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Colonization history and clonal richness of asexual *Daphnia* in periglacial habitats of contrasting age in West Greenland

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Summary

1. Due to climate change, Arctic ice sheets are retreating. This leads to the formation of numerous new periglacial ponds and lakes, which are being colonized by planktonic organisms such as the water flea *Daphnia*. This system provides unique opportunities to test genotype colonization dynamics and the genetic assemblage of populations. Here, we studied clonal richness of the *Daphnia pulex* species complex in novel periglacial habitats created by glacial retreat in the Jakobshavn Isbræ area of western Greenland.

2. Along a 10 km transect, we surveyed 73 periglacial habitats out of which 61 were colonized by *Daphnia pulex*. Hence, for our analysis, we used 21 ponds and 40 lakes in two clusters of habitats differing in age (estimated <50 years vs. >150 years). We tested the expectation that genetic diversity would be low in recently formed (i.e. young), small habitats, but would increase with increasing age and size.

3. We identified a total of 42 genetically distinct clones belonging to two obligately asexual species of the *D. pulex* species complex: *D. middendorffiana* and the much more abundant *D. pulicaria*. While regional clonal richness was high, most clones were rare: 16 clones were restricted to a single habitat and the five most widespread clones accounted for 68% of all individuals sampled. On average, 3·2 clones (range: 1–12) coexisted in a given pond or lake. There was no relationship between clonal richness and habitat size when we controlled for habitat age. Whereas clonal richness was statistically higher in the cluster of older habitats when compared with the cluster of younger ponds and lakes, most young habitats were colonized by multiple genotypes.

4. Our data suggest that newly formed (periglacial) ponds and lakes are colonized within decades by multiple genotypes via multiple colonization events, even in the smallest of our study systems (4 m^2).

Key-words: asexuality, climate change, clonal diversity, colonization, *Daphnia middendorffiana*, *Daphnia pulex*, genetic diversity, glacial retreat, novel habitats

Introduction

The assembly of populations and communities in natural landscapes is governed by several processes, including dispersal and colonization, and environmental sorting by both abiotic and biotic factors (Hanski & Gaggiotti 2004; Leibold *et al.* 2004; Holyoak *et al.* 2005). For organisms to establish in a population or community, they must first arrive at their respective new habitats. Indeed, dispersal rates, arrival order and dispersal limitation are viewed as key processes in structuring populations (Hanski & Gaggiotti 2004; Orsini *et al.* 2013b) and communities (Leibold *et al.* 2004; De Bie *et al.* 2012). Especially in the context

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of human-induced habitat alterations and climate change, understanding at which rate novel habitats are being colonized and whether or not founding populations harbour much genetic variation is crucial (Sheldon, Yang & Tewksbury 2011; Urban *et al*. 2012; Travis *et al*. 2013).

Successful colonization implies that a species can establish in a habitat in which it has not yet occurred. Colonization of newly formed aquatic habitats is relatively easy to monitor because they are free of established populations including diapausing (dormant) propagules, so that one can trace in a relatively straightforward way the first arrival of the different species (and genotypes) that result in the assembly of communities and populations (Louette & De Meester 2004; Louette *et al*. 2007; Louette, De Meester & Declerck 2008). However, establishment of whether colonization involves just one or multiple individuals during an initial stage and whether it is subsequently supported by regular additional immigrations leading to a higher genetic diversity is more difficult, but has important consequences regarding the evolutionary potential of the resident population (Visser 2008) and the genetic structure of the metapopulation in the landscape (Kremer *et al*. 2012; Orsini *et al*. 2013a).

