

## Local adaptation in rotifer populations

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**Abstract** The adaptation of organisms to their environment has been a subject of study for a long time. One method to study adaptations in populations involves comparing contemporary populations of the same species under different selective regimes, in what is known as a ‘local adaptation’ study. A previous study of the cyclically parthenogenetic rotifer *Brachionus plicatilis* found high heritabilities for some life-history traits. Some of these life-history traits significantly differed among six populations from Eastern Spain and data suggested some traits to have higher evolutionary rates than neutral genetic markers. Here, by studying the same *B. plicatilis* populations, we examine the variation and possible local adaptation of their main life-history traits, closely related to fitness, in relation to habitat salinity and temperature. These environmental factors have been shown to play a key role in the ecological differentiation among co-generic species of *B. plicatilis*. The results obtained in this study show that: (1) the seasonality of rotifer populations from Eastern Spain has profoundly influenced sexual reproduction strategies; (2) salinity is probably a key factor in the ecological specialization of some populations; and (3) rotifer populations harbour high variability in their fitness components.

**Keywords** *Brachionus plicatilis* · Local adaptation · Temporary environments · Diapausing egg bank

### Introduction

The adaptation of organisms to their environment has been a subject of study for a long time (Darwin 1859; Williams 1966; Ridley 1996). The direct method to look for evidence of adaptation in natural populations is to monitor populations in newly colonized areas

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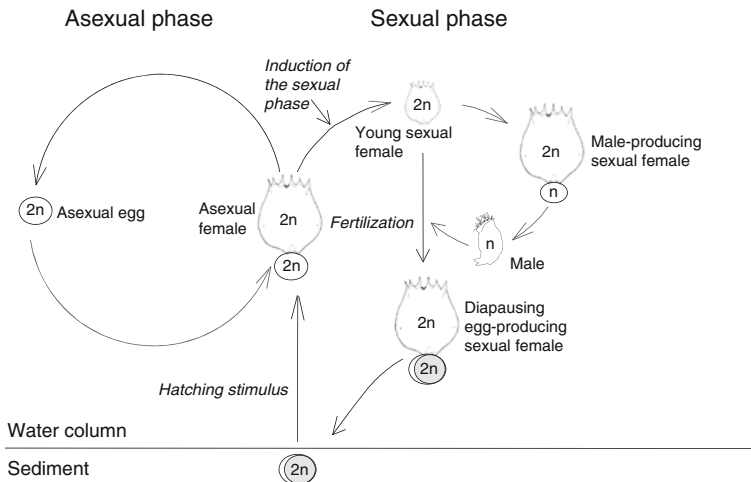
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(putatively unadapted) over time, testing for an increase in fitness. This is, however, a long-term task for most animals. An indirect way to study adaptation is to compare contemporary natural populations under supposedly different selective regimes, in what is known as local adaptation studies (Kawecki and Ebert 2004). For local adaptation to arise, certain requirements must be met (Kawecki and Ebert 2004): (1) existence of divergent selection pressure in the different habitats, (2) existence of “genotype  $\times$  habitat” interaction, phenomena that should result in (3) local genotypes being on average better adapted than foreign ones in their local habitat (“local vs. foreign criterion”). If the latter condition is not fulfilled, local adaptation has not occurred, although it may be underway but not yet complete. Several causes, such as high gene flow among habitats (causing homogenization between habitats) and genetic drift (causing non-adaptive heterogeneity between habitats), may prevent local adaptation from taking place.

The importance of local adaptation in lake-dwelling cyclical parthenogens (rotifers and cladocerans) has received a great impetus in the last two decades (for a review see De Meester 1996; Lynch et al. 1999; Declerck et al. 2001; Morgan et al. 2001; De Meester et al. 2002) and was fuelled by the proposal of the Monopolization hypothesis. This hypothesis states that numerical superiority of prior residents and rapid adaptation to local selective regimes override dispersal and strongly hinder gene flow, thus causing populations to diverge (De Meester et al. 2002), which explains the high population differentiation found in molecular markers in lake-dwelling zooplankters.

