Strong intraspecific variation in genetic diversity and genetic differentiation in *Daphnia magna*: the effects of population turnover and population size

B. WALSER*† and C. R. HAAG*†

Department of Biology, Section Ecology and Evolution, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland, †Tvärminne Zoological Station, SF-10900 Hanko, Finland

Abstract

Theory predicts that genetic diversity and genetic differentiation may strongly vary among populations of the same species depending on population turnover and local population sizes. Yet, despite the importance of these predictions for evolutionary and conservation issues, empirical studies comparing high-turnover and low-turnover populations of the same species are scarce. In this study, we used *Daphnia magna*, a freshwater crustacean, as a model organism for such a comparison. In the southern/central part of its range, *D. magna* inhabits medium-sized, stable ponds, whereas in the north, it occurs in small rock pools with strong population turnover. We found that these northern populations have a significantly lower genetic diversity and higher genetic differentiation compared to the southern/central populations. Total genetic diversity across populations was only about half and average within-population diversity only about a third of that in southern/central populations. Moreover, an average southern population contains more genetic diversity than the whole metapopulation system in the north. We based our analyses both on silent sites and microsatellites. The similarity of our results despite the contrasting mutation rates of these markers suggests that the differences are caused by contemporary rather than by historical processes. Our findings show that variation in population turnover and population size may have a major impact on the genetic diversity and differentiation of populations, and hence may lead to differences in evolutionary processes like local adaptation, hybrid vigour and breeding system evolution in different parts of a species range.

Keywords: Population turnover, genetic diversity, genetic differentiation, *Daphnia magna*

Introduction

Geographically widespread species have to adapt to different types of habitats. As a consequence, population turnover and local population sizes may strongly vary throughout a species range. This can lead to substantial differences in genetic diversity and genetic differentiation within the same species. In fragmented habitats, populations are subdivided and only connected by migration. This may increase the extinction risk of local subpopulations (Hanski 1999), in some cases leading to

turnover dynamics where local extinction and recolonization alternate. Theoretical studies predict that metapopulations with strong turnover dynamics have a reduced genetic diversity compared to stable and structurally uniform populations owing to restricted gene flow and genetic bottlenecks during colonization. In addition, turnover dynamics are predicted to increase differentiation among subpopulations because of genetic drift and strong founder effects (McCauley 1991; Boileau *et al.* 1992; Hastings & Harrison 1994; Harrison & Hastings 1996; Hanski & Gilpin 1997; Hanski 1999; Pannell & Charlesworth 1999, 2000).

Empirical studies of metapopulations have yielded some support for these predictions. In insects, Saccheri

Correspondence: Barbara Walser, Fax +41 26 300 96 98;
E-mail: barbara.walser@unifr.ch

et al. (1998) showed a correlation between heterozygosity and extinction rate in a metapopulation system of a butterfly, and Massonnet *et al.* (2002) found high extinction rates and strong degrees of genetic differentiation between local populations of aphids. Freeland *et al.* (2000) found evidence for a metapopulation existing over a large spatial scale in a freshwater bryozoan. Other studies show a decrease in genetic differentiation and an increase in genetic diversity with increasing time since colonization (Giles & Goudet 1997; Haag *et al.* 2005). This supports the idea that turnover decreases genetic diversity and increases genetic differentiation, and that these effects are alleviated by subsequent gene flow. However, differences in genetic diversity and genetic differentiation between high-turnover and low-turnover populations of the same species have rarely been investigated, even though many species show fragmented populations and turnover dynamics only in parts of their distribution.

One species that exists in such contrasting systems is the small freshwater crustacean *Daphnia magna*. It is widely distributed in Europe, and in most of its range, it inhabits medium-sized, stable ponds. In contrast, northern populations mainly occur in small rock pools along the coast. Ecological and demographic studies in the archipelago of southern Finland have shown that these populations exhibit strong turnover dynamics with high rates of extinction and recolonization (Ranta 1979; Hanski & Ranta 1983; Pajunen 1986; Pajunen & Pajunen 2003). Genetic studies of the same metapopulation found strong founder effects and restricted gene flow both between ponds on the same island as well as between ponds on different islands (Haag *et al.* 2006). In contrast to these high-turnover populations in the north, no turnover dynamics have been reported for the populations of southern and central Europe (Vanoverbeke & De Meester 1997; Vanoverbeke *et al.* 2007).

To investigate potential differences in genetic diversity and genetic differentiation between these two systems, we sampled populations across a wide geographical range in Europe. Our sampling includes both populations from rock pools as well as from medium-sized ponds. Habitat ecology and geographical site are strongly correlated in *D. magna*, as rock pool populations with high turnover are found in northern Europe, whereas large, non-turnover populations are found in southern and central Europe. Because geography also correlates with postglacial colonization history, it is important to distinguish between contemporary and historic influences when comparing genetic diversity and differentiation between the two systems (Hewitt 1999; Petit *et al.* 2003; Obbard *et al.* 2006). In our study, this was particularly important because both turnover and potential bottlenecks during postglacial colonization are predicted to lead to reduced diversity in the north.

However, equilibration times of genetic diversity after a bottleneck may differ substantially among loci because for populations that expand after a genetic bottleneck, the time to reach equilibrium depends mostly on the influx of new mutations (Crow & Aoki 1984; Pannell & Charlesworth 1999), which varies among loci. The maximum time to go halfway to a new equilibrium ($t_{0.5}$) is approximately $\ln(2)/[2uK/(K-1)]$, where u is the mutation rate and K is the number of alleles (Crow & Aoki 1984). This is a maximum estimate, as the time can be shorter, depending on effective population sizes N and number of populations n ($t_{0.5} = \ln(2)/[2uK/(K-1) + 1/(2Nn)]$ if the term $1/(2Nn)$ cannot be neglected (Crow & Aoki 1984), migration rates m among populations ($t_{0.5} = \ln(2)/[2uK/(K-1) + 2m/(n-1)]$ if $m \ll 1/N$ and if the term $2m/(n-1)$ cannot be neglected (Crow & Aoki 1984), and immigration of alleles from outside the newly colonized region (which are an additional source of genetic variation similar to new mutations, Allendorf & Luikart 2007). The equilibration time is also much shorter if colonization leads to a permanent reduction in the effective population size (rather than to a bottleneck with subsequent expansion) or if turnover rate is increased in the new habitat (Pannell & Charlesworth 1999).

