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Selection on life-history traits and genetic population divergence in rotifers

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Abstract

A combination of founder effects and local adaptation – the Monopolization hypothesis – has been proposed to reconcile the strong population differentiation of zooplankton dwelling in ponds and lakes and their high dispersal abilities. The role genetic drift plays in genetic differentiation of zooplankton is well documented, but the impact of natural selection has received less attention. Here, we compare differentiation in neutral genetic markers (F_{ST}) and in quantitative traits (Q_{ST}) in six natural populations of the rotifer *Brachionus plicatilis* to assess the importance of natural selection in explaining genetic differentiation of life-history traits. Five life-history traits were measured in four temperature × salinity combinations in common-garden experiments. Population differentiation for neutral genetic markers – 11 microsatellite loci – was very high (F_{ST} = 0.482). Differentiation in life-history traits was higher in traits related to sexual reproduction than in those related to asexual reproduction. Q_{ST} values for diapausing egg production (a trait related to sexual reproduction) were higher than their corresponding F_{ST} in some pairs of populations. Our results indicate the importance of divergent natural selection in these populations and suggest local adaptation to the unpredictability of *B. plicatilis* habitats.

Introduction

Evidence for the effects of selection on genetic differentiation can be drawn from quantitative trait analysis, as they may be related to fitness (Kawecki & Ebert, 2004). Neutral processes are expected to drive differentiation in quantitative traits in parallel to neutral markers (Lande, 1992), therefore a comparison between differentiation in neutral markers and quantitative traits can disclose whether selection plays a role in the genetic differentiation of the latter (Merilä & Crnokrak, 2001). This comparison is commonly made with the expectation that, if differentiation in quantitative traits occurred by neutral processes, then Q_{ST}, an index of differentiation in quantitative traits, and F_{ST}, an index of differentiation in neutral genetic markers, should have the same expectations; conversely, a greater value of Q_{ST} would indicate the action of divergent selection (Spitze, 1993; Merilä & Crnokrak, 2001; Mckay & Latta, 2002).

The zooplankton of lakes and ponds – island-like habitats – often disperse through diapausing stages. Populations of these organisms can be used as a model to study how differentiation can evolve among demes of small organisms with high dispersal ability (De Meester *et al.*, 2004), which is crucial to understand speciation and the match between environment and phenotype. Gene flow was considered to be the driving force behind the evolution of zooplankton due to its assumed high dispersal capability. Accordingly, little attention was paid to differentiation in neutral genetic markers in these organisms, and many taxa were thought to be essentially cosmopolitan (Darwin, 1859; Mayr, 1963). However, recent research into rotifers (Gómez *et al.*, 2000, 2002, 2007; Ortells *et al.*, 2000) and cladocerans (Lynch & Spitze, 1994; De Meester, 1996; Pálsson, 2000; Pfrender

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et al., 2000) has provided evidence of high genetic population divergence in neutral markers on both local and regional scales. The Monopolization hypothesis was put forward to explain this paradox between high dispersal ability and high population differentiation, stating that a combination of two factors – (1) strong, persistent founder effects and (2) a rapid build-up of local adaptation – contributes to strongly reduce the impact of dispersal once a population is established (De Meester *et al.*, 2002). The hypothesis accounts for the demographic features of zooplankton, i.e. very fast population growth rates, and that often reach very large populations even in moderately sized ponds (Miracle & Serra, 1989; Gómez *et al.*, 1995; Hairston, 1996; Ortells *et al.*, 2003; García-Roger *et al.*, 2006b). The Monopolization hypothesis is built on the results of Boileau *et al.* (1992) regarding the persistence of founder effects in these populations, as they can be founded by a few propagules capable of rapid population growth. The founder effect is long-lasting due to the dilution of new immigrants in a very large population – a numerical effect. Founders are either active in the water column or, during the adverse periods, inactive in the diapausing egg bank. The rapid build-up of local adaptation, the second proposition of the hypothesis, means that local residents will enjoy fitness advantages compared with later immigrants, and this will contribute to reinforcing founder effects and maintenance of the high neutral genetic differentiation among populations.

The monogonont rotifer *Brachionus plicatilis* (Müller, 1786) has been shown to exhibit strong population divergence in neutral genetic markers in the Iberian Peninsula (Gómez *et al.*, 2000, 2002). *Brachionus plicatilis* inhabits salt lakes and coastal lagoons (Walker, 1981), highly seasonal and unpredictable habitats, and populations vary from very ephemeral to almost permanent in these habitats (Ortells *et al.*, 2003; Lapesa, 2004). This species is a cyclical parthenogen combining asexual and sexual reproduction, whose life cycle begins when asexual females hatch from diapausing eggs in the sediment banks (Pourriot & Snell, 1983; Wallace & Snell, 1991). During the asexual phase, females proliferate by ameiotic parthenogenesis. This phase lasts for an unspecified number of generations until population density triggers the initiation of sexual reproduction (Carmona *et al.*, 1993, 1994; Stelzer & Snell, 2003). When the sexual phase starts, asexual females begin to parthenogenetically produce sexual and asexual daughters. The sexual females produce haploid eggs which hatch into dwarf males if unfertilized but if fertilized become diapausing eggs that can resist adverse conditions (e.g. desiccation) and accumulate in the sediment (García-Roger *et al.*, 2006a). When favourable conditions resume, a fraction of the viable diapausing eggs will hatch. The remaining unhatched eggs form egg banks, and can survive for decades in the sediment (Brendonck & De Meester, 2003; García-Roger *et al.*, 2006b). During the asexual growth phase, populations may grow exponentially and generation time is very short, thus *B. plicatilis* population sizes can almost double in half a day (Miracle & Serra, 1989).