Several papers have focussed on seasonal, rather than local, adaptation in rotifers (King 1972, 1977; Gómez et al. 1995). However, since gene flow was not found among seasonally adapted genotypes, these are currently recognized as different species, belonging to a cryptic species complex (Ciros-Pérez et al. 2001; Gómez et al. 2002). Therefore, the occurrence of within-species ecological specialization in rotifers remains unknown. In temperate climates, cyclically parthenogenetic rotifer populations (Wallace et al. 2006; Fig. 1) are not active in the water column throughout the year. Typically, every year the active population is initiated by the hatching of diapausing eggs from the sediment of the pond or lake. Hatchlings are diploid asexual females, which reproduce by ameiotic parthenogenesis. After a period of clonal propagation, a sexual phase starts when, induced by environmental factors such as population density (Carmona et al. 1993, 1995; Stelzer and Snell 2003; Snell et al. 2006), asexual females start to parthenogenetically produce both sexual and asexual daughters. Sexual females produce haploid eggs. If a sexual female is not inseminated her unfertilized eggs develop into haploid males. If inseminated, her fertilized sexually-produced eggs result in a diploid embryo, the so-called diapausing egg. These eggs are dormant, tolerate harsh conditions, including desiccation, and tend to rest in the sediment. Only a fraction of the diapausing eggs hatches every year, so an egg bank in the sediment is created. This life cycle is considered an adaptation to time-varying environments. During this cycle, fitness can be conceptualized as composed by short-term components, affecting clonal propagation, and long-term components, affecting sexual reproduction and hence between-year survival. For instance, intrinsic growth rate ( $r$ ) is frequently considered a short-term fitness measure of the clone, representing its ability to grow in a particular environment (Stelzer 2005). On the other hand, diapausing egg production is considered a long-term fitness measure as these eggs are the only way to survive adverse periods throughout years. It is worthy to note that a trade-off between sexual and asexual reproduction exists (Snell 1987; Serra et al. 2004, 2005), since early initiation of sex depresses clonal propagation.

The rotifer *Brachionus plicatilis* inhabits salt and brackish waters (Walker 1981). Several facts suggest salinity is a critical condition for this species. First, *B. plicatilis* is not



**Fig. 1** Typical biological cycle of monogonot rotifers. Modified from Ciroso-Pérez et al. (2001) and Lapesa (2004)

an osmoconformer (Lowe et al. 2005), so that regulation costs are expectable. Second, *B. plicatilis* belongs to a cryptic species complex, whose species are adapted differentially to salinity (Gómez et al. 1997; Ortells et al. 2003). Third, ca. 86% of the described species of rotifers are freshwater (Fontaneto et al. 2006), so that the ability to inhabit salt waters seems to be a derived feature in these *Brachionus*, requiring special physiological adaptations. Moreover, response to salinity has been documented to be dependent on temperature (Miracle and Serra 1989).

In a recent study, Campillo et al. (2009) compared variation in neutral markers and life-history traits in clones from six *B. plicatilis* populations from Eastern Spain. Life-history traits measured reproductive capability and were estimated for each clone in four laboratory environments combining temperature and salinity conditions. These results suggested that some life-history traits evolved at higher rates than neutral markers, as expected from local adaptation. However, in Campillo et al. (2009) no explicit analysis was made on the relationships between the response of life-history traits to the experimental conditions (salinity and temperature) and the conditions in the ponds where the populations were sampled. This analysis is crucial to draw conclusions on local adaptation, and is the aim of the present study. We use the raw data base of Campillo et al. (2009), taking advantage of the fact that the studied ponds represent a remarkably wide range of temperature and, particularly, salinity. For instance, in one of the six ponds studied — Salada de Chipana — the recorded salinities are twofold the salinities in the other ponds. Nonetheless, the conditions which were kept constant in the experiment conducted by Campillo et al. (2009) — e.g., food or growth period —, might be more similar to the conditions in some ponds than in others. This is a caveat affecting many local adaptation studies, which cannot be performed in natural conditions. We carefully analyse the evidence for local adaptation in the data set out by Campillo et al. (2009) by studying variation in five life-history traits. With this aim, we compute new life-history traits more commonly used in the ecological literature, and address their variation in the laboratory environment with respect to the temperature and salinity of the populations' habitats. Our aim is to assess the hypothesis that, if salinity — either related to temperature or not — plays a major role in the

adaptation of rotifer populations to local conditions, those populations coming from the higher salinity ponds would perform better at the higher salinity treatments in our experiment.

