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Adaptation in response to environmental unpredictability

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Understanding how organisms adaptively respond to environmental fluctuations is a fundamental question in evolutionary biology. The Mediterranean region typically exhibits levels of environmental unpredictability that vary greatly in habitats over small geographical scales. In cyclically parthenogenetic rotifers, clonal proliferation occurs along with occasional bouts of sex. These bouts contribute to the production of diapausing eggs, which allows survival between growing seasons. Here, we studied two diapause-related traits in rotifers using clones from nine *Brachionus plicatilis* natural populations that vary in the degree of environmental unpredictability. We tested the hypothesis that the level of environmental unpredictability is directly related to the propensity for sex and inversely related to the hatching fraction of diapausing eggs. We found significant levels of genetic variation within populations for both traits. Interestingly, a positive correlation between pond unpredictability—quantified in a previous study from satellite imagery—and the propensity for sex was found. This correlation suggests a conservative, bet-hedging strategy that provides protection against unexpectedly short growing seasons. By contrast, the hatching fraction of diapausing eggs was not related to the level of environmental predictability. Our results highlight the ability of rotifer populations to locally adapt to time-varying environments, providing an evolutionarily relevant step forward in relating life-history traits to a quantitative measure of environmental unpredictability.

1. Introduction

Understanding how organisms adaptively respond to environmental fluctuations is a fundamental question in evolutionary biology and has motivated a growing body of research (e.g. [1–3]). This is not surprising, given that all natural habitats experience some degree of environmental fluctuation. Environmental fluctuations can be decomposed into predictable and unpredictable components, and human activity often causes an increase of the latter [4–6]. Environmental fluctuations and their degrees of predictability are evolutionarily relevant because they are expected to produce diverging adaptive responses in organisms.

There are several ways by which organisms evolutionarily respond to unpredictability [7]. The best known of these ways is through natural selection that acts recurrently on the heritable variation among individuals; this process is named adaptive tracking or genetic evolution (e.g. [8,9]). Phenotypic plasticity, another mode of adaptive response, occurs when individuals modify their responses according to environmental conditions without changing their genetics [10]. Bet hedging constitutes a third way and occurs when a genotype increases the geometric mean of fitness at the cost of a decrease in the arithmetic mean, thus reducing fitness variance [11]. There are two main modes of bet hedging: diversified and conservative [11]. Diversified bet hedging is a transgenerational effect that occurs when a single genotype produces different phenotypes in its offspring in advance of future unpredictable conditions [12]. By contrast, under conservative bet hedging, a genotype sacrifices expected fitness to reduce temporal variance in reproductive success by employing a single low-risk phenotype

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across all possible environmental future scenarios [13]. All of these strategies are non-exclusive, and combinations of them may occur simultaneously in natural populations [9]. Although bet hedging is well addressed theoretically [14,15], empirical evidence in the wild remains scarce [7].

To understand adaptation to unpredictability, it is informative to study populations from an array of habitats with different fluctuating environments [16]. The Mediterranean region typically exhibits very different environmental regimes over small geographical scales. For example, Mediterranean water bodies are characterized by strong seasonality and temporal unpredictability at several timescales [17]. By using data recorded from remote sensing (27 years of Landsat TM/ETM + data), Franch-Gras *et al.* [18] were able to acquire a quantitative, long-term time series of water-surface area in a set of shallow, non-permanent water bodies situated in the east of the Iberian Peninsula. They measured the predictable and unpredictable components of the fluctuation in water-surface area. Interestingly, they found a wide range of unpredictability with respect to environmental features at scales relevant to small aquatic invertebrates. Cyclically parthenogenetic rotifers are common inhabitants of Mediterranean ponds and lakes. These rotifers are facultatively sexual, combining clonal proliferation with occasional bouts of sexual reproduction. In this temperate region, rotifer populations are temporal; i.e. they are not active year-round, and they colonize the water column during the so-called planktonic growing season. Typically, each growing season, the active population is initiated by the hatching of diapausing eggs from the pond sediment [19,20]. Hatchlings are asexual females that parthenogenetically produce subitaneous eggs, which hatch into genetically identical daughters, thus producing clones. Sex initiates once a population density threshold is reached, which triggers asexual females to produce sexual daughters as some fraction of their offspring (e.g. [19–21]). Thus, both sexual and asexual reproduction occur simultaneously. Sexual females produce meiotic haploid eggs that develop into haploid males if they remain unfertilized and into diploid diapausing eggs if they are fertilized. Unlike asexual eggs, these sexually produced eggs are dormant embryos; therefore, sex is associated with diapause. Diapausing eggs settle in the sediment and remain dormant for a period of variable duration [22,23]. Under suitable conditions, diapausing egg hatching is induced, and a new growing season starts. However, not all diapausing eggs hatch in the season following their production [23]. The unhatched eggs often show prolonged dormancy and accumulate, forming diapausing egg banks where they can remain viable for decades or even centuries [24–26]. These banks allow rotifer populations to overcome unsuitable environmental conditions.

