The interplay between colonization history and gene flow in passively dispersing zooplankton: microsatellite analysis of rotifer resting egg banks

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Abstract

Zooplanktonic organisms that disperse passively as diapausing eggs often exhibit surprisingly strong population subdivision given their high colonization ability. Here we attempt to disentangle the impacts of colonization history and gene flow on these organisms by studying the population genetic structure of the rotifer *Brachionus plicatilis*. The resting egg banks of *B. plicatilis* in fourteen salt lake populations in the Iberian Peninsula were examined using seven microsatellite loci. A remarkably high degree of geographical structuring was found ($F_{st} = 0.43$), with a significant pattern of isolation by distance. Microsatellite loci were in genetic equilibrium, ruling out inbreeding as an important force in population structuring. Comparisons are drawn with previously published phylogeographical data. Surprisingly, introgression of nuclear genes was detected in neighbouring populations with divergent mtDNA haplotypes. These results stress the long lasting impact of colonization history and the modulating effect of gene flow at local scales in these organisms.

Introduction

The last two decades have witnessed a revolution in the way we regard the population structure of aquatic continental planktonic species. What were formerly considered as geographically invariant, cosmopolitan species are now seen as species complexes with a high degree of regional endemism (Gómez & Snell, 1996; Hebert, 1998). With the development of techniques for rapid screening of polymorphic genetic markers in natural populations of these taxa, a general pattern has emerged of high genetic differentiation among populations, even at small geographical scales (De Meester, 1996a; Hebert, 1998). This level of population subdivision is surprising given the high dispersive potential of their diapausing propagules (see review in De Meester, 1996a). Several lines of evidence support the high, often long-range, dispersal ability in these organisms via wind or waterfowl. First, virtually all planktonic inland invertebrates possess a diapausing stage at some phase in their life cycle, called resting egg, ephippial egg or cyst, that can withstand desiccation, freezing and other environmental extremes (Gilbert, 1974; Cáceres, 1997a). In addition, in cyclically parthenogenetic zooplankters, these eggs hatch into parthenogenetic females, constituting ideal colonization propagules. These eggs often accumulate in high numbers in lake and pond sediments forming resting egg banks (Hairston, 1996; Hairston & Cáceres, 1996). Secondly, rotifers, cladocerans and copepods are rapid colonisers of new ponds, in some cases colonization taking only some weeks (Jenkins, 1995). Finally, molecular assessments of obligatorily parthenogenetic clones in *Daphnia* and ostracods indicate rapid colonization of newly available habitats (Chaplin & Ayre, 1997; Weider *et al.*, 1999b).

The underlying causes shaping the high genetic diversification of aquatic invertebrate populations in the face of potentially high levels of gene flow remain

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controversial, and arguments based on either selection or demographic processes have been favoured by different authors (Boileau *et al*., 1992; De Meester, 1996a). Local selection coupled with strong pre-emptive competition and outbreeding depression (the 'priority effect') has been suggested as an explanation for this apparent paradox (De Meester, 1996a). Local selection, especially in response to predators and parasites, has found experimental support in *Daphnia* and in copepods (Hebert & Moran, 1980; Hairston & Olds, 1984, 1987; Ebert, 1994; De Meester, 1996b; Boersma *et al*., 1999; Cousyn *et al*., 2001). In addition, fluctuating selection (Lynch, 1987) has been invoked to cause a pattern of population genetic diversification similar to a drift effect (but see Lynch *et al*., 1999). However, the effects of founding events, population age, and life history and other ecological traits (such as intrinsic population growth rates), have also been advocated to have a dramatic impact on pond organism population structure (Boileau *et al*., 1992; Pálsson, 2000). Other factors that might influence population structure in these organisms are inbreeding (in the form of selfing or mating among clone-mates), drift (through a limited number of resting egg hatching) (Berg & Lascoux, 2000), and intragenomic interactions such as selection at linked loci, associative overdominance and background selection especially during population founding or in small populations in cyclical parthenogens (Pálsson, 2001). To discriminate among such processes, it is crucial to analyse the population genetic structure of pond organisms in the light of a thorough knowledge on population sizes, patterns of sexual reproduction, dispersal ability and, most importantly, population history. These inferences will be compounded by (1) complex life cycles; (2) the presence of a resting egg bank, which is often neglected in population genetic studies, although it is known to affect the ecology and evolution of pond organisms (Hairston, 1996; Cáceres, 1997b; Brendonck *et al*., 1998); and (3) the difficulty of direct estimates of dispersal, as a result of small size and short life span.

Monogonont rotifers are cyclically parthenogenetic metazoans constituting important components of non-marine aquatic systems. They disperse passively through sexually produced resting eggs, an adaptation to survival during adverse conditions, which accumulate in resting egg banks (Gilbert, 1974, 1993; Pourriot & Snell, 1983; Snell *et al*., 1983; Marcus *et al*., 1994). Parthenogenetic reproduction takes place during favourable growth periods, and sexual reproduction is induced by a chemical produced at high population densities (Carmona *et al*., 1993).

Brachionus plicatilis is one of the most thoroughly studied rotifer species from a molecular ecological perspective. This species belongs to a sibling species complex inhabiting brackish and saline lakes and ponds world-wide, which are patchy, isolated habitats. This species complex, and in particular *B. plicatilis*, has recently been the focus of intensive study in the Iberian Peninsula (Gómez *et al*., 1995, 1998, 2000; Serra *et al*., 1998; Gómez & Carvalho, 2000), yielding comprehensive information on its ecology and demography, mating patterns, sexual strategies and population clonal structure. Furthermore, the populations investigated in the present study have been the focus of a parallel mtDNA phylogeography analysis (Gómez *et al*., 2000), which yielded important insights into population history. Partial sequencing of the mitochondrial cytochrome oxidase I gene (COI) revealed a strong phylogeographical structure, with two main mtDNA lineages, 'northern' and 'southern' (see Fig. 1), with a 2.8% sequence divergence between them, which suggested survival of populations in two or three glacial refugia, at least one of which was located in the Iberian Peninsula (Gómez *et al*., 2000).

An analysis based on several unlinked nuclear loci is essential for a characterization of population structure of these organisms, and for an understanding of the importance of current gene flow vs. historical forces genome-wide. Microsatellite loci have become the marker of choice for many types of population genetic studies as they are highly polymorphic, codominant genetic markers, abundant and ubiquitous in eukaryote genomes (Jarne & Lagoda, 1996; Goldstein & Schlötterer, 1999) and molecular techniques exist for their use in individual resting eggs (Gómez & Carvalho, 2000; Reid *et al*., 2000). Here, we report on the investigation of the population genetic structure of *B. plicatilis* in the Iberian Peninsula using seven polymorphic microsatellite loci. As planktonic populations can be ephemeral and undergo strong seasonal changes in allele frequencies (e.g. Carvalho & Crisp, 1987) we screened individual resting eggs retrieved from resting egg banks to maximize sampling success and to avoid confounding temporal clonal dynamics with interpopulational differentiation.

Materials and methods

Sample collection

Forty-seven salt lakes, ponds and brackish lagoons covering the five endorheic basins in the Iberian Peninsula and the coastal chain were sampled in 1998 and 1999 (see Gómez *et al*., 2000, for details). Superficial mud was collected using a scoop from the deepest part of each habitat. When water was present, plankton samples were collected using a plankton net made with 30 μm pore Nytal mesh and preserved in 95% ethanol. Sediment and ethanol samples were stored in dark and cool conditions until required.

