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THE ROLE OF PONDS

Colonization of *Daphnia magna* in a newly created pond: founder effects and secondary immigrants

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Abstract In habitats recently colonized by cyclical parthenogens, founder events lead to genetic differences between populations that do not erode quickly despite ongoing dispersal. By comparing the genetic composition during initial colonization with that of the diapausing egg bank at a local scale, we here present the relative contribution of the founding clones to the build-up of genetic diversity and differentiation of a newly established cladoceran population. We monitored the population genetic structure of *Daphnia magna* in one newly created pond as well as the diapausing egg banks of four water bodies in the neighbouring area. Our population was founded by four individuals. After the first growing season, the largest contribution to the sexually produced resting egg bank came from only two clones. Descendants of initially rare clones and potentially also additional immigrant clones profited from outbreeding vigour and increased their frequency during the first few years after colonization. Beyond this, no further significant changes in genetic structure were observed in the egg bank. At this point, priority effects became fully operational and led to sustained population genetic differentiation from nearby ponds. Our results support that colonization dynamics strongly influence within and among population genetic variation and evolutionary potential of populations.

Keywords Cladocera · Ecological genetics · Egg bank · Monopolization hypothesis · Temporary ponds

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Introduction

The notion of panmixia among sexual populations unified by contemporary gene flow as proposed by Darwin (1859) has been controversial since genetic approaches allowed the study of population genetic composition in detail. This traditional view has been challenged by observations that gene flow among natural populations is generally too low to prevent divergence through local adaptation or genetic drift (Morjan & Rieseberg, 2004). In addition, this day population structure may be largely the result of historical recolonization events rather than from ongoing gene flow (De Gelas & De Meester, 2005). The quantification of dispersal and contemporary gene flow and its influence on genetic variation within and among populations can gain from studies on colonization of new habitats, where the earliest stages of population foundation can be monitored (Louette et al., 2007; Ortells et al., 2012).

In cyclical parthenogenetic cladocerans and rotifers, the colonization of new habitats and dispersal among established habitats is associated with a sexually produced dormant stage that enables dispersal through time and space (Bohonak & Jenkins, 2003). This dormant stage—the diapausing egg—has a high potential to colonize and fill habitats from even a few propagules because after hatching, parthenogenetic reproduction allows for rapid population growth (Hebert, 1987; Boileau et al., 1992; De Meester et al., 2002). In the extreme, new populations can be entirely established from a single colonizing individual. Despite the high dispersal potential, pronounced genetic differentiation is common even among neighbouring populations of cladocerans and rotifers (e.g., Vanoverbeke & De Meester, 1997; Haag et al., 2005; Thielsch et al., 2009; Orsini et al., 2013). To explain this paradox, the "monopolization hypothesis" (De Meester et al., 2002) suggests that genetic differentiation among populations is due to strong and long-lasting founder effects. Early colonizing individuals should have the potential to abort subsequent immigrant establishment because of a numerical advantage due to high population growth rates (Boileau et al., 1992) and the buildup of an extensive diapausing egg bank (Brendonck & De Meester, 2003) combined with the capacity for rapid local adaptation (Hairston et al., 1999; Cousyn et al., 2001).

Nevertheless, successful establishment of secondary immigrants can be enhanced by a reduced fitness of the resident genotypes due to inbreeding depression resulting from selfing (Whitlock et al., 2000; Ebert et al., 2002), when the founding population descends from a low number of colonizing genotypes. A decline in fitness-associated traits in individuals with high inbreeding coefficients has been supported in many studies (Charlesworth & Charlesworth, 1987; Lynch & Walsh, 1998) and is known to be severe in *Daphnia* populations (De Meester, 1993; Deng, 1997; Deng & Lynch, 1997; Haag et al., 2002). The selective advantage of outbred offspring should increase the frequency of colonizers with initially low frequencies or allow for secondary immigration until a certain minimum genetic diversity has been reached (Ebert et al., 2002; Louette et al., 2007; Ortells et al., 2012). The latter would result in an increase in effective gene flow (Ingvarsson & Whitlock, 2000); an opposite effect from that predicted by the monopolization hypothesis, at least during the initial stages of population establishment. For how long populations are still open to immigration of novel genotypes or how the relative importance of monopolization versus immigrant success changes over time remains poorly studied.