The aim of this study was to assess the extent to which natural selection can account for genetic differentiation of life-history traits in *B. plicatilis*. To do so, we applied a Q_{ST} vs. F_{ST} approach. Although some authors have stressed (e.g. Merilä & Crnokrak, 2001) that the order comparison between Q_{ST} and F_{ST} is only appropriate when using the so-called narrow-Q_{ST}, which is based on additive genetic variances (Leinonen *et al.*, 2008), in this study only broad-Q_{ST} could be estimated from total genetic variances due to biological and logistical constraints. Broad-Q_{ST} has been used in previous studies of genetic differentiation in zooplankton (Lynch *et al.*, 1999; Morgan *et al.*, 2001). Rotifer clones were grown in a common-garden experiment under four combinations of temperature and salinity in order to test whether populations differed in their response to experimental environments representing a range similar to that found in natural populations. In addition, population divergence in neutral genetic markers was estimated in the same populations using 11 microsatellites, seven developed by Gómez *et al.* (1998) and four newly developed in this study.

Materials and methods

Populations and clones studied

Rotifer diapausing egg banks were sampled in four inland ponds: Balsa de Santed 1 (SA1), Hoya Rasa (HOY), Salada de Chiprana (CHI) and Salobrejo (SAL); and in two coastal ponds: Poza Sur de Torreblanca (TOS) and Hondo Sur (HOS) in Eastern Spain during summer 2003 (Table 1). The six ponds were chosen as they differed in their ecogeographical conditions and were known to hold *B. plicatilis* populations (Ortells *et al.*, 2000).

A sample was taken with a Van Veen grab (Eijkelkamp Agrisearch Equipment, Giesbeck, The Netherlands) from the superficial sediment of each pond. Samples were stored for at least 1 month in the dark at 4 °C in order to ensure the completion of the obligate period of dormancy for all *B. plicatilis* diapausing eggs present (Hagiwara & Hino, 1989). Diapausing eggs were isolated from the sediment using a sugar flotation technique (Gómez & Carvalho, 2000). In order to obtain experimental clones, healthy-looking diapausing eggs were selected as unhealthy-looking eggs have very low hatching rates due to embryo mortality or nonviability (García-Roger *et al.*, 2005). The selected eggs were isolated individually in 96-multiwell plates (Nunc™, Nalge Nunc Int., Roskilde, Denmark) and induced to hatch under the following conditions: 200 μL of 6 g L^{-1} salinity artificial seawater (Instant Ocean®; Aquarium Systems, Inc., Mentor, OH, USA), 25 °C, and constant illumination (150–170 μmol quanta m^{-2} s^{-1}). Eggs were checked every 24 h for a maximum of 3 weeks. Once a diapausing egg hatched, the

Table 1 Features of the ponds from which *Brachionus plicatilis* samples were taken. Data from García-Roger (2006) and García-Roger *et al.* (2006b). Geographical coordinates according to *Datum* WGS 84.

Pond or lake (acronym)	Geographical coordinates	Area (km^2)	Depth (m)	Hydroperiod pattern	Temperature (°C)	Salinity (g L^{-1})	Location
Hondo Sur (HOS)	38° 10.004′ N 0° 44.007′ W	0.20	0.8	Semi-permanent	15–25	8–21	Coastal
Poza Sur (TOS)	40° 08.715′ N 0° 10.059′ E	0.01	1.0	Seasonal	20–25	10–32	Coastal
Balsa de Santed 1 (SA1)	41° 00.975′ N 1° 32.477′ W	0.02	< 1.0	Ephemeral	10–20	17–20	Inland
Hoya Rasa (HOY)	38° 47.075′ N 1° 25.620′ W	0.08	< 1.0	Seasonal	n/a	n/a	Inland
Salada de Chiprana (CHI)	41° 14.417′ N 0° 10.874′ W	0.23	5.0	Permanent	15–25	32–47	Inland
Salobrejo (SAL)	38° 54.765′ N 1° 28.275′ W	0.36	0.8	Semi-permanent	8–25	8–30	Inland

n/a, data not available.

neonate female was fed on *Tetraselmis suecica* algal culture in 12 g L^{-1} salinity artificial seawater and allowed to grow and reproduce parthenogenetically. Three to four days later, the individuals were transferred to a test tube with a total volume of 40 mL of 12 g L^{-1} salinity artificial seawater, maintained at 25 °C, constant illumination (35 μmol quanta m^{-2} s^{-1}), and fed *T. suecica*. As *B. plicatilis* belongs to a cryptic species complex, the experimental clones were taxonomically classified initially by morphological identification (Ciros-Pérez *et al.*, 2001), which was subsequently confirmed by genetic analysis of cytochrome *c* oxidase subunit I (COI) based on PCR-RFLP (Campillo *et al.*, 2005). Stock cultures were individually prepared for a total of 180 isolated clones of *B. plicatilis* (30 per pond), culture conditions being 22.5 g L^{-1} salinity artificial seawater, 22.5 °C, constant illumination (35 μmol quanta m^{-2} s^{-1}), with live *T. suecica* dispensed *ad libitum* as food (volume culture 0.5 L). These salinity and temperature conditions represent the intermediate range of the experimental culture conditions (see below).

Life-history trait experiment

Experimental design

A laboratory common-garden experiment was performed by culturing the 30 clones for each of the six populations of *B. plicatilis* under four experimental environments, obtained by combining two temperatures (20 and 25 °C) and two salinities (15 and 30 g L^{-1}). The four experimental environments were selected because they represent a range of conditions experienced by natural populations of this species, and our aim was to broadly explore fitness responses. *Brachionus plicatilis* habitats differ in salinity and temperature (Ortells, 2002; Lapesa, 2004; García-Roger *et al.*, 2006b) and it is known that species from the *B. plicatilis* species complex are also ecologically specialized according to these parameters (Gómez *et al.*, 1997; Ortells *et al.*, 2003). Therefore, we expected population fitness differences across the parameter range. In each of these four experimental environments, five life-history traits were measured (see below). For each experimental combination (6 populations × 30 clones per population × 2 temperatures × 2 salinities) four replicate cultures were prepared; two of them were short-term growth replicates for measuring four life-history traits (female production, female-producing eggs, male production and male-producing eggs), and the other two were long-term growth replicates for measuring an additional life-history trait (diapausing egg production). This gave a total of 2880 experimental cultures.