Natural populations confined in shallow, non-permanent ponds are expected to be adapted to and strongly driven by inundation patterns [27–29]. This is probably the case for rotifer populations that inhabit Mediterranean water bodies. These populations can be conceived as a collection of annual clones that cross sexually to produce diapausing eggs, some of which will hatch in the next growing season [30,31]. The number of diapausing eggs produced is a component of clonal fitness as these eggs are the only way to survive unsuitable water column conditions between growing seasons [32]. As the length of the growing season affects diapausing egg production, among-year fluctuations in the duration of the growing season can provide insight into how environmental unpredictability affects fitness. For instance, an unexpected critically short growing season may cause the density threshold for sex initiation to be unmet. Failure of a clone to reproduce sexually means no production of diapausing eggs and, consequently, zero fitness. The end of the growing season in rotifer populations can arise not only due to abiotic factors (e.g. drought, extreme salinity, extreme temperature) but also due to biotic factors (e.g. occurrence of competitors and predators). Randomness in these ecological factors will determine the variance of the growing season and hence the uncertainty the population will encounter. Such unpredictable fluctuations are expected to be powerful drivers of life-history traits and might be increasingly important in periods of climate change. Several studies have reported high levels of genetic variation in diapause-related traits in rotifers [30,33–36]. A few other studies have found signatures of local adaptation in these traits [21,37]. Nevertheless, the relationships between life-history traits related to diapause and the degree of environmental unpredictability in natural populations of aquatic organisms remain largely unknown, especially because quantifying predictability is a complex issue [18,38]. This difficulty can be partly overcome in the laboratory by simulating different patterns of predictability. In a recent study using an experimental evolution approach, laboratory rotifer populations showed a rapid adaptive response in diapause-related traits under two contrasting selective regimes of environmental predictability [39]. These selection experiments are important because they show that traits evolve as expected in relation to environmental unpredictability after controlling for other factors in laboratory conditions. However, they do not provide unambiguous evidence about whether such an evolution has occurred in the wild, where levels of unpredictability might be different from the experimental ones, and adaptation to unpredictability might be traded off by adaptation to other concomitant conditions or counterbalanced by non-selective evolutionary forces, namely migration.

There are two key life-history traits in the rotifer life cycle that are associated with the entrance to and exit from dormancy: (i) the propensity for sex, which is inversely related to the density threshold for sex initiation [30,33] and is a proxy of the timing of diapausing egg production [40]; and (ii) the diapausing egg hatching fraction. Both traits have been proposed as instances of bet-hedging strategies that might interact to reduce the risks associated with environmental unpredictability [41].

The propensity for sex has been proposed as a case of conservative bet hedging in rotifer populations that inhabit unpredictable habitats [40]. When there is uncertainty regarding the onset of unsuitable periods, a low-risk strategy might be to produce diapausing eggs as soon as possible to avoid an unexpectedly short growing season [32]. However, if the growing season is not short, early investment in sex and diapause will reduce the rate of clonal proliferation, thereby resulting in lower fitness [30,42]. These considerations lead to the prediction that the propensity for sex will increase with increasing unpredictability. However, in testing this prediction, a confounding factor should be considered: if the growing seasons are predictably short, a high propensity for sex is also expected to evolve.

In contrast, diapausing egg hatching has been proposed in several theoretical studies as a form of diversified bet hedging. As rotifers cannot predict whether a particular growing season will be sufficiently long to complete the life cycle and ensure