## Materials and methods

### Populations and clones studied

Rotifer populations from Hondo Sur (HOS), Poza Sur de Torreblanca (TOS), Balsa de Santed 1 (SA1), Hoya Rasa (HOY), Salada de Chiprana (CHI) and Salobrejo (SAL) were studied (Table 1). Pond selection was intended to comprise an environmental gradient — salinity, temperature — when *B. plicatilis* populations are present in the water column, as well as different hydroperiods. Pond sampling, clone founding, pre-experimental culture, experimental design and experimental set-up are described in detail in Campillo et al. (2009). Briefly, rotifer diapausing eggs were collected from pond sediments, and a total of 180 *B. plicatilis* clones were founded (30 per population; species identification tested by molecular markers). The experimental design combined two temperatures (20 and 25°C) and two salinities (15 and 30 g L<sup>-1</sup>), which are in the range of conditions experienced by our rotifers in the wild (Ortells 2002; Lapesa 2004; García-Roger 2006). For each experimental environment and clone, four cultures were performed. Two of them were short-term growth replicates to estimate four life-history traits (Table 2) and the other two were long-term growth replicates to estimate diapausing egg production. This design yields 2,880 experimental cultures (6 populations × 30 clones × 2 temperatures × 2 salinities × 4 replicates). Pre-experimental cultures were mass cultured under constant illumination (35 μmol quanta m<sup>-2</sup> s<sup>-1</sup>), at 22.5°C temperature, 22.5 g L<sup>-1</sup> salinity artificial seawater (Instant Ocean®, Aquarium Systems, Mentor, OH, USA) and fed with frozen *Tetraselmis suecica* in order to minimize food quality variation. Except for salinity, temperature and light, these conditions were the same as in the experiment, where the initial concentration of *T. suecica* was 10<sup>5</sup> cells ml<sup>-1</sup> and rotifers were grown in the dark. Each experimental culture was started by transferring 12 egg-carrying asexual females

**Table 1** Main characteristics of the studied ponds

Pond or lake	Area (km <sup>2</sup> )	Depth (m)	Hydroperiod pattern	Temperature (°C)	Salinity (g L <sup>-1</sup> )	Salinity classification <sup>a</sup>
Hondo Sur	0.20	0.8	Semi-permanent	15–25	8–21	Mesohaline
Poza Sur	0.01	1.0	Seasonal	20–25	10–32	Polyhaline
Balsa de Santed 1	0.02	<1.0	Ephemeral	10–20	17–20	Polyhaline
Hoya Rasa	0.08	<1.0	Seasonal	21.8 <sup>b</sup>	15.2 <sup>b</sup>	Mesohaline
Salada de Chiprana	0.23	5.0	Permanent	15–25	32–47	Hyperhaline
Salobrejo	0.36	0.8	Semi-permanent	8–25	8–30	Polyhaline

Data from García-Roger (2006) and García-Roger et al. (2006), except when indicated otherwise

<sup>a</sup> After the classification proposed in Montes and Martino (1987) with modifications

<sup>b</sup> Hilary A. Smith (pers. comm.; data from one single observation)

**Table 2** Life-history traits

Life-history trait	Formula	Units	Ecological meaning	Related LHTs in Campillo et al. (2009)
Egg ratio (ER)	(asexual eggs + 0.8 male eggs + 5 diapausing eggs at $t = 4$ )/females at $t = 4$	Egg equivalents/female	Ability of an individual to convert its energy into eggs	Production of female-producing eggs, Production of male-producing eggs
Mixis index ( $m$ )	(male eggs + diapausing eggs at $t = 4$ )/total eggs	Adimensional	Investment in sexual reproduction in the population	Production of male-producing eggs
Male production (MP)	Males at $t = 4$ /females at $t = 2$	Adimensional	Amount of sexual reproduction in the population	Male production
Intrinsic growth rate ( $r$ )	$[\ln(\text{females at } t = 4) - \ln(\text{females at } t = 2)]/2$	$d^{-1}$	Amount of asexual reproduction occurring in a population (a short-term fitness measure)	Female production
Diapausing egg production (DE)	Total diapausing eggs	Eggs/replicate	Long-term fitness component	Diapausing egg production

Time ( $t$ ) is expressed in days. Diapausing egg production was obtained from long-term cultures, and the other life-history traits from the short-term cultures. To calculate the egg ratio (ER), since the different types of eggs are not equally costly to produce, we assigned a weight in the calculation to each type of egg according to previous studies (Serra 1987; Serra et al. 2005)

from the corresponding pre-experimental culture to 50 mL of artificial seawater in the experimental conditions. The experimental rotifer cultures were prepared in several batches due to practical constraints and full randomization was prevented by the timing of hatching of the diapausing eggs, which provided the experimental clones. To do so, we randomly selected a group of clones for each batch, testing the four experimental environments with their replicates. The cultures in a batch were randomly placed in the experimental growing chamber. This procedure was repeated for each batch until getting the experimental results for the 180 clones. In the short-term growth cultures, females were counted after two days (intended to be an additional acclimatization to the experimental conditions). On day 4, the culture period concluded, and the numbers of females, males, female-producing eggs and male-producing eggs were counted. In the long-term cultures, on day 4 the cultures were fed again with frozen *T. suecica* ( $10^5$  cells  $\text{ml}^{-1}$ ), and left to grow until their extinction, which happened one to two months later due to starvation. During this period, the population could achieve high densities, and sexual reproduction was expected to be induced (Carmona et al. 1993, 1994; Stelzer and Snell 2003). After the extinction of the active population, the diapausing eggs were counted. From all these counts, five life-history traits were computed for each experimental combination (Table 2).