If indeed historical events during postglacial colonization are still the main determinants of today's genetic diversity patterns, then $t_{0.5}$ should not be much shorter than the timing of postglacial colonization. To estimate maximum equilibration time, we used two types of genetic markers with strongly contrasting mutation rates: silent sites of housekeeping genes and microsatellites. Even though their exact mutation rates are not known for *D. magna*, estimates exist for other invertebrates. Assuming a microsatellite mutation rate of $u = 7.11 \times 10^{-5}$ as estimated for *Daphnia pulex* (Seyfert *et al.* 2008) and $K = 10$ alleles per locus, maximum $t_{0.5} = 8912$ generations or 1800 years, assuming a minimum of five generations per year (Ebert 2005). We used conservative estimates both for mutation rate (Ellegren 2000; Seyfert *et al.* 2008) and for numbers of generations per year; hence, the true equilibration time for microsatellites may be even shorter. In contrast, $t_{0.5} = 34.7$ million generations (about 7 million years) for silent sites, assuming a mutation rate of $u = 10^{-8}$ (Haag-Liautard *et al.* 2007) and $K = 2$ alleles per locus. It is unknown when *D. magna* reached northern Europe, but the likely time window of colonization can be inferred from the European postglacial colonization history. Around 20 000 years ago, when the ice cover started to retreat (Clark *et al.* 2009), parts of southern/central Europe were already ice-free and the recolonization of the species was in progress (Hewitt 2000). The large-scale

recession of the Scandinavian ice sheet, however, started only 14 600 years ago (Rinterknecht *et al.* 2006), and it is thus unlikely that *D. magna* colonized the southern margins of this region long before this moment and the spread further north occurred probably even more recently. However, given their rapid colonization of empty habitats and treeless islands (Pajunen & Pajunen 2003, 2007; Louette & De Meester 2004), it is likely that postglacial colonization started soon afterwards and was completed before 7800 years ago when the trees reached northernmost Scandinavia (Willis *et al.* 1998).

Comparing the maximum equilibration times with the likely timing of postglacial colonization, it appears possible that genetic signatures of this colonization are still detectable in the silent sites, but microsatellites should have had time to substantially recover from a bottleneck. Hence, if patterns of genetic diversity were still influenced by postglacial colonization history, we would expect to see dissimilar patterns between the two types of markers, with a stronger difference in genetic diversity between the northern and the southern/central populations in the silent site data compared to the microsatellite data. In contrast, if the two types of markers showed similar patterns of diversity between the regions, this would suggest that both have had time to approach a new equilibrium and hence that patterns of genetic diversity are mainly influenced by contemporary rather than historical processes.

In the current study, we investigated differences in the genetic population structure between metapopulations and non-metapopulations of *D. magna* from different geographical regions. Understanding intraspecific differences in the genetic population structure is important in order to understand potentially different outcomes of evolutionary processes (e.g. the efficacy of selection) in different parts of a species distribution as well as for conservation issues. However, when comparing populations on a large scale, it is important to take into account potential confounding factors owing to differences in colonization history. By using two types of markers with contrasting mutation rates, we made an attempt to disentangle historical vs. contemporary processes—an approach that might be applicable also to other geographically widespread species with sufficiently small generation times.

Materials and methods

Study system

Daphnia magna is a small, filter-feeding freshwater crustacean. It reproduces by cyclical parthenogenesis where phases of clonal reproduction are intermitted by sexual reproduction, the latter leading to the production of drought-resistant and freezing-resistant resting eggs (Hobaek & Larsson 1990; Kleiven *et al.* 1992). These resting eggs are also thought to be the main dispersal and colonization stage because they can be carried by wind, water or birds (Ranta 1979; Figuerola *et al.* 2003, 2005). Owing to clonal reproduction, colonization even by a single resting egg is sufficient to establish a population and populations have the potential to grow rapidly during the clonal phase. *D. magna* is widely distributed in the northern hemisphere and occurs in different types of habitats. In northern Europe, populations exist in highly dynamic metapopulation systems and have estimated yearly extinction rates of 17% (Bengtsson 1989; Pajunen & Pajunen 2003). These populations do not have long-lasting resting egg banks (as they tend to lose their sediment owing to ice movements and winter storms), and the likelihood of recolonization of a pond depends strongly on the distance to the nearest pond occupied by *D. magna* (Pajunen & Pajunen 2003). This indicates that most recolonizations take place by immigration from other ponds rather than by hatching of a few remaining local resting eggs. In contrast, the southern and central European populations are mainly stable (Vanoverbeke *et al.* 2007). The current study only includes populations with at least one sexual cycle per year to avoid potentially confounding effects of strong clonal selection, which are possible in populations with permanent growing conditions (De Meester *et al.* 2006).

Sampling design

We investigated a total of 71 *Daphnia magna* individuals from 12 populations (Table 1, Figure S1 in supporting information) at 32 microsatellite loci and six housekeeping genes. Our design followed theoretical recommendations (Nei 1987; Kalinowski 2005) that suggest to maximize numbers of loci rather than numbers of individuals to optimize power for the estimates of genetic differentiation when differentiation is strong, as it is expected in *Daphnia* (Vanoverbeke & De Meester 1997; Haag *et al.* 2005; Thielsch *et al.* 2009). Seven of these 12 populations originated from rock pools in northern Europe and five populations originated from medium-sized ponds in southern and central Europe (Table 1).