Global warming is causing rapid ice sheet and glacial retreat in polar as well as in alpine regions (for a review see Barry 2006). Ice sheet and glacial retreat result in the opening of new ice-free (i.e. periglacial) areas for the colonization of organisms. These newly formed ice-free regions are typically rich in ponds and lakes, resulting in numerous novel habitats that can be colonized by aquatic organisms. The colonization process is especially interesting in ponds and lakes, as they represent isolated habitats in a matrix of inhospitable terrestrial environments (De Meester *et al*. 2005). If a species colonizes a suitable new habitat, it can rapidly build up a local population, but its local success provides no guarantee that a nearby habitat will also be colonized. Due to habitat isolation, each colonization of a pond or lake is a discrete process subject to stochasticity. Because of the isolated nature of inland lentic waters, many aquatic organisms have developed effective dispersal strategies, involving both active (e.g. flying adults of many aquatic insects) as well as passive (dormant stages that are carried by wind, water or animal vectors) dispersal (reviewed by Bilton, Freeland & Okamura 2001).

One of the most prominent and rapid retreats of ice sheets is Jakobshavn Isbræ in western Greenland. The glacial retreat in Jakobshavn Isbræ during the past 200 years has been well documented (Weidick *et al*. 1990; see also Fig. 1). It has been under continuous recession since 1850, with a rapid acceleration in the past two to three decades exposing new land with lakes and ponds (Sohn, Jezek & van der Veen 1998). The new periglacial ponds and lakes created by glacial retreat offer an interesting playground to study colonization processes and the resulting genetic structure of local populations.

There is ample literature on colonization of newly created habitats by cladocerans including *Daphnia* (Louette & De Meester 2004, 2005; Louette *et al*. 2007; Ortells *et al*. 2014), copepods and rotifers (Frisch & Green 2007; Frisch *et al*. 2012), toads (Pearl & Bowerman 2006), benthic macroinvertebrates (Kim *et al*. 2014), newts (Arntzen & Teunis 1993) and vascular plants (Järvinen & Ranta 1987). These studies are, however, all from temperate climate zones. The rates at which aquatic organisms colonize newly formed habitats range from a few months to years after its creation and is taxon-dependent (Layton & Voshell 1991; Louette & De Meester 2004, 2005). Louette *et al*.(2007) reported newly created ponds in Belgium were colonized by only 1–3 *Daphnia* clones soon after their creation. Experimental studies by Frisch *et al*. (2012), in southern Spain, showed that new ponds in a region with very high population densities of birds are colonized by zooplankton at a mean rate of 0·09 species per day. In addition, they showed the rate at which new ponds are colonized by cladocerans (0·028 species per day) was higher than that of rotifers or copepods, which colonize new ponds at a mean rate of 0·05 and 0·015 species per day, respectively. However, studies reporting the build-up of genotypic or species richness of aquatic organisms with time are non-existent from the Arctic zone.

Tundra ponds and lakes in the high Arctic are often inhabited by obligately asexual lineages of the *Daphnia pulex* species complex (Weider *et al*. 1996). This species complex shows geographic parthenogenesis, with populations in warm and cold temperate zones being cyclically parthenogenetic, while the high Arctic is dominated by asexual lineages: *Daphnia pulicaria* and *D. middendorffiana* (Decaestecker, De Meester & Mergeay 2009). The landscape genetic structure of obligately parthenogenetic species in the high Arctic has been extensively examined in earlier studies, which have reported both widespread occurrence of specific haplotypes, as well as co-occurrence of multiple clones in single habitats (Ward *et al*. 1994; Weider & Hobak 1997; Weider & Hobæk 2003). Typically, ponds were found to be inhabited by 1–6 clones (Ward *et al*. 1994; Weider *et al*. 1996). These studies did not report on genetic diversity in young habitats but rather focus on landscapes that have been colonized during the course of the last 8000 years. Their observation of the occurrence of widespread clones and the simple structure of local populations that are composed of a limited number of clones provides an attractive setting to study colonization dynamics of novel ponds at a much shorter time frame.