Resting egg isolation, DNA extraction and microsatellite genotyping

We followed the procedures detailed in Gómez & Carvalho (2000) and Gómez *et al*. (2000) to isolate resting

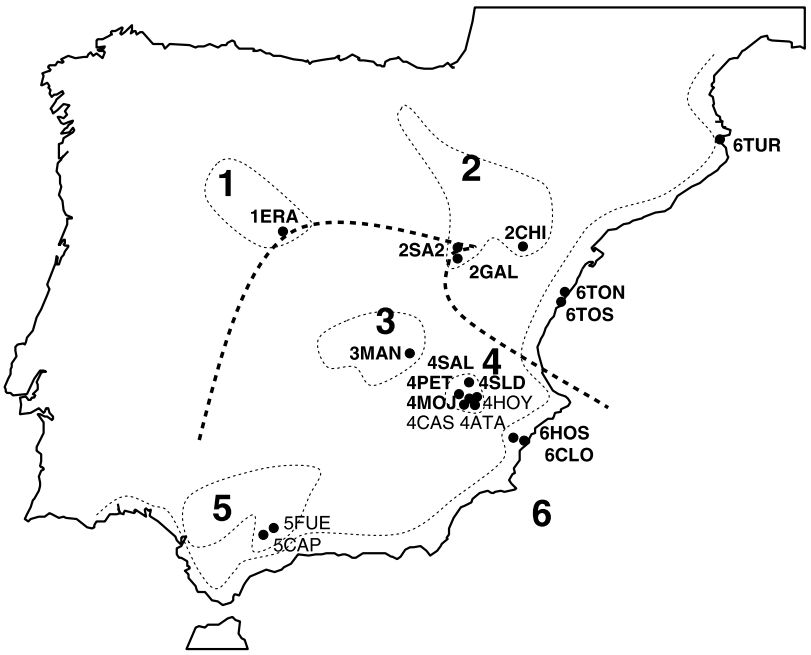

Fig. 1 Map showing the sampling site locations. The thin broken lines represent the approximate limits of the inland basins and the coastal chain of lagoons. Basins: 1, Duero; 2, Ebro; 3, Guadiana; 4, Júcar-Segura; 5, Guadalquivir; 6, coastal chain. The thick broken line indicates the boundary between northern and southern clades according to mtDNA analysis (Gómez *et al*., 2000). Ponds in bold were used in the genetic analysis.

eggs from pond sediments and to recognize *B. plicatilis* resting eggs. When available, 20–50 resting eggs were isolated per pond and DNA extracted from individual eggs using CHELEX (Gómez & Carvalho, 2000).

Because of the minute amounts of DNA in rotifer eggs, microsatellite PCR amplifications were set up in a one-way-flow containment laboratory in which no PCR had previously been carried out. All samples were genotyped for seven *B. plicatilis* trinucleotide microsatellite loci, *Bp1b*, *Bp2*, *Bp3*, *Bp3c*, *Bp4a*, *Bp5d* and *Bp6b* as described in Gómez *et al*. (1998). These microsatellite loci are species-specific for *B. plicatilis* in the conditions used in this study, and do not amplify any sibling species. The 10 μL PCR amplifications contained: 2 μL template DNA, 1.5 mM $MgCl_2$ (3 mM for locus *Bp2*), 200 μM of each nucleotide, 2.5 μM of each primer (reverse primer end-labelled with Cy5), 16 mM $(NH4)_2SO_4$, 67 mM Tris-HCl (pH 8.8 at 25 °C), 0.01% Tween-20 buffer, and 0.125 U of *Taq* DNA polymerase. The resulting PCR products were resolved on 6% acrylamide gels using an automatic sequencer (ALF*express*^{TM}, Pharmacia, Amersham Pharmacia Biotech, UK), along with appropriate internal size markers (van Oppen *et al*., 1997). Allele sizes were scored using the program AlleleLinks^{TM} (Pharmacia).

Statistical analyses

We used the BIOSYS-1 package (Swofford & Selander, 1981) to calculate allelic frequencies, mean number of alleles per locus (A), observed heterozygosity (H_o) and expected under Hardy–Weinberg assumptions (H_e, unbiased heterozygosity estimate based on conditional expectations, Nei, 1978). Tests for deviations from Hardy–Weinberg proportions, heterozygote deficiencies, genotypic linkage equilibrium and genic heterogeneity among populations were estimated using the computer program GENEPOP version 3.2 (Raymond & Rousset, 1995). A Markov chain method was used in loci with five or more alleles in the population. Estimates of F_{ST} (θ), F_{IS} (f), and their significance per population over all loci were calculated according to Weir & Cockerham (1984) using FSTAT (Goudet, 2000). Whenever significance was assigned to multiple tests, the sequential Bonferroni technique (Rice, 1989) was used to calculate corrected P-values.

A regression analysis of F_{ST} vs. the logarithm of the geographical distance for all pairs of populations was made to test for the correlation between population differentiation and geographical distance (isolation by distance) using GENEPOP. A Mantel test with 1000 permutations was used to test the significance of this correlation. GENEPOP was also used to test for the correlation between nuclear microsatellite divergence between populations (pair-wise F_{st}) and two measures of the mitochondrial DNA sequence divergence between populations: D_a, the number of net nucleotide substitutions per site between populations with Jukes & Cantor correction (Nei, 1987), and N_{st} (Lynch & Crease, 1990), which measures the level of population sequence differentiation for mtDNA variation, presented in Gómez *et al*. (2000).

Tree building is not the optimal way to represent patterns of relationships among populations, due to the absence of a strictly bifurcating pattern in a nongenealogical dataset such as unlinked microsatellite allele frequencies (i.e. multiple gene trees are combined) and to the occurrence of gene flow among populations

(Templeton, 1998). Therefore, we used an ordination technique, Principal Components Analysis (PCA), to visualize the level of similarity between the populations. The program PCA-General vs. 1.2 (Goudet, 1999) was used to perform PCA on the microsatellite genotypic data from the seven loci in the 14 populations.

The program Arlequin ver. 2.0 (Schneider *et al*., 2000) was used to perform a hierarchical analysis of molecular variance (AMOVA; Michalakis & Excoffier, 1996) using an *a priori* design which divided all populations in two groups corresponding with the dominant mtDNA lineages (northern or southern) found in every population. A nonparametric permutation procedure (10 000 permutations) was used to assess population genetic variability explained by each level of the analysis.

As resting eggs accumulate in sediments they probably represent products of independent sexual reproduction events, and therefore deviations from Hardy–Weinberg proportions should be most probably because of (a) the admixture of resting eggs produced in different years with different allelic compositions (Wahlund effect) or (b) resting eggs that are the product of sexual reproduction of different seasonal swarms of related clones (inbreeding). Another possible cause of deviations from Hardy–Weinberg is the presence of null alleles. All three factors will cause heterozygote deficiencies. We, therefore, used one-tailed Hardy–Weinberg tests with a H_1 of heterozygote deficiency as implemented in GENEPOP. The multilocus *P*-value was calculated using Fisher's combined probability test. Both Wahlund effect and inbreeding are expected to cause heterozygote deficiencies at all loci, whereas null alleles are expected to affect particular loci. For those loci displaying significant heterozygote deficiencies we estimated the frequency of putative null alleles. In order to do this, we first estimated the frequency of each observed allele size class using the heterozygote genotype frequencies and assuming Hardy–Weinberg equilibrium. Then, the frequency of a putative null allele was estimated from the deviation of observed and expected homozygote frequencies, again assuming Hardy–Weinberg proportions. Although this method will give only approximate estimates, the more biased the more the population suffers from inbreeding, it is sufficient for our purposes and we were careful to use it only when the other loci were in Hardy–Weinberg proportions.

Results

A total of 295 *B. plicatilis* resting eggs and individuals retrieved from 19 lakes and ponds (see Fig. 1) were typed for the seven microsatellite loci. To this dataset we added a sample of 145 resting eggs previously typed for the same set of microsatellite loci in a temporal population analysis of one of these lakes, 6TOS (Gómez & Carvalho, 2000). Five populations with less than nine resting eggs retrieved were not included in the genetic analysis (see Table 1).

Genetic diversity within populations

The number of polymorphic loci ranged from three to seven per population and the number of alleles from 6 to 13 per locus, with a total number of 63 alleles in the global sample (see Appendix 1). Each locus was monomorphic in at least one population. Four coastal populations (6HOS, 6CLO, 6TON and 6TOS) were the most diverse genetically. They were polymorphic for all seven loci with mean heterozygosity ranging from 0.45 to 0.66. Three inland populations (4SAL, 4CHI and 2SA2) were the most genetically depauperate, with only three polymorphic loci and mean heterozygosity from 0.17 to 0.19. There were 23 alleles found in single populations (private alleles), with average allele frequency of 0.11 (range from 0.009 to 0.611).