## Data analysis

We used a mixed hierarchical-factorial design, with the factors: salinity, temperature, population, clone within population and all the possible interactions. All factors were treated as fixed except clone, which was considered random. For each life-history trait, an ANOVA was performed to test for the significance of the above-cited factors using SPSS v. 13.0 (SPSS Inc., Chicago, IL, USA). For diapausing egg production and the intrinsic growth rate, which are two life-history traits highly related to fitness, the slope of the line corresponding to the life-history trait vs. salinity was computed for each clone and the distribution of this slope was computed for each population at each experimental temperature.

Correlation analysis was applied to explore the relationship between (1) diapausing egg production, intrinsic growth rate and mixis index, with (2) the habitat features pond surface, pond depth and hydroperiod duration. Though “hydroperiod duration” is a ranked variable (values: short, medium and long-duration; García-Roger 2006), a continuous variable underlies, so values were coded as 1, 2 and 3 for the correlation analyses. Since the maximum value for depth was only known for two ponds, this factor was not used in the correlations.

## Results

Salinity, temperature and their interaction had a statistically significant effect on all life-history traits, except for the effect of salinity on mixis index and temperature on the intrinsic growth rate when the correction for multiple comparisons was applied (Table 3). The factor “population” was significant in all life-history traits. Diapausing egg production had a significant “population  $\times$  salinity” interaction (Table 3).

Life-history traits tended to have higher values at the lowest salinity (15 g L<sup>-1</sup>; Fig. 2). A noticeable exception associated to the “salinity  $\times$  temperature” interaction is that the mixis index was higher at 30 than at 15 g L<sup>-1</sup>, when tested at 25°C. Temperature showed more complex patterns. Egg ratio and mixis index showed lower values at 25°C than at 20°C. The values of other life-history traits (male production, intrinsic growth rate and diapausing egg production) increased in line with temperature (Fig. 2). In general, the combination of temperature and salinity causing the highest life-history trait values was 25°C and 15 g L<sup>-1</sup>, except for egg ratio and mixis index, the highest values being found at 20°C and 15 g L<sup>-1</sup> (Fig. 2).

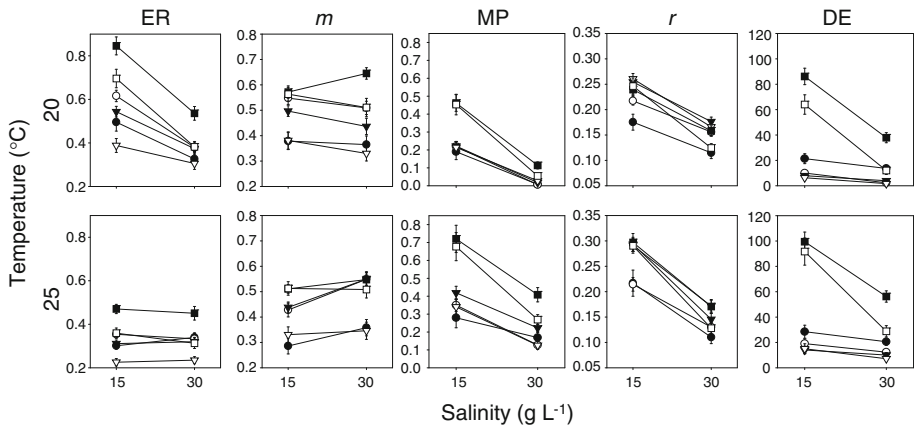
The population having the highest values for most life-history traits in the four experimental environments was Balsa de Santed 1, followed by Poza Sur (Fig. 2). For the intrinsic growth rates, Hondo Sur and Hoya Rasa were the two populations showing the highest values at 20°C whereas for Hondo Sur and Balsa de Santed 1 this occurred at 25°C. On the other hand, Salada de Chiprana was the population with the lowest intrinsic growth rate in all experimental environments. With respect to diapausing egg production, Balsa de Santed 1 and Poza Sur were the two populations with the highest values, their productions being very high in all experimental environments except at 20°C and 30 g L<sup>-1</sup>, where Salada de Chiprana corresponded to greater diapausing egg production than Poza Sur. The lowest production was exhibited by Hondo Sur, Salobrejo and Hoya Rasa, whereas Salada de Chiprana exhibited intermediate diapausing egg production.

