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Review Paper

Modes, mechanisms and evidence of bet hedging in rotifer diapause traits

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Abstract In this contribution, we review our knowledge on bet-hedging strategies associated with rotifer diapause. First, we describe the ecological scenario under which bet hedging is likely to have evolved in three diapause-related traits in monogonont rotifer populations: (1) the timing of sex (because diapausing eggs are produced via sexual reproduction), (2) the sexual reproduction ratio (i.e. the fraction of sexually reproducing females) and (3) the timing of diapausing egg hatching. Then, we describe how to discriminate among bet-hedging modes and discuss which modes and mechanisms better fit the variability observed in these traits in rotifers. Finally, we evaluate the strength of the empirical evidence for bet hedging in the scarce studies available, and we call for the need of research at different levels of biological complexity to fully understand bet hedging in rotifer diapause.

Keywords Bet hedging · Diapause · Rotifers · Unpredictability

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Introduction

Monogonont rotifers typically inhabit time-varying habitats and produce diapausing eggs to overpass recurrent adverse periods between favourable planktonic growing seasons (Ricci, 2001; Schröder, 2005). Diapausing eggs are the only link between one season and the next; therefore, the number of diapausing eggs produced at the end of a growing season is typically considered the appropriate measure of fitness in rotifer clonal lineages (Serra & King, 1999; Serra et al., 2004; Campillo et al., 2011). Interestingly, many rotifer populations from temperate latitudes live in temporary habitats (Walsh et al., 2014), which are often characterized by year-to-year fluctuations in the length of the planktonic growing season (García-Roger et al., 2014). The end of the growing season may be related to changes in the physical environment (e.g. presence/absence of water) or in biotic factors (e.g. presence/absence of competitors or predators), and it imposes habitat regimes for rotifers. These regimes vary from highly predictable (i.e. when the length of the growing season extends regularly among seasons) to highly unpredictable (i.e. when there is great among-season variance in the length of the growing season). Such unpredictability may occasionally lead to a failure in the production of a new cohort of diapausing eggs (for example, if the length of the growing season is unexpectedly short), i.e. a zero-fitness event (Martínez-Ruiz & García-Roger, 2015). Under these circumstances, one of a variety of bet hedging strategies in

diapause-related traits may be selected to avoid the risk of complete reproductive failure in any generation.

Almost without exception, the production of diapausing eggs is coupled with sexual reproduction in monogonont rotifers (Gilbert & Schreiber, 1998; Ricci, 2001; Schröder, 2005). Sexual reproduction is initiated by the appearance of sexual females followed by male production, insemination and diapausing egg production. After revisiting the existing rotifer literature, we found that bet hedging has been proposed to evolve in at least three diapause-related traits: (1) the timing of sex, which in practice is used as a proxy of the timing of diapausing egg production and typically measured as the population density threshold for sexual reproduction initiation (i.e. the lowest the threshold, the earliest the timing of sex; Carmona et al., 1995; Schröder & Gilbert, 2004; Serra et al., 2004; Schröder, 2005; Carmona et al., 2011); (2) the sexual reproduction ratio (best known as the mixis ratio and computed as the fraction of sexually reproducing females in the population) (Gilbert, 2003; Fussmann et al., 2007; Gilbert & Schröder, 2007) and (3) the timing of diapausing egg hatching (i.e. the fraction of diapausing eggs hatching at the beginning of a new growing season) (Schröder, 2005; García-Roger et al., 2014). However, because bet-hedging traits must fulfil a set of conditions (see below) and there are various possible modes of bet hedging (Oloffson et al., 2009; Childs et al., 2010), the proper identification of the above-mentioned traits as bet-hedging traits is still uncertain.

Modes of bet hedging and strength of evidence of candidate bet-hedging traits

Theoreticians distinguish between two basic modes of bet hedging (for review, see Childs et al., 2010), which are typically referred to as conservative and diversified bet hedging (Seger & Brockmann, 1987; Philippi & Seger, 1989). These modes result from risk avoidance at the individual level or risk spreading among related individuals of the same genotype, respectively. Under conservative bet hedging, an individual sacrifices expected fitness to reduce temporal variance in reproductive success by employing a single, generalist strategy across all possible environmental scenarios (Philippi & Seger, 1989; Simons & Johnston, 2003). On the other hand, diversified bet hedging is conceived

as a trans-generational effect that occurs when a single 'genetic' individual—say a genotype or a rotifer clonal lineage—produces different phenotypes in its offspring in advance of future unpredictable conditions (Simons, 2011). At least two sub-modalities are possible for diversified bet-hedging strategies (Childs et al., 2010). These sub-modalities differ on whether diversification occurs within a cohort (i.e. among the offspring derived from one genotype during a single reproductive event) or among cohorts (i.e. when parents vary the characteristics of their offspring stochastically across reproductive events in what is called 'adaptive coin-flipping' bet hedging). It is worth to noting that both strategies involve risk spreading among the offspring of a single genotype.