Experimental set-up

To minimize variation in food quality, rotifers were fed frozen *T. suecica*. Several batches of algal culture were concentrated by centrifugation and blended in order to produce a homogeneous mixture of algae at 5×10^7 cells mL^{-1}. This mixture was dispensed in vials and these were stored at −80 °C until use. The experimental rotifer cultures were prepared in several batches due to practical constraints; moreover, full randomization was prevented by the timing of hatching of the diapausing eggs used to establish the experimental clones. Nevertheless, all cultures in a batch were randomly placed in the experimental growing chamber.

In order to acclimatize the rotifers to the experimental food type, cultures (one per clone, a total of 180 cultures) were prepared by transferring rotifer females from the corresponding stock culture at an initial density of 0.7 females mL^{-1} to flasks with 1 L of culture medium with 10^5 cells mL^{-1} of frozen *T. suecica* (salinity and temperature conditions as in stock cultures; notice that these conditions are the intermediate range of the experimental conditions). No additional food was provided to these cultures and they were allowed to grow without further manipulation. On day 5, from each of these 180 cultures, 16 cultures were prepared, corresponding to four experimental environments × two replicate short-term cultures × two replicate long-term cultures. Cultures

started with 12 randomly chosen egg-carrying asexual females placed in 50 mL of growth medium (10^5 cells mL^{-1} of frozen *T. suecica* as food) in Petri dishes and kept in the dark at the experimental salinity-temperature conditions.

Animals in the short-term cultures were counted at day 2. The first 2 days were considered a pre-experimental period. During these 2 days, rotifers grew exponentially thus no density-dependent effects were expected to occur (density range at day 2 was 0.782–0.952 females mL^{-1}, much lower than the maximum densities usually achieved in the experimental conditions). This 2-day pre-experimental period allowed the rotifers to acclimatize to the experimental conditions and reproduce in them. The day 2 count was used to standardize two life-history traits (see below). At the moment of counting, most of the individuals had been born at the experimental conditions. However, individuals from the stock cultures were also present and therefore some dependence between replicate cultures and between experimental environment cultures cannot be ruled out. This experimental design, however, was imposed by practical constraints given the number of cultures required. On day 4 of the experiment, the two short-term growth replicate cultures of each experimental environment were fixed with formaldehyde (4% final concentration) and females, males, female-producing eggs and male-producing eggs were counted to estimate life-history traits. Male-producing and female-producing eggs can be identified according to the size of the eggs carried by the females (Carmona *et al.*, 1995). This estimation of life-history traits based on 2-day experiment counts has been used in previous rotifer studies (Snell & Moffat, 1992; Snell & Carmona, 1995). The long-term cultures were fed again on day 4 of the experiment with 10^5 cells mL^{-1} of frozen *T. suecica* and kept in the same experimental conditions until the natural extinction of the cultures (approx. 1–2 months later). During this period, sexual reproduction was expected to be induced in the culture by the increase in population density (Carmona *et al.*, 1993, 1994; Stelzer & Snell, 2003). After extinction of the culture, diapausing eggs, which accumulate without hatching, were counted.

As a result, five life-history traits were measured in each experimental combination: (1) female production (FP), defined as the total number of females counted on day 4 of the experiment divided by the number of females on day 2; (2) male production (MP), defined as the total count of males counted on day 4 divided by the number of females on day 2; (3) female-producing eggs (FEP), defined as the count of female-producing eggs on day 4 divided by the number of females on day 4; (4) male-producing eggs (MEP), defined as the count of male-producing eggs on day 4 divided by the number of females on day 4; (5) diapausing egg production (DE), defined as the total number of diapausing eggs produced from the descendants of the 12 initial females. Life-history traits 1–4 were computed from the short-term cultures, and trait 5 from the long-term culture. We analysed each life-history trait in each experimental environment, which gave 20 trait × environment combinations. To refer to each trait × environment combination, we have added extra digits to the symbol of the corresponding life-history trait; for example, in FP2015, FP refers to female production, the first two digits indicate the experimental temperature and the second two digits refer to the experimental salinity.

Data analysis

We performed nested analyses of variance (ANOVAS) (three sources of variance: 'population', 'clone' nested within 'population' and 'replicate' nested within 'clone') for each trait × environment combination, in order to test the statistical effects on those 16 trait × environment combinations associated with the following life-history traits: female production (log-transformed), female-producing eggs, male production (log-transformed), and male-producing eggs. These analyses were performed using SPSS 13.0 (SPSS Inc., IL, Chicago, USA). Salinity and temperature were not included as ANOVA effects, but these environmental factors were used to create response variables by combining them with life-history traits (see this use with a different purpose in Via & Lande, 1985). For instance, FP2015 and FP2515 are treated as two different variables. In this way, ANOVAS could be used to test divergence associated with the Q_{ST} index (see below), as Q_{ST} was computed for each of the 20 trait × environment combinations. For the four trait × environment combinations associated with diapausing egg production, generalized linear models (GLMs; Nelder & Wedderbur, 1972) were carried out with the same hierarchical structure as the ANOVAS described above. Poisson distribution of data and the square root as link function were assumed. These analyses were carried out using R 2.1.1 (Ihaka & Gentleman, 1996). A Principal Component Analysis (PCA) was performed using SPSS 13.0 (SPSS Inc.) on the 20 trait × environment combinations as variables, and the 180 clones as observations.

Broad-sense heritability (H^2) for each trait × environment combination and population was estimated (female production and male production, log-transformed; diapausing egg production, square-root transformed). This yielded a total of 120 H^2 estimates (6 populations × 5 life-history traits × 4 environments). For each estimate we followed the procedure for clonal organisms described in Lynch & Walsh (1998). Briefly, after a nested ANOVA with among-clone and within-clone (replicates) as the two sources of variance, genetic variance was estimated as the among-clone variance component (degrees of freedom for these variances ranged from 26 to 29 due to missing data) and environmental variance was estimated as the error variance component (degrees of freedom for these variances ranged from 24 to 30 due to missing data). Computations were performed using the 'Variance

Components' routine (ANOVA method) in SPSS 13.0 (SPSS Inc.).