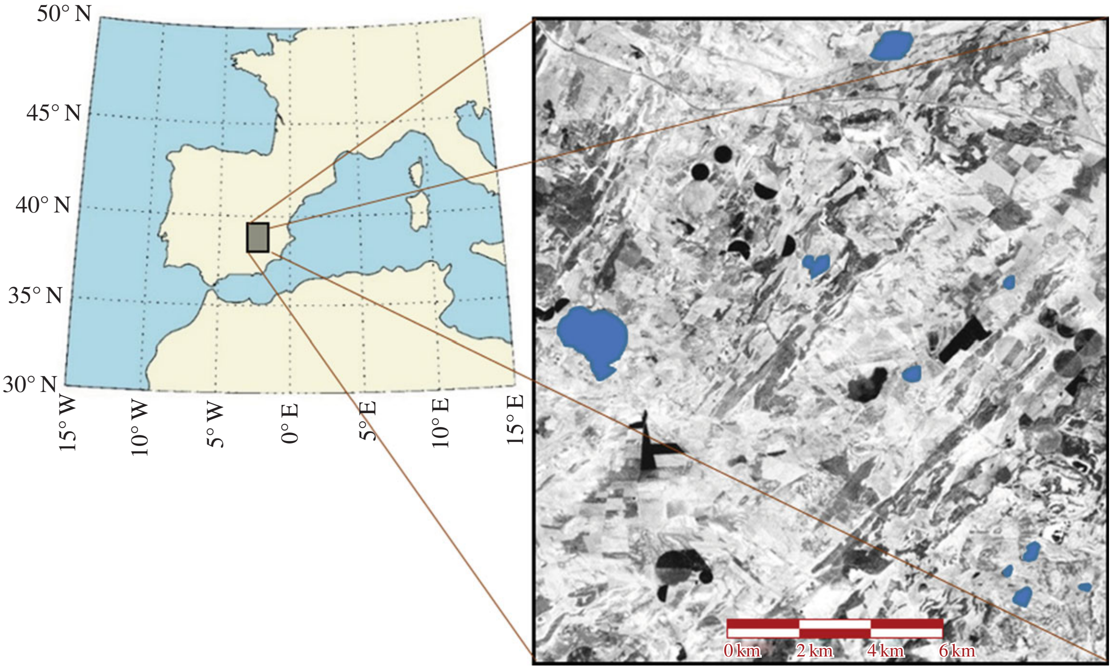

Figure 1. Location of the region (38°55.4′ to 38°41.803′ N and 1°47.32′ to 1°24.26′ W), with the studied ponds highlighted in blue.

diapausing egg production, intermediate hatching rates are expected in habitats with both long growing seasons (where, ideally, all of the eggs would hatch; i.e. good seasons) and unexpectedly short growing seasons (where, ideally, no egg would hatch; i.e. bad seasons). According to bet-hedging theory, the optimal hatching fraction should equal the frequency of good seasons [14] (reviewed in [43]). For example, in this 'good versus bad growing season' scenario, a hatching fraction of approximately 0.5 (i.e. intermediate) would be expected in a completely unpredictable habitat, because the frequency of good seasons would be somewhere around 0.5 [14].

Here, we study whether natural rotifer populations locally adapt to the degree of environmental unpredictability by evolving diverging strategies for both the propensity for sexual reproduction and the timing of diapausing egg hatching. We analysed 270 clones from nine populations of *B. plicatilis* from habitats in which environmental unpredictability has previously been quantified by Franch-Gras *et al.* [18]. While most studies on bet hedging typically focus on single traits [12], our study addresses whether bet hedging has evolved in either or both diapause-related traits in response to a natural gradient of environmental predictability and tests for the predictions from the well-established theory [14,15] in a field where empirical evidence is scarce [7]. Specifically, we hypothesize that environmental unpredictability will select for (i) a high propensity for sex (early diapausing egg production) and (ii) intermediate diapausing egg hatching fractions.

2. Material and methods

(a) Study populations and clone establishment and maintenance

To obtain a collection of experimental clones directly from the field, diapausing egg banks of *B. plicatilis* were sampled in nine saline inland ponds located in the east of the Iberian Peninsula (figure 1). The degree of environmental predictability and hydroperiod length at these sites were previously quantified by Franch-Gras *et al.* [18] from satellite data obtained over a 27-year period (table 1). To quantify environmental predictability, they described different metrics associated with different organisms. Here, we chose a predictability metric derived from Colwell's approach [45] and based on the presence/absence of water (as a state variable). This has the advantage of being a simple, parameter-free metric that has been proposed as appropriate for aquatic invertebrates [18]. Briefly, the metric quantifies unpredictability as the deviation from the product of marginal probabilities in a contingency table of state versus month (cells: counts with water presence); therefore, the main source of environmental predictability is the inter-annual repetition of the within-year pattern of the presence of water. An assumption of this metric is that unpredictability in the presence/absence of water correlates with unpredictability in a suite of biotic and biotic factors (e.g. temperature, ionic concentration, food supply, competition, predation) beyond simply hydroperiod.