To invoke bet hedging, either conservative or diversified, a candidate trait is required to reduce temporal variance in fitness across generations at the expense of decreasing the arithmetic mean of fitness. By doing so, the candidate bet-hedging trait would maximize the geometric mean of fitness (Gillespie, 1974; Childs et al., 2010; Starrfelt & Kokko, 2012). Therefore, bet-hedging traits are apparently sub-optimal under the averaged environmental conditions, but they are adaptive over long time scales. Although this can be seen as a simple condition, empirical assessments of bet hedging are infrequent because quantitative tests of optimality are required across temporal sequences of environmental changes, which is still a challenge (Simons, 2011).

Claims of bet hedging in diapause-related traits are scarce but not rare in the rotifer literature (García-Roger et al., 2014). After a search in the Thomson ISI Web of Science® using combinations of title and topic searches that included '(bet hedg* OR bet-hedg*) AND (rotifer*) AND (diapaus* OR resting egg) AND (sex* OR mixis)', we found a total of 16 records for the period 1970–2014. However, the putative bet hedging in these records was not always supported by adequate tests. Interestingly, Simons (2011) has recently suggested an ordinal scale of six categories to define increasing levels of empirical evidence in candidate bet-hedging traits, which were adapted to whether studies are performed at the within- or among-population level (Table 1). The categories are cumulative in the sense that each category includes the criteria from the previous ones.

Next, we discuss which bet-hedging mode better fits the variability observed in the three diapause-related rotifer traits mentioned above. We also discuss

Table 1 Categories of empirical evidence for candidate bet-hedging traits, modified from Simons (2011)

Category	Within-population level	Among-population level
I	Recognition of a candidate bet-hedging trait	Recognition of a candidate bet-hedging trait
II$_a$	Observation of unpredictable variation at any environmental factor	Observation of unpredictable variation at any environmental factor differing across habitats
II$_b$	Existence of within-genotype phenotypic variability in the candidate bet-hedging trait	Population differentiation in trait expression
III	II$_a$ + II$_b$	II$_a$ + II$_b$
IV	Demonstration of different fitness consequences for the phenotypes under consideration	Demonstration of different fitness consequences for population strategies across habitats
V	Positive test of a selective advantage of the trait under unpredictable fluctuating selection regime	Selective advantage of population strategies in respective habitats
VI	Quantitative fit of trait expression to the degree of unpredictability	Quantitative fit of population strategies to the degree of unpredictability in respective habitats

the underlying mechanisms promoting bet hedging and evaluate the strength of evidence in favour of bet hedging for each candidate trait following the criteria proposed by Simons (2011).

The timing of sex

The timing of sex has been proposed as an instance of the conservative bet-hedging trait in rotifer populations inhabiting unpredictable environments (Carmona et al., 1995; Serra & King, 1999; Schröder & Gilbert, 2004; Serra et al., 2004; Gilbert, 2007a). If the end of the growing season—or the nutritional conditions required for diapausing egg production (Gilbert, 2010)—cannot be predicted by rotifers, then producing diapausing eggs as soon as possible can avoid the risk of a zero-fitness event. This strategy may seem sub-optimal because a rotifer clone producing diapausing eggs too early, while favourable conditions still prevail would experience a selective disadvantage because an investment in diapause results in a direct reduction of the current population growth rate (Carmona et al., 2009; Serra & Snell, 2009). However, clones producing diapausing eggs too late will probably not survive an unexpectedly early end of the growing season. Accordingly, a conservative bet-hedging clone would overcome the disadvantage of producing, on average, a lower yield of diapausing eggs by reducing the variance in diapausing egg production across growing seasons.