Understanding colonization dynamics of novel habitats associated with glacial retreat can help us to understand and model the expected dynamics of population and community assemblages under future climate change. In this study, we compare clonal richness in two clusters of periglacial ponds and lakes near the tongue of the Jakobshavn Isbræ, western Greenland, to establish how rapidly ponds are colonized and whether they are colonized by

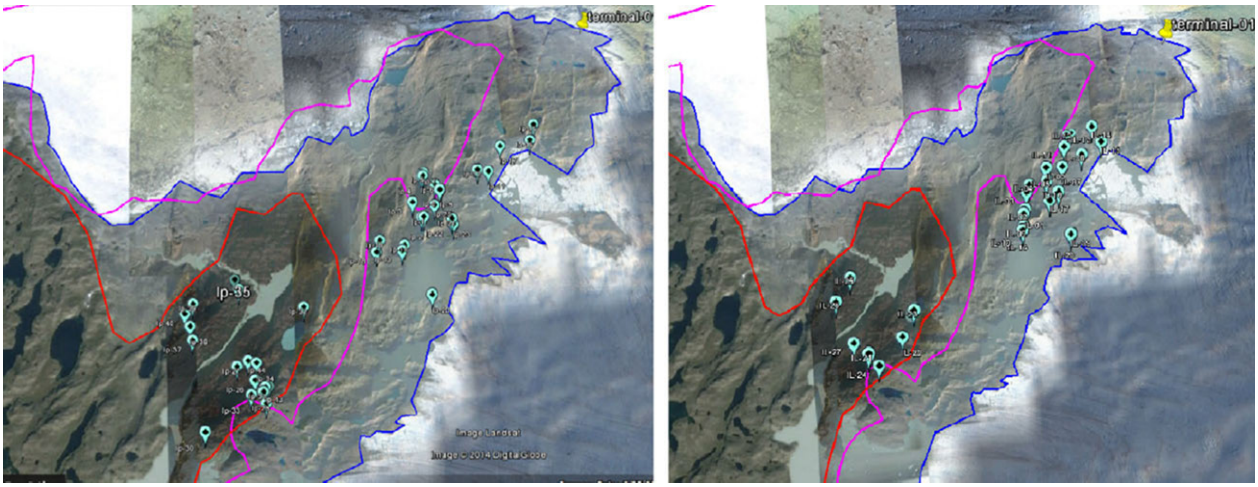

Fig. 1. Two-way retreat of glacial terminus at Jakobshavn Isbrae near Ilulissat: red line 1850 AD; pink line since 1953; blue line the current mouth (margin) of ice sheet. Position of lines adopted from Weidick *et al.* (1990) and Csatho *et al.* (2008); background map is taken from Landsat image ©2014 digital globe. Pointers indicate lakes (right plot) and ponds (left plot) sampled.

multiple genotypes. Our study focuses on differences in clonal richness at a micro-geographic scale by comparison of clusters of habitats differing in age since glacial retreat. According to the theory of island biogeography, larger habitats (or islands) accumulate a higher number of species (MacArthur & Wilson 1967; Losos & Ricklefs 2009), and a similar genetic diversity to population size relationship is expected from genetic diversity theory (Kimura 1968). We tested the hypothesis that clonal richness increases with habitat age and habitat size. The main objective of our analysis was to elucidate the colonization dynamics of newly created habitats formed via climate change and to derive input to models and concepts on population and community assembly dynamics.

Materials and methods

STUDY AREA

Greenland was nearly entirely glaciated during the late Pleistocene epoch and started to deglaciate during the early Holocene (Sohn, Jezek & van der Veen 1998; Csatho *et al.* 2008). Currently, a large part of Greenland is still ice-capped. The Greenland Ice Sheet is progressively melting as a result of global warming. The Jakobshavn Isbræ in the Ilulissat region has been studied extensively with regard to climate change, and the glacial retreat during the past 200 years is amply documented (Weidick *et al.* 1990). This provides us with approximate ages of ponds and lakes in the region.