The factor “clone” was significant for all life-history traits except for the intrinsic growth rate (after Dunn–Šidák correction, Table 3), meaning that there was genetic

**Table 3** Hierarchical factorial ANOVA for the life-history traits

Life-history trait														
Egg ratio			Mixis index			Male production			Growth rate			Diapausing egg production		
<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
S	1,174	80.4	<0.01*	1,175	4.4	0.04	1,170	306.3	<0.01*	1,170	201.73	1,174	47.41	<0.01*
T	1,173	189.9	<0.01*	1,174	7.6	0.01*	1,170	331.0	<0.01*	1,170	5.85	1,173	65.97	<0.01*
P	5,172	14.4	<0.01*	5,172	8.8	<0.01*	5,169	10.3	<0.01*	5,170	6.13	5,173	36.76	<0.01*
C	172,98	2.9	<0.01*	172,53	6.1	<0.01*	169,120	3.4	<0.01*	169,148	1.34	173,201	1.92	<0.01*
S x T	1,172	96.2	<0.01*	1,173	14.2	<0.01*	1,169	46.9	<0.01*	1,169	16.31	1,174	8.14	<0.01*
P x S	5,173	2.8	0.02	5,174	2.2	0.05	5,170	2.2	0.06	5,170	1.78	5,173	7.80	<0.01*
P x T	5,174	2.6	0.03	5,174	1.9	0.10	5,169	2.4	0.04	5,169	1.82	5,173	1.69	0.14
C x S	172,170	1.3	0.03	172,171	0.8	0.87	169,167	2.0	<0.01*	169,167	2.46	173,172	4.77	<0.01*
C x T	172,170	1.2	0.08	172,171	1.1	0.23	168,167	1.1	0.23	168,167	1.32	172,172	1.86	<0.01*

Egg ratio, male production and diapausing egg production: square-root transformed data; mixis index: Arcsin transformed data. *S* salinity, *T*, temperature, *P* population, *C* clone. Triple interactions were dropped. *P*-values are given with no correction for multiple tests. \*Significance after Dunn–Šidák correction, taking into account that five variables were tested, is also shown (nominal  $\alpha = 1 - (1 - 0.05)^{1/5} = 0.0102$ )



**Fig. 2** Reaction norms of the life-history traits for the six populations in each experimental environment. *ER* egg ratio (egg equivalents/female), *m* mixis index, *MP* male production, *r* intrinsic growth rate ( $d^{-1}$ ), *DE* diapausing egg production (eggs/replicate). Each value is the population mean ( $\pm$ SE). —□—: Poza Sur; —■—: Balsa de Santed 1; —▽—: Hondo Sur; —▼—: Hoya Rasa; —○—: Salobrejo; —●—: Salada de Chiprana

**Table 4** Proportion of clones with positive slopes of the intrinsic growth rate and diapausing egg production response lines in relation to salinity in the six populations and two temperatures studied

Population	Intrinsic growth rate		Diapausing egg production	
	20°C (%)	25°C (%)	20°C (%)	25°C (%)
Hondo Sur	17	13	17	23
Poza Sur	10	11	7	13
Balsa de Santed 1	17	17	23	20
Hoya Rasa	13	7	27	33
Salada de Chiprana	32	29	41	41
Salobrejo	21	21	13	40

variability within populations. ‘Clone  $\times$  salinity’ interaction was statistically significant for male production, intrinsic growth rate and diapausing egg production, while diapausing egg production was significantly affected by the interaction between clone and temperature.

Since our ANOVA detected interactions between population and clone effects with salinity, we explored these interactions by computing the slope of the response lines of both intrinsic growth rate and diapausing egg production to salinity for each clone (see Methods). The higher the slope, the better the adaptation to high salinity. Table 4 shows the percentage of clones displaying a positive response to salinity. In all six populations, the majority of clones had negative slopes for intrinsic growth rate. According to the ANOVA, slopes did not vary significantly among populations, though Salada de Chiprana had the highest percentage of positive slopes (i.e., clones favoured by salinity) at both temperatures. In the case of diapausing egg production, again, the majority of clones from the six populations showed negative slopes (less production of diapausing eggs at 30  $g L^{-1}$  than at 15  $g L^{-1}$ ; Table 4). The percentage of clones with positive slopes ranged from 7% in Poza Sur at 20°C to 41% in Salada de Chiprana at both 20°C and 25°C. In this case, these slopes differed significantly among populations (“population  $\times$  salinity” interaction, Table 3).