A sample from the uppermost 10 cm of sediment at each of the nine ponds was obtained with a Van Veen grab (Eijkelkamp Agrisearch Equipment, Giesbeck, The Netherlands) in September 2013. Diapausing eggs were isolated from the sediment using a sugar flotation technique [31], and diapausing eggs that looked healthy [46] were transferred individually to wells in 96-multiwell plates (NuncTM, Nalge Nunc Int., Roskilde, Denmark). Eggs were induced to hatch under standard hatching conditions: 25°C, 6 $\mathrm{g\,l^{-1}}$ (artificial saline water, Instant Ocean; Aquarium Systems, Inc., Mentor, OH, USA) and constant illumination (photosynthetically active radiation: 35 μmol photons $\mathrm{m^{-2}\,s^{-1}}$) (details in [23,26,46]). Hatchlings were monitored every 24 h for a maximum of three weeks. Clonal lines were established by asexual proliferation of the resulting neonate females. Inadvertent selection in favour of clonal lines with high hatchability is unlikely. At the sediment depth sampled—which integrates several years [26]—eggs induced to hatch in the laboratory are a mixture of different cohorts, so that, if variation in timing of hatching occurs, the hatched eggs should include early hatchers and late hatchers (i.e. those eggs that remained in diapause when cues inducing hatching occurred in their habitats). Thirty clones from each field population were founded and maintained in 15 ml stock cultures at 12 $\mathrm{g\,l^{-1}}$ salinity and 20°C. Every week, half of each clone's culture volume was refreshed with fresh medium. This medium was f/2-enriched saline water [47] in which the microalga *Tetraselmis*

Table 1. Features of the studied ponds (adapted from Franch-Gras *et al.* [18]), populations' nuclear genetic diversity (expected heterozygosity, from [44]) and broad-sense heritability of propensity for sex of the studied populations.

pond/population	acronym	area (m^2)	hydroperiod (fraction of the year flooded)	estimated environmental predictability	broad-sense heritability of propensity to sex	expected heterozygosity
Pétrola	PET	1 190 000	1.00	1.00	0.11	0.44
Salobralejo	SAL	237 000	1.00	1.00	0.36	0.27
Atalaya de los Ojicos	ATA	47 000	0.93	0.75	0.49	0.25
Hoya Rasa	HYR	40 000	0.87	0.66	0.35	0.38
Hoya Chica	HYC	32 000	0.51	0.12	0.58	—
La Campana	CAM	29 000	0.63	0.11	0.30	0.41
Hoya del Monte	HMT	15 800	0.51	0.19	0.16	0.41
Hoya Yerba	HYB	1060	0.23	0.34	0.30	0.41
Hoya Turnera	HTU	130	0.07	0.70	0.68	0.26

suecica (Microalgae Culture Collection of ICMAN-CSIC, Spain) had been grown as rotifer food. Unless otherwise indicated, pre-experimental and experimental rotifer culture media and conditions were the same as for the stock cultures (hereafter, 'standard conditions'). Clonal lines were identified to the species level by genetic analysis of cytochrome *c* oxidase subunit I (COI) based on PCR-RFLP [48] because *B. plicatilis* belongs to a cryptic species complex. Data on the unbiased nuclear genetic diversity for these populations are available from [44] (range 0.25–0.44, table 1). Genetic diversity and pond unpredictability were not significantly correlated ($R^2 = 0.23$; $p = 0.22$).

(b) Characterization of life-history traits: propensity for sex

Genetic variation in the propensity for sex was studied by conducting 810 bioassays (9 populations × 30 clones × 3 replicates) following the procedure of Carmona *et al.* [30] with minor modifications as described below.

(i) Pre-experiment

To control for maternal effects and avoid the early induction of sexual reproduction, each rotifer clone was pre-cultured at low density under standard conditions in darkness over three generations [49]. To accomplish this, three asexual females that each carried two asexual eggs were individually sampled from each stock clonal culture and transferred to Petri dishes with 40 ml of culture medium (initial microalgae concentration: 250 000 cells ml^{-1}). After 24 h, daughters had been produced. Next, all of the females were removed except for one neonate female (F1) in each dish. Each of these females produced daughters after 48 h. Next, a single neonate female from the second generation (F2) of each replicate was transferred individually into a new Petri dish containing 40 ml of fresh culture medium. This procedure was repeated to obtain F3 neonate females and begin the bioassay. Through this approach, the three experimental replicates had independent pre-experimental conditions.