Individuals were hatched from resting eggs or were sampled just after hatching in nature. As resting eggs are produced sexually in *D. magna*, our sample does not include copies of the same clone. The individuals were used to establish clonal lines by placing them singly into 300 ml of artificial *Daphnia* medium (Klüttgen *et al.* 1994) and allowing them to reproduce clonally. The cultures were fed daily with unicellular algae (*Scenedesmus* sp.).

Table 1 List of the 12 *Daphnia magna* populations used in this study

Abbreviation	Locality	N	Latitude	Longitude	System
AST	Astrakhan, Russia	5	N45°54′13.00″	E47°39′23.00″	Southern/Central
BOL	Bolshoi Asafiy, Russia	6	N66°25′30.00″	E33°50′00.00″	Northern
ISM	Ismaning, Germany	10	N48°12′24.94″	E11°42′12.55″	Southern/Central
K	Tvärminne (Island K), Finland	5	N59°49′25.62″	E23°15′09.30″	Northern
KMG	Tvärminne (Island Kummelgrundet), Finland	5	N59°49′18.54″	E23°12′20.04″	Northern
LVR	Langerodevijver, Belgium	2	N50°49′42.08″	E04°38′20.60″	Southern/Central
MOS	Moscow, Russia	6	N55°45′48.65″	E37°34′54.00″	Southern/Central
N	Tvärminne (Island Storgrundet), Finland	10	N59°49′19.62″	E23°15′38.28″	Northern
SE1	Tvärminne (Island Segelskär), Finland	6	N59°45′48.00″	E23°22′24.30″	Northern
SOE	Östhammar (Söderboda hamn), Sweden	5	N60°26′55.56″	E18°23′12.78″	Northern
SRO	West of Pisa, Italy	5	N43°41′31.00″	E10°17′20.00″	Southern/Central
VR1	Vääränmaanruskea, Finland	6	N60°16′17.82″	E21°53′46.74″	Northern

N, number of individuals.

Sequencing of housekeeping genes

We extracted genomic DNA from 5 to 10 genetically identical individuals per line using the DNeasy blood and tissue kit (Qiagen) and sequenced single amplicons (439–672 bp) from six genes including four metabolic enzymes, a translation initiation factor and a nuclear receptor protein (Table 2). Primers (Table 2) had either been designed earlier (Haag *et al.* 2009) or were modified and newly designed with Primer3 (Rozen & Skaletsky 2000). PCR reactions were performed according to the standard protocols using TopTaq polymerase (Qiagen), and cleaned PCR products were directly sequenced (Macrogen Inc., South Korea). The sequences are deposited on GenBank (Accession numbers see Table S1 in supporting information). Despite several repetitions including new DNA extractions, we failed to obtain high-quality sequences for a few individuals and thus had to exclude these from the analyses (*Got:* one individual from BOL; *Ldh:* one individual from AST; *Usp:* three individuals from BOL, two from AST). We cannot exclude that this has induced a slight bias in diversity estimates for populations BOL and AST, but the results for these three loci were very similar to the results for the loci at which all individuals were successfully amplified.

Microsatellite genotyping

We genotyped all individuals at 32 microsatellite markers assembled in four multiplex sets (Molecular Ecology Resources Primer Development Consortium *et al.* 2010). We set-up reactions of 10μL, using the Type-it Multiplex PCR kit (Qiagen) with cycling conditions according to the recommendations of the manufacturer. Fragments were run on an ABI Prism 3130 Genetic Analyser (Applied Biosystems), and fragment lengths were ana-

lyzed using GeneMapper Software version 4.0 (ABI Prism) with Gene Scan-500 LIZ as size standard. Ambiguous genotypes were repeated. Despite two attempts, we were unable to obtain clear microsatellite genotypes for one individual from population BOL (probably due to low DNA quality) and thus excluded this individual from the analyses (microsatellite genotypes see Table S2 in supporting information).

Analysis of sequence data

Sequences were aligned and manually edited using Sequencher version 4.8 (Gene Codes) and BioEdit (Hall 1999), and primer sequences were removed. We used DnaSP version 5.00.06 (Librado & Rozas 2009) to analyze diversity based on pairwise differences (π) or the number of segregating sites (θ). We used subscripts to designate diversity indices that are based on all sites (*all*) or only on silent sites (*sil*, non-coding and synonymous combined). In addition, we used subscripts to distinguish total nucleotide diversity across populations (π_T), average within-population nucleotide diversity (π_S), the between-population component of diversity (π_{T-S}, a measure of the absolute degree of population differentiation), the divergence between pairs of haplotypes sampled from two different populations (π_B) and π_D, the difference between π_B and π_S (Charlesworth 1998). All the latter estimates were based on silent sites only and were estimated for each of the two groups (northern and southern/central populations) separately. We used DnaSP to estimate π_T by pooling individuals from all populations within each of the two groups. As sample size does not correspond with population size in our study, we weighted populations equally as suggested by Charlesworth (1998) by using ten sequences from each population, which were randomly chosen if more sequences were available. For *Ldh*, we had only

Table 2 List and general properties of the six nuclear loci analyzed

Locus	Abbreviation	Characterization	Primer forward 5' > 3'	Primer reverse 5' > 3'	References	Average sequence length (bp)	Polymorphic sites
Eukaryotic translation initiation factor 2γ	*Eif*	Translation initiation factor	TGCGCTTCAAAAATGAGTTG	TGAGCCGAGATTGGGATAAC	Haag *et al.* 2009	615	13
Enolase	*Eno*	Metabolic enzyme	ATTGCTGATTTGGCTGGAAC	CAATTGAGACCATGGGGAAC	Haag *et al.* 2009	631	18
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	Metabolic enzyme	CAATGGTCACCACATTCAGG	AATATTTTGGGCAGCACCAC	Haag *et al.* 2009	439	14
Glutamine-oxaloacetic transaminase	*Got*	Metabolic enzyme	AAACCTTGGAGTTGGGAGGT ACA	CGATCCAAATCTCCAGATGC	Newly designed	581	14
L-lactate dehydrogenase	*Ldh*	Metabolic enzyme	ACTGGCATTGGTTGATGTGA	ATTGCCCAAGAAGTGTGTCC	Newly designed	672	8
Ultraspiracle	*Usp*	Nuclear receptor protein	ACAATTCCAATGGCTCCAAG	TTGACTCCAACAGAGCGATG	Newly designed	641	18

eight sequences per population and for *Usp* only six. We discarded the population LVR from these analyses because we had only four sequences per locus from this population. Additionally, we used averaged estimates of π_{sil} to estimate π_S. Here, we included all sequences and all populations. The indices π_{T-S}, π_D and π_B were estimated as described in Charlesworth (1998) and used to compute a π-based estimate of F_{ST} (equation 3a in Charlesworth 1998). All indices (including the F_{ST} estimate) were estimated separately for each locus and then averaged across loci, giving equal weight to each locus.