Population genetic equilibrium and inbreeding

Only 3 of 14 populations showed significantly positive multilocus F_{is} values (Table 2). In two of these populations, 3MAN and 4SLD (F_{is} of 0.291 and 0.161, respectively), this was caused by the strong deviations from Hardy–Weinberg equilibrium in single locus. Therefore, the occurrence of null alleles in these loci could be responsible for the observed heterozygote deficiencies. For 3MAN, a putative null allele in *Bp6b* would have an estimated mean frequency of 0.22, and for 4SLD, a null allele in *Bp3* would have an estimated mean frequency of 0.10. However, in the third population, 2GAL, inbreeding may have caused a significant population substructuring. Here, three loci (*Bp3*, *Bp1b* and *Bp5d*) displayed significant heterozygote deficiencies. In addition, two of these loci (*Bp3* and *Bp5d*) were in strong linkage disequilibrium in this population. In summary, these data indicate that with the exception of population 2GAL, all our study populations are in equilibrium and do not show evidence of inbreeding.

Population genetic structure

Marked genetic differentiation was detected among populations (Table 3), with multilocus F_{st} values ranging from 0.08 to 0.68. Multilocus genic differentiation tests were highly significant for all the population pair-wise comparisons, even for geographically very close populations, although some population pairs were not significantly different after Bonferroni correction (Table 3). Global F_{st} was 0.43 (SE 0.026, obtained through jack-knifing over loci).

This pattern of strong genetic differentiation was modulated by geographical distance. Populations showed a strong correlation between F_{st} and interpopulation distance (see Fig. 2) with an R^2 of 0.4271. A Mantel test for correlation of F_{st} with the logarithm of distance was highly significant ($P = 0.003$).

Table 1 Sampling site locations and number of *Brachionus plicatilis* eggs sampled from every site.

Code	Site	Location	mtDNA haplotypes	Sample size and type
1ERA	Laguna de las Eras	41°10′N 4°35′W	N (S)	15 (RE)
2GAL	Laguna de Gallocanta	40°59′N 1°31′W	N	36 (RE)
2SA2	Balsa de Santed II	41°01′N 1°30′W	S (N)	20 (RE)
2CHI	Salada de Chiprana	41°14′N 0°11′W	N	9 (RE)
3MAN	Laguna de Manjavacas	39°25′N 2°53′W	S	17 (RE)
4PET	Laguna de Pétrola	38°50′N 1°34′W	S	15 (RE)
4SAL	Laguna del Salobrejo	38°55′N 1°27′W	S	10 (RE)
4SLD	Laguna del Saladar	38°48′N 1°25′W	S	20 (RE)
4MOJ	Laguna de Mojón Blanco	38°48′N 1°26′W	S	19 (RE)
6TUR	Estany de En Turies	42°15′N 3°06′E	N	11 (RE)
6TON	Poza Norte	40°10′N 0°10′E	N	55 (RE + PL)
6TOS	Poza Sur	40°10′N 0°10′E	N	155 (RE)
6HOS	Hondo Sur	38°12′N 0°43′W	–	15 (PL)
6CLO	Clot de Galvany	38°16′N 0°31′W	S	27 (RE)

The mitochondrial lineage recorded in each pond is also indicated. RE, sample from resting eggs; PL, sample from plankton. MtDNA haplotypes are N (northern lineage) and S (southern lineage). The haplotype lineage in parentheses is the one in lower frequency. Acronyms of sample location indicate the basin (Fig. 1) and a code for the pond.

A Mantel test showed no significant correlation between nuclear (F_{st} obtained from the microsatellite loci) and mtDNA (N_{st} and D_a based on cytochrome oxidase sequence data from Gómez *et al*., 2000) patterns of population differentiation ($P = 0.46$ and 0.42, respectively). A further level of analysis was possible using the pattern of differentiation observed in a previous study of mtDNA haplotypes (Gómez *et al*., 2000). Here, each lake possessed a dominant haplotype belonging to one of two divergent lineages. These haplotypes were often most closely related to others in the same region but in some instances they were more divergent from neighbouring lakes than some distant ones (see the distribution of the two mtDNA haplotype lineages in Fig. 1). An AMOVA was performed to test the effect of this mtDNA subdivision on microsatellite population structure. The level of the hierarchy was defined by the lineage of the dominant mtDNA haplotype (north or south, Gómez *et al*., 2000) found in each population. Surprisingly, dominant mtDNA haplotype explained only 6% of the microsatellite variation and was not statistically significant ($P = 0.32$). Variation caused by population explained 38.61% of the variance, and within population variation explained 55.06% of the variance, both highly statistically significant.

The PCA on the 14 populations performed to evaluate the patterns of relationship between populations (see Fig. 3) showed only one significant axis. The F_{st} retained by the first axis, x, equals 0.22 (P-value 0.008); the second axis, y, 0.06 (P-value 0.58). Although all populations were significantly different from each other (see above), the populations clustered in three main geographically meaningful groups: A 'northern inland' group contained the four lake populations from basins 1 and 2; a 'coastal' group included the five lake populations from the coast; and a 'southern inland' group contained the five lake populations from basins 3 and 4.

Discussion

The striking global morphological similarity of freshwater invertebrates around the world, already noticed by Darwin (1859), promoted the view that ongoing dispersive processes were responsible for their cosmopolitan distributions and the absence of geographical diversification. This paradigm has been recently challenged with the introduction of molecular markers and, as high geographical diversification has been found in almost every zooplanktonic organism studied (Innes, 1991; Boileau *et al*., 1992; Hebert, 1998; but see Naihong *et al*., 2000), the focus has been shifted towards a less descriptive, more analytical approach to understand the causes involved.

The present study has revealed that the nuclear gene pool of a salt lake rotifer is highly fragmented at a regional scale. The high levels of population structure suggest a persistent effect of population colonization history. This 'founder effect', most probably together with local adaptation, helps to solve the paradox of high population differentiation in the face of high potential gene flow: zooplanktonic organisms might well be good colonizers, but other processes attenuate effectively the impact of ongoing gene flow. We provide population data directly derived from resting egg banks, which potentially removes biases created by fluctuating gene frequencies in single season populations (Carvalho & Crisp, 1987) and potential hatching biases, unavoidable when working on allozyme loci on hatchlings.

Table 2 Genetic variability at seven microsatellite loci in 14 *Brachionus plicatilis* populations.

	Mean sample size per population	Mean number of alleles per locus	Number of polymorphic loci	H_o	H_e	F_{is}	H–W probability (H_1: heterozygote deficit)	Linkage disequilibrium
6TON	54.4	3.9	7	0.454	0.451	−0.006	n.s.	9**(2)/21** Bp1b–Bp3c, Bp1b–p4a
	(0.3)	(0.5)		(0.076)	(0.072)			
6TOS	136.1	3.7	7	0.447	0.459	0.027	n.s.	**0/21**
	(13.6)	(0.4)		(0.064)	(0.068)			
6TUR	8.7	2.3	5	0.417	0.377	−0.113	n.s.	**1(0)/15**
	(0.5)	(0.4)		(0.108)	(0.085)			
6CLO	26.9	4.4	7	0.634	0.648	0.023	n.s.	**1(0)/21**
	(0.1)	(0.4)		(0.040)	(0.036)			
6HOS	14.1	3.6	7	0.660	0.624	−0.060	n.s.	**4(0)/21**
	(0.3)	(0.4)		(0.050)	(0.028)			
1ERA	13.7	2.0	5	0.229	0.298	0.241	n.s.	**1(0)/10**
	(0.6)	(0.3)		(.070)	(0.087)			
2GAL	35.4	3.1	7	0.337	0.402	0.163	*Bp3*, *Bp1b*, *Bp5d* $P < 0.01$	2**(1)/21** Bp3–Bp5d
	(0.3)	(0.4)		(0.078)	(0.072)			
2SA2	19.6	2.3	3	0.186	0.184	−0.007	n.s.	**0/10**
	(0.2)	(0.5)		(0.084)	(0.083)			
2CHI	9.4	1.7	3	0.161	0.181	0.113	n.s.	**0/10**
	(0.4)	(0.2)		(0.082)	(0.067)			
3MAN	13.7	2.7	5	0.249	0.347	0.291	**Bp6b**, $P < 0.001$	**2(0)/10**
	(1.3)	(0.5)		(0.080)	(0.095)			
4SAL	9.0	1.9	3	0.167	0.251	0.350	n.s.	**0/3**
	(0.3)	(0.5)		(0.081)	(0.124)			
4MOJ	17.6	2.7	5	0.410	0.382	−0.077	n.s.	**0/10**
	(0.6)	(0.7)		(0.140)	(0.125)			
4PET	14.3	2.3	4	0.314	0.319	0.015	n.s.	2**(0)/7**
	(0.4)	(0.6)		(0.121)	(0.118)			
4SLD	18.9	2.1	4	0.237	0.282	0.161	**Bp3**, $P < 0.01$	1**(0)/6**
	(0.5)	(0.5)		(0.116)	(0.116)			