Q_{ST} was estimated between each pair of populations and for each 20 trait × environment combinations following the procedure of Morgan *et al.* (2001). The Q_{ST} computed was based on the total genetic variance (i.e. it is a broad-Q_{ST}) and defined as $Q_{ST} = V_{GB}/(V_{GB} + 2V_{GW})$, where V_{GB} is the genetic component of the variance between population means and V_{GW} is the average genetic variance within populations (i.e. including additive variance and the variance due to interactions). Broad-Q_{ST} is expected to be equal or lower than narrow-Q_{ST} (i.e. that based on additive genetic variance). The index of differentiation in quantitative traits (Q_{ST}) can depend largely on the experimental conditions under which organisms are tested (Gomez-Mestre & Tejedo, 2004), which motivates computing Q_{ST} from different experimental environments. As a global index of differentiation, pairwise population Q_{ST} values were also computed for an integrated life-history trait. This integrated trait was the first component obtained from a PCA performed on the matrix of 180 cases (clones) × 20 variables (life-history trait × environment combinations).

Neutral marker analysis

Our experimental populations were screened for genetic variation at neutral markers in diapausing eggs of *B. plicatilis* isolated from the same sediment samples as used for the life-history study. DNA from individual diapausing eggs was extracted using HotSHOT (Truett *et al.*, 2000) following the method of Montero-Pau *et al.* (2008) for zooplanktonic diapausing eggs. The DNA was then ready for PCR amplification. The individually extracted diapausing eggs were identified as belonging to *B. plicatilis* by PCR amplification of the species-specific *Bp1b* microsatellite locus (Gómez *et al.*, 1998).

For each pond a sample of 30 *B. plicatilis* diapausing eggs – 17 in the case of Hoya Rasa, where no more eggs could be obtained – was screened for 11 microsatellite loci. The loci included the seven microsatellite loci already available for *B. plicatilis* (Gómez *et al.*, 1998) and four additional ones developed in the present study (see below). For the former set of loci, we followed the PCR conditions described in Gómez *et al.* (1998). Reactions were separated in a CEQ™ 8000 Genome Analysis System (Beckman-Coulter™, Fullerton, CA, USA) and all peaks were scored by eye using the software provided by the manufacturer.

Four additional microsatellite loci were developed following the procedure of Hammond *et al.* (1998) with minor modifications. Enriched libraries were created and positive colonies screened for microsatellite motifs following the PCR-based isolation of microsatellite array (PIMA) method, a PCR-based method to detect microsatellites (Lunt *et al.*, 1999). Of the 242 colonies screened, 32 yielding an extra band in the gel when using the PIMA primer were then sequenced on a CEQ™ 8000 Genome Analysis System (Beckman-Coulter™). Nine of the sequenced colonies presented one or two microsatellites and four of these microsatellites were polymorphic and could be optimized: *Bp7*, *Bp8*, *Bp9* and *Bp10* (Table 2). All four loci were amplified separately in total reaction volumes of 10 μL, containing 2 μL of template DNA, 250 μM of each nucleotide, 0.5 μM of each primer, 1X BIOTOOLS buffer (producing a final 2 mM concentration of $MgCl_2$), and 0.75 U of Taq polymerase (BIOTOOLS). A Mastercycler™ (Eppendorf, Hamburg, Germany) was used for PCR amplification with the following cycling profile: one cycle of 3 min at 94 °C, 40 cycles of 30 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C and a final cycle of 7 min at 72 °C. Eggs were genotyped with an ABI 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). All peaks were scored by eye using Peak Scanner™ 1.0 Software (available at http://www.appliedbiosystems.com). As the four microsatellite loci developed in this study displayed certain difficulties in genotyping, we performed a new and blind re-analysis of 10% of the clones for these loci from the amplification step (Bonin *et al.*, 2004) in order to obtain an estimation of the genotyping error.

MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was run to check for typing errors, null alleles, evidence of stuttering and large allele dropout. The Microsatellite toolkit (Park, 2001) was used to calculate sample size and mean number of alleles per locus. The program was also

Table 2 Microsatellite loci for *Brachionus plicatilis* developed in this study.

Locus and EMBL accession number	Repeat motif	Primer sequences (5′–3′)	Size range (bp)	*A*	H_E
Bp7	$(TC)_7$	F: ATCAACTAATATGTGACAAGACAAC	165–191	8	0.570
AM882507		R: TAAAGTATTAAAAGCCAAGATAACG			
Bp8	$(CCAACG)_7(CCAACA)_3$	F: GAGTTTTTTCAACGCTATCGC	186–301	13	0.565
AM882508		R: TGCCAAATTGATACTTTTTGC			
Bp9	$(GA)_5CA(GA)_8$	F: AGCAGGTTTTTGTACGTCTGG	279–287	5	0.561
AM882509		R: TCTCTTCACACACAAGCAACG			
Bp10	$(TG)_{10}$	F: GATCAACTAAAAATGTTCAAGG	392–454	10	0.778
AM882510		R: TAGAACAAAACAAAAAGGTG			

Annealing temperature: 55 °C. F, forward primer; R, reverse primer; *A*, number of alleles per locus; H_E, overall expected heterozygosity.

used to create the files required to run 'GENEPOP on the web' and Arlequin 3.1. 'GENEPOP on the web' (Raymond & Rousset, 1995) was used to check for deviations from Hardy–Weinberg equilibrium and for calculating inbreeding index, F_{IS}, for each population. Finally, Arlequin 3.1 (Excoffier *et al.*, 2005) was used to calculate pairwise F_{ST} estimates among populations and expected heterozygosities (H_E). A PCA of the microsatellite data was performed with the program PCA-General V. 1.2 (Goudet, 1999) in order to visualize the relationships among populations.