(ii) Bioassay

Each F3 neonate female was transferred individually to a Petri dish containing 15 ml of fresh culture medium (initial microalgae concentration: 500 000 cells ml^{-1}). The F3 females were allowed to reproduce and proliferate and were monitored under a stereomicroscope every 12 h until the first male was observed. Then, the culture was fixed with Lugol's solution (final concentration 4%), and the population density was recorded as an inverse measure of the propensity for sex [33]. Additionally, the time for the appearance of males was recorded. The record (time and density) of first male appearance is a standard proxy of sex initiation [30,50,51]. It is more reliable than recording the appearance of sexual females because asexual and sexual females can only be differentiated based on their egg size. The bioassay using the appearance of the first male is highly reproducible [30]. Male appearance lags approximately 3 days after actual sexual reproduction initiation (i.e. when the development of a parthenogenetic egg into a sexual female is first triggered; [33]). Variation in this time lag has not been documented, the assumption being that it is negligible (hours, at most) when compared with biologically significant variation in sex propensity among genotypes (days; e.g. [30]).

(c) Characterization of life-history traits: diapausing egg hatching fraction

Estimation of the diapausing egg hatching fraction was performed by randomly selecting from each pond a subset of 10 clones that were used in the propensity-for-sex experiment. In total, we conducted 8640 bioassays (96 diapausing eggs × 10 clones × 9 populations).

(i) Diapausing egg production

To estimate hatching fraction, a high number of diapausing eggs were produced under laboratory conditions for each clone by intraclonal sexual reproduction within a narrow time window (4 days). Twenty ovigerous, asexual neonate females of each stock clone were transferred into a Petri dish containing 40 ml of culture medium (initial microalgae concentration: 500 000 cells ml^{-1}), and parthenogenetic proliferation and sexual reproduction were allowed. Microalgae density was maintained at over 250 000 cells ml^{-1} by adding highly concentrated (centrifuged) algae, and cultures were inspected every 24 h until the first mature diapausing egg was observed in any clone. Then, all of the cultures were maintained for 4 additional days. On the 4th day, mature diapausing eggs that looked healthy (types I and II; see [46]) were collected and cleaned with 6 g l^{-1} of saline water. Most of the clones (83.3%) had produced 96 diapausing eggs by this stage; however, for the remaining clones, 8 additional days, under the same production conditions and collecting diapausing eggs every 4 days, were required to obtain 96 diapausing eggs. Immediately following collection, the diapausing eggs were individually transferred to wells in 96-multiwell plates and incubated under standard hatching conditions. As shown in some recent studies in *Brachionus* [23,52,53], a long dormancy period after the production of diapausing eggs before hatching is not necessarily required.

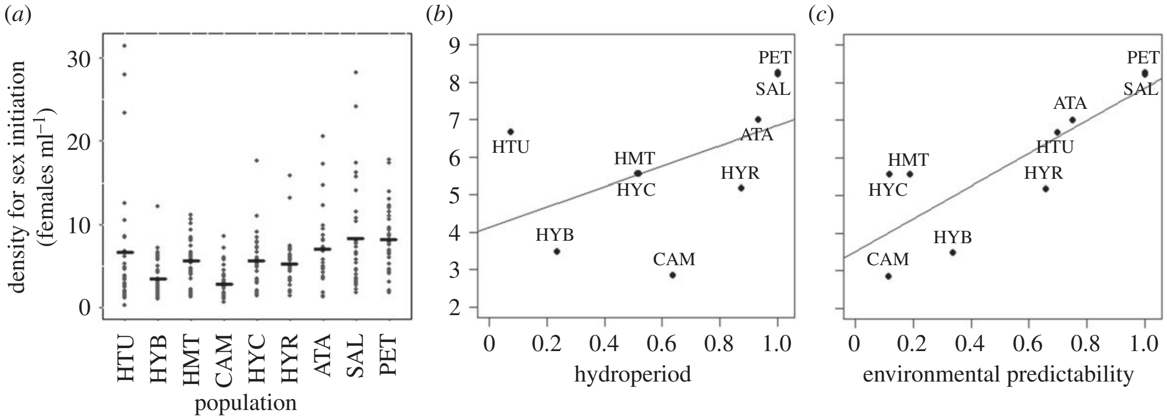

Figure 2. Propensity for sex in each pond as indicated by the population density for sex initiation. (*a*) Clonal means (dots) and population means (horizontal bars) as estimated for the nine populations. (*b*) Relationship between the estimated population means and the pond hydroperiod (fraction of the year flooded). The solid line indicates the least-squares regression fitting. (*c*) Relationship between the estimated population means and the estimated environmental predictability in the ponds. The solid line indicates the least-squares regression fitting.