There is empirical evidence that at least for recently colonized habitats (less than 5 years) founder events occur and lead to population genetic differentiation that does not erode quickly despite ongoing dispersal (Haag et al., 2006; Louette et al., 2007; Ortells et al., 2012). However, these studies were performed on active populations and only indirectly link the build-up of genetic diversity in the diapausing egg bank to the initial colonization and subsequent immigration. In this study, we directly compare the genotypic composition during initial colonization with the build-up of genetic diversity and differentiation in the diapausing egg bank, which provides the genetic reservoir from which future generations emerge. We carried out a detailed temporal analysis of the genetic structure of *Daphnia magna* in one newly created pond studied by Louette et al. (2007), both of the active population during the first growing season and of the genetic composition of the diapausing egg bank over subsequent growing seasons. Our aim was twofold: first, to document the initial stages of population establishment with highly polymorphic genetic markers. Specifically, we identified the number of initial colonizers in the active population during the first year, monitored the contribution of each colonizer into the diapausing egg bank and tested for changes in the allelic composition during three following years. We used microsatellite markers and frequent sampling in time to improve the genetic and temporal resolution previously obtained from an allozyme study (Louette et al., 2007). Similar studies have proven the efficiency of microsatellites in detecting new alleles (Ortells et al., 2012). Despite the high dispersal capacity of *D. magna*-diapausing stages (Louette & De Meester, 2005), we expected to corroborate previous evidence for a low number of initial colonizing genotypes and a low establishment success of secondary immigrants (Louette et al., 2007). Second, as the process of colonization is strongly influenced by regional dynamics (e.g., the presence of source populations and their genetic diversity), we monitored the diapausing egg banks of several water bodies containing a population of *D. magna* in the neighboring area. In order to determine to what extent founder effects after colonization result in divergence among populations, we measured interpopulation genetic differentiation over time. If newly established populations remain open to new immigrants, then the among-population genetic differentiation is expected to decline with time. If there is limited gene flow, genetic differentiation is expected to be maintained.

Methods

Pond and local area characteristics

Damme pond (51°14′59.6″N, 03°16′22.4″E; Fig. 1) was created as part of a conservation project in late September 2001 in a grassland near the town of Damme (Flanders; Belgium) and filled by rain and groundwater within 2 months. The pond is small, shallow, and circular (Table 1) and may dry in the summer and freeze in the winter. The soil of the pond presumably contained no previous diapausing egg banks as it was not connected with other inundated areas. This expectation was confirmed by an analysis of the build-up of the diapausing egg bank (Vandekerkhove et al., 2005a). Thus, colonization could have occurred by dispersal via wind or animal vectors (Louette & De Meester, 2004), and possibly by unintentional human-mediated transport (Waterkeyn et al., 2010). The colonization history of Damme pond has been monitored for 4 years (Louette & De Meester, 2004, 2005). It was colonized within 3 months after its creation by *Daphnia atkinsoni*, and 3 months later by *D. magna* and *Simocephalus vetulus*, although the latter subsequently disappeared (Louette & De Meester, 2004).

Fig. 1 Location of Damme pond (*star* in the map of Belgium) and the neighbouring ponds studied numbered 1–12

The local study area which includes Damme is within a main wintering region for geese and waterfowl and is exposed to wind. We explored the landscape in search for water bodies close to Damme, all included in an area within 2 km distance. We found 12 water bodies (ditches, ponds and puddles; named reg1–reg12 after "regional") whose average size and perimeter are shown in Table 1. The closest pond to Damme was reg6, and the furthest, reg11 (Fig. 1).

Population sampling

The active population of *D. magna* in the water column was sampled monthly during the first growing season.

Table 1 Morphometric features of the studied ponds

Pond	Area (m^2)	Perimeter (m)
Damme	180	50
reg1	124	65
reg2	421	99
reg3	170	51
reg4	1,565	164
reg5	252	60
reg6	105	38
reg7	400	83
reg8	165	47
reg9	107	39
reg10	130	45
reg11	480	81
reg12	1,726	366

We here refer to "growing season" as the time interval for which a population is continuously present in the water column until it becomes undetectable for at least 3 months (see Louette et al., 2007). Sampling started on first detection of *D. magna* in May 2002 and ended in May 2003, when the population disappeared from the water column and did not appear again for 4 months. Quantitative samples were collected with a tube sampler (length: 1.5 m; Ø 0.75 mm) at randomly chosen locations. Density estimates from these samples are reported in Louette & De Meester (2004). Qualitative samples were also collected with a tube sampler and preserved in 90% ethanol for further genetic analyses. From these samples, we annotated any evidence of sexual reproduction (ephippia or males).

The diapausing population of *D. magna* was sampled in Damme in one single event per year (from 2002 to 2006), as it integrates spatio-temporal heterogeneity in one sampling effort (Vandekerkhove et al., 2005b). The regional ponds were screened for the presence of *D. magna* diapausing eggs in 2005. Sampling of the sediment was performed when *D. magna* was absent from the water column to ensure that the whole population was in the diapausing stage. Sediment was taken from superficial layers (5 cm) at different sampling points (4–10 depending on pond size) along the water basin to prevent bias due to patchy egg banks. We used a hand-operated sediment core sampler composed of an acrylic tube (diameter: 5.2 cm). Samples were kept in cold (4°C) and dark conditions until processed. Sediment was filtered over a 250-μm mesh and the residue was transferred to Petri dishes with distilled water. Ephippia (which contain at most two diapausing eggs) were picked from the residue with forceps under binocular lenses and frozen at −20°C for genetic analysis. We found no ephippia belonging to *D. magna* in the sediments of Damme in 2002 and sediment samples from 2004 were regrettably lost. We obtained sufficient numbers of eggs for genetic analysis from the sediments of Damme in 2003, 2005, and 2006. Among the 12 neighbouring ponds sampled in 2005 only five contained *D. magna* (reg4, reg7, reg8, reg10 and reg12), although reg7 was excluded from analyses due to small sample size (less than 10 eggs). In 2006, reg4 was sampled again as we considered it a very likely source of colonization, at least among the four analysed local ponds. This was based on the fact that the other three ponds were genetically less diverse than Damme, and that two alleles were exclusively found in reg4 and Damme pond (see "Results").