To test whether F_{ST} differed between the two groups (more precisely, whether F_{ST} among populations within groups differed between the two groups), we used the software FSTAT 2.9.3 (Goudet 2001) and performed a permutation test (10 000 permutations) by randomly assigning populations either to the north or to the south/central group while keeping the number of populations per group constant. We did this separately for each locus, weighing populations equally by using the same data as above, and then combined *P*-values of the different loci according to Fisher (1925). To make sure that the same hypothesis was tested for each locus, we used a one-sided test, assessing whether differentiation among northern populations was significantly higher than among southern/central populations, but an approximate *P*-value for a two-sided test can easily be obtained by multiplying the one-side *P*-value by two. The estimation of F_{ST} in FSTAT treats each polymorphic site as a separate locus, which essentially assumes free recombination between sites. Despite the fact that this assumption is almost certainly violated within each locus, this is a problem only for testing $F_{ST} > 0$, not for estimating F_{ST} (Hudson *et al.* 1992). We used this method only to estimate F_{ST} in the real sample of northern and southern/central populations and in many permuted samples (as described above), but did not test whether F_{ST} within each group was significantly larger than zero. The latter was shown convincingly in the microsatellite data (see below). Hence, the permutation test implemented in FSTAT provided a convenient way to test for differences in within-group differentiation between the two groups, even for the nucleotide diversity.

Analysis of microsatellite data

We used FSTAT to estimate pairwise F_{ST} and expected heterozygosity (H_S), an estimate of genetic diversity robust to small sample size (Nei 1978, 1987). Additionally, we used FSTAT to calculate the overall expected heterozygosity per group (H_T). We estimated all parameters for each population and compared them between the two groups of populations (10 000 permutations,

two-tailed tests). Additionally, we analyzed isolation by distance using Mantel tests and by plotting $F_{ST}/(1 - F_{ST})$ against the logarithm of geographic distance for each pair of populations (Rousset 1997).

Results

Marker polymorphism

Across the six loci, we obtained about 3550 base pairs of sequence per individual (Table 3). The number of polymorphic sites per locus ranged from 9 (*Ldh*) to 24 (*Usp*). Averaged across all loci, 90.3% of the polymorphisms were at silent sites (synonymous sites or non-coding sites) and 9.7% at non-synonymous sites (Table 3). 66 of the 71 individuals were heterozygous at least at one of the 32 microsatellite loci. The most polymorphic locus had 14 alleles across all populations, whereas three loci had only three alleles.

Genetic diversity within populations

Genetic diversity in housekeeping genes based on pairwise differences (π) averaged across loci and populations was 0.0022 and average θ based on the number of polymorphic sites was 0.0018. Calculated for silent sites only, π_{sil} was 0.0048 and θ_{sil} was 0.0041. The mean expected heterozygosity H_S within populations (micro-

satellite data) was 0.265 (Table 3). The two measures of within-population genetic diversity (π_{sil} based on silent sites and H_S based on microsatellites) were strongly correlated ($R^2 = 0.71565$, $P < 0.0005$), indicating clear and consistent differences in genetic diversity among populations (Figure 1). This implies that small sample size is not an important issue in our genetic diversity data.

Northern rock-pool populations had lower within-population nucleotide diversities and expected heterozygosities than southern/central non-rock-pool populations. Average within-population diversity was $\pi_{sil} = 0.0025$ for northern populations and $\pi_{sil} = 0.0080$ for southern/central populations based on silent site data, and $H_S = 0.135$ and $H_S = 0.448$ based on microsatellite data. In both cases, the difference between northern and southern/central populations was significant ($P = 0.014$ and $P = 0.002$, respectively, Table 3). Furthermore, total nucleotide diversity across all northern populations ($\pi_T = 0.0058$) was smaller than across southern populations ($\pi_T = 0.0104$, Table 4). The same was true for the comparable parameter H_T based on microsatellite data ($H_T = 0.414$ and $H_T = 0.689$, for northern and southern/central populations, respectively).

Genetic differentiation among populations

Genetic differentiation among northern populations as estimated with FSTAT ($F_{ST} = 0.699$, microsatellite data)

Table 3 Sequence length and measures of genetic diversity across the six nuclear loci, as well as average expected heterozygosity at 32 microsatellite loci, listed for each population