(ii) Bioassay

Diapausing eggs were inspected every 24 h over a 28-day period for the presence of hatchlings and deterioration. The unhatched fraction of diapausing eggs that looked healthy after that period (see [46]) was dried out and held in darkness at 4°C for 28 days. After that period, diapausing eggs were induced to hatch under the same hatching conditions to confirm viability. The diapausing eggs hatching fraction was calculated as the number of hatched eggs in the first incubation period out of those that looked healthy. The deterioration state (healthy versus deteriorated) of the diapausing eggs was determined by the percentage of embryo integrity and visual aspect (see [46]). The fraction of deteriorated diapausing eggs (i.e. excluded diapausing eggs) ranged from 0.24 to 0.53 across populations.

(d) Data analysis

Generalized linear mixed-effects models (GLMMs) were used to test for differences among populations in both life-history traits. The propensity for sex was analysed using a Poisson distribution of errors and log link function. For the diapausing egg hatching fraction, a binomial distribution of errors and the logit function were used. For both life-history traits, hydroperiod and predictability were included as fixed-effect continuous predictors (factors), whereas population (nine levels) and clone nested within population ($n = 30$ and 10 per population for propensity for sex and hatching fraction, respectively) were considered as random-effects factors. In both analyses, we used maximum-likelihood (ML) ratio tests to determine the structure of fixed effects by alternatively dropping hydroperiod or predictability against a full model including all effects. Then, we tested the significance of random effects by means of Restricted maximum-likelihood (REML) ratio tests. GLMMs were performed in R v. 4.1.1. [54] by using the glmer function of the lme4 package [55].

Within-population genetic variation in the propensity for sex was measured using broad-sense heritability (H^2, i.e. the ratio of the among-clone variance to the total within- and among-clone variance), which is the relevant measure for selection during clonal proliferation [56]. H^2 was estimated according to Pfrender & Lynch [57]. H^2 for the hatching fraction was not calculated because variance components cannot be reliably estimated under a binomial distribution of errors [58].

3. Results

(a) Propensity for sexual reproduction

Clones of *B. plicatilis* showed wide variation in density and the timing for sex initiation (figure 2), with ranges of clone means of 0.2–31.4 females ml^{-1} and 3.5–14 days, respectively. These two traits are highly correlated (overall Pearson correlation coefficient: 0.754, $p < 0.0001$). Propensity for sex differed significantly among populations and among clones within populations (table 2). Broad-sense heritability values were significant in most populations (7 out of 9; table 1) and were not significantly correlated with unpredictability or hydroperiod length.

Table 2. Summary of the generalized linear mixed-effects model (GLMM) results on the propensity for sex and the diapausing egg hatching fraction with one degree of freedom.

	propensity for sex		diapausing egg hatching fraction	
effect	χ^2	p	χ^2	p
predictability	4.08	0.043*	0.43	0.511
hydroperiod	1.25	0.262	1.58	0.208
population	11.61	<0.001*	3.57	0.058
clone (population)	22288.0	<0.001*	1140.9	<0.001*

*p-value < 0.05.

The propensity for sex was significantly affected by the degree of predictability of each pond after controlling for hydroperiod (table 2 and figure 2).

(b) Diapausing egg hatching fraction

The clones of *B. plicatilis* showed wide variation in diapausing egg hatching fraction, which ranged from 0 to 100% among clones (figure 3). Hatching fraction differed significantly among clones but not among populations (table 2). Differences among clones within populations indicated that heritable variation existed, although H^2 estimates could not be obtained. Diapausing egg hatching fraction was neither significantly affected by predictability nor hydroperiod (table 2).

4. Discussion

Diapause-related traits are proposed to be important fitness components, and they are expected to be subject to strong