Results

Life-history trait experiment

Statistically significant differences among populations were found in 15 of 20 trait × environment combinations, the differences involving all the combinations that include traits related to sexual reproduction (male production, male-producing eggs and diapausing egg production; Table 3). Balsa de Santed 1 and Poza Sur populations tended to have the highest values for most of the trait × environment combinations, particularly in those traits related to sexual reproduction (see, for instance, diapausing egg production on Table 3). Accordingly, in the PCA performed on trait × environment combinations as variables (Fig. 1, upper panel), the means of Balsa de Santed 1 and Poza Sur populations showed the highest values for the first component (PC1; explained variance, 34%), which is correlated mainly with sexual traits. The second PCA component (PC2; explained variance, 11%) was mainly correlated with asexual growth traits, for which the Salobrejo population showed low values (e.g. FP2515).

Among-clone within-population variation was statistically significant for all trait × environment combinations (Table 3), indicating that all populations harboured significant broad-sense heritability values for these parameters. Broad-sense heritabilities were rather high, with an average over all trait × environment combinations and populations of 0.674 (Table 4). Broad-sense heritability values tended to be higher for sexual traits (Table 4).

Average Q_{ST} values of life-history traits related to asexual reproduction (female production, 0.055; female-

Table 3 Mean phenotypic values (SE) of the trait × environment combinations for six *Brachionus plicatilis* populations. *P*-values for differences among populations were computed after hierarchical ANOVAS (clones nested within populations) on log female production, female-producing eggs, log male production and male-producing eggs, and after GLMs (clones nested within populations, Poisson distribution, and 'sqrt' link function) for diapausing egg production. The effect 'clone within population' was always significant ($P < 0.001$). Trait × environment combinations are termed as shown by the following instance: FP2015 refers to female production at 20 °C and 15 g L^{-1}.

Life-history trait	Population						
Trait × environment combination	TOS	SA1	HOY	HOS	CHI	SAL	*P*-value of population
Female production (FP)							
FP2015	1.684 (0.057)	1.641 (0.038)	1.682 (0.032)	1.707 (0.037)	1.455 (0.041)	1.556 (0.027)	0.001**
FP2030	1.293 (0.022)	1.387 (0.027)	1.436 (0.025)	1.414 (0.032)	1.275 (0.028)	1.376 (0.023)	0.001**
FP2515	1.827 (0.054)	1.873 (0.062)	1.839 (0.052)	1.818 (0.043)	1.649 (0.080)	1.566 (0.041)	0.004*
FP2530	1.320 (0.037)	1.417 (0.043)	1.361 (0.030)	1.433 (0.035)	1.267 (0.031)	1.312 (0.025)	0.183
Female-producing eggs (FEP)							
FEP2015	0.298 (0.019)	0.348 (0.015)	0.296 (0.017)	0.229 (0.014)	0.299 (0.022)	0.297 (0.017)	0.015*
FEP2030	0.189 (0.015)	0.200 (0.012)	0.206 (0.013)	0.204 (0.018)	0.197 (0.012)	0.189 (0.011)	0.962
FEP2515	0.170 (0.009)	0.242 (0.010)	0.180 (0.013)	0.146 (0.010)	0.226 (0.014)	0.217 (0.012)	< 0.001**
FEP2530	0.160 (0.017)	0.200 (0.010)	0.160 (0.013)	0.153 (0.012)	0.209 (0.012)	0.157 (0.013)	0.031*
Male production (MP)							
MP2015	0.453 (0.057)	0.462 (0.046)	0.221 (0.026)	0.214 (0.033)	0.186 (0.043)	0.219 (0.028)	< 0.001**
MP2030	0.055 (0.009)	0.114 (0.019)	0.018 (0.004)	0.028 (0.007)	0.008 (0.002)	0.006 (0.003)	< 0.001**
MP2515	0.677 (0.078)	0.720 (0.076)	0.419 (0.037)	0.341 (0.041)	0.280 (0.056)	0.350 (0.036)	< 0.001**
MP2530	0.269 (0.028)	0.408 (0.040)	0.223 (0.022)	0.126 (0.023)	0.169 (0.029)	0.127 (0.020)	< 0.001**
Male-producing eggs (MEP)							
MEP2015	0.492 (0.045)	0.587 (0.045)	0.306 (0.021)	0.200 (0.032)	0.245 (0.035)	0.396 (0.029)	< 0.001**
MEP2030	0.241 (0.026)	0.416 (0.033)	0.205 (0.022)	0.114 (0.018)	0.162 (0.023)	0.233 (0.018)	< 0.001**
MEP2515	0.224 (0.024)	0.284 (0.022)	0.140 (0.011)	0.092 (0.012)	0.094 (0.011)	0.171(0.014)	< 0.001**
MEP2530	0.176 (0.018)	0.275 (0.019)	0.194 (0.015)	0.098 (0.014)	0.158 (0.023)	0.213 (0.019)	< 0.001**
Diapausing egg production (DE)							
DE2015	64.018 7.642)	86.155 (6.484)	7.933 (1.615)	6.305 (1.777)	22.088 (4.013)	10.000 (2.328)	< 0.001**
DE2030	12.018 (3.098)	37.864 (3.968)	3.983 (1.379)	1.667 (0.662)	14.140 (2.213)	2.333 (0.637)	< 0.001**
DE2515	91.684 (10.713)	99.441 (7.645)	14.067 (3.012)	15.033 (3.890)	29.466 (5.281)	19.050 (3.660)	< 0.001**
DE2530	28.789 (4.438)	56.241 (4.441)	10.050 (1.939)	7.167 (1.622)	21.281 (2.844)	12.333 (1.829)	< 0.001**

*$P < 0.05$ (no Dunn–Šidák correction); **$P < 0.003$ (Dunn–Šidák correction).