SAMPLING

During an expedition in July–August 2012 to the area close to the tongue of the Jakobshavn Isbræ near Ilulissat, a total of 73 ponds and lakes were surveyed in the neighbourhood of two basecamps: one close to the ice sheet representing a recently deglaciated area (coordinates basecamp: 69°05′27″ N and 49 ° 47′46″ E) and another area further away from the ice sheet representing an area that was deglaciated more than 150 years ago (69°03′33″ N and 49 ° 54′31″ E). Based on maps of the ice sheet provided by Weidick *et al.* (1990) and Csatho *et al.* (2008; see Fig. 1), the age of the pond and lake clusters was determined to be <50 years and >150 years, respectively. The survey included 46 ponds and 27 lakes of which 25 ponds and 20 lakes were located in the recently deglaciated region, and 21 ponds and seven lakes in the region that was deglaciated >150 years ago. A total of 40 of the ponds and 21 of the lakes were colonized by *Daphnia pulex* sensu lato (s.l.) (Appendix S1, Supporting Information shows the locations of all sample sites; Appendix S2 provides coordinates; and Appendix S3 shows box plots on environmental variables measured in the ponds and lakes in the old and young cluster). From each population, we sampled, when possible, at least 20 *D. pulex* individuals by randomly picking out adults from a sample of living animals with a glass pipette. This was done in the field or immediately upon return to the basecamp. Some of the ponds and lakes were found to harbour both melanic and non-melanic (translucent) individuals (Hebert & Emery 1990). In these cases, we isolated 20 individuals from each phenotypic category, when possible.

GENETIC ANALYSIS

A total of 1420 individuals, on average 20 individuals per population, were subjected to genetic analysis at nine microsatellite loci described by Colbourne *et al.* (2004), according to the protocol described in Mergeay (2005). Six of these loci have been used previously to estimate the ploidy level in *Daphnia pulex* s.l. (Aguilera *et al.* 2007; Vergilino, Belzile & Dufresne 2009). Genomic DNA was extracted from individual *Daphnia* using the HotSHOT method of Montero-Pau, Gómez, & Muñoz (2008), and sample DNA amplifications were carried out in a final reaction volume of 11 μL containing 1 μL of template genomic DNA and 10 μL of a mixture of 0·1–0·2 μM of each primer and 1x QIAGEN Multiplex PCR Master Mix buffer (QIAGEN®, Hilden, Germany). PCR amplification cycling conditions included an initial denaturation step at 95 °C for 15 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and a final elongation step at 60 °C for 30 min.

Amplified PCR products were separated on an ABI3130 capillary sequencer (Applied Biosystems®, Foster City, CA, USA). Allele sizes (fragment lengths) were determined by comparison with an internal Liz500 size standard using the software GENEMAPPER (GeneMapper® v4.0; Applied Biosystems®).

In addition to microsatellite amplification, we also performed a sequencing analysis of a 550 bp fragment of the cytochrome oxidase subunit I (COI) gene using the universal primers LCOI49 and HCO2918, following Folmer *et al.* (1994). We did not screen all individuals, but given the asexual reproductive mode of the populations inhabiting the ponds and lakes in the region (see further), we randomly selected one to five individuals from each multilocus genotype for sequencing.

DATA ANALYSIS

Cytochrome oxidase subunit I sequences were analysed using the Basic *Local Alignment Search Tool* (BLAST) online (http://blast.ncbi.nlm.nih.gov) to identify species identity of the different multilocus genotypes. The ploidy level of individuals was determined using the maximum number of alleles per locus and the relative height of peaks following Mergeay *et al.* (2008) and Vergilino, Belzile & Dufresne (2009). Given that we were dealing with obligately asexual species, we identified clones as multilocus genotypes, grouping all individuals with the same genotype at all nine loci into the same multilocus genotype. Therefore, our values of clonal richness should be viewed as conservative estimates.