**Table 5** Pearson's correlation coefficients between diapausing egg production (DE), mixis index ( $m$ ) and intrinsic growth rate ( $r$ ) in each studied population. Ranges for these life-history traits are also shown

Population	Correlation			Range		
	DE vs. $r$	DE vs. $m$	$r$ vs. $m$	DE (eggs/replicate)	$m$	$r$ ( $d^{-1}$ )
Hondo Sur	0.221**	0.113	-0.220**	1.67–15.03	0.330–0.381	0.166–0.291
Poza Sur	0.464**	0.078	-0.034	12.02–91.68	0.508–0.563	0.124–0.290
Balsa de Santed 1	0.490**	0.011	-0.069	37.86–99.44	0.511–0.645	0.158–0.297
Hoya Rasa	0.117	0.153*	-0.069	3.98–14.07	0.436–0.549	0.145–0.293
Salada de Chiprana	0.453**	0.279**	-0.048	14.14–29.47	0.286–0.379	0.110–0.217
Salobrejo	0.317**	0.049	-0.214*	2.33–19.05	0.428–0.549	0.130–0.217

\*  $0.001 < p < 0.05$ ; \*\*  $p < 0.001$

When we analyzed the life-history traits averaged over clones for each population and experimental combination, we found a significant correlation between diapausing egg production and intrinsic growth rate (Pearson's  $r = 0.445$ ,  $n = 24$ ,  $P = 0.029$ ). Diapausing egg production was also positively correlated with mixis index (Pearson's  $r = 0.416$ ,  $n = 24$ ,  $P = 0.043$ ). The correlation between intrinsic growth rate and mixis index was very low and non-significant (Pearson's  $r = -0.021$ ,  $n = 24$ ,  $P = 0.923$ ). Within-population relationships between these variables are shown in Table 5, in which we can also observe the low variation in the mixis index exhibited by a population, regardless of the experimental combination, as well as the lack of association between the mixis index and the intrinsic growth rate. In Table 5, some patterns regarding diapausing egg production can be observed. For similar mixis index values, higher intrinsic growth rates are generally associated with higher diapausing egg production. This tendency is remarkable in Poza Sur and Balsa de Santed 1, but not in Hoya Rasa. The combination of low intrinsic growth rate and low mixis index results in low diapausing egg production (Salada de Chiprana). When the intrinsic growth rate is low and the mixis index is high, diapausing egg production is also low (e.g., Salobrejo population). The opposite situation, i.e. high intrinsic growth rate and low mixis index, also resulted in low diapausing egg production (Hondo Sur).

Some life-history traits, averaged over clones within population and experimental conditions, showed a significant relationship with quantifiable characteristics of the habitat (surface, depth and hydroperiod duration). We found a significant and negative relationship between the mixis index and both hydroperiod duration (Pearson's  $r = -0.690$ ,  $n = 24$ ,  $P < 0.001$ ) and pond depth (Pearson's  $r = -0.514$ ,  $n = 16$ ,  $P = 0.04$ ). In contrast, the mixis index was not significantly correlated to pond surface (Pearson's  $r = -0.349$ ,  $n = 24$ ,  $P = 0.09$ ). Diapausing egg production was also negatively correlated with hydroperiod duration (Pearson  $r = -0.671$ ,  $n = 24$ ,  $P < 0.001$ ) and pond surface (Pearson's  $r = -0.563$ ,  $n = 24$ ,  $P = 0.004$ ), but not with pond depth (Pearson's  $r = 0.004$ ,  $n = 16$ ,  $P = 0.989$ ). None of these three habitat characteristics showed a correlation with the intrinsic growth rate.

## Discussion

In this study we address local adaptation in rotifers collected from their natural habitats. Our results provide information on the response of the rotifer model species *B. plicatilis* to

salinity and temperature, suggesting that some traits of the studied populations show signs of local adaptation.

There are a number of studies on the response of what was considered a single species — *B. plicatilis* — to salinity and temperature, sometimes showing contrasting patterns (Miracle and Serra 1989). However *B. plicatilis*, formerly described as a taxon, is now known to be a species complex, including the species *B. plicatilis sensu stricto*. Therefore, our study provides relevant information on the response of the model species *B. plicatilis* to salinity and temperature, since (1) clones were identified molecularly, and (2) our study was based on a number of clones and six populations.