DNA extraction and microsatellite amplification

Genomic DNA was obtained by embedding individual *Daphnia* and single diapausing eggs separated from the ephippium in 100 μl DTT-buffer (0.08 M NaAc pH 5.2 and 1 M DTT in 10× Reaction Buffer $MgCl_2$-free). After adding 0.5 μl of Proteinase K, samples were incubated for 1 h at 56°C followed by 10 min at 95°C and a gentle centrifugation. The resulting DNA preparation was stored at −20°C until analysis.

We used seven microsatellite markers accessible in GenBank: Dma3R (AF291910), Dma11 (AF291911), Dma12 (AF291912), Dma15 (EU131363) and three non-described microsatellites developed in the Laboratory of Aquatic Ecology, Evolution and Conservation (KULeuven) in cooperation with Hull University (S5157, S6-199 and S6-38; C. Clabby, unpubl.). Forward primers were labelled with a fluorescent dye to enable the use of an automated sequencer. Sets of loci were amplified simultaneously with the Qiagen® Multiplex PCR Kit (Qiagen). The total reaction volume (10 μl) consisted of 1× PCR Master Mix (3 mM $MgCl_2$), 1 μl of DNA and 0.2 μM of each primer. PCR amplifications commenced with an initial step of 15 min at 95°C, followed by 30 cycles of 30 s at 94°C, 90 s at 56°C and 90 s at 72°C with a final elongation step of 10 min at 72°C. Allele sizes were scored relative to an internal GeneScan 500LIZ size standard and visualized on an ABI 3130 Avant Genetic Analyzer (Applied Biosystems). Multilocus genotypes (MLGs) were obtained using Gene Mapper 3.7 (Applied Biosystems). Micro-checker (van Oosterhout et al., 2004) was used to check for the presence of null alleles and scoring errors due to stuttering or large allele dropout through the data set. There was no evidence of such artefacts in the water column samples and instances of null alleles in the egg banks were rare and not consistently associated with a specific locus or population. We therefore retained all polymorphic loci for analysis.

Data analysis

Genetic structure was evaluated based on allele, single locus genotype and MLG frequencies. We calculated allele frequencies for each sample of the active population and the egg bank. The presence and fate of rare alleles in Damme pond throughout the study period were compared with the regional ponds. To test the hypothesis that sexual offspring of rare clones have a fitness advantage due to outbreeding vigour, we monitored the fate of those alleles that were least frequent (never above 20%) in the egg bank during the first year of colonization of Damme. We compared the frequencies of these rare alleles between 2003 and 2005 and between 2005 and 2006 using a Wilcoxon matched pairs test with PASW Statistics (SPSS Inc).

The following parameters of genetic diversity were measured over loci: observed heterozygosity (H_o), expected heterozygosity (H_e) with GeneAlEx 6.3 (Peakall & Smouse, 2006) and allelic richness (A) and inbreeding coefficient (F_{is}) with FSTAT 2.9.3.2 (Goudet, 2002). We examined significant levels of F_{is} and linkage disequilibrium between all pairs of loci with permutations based on the minimum sample size available with FSTAT 2.9.3.2. Multiple comparisons were accounted for by sequential Bonferroni correction (Rice, 1989). Whenever sample size was too small (less than 10 eggs or individuals), data were removed from the genetic analysis.

Multilocus genotypes are indicative of clonal lineages during the first growing season after colonization. If the number of MLGs found in the pond before sexual reproduction is significantly lower than the resolution of the markers used (expected number of MLGs under Hardy–Weinberg equilibrium), this is a good indicator of the number of individuals that colonized the habitat and settled successfully (Louette et al., 2007). We calculated the number of MLGs as a measure of clonal richness and estimated clonal diversity (CD) expressed as the inverse of Simpson index, which combines richness and evenness (Lande, 1996). We used temporal samples to calculate deviations from expected MLG and CD under HW-equilibrium using randomisation tests implemented in Hwclon (De Meester & Vanoverbeke, 1999). Once sexual reproduction occurs, MLGs are recombined and analysis at multilocus genotypic level is no longer indicative of clonal lineages. Yet, for our diapausing egg bank samples we calculated expected CD to provide with the maximum number of genotypes that can be detected, as an estimation of the power of the genetic markers used.

Genetic structure was assessed by means of F statistics (Weir & Cockerham, 1984). In order to test for the stability of the genetic structure in the egg bank over time, we calculated pairwise fixation indexes (using jack-knifing over loci for each pairwise estimate of fixation index—F_{st}) among the egg bank samples in Damme obtained in different years and tested for significant genetic differentiation with exact tests using TFPGA 1.3. We also calculated pairwise genetic differentiation (jackknifing over loci for each pairwise estimate of F_{st}) among the regional ponds in 2005 and tested for significant genetic differences with FSTAT 2.9.3.2. The correlation between genetic and geographic distance was analysed with a Mantel test in the Isolation By Distance Web Service version 3.23 (Jensen et al., 2005). We used 1000 randomizations and Rousset's distance measure ($F_{st}/(1 - F_{st})$), which takes into account the maximum value of fixation index (F_{st}) attainable according to average heterozygosity. Additionally, we measured F_{st} values between Damme and reg4 in 2005 and in 2006, and tested whether these values differed with time with the among-groups comparison option implemented in FSTAT 2.9.3.2.

