to the finding of high sexual reproduction levels in good environments. Using computer simulation,

Serra and King (1999) also found that an intermediate strategy—that is, simultaneous allocation into asexual and sexual offspring—was better than a big-bang strategy. In summary, intermediate sexual reproduction patterns and extended periods of sexual reproduction are expected if rotifer populations grow density-dependently.

The optimal pattern of sexual reproduction when the length of the population growth period is uncertain and cannot be predicted by the rotifers was discussed by Serra and King (1999; Table 12.2). They used an analogy between individual resource allocation to reproduction and somatic growth and the allocation of resources to sexual and asexual daughters. Then, by analogy with the theory of life history optimization in randomly varying habitats, they proposed that a bet-hedging strategy should be optimal. An intermediate sexual reproduction ratio is a way to produce at least a few resting eggs during bad years while not stopping parthenogenetic growth during good years. This would imply an extended period of sexual reproduction. Evidence in support of this expectation was found in a field study of two sympatric populations of congeneric species with different seasonal distributions (Carmona *et al.* 1995). The winter species—supposedly living in a less predictable habitat—showed sexual reproduction almost continually, in contrast to the pattern found in the summer species.

In randomly varying habitats, early sex seems to be optimal. However, early sex, despite occurring at intermediate ratios, implies a cost for population growth that is necessary in order to protect against unpredictable years with short growth periods leading to resting egg failure. An alternative protection against total failure of sexual reproduction in a given year is the resting egg bank found in the sediments. It is well known that rotifer resting eggs can remain viable for decades (Marcus *et al.* 1994). Not all of the resting eggs that are produced in one year hatch when good conditions occur. As a consequence of this bet-hedging tactic, the risks of delayed sex may be rewarded by an increased output of resting eggs when the bet is won, and extinction is avoided because of the resting egg bank when the bet is lost. These arguments have been accounted for in recent developments of the

dormancy theory, in which both optimal production of resting stages and optimal rates of dormancy have been analyzed simultaneously (Spencer *et al.* 2001).

### **The impact of environmental change on the timing and proportion of sex**

In monogonont rotifers, environmental change can have a stronger effect on sexual reproduction than on asexual reproduction. This is because sexual reproduction takes longer to complete than asexual reproduction and requires more resources. Sexual reproduction consists of several steps in a cascade of events from sex initiation to resting egg production (Table 12.3). The entire process spans three generations and takes several days to complete. Laboratory experiments with *Brachionus calyciflorus* at 25 °C demonstrate that under optimal conditions sexual reproduction takes about 4 days to form resting eggs (Preston *et al.* 2000). If environmental conditions deteriorate during this period, resting egg formation could fail (Serra and Carmona 1993). A switch from asexual to sexual reproduction, therefore, is a commitment that must culminate in resting egg formation; otherwise the original parental females will die without leaving progeny.

In addition to the time required for individual females to switch their reproductive physiology to resting egg production, time is required for a rotifer population to reach a density sufficient to trigger sex. When a population is re-established each year, newly hatched rotifers from the resting egg bank reproduce asexually, growing to considerable densities because resources are initially abundant. Sex is triggered as populations reach densities of 1–100/l. It takes rotifer populations several days to grow from the low densities following resting egg hatching to the density threshold that triggers sex. As an example, at low population densities and in the absence of competition, the instantaneous population growth rate of *Brachionus* can approach 1 d<sup>-1</sup> (Snell and Serra 2000). With such a growth rate, it would take a rotifer population 9–10 days to grow from a resting egg hatching density (e.g. 0.01/l) to a density sufficient to trigger sex (100/l). Adding to this the time required for females to form resting

**Table 12.3** Life cycle stages needed for resting egg production, and fitness losses associated with environmental stressors

Stage	Environmental stressor	Fitness loss
Parthenogenetic reproduction to sexual reproduction threshold	1. Suboptimal temperature, salinity, food level, etc.; pollutants, endocrine disruptors 2. Episodic catastrophes truncate population density before threshold	1. Slow population growth due to reduced birth rate and/or death rate 2. Insufficient time for population growth to reach sexual reproduction threshold
Sex initiation	1. Pollutants, endocrine disruptors 2. Episodic catastrophes truncate sexual reproduction development 3. Suboptimal environment; pollutants, endocrine disruptors	1. Interference with chemical signals of population density 2. Insufficient time for transition from asexual to sexual females 3. Reduced sexual reproduction ratio (production of sexual daughters)
Fertilization	1. Pollutants reduce swimming speed 2. Pollutants, endocrine disruptors 3. Poor quality diet, pollutants, endocrine disruptors	1. Low male–female encounter probability 2. Low recognition probability 3. Low male fertility
Resting egg production and quality	1. Poor quality diet, pollutants, endocrine disruptors	1. Low fertilized female fecundity and low hatching percentage

eggs after receiving a stimulus to initiate sexual reproduction, we get a minimum total of about 2 weeks required for resting egg formation.

Some evidence indicates that sexual reproduction also requires special resources. Rapid asexual reproduction was observed when *Brachionus plicatilis* was fed the alga *Chlorella* (Snell and Hoff 1987). However, when sex was triggered in these populations, resting egg production failed. The source of this failure was traced to male fertility. Adding the alga *Tetraselmis* to the diet of the maternal sexual females restored fertility to their sons. These data suggest that as yet unidentified nutrients are required for male fertility, but not for female fertility. Food limitation also appears to differentially affect sexual and asexual females so that sexual females have a higher threshold food concentration (Snell and Boyer 1988).