Table 2 summarizes the strength of evidence from tests of bet hedging associated with the timing of sex following Simons' criteria (2011). Although theoretical work supports the advantage of a conservative 'Early sex' strategy in unpredictable habitats (Serra et al., 2004), to the best of our knowledge, no study has yet compared the geometric mean fitness across generations of rotifer clones that differ in their timing of sex, which could be performed in experimentally manipulated planktonic growing seasons of unpredictable length. In the absence of this type of study, evidence for bet hedging in this trait seems restricted and sub-optimal, as it has been observed at the genotype level in qualitative agreement with the expectations from bet-hedging theory.

Although it is not definitive, strong evidence in favour of bet hedging in this trait would arise from population differentiation in the timing of sex associated with a gradient of environmental predictability (i.e. earlier sex as the habitat becomes more unpredictable). Interestingly, a high propensity for sexual reproduction has been reported in a *Brachionus calyciflorus* Pallas, 1766, population inhabiting a highly unpredictable pond in Patagonia (Gilbert & Dieguez, 2010). This study reaches up to Category II$_a$, as it does not hold an among-population comparison. Additionally, several other empirical studies have reported variability in the timing of sex among rotifer populations of different species and from different habitats (Category II$_b$; Schröder & Gilbert, 2004; Schröder et al., 2007; Gabaldón & Carmona, 2015), but they fail to look at the match between this

Table 2 Categories of bet-hedging evidence in the timing of sex in rotifers

Category	Description	Type of study	Species	References
I	Proposal of the timing of sex as a candidate bet-hedging trait	T	–	Serra & King (1999), Gilbert (2007a)
II$_a$	Observation of unpredictable variation in hydroperiod length in a single habitat	W	*B. calyciflorus* Pallas, 1766	Gilbert & Dieguez (2010)
			B. manjavacas Fontaneto et al., 2007	Gabaldón & Carmona (2015)
			B. plicatilis Müller, 1786	Gabaldón & Carmona (2015)
	Observation of unpredictable variation in hydroperiod length across habitats	A	*B. plicatilis*	Franch-Gras et al. (in prep.)
II$_b$	Among-population variability in the timing of sex	A	*B. angularis* Gosse, 1881	Schröder & Gilbert (2004)
		A	*B. calyciflorus*	Schröder & Gilbert (2004)
		A	*E. senta**	Schröder & Gilbert (2004), Schröder et al. (2007)
		A	*R. frontalis*	Schröder & Gilbert (2004)
		A	*Hexarthra* spp. Schmarda, 1854	Schröder et al. (2007)

Only categories for which there is any type of evidence are presented. Type of study: *T* theoretical, *A* among-populations empirical study, *W* within-population empirical study

* This species is not *E. senta* sensu stricto, but a species belonging to the *E. senta*-species complex as described by Schröder & Walsh (2007)

variability and the degree of environmental unpredictability.

We conclude that evidence for bet hedging in the timing of sex is still weak, likely because of several reasons: (1) many studies often lack accurate measurements for habitat predictability—due to the fact that appropriate metrics for the organism on focus and long-term monitoring are required (García-Roger et al. 2014), (2) confounding effects such as the possible relationship between growing season length and predictability are not dissected (e.g. a short, but predictable, growing season, would select for early sex too) and (3) the fixation of the long-term optimal strategy can be partially avoided by selection during the clonal growing phase, as each growing season acts as a selective regime (Carmona et al., 2009). In this sense, some studies have reported the existence of genetic (among-clone) variation of the timing of sex within rotifer populations (Aparici et al., 2001; Gilbert, 2002; Carmona et al., 2009; Gabaldón & Carmona, 2015). The finding of a substantial amount of genetic variation in sex propensity is particularly

relevant, given the effect of the timing of sex on individual fitness (reviewed in Serra et al., 2004).

The sexual reproduction ratio

The sexual reproduction ratio is extremely variable (e.g. Carmona et al., 1994, 1995; Snell & Boyer, 1988; Gilbert, 2002; Schröder & Gilbert, 2004; Gilbert & Schröder, 2007; Carmona et al., 2009). However, despite this variability, the sexual reproduction ratio is almost never 100%, and commonly, it is not higher than 30%; thus, some fraction of the population continues reproducing asexually when sexual reproduction is initiated (Gilbert, 1974; Pourriot & Clément, 1975; Carmona et al., 1995; Schröder, 2001; Gilbert, 2002; Schröder, 2005). This partial response to the induction of sexual reproduction has been reported at the clone level too, and it is described as an intriguing phenomenon because all females within a clonal lineage and their oocytes are genetically identical (Gilbert, 2003; Gilbert & Schröder,