Results

Allele frequencies for the analysed loci are reported in Table 2. In general, we detected one (S5-157) to six (Dma 11) alleles per locus. The distribution of the 23 alleles detected at the local scale is shown in Fig. 2.

Table 2 Allele frequencies of the analysed loci in *D. magna* populations observed in Damme during the study period in the water column (wc, averaged over sampling dates) and in the diapausing egg bank (deb) of Damme and the surrounding ponds (reg)

Sample locus	Allele	wc 2002–2003	deb 2003	deb 2005	deb 2006	reg4 2005	reg4 2006	reg8 2005	reg10 2005	reg12 2005
Dma11	*N*	501	46	68	39	83	50	38	71	29
	155	0.049	0.011	0.169	0.154	0.060	0.090	0.000	0.000	0.000
	159	0.398	0.413	0.243	0.167	0.337	0.170	0.605	0.465	0.310
	161	0.154	0.109	0.243	0.282	0.187	0.450	0.013	0.014	0.207
	163	0.000	0.000	0.022	0.064	0.024	0.060	0.316	0.380	0.414
	169	0.399	0.467	0.324	0.333	0.380	0.210	0.066	0.141	0.069
	177	0.000	0.000	0.000	0.000	0.012	0.020	0.000	0.000	0.000
Dma12	*N*	501	47	71	41	87	53	39	73	31
	136	0.399	0.309	0.303	0.171	0.368	0.189	0.295	0.155	0.500
	138	0.101	0.128	0.204	0.329	0.098	0.264	0.038	0.041	0.129
	142	0.399	0.543	0.289	0.329	0.420	0.274	0.269	0.466	0.258
	146	0.000	0.000	0.000	0.000	0.052	0.179	0.218	0.041	0.097
	148	0.101	0.021	0.204	0.171	0.063	0.094	0.179	0.297	0.016
Dma15	*N*	501	46	70	39	87	50	39	73	31
	129	0.049	0.000	0.229	0.154	0.034	0.130	0.000	0.000	0.032
	131	0.552	0.467	0.550	0.654	0.615	0.800	0.744	0.596	0.935
	133	0.399	0.533	0.221	0.192	0.351	0.070	0.256	0.404	0.032
Dma3R	*N*	501	44	65	40	82	51	40	71	29
	188	0.559	0.500	0.785	0.825	0.604	0.794	0.675	0.901	0.862
	198	0.441	0.500	0.215	0.175	0.396	0.206	0.325	0.099	0.138
S5-157	*N*	501	44	72	42	84	49	37	72	30
	123	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
S6-199	*N*	501	46	56	40	84	50	37	72	30
	163	0.449	0.457	0.384	0.413	0.476	0.450	0.635	0.160	0.450
	165	0.500	0.478	0.429	0.388	0.256	0.290	0.351	0.618	0.167
	167	0.051	0.065	0.188	0.200	0.268	0.260	0.014	0.222	0.383
S6-38	*N*	501	48	71	42	89	51	41	74	31
	104	0.496	0.167	0.232	0.310	0.348	0.451	0.085	0.270	0.419
	108	0.503	0.813	0.768	0.690	0.618	0.471	0.915	0.730	0.581
	110	0.001	0.021	0.000	0.000	0.022	0.069	0.000	0.000	0.000
	112	0.000	0.000	0.000	0.000	0.011	0.010	0.000	0.000	0.000

Most alleles were shared across populations, 18 alleles were present in all ponds but only reg4 presented all alleles detected. Moreover, two alleles were found exclusively in reg4 (Dma11—177 and S6-38—112), and another two were exclusive of reg4 and Damme (Dma11—155 and S6-38—110), although the latter disappeared in the following years in Damme. All alleles in Damme pond were present in at least one of the nearby ponds, but one allele was shared by all local ponds except Damme (Dma12—146). During the first growing season, 19 alleles were found in the active population, and only one of them (also found in reg4 and reg12) was not represented in the egg bank that year but reappeared in the following years (Dma15—129). In the Damme egg bank samples of 2005 and 2006, a new allele was detected in locus Dma11 (Dma11—163) at low frequencies (2% in 2005 and 6% in 2006; Table 2). This allele was present at low frequencies in reg4 (6%) and at moderate frequencies in the other local ponds (30–40%). Rare alleles that

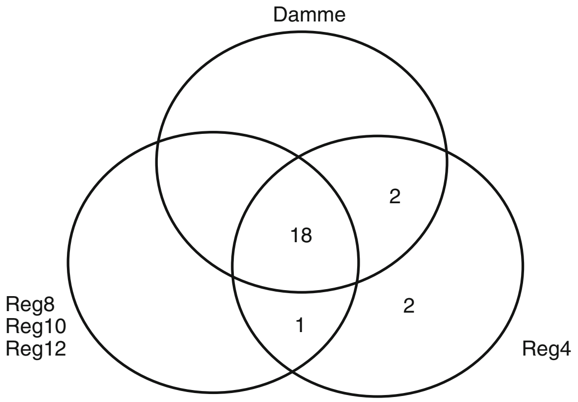

Fig. 2 *Venn diagram* showing the number of alleles detected in the ponds in the studied region based on all records (egg bank and active populations). As a potential source pond, reg4 is plotted separately from the rest of regional ponds

were present in 2003 in the diapausing egg bank of Damme pond at frequencies below 20% significantly increased in frequency in 2005 (Wilcoxon matched pairs test: $Z = 2.547$; $P = 0.01$; $n = 9$), but no difference was detected in their frequencies between 2005 and 2006 ($Z = 0.980$; $P = 0.33$), although a tendency to increase in 2006 can still be observed in Fig. 3.