Clonal richness (CR) was estimated as the number of unique multilocus genotypes found per population. Even though we tried to standardize sample sizes, some variation remained. We therefore also took sample size into account and calculated relative clonal richness (*R*) following Arnaud-Haond *et al.* (2007):

$$R = \frac{(G-1)}{(N-1)},$$

where G is the number of multilocus genotypes detected in a sample, N is the total number of individuals genotyped from that particular population, and R is relative clonal richness. The value for R ranges from 0 (monoclonal) to 1 (i.e. all individuals analysed represent distinct clonal lineages).

We estimated clonal diversity (CD), which represents the effective number of clones in a population, as the inverse of Simpson's measure of concentration (i.e. $CD = 1/\sum P_i^{2,}$ where P_i is the frequency of the ith clone in a population).

DIVERSITY PARTITIONING

We partitioned alpha (α), beta (β) and gamma (γ) diversity of clones, both as clonal richness and clonal diversity. We calculated beta diversity multiplicatively ($\alpha * \beta = \gamma$), as gamma diversity divided by mean alpha diversity, with all samples being equally weighted as applied in the R-software package vegetarian (Jurasinski, Retzer & Beierkuhnlein 2009). The true diversity measures of alpha, beta and gamma were calculated as $D = 1/\sum p^q$, where D is Jost's true diversity and q is the order of diversity with $q = 0$ and $q = 1$ referring to clonal richness (CR) and clonal diversity (as numbers equivalent of Shannon's index, see Jost 2007), respectively. We compared clonal richness (CR) and clonal diversity ($q = 1$) of pre-defined groups (ponds vs. lakes, young vs. old systems) and tested for significant differences using a two-way analysis of variance (ANOVA).

We also applied additive partitioning to clonal richness at four hierarchical spatial scales: habitat ($n = 61$), habitat groups ($n = 4$), age group/water body type ($n = 2$), and the total set of sampled habitats in the Jakobshavn Isbræ region of Greenland. This enabled us to directly calculate and compare the contribution of alpha (α) and beta (β) diversity to total diversity (γ).

Results

Out of the total set of 40 ponds used in this study, 21 were inhabited by melanic *Daphnia* and 15 by non-melanic ('translucent') animals. In four of the ponds (Ip-3, Ip-4, Ip-6 and Ip-11), melanic and translucent morphs co-occurred (see Appendix S2). Out of the 21 lakes, five were colonized by melanic morphs and 14 by translucent morphs. Three lakes (IL-7, IL-12 and IL-13) were found to harbour both melanic and translucent colour morphs (see Appendix S2). In lake IL-10, only one (translucent) individual was found, and this population therefore was excluded from further population genetic analyses.

Forty-two unique multilocus genotypes (hereafter referred to as clones) were detected in the 61 populations surveyed. On the basis of COI barcodes, 39 of the clones were identified as *D. pulicaria*, while three were *D. middendorffiana* (Fig. 2; see Mergeay *et al.* 2008) for an account of taxonomic issues with this nomenclature). All *D. middendorffiana* were melanic, whereas clones assigned to *D. pulicaria* contained both melanic and translucent individuals in both ponds and lakes. A total of 31 clones were observed in lakes and 27 in ponds. Of these, 15 uniquely occurred in lakes and 11 uniquely in ponds. Thirty-three clones were identified as triploids based on the criterion that they contained at least one locus with three alleles; four clones were identified as tetraploid and five as putatively diploid. All of our analyses were done both with and without the few *D. middendorffiana* clones, yielding the same overall results. The result of the analyses presented here is based on the dominant (i.e. *D. pulicaria*) species unless otherwise specified.

All except four of the 42 clones were represented by more than one individual and 26 clones were found in more than one population. Clones strongly differed in relative abundances (Fig. 2), and the five most widespread clones (M1, M2, M3, M4 and M5) constituted 68% of all individuals genotyped. Clone M3 occurred in 18 ponds and six lakes, whereas M1 was detected in 17 ponds and five lakes. Clone M1 was the most abundant clone in ponds (210 out of the 900 individuals being genotyped from ponds) followed by M3 (125 individuals); M5 was the most dominant clone in lakes, representing 149 out of the 500 individuals being genotyped.