2007; Fussmann et al., 2007). Consequently, intermediate sexual reproduction ratios have been regarded as evidence of a diversified bet-hedging strategy (sensu Seger & Brockmann, 1987) that would allow for the maintenance of both reproductive modes within a clonal lineage (Serra & King, 1999). Intermediate sexual reproduction ratios may evolve in unpredictable habitats, where early sex ensures the production of at least some diapausing eggs. It should be noted that an early sex initiation could have a significant cost if the planktonic growing season was to end up being long, but this cost could be reduced if early sex initiation is balanced by an intermediate sexual reproduction ratio. Modelling by Serra et al. (2005) showed that sexual reproduction ratios as low as 14% maximized diapausing egg production in habitats with unpredictable growing season length. Indeed, such ratio and a threshold of 70 individuals l^{-1} were revealed as an evolutionary stable strategy.

Evidence for bet hedging in the sexual reproduction ratio is summarized in Table 3. This evidence is restricted to the observation of within-genotype variability in the trait (Category II_b), which has been reported in a considerable number of rotifer species. Unfortunately, only a single study by Campillo et al. (2011) has focused on among-population variation, but it had no direct measure of the degree of habitat unpredictability.

The description of a proximate mechanism responsible for the within-genotype variation observed in the sexual reproduction ratio is still in progress (Gilbert 2003, 2007a, b; Fussmann et al., 2007). Such an underlying mechanism would help in differentiating between bet-hedging sub-modalities. Sexual offspring production haphazardly varies among females of the same clone in several rotifer species (Gilbert, 2007b; Gilbert & Schröder, 2007; Fussmann et al., 2007) and among maternal age at the within-female level (Pourriot & Rougier, 1976; Rougier & Pourriot, 1977; Pourriot & Rougier, 1986; Carmona et al., 1994; Gilbert & Schröder, 2007; Fussmann et al., 2007). The most prevalent pattern here is one of the decreasing propensities to produce sexual offspring with maternal age. Therefore, at least two different mechanisms seem to jointly operate to generate diversification in the reproductive mode of the offspring of rotifer females. In any case, whatever the mechanism underlying this variability in the sexual reproduction ratio (i.e. either 'coin-flipping' or non-genetic maternal

effects), a condition for bet hedging is that diversification must be expressed in response to environmental factors without predictive value for the future environment (see Menu & Desouhant, 2002; Evans & Dennehy, 2005; Crean & Marshall, 2009). Currently, this concept has not yet been proved.

The timing of diapausing egg hatching

Not all rotifer diapausing eggs hatch in the season following their production. Consequently, bet hedging in the timing of diapausing egg hatching has been claimed (Schröder, 2005; García-Roger et al., 2006a). Most of our knowledge on the optimum duration of diapause in rotifers comes from mathematical models (for review see García-Roger et al., 2014), which are mainly inspired by the seminal work by Cohen (1966) on germination in plant seeds. In his original model, Cohen (1966) considered two types of years (favourable vs. adverse) occurring with a certain fixed probability and showed that optimal germination/hatching rates should be proportional to the probability of experiencing a favourable year. In an ecological scenario where the habitat is unsuitable during periods of varying predictability, a fraction of diapausing eggs is expected to remain in diapause at the beginning of a new planktonic growing season, unresponsive to the environmental stimuli promoting hatching. Thus, this pattern ensures that some eggs will survive in the sediments until the following growing season.

Empirical evidence for bet hedging in the timing of diapausing egg hatching is summarized in Table 4. Beyond the observation of different durations of diapause in diapausing eggs of natural rotifer populations, which forms a very preliminary level of evidence (Category I; see for instance García-Roger et al., 2006b), Schröder (2005) suggested that producing diapausing eggs with more or less prolonged diapauses might be a case of diversified bet hedging if 'Early' (diapausing eggs being ready to hatch immediately after production) and 'Late' (diapausing eggs remaining in diapause in the egg bank) hatchers are produced from the same clone. Interestingly, this author observed that not all diapausing eggs produced from single clones in *Epiphanes senta* Müller, 1773, *Rhinoglena frontalis* Ehrenberg, 1853, and *B. calyciflorus* (Schröder, 1999, 2005) hatched when subjected to a first event of hatching stimulus. Thereafter, in a