Estimates of allelic (averaged over loci) and genotypic diversity are summarised in Table 3. Most water column samples during the first growing season (except May 2002) were characterized by significant heterozygote excess (negative F_{is} values) and linkage disequilibrium (63 out of 121 comparisons). Contrarily, genetic equilibrium was common in the diapausing egg banks of Damme, consistent with what would be expected in a randomly mating sexual population. The exception was a significant heterozygote deficit in locus Dma15 in the egg bank of 2006. The egg banks of the regional ponds sampled in 2005 were also generally in equilibrium, except reg4, which presented heterozygote excess in four loci and significant linkage disequilibrium in 3 locus combinations (out of 60) and reg10, which also showed one locus-pair in linkage disequilibrium and heterozygote deficit in one locus. In the analysis of reg4 in 2006, in contrast to the previous year, there was HW-equilibrium at all loci and only one out of 36 test combinations showed genetic linkage. Gene diversity (expected heterozygosity) levels in 2005 were similar between reg4 and Damme (0.50 and 0.49 in 2005; 0.48 both in 2006) compared to the other three ponds (0.39–0.41 in 2005).

Four distinct MLGs were detected (out of 501 individuals) throughout the growing season with an average MLG diversity of two per sampling date. This observed number of MLGs and CD was significantly lower than the expected values obtained using randomization tests (Table 3). Contrarily, in all the diapausing egg banks analysed, the number of MLG and the CD observed were high and no different from that expected in a sexually produced population. Figure 4 shows the relative frequency of the four MLGs detected in the active population during the first growing season. Although, they were all present early in the season (May 2002), two of them (clones 2 and 4) disappeared after September 2002 despite representing 20–40% of the total population on earlier sampling dates. Throughout the season, there was one dominant MLG (clone 1) with relative frequencies ranging between 50 and 100%. Evidence for sexual reproduction (females carrying ephippia and/or males in the active population) was observed from May to September 2002 and then again in May 2003, coinciding with some population density peaks (81.7 ind l^{-1} in May 2002, 122.0 ind l^{-1} in September 2002 and 69.2 ind l^{-1} in May 2003; see also Louette & De Meester, 2004). On average, during these periods clone 1 and 2 had the highest relative frequencies in the population (Fig. 4). When comparing the frequency of unique alleles between the founding clones with those found in the egg bank of 2003, we could infer that most of the contribution to the egg bank came from clones 1 and 2, as the unique alleles of clone 3 (Dma11—155 and Dma15—129) and clone 4 (S6-38—110) had low frequencies or were even absent (clone 3, allele Dma15—129). However, allele Dma15—129 reappeared in subsequent years at high frequencies, coinciding with a comparable increase in allele Dma11—155, the other unique allele for clone 3 (see Table 2).

Genetic differentiation among the egg bank samples of 2003, 2005 and 2006 in Damme pond was significant ($F_{\mathrm{st}} = 0.049 \pm 0.016$ SE; $\chi^2 = 90.925$, df = 12; $P < 0.01$). Population differentiation among the egg banks at the local scale was significant both when Damme pond was included ($F_{\mathrm{st}} = 0.086 \pm 0.013$ SE; $\chi^2 = 118.842$; df = 12; $P < 0.01$), or when Damme pond was excluded ($F_{\mathrm{st}} = 0.101 \pm 0.015$ SE; $\chi^2 = 118.842$; df = 12; $P < 0.01$). The maximum value of F_{st} attainable in the local area according to average heterozygosity was 0.5. Pairwise population comparisons based on F_{st} were significantly different from zero in all cases (Table 4), with no significant correlation between genetic differentiation and geographic distance (Mantel test: $Z = 707{,}938$; $P = 0.37$; $n = 10$). The diapausing egg banks of reg4 and Damme pond for the two sampled years were significantly differentiated (2005: $F_{st} = 0.034 \pm 0.007$ SE, $\chi^2 = 80.658$, df = 12, $P < 0.01$; 2006: $F_{st} = 0.019 \pm 0.009$ SE, $\chi^2 = 50.917$, df = 14, $P < 0.01$). F_{st} values between both ponds did not significantly differ among years ($P = 0.63$).