Mean sample size per population and mean number of alleles per locus (SE in parentheses). Mean heterozygosity (H_o, direct-count heterozygosity, H_e, expected heterozygosity). F_{is} is the multilocus heterozygote deficit within populations. Multilocus Hardy–Weinberg probability for every population when H_1 is a heterozygote deficit (single locus U-score tests, with Markov chain method, *P*-values combined using Fisher's method), when the multilocus test was significant, individual loci showing H–W deviations are shown as well. Individual loci showing significant heterozygote deficiency are shown. Significant results after applying the sequential Bonferroni correction (Rice, 1989) are shown in bold.

We have built on sound previous knowledge of population history obtained through mtDNA phylogeography (Gómez *et al.*, 2000) and ecological and demographic processes in this rotifer. The high level of population differentiation found using nuclear microsatellite markers is in agreement with the mtDNA analysis in these rotifer populations, although some discrepancies are apparent. Phylogeographical analysis of mtDNA sequence haplotypes indicated a strong population structure created in part through several past subdivision events and maintained by restricted gene flow via isolation by distance (Gómez *et al.*, 2000). MtDNA analysis showed very high population differentiation and very little intrapopulation variation with most haplotypes localized in single ponds. The microsatellite analyses reinforce the marked population structure and the pattern of isolation by distance. In addition, microsatellites show a similar level of population geographical partitioning, but, as expected due to the higher rate of drift in mtDNA (Birky *et al.*, 1989; Hedrik, 2000), and probably because of the strong bottleneck during historical population foundation, higher estimates were detected for population subdivision using mitochondrial than nuclear DNA markers. This phenomenon has been also reported in *Daphnia* (Lynch *et al.*, 1999). In spite of the high level of population structure found in both sets of markers, no correlation was found between the degree of differentiation estimated for nuclear and mitochondrial genomes. This lack of correlation could be due to a number of factors. First, the high mutation rate of microsatellite loci combined with their often step-wise mutation model could lead to a substantial level of allele homoplasy (Estoup & Angers, 1998) and the occurrence of new unique alleles in populations, which would decrease

Table 3 Genetic differentiation between *Brachionus plicatilis* populations.

	6TON	6TOS	6TUR	6CLO	6HOS	1ERA	2GAL	2SA2	2CHI	3MAN	4SAL	4MOJ	4PET	4SLD
6TON	–	6	6	6	6	7	7	6	7	7	7	6	7	6
6TOS	0.09**	–	5	7	7	7	7	7	7	7	7	6	7	6
6TUR	0.38**	0.32**	–	7	6	7	7	6	7	5	5	7	7	7
6CLO	0.26**	0.27**	0.34**	–	5	7	7	7	7	7	7	7	7	7
6HOS	0.20**	0.21**	0.30**	0.15**	–	7	6	7	7	7	7	7	7	6
1ERA	0.59**	0.58**	0.62**	0.43**	0.45**	–	6	5	6	6	5	7	6	6
2GAL	0.51**	0.51**	0.54**	0.37**	0.37**	0.42**	–	6	5	6	6	6	6	5
2SA2	0.59**	0.57**	0.68**	0.47**	0.49**	0.56**	0.16 n.s.	–	5	5	5	6	5	6
2CHI	0.59**	0.58**	0.68**	0.42**	0.45**	0.57*	0.41**	0.63**	–	5	4	4	3	4
3MAN	0.51**	0.50**	0.50**	0.41**	0.39**	0.51**	0.45**	0.60**	0.49*	–	5	6	4	4
4SAL	0.52**	0.50**	0.50**	0.43**	0.44**	0.58**	0.49**	0.67**	0.58**	0.31*	–	4	4	4
4MOJ	0.43**	0.43**	0.53**	0.34**	0.33**	0.50**	0.35**	0.52**	0.33 n.s.	0.25 n.s.	0.32**	–	4	4
4PET	0.45**	0.44**	0.48**	0.40**	0.38**	0.52**	0.42**	0.56**	0.46**	0.26 n.s.	0.17 n.s.	0.15**	–	3
4SLD	0.46**	0.44**	0.55**	0.41**	0.40**	0.57**	0.45**	0.62**	0.46**	0.32**	0.33**	0.08**	0.08 n.s.	–

The lower matrix shows pairwise F_{st} values (θ) per population (Weir & Cockerham, 1984), and their significance after sequential Bonferroni correction (Rice, 1989). The upper matrix shows the number of rejected single locus χ^2 tests ($P < 0.05$) for homogeneity of allele frequencies.
*$P < 0.05$, **$P < 0.001$.

correlations between mitochondrial and nuclear loci. As microsatellite allele homoplasy will lead to an underestimation of among population differentiation (Angers *et al*., 2000) our main conclusions will not be affected. In contrast, mutation might be expected to lead to new and unique microsatellite alleles in individual ponds. Indeed, private alleles are common in these rotifer populations representing 37% of all alleles, some of them present in high frequency, and they could contribute significantly to this lack of correlation. Another possible factor is the strong founder effect that seems to accompany the initial establishment of rotifer populations, as strong genetic drift is expected during colonization, with probably founding of single individuals. Finally, differences in male and female mediated gene flow are possible (see below) as we observed several populations which differed in their mtDNA haplotype composition although they were more similar in their microsatellite allele frequencies.

Additional studies on the phylogeographical patterns are needed in continental aquatic invertebrates in order to discriminate between the impact of historical and ongoing population processes on population genetic structure. In spite of the potential benefits to be gained from population structure analysis in the context of a well-understood phylogeographical history, just some studies exist, mostly in *Daphnia*, comparing nuclear (often allozyme loci) and mtDNA results (Crease *et al*., 1997; Taylor *et al*., 1998; Weider *et al*., 1999a,b). For example, Weider and collaborators' extensive study of a circumpolar *Daphnia* species complex, regarded as obligate parthenogens, revealed two major geographically concordant mtDNA clades and the occurrence of 'hybrid populations' with nuclear introgression in a zone of secondary contact between both clades. Allozyme analysis revealed that some clones were widespread, most probably the product of colonization of previously ice-covered areas (Weider *et al*., 1999b). These studies have revealed clear phylogeographical patterns with instances of vicariance, long distance dispersal, post-glacial colonization and introgression, which are critical for an understanding of the current processes shaping the genetic diversity in these organisms.