In general, increasing salinity from 15 to 30 g L<sup>-1</sup> affected life-history traits negatively. This was the case for all life-history traits when rotifers were cultured at 20°C, and almost all life-history traits when rotifers were cultured at 25°C, except for egg ratio and mixis index. This general and negative effect of salinity was in agreement with previous findings, which showed that *B. plicatilis* rotifers spend more metabolic energy at high salinity since this species is not an osmoconformer (Lowe et al. 2005). In the majority of populations, the mixis index was negatively affected by high salinity at 20°C, in agreement with previous reports of salinity inhibition of sexual reproduction in species of the same complex (Lubzens et al. 1980; Lubzens 1981; Pourriot and Snell 1983; Lubzens et al. 1985; Serra 1987; Hino and Hirano 1988; Pozuelo and Lubián 1993). However, at 25°C we did not observe a decline in the mixis index on increasing salinity. The proportion of sexual daughters is not necessarily maximized or minimized, but is expected to be optimized by natural selection to produce the maximum number of diapausing eggs (King and Serra 1998). The contrasting patterns between 20 and 25°C would suggest the importance of the interaction between temperature and salinity on shaping rotifer life-history traits.

Temperature tended to increase life-history trait values, which is expected from metabolism acceleration (Miracle and Serra 1989 and references herein). As expected, the intrinsic growth rate was higher at 25°C than at 20°C, as found, for instance, by Miracle and Serra (1989) and Yin and Zhao (2007). With respect to sexual reproduction, Kogane et al. (1997) found the percentage of sexual females to be maximized after a long period at low temperature (12°C), although diapausing egg formation was favoured at high temperature (25°C). The latter study is in agreement with our results, since we found that on average populations had a higher diapausing egg production at 25°C (average: 33 eggs/replicate) than at 20°C (average: 21 eggs/replicate).

Saline lakes harbour quite a low richness in plankton species, suggesting environmental adversity and the need for special adaptations. Temperature, and in particular salinity have been shown to be among the most important factors promoting ecological specialization between the species of the *B. plicatilis* complex (Gómez et al. 1997; Serra et al. 1998; Ortells et al. 2003), allowing seasonal succession of species in a single pond, and likely playing a role in the diversification of the genus. However, no studies have considered both within and among-population variation in any of these species to date. *B. plicatilis* populations have been found in ponds with widely varied ecological conditions, including temperature and salinity (Ortells et al. 2003; Lapesa 2004), but it was unknown whether local adaptation played a role in the ability of this species to occupy its habitat range.

Our findings suggest adaptation to high salinity has taken place in the *B. plicatilis* population of Salada de Chiprana, a hyperhaline pond. This conclusion is suggested by at least three observations: (1) this population harboured the highest percentage (ca. 30%) of clones growing better at high salinity than at low salinity, as shown by their intrinsic growth rate; (2) it showed the highest percentage (41%) of clones producing more diapausing eggs at high than at low salinity; and (3) it showed the second highest diapausing

egg production at 20°C and 30 g L<sup>-1</sup>, an experimental environment similar to the natural conditions of this pond. Notice that diapausing egg production is a very important long-term fitness component of these temporary rotifer populations. Nevertheless, Salada de Chiprana was not the population with the largest life-history trait averages at the highest salinity studied and thus, if we apply the criteria of Kawecki and Ebert (2004) on local adaptation (see Introduction), we cannot confirm that the Salada de Chiprana population is locally adapted to salinity. We suggest, however, that the laboratory conditions in our experiments did not favour Salada de Chiprana clones, so its adaptation to high salinity is not shown by simple inspection of Fig. 2. However, the diapausing egg production of this population remained constant when salinity increased, while other populations with higher diapausing egg production were very sensitive to salinity increase. Other indices for differential adaptation to salinity are provided by Poza Sur and Balsa de Santed 1 populations, which had relatively high fitness components at low salinity. These ponds are not classified in the lower salinity classes (Table 1). However, their small size could make them vulnerable to the effect of occasionally heavy rain and floods, which would decrease salinity levels. We know that the *B. plicatilis* Poza Sur population is a winter population (Gómez et al. 1995), a period when flooding and drops in salinity are likely.

Our results also suggest differential adaptation to ephemeral and unpredictable habitats, although our experiment was not designed with this in mind. Balsa de Santed 1 and Poza Sur had the highest values of diapausing egg production in the majority of experimental environments, and they are also the smallest ponds (Campillo et al. 2009). It has been proposed that rotifer populations in unpredictable habitats would invest early in sexual reproduction during their annual growth cycle, in what is known as a bet-hedging strategy (Carmona et al. 1995; Serra and King 1999; Serra et al. 2004, 2005). Our experimental design, which involved a short period of population growth, with food provided only at the beginning of the experiment, should resemble the ecology of an ephemeral site. Correspondingly, populations better adapted to ephemeral/unpredictable habitats are expected to have high fitness, particularly high production of diapausing eggs, under our laboratory conditions. Consistently, the Hondo Sur population, which seems to be less episodic (Campillo et al. 2009), had much lower diapausing egg production at all experimental environments tested.  $Q_{ST} - F_{ST}$  analysis (Campillo et al. 2009) supports the hypothesis that the degree of environmental unpredictability has shaped the patterns of diapausing egg production, since this fitness component seems to have evolved at a higher rate than neutral markers (Hondo Sur vs. Poza Sur, Hondo Sur vs. Balsa de Santed 1, and Hoya Rasa vs. Balsa de Santed 1).