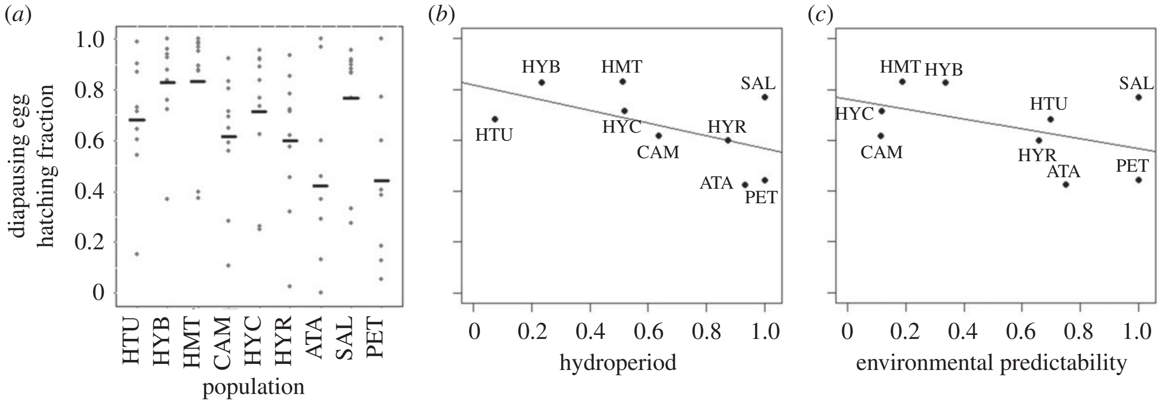

Figure 3. Diapausing egg hatching fraction in each pond. (*a*) Observed clonal hatching fractions (dots) and population means (horizontal bars) as estimated for the nine populations. (*b*) Relationship between the estimated population means and pond hydroperiod (fraction of the year flooded). The solid line indicates the least-squares regression fitting. (*c*) Relationship between the estimated population means and pond environmental predictability. The solid line indicates the least-squares regression fitting.

selection [59]. Accordingly, within-species adaptive divergence in the response to local conditions is expected where gene flow and genetic drift do not counterbalance selection. Here, we demonstrate that the degree of environmental predictability strongly correlates with the propensity for sex in rotifer populations, with rotifer populations that inhabit more unpredictable environments being more prone to reproduce sexually. Patterns of differentiation among populations in diapause-related traits have been found in other studies of cyclical parthenogens, including cladocerans [16,60,61], aphids [62,63] and rotifers [21,64]. Thus, our research extends previous studies showing that populations are locally adapted to the environment in the timing of diapausing egg production. Moreover, these studies did not investigate whether these patterns are correlated with the environmental unpredictability of the habitats of these organisms. The present study provides this correlational evidence across a well-established gradient of environmental unpredictability occurring in the wild, pointing to local adaptation in a small geographical range (240 km^2).

Our results are consistent with the theoretical prediction that environmental unpredictability selects for early timing of the production of diapausing stages [32,65,66], as propensity for sex is a major factor influencing diapausing egg production in cyclically parthenogenetic rotifers. As rotifer response was measured in many clones under laboratory conditions, our experimental design allows us to conclude that the differential response among populations is genetically based and is thus shaped by evolutionary forces, probably natural selection. The apparent suboptimality of early sex may be explained as a bet-hedging strategy that has evolved in response to environmental unpredictability. Even if diversifying bet-hedging strategies might be involved in the rotifer sexual phase (e.g. betting for sexual and asexual offspring after sex initiation), the pattern found here implies a conservative strategy, as we analysed the average value for sex initiation of a genotype (i.e. variation within genotype was not considered). A low-risk strategy consisting of early sex protects against unexpectedly short growing seasons (e.g. due to drought or the occurrence of predators) by ensuring that some diapausing egg production occurs despite the cost to the current growth rate in long growing seasons. Delay of sex would be fatal if the habitat becomes unsuitable when reproduction is still exclusively asexual or when sexual reproduction is incomplete and no diapausing eggs have yet been produced. Thus, according to bet-hedging theory [7], by reducing the variance in diapausing egg production across growing seasons, a higher propensity for sex would overcome the disadvantage of producing a lower average yield of diapausing eggs. A high propensity for sex could also be expected to evolve as a response to predictable short hydroperiods [51]. However, this latter explanation appears unlikely in our case because our analysis revealed a highly significant correlation between predictability and propensity for sex when the effect of hydroperiod length was controlled for. Nevertheless, unpredictability should select for early sex because short hydroperiods may occur, and these events have an overwhelming effect on shaping optimal investment in sex and diapause.

A previous experimental evolution study using the same model organism showed that a selective regime simulating environmental unpredictability rapidly selected for early sex in multi-clonal, highly diverse populations created in the laboratory [39]. Now, our study has identified the degree of environmental unpredictability as an important contributor to explain the existing patterns of timing of sex in nature. Unpredictability works as an effective selective factor in the studied rotifer natural populations. Their evolutionary effects are not traded off by adaptation to unknown selective factors that might act in the wild, and not counterbalanced by other evolutionary forces as migration or genetic drift. Of most importance when comparing with the results in [39], our results indicate that the studied natural populations harboured enough genetic diversity to fuel adaptation to unpredictability.