Mean clonal richness per habitat was 3·20 and did not differ between lakes and ponds (Table 1; two-way ANOVA test). However, there was a significant effect of lake and

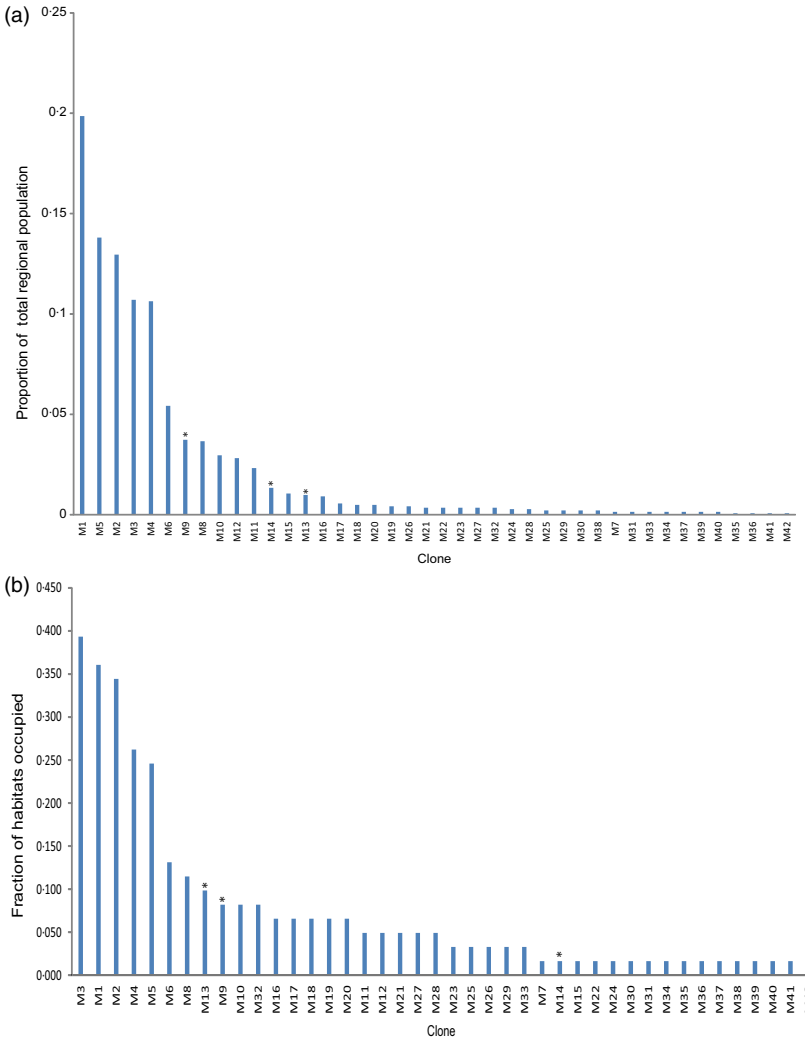

Fig. 2. Relative abundances of the 42 clones detected in our survey: (a) proportion of the total regional population that each clone represents for the 42 clones ranked from the most to the least abundant clone; (b) proportion (frequency of occurrence) of habitats in which specific clones were detected. Clones identified as *D. middendorffiana* are indicated with *.