Table 3 Categories of bet-hedging evidence in the sexual reproduction ratio in rotifers

Category	Description	Type of study	Species	References
I	Proposal of intermediate sexual reproduction ratios as a candidate bet-hedging trait	T	–	Serra & King (1999), Serra et al. (2004)
II$_b$	Existence of phenotypic variability in the sexual reproduction ratio at the within-genotype level	W	N. copeus Ehrenberg, 1834	Pourriot & Clèment (1975)
		W	A. sieboldi Leydig, 1854	Kabay & Gilbert (1977)
		W	B. plicatilis	Hino & Hirano (1977), Snell & Boyer (1988), Carmona et al. (1994)
		W	S. pectinata Ehrenberg, 1832	Gilbert & Schreiber (1998)
		W	B. calyciflorus	Gilbert (1963, 2002, 2003, 2007b), Gilbert & Schröder (2007), Fussmann et al. (2007)
		W	B. angularis	Gilbert & Schröder (2007)
			E. ukera Schröder & Walsh, 2007	Gilbert & Schröder (2007)
			R. frontalis	Gilbert & Schröder (2007)
	Among-population variability in the sexual reproduction ratio	A	B. plicatilis	Campillo et al. (2011)

Only categories for which there is any type of evidence are presented. Type of study: *T* theoretical, *A* among-populations empirical study, *W* within-population empirical study

second exposure to identical hatching conditions, a fraction of 'Late' diapausing eggs still hatched. This pattern has been recently observed for *B. plicatilis* Müller, 1786, too (Martínez-Ruiz & García-Roger, 2015). All these studies provide empirical evidence at the Category II$_b$ level.

Stronger evidence (Category III) arises from a recent study by García-Roger et al. (2014), in which a significant positive correlation was found between the hatching fraction of diapausing eggs from different *B. plicatilis* populations (initially reported by García-Roger et al., 2006b) and a proxy for predictability of the ponds that these populations inhabit. That proxy was based on the assumed positive relationship between habitat predictability and diversity (Slobodkin & Sanders, 1969) and only considered a predictability gradient that varied from unpredictable to predictably good habitats. While the pattern found was consistent with theoretical predictions, it is worth noting that the gradient from unpredictable to predictably bad habitats remains unexplored, and more accurate ways of measuring predictability are necessary.

Little is known about the proximate mechanisms that lead to within-genotype variability in the duration of diapause. Martínez-Ruiz & García-Roger (2015) proposed that diversification in the timing of diapausing egg hatching could occur through the following ways: (1) Each inseminated sexual female within a clone, as a result of an independent reproductive event, may probabilistically produce a single phenotype (all 'Early' or all 'Late', in Schröder's terminology; see Fig. 1a), which would be an instance of the 'adaptive coin-flipping' modality of bet hedging operating at the mother level (Oloffson et al., 2009; Childs et al., 2010). (2) Each inseminated sexual female within a clone may produce resting eggs with different phenotypes ('Early' and 'Late'; see Fig. 1b, c). Note here that the authors considered each insemination as a single reproductive event.

Table 4 Categories of bet-hedging evidence in the timing of diapausing egg hatching in rotifers

Category	Description	Type of study	Species	References
I	Proposal of the duration of diapause as a candidate bet-hedging trait	T	–	Schröder (2005), García-Roger et al. (2006a, 2014)
II$_a$	Observation of unpredictable environmental variation across habitats	A	*B. plicatilis*	García-Roger et al. (2014)
II$_b$	Existence of phenotypic variability in the timing of diapausing egg hatching at the within-genotype level	W	*E. senta* Müller, 1773	Schröder (1999)
		W	*R. frontalis*	Schröder (2005)
		W	*B. calyciflorus*	Schröder (2005)
		W	*B. plicatilis*	Martínez-Ruiz & García-Roger (2015)
	Among-population variability in the timing of diapausing egg hatching (i.e. population differentiation in the hatching fraction of diapausing eggs)	A	*B. plicatilis*	García-Roger et al. (2006b)
III	Observation of unpredictable environmental variation across habitats + Population differentiation in the hatching fraction of diapausing eggs	A	*B. plicatilis*	García-Roger et al. (2014)

Only categories for which there is any type of evidence are presented. Type of study: *T* theoretical, *A* among-populations empirical study, *W* within-population empirical study

Martínez-Ruiz & García-Roger (2015) tracked the offspring of individual inseminated females from controlled crosses in clonal lineages of *B. plicatilis* and tested for an effect of egg laying order on the hatching phenotype ('Early' vs. 'Late'). These authors have found a significant effect of diapausing egg laying order on the probability of being an 'Early' or 'Late' hatcher expressed at the within-female level, but no differences among females within clones were observed (i.e. all females exhibited the same, but variable, response). This finding supports a diversification mechanism mediated by a non-anticipatory maternal effect.