Discussion

There were at least four females of *D. magna* arriving in Damme pond shortly after its creation. This study confirms the low number of founders observed by Louette et al. (2007), who detected two MLGs in Damme pond with three allozyme polymorphic loci. With highly polymorphic microsatellites we only detected four MLG. The low number of colonizers is consistent with other studies on cladoceran populations colonizing new habitats (Hairston et al., 1999; Ebert et al., 2002; Haag et al., 2002; Ortells et al., 2012). We here not only demonstrate that the pond was colonized by a low number of clones, but that this number remained low for the entire first growing season. We are confident that the population established with a very low number of founders and that further establishment during the growing season was strongly impeded for several reasons. First, despite the high number of individuals analysed in the active population (501) and the high polymorphism of microsatellite markers, no additional MLGs were detected in the 12 monthly samples after foundation. Second, our study with neutral markers detected strong deviations from HW-equilibrium (excess of heterozygotes) and significant linkage disequilibrium between loci throughout the growing season. This is evidence of strong clonal structure and shows that the low observed MLG diversity in the first growing season is not caused by a lack of resolution of the markers but truly reflects low CD in the studied pond (Vanoverbeke & De Meester, 2010). With the given allelic diversity and sample sizes, up to 70 distinct MLGs could be detected. Third, first colonizers reached the habitat in April, and as evidenced by the high densities detected at that time (see also Louette et al., 2007), conditions may have been optimal for high population growth and reproduction, allowing for a rapid monopolization of pond resources and preventing further establishment of later immigrants by competitive exclusion. It is important to highlight that only a couple of clones established at high densities the first year despite the fact that many more migrants may have arrived. These strong priority effects might even have prevented better adapted but later arriving genotypes to establish. Finally, the diapausing egg bank formed after the first growing season (i.e., after the first episode of sexual reproduction) contained no alleles different from those present in the founding

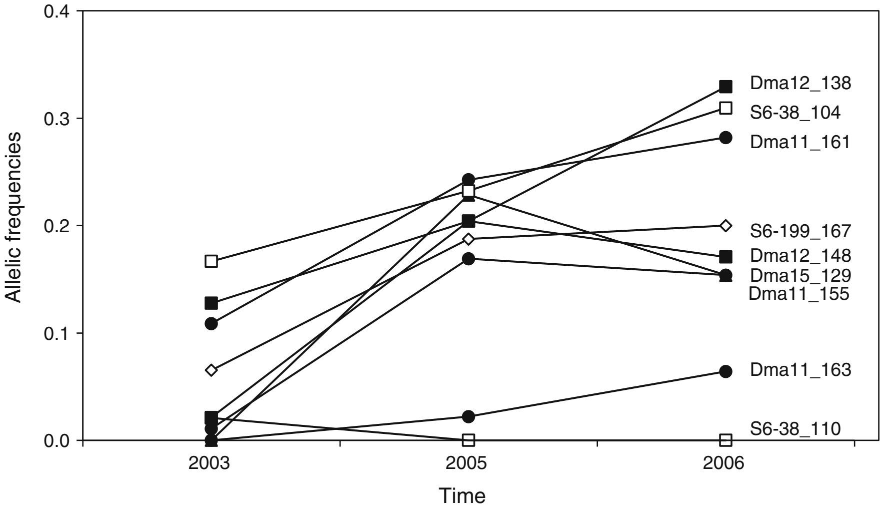

Fig. 3 Change over time in the frequency of rare alleles for the *D. magna* population in Damme pond. We considered rare alleles when they represented less than 20% for each correspondent loci in the first dormant egg bank. Allele size of each locus is shown on the *right*

Table 3 Genetic diversity measures at single locus (averaged over loci, SE between brackets) and MLG level