Table 1. Results of two-way ANOVA testing for the effect of age (young/old) and habitat type (lake/pond) on diversity measures (i.e. clonal richness and clonal diversity) of *Daphnia* populations in the Jakobshavn Isbræ region of Greenland

	d.f.	Sum Sq.	Mean Sq.	*F* value	Pr(>*F*)
Response: Clonal Richness (CR)					
Age	1	50·578	50·578	12·2149	0·0009***
Habitat	1	0·599	0·599	0·1446	$0·7052^{ns}$
Age × Habitat interaction	1	4·446	4·446	1·0738	$0·3045^{ns}$
Residuals	57	236·017	4·141		
Response: Clonal diversity (^{1}D)					
Age	1	17·371	17·3713	8·7934	0·0044**
Habitat	1	0·174	0·1745	0·0883	$0·7674^{ns}$
Age × Habitat interaction	1	2·385	2·3845	1·2071	$0·2765^{ns}$
Residuals	57	112·603	1·9755		

Signif. codes: ***$P < 0·001$; **$P < 0·01$; ns, non-significant.

pond age on clonal richness (Table 1, Figs 3 and 4a). Figure 3a shows a clear difference in clonal richness between habitats in the old and young cluster, while Fig. 3c shows a gradient in clonal richness along a north-east/south-west axis, which likely corresponds to a gradient of age since the retreat of the glacier tongue (see Fig. 1). Within clusters, especially within the old cluster, there was also a north-west/south-east gradient of clonal richness (Fig. 3b), which corresponds to a distance gradient to the margin of the ice sheet (see also Fig. 1).

Partitioning regional clonal richness (γ) into a within- (α) and an among-population (β) component revealed that a large part (91·5%) of the regional clonal richness was determined by differences in clonal identities among habitats (see Appendix S4). Mean within-population clonal richness (α richness) in young systems was lower than in old systems (Table 2). Among-population diversity tended to be lower in young compared to older lakes and ponds (Table 2, see also Appendix S4). There was no significant overall correlation between clonal richness and habitat area (Fig. 5a; $r_s = -0·036$, $P = 0·44$). There was also no significant correlation between clonal richness and habitat area when separate analyses were conducted for young and old habitats ($r_s = -0·157$, $P = 0·83$ and $r_s = -0·053$, $P = 0·59$, respectively, see Appendix S5).

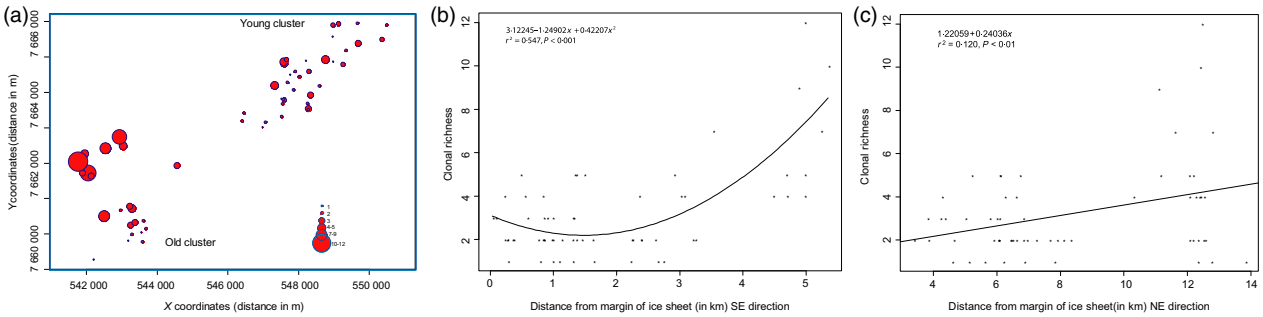

Fig. 3. Pattern of clonal richness of *D. pulicaria* populations inhabiting ponds and lakes sampled in the Jakobshavn Isbræ region of Greenland. (a) Clonal richness with respect to age cluster (old cluster approximately 150 years old and young cluster less than 50 years old); the size of the circle is proportional to clonal richness; (b) relationship between clonal richness and distance from ice sheet (in km) along the north-west/south-east axis (i.e. distance to the side of the ice sheet); (c) relationship between clonal richness and distance from ice sheet (in km) along the north-east/south-west axis (i.e. distance to tongue of the glacier). '*x*' in the equations in both b and c represents the distance from the ice sheet in km. In each case, we show the best fit from a quadratic or linear relationship.