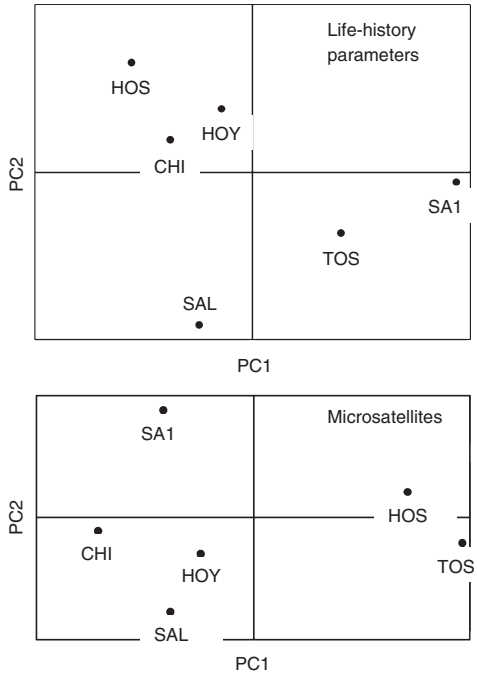

Fig. 1 Upper panel: mean values of the six populations in the space defined by the two principal components obtained by Principal Component Analysis (PCA) on trait × environment combinations (as variables) of clones. Correlation coefficients between principal components and trait × environment combinations: $r_{PC1\text{-}MP2015} = 0.754$, $r_{PC1\text{-}MP2030} = 0.573$, $r_{PC1\text{-}MP2515} = 0.727$, $r_{PC1\text{-}MP2530} = 0.764$, $r_{PC1\text{-}MEP2015} = 0.823$, $r_{PC1\text{-}MEP2030} = 0.710$, $r_{PC1\text{-}MEP2515} = 0.662$, $r_{PC1\text{-}MEP2530} = 0.703$, $r_{PC1\text{-}DE2015} = 0.749$, $r_{PC1\text{-}DE2030} = 0.644$, $r_{PC1\text{-}DE2515} = 0.773$, $r_{PC1\text{-}DE2530} = 0.707$, $r_{PC2\text{-}FP2015} = 0.564$, $r_{PC2\text{-}FP2515} = 0.657$, $r_{PC2\text{-}FEP2015} = 0.686$, $r_{PC2\text{-}FEP2530} = 0.528$; (only $|r| > 0.5$ are shown). Lower panel: means of the six populations in the space defined by the two principal components obtained by PCA with microsatellite loci as variables. For population acronyms, see Table 1.

producing eggs, 0.040) were lower than Q_{ST} values of life-history traits related to sexual reproduction (male production, 0.109; male-producing eggs, 0.120; and diapausing egg production, 0.222). Global population differentiation, as measured by Q_{ST} computed on the integrated life-history trait, was 0.218 when averaged over population pairs (Table 5).

Neutral marker analysis

A mean of 27.5 individuals (diapausing eggs from the sediment) were analysed per population (sample size range: 17–30; Table 4). The mean number of alleles per locus ranged from 2.09 for Salada de Chiprana to 4.46 for Hondo Sur. Reanalysis of the four new microsatellite loci developed in the present study yielded the following genotyping error estimations: 0% (*Bp7* and *Bp8*), 6% (*Bp9*) and 7% (*Bp10*). In Hondo Sur, loci *Bp5d* and *Bp9* had an estimated frequency of null alleles of 0.12 and 0.16 respectively. Both Poza Sur and Salada de Chiprana showed evidence of a null allele for locus *Bp10* with frequencies of 0.15 and 0.13. Salobrejo showed evidence of a null allele for locus *Bp8* with a frequency of 0.133. Despite the likely presence of null alleles in some loci for some populations, no population departed significantly from Hardy–Weinberg proportions (Table 4), indicating no evidence for inbreeding or Wahlund effects.

Both coastal populations (Poza Sur and Hondo Sur) showed higher genetic diversity than inland populations, as shown by levels of H_E. All pairwise F_{ST} estimates between populations (Table 5) were highly significant ($P < 0.001$) and ranged from 0.213 (Hondo Sur-Poza Sur) to 0.606 (Poza Sur-Salada de Chiprana), confirming the high genetic structure previously found in *B. plicatilis* in Eastern Spain (Gómez *et al.*, 2002).

A PCA on microsatellite data clustered the two coastal populations, Hondo Sur and Poza Sur, together (Fig. 1, lower panel), whereas the southern inland populations (Hoya Rasa and Salobrejo) plus a northern inland population (Salada de Chiprana) also tended to be associated. The first axis, which separated both coastal ponds, explained 42% of variance, and the second axis, which separated Balsa de Santed 1 from the rest, explained 27% of the variance (Fig. 1).

Comparison between neutral markers and life-history traits

The PCAs performed independently on trait × environment combinations and neutral markers yielded different clustering of the populations. Both coastal ponds tended to group together when microsatellites were used as variables but they were quite separated when trait × environment combinations were used as variables. Balsa de Santed 1 tended to cluster together with Poza Sur for trait × environment combinations, but this grouping was not kept for microsatellites.

Within populations, no significant correlation was found between H_E and H^2. Correlation coefficients were: 0.476 ($P = 0.339$) for female production, 0.155 ($P = 0.767$) for production of female-producing eggs, 0.378 ($P = 0.458$) for male production, 0.539 ($P = 0.270$) for production of male-producing eggs and 0.000 ($P = 0.995$) for diapausing egg production; the correlation between H_E and global H^2 was 0.543 ($P = 0.265$). Between-population differentiation in microsatellites and in life-history traits are compared in Fig. 2. All the analyses comparing neutral markers and life-history traits were repeated after excluding the two microsatellite loci for which genotyping errors were detected and we found no qualitatively different effect with respect to the analysis using all the microsatellite loci.

Most Q_{ST} estimates were lower than the corresponding F_{ST} estimates. No Q_{ST} for traits related to asexual reproduction exceeded the corresponding estimate of F_{ST}. In

Table 4 Mean H^2 of the life-history traits and main features of the microsatellite analysis in the six populations of *Brachionus plicatilis*. For population acronyms, see Table 1.