Inbreeding in rotifer populations

The present microsatellite dataset and recent allozyme data (Gómez *et al*., 1995; Ortells *et al*., 2000) suggest that inbreeding is not widespread in rotifer populations. Further support for this conclusion comes from a temporal study of rotifer clonal structure and dynamics (Gómez & Carvalho, 2000). The high number of clones found in rotifer populations even at the end of a

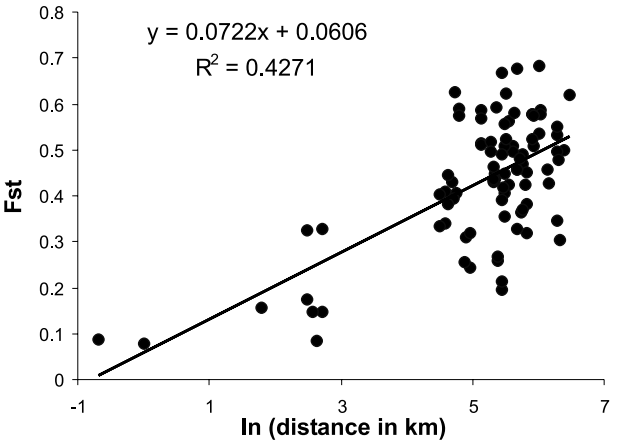

Fig. 2 Linear regression of F_{st} against log of distance between all pairs of *Brachionus plicatilis* populations. The adjusted regression equation and R^2 is shown inside the plot.

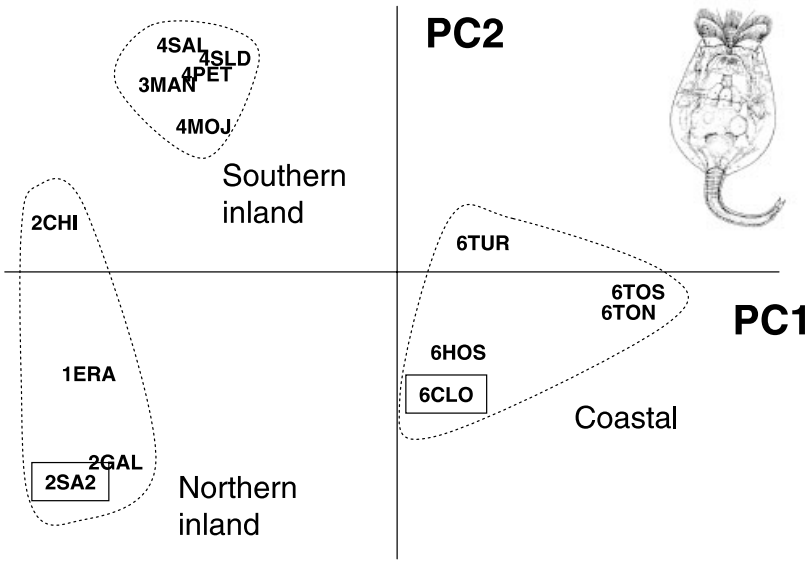

Fig. 3 Principal components analysis of the 14 *Brachionus plicatilis* populations. The populations in frames represent those that do not group with populations with their dominant mtDNA haplotype lineage.

parthenogenetical growth cycle, indicates that clonal selection causes little erosion of genetic diversity, with few deviations from the genetic equilibrium and clonal diversity generated by each bout of sexual reproduction (Gómez & Carvalho, 2000). This will mean that a high number of clones are present when sexual reproduction occurs. However, in a recent theoretical approach Berg & Lascoux (2000) studied equilibrium situations for an infinite island null model adapted for the cyclically parthenogenetical *Daphnia* life cycle. This study suggested that significant population structuring would occur if inbreeding is strong because of (1) mating between clonemates (equivalent to selfing); (2) reproduction occurring primarily within seasonally adapted clonal swarms; (3) random associations between neutral and selected genes. In spite of this, many allozyme studies in *Daphnia* have failed to provide evidence for inbreeding, with Hardy–Weinberg equilibrium being general in cyclical parthenogenetical species (De Meester, 1996a), and the uncoupling of male and sexual female production helps to reduce inbreeding (De Meester & Vanoverbeke, 1999). The occurrence of severe inbreeding depression caused by selfing in *Daphnia* and rotifers (see review in De Meester & Vanoverbeke, 1999) also indicates that strong inbreeding does not occur in natural populations. Combined with our data this suggests that inbreeding is not a general cause of pond invertebrate population structure when a sexual phase is present in the life cycle.

Drift in rotifer populations

Recurrent genetic drift has repeatedly been discarded as a factor responsible for the high population subdivision in pond invertebrate populations (Lynch, 1987; Carvalho, 1994; but see Berg & Lascoux, 2000). These organisms often reach very high population sizes, and we do not have evidence for low effective population sizes. Rotifers in particular, exhibit high population growth rates and they usually maintain very large population sizes (with densities of 10^2–10^3 individuals per litre), even in small habitats (Nogrady *et al*., 1993; Gómez, 1996). Although fluctuations in population density of several orders of magnitude are not rare, the presence of a resting egg bank protects against extinction in poor recruitment years and might cause a strong resilience to selection and drift ('slowed evolution'; Hairston & De Stasio, 1988) or competitive exclusion (Cáceres, 1997b) due to the extension of generation times and the high numbers involved (Hairston, 1996). Effective population size in rotifers is related to the number of different clones that reproduce sexually every season and this is determined by the number of hatching resting eggs and the pattern of selection in relation to patterns of sexuality. Indirect evidence for massive resting egg hatching at the beginning of the growth season and very high numbers of clones in natural populations exists (Gómez *et al*., 1995; Gómez & Carvalho, 2000), supporting the existence of high effective population sizes in rotifers.

Drift in the form of recurrent population bottlenecks might, therefore, be negligible, in the face of the staggering sizes of the resting egg banks and populations, and the role of drift might be important only at the time of population founding (see below). The relevance of data on effective population sizes prompts for the estimation of the number of hatchlings per population, clonal diversity and population sizes during sexual reproduction, as well as the size and dynamics of the resting egg bank.

Population history and founding events

The time required for a set of populations to reach migration-drift equilibrium in an island model is

increased by low migration rates and large population sizes (Boileau *et al*., 1992). When populations grow rapidly in size after founding from few individuals, as often happens with pond invertebrates, the allele frequency divergence established during colonization history is resistant to decay by migration (Boileau *et al*., 1992). Therefore, the existing levels of divergence may closely approximate those established during population founding. The mtDNA analysis gave a rough estimate of population differentiation of less than 2 million years in the Iberian Peninsula. What this means in terms of rotifer generations is difficult to estimate, as we know very little about resting egg bank dynamics. The effect of resting egg banks in increasing generation time (Hairston & De Stasio, 1988) might mean that rotifer generation time could well be over a year. In addition, many, if not most, of the ponds studied here are of late Pleistocene, or early Holocene origin (15 000–10 000 years before present). Therefore, rotifer populations in this area are probably young and thus are not at migration-drift equilibrium, and are shaped in their genetic structure by differences generated during their founding event. The F_{st} value found in these rotifer populations indicates that populations were initially established by a single founder (see Boileau *et al*., 1992), which is consistent with rotifer biology, in which resting eggs, the dispersing propagule, hatch into parthenogenetic females which show very high reproductive rates (Gómez *et al*., 1997). These results indicate that founding events might be critical in setting the level of variation in rotifer populations. It does not follow, however, that migration is rare or unimportant, but that after the initial founding event and because of the rapid increase in population size immediately after colonization, and the local adaptation ensuing, migrants will have very little effect on the level of genetic differentiation of populations. These results agree with similar patterns found in phylogeographical studies in copepods (Boileau & Hebert, 1991) and cladocerans (Taylor *et al*., 1998; Weider *et al*., 1999a,b; Cox & Hebert, 2001) that indicate that the patterns created during population recolonization after Ice Age range contractions persist today. This strong and persistent founding effect might also explain why bird migratory flyways exert so little impact on the phylogeographical patterns of pond invertebrates (Crease *et al*., 1997; Gómez *et al*., 2000; Straughan & Lehman, 2000).