The time and resources required for sexual reproduction constrain its occurrence in a growing season and determine the optimal allocation to asexual and sexual reproduction. Therefore, an important

question is, how does environmental change alter the time and resources required for sexual reproduction in rotifers? If the time necessary for sexual reproduction is lengthened or the resources available for sexual reproduction are reduced, resting egg production could be seriously diminished (Table 12.3). The size and stability of the resting egg bank determines the long-term persistence of monogonont rotifer populations (Snell and Serra 2000).

The effects of a variety of environmental changes on sexual reproduction can be predicted. For example, Snell and Serra (2000) simulated the effect of increases in the frequency and severity of catastrophic population crashes on resting egg bank dynamics and the probability of population extinction. Catastrophes could result from events like an early period of cold weather, rapid oxygen depletion, a sudden bloom of cyanobacteria, or acute exposure to toxicants in runoff or effluent. Catastrophes occurring once every 5 years in the Snell–Serra model had little effect on the probability

of population extinction over 100 years. However, a rotifer population experiencing three catastrophic crashes per year is virtually certain to go extinct within 100 years. These results stress the buffering effect of the resting egg bank, but also how that effect can be altered. A series of several bad years in a row could tip the balance toward long-term depletion of the resting bank and send a population towards extinction. Natural rotifer populations are often food limited, even in nutrient-rich systems (Merriman and Kirk 2000). Resource competition is probably common (Grover 1997). If rotifer species typically exist in marginal resource conditions, environmental change resulting from climate cycles of increased or decreased temperature or drying could further reduce opportunities for resting egg production. Several rotifer species are susceptible to cyanobacteria toxins, which can alter rotifer abundance and species composition (Gilbert 1996). Environmental changes increasing the frequency or intensity of cyanobacterial blooms would almost certainly reduce opportunities for rotifers to produce resting eggs.

Humans have accelerated the rate of environmental change in many ecosystems. In aquatic communities, human activities have increased the mean annual temperature of surface waters and increased the frequency of extreme temperature events (Moore *et al.* 1996). Pesticide exposures alter the composition of zooplankton communities (Hanazato 1991) and represent yet another source of catastrophic mortality for rotifer populations. For many temperate rotifer populations, resting egg production is usually highest in spring and summer when exposures to pollutants in runoff and effluents are highest. Small reductions in population growth rate from toxicant exposures can seriously reduce resting egg production. Reductions in growth rate of only 20 percent caused resting egg bank size to decline over 100 years (Snell and Serra 2000). When assessing the potential negative effects of these changes, testing for the effects on asexual reproduction is not enough. The effect of environmental change on sexual reproduction is probably more important for determining long-term population survival. The resting egg bank can buffer transient negative effects, but

sexual reproduction is expected to be more sensitive to environmental changes. Moreover, the evolutionary adjustment of sexual reproduction to environmental challenges is expected to be more difficult, due to its higher complexity.

The reliance of sexual reproduction on chemical communication may further make it susceptible to pollutants. Sexual reproduction requires chemical signals about population density, male recognition of conspecific females through contact chemoreception, and sperm-egg fusion in the pseudocoelom (Snell 1998). Chemical communication systems are vulnerable to interference from pollutants, but these effects are currently poorly understood in aquatic systems. A new class of pollutants gaining attention in invertebrates is endocrine disruptors. Compounds known to have endocrine disrupting activity in other animals have effects in rotifers (Preston *et al.* 2000). The effect of some vertebrate endocrine chemicals on rotifer reproduction has also been demonstrated (Gallardo *et al.* 1997).

Perhaps a more direct effect of pollutants is on rotifer swimming speed. The probability of encounter between male and female rotifers is critical for determining the proportion of sexual females that get fertilized (Snell and Garman 1986). A key parameter determining encounter probabilities is male and female swimming speed. Exposures to 110–330  $\mu\text{g}$  pentachlorophenol/l for 30 min reduced female swimming speed 20–30 percent in four of seven rotifer species (Preston *et al.* 1999).

In summary, environmental change poses one of the biggest challenges to the long-term persistence of rotifer populations. Sexual reproduction seems to be the point in the rotifer life cycle that is most vulnerable to disruption. A variety of anthropogenic activities lengthen the time required for sexual reproduction, reduce resource availability, or truncate population growth cycles, diminishing resting egg formation. Reductions in deposition into resting egg banks can tip the balance towards population extinction.

### Final remarks

To integrate some of the above points, let us consider an idealized population starting from an

single resting egg that hatches to produce a “stem female.” The stem female and her descendants, for at least several generations, will all be asexual females. During this phase the population increases in size through **clonal reproduction**. Eventually, an environmental signal will trigger the production of sexual females, but as discussed above, not all females respond to this signal. As we know from both theory and observation, the sexual reproduction ratio will be intermediate in many conditions. This ratio commits a part of the population to sexual reproduction while the other part continues to reproduce asexually as might be expected under a “bet-hedging” scenario. Note that half of the sexual females are expected to produce males while the other half produce resting eggs (Aparici *et al.* 1998). If the asexual fraction of the population that is engaging in the bet-hedging strategy becomes divided at a later time into sexual and asexual fractions, the overall production of resting eggs will increase.

This simplified clonal growth cycle suggests an interpretation of the “cost of sex” concept from the point of view of a particular **clone**. The parthenogenetic phase would be a way to produce a larger

number of sexual females, and eventually a higher resting egg output. However, given that each resting egg produced needs half of the sexual females to be male producing, the cost of sex for the whole cycle (from resting egg to resting egg) is  $1/2$  in the sense that twice as many resting eggs could be produced if they could be formed asexually.

Population biologists studying rotifers are nearing an understanding of the key evolutionary processes shaping the life history of these fascinating cyclical parthenogens. We note with more than passing interest that the analyses presented here are critically dependent on approximately equal measures of results from field, laboratory, and theoretical studies. This is precisely the triad of “modern” population studies that Thomas Park called for well over 50 years ago and that remains as urgently needed today as it was then.

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