Sample	*N*	*A*	H_o	H_e	F_{is}	MLG	ExpMLG	CD	ExpCD
Water column									
Apr 2002	7.00	2.00	0.86	0.43	−1.00*	1	7.0*	1.0	6.9*
	(0.00)	(0.00)	(0.14)	(0.07)	(0.00)				
May 2002	12.00	2.79	0.64	0.47	−0.34	4	12.0*	2.2	12.0*
	(0.00)	(0.82)	(0.13)	(0.09)	(0.09)				
Jun 2002	26.00	2.45	0.75	0.47	−0.58*	2	25.8*	1.5	25.6*
	(0.00)	(0.78)	(0.13)	(0.08)	(0.05)				
Jul 2002	48.00	2.00	0.85	0.43	−1.00*	1	45.2*	1.0	42.9*
	(0.00)	(0.00)	(0.14)	(0.07)	(0.00)				
Aug 2002	13.00	2.91	0.81	0.48	−0.57*	2	13.0	1.5	13.0*
	(0.00)	(0.82)	(0.14)	(0.08)	(0.03)				
Sep 2002	109.00	2.48	0.69	0.47	−0.53*	2	106.0*	1.9	103.2*
	(0.00)	(0.80)	(0.14)	(0.09)	(0.04)				
Oct 2002	78.00	2.38	0.84	0.45	−0.85*	2	74.5*	1.1	71.3*
	(0.00)	(0.34)	(0.14)	(0.08)	(0.00)				
Nov 2002	19.00	2.37	0.84	0.45	−0.87*	2	18.8*	1.1	18.6*
	(0.00)	(0.33)	(0.14)	(0.07)	(0.01)				
Dec 2002	31.00	2.94	0.76	0.51	−0.50*	2	30.9*	1.8	30.9*
	(0.00)	(0.84)	(0.14)	(0.09)	(0.02)				
Jan 2003	48.00	2.96	0.70	0.49	−0.40*	2	47.9*	2.0	47.7*
	(0.00)	(0.88)	(0.15)	(0.10)	(0.02)				
Mar 2003	47.00	2.00	0.86	0.43	−1.00*	1	44.3*	1.0	42.1*
	(0.00)	(0.00)	(0.14)	(0.07)	(0.00)				
May 2003	62.00	2.23	0.85	0.44	−0.91*	3	58.9*	1.1	56.1*
	(0.00)	(0.19)	(0.14)	(0.07)	(0.00)				
Egg bank									
deb 2003	46.43	2.65	0.44	0.44	−0.01	40	41.0	38.3	40.0
	(0.53)	(0.11)	(0.08)	(0.08)	(0.02)				
deb 2005	67.57	2.86	0.47	0.49	0.04	52	51.9	52.0	51.7
	(2.12)	(0.17)	(0.10)	(0.10)	(0.01)				
deb 2006	40.43	2.86	0.40	0.48	0.15	38	37.9	38.0	37.8
	(0.48)	(0.05)	(0.08)	(0.10)	(0.03)				
Regional ponds									
reg4 2005	85.86	3.60	0.66	0.50	−0.34	53	74.5	26.9	74.1
	(1.08)	(1.30)	(0.11)	(0.09)	(0.02)				
reg4 2006	50.57	3.39	0.45	0.48	0.07	44	43.9	44	43.7
	(0.48)	(0.05)	(0.11)	(0.10)	(0.01)				
reg8 2005	39.43	2.92	0.34	0.39	0.12	33	35.2	30.9	34.5
	(0.65)	(1.23)	(0.09)	(0.09)	(0.04)				
reg10 2005	72.71	2.93	0.42	0.41	−0.02	61	67.5	55.7	65.2
	(0.52)	(1.18)	(0.09)	(0.09)	(0.02)				
reg12 2005	30.29	3.15	0.46	0.40	−0.07	27	27.7	26.1	27.3
	(0.36)	(1.15)	(0.13)	(0.11)	(0.07)				

Samples were obtained during the first growing season after colonization and in the diapausing egg banks of three posterior years, as well as from the diapausing egg banks of four nearby ponds

N sample size, *A* Allelic richness, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} inbreeding coefficient, *MLG* number of multilocus genotypes observed in the sample, *ExpMLG* expected number of MLGs under Hardy–Weinberg equilibrium, *CD* observed clonal diversity (inverse of Simpson index), *ExpCD* expected clonal diversity under HW-equilibrium

Significant departures from HW-equilibrium at level 0.05 are indicated with an asterisk

Fig. 4 Frequency of the clones found in the water column during the first growing season of *D. magna* in Damme pond

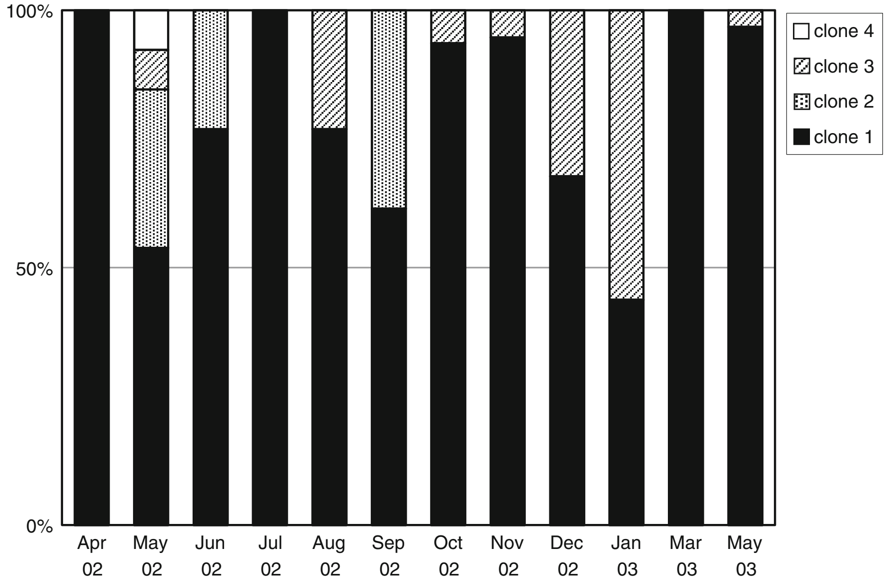

Table 4 Matrix of geographic and genetic distances among Damme and the regional ponds in 2005

	Damme	reg4	reg8	reg10	reg12
Damme	0	0.0345*	0.0894*	0.0788*	0.0944*
reg4	461	0	0.0847*	0.1030*	0.0845*
reg8	194	552	0	0.0990*	0.1144*
reg10	77	538	179	0	0.1310*
reg12	1,210	1,577	1,305	1,160	0

Below diagonal: geographic distance (*m*). Above diagonal: pairwise genetic differentiation

* Significant F_{st}. P values were obtained after 200 permutations and adjusted for multiple comparisons ($P_{5\%} = 0.005$)

clones. Caution should be taken here, as this may suggest that no further clones established, but also that they may have been not detected in our samples (e.g., due to low frequencies). Nevertheless, even the latter indicates that only a few colonizing clones imposed strong priority effects and significantly contributed to the population genetic structure in the diapausing egg bank.