Isolation by distance and the importance of local migration

The present data provide one of the clearest patterns of isolation by distance in a planktonic lake invertebrate. Resting eggs are purportedly transported by wind, aquatic insects, waterfowl and flooding or waterways connecting water bodies. Iberian salt lakes are located in arid areas, and are widely spaced in closed individual endorheic basins, where direct water connections are unlikely to be avenues for gene flow, leaving as the most likely vectors wind and waterfowl or aquatic insects. These processes, and therefore dispersal events, are likely to be stronger on a local scale and are expected to create a pattern of isolation by distance. In the face of the strong resilience of initial gene frequencies to change, the pattern of isolation by distance will build up from closer to more distant ponds and will affect the entire system given sufficient time (Hutchison & Templeton, 1999). The results for microsatellite loci presented here reinforce the conclusions obtained using a mitochondrial marker (Gómez *et al*., 2000), which showed the effects of a restricted gene-flow modulated by isolation by distance. Our conclusion is, therefore, that ongoing migration has managed to erode to some extent the level of differentiation. The fact that the level of microsatellite differentiation is correlated with geographical distance, but not with the level of mtDNA differentiation helps to support the notion that the pattern of isolation by distance has been created by ongoing restricted gene flow, as opposed to being established during the colonization process, supporting the vagility of rotifer resting eggs on a localized scale. Clear patterns of isolation by distance are rare in zooplanktonic organisms (Lynch & Spitze, 1994). The strongest patterns have been found for zooplankters inhabiting large lakes, but the F_{st} involved were low (Gießler, 1997; Naihong *et al*., 2000). However, weaker or no patterns of isolation by distance have been found in pond *D. pulex* (Innes, 1991; Pálsson, 2000). These results are usually interpreted as indicating an already high pattern of differentiation among neighbouring pond populations.

Nuclear vs. mitochondrial gene flow: is rotifer gene flow female mediated?

Because planktonic rotifers, as well as many other pond invertebrates, are unable to disperse actively, migration events between isolated water bodies are mediated by resting eggs. In the case of cyclically parthenogenetical rotifers and cladocerans, resting eggs are produced sexually and always hatch into parthenogenetical females. Therefore, as all migration is female mediated, we would expect a close correlation in the differentiation patterns between mitochondrial and nuclear DNA. Surprisingly, no correlation between estimates of population differentiation using mitochondrial and nuclear markers was found. Although the results of the PCA on nuclear DNA markers revealed the occurrence of three major population groupings (southern inland, northern inland and coastal), with an overall similarity with the mtDNA results (see nested design in Gómez *et al*., 2000), the relationships of these three groups were different, as the close relationship between the two mtDNA northern groups is not supported by microsatellites. In addition, in the microsatellite PCA, the ponds, 2SA2 and 6CLO, which were dominated by southern mtDNA haplotypes, group

with geographically closer ponds bearing northern haplotypes, indicating that they may form part of a contact zone. Thus, 6CLO appears in the 'coastal' grouping together with northern haplotype ponds 6TUR, 6TOS and 6TON. Similarly, 2SA2, which contains predominantly southern mtDNA haplotypes, appeared very close to 2GAL, a nearby pond dominated by northern haplotypes. The lack of correlation between mtDNA phylogeography and nuclear DNA is often attributed to differences in the level of male and female gene flow (Avise, 2000). This lack of correlation between mitochondrial and nuclear gene flow was unexpected, as, in principle, all gene flow in rotifers is female mediated. However, to make an impact on the mitochondrial genetic structure of the population, the females hatching from the dispersing resting eggs must produce resting eggs in their first season. If the clones produce only males, then nuclear genes alone will be transmitted to the following generations and their mitochondrial genome lost. Indeed there is evidence of male-only producing clones in *Daphnia* (Ferrari & Hebert, 1982; Hobæk & Larsson, 1990). In such cases, there is potential for male-mediated and female-mediated gene flow in resting-egg dispersing cyclical parthenogens. If this is occurring in the contact zone between northern and southern mitochondrial haplotypes we might be witnessing a pattern of nuclear (male mediated) introgression from the northern group. This pattern could be caused by an earlier induction of sexual reproduction (Aparici *et al.*, 1996) or by a pattern of asymmetric mating. These results suggest that the occurrence of 'male' mediated gene flow should be taken into account in studies of organisms with complex life cycles, even when the dispersive propagule is female.

Conclusions

This study has found extreme population subdivision in a pond invertebrate, the rotifer *B. plicatilis*. The demographic patterns and clonal population dynamics of this species excludes inbreeding and genetic drift as significant ongoing evolutionary forces shaping genetic structure. Populations are probably far from migration-drift equilibrium because of the persistent consequences of colonization events on gene frequency divergence; the level of population differentiation found is consistent with initial colonization of single resting eggs from neighbouring populations. The effects of local migration, however, have shaped genetic differentiation to the point that strong isolation by distance has developed. Surprisingly, comparisons with mtDNA results indicate a marked process of nuclear introgression, which suggests male-mediated gene flow. The patterns of isolation by distance, and the nuclear introgression between populations with divergent dominant mtDNA lineages suggest that past historical colonization events are not the only source of interpopulation differentiation because gene flow has a role as well. Finally, this study demonstrates the potential for examining the molecular ecology of resting egg banks, opening an avenue of research in a number of ephemeral, passively dispersing aquatic organisms.

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References

Angers, B., Estoup, A. & Jarne, P. 2000. Microsatellite size homoplasy, SSCP, and population structure: a case study in the freshwater snail *Bulinus truncatus*. *Mol. Biol. Evol.* **17**: 1926–1932.

Aparici, E., Carmona, M.J. & Serra, M. 1996. Polymorphism in bisexual reproductive patterns of cyclical parthenogens. A simulation approach using a rotifer growth model. *Ecol. Modelling* **88**: 133–142.

Avise, J.C. 2000. *Phylogeography*. Harvard University Press, New York, 447 pp.

Berg, L.M. & Lascoux, M. 2000. Neutral genetic differentiation in an island model with cyclical parthenogenesis. *J. Evol. Biol.* **13**: 488–494.

Birky, C.W., Fuerst, P. & Maruyama, T. 1989. Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparisons to nuclear genes. *Genetics* **121**: 613–627.

Boersma, M., De Meester, L. & Spaak, P. 1999. Environmental stress and local adaptation in *Daphnia magna*. *Limnol. Oceanogr.* **44**: 393–402.

Boileau, M.G. & Hebert, P.D.N. 1991. Genetic consequences of passive dispersal in pond-dwelling copepods. *Evolution* **45**: 721–733.

Boileau, M.G., Hebert, P.D.N. & Schwartz, S.S. 1992. Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. *J. Evol. Biol.* **5**: 25–39.

Brendonck, L., De Meester, L. & Hairston, N. Jr. 1998. *Evolutionary and Ecological Aspects of Crustacean Diapause. Arch.*

Hydrobiol. Spec. Issues Advanc. Limnol. 52. E. Schweizerbart'sche-Verlagsbuchhandlung, Stuttgart.

Cáceres, C.E. 1997a. Dormancy in invertebrates. *Invert. Biol.* **116**: 371–383.

Cáceres, C.E. 1997b. Temporal variation, dormancy and coexistence: a field test for the storage effect. *Proc. Nat. Acad. Sci. USA* **94**: 9171–9175.

Carmona, M.J., Serra, M. & Miracle, M.R. 1993. Relationships between mixis in *Brachionus plicatilis and* preconditioning of the culture medium by crowding. *Hydrobiologia* **255/256**: 145–152.

Carvalho, G.R. 1994. Genetics of aquatic clonal organisms. In: *Genetics and Evolution of Aquatic Organisms* (A.R. Beaumont, ed.), pp. 291–323. Chapman & Hall, London.

Carvalho, G.R. & Crisp, D.J. 1987. The clonal ecology of *Daphnia magna* (Crustacea, Cladocera).1. Temporal changes in the clonal structure of a natural-population. *J. Anim. Ecol.* **56**: 453–468.

Chaplin, J.A. & Ayre, D.J. 1997. Genetic evidence of widespread dispersal in a parthenogenetic freshwater ostracod. *Heredity* **78**: 57–67.

Cousyn, C., De Meester, L., Colbourne, J.K., Brendonk, L., Verschuren, D. & Volkaert, F. 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in absence of neutral genetic changes. *Proc. Nat. Acad. Sci. USA* **98**: 6256–6260.