Remarks

To date, the level of empirical evidence in favour of bet hedging, according to Simons' criteria (2011), has reached Category III in the timing of diapausing egg hatching, but only Category II has been reached in both the timing of sex and the sexual reproduction ratio. Our review reveals that the studied traits have a low level of support of empirical evidence and that more specific evidence is still needed for all of them. Namely, more evidence is needed for the establishment of fitness consequences associated with the expression of each diapause-related trait (Category IV), the demonstration of selective advantage of the traits studied against non-bet-hedging alternatives under unpredictable regimes (Category V), and the finding of a match between bet-hedging trait expression and the degree of unpredictability (Category VI).

Empirical studies of bet hedging in other aquatic organisms displaying diapause are also limited in number. Notwithstanding, there are some remarkable cases where the upper category of evidence has been reported (Simons, 2011). Here, we extended the review of Simons (2011) for the period 2010–2015. For this purpose, we used a search engine in the Thomson ISI Web of Science® similar to that described above, only changing the organism under study to cladocerans, copepods and anostracans, respectively. Studies of cyclic parthenogenetic cladocerans were scarce—only three—and paid attention to either the timing of diapause (Cáceres & Tessier, 2003) or the timing of
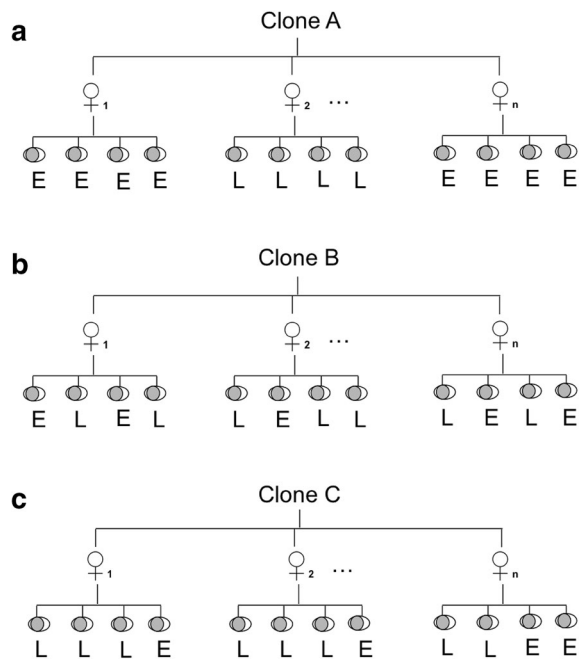
Fig. 1 Modes of diversified bet hedging for the timing of diapausing egg hatching in rotifers. **a** Diversification occurring among females within the same clone; **b** diversification haphazardly occurring within the offspring of single females within a clone; and **c** diversification (E for 'Early' and L for 'Late' hatchers) occurring within the offspring of a single female within a clone following a trend (e.g. a monotonic variation with mother age so that E diapausing eggs are typically produced at advanced laying order)

diapausing egg hatching (De Meester & De Jager, 1993; Vanoverbeke & De Meester, 2009). In both cases, the level of empirical evidence met Category II$_b$. Studies of anostracans typically dealt with the timing of diapausing egg hatching and were most prevalent in Category III (Hildrew, 1985; Saiah & Perrin, 1990; Simovich & Hathaway, 1997; Dumont & Ali, 2004; Vanschoenwinkel et al., 2010), with a remarkable exception in *Branchinecta sandiegonensis* Fugate, 1993, for which hatching fractions in different populations were found to be optimal given their respective frequency patterns of pool filling, so that Category VI was reached (Philipi et al., 2001). Similarly in copepods, it has been found that the timing of diapausing egg production in *Diaptomus sanguineus* Forbes, 1876, is in close agreement with a simulation-derived evolutionary stable strategy, thus reaching the highest level of empirical evidence for a conservative bet-hedging trait (Category VI). This study was however restricted to the analysis of a single population

(Hairston & Munns, 1984; see also Hairston & Olds, 1984).