| Population | System | N | Sequence data | | | | | | | | | Microsatellite data |
| | | | Sequence length (nt) | | Polymorphic sites | | Genetic diversity | | | | | |
			Non-coding	Coding	S_{all}	S_{sil}	π_{all}	π_{sil}	θ_{all}	θ_{sil}		H_S
AST	Southern/Central	5	796	2700	36.0	32.0	0.0045	0.0103	0.0039	0.0091		0.682
ISM	Southern/Central	10	812	2748	30.0	28.0	0.0029	0.0063	0.0023	0.0052		0.456
LVR	Southern/Central	2	813	2748	23.0	20.0	0.0035	0.0073	0.0035	0.0073		0.359
MOS	Southern/Central	6	812	2745	27.0	24.0	0.0033	0.0073	0.0026	0.0058		0.446
SRO	Southern/Central	5	813	2818	26.0	25.0	0.0034	0.0087	0.0026	0.0065		0.296
Average		6	809	2752	28.0	26.0	0.0035	0.0080	0.0030	0.0068		0.448
BOL	Northern	6	812	2751	34.0	30.0	0.0038	0.0083	0.0032	0.0069		0.162
K	Northern	5	813	2751	8.0	7.0	0.0004	0.0008	0.0008	0.0013		0.154
KMG	Northern	5	813	2751	9.0	8.0	0.0012	0.0024	0.0009	0.0017		0.159
N	Northern	10	813	2751	9.0	8.0	0.0012	0.0024	0.0007	0.0013		0.137
SE1	Northern	6	817	2736	14.0	13.0	0.0016	0.0033	0.0015	0.0035		0.241
SOE	Northern	5	813	2751	1.0	1.0	0.0001	0.0001	0.0001	0.0002		0.017
VR1	Northern	6	813	2751	0.0	0.0	0.0000	0.0000	0.0000	0.0000		0.076
Average		6	813	2749	11.0	10.0	0.0012	0.0025	0.0010	0.0021		0.135

N, number of individuals; S_{all}, total number of segregating sites; S_{sil}, number of segregating silent sites; π_{all}, diversity based on pairwise differences at all sites; π_{sil}, diversity based on pairwise differences at silent sites; θ_{all}, diversity based on the number of all segregating sites; θ_{sil}, diversity based on the number of segregating silent sites; H_S, expected heterozygosity.
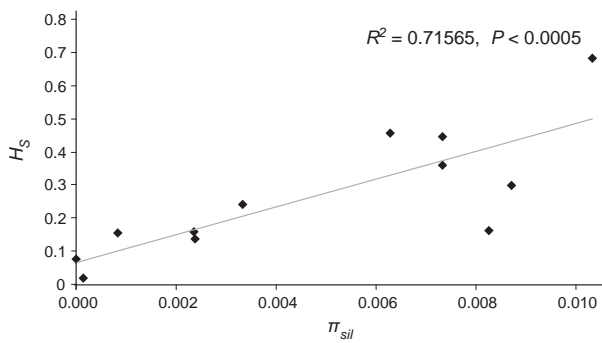
Fig. 1 Correlation between nucleotide diversity at silent sites of housekeeping genes (π_{sil}) and expected heterozygosity at microsatellites (H_S). Each point represents one population.

was significantly higher than among southern/central populations ($F_{ST} = 0.382$, $P = 0.003$, Table 4). The F_{ST} estimate based on π_S and π_T of the nucleotide diversity was similar to the one obtained for microsatellites (Table 4) and again significantly different between the two groups ($P = 0.044$). Isolation by distance based on microsatellite data was significant for the northern populations ($R^2 = 0.3946$, $P < 0.0023$) but not for the southern/central populations ($R^2 = 0.0743$, $P = 0.4461$, Figure 2).

Discussion

This study shows that *Daphnia magna* populations inhabiting rock pools in northern Europe have strongly reduced levels of genetic diversity and increased levels of genetic differentiation compared to populations inhabiting medium to large ponds in southern and central Europe. These findings are consistent with the theoretical expectations that high-turnover dynamics and reduced population size increase genetic drift and hence lead to reduced genetic diversity within subpopulations and increased differentiation among them. Our results are also congruent with other empirical studies of metapopulations. Massonnet *et al.* (2002) showed that frequent events of extinction and recolonization in the aphid species *Macrosiphoniella tanacetaria* lead to high differentiation even among very close populations. Several studies found a correlation between population age and the degree of differentiation, with younger populations being more differentiated than older ones (Giles & Goudet 1997; Haag *et al.* 2005; Garcia-Verdugo *et al.* 2010). This implies that the high degrees of genetic differentiation among the rock-pool populations reflect a high-turnover rate, which prevents subsequent gene flow to reduce the effects of colonization bottlenecks on the genetic population structure.

Table 4 Comparison of nucleotide diversity and F_{ST} among the two systems

System	Sequence data						Microsatellite data		
	π_T	π_{T-S}	π_S	π_B	π_D	F_{ST}	H_O	H_S	F_{ST}
Northern	0.0058	0.0034	0.0025	0.0064	0.0039	0.5221	0.081	0.135	0.699
Southern/Central	0.0104	0.0024	0.0080	0.0112	0.0032	0.2817	0.364	0.448	0.382
P						0.0220	0.002	0.002	0.003

π_T, total diversity; π_{T-S}, between-population component of diversity; π_S, mean within-population diversity; π_B, divergence between a pair of alleles sampled from two different populations; π_D, difference between π_B and π_S; H_O, observed heterozygosity; H_S, expected heterozygosity.
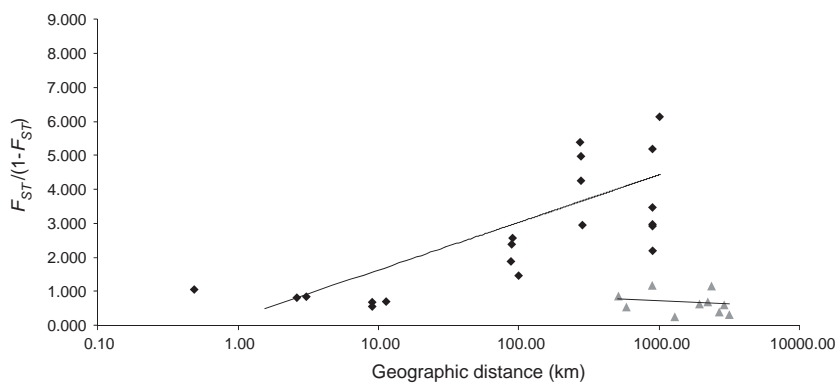

Fig. 2 Analysis of isolation by distance: $F_{ST}/(1 - F_{ST})$ against geographic distance (km) for each pair of populations within each group (black diamonds = northern populations; grey triangles = southern/central populations).