	Population						
	TOS	SA1	HOY	HOS	CHI	SAL	Mean
Broad-sense heritability (H^2)							
Sample size	29	30	30	30	28	28	29.2
FP	0.595	0.597	0.463	0.603	0.618	0.462	0.556
FEP	0.707	0.610	0.672	0.581	0.587	0.549	0.618
MP	0.705	0.869	0.649	0.750	0.434	0.546	0.640
MEP	0.675	0.785	0.608	0.822	0.727	0.635	0.708
DE	0.697	0.818	0.834	0.828	0.814	0.752	0.828
Global	0.698	0.741	0.650	0.719	0.638	0.597	0.674
Microsatellite loci							
Sample size	30	29	17	30	30	29	27.5
Number of polymorphic loci	9	7	10	10	7	9	8.667
Mean number of alleles per locus	3.818	2.364	2.182	4.455	2.091	2.364	2.879
Heterozygosity (H_E)	0.468	0.270	0.269	0.616	0.267	0.238	0.355
Inbreeding index (F_{IS})	0.010	−0.030	0.068	0.019	−0.028	−0.103	−0.011
P-value H–W*	0.188	0.732	0.197	0.182	0.110	0.928	

**P*-value for deviation from Hardy–Weinberg test with H_1 the heterozygote deficit.

contrast, for the life-history trait diapausing egg production – i.e. related to sexual reproduction – the pairs Hondo Sur-Poza Sur, Hondo Sur-Balsa de Santed 1, and Hoya Rasa-Balsa de Santed 1 showed $Q_{ST} > F_{ST}$ (Fig. 2, diapausing egg production, points encircled with dashed line).

Discussion

In order to explain the high genetic divergence in molecular markers found in zooplankton populations dwelling in ponds and lakes, a hypothesis invoking local adaptation has been proposed (i.e. the Monopolization hypothesis). This hypothesis should be tested in several taxa that are representative of a range of life histories; for instance, differing in generation times (De Meester *et al.*, 2002). To our knowledge, this is the first report of differentiation in life-history traits among local populations of a rotifer species. Despite the high dispersal capability attributed to zooplankton (Jenkins & Buikema, 1998; De Meester *et al.*, 2002, 2004; Louette & De Meester, 2004), our results show that rotifer populations are highly differentiated in both neutral markers and life-history traits. Differentiation among eastern Spain *B. plicatilis* populations in neutral molecular markers was found to be high (F_{ST} = 0.482), corroborating results of previous studies in the area (F_{ST} = 0.430; Gómez *et al.*, 2002). Likewise, the differentiation structure revealed in this study by the PCA roughly coincides with the three clusters (one coastal, one northern inland and one southern inland) found by Gómez *et al.* (2002); however, the two northern inland populations, Balsa de Santed 1 and Salada de Chiprana, were not associated in our study (i.e. they did not cluster together in the same group in the PCA analysis).

Regarding life-history traits, we found significant differentiation among populations with respect to almost all life-history traits, irrespective of the experimental environment they were measured in. Some extent of differentiation related to asexual reproduction was also observed. This is the case of the Salobrejo population, which showed low female production at 25 °C and 15 g L^{-1} salinity compared with the rest of the populations. However, our results revealed that population differentiation is much higher in those traits related to sexual reproduction. As most rotifer populations are temporary, and sex is necessary for diapause, the overall sexual egg (i.e. diapausing egg) production is an important fitness component. There were three pairwise population comparisons in the Q_{ST}–F_{ST} analysis for diapausing egg production that yielded higher Q_{ST} values than their corresponding F_{ST} estimates (i.e. more genetic divergence in the trait than expected by neutral genetic drift only). These higher Q_{ST} involved the following

Table 5 Pairwise Q_{ST} calculated from an integrated life-history trait (above the diagonal) and F_{ST} (below the diagonal) estimates among *Brachionus plicatilis* populations based on 11 microsatellite loci. All F_{ST} estimates were significant ($P < 0.001$). For population acronyms, see Table 1.

	TOS	SA1	HOY	HOS	CHI	SAL
TOS		0.106	0.279	0.423	0.234	0.255
SA1	0.586		0.459	0.551	0.408	0.437
HOY	0.429	0.481		0.083	−0.013	−0.019
HOS	0.213	0.424	0.351		0.023	0.065
CHI	0.606	0.545	0.465	0.461		−0.016
SAL	0.536	0.603	0.454	0.487	0.566	

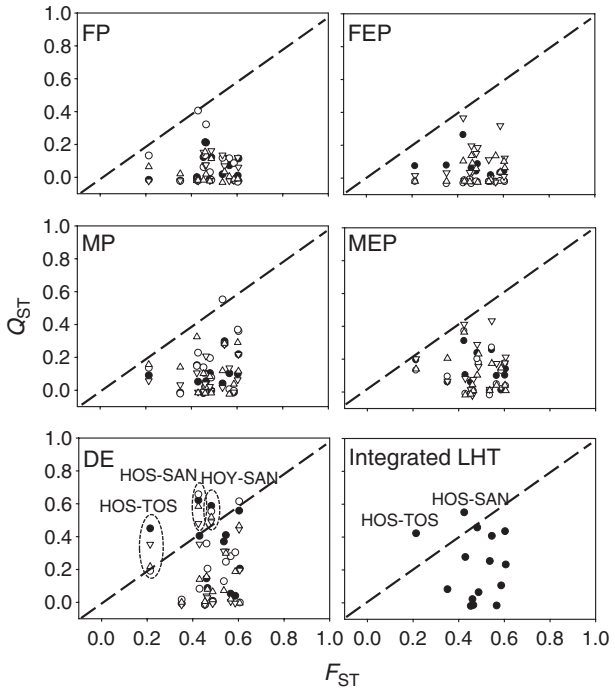

Fig. 2 Relationship between Q_{ST} estimated for the life-history traits studied and F_{ST} estimates. A comparison using Q_{ST} values computed from an integrated life-history trait is also shown. Solid circles: Q_{ST} (20 °C; 15 g L^{-1}); open circles: Q_{ST} (20 °C; 30 g L^{-1}); triangles down: Q_{ST} (25 °C; 15 g L^{-1}); triangles up: Q_{ST} (25 °C; 30 g L^{-1}); dashed line: $Q_{ST} = F_{ST}$. Points encircled by a dashed line, most $Q_{ST} > F_{ST}$: HOS-SAN and HOY-SAN: all Q_{ST} higher than F_{ST}; HOS-TOS: Q_{ST} (20 °C; 15 g L^{-1}), Q_{ST} (25 °C; 15 g L^{-1}) and Q_{ST} (25 °C; 30 g L^{-1}). For life-history trait acronyms, see Table 3. For details, see the text.