Whereas the rapid build-up of priority effects during the first growing season might impede establishment of new immigrants shortly after founding, the population might as well be partly released from these effects at the onset of subsequent growing seasons. As a consequence of the low number of colonizing clones, there is a high probability for inbreeding within dominant clones during sexual reproduction, resulting in high levels of inbreeding depression in the next generation. As a result, descendants of outbred crosses, produced by either mating with immigrants or with less frequent local clones, might have a fitness advantage over the inbred offspring (Whitlock et al., 2000; Ebert et al., 2002). In our study pond, genetic diversity increased from 2003 to 2005 mainly due to equalized frequencies of initially established alleles. Indeed, rare alleles in the first diapausing egg bank formed after sexual reproduction tended to increase in frequency through time. In addition, one new allele was detected in the 2005 egg bank sample, evidencing either the arrival of new genotypes or the increase in descendants of rare, previously undetected clones. Yet the relative frequency of this new allele was low (2%), supporting the idea that the early colonizers largely determined population genetic structure at least in early years. In 2006, we found no further evidence of the introduction of new alleles or a significant increase in the frequency of rare alleles. This could suggest that an effect of inbreeding depression facilitating genetic admixture was, already after a few growing seasons, no longer strong. However, unless experimentally demonstrated (e.g., Declerck et al., 2001), the role of inbreeding depression in reducing the numerical advantage of the founders or that of local adaptation enhancing it, cannot be quantified.

By analysing the egg bank formed after the first growing season it was possible to trace back the relative contribution of the founding clones. Most of this contribution to the egg bank came from clones 1 and 2, despite the fact that clone 2 was only detected in May, June and September 2002. However, May and September were the months with the highest density peaks, when a high number of ephippia were presumably produced, as high population densities induce sexual reproduction in *Daphnia* (De Meester et al., 2004). Ephippia were indeed observed in our samples from May to September that year. Based on the unique alleles of the founding clones we detected only a small contribution of clones 3 and 4 in the egg bank of 2003. This is rather striking given the presence of ephippia in samples when clone 3 was present at relatively high frequencies in the water column. However, descendant from this clone reappeared in subsequent years at high frequencies, coinciding with a parallel increase in alleles unique to this founding clone. This is suggestive for the fact that this clone benefited from outbreeding vigour and that allele Dma115—129 (a unique allele for this clone among the founding clones) was not reintroduced from the region but remained present at low frequencies. The one allele that was not present in the founding clones and was probably introduced by subsequent immigration, allele 163 at locus Dma11, remained at low frequencies in the following year, indicating that the impact of immigration was indeed low after initial colonization.

Despite the short distance among the focal pond and the four analysed ponds from the neighbourhood, levels of F_{st} were significant and moderately high. Other studies on newly colonized habitats (Haag et al., 2006; Louette et al., 2007; Ortells et al., 2012) reported higher levels of genetic differentiation. The use of diapausing egg banks instead of samples of the active population (which are a snapshot of the clonal composition) may explain the slightly lower levels of F_{st} reported in this and similar studies (Orsini et al., 2013). Reg4 was considered the most possible candidate source pond, at least among the four analysed ponds. Two reasons lie behind this assumption: its higher allelic richness (2 exclusive alleles and two alleles only shared with Damme and not in the other three ponds) and gene diversity levels (expected heterozygosity) similar to Damme. However, had we expanded the area of regional ponds, we could have probably found further sources of colonization. Over time, genetic differentiation between populations may decay due to gene flow among the populations counteracting the initial founder effects. In addition, the increase in expected heterozygosity due to a higher evenness among alleles as a result of outbreeding vigour may result in a slight decay of genetic differentiation between populations (Louette et al., 2007; Ortells et al., 2012). Although we found both evidence of previously undetected alleles and of equalized allele frequencies, F_{st} with reg4 did not decay significantly over time. Further evidence of limited gene flow after initial colonization comes from the existence of exclusive alleles of *D. magna* in the different regional ponds. However, caution is needed in interpreting these results as only one allele (DMA12—146) that had relatively high frequency in the region (>10% in some of the ponds) was not detected in Damme pond. The fact that most of the regional allelic diversity was present in Damme allows for the possibility that some migrants established in the pond but were not detected. In addition, new alleles may have arrived in our study pond but remained undetected in our field samples.

To our knowledge, this is the first study reporting on the relative contribution of the founding clones to the build-up of genetic diversity and differentiation of a newly established population of cyclical parthenogens. In summary, our results indicate that the Damme population was founded by only a few *D. magna* individuals, which prioritized resources during the first growing season. Although our data suggest that, aided by outbreeding vigour, some additional migrants may have arrived shortly after this initial colonization, their contribution remains minimal and the population genetic structure remains largely determined by the few founding genotypes throughout the study period. No significant changes in the genetic structure of the diapausing egg banks were observed after one or two growing seasons. As predicted by the monopolization hypothesis, at this point, priority effects may have become fully operational strongly reducing gene flow and leading to patterns of sustained population genetic differentiation with nearby ponds. Accordingly, our results evidence that colonization dynamics can strongly influence within and among population genetic variation (Boileau et al., 1992; De Meester et al., 2002).

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