Our results are based on both silent sites and microsatellites, and the proportional differences in genetic diversity and in genetic differentiation were notably similar for the two types of markers, which differ strongly in mutation rate. The latter is reflected in the much lower absolute measures of genetic diversity in the silent site data compared with the microsatellite data. As explained in the introduction, the similarity of the results using markers that strongly differ in mutation rates implies that ongoing contemporary processes rather than historical factors are mainly responsible for the differences in genetic differentiation and genetic diversity between northern and southern/central populations.

Among the contemporary factors, both population size and turnover could play a role. Both have been shown to affect genetic diversity and genetic differentiation in *Daphnia* (Haag *et al.* 2006; Vanoverbeke *et al.* 2007). However, whereas reduced population size only leads to a reduction in the genetic diversity within populations (through genetic drift within populations), turnover may reduce the genetic diversity of the whole metapopulation system (through genetic drift across the entire system, Pannell & Charlesworth 1999, 2000). To evaluate these two possibilities, we compared the estimates of total diversity (π_T) and mean within-population diversity (π_S). According to Pannell & Charlesworth (1999), population turnover causes a reduction both in π_T and π_S, which is what we observed in our data: π_S in the northern pool populations was considerably lower than in the southern/central populations, and π_T was only about half of the value of the southern/central populations. Moreover, π_T in the northern populations was even lower than π_S in the southern/central populations. This was also true for H_S and H_T in the microsatellite data and suggests that the whole metapopulation system in the north contains less genetic diversity than an average single population in southern/central Europe. Because again the same pattern was observed in silent sites and microsatellites, our data imply that contemporary rather than historical factors contribute to the reduction in total genetic diversity in the north. We suggest that this reduction is caused by the different rates of population turnover observed between the two regions.

The estimates of π_S can also be used as an estimator of $\theta = 4N_e\mu$, but only in populations with no turnover (Pannell & Charlesworth 1999). Thus, estimates of the mutation effective population size (Whitlock & Barton 1997) of *D. magna* should use π_S from the southern/central populations, not from the northern ones. Using average $\pi_S = 0.008$ (Table 4) and a mutation rate of 10^{-8} (as in Pannell & Charlesworth 1999; Haag *et al.* 2009) results in an estimate of $N_e = 200\,000$ (see also Haag *et al.* 2009; Omilian & Lynch 2009).

In natural populations of *Daphnia magna*, pond size and turnover dynamics are correlated: The northern high-turnover populations mainly live in small ponds (Pajunen & Pajunen 2003; Haag *et al.* 2005), whereas the southern/central low-turnover populations inhabit larger ponds (Vanoverbeke *et al.* 2007). Therefore, it is possible that differences in pond size may also contribute to the strong differences in genetic diversity and differentiation between northern and southern/central populations. The effect may either be indirect because pond size likely affects the rate of population turnover or direct through its probable effect on population size and thus genetic drift. Indeed, Vanoverbeke *et al.* (2007) found lower genetic diversity and stronger differentiation in populations from small water bodies compared to larger ponds in central Europe. Similarly, Haag *et al.* (2005) found significant correlations between pond volume and genetic diversity and differentiation in *Daphnia longispina* from the Finnish metapopulation, but not in *D. magna* (although marker resolution for the latter species was low). These findings suggest that in *D. magna* populations inhabiting small ponds, genetic drift may not be limited to colonization bottlenecks but may rather be an effect of pond size. Thus, it is likely that differences in pond size also contribute to some degree to decreased within-population diversity and increased genetic differentiation in the north, but the effects on total diversity discussed above are only predicted if turnover plays a significant role.

The significantly higher genetic differentiation among northern populations compared to southern/central populations was true despite the fact that the average geographic distances among northern populations were smaller than among the southern/central populations. Indeed, the differences in genetic differentiation between northern and southern/central populations are even more pronounced if similar distances are compared (Fig. 2). Isolation by distance was found in northern, but not in southern/central populations. This may be explained by the fact that no nearby ponds were sampled in southern/central Europe (Palsson 2000; Haag *et al.* 2006). Thus, we cannot exclude that isolation by distance also exist in the southern/central populations.

De Meester *et al.* (2002) explained the strong differentiation among nearby populations of *Daphnia* by the 'monopolization hypothesis'. They argued that strong founder effects lead to strong initial differentiation and that local adaptation impedes the successful establishment of new immigrants and thus reduces subsequent gene flow. Additionally, they stated that large resting egg banks provide a source of numerical advantage of the local residents. This hypothesis may very well

explain differentiation among populations in southern/central Europe. However, the rock pools in northern Europe often fail to establish a resting egg bank, as they lose their sediment during winter (Pajunen & Pajunen 2003). Moreover, the strong genetic drift in these populations during and after colonization likely counteracts local adaptation (Whitlock 2000). Indeed, Altermatt *et al.* (2007) found no local adaptation of *D. magna* to their parasites in the Finnish metapopulation, but rather local maladaptation. This resulted in a reduced competitive fitness of local residents relative to immigrants because the local residents suffered more from the resident parasites than the immigrants. Additionally, Ebert *et al.* (2002) showed that these immigrants have a further strong selective advantage as offspring from crosses between residents and immigrants have an increased fitness owing to hybrid vigour. This effect can be rather strong and increases effective gene flow per immigrant several times compared to the nominal migration rate (Ebert *et al.* 2002). This is in contrast to southern/central populations, where immigrants are thought to have a strong disadvantage owing to locally adapted residents and owing to priority effects (the monopolization hypothesis, De Meester *et al.* 2002).