populations: (1) Hondo Sur and Poza Sur, (2) Hondo Sur and Balsa de Santed 1 and (3) Hoya Rasa and Balsa de Santed 1. Balsa de Santed 1 and Poza Sur are the two populations with the highest production of diapausing eggs, and Hondo Sur and Hoya Rasa are those with the lowest production. This suggests that divergent selection has acted on these two groups of populations. Optimal patterns in sexual reproduction investment are expected to be dependent upon the environmental conditions. That is, rotifers show different strategies for sex initiation in different environments and thus, sexual traits are good candidates to study local adaptation processes. For instance, populations subjected to growing seasons of short and uncertain length should be selected for early sex initiation (Carmona *et al.*, 1995; Serra & King, 1999; Schröder *et al.*, 2007). The remarkable differentiation in the sexual response found in our study can be interpreted as a result of selection for different life cycle patterns in different ponds. Our experimental design, which did not exceed 2 months of population growth, with food provided only at the beginning of the culture period, should resemble the ecology of a site where suitable conditions are ephemeral within an annual cycle. Accordingly, populations that are better adapted to such ephemeral habitats are expected to have higher fitness in our experimental conditions (e.g. higher production of diapausing eggs). Interestingly, Balsa de Santed 1 and Poza Sur populations – two populations with high values in sex-related life-history traits – inhabit small ponds, a feature that is likely to be correlated with the effect of physical perturbations which could cut short the permanence of a population in the water column (e.g. sudden salinity drops due to rainfall or rapid drying-up of the pond), and so to more environmental variance. On the contrary, rotifers from Hondo Sur produced the lowest number of diapausing eggs. In this coastal lagoon *B. plicatilis* has a long growing season, and thus, sexual reproduction is not expected at the beginning of the growing cycle. These results suggest that specific populations are adapted to features of their local ecological regime.

It is generally accepted that when narrow-Q_{ST} is lower than F_{ST}, then stabilizing selection is acting between populations. Other authors (e.g. Edmands & Harrison, 2003) have proposed that fluctuating environments could lead also to a $Q_{ST} < F_{ST}$ pattern. However, we computed broad-Q_{ST} values instead of narrow-Q_{ST}, and the former is expected to be lower than the later if dominance or epistatic variances exist, as within-population genetic variance is a term in the denominator for Q_{ST} computation. Therefore, although we found higher values for F_{ST} than for Q_{ST} for most life-history traits and pairwise population comparisons, this should be regarded as a still weak suggestion for stabilizing selection on those traits. Nevertheless, we hypothesize that, due to the high environmental fluctuation in salinity and temperature, typical of the sites studied, the environmental range for these conditions to which a particular population is exposed might be similar to the environmental range found at a more regional scale, that is, encompassing several populations, which would result in weak divergent selection. Note however that our finding of $Q_{ST} > F_{ST}$ between some pairs of populations in traits related to sexual reproduction would contrast with the hypothesized low divergent selection indicating that populations might not adapt to particular values of salinity or temperature but to other ecological features of their habitats not included in our design (length of growing season, unpredictability, etc).

The high genetic differentiation in neutral markers found in zooplankton populations can be explained as a resistance-to-decay founder effect, due to fast growth and large population sizes after a bottleneck in population foundation by a few individuals (Boileau *et al.*, 1992). The Monopolization hypothesis (De Meester *et al.*, 2002) extended this explanation to include rapid local adaptation resulting in selection against new immigrants after the population founding event. Therefore, the high levels of population differentiation established when

populations are founded would be maintained by a combination of numerical effects and local adaptation. Cyclical parthenogens indeed show a high potential to respond to natural selection (Lynch & Gabriel, 1983; see review in De Meester, 1996), and rapid changes in ecologically relevant traits have been reported in response to changes in selective pressure within populations (Cousyn *et al.*, 2001). However, empirical data on the actual occurrence of local adaptation are scarce, and focus on cladocerans (reviewed in De Meester, 1996; Declerck *et al.*, 2001; Declerck & Weber, 2003). Our results provide the first evidence for patterns that suggest local adaptation in rotifers. Although our results showing local adaptation are consistent with the expectations of the Monopolization hypothesis, they do not show that local adaptation has been instrumental in maintaining the high levels of genetic differentiation observed. As explained above, the Monopolization hypothesis assigns a role to both local adaptation and numerical effects in the genetic differentiation in neutral markers. However, this hypothesis has not been formalized and is not explicit about the relative role of numerical effects and local adaptation in terms of maintaining genetic population divergence in neutral markers. The relative importance of both factors might differ among populations and among species, depending, amongst other factors, on population size, heritability and the strength of local selection patterns. By modelling the Monopolization hypothesis, insights into the role of these features could be obtained.

In summary, we have presented the first experimental analysis on population differentiation for ecologically relevant traits in rotifers, and our results shed light on how genetic variation is shaped in small organisms characterized by large population sizes, mixed reproduction modes and short generation times. Our results reveal very strong genetic differentiation for both neutral markers and life-history traits in temporary salt-lake rotifer populations. Neutral genetic differentiation is likely to have been caused by founder events. We observed very high heritability, which shows that populations have the potential to respond to local selection. The pattern of relationships between F_{ST} and Q_{ST} between certain populations indicates directional selection and suggests that local adaptation has played a role in the differentiation of life-history traits in these rotifers, particularly in those traits related to sexual reproduction.

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