The significant levels of genetic variation we found within our populations in the propensity for sex may be the consequence of both fluctuating selection and the buffering effect on genetic variation that is provided by the diapausing egg banks [67]. Adaptive tracking may be acting simultaneously with bet hedging in these fluctuating environments. Nevertheless, if the rate of change in the environment is high, the mean phenotype selected through adaptive tracking may lag behind the optimum, and bet-hedging strategies may become more important [7,9,68]. However, adaptive tracking of the propensity for sex appears to be less likely in our populations because no relation between unpredictability and the heritability of this trait was found.

We found intermediate hatching fractions (range: 44–88%) in all populations as well as among-population genetic differentiation in relation to this trait. However, in contrast with our expectations, we found non-significant negative relationships of this trait with both environmental predictability and hydroperiod. Because these non-significant relationships had directions opposite to the predicted ones, a lack of statistical power is unlikely to explain the rejection of our directional hypotheses. Several factors might explain the observed lack of association between unpredictability and the hatching fraction of diapausing eggs. First, because clones were produced by intra-clonal crosses, inbreeding depression could be affecting the hatching fractions [69]. Inbreeding depression caused by intra-clonal crosses is expected in populations where clones normally do not inbreed: that is, in genetically diverse populations. Our populations embrace a range of genetic diversity. However, such diversity does not correlate with environmental unpredictability. Thus, although inbreeding depression could be causing some noise in our results, we think that it is very unlikely it could counterbalance the hypothesized effect of unpredictability on hatching fractions. Second, theoretical work has suggested that organisms that hedge their bets successfully with one strategy do not need to bet hedge to the same extent with another [41]. This phenomenon might occur here, with a high propensity for sex being sufficient to avoid the risk of zero-fitness growing seasons, particularly if every population has intermediate hatching fractions. Although further evidence might be needed to discard an effect of unpredictability on the hatching fraction in the wild, our findings highlight the importance of studying multiple traits involved in the same strategy to get a sensible test for bet-hedging [12]. Third, a recent study in laboratory populations has addressed the effects of two selective regimes, predictable and unpredictable, on these two traits using an experimental evolution approach [39]. That study found a rapid adaptation of hatching fractions to experimental conditions. Accordingly, the hatching fraction might adaptively track rows of similar growing seasons, instead of being optimized to the predictability of the overall time series of growing seasons. We know that, in our system, the presence of water is weakly correlated across population habitats [18]. Thus, if genetic tracking is intense, it might result in divergence among populations in relation to this trait.

The present study reveals that rotifers are able to locally diverge in diapause-related traits even within a small geographical range (240 km^2) despite their potential for widespread genetic exchange through the passive dispersal of diapausing eggs [67]. This finding is in agreement with the genetic differentiation observed in neutral and ecologically relevant traits among populations of cyclical parthenogenetic zooplankters at local scales [64,67,70–73]. These populations are likely to adapt within short time spans [74], with their huge local abundances diluting the effect of immigrants. Several studies have indeed shown rapid evolution in response to environmental changes in natural populations of cladocerans (e.g. [75–77]).

Our work supports the expectation that wild populations of *B. plicatilis* can develop evolutionary responses to face environmental unpredictability. Given that scenarios of increased environmental variability are expected to occur in the near future [4], the persistence of rotifer natural populations under these circumstances may depend on the evolution of bet hedging in key life-history traits [7,12,78]. Therefore, a comprehensive understanding of the role of bet-hedging strategies is necessary for predicting population responses to environmental change [79]. Our study contributes to this understanding by relating two potentially bet-hedging life-history traits with a quantitative measure of environmental unpredictability and showing how they interact in nature. This makes our contribution particularly relevant in a field of study—bet-hedging strategies—with strong theoretical development [14,15] but where empirical evidence is still scarce, especially in natural populations [7]. A next step is understanding and identifying the molecular mechanisms underlying response variation, which will further increase our knowledge of how organisms adapt to unpredictable habitats.

Data accessibility. Raw data and the R script are available via Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5vq2h [80].

Authors' contributions. L.F.-G. performed sampling and experimental work with support from E.M.G.-R. and M.J.C., participated in the experimental design, implemented statistical data analyses with the aid of E.M.G.-R., M.S. and M.J.C. and prepared the manuscript's first draft. E.M.G.-R., M.S. and M.J.C. conceived, designed and coordinated the study. All authors discussed the results, contributed to subsequent manuscript drafts and approved the submitted version.

Competing interests. We declare we have no competing interests.

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