The number of species in which there is any evidence of bet hedging also provides insight into the prevalence of bet hedging in rotifers. Interestingly, while the timing of diapausing egg hatching has reached a higher category than the two other traits, evidence in the first trait is restricted to fewer rotifer species. This result is not surprising because high categories of empirical evidence demand for very challenging and complex studies, which can only be afforded by using a restricted number of adequate model species. In this sense, *B. calyciflorus* and *B. plicatilis* arise as promising model species for testing bet hedging in diapause-related traits after being studied in 18 out of 30 (60%) assays.

Information on the mechanisms for within-genotype variability needed for diversified bet-hedging traits is also scarce. Gilbert (2003, 2007b) stressed that the oocytes of the same female can develop into either sexual or asexual females, thus making within-genotype diversification possible. However, this phenomenon could be generated by 'adaptive coin-flipping' plasticity at the female level mediated by haphazard variation in the physiology of the mother, or it could alternatively be generated by any type of non-haphazard, non-genetic maternal effect (e.g. female age; Carmona et al., 1994; Gilbert & Schröder, 2007; Fussmann et al., 2007). Notwithstanding, although the phenomenon of intermediate sexual reproduction ratios is puzzling from a mechanistic point of view, it is a clear candidate for diversified bet hedging, as it consists of probabilistic risk spreading among individuals of the same genotype.

In the case of the timing of diapausing egg hatching, the study by Martínez-Ruiz & García-Roger (2015) points to non-anticipatory maternal effects (see Marshall & Uller, 2007; Crean & Marshall, 2009) as the most likely mechanism for diapause duration in rotifers, although other mechanisms of bet hedging contributing to intra-clonal variability cannot be completely ruled out. For instance, 'coin-flipping' could also occur at the embryo level to introduce some noise to the observed maternal-mediated pattern. In this sense, we consider modes and sub-modalities of bet hedging, not as alternatives to explain variability in diapause duration or an incomplete sexual reproduction induction, but as concomitant sources to generate a diverse response. A single trait being able to mediate

different types of bet hedging has been previously suggested in plants in the case of the timing of flowering, where the evolution of the trait results from a balance between conservative and diversified bet-hedging components (Rees et al., 2004, 2010; Childs et al., 2010).

Other diapause traits, such as the quantity or quality of diapausing eggs, could also be considered as potential candidate bet-hedging traits. However, we did not obtain any hit after implementing them in our search criteria and so they were not included in this analysis. Egg size can be considered as a proxy of egg quality (i.e. mediated by the amount of reserves). In relation to the evolution of this trait in unpredictable environments, it has been hypothesized a conservative bet hedging consisting in producing eggs larger than would be the optimum in a predictable environment with the same long-term mean quality (Einum & Fleming, 2004). As mentioned above, to our knowledge, this prediction has not been tested yet in rotifers.

Typically, bet hedging is considered in the context of a single trait. However, in the real world, more than one trait can be involved in the adaptation to environmental unpredictability, as several traits can mediate the same bet-hedging response (Wilbur & Rudolf, 2006; Childs et al., 2010). For instance, both a conservative bet-hedging strategy in the timing of sex and a diversified strategy in the duration of diapause likely serve the same objective of avoiding the risk of a complete reproductive failure in habitats with an unpredictable length of the growing season. Because both traits have essentially the same bet-hedging role, it is interesting to ask which should evolve or whether selection of both traits is possible (Wilbur & Rudolf, 2006). For instance, if the sediments are risky because deterioration in the egg banks is very high (García-Roger et al., 2006a, c), then an early induction of sex to ensure the production of a new cohort of diapausing eggs would be a less costly bet-hedging strategy. In this review, we have identified four rotifer species (*B. calyciflorus*, *B. plicatilis*, *E. senta* and *R. frontalis*) for which evidence of bet hedging exists in both the timing of sex and the timing of diapausing egg hatching (see Tables 2, 4). However, evaluating the most efficient strategy, or a possible coexistence between traits, requires the assessment of both traits within the same population, which is a condition that has not been accomplished in any species at the moment. Moreover,

such assessments should be performed by means of experimental approaches that allow for testing bet hedging at the highest level of empirical evidence (Simons, 2011). From here, we emphasize the need to perform such studies to provide definitive support for the existence of bet hedging in rotifer diapause-related traits in response to year-to-year unpredictability in the planktonic growing season length.

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