Cox, A.J. & Hebert, P.D.N. 2001. Colonisation, extinction and phylogeographic patterning in a freshwater crustacean. *Mol. Ecol.* **10**: 371–386.

Crease, T.J., Lee, S.-L., Yu S.-K., Spitze, K. & Lehman, N. & Lynch, M. 1997. Allozyme and mtDNA variation in populations of the *Daphnia pulex* complex from both sides of the Rocky Mountains. *Heredity* **79**: 242–251.

Darwin, C.R. 1859. *The Origin of Species by Means of Natural Selection*. John Murray, London.

De Meester, L. 1996a. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience* **3**: 385–399.

De Meester, L. 1996b. Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, *Daphnia magna*. *Evolution* **50**: 1293–1298.

De Meester, L. & Vanoverbeke, J. 1999. An uncoupling of male and sexual egg production leads to reduced inbreeding in the cyclical parthenogen *Daphnia. Proc. Royal Soc. London. Series B.* **266**: 2471–2477.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science* **265**: 1084–1086.

Estoup, A. & Angers, B. 1998. Microsatellites and Minisatellites for Molecular Ecology. In: *Advances in Molecular Ecology* (G.R. Carvalho, ed.), pp. 55–86. IOS Press, Amsterdam.

Ferrari, D.C. & Hebert, P.D.N. 1982. The induction of sexual reproduction in *Daphnia magna* – genetic differences between arctic and temperate populations. *Can. J. Zool.* **60**: 2143–2148.

Gießler, S. 1997. Gene flow in the *Daphnia longispina* hybrid complex (Crustacea, Cladocera) inhabiting large lakes. *Heredity* **79**: 231–241.

Gilbert, J.J. 1974. Dormancy in rotifers. *Trans. Am. Microsc. Soc.* **93**: 490–513.

Gilbert, J.J. 1993. Rotifera. In: *Reproductive Biology of Invertebrates*, Vol. VI, Part A. *Asexual Propagation and Reproductive Strategies.* (K.G. Adiyodi & R.G. Adiyodi, eds), pp. 231–263. Oxford & IBH Publishing Co, New Delhi, India.

Goldstein, D.B. & Schlötterer, C. 1999. *Microsatellites: Evolution and Applications*. Oxford University Press, Oxford.

Gómez, A. 1996. *Ecological genetics and mate recognition systems in sympatric rotifer populations*. PhD Thesis. Universitat de Valèncía, València.

Gómez, A. & Carvalho, G.R. 2000. Sex, parthenogenesis and the genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Mol. Ecol.* **9**: 203–214.

Gómez, A. & Snell, T.W. 1996. Sibling species in the *Brachionus plicatilis* species complex. *J. Evol. Biol.* **9**: 953–964.

Gómez, A., Temprano, M. & Serra, M. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *J. Evol. Biol.* **8**: 601–622.

Gómez, A., Carmona, M.J. & Serra, M. 1997. Ecological factors affecting gene flow in the *Brachionus plicatilis* species complex (Rotifera). *Oecologia* **111**: 350–356.

Gómez, A., Clabby, C. & Carvalho, G.R. 1998. Isolation and characterisation of microsatellite loci in a cyclically parthenogenetic rotifer, *Brachionus plicatilis. Mol. Ecol.* **7**: 1619–1621.

Gómez, A., Carvalho, G.R. & Lunt, D.H. 2000. Phylogeography and regional endemism of a passively dispersing zooplankter: mtDNA variation of rotifer resting egg banks. *Proc. Royal Soc. Lond. B.* **267**: 2189–2197.

Goudet, J. 1999. PCA-GEN for windows V. 1.2. [WWW document]. URL *http://www.unil.ch/izea/softwares/pcagen.html*

Goudet, J. 2000. *FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices* Version 2.9.1. Updated from Goudet 1995). [WWW document] http://www.unil.ch/izea/softwares/fstat.html

Hairston, N.G. 1996. Zooplankton egg banks as biotic reservoirs in changing environments. *Limnol. Oceanogr.* **41**: 1807–1092.

Hairston, N.G. & Cáceres, C.E. 1996. Distribution of crustacean diapause: Micro and macroevolutionary pattern and process. *Hydrobiologia* **320**: 27–44.

Hairston, N.G. & De Stasio, B.T. Jr. 1988. Rate of evolution slowed by a dormant propagule pool. *Nature* **336**: 239–242.

Hairston, N.G. & Olds, E.J. 1984. Population differences in the timing of diapause: adaptation in a spatially heterogeneous environment. *Oecologia* **61**: 42–48.

Hairston, N.G. & Olds, E.J. 1987. Population differences in the timing of diapause: a test of hypotheses. *Oecologia* **71**: 339–344.

Hebert, P.D.N. 1998. Variable environments and evolutionary diversification in inland waters. In: *Advances in Molecular Ecology* (G.R. Carvalho, ed.), pp. 267–290. NATO Science Series, IOS Press, Amsterdam.

Hebert, P.D.N. & Moran, C. 1980. Enzyme variability in natural populations of *Daphnia carinata* King. *Heredity* **45**: 313–321.

Hedrik, P.W. 2000. *Genetics of Populations*, 2nd edn. Jones & Bartlett, Boston.

Hobæk, A. & Larsson, P. 1990. Sex determination in *Daphnia magna*. *Ecology* **71**: 2255–2268.

Hutchison, D.W. & Templeton, A.R. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**: 1898–1914.

Innes, D.J. 1991. Geographic patterns of genetic differentiation among sexual populations of *Daphnia pulex*. *Can. J. Zool.* **69**: 995–1003.

Jarne, P. & Lagoda, P.J.L. 1996. Microsatellites, from molecules to populations and back. *Trends Ecol. Evol.* **11**: 424–429.

Jenkins, D.G. 1995. Dispersal-limited zooplankton distribution and community composition in new ponds. *Hydrobiologia* **313**: 15–20.

Lynch, M. 1987. The consequences of fluctuating selection for isozyme polymorphism in *Daphnia*. *Genetics* **115**: 657–669.

Lynch, M. & Crease, T.J. 1990. The analysis of population survey data on DNA sequence variation. *Mol. Biol. Evol.* **7**: 377–394.

Lynch, M., Pfrender, M., Spitze, K., Lehman, N., Hicks, J., Allen, D., Latta, L., Ottene, M., Bogue, F. & Colbourne, J. 1999. The quantitative and molecular genetic architecture of a subdivided species. *Evolution* **53**: 100–110.

Lynch, M. & Spitze, K. 1994. Evolutionary genetics of *Daphnia*. In: *Ecological Genetics* (L.A. Real, ed.) Princeton University Press, Princeton, NJ, pp. 109–128.

Marcus, N.H., Lutz, R., Burnett, W. & Cable, P. 1994. Age, viability and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. *Limnol. Oceanogr.* **39**: 154–158.

Michalakis, Y. & Excoffier, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* **142**: 1061–1064.

Naihong, X., Audenaert, E., Vanoverbeke, J., Brendonk, L., Sorgeloos, P. & De Meester, L. 2000. Low among-population genetic differentiation in Chinese bisexual *Artemia* populations. *Heredity* **84**: 238–243.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nogrady, T., Wallace, R.L. & Snell, T.W. 1993. Rotifera, Vol. 1: biology, ecology and systematics. In: *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World* (H.J.F. Dumont, eds). SPB Academic Publishing bv, Le Hague.

van Oppen, M.J.H., Rico, C., Deutsch, J.C., Turner, G.F. & Hewitt, G.M. 1997. Isolation and characterisation of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Mol. Ecol.* **6**: 387–388.

Ortells, R., Snell, T.W., Gómez, A. & Serra, M. 2000. Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Arch. Hydrobiol.* **149**: 529–551.

Pálsson, S. 2000. Microsatellite variation in *Daphnia pulex* from both sides of the Baltic sea. *Mol. Ecol.* **9**: 1075–1088.

Pálsson, S. 2001. The effect of deleterious mutations in cyclically parthenogenetic organisms. *J. Theor. Biol.* **208**: 201–214.