In addition, our study suggests a compromise among life-history traits. Diapausing egg production is positively and significantly correlated to intrinsic growth rate and mixis index, which is to be expected since production of diapausing eggs is increased by population abundance and by investment in sex. However, correlation between intrinsic growth rate and mixis index was negative, showing that sex has a cost for parthenogenetic proliferation. This suggests that mixis index should be optimized to maximize diapausing egg production. A remarkable finding is that mixis index values are placed in quite a narrow interval in each of the studied populations. These within-population conservative values of sexual reproduction also suggest the existence of different sexual investment strategies in each population. According to the life cycle theory (Stearns 1992), some authors have predicted that the patterns of sexual reproduction must be compromised depending on habitat predictability conditions (Carmona et al. 1995; Serra and King 1999). Interestingly, we found the highest values of mixis index in rotifer populations dwelling in small ponds and with a short hydroperiod (Poza Sur and Balsa de Santed 1). As stated

above, this kind of habitat is considered particularly unstable, sensitive to environmental variation, and risky. In these environments, rotifers are expected to initiate early and continuous sexual reproduction (Carmona et al. 1995; Serra and King 1999).

The Monopolization hypothesis (De Meester et al. 2002) assigns a significant role to local adaptation in the maintenance of the high level of neutral genetic differentiation found among populations of aquatic invertebrates. In recent times, several studies on cladocerans, mainly *Daphnia* sp., have shown rapid adaptation to both biotic and abiotic factors (Hairston Jr et al. 1999; Cousyn et al. 2001; Declerck et al. 2001; Michels and De Meester 2004; Sarnelle and Wilson 2005). However, in the case of rotifers in Spanish Mediterranean ponds and lakes, environmental fluctuation seems to constrain adaptation to local conditions to some extent, at least in relation to temperature and salinity. Many ponds might overlap in their salinity and temperature conditions, which could favour high ecologically relevant genetic variation, as found in our results. This would result in adaptation to these factors occurring less frequently than found for other factors in cladoceran populations inhabiting relatively constant environments. According to our hypothesis, differential specialization in diapausing egg production patterns does occur in rotifer populations. This can be explained in that these patterns are likely optimized by integrating long time periods, thereby integrating the environmental fluctuation in a locality.

Could the levels of adaptation, suggested by our results, have played a role in maintaining differentiation in genetic markers, as the Monopolization hypothesis states? Although a definitive answer to this question cannot be given here, we suggest that temperature and salinity are not important factors in the differentiation of neutral markers within the biological species *B. plicatilis*. This contrasts with the role played by salinity and temperature in the ecological divergence between species in the *B. plicatilis* complex (Serra et al. 1998; Ortells et al. 2003; Montero-Pau et al. submitted). Our suggestion is based on the finding that while salinity proved important for only one population, deep differentiation in genetic markers occurred among all studied populations (Campillo et al. 2009). Furthermore, Campillo et al. (2009) did not observe among-population correlation among levels of genetic differentiation in molecular markers and levels of genetic differentiation in life-history traits. Nevertheless, here, we analyzed only two potential factors for local adaptation. Moreover, as a by-product of our experimental design, which aimed to study salinity and temperature effects, our results also suggest differentiation in sexual reproduction patterns in relation to pond size, a feature related to the levels of environmental fluctuation. Studies on local adaptation of rotifers were unfeasible before recognition of the cryptic species problem in these organisms (Gómez et al. 2002; Campillo et al. 2005; Suatoni et al. 2006; Fontaneto et al. 2007). Currently available accurate taxonomic tools provide better information on the ecology of specific species, which allows the study of local adaptation in additional traits. This may shed light on the implication of local adaptation for the differentiation in neutral genetic markers.

#### Final remark

Our findings in this study — in combination with the results in Campillo et al. (2009) — suggest: (1) temporality of rotifer populations from Spain has exerted an impact on their strategies of sexual reproduction, as shown by diapausing egg production in Balsa de Santed and Poza Sur populations (see also Campillo et al. 2009); (2) in some populations, salinity is a key factor in ecological specialization, and thus in local adaptation, as shown by the Salada de Chiprana population, although many populations can share a similar

response to salinity; and (3) rotifer populations harbour high variability in fitness components.

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