Our results suggest that genetic drift is a much stronger evolutionary force in the northern compared to the southern/central populations of *D. magna*. This implies that strongly deleterious alleles may be partially purged from the northern populations ('purging by drift', Glémin 2003), whereas mildly deleterious alleles may accumulate or even go to fixation ('drift load', Glémin 2003). The hybrid vigour observed in crosses between northern populations is likely a consequence of this drift load (Whitlock *et al.* 2000). Purging of highly deleterious alleles and fixation of mildly deleterious alleles are also predicted to reduce levels of inbreeding depression (Kimura *et al.* 1963; Lynch *et al.* 1995; Wang *et al.* 1999), which is an important evolutionary factor, notably for breeding system evolution (Lande & Schemske 1985). Some populations of *D. magna* show a breeding system polymorphism resembling gynodioecy, with some genotypes producing both sexes and others producing only females (Galimov *et al.* 2011). This breeding system polymorphism also occurs in other *Daphnia* species and had been explained by the fact that female-limited genotypes always undergo outbreeding and thus suffer less from inbreeding depression (Innes & Dunbrack 1993). It is possible that the same is true in *D. magna*, especially because this breeding system polymorphism has only been found in southern/central populations where inbreeding depression is expected to be strong (Galimov *et al.* 2011; Y. Galimov, personal communications).

Conclusion

In this study, we found empirical support for the theoretically predicted effects of turnover dynamics on genetic diversity and genetic differentiation. To our knowledge, this is one of the first studies documenting such strong differences in genetic variation between high-turnover and low-turnover populations of the same species. Our results imply that evolutionary processes may differ substantially in different parts of a species range. These findings may be particularly important in model organisms like *Daphnia magna*, which are frequently used to investigate general questions in evolutionary biology and population genetics. Our data suggest that by focusing only on one part of the species range, many important evolutionary processes may be missed or it may be neglected that conclusions do not apply to the whole species range. The same should be considered also in other species that live in environments with different degrees of turnover.

Finally, understanding the ongoing processes that influence genetic diversity and genetic differentiation in natural populations is not only of interest for evolutionary biology, but also relevant from a conservation point of view, as it underlines the importance to maintain sufficient gene flow and to support the genetic diversity of subdivided populations.

References

Allendorf FW, Luikart G (2007) *Conservation and the Genetics of Populations*. Blackwell Publishing, Oxford.

Altermatt F, Hottinger J, Ebert D (2007) Parasites promote host gene flow in a metapopulation. *Evolutionary Ecology*, **21**, 561–575.

Bengtsson J (1989) Interspecific competition increases local extinction rate in a metapopulation system. *Nature*, **340**, 713–715.

Boileau MG, Hebert PDN, Schwartz SS (1992) Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. *Journal of Evolutionary Biology*, **5**, 25–39.

Charlesworth B (1998) Measures of divergence between populations and the effect of forces that reduce variability. *Molecular Biology and Evolution*, **15**, 538–543.

Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J (2009) The last glaciation maximum. *Science*, **325**, 710–714.

Crow JF, Aoki K (1984) Group selection for a polygenic behavioural trait: estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 6073–6077.

De Meester L, Gomez A, Okamura B, Schwenk K (2002) The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica*, **23**, 121–135.

De Meester L, Vanoverbeke J, De Gelas K, Ortells R, Spaak P (2006) Genetic structure of cyclic parthenogenetic zooplankton populations—a conceptual framework. *Archiv Fur Hydrobiologie*, **167**, 217–244.

Ebert D (2005) Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda, Maryland. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books.

Ebert D, Haag C, Kirkpatrick M *et al.* (2002) A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science*, **295**, 485–488.

Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics*, **16**, 551–558.

Figuerola J, Green AJ, Santamaria L (2003) Passive internal transport of aquatic organisms by waterfowl in *Donana*, south-west Spain. *Global Ecology and Biogeography*, **12**, 427–436.

Figuerola J, Green AJ, Michot TC (2005) Invertebrate eggs can fly: evidence of waterfowl-mediated gene flow in aquatic invertebrates. *American Naturalist*, **165**, 274–280.

Fisher RA (1925) *Statistical Methods for Research Workers*, 1st edn (14th ed. 1970). Oliver and Boyd, Edinburgh.

Freeland JR, Noble LR, Okamura B (2000) Genetic consequences of the metapopulation biology of a facultative sexual freshwater invertebrate. *Journal of Evolutionary Biology*, **13**, 383–395.

Galimov Y, Walser B, Haag CR (2011) Frequency and inheritance of non-male producing clones in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen? *Journal of Evolutionary Biology*, **24**, 1572–1583.

Garcia-Verdugo C, Forrest AD, Fay MF, Vargas P (2010) The relevance of gene flow in metapopulation dynamics of an oceanic island endemic, *Olea europaea* subsp. *guanchica*. *Evolution*, **64**, 3525–3536.

Giles BE, Goudet J (1997) Genetic differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. *American Naturalist*, **149**, 507–526.

Glémin S (2003) How are deleterious mutations purged? Drift versus nonrandom mating. *Evolution*, **57**, 2678–2687.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from: http://www.unil.ch/izea/softwares/fstat.html.

Haag CR, Riek M, Hottinger JW, Pajunen VI, Ebert D (2005) Genetic diversity and genetic differentiation in *Daphnia* metapopulations with subpopulations of known age. *Genetics*, **170**, 1809–1820.

Haag CR, Riek M, Hottinger JW, Pajunen VI, Ebert D (2006) Founder event as determinants of within-island and among-island genetic structure of *Daphnia* metapopulations. *Heredity*, **96**, 150–158.

Haag CR, McTaggart SJ, Didier A, Little TJ, Charlesworth D (2009) Nucleotide polymorphism and within-gene recombination in *Daphnia magna* and *D. pulex*, two cyclical parthenogens. *Genetics*, **182**, 313–323.

Haag-Liautard C, Dorris M, Maside X *et al.* (2007) Direct estimation of per nucleotide and genomic deleterious mutation rates in *Drosophila*. *Nature*, **445**, 82–85.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hanski I (1999) *Metapopulation Ecology*. Oxford University Press, Oxford.

Hanski I, Gilpin M (1997) *Metapopulation Biology: Ecology, Genetics and Evolution*. Academic Press, San Diego, California.