Pourriot, R. & Snell, T.W. 1983. Resting eggs in rotifers. *Hydrobiologia* **104**: 213–224.

Raymond, M. & Rousset, F. 1995. GENEPOP: a population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Reid, V., Carvalho, G.R. & George, D.G. 2000. Molecular genetic analysis of *Daphnia* in the English lake district: species identity, hybridisation and resting egg banks. *Freshwat. Biol.* **43**: 247–254.

Rice, W.R. 1989. Analysing tables of statistical tests. *Evolution* **43**: 223–225.

Schneider, S., Roessli, D. & Excoffier, L. 2000. *Arlequin*, Version 2.000: *A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory*. University of Geneva, Switzerland. [WWW document] URL. http://anthro.unige.ch/arlequin

Serra, M., Gómez, A. & Carmona, M.J. 1998. Ecological genetics of *Brachionus* sibling species. *Hydrobiologia* **387/388**: 373–384.

Snell, T.W., Burke, B.E. & Messur, S.D. 1983. Size and distribution of resting eggs in a natural population of the rotifer *Brachionus plicatilis*. *Gulf Res. Rep.* **7**: 285–287.

Straughan, D.J. & Lehman, N. 2000. Genetic differentiation among Oregon lake populations of the *Daphnia pulex* species complex. *J. Hered.* **9**: 8–17.

Swofford, D.L. & Selander, R.B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**: 281–283.

Taylor, D.J., Finston, T.L. & Hebert, P.D.N. 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution* **52**: 1648–1670.

Templeton, A.R. 1998. Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* **7**: 381–397.

Weider, L.J., Hobæk, A., Colbourne, J.K., Crease, T.J., Dufresne, F. & Hebert, P.D.N. 1999a. Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in Arctic *Daphnia*. *Evolution* **53**: 777–792.

Weider, L.J., Hobæk, A., Hebert, P.D.N. & Crease, T.J. 1999b. Holarctic phylogeography of an asexual species complex II. Allozyme variation and clonal structure in Arctic *Daphnia*. *Mol. Ecol.* **8**: 1–13.

Weir, B.S. & Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

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Appendix 1 Allele frequencies and sample sizes for seven microsatellite loci in the 14 *Brachionus plicatilis* populations.

Allele	6TON	6TOS	6TUR	6CLO	6HOS	1ERA	2GAL	2SA2	2CHI	3MAN	4SAL	4MOJ	4PET	4SLD
Bp3(N)	55	155	9	27	13	14	36	20	10	8	8	17	12	18
113	0	0.113	0.389	0.019	0.385	0	0	0	0	0	0	0	0	0
140	0	0	0.556	0.056	0.423	1	0.861	0.975	1	1	1	0.794	1	0.889
143	0.582	0.297	0	0.537	0.154	0	0.139	0	0	0	0	0.206	0	0.111
152	0.418	0.581	0.056	0.389	0.038	0	0	0	0	0	0	0	0	0
158	0	0	0	0	0	0	0	0.025	0	0	0	0	0	0
161	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0
Bp2(N)	55	134	8	27	14	11	36	19	8	10	9	15	15	20
115	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	0.127	0	0.444	0.429	0.091	0.542	0.079	1	0.05	0	0.567	0.133	0.55
137	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0.864	0.821	1	0.204	0.464	0	0.319	0.737	0	0	0.167	0.167	0.433	0.35
149	0	0	0	0.259	0.071	0.364	0.056	0.026	0	0	0	0	0	0
152	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0.025
155	0	0.004	0	0	0	0	0	0	0	0	0	0	0	0
158	0.109	0.049	0	0	0.036	0.545	0.069	0.158	0	0.05	0.611	0	0.233	0.075
167	0	0	0	0.074	0	0	0	0	0	0.1	0.222	0	0.167	0
176	0	0	0	0.019	0	0	0	0	0	0	0	0	0	0
184	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0
186	0	0	0	0	0	0	0	0	0	0.8	0	0.267	0.033	0
Bp1b(N)	54	155	11	26	15	15	36	20	10	16	8	19	14	19
238	0.056	0.01	0	0.038	0.1	0	0.236	0	0	0	0	0	0	0
244	0.787	0.755	0.591	0.5	0.533	0	0	0	0	0	0	0	0	0
247	0.139	0.016	0	0	0	0	0	0	0	0	0	0	0	0
250	0.019	0.219	0.409	0.192	0.133	0	0	0	0	0	0	0	0	0
253	0	0	0	0.269	0.233	0.767	0.653	1	0.95	1	1	1	1	1
256	0	0	0	0	0	0	0.111	0	0	0	0	0	0	0
259	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0
277	0	0	0	0	0	0.233	0	0	0	0	0	0	0	0
Bp4a (N)	55	153	9	27	15	15	35	19	10	16	10	19	14	18
174	0	0	0.611	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0.019	0.333	0.2	0.057	0.289	0	0	0	0	0	0
183	0	0	0.111	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0.05	0	0	0	0	0
192	0	0	0	0	0	0.8	0.129	0	0	0	0	0	0	0
198	0.427	0.17	0	0	0	0	0.029	0.053	0.95	0.688	1	1	1	1
201	0	0	0	0.389	0	0	0	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0.786	0.632	0	0.094	0	0	0	0
213	0.264	0.252	0.222	0.241	0.433	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0.026	0	0.219	0	0	0	0
222	0.082	0.542	0	0	0	0	0	0	0	0	0	0	0	0
228	0.082	0.01	0.056	0.352	0.233	0	0	0	0	0	0	0	0	0

237	0.145	0.026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bp6b(N)	54	153	8	27	14	13	36	20	10	17	10	16	15	20		
116	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0		
118	0	0	0	0	0	0	0.153	0	0	0	0	0	0	0		
121	0.88	0.915	0.25	0.407	0.571	0	0.292	0.125	0	0.324	0.25	0.875	0.733	1		
124	0.028	0	0.75	0.056	0	0.538	0	0	0.2	0.559	0.4	0	0.133	0		
127	0.093	0.085	0	0.537	0.429	0.462	0.556	0.875	0.8	0.118	0	0.125	0.133	0		
136	0	0	0	0	0	0	0	0	0	0	0.15	0	0	0		
Bp3c(N)	53	56	9	27	14	13	35	20	10	15	9	19	15	20		
172	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0		
187	0	0	0	0	0	0	0	0	0.85	0	0	0.184	0	0.1		
190	0	0	0	0.185	0	0.846	0.043	0	0	0	0	0.132	0	0.25		
193	0.481	0.366	0	0.222	0.464	0	0	0	0	0.333	0	0.184	0	0		
196	0.179	0.143	0.278	0.185	0.286	0	0	0	0	0	0	0	0	0		
199	0	0	0	0.148	0	0	0.957	1	0.15	0.033	0.278	0.316	0.1	0		
202	0.009	0.027	0	0	0	0	0	0	0	0.567	0	0.105	0.3	0.3		
205	0.321	0.464	0.722	0.241	0.107	0	0	0	0	0.067	0.722	0.079	0.6	0.35		
211	0	0	0	0	0.036	0	0	0	0	0	0	0	0	0		
220	0	0	0	0.019	0.107	0	0	0	0	0	0	0	0	0		
223	0	0	0	0	0	0.115	0	0	0	0	0	0	0	0		
229	0	0	0	0	0	0.038	0	0	0	0	0	0	0	0		
Bp5d(N)	55	147	7	27	14	15	34	19	8	14	9	18	15	17		
230	0	0	0	0.019	0	0	0	0	0	0	0	0	0	0		
233	0	0	0	0	0	0	0.088	0	0	0	0	0	0	0		
236	0.2	0.48	0.929	0.13	0.214	0	0.162	0.026	0.313	0.75	1	0.417	0.6	0.676		
239	0.682	0.463	0.071	0.148	0.643	0	0	0	0	0.071	0	0.111	0	0		
242	0.118	0.058	0	0.074	0.143	1	0.75	0.974	0.688	0.179	0	0.361	0.4	0.324		
245	0	0	0	0.63	0	0	0	0	0	0	0	0.111	0	0		