Hanski I, Ranta E (1983) Coexistence in a patchy environment: three species of *Daphnia* in rock pools. *Journal of Animal Ecology*, **52**, 263–279.

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution*, **11**, 180–183.

Hastings A, Harrison S (1994) Metapopulation dynamics and genetics. *Annual Review of Ecology and Systematics*, **25**, 167–188.

Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hobaek A, Larsson P (1990) Sex determination in *Daphnia magna*. *Ecology*, **71**, 2255–2268.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583–589.

Innes DJ, Dunbrack RL (1993) Sex allocation variation in *Daphnia-pulex*. *Journal of Evolutionary Biology*, **6**, 559–575.

Kalinowski S (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity*, **94**, 33–36.

Kimura M, Maruyama T, Crow JF (1963) The mutation load in small populations. *Genetics*, **48**, 1303–1312.

Kleiven OT, Larsson P, Hobaek A (1992) Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos*, **65**, 197–206.

Klüttgen B, Dulmer U, Engels M, Ratte HT (1994) ADaM, an artificial freshwater for the culture of zooplankton. *Water Research*, **28**, 743–746.

Lande R, Schemske DW (1985) The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution*, **39**, 24–40.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Louette G, De Meester L (2004) Rapid colonization of a newly created habitat by cladocerans and the initial build-up of a *Daphnia*-dominated community. *Hydrobiologia*, **513**, 245–249.

Lynch M, Cornery J, Bürger R (1995) Mutation accumulation and the extinction of small populations. *American Naturalist*, **146**, 489–518.

Massonnet B, Simon JC, Weisser WW (2002) Metapopulation structure of the specialized herbivore *Macrosiphoniella tanacetaria* (Homoptera, Aphididae). *Molecular Ecology*, **11**, 2511–2521.

McCauley DE (1991) Genetic consequences of local population extinction and recolonization. *Trends in Ecology and Evolution*, **6**, 5–8.

Molecular Ecology Resources Primer Development Consortium, Agostini C, Agudelo PA, Ba K *et al.* (2010) Permanent genetic resources added to molecular ecology resources database 1 October 2010–30 November 2010. *Molecular Ecology Resources*, **11**, 418–421.

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.

Obbard DJ, Harris SA, Pannell JR (2006) Sexual systems and population genetic structure in an annual plant: testing the metapopulation model. *American Naturalist*, **167**, 354–366.

Omilian AR, Lynch M (2009) Patterns of intraspecific DNA variation in the *Daphnia* nuclear genome. *Genetics*, **182**, 325–336.

Pajunen VI (1986) Distributional dynamics of *Daphnia* species in a rock-pool environment. *Annales Zoologici Fennici*, **23**, 131–140.

Pajunen VI, Pajunen I (2003) Long-term dynamics in rock pool *Daphnia* metapopulations. *Ecography*, **26**, 731–738.

Pajunen VI, Pajunen I (2007) Habitat characteristics contributing to local occupancy and habitat use in rock pool *Daphnia* metapopulations. *Hydrobiologia*, **592**, 291–302.

Palsson S (2000) Microsatellite variation in *Daphnia pulex* from both sides of the Baltic Sea. *Molecular Ecology*, **9**, 1075–1088.

Pannell JR, Charlesworth B (1999) Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonization. *Evolution*, **53**, 664–676.

Pannell JR, Charlesworth B (2000) Effects of metapopulation processes on measures of genetic diversity. *Philosophical Transactions of the Royal Society London Series B*, **355**, 1851–1864.

Petit RJ, Aguinagalde I, de Beaulieu JL *et al.* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Ranta E (1979) Niche of *Daphnia* species in rock pools. *Archiv für Hydrobiologie*, **87**, 205–223.

Rinterknecht VR, Clark PU, Raisbeck GM *et al.* (2006) The last deglaciation of the southeastern sector of the Scandinavian Ice Sheet. *Science*, **311**, 1449–1452.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, **132**, 365–386.

Saccheri IM, Kuussaari M, Kankare P *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Seyfert AL, Cristescu MEA, Frisse L *et al.* (2008) The rate and spectrum of microsatellite mutation in *Caenorhabditis elegans* and *Daphnia pulex*. *Genetics*, **178**, 2113–2121.

Thielsch A, Brede N, Petrusek A, De Meester L, Schwenk K (2009) Contribution of cyclic parthenogenesis and colonization history to population structure in *Daphnia*. *Molecular Ecology*, **18**, 1616–1628.

Vanoverbeke J, De Meester L (1997) Among-populational genetic differentiation in the cyclical parthenogen *Daphnia magna* (Crustacea, Anomopoda) and its relation to geographic distance and clonal diversity. *Hydrobiologia*, **360**, 135–142.

Vanoverbeke J, De Gelas K, De Meester L (2007) Habitat size and the genetic structure of a cyclical parthenogen, *Daphnia magna*. *Heredity*, **98**, 419–426.

Wang JL, Hill WG, Charlesworth D, Charlesworth B (1999) Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genetical Research*, **74**, 165–178.

Whitlock MC (2000) Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution*, **54**, 1855–1861.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. *Genetics*, **146**, 427–441.

Whitlock MC, Ingvarsson PK, Hatfield T (2000) Local drift load and the heterosis of interconnected populations. *Heredity*, **84**, 452–457.

Willis KJ, Bennett KD, Birks JB (1998) The late quaternary dynamics of pines in Europe. In: *Ecology and Biogeography of Pinus* (ed. Richardson DM), pp. 107–121. Cambridge University Press, Cambridge.

Data accessibility

Sequence data: GenBank accession numbers (JN994173–JN994591) including allocation to individuals uploaded as Table S1 (Supporting Information). Microsatellite genotypes: uploaded as Table S2 (Supporting Information).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 GenBank accession numbers.

Table S2 Microsatellite genotypes.

Fig. S1 Map of sampling locations. Filled triangles; southern/central populations. Open triangles: northern populations.