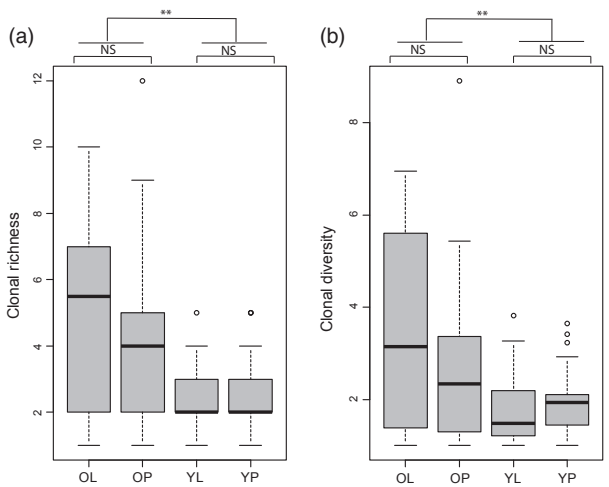

Fig. 4. Boxplot of clonal richness (a) and Simpson clonal diversity (b) of ponds and lakes in the Jakobshavn Isbræ region of Greenland. Age cluster abbreviations: old lake (OL), old pond (OP), young lake (YL) and young pond (YP). Boxes represent the interquartile range; whiskers represent the minimum and maximum observations. Significance: ** = $P < 0{\cdot}01$; ns = non-significant.

The pattern for clonal diversity largely mimicked that observed for clonal richness. Average clonal diversity across all studied populations was 2·0. Clonal diversity was significantly different between young and old habitats (Table 1, Fig. 4b), but did not differ between ponds and lakes (Table 1, Fig. 4b). There was no significant correlation between clonal diversity and habitat area (Fig. 5b; $r_s = -0{\cdot}088$, $P = 0{\cdot}75$). Also, there was no significant correlation detected when separate analyses were carried out for young and old habitats ($r_s = -0{\cdot}229$, $P = 0{\cdot}92$ and $r_s = -0{\cdot}097$, $P = 0{\cdot}67$, respectively; see Appendix S5).

Discussion

The key results of our analysis are that (i) water bodies in the Jakobshavn Isbræ area of western Greenland harbour a regionally diverse set of asexually reproducing *Daphnia* clones; (ii) most water bodies, even the youngest ones, have been colonized by multiple clones; (iii) clonal diversity is not related to either habitat size or habitat type, but (iv) is related to the age of the habitats, with the populations in the older cluster (>150 years) of ponds and lakes being genetically more diverse than those in the ponds and lakes <50 years old. In the following, we discuss each of these observations in relation to our hypotheses and the results reported in previous studies and reflect on the implications with respect to responses to climate change and ice-sheet retreat.

We observed two obligately asexual species of the *Daphnia pulex* spp. complex in the ponds and lakes sampled in the Jakobshavn Isbræ region. Of these, *D. pulicaria* is by far the more common, but three clones of the melanic species *D. middendorffiana* were also detected. We observed both melanic and translucent *D. pulicaria* genotypes in both ponds and lakes, although there was a tendency for higher occurrence of melanic genotypes in ponds and more translucent genotypes in lakes. The latter observation is in line with expectations as the greater depth of the lakes may allow avoidance of UV radiation by diel vertical migration (Hebert & Emery 1990). Most genotypes were triploid, while a minority of the clones were tetraploid or (putatively) diploid. This is in concordance with previous circum-Arctic studies where occurrence of triploid and tetraploid clones of the *Daphnia pulex* spp. complex has been reported based on allozymes (Ward *et al.* 1994; Dufresne & Hebert 1995; Weider *et al.* 1996, 1999a,b; Weider & Hobak 1997; Weider & Hobæk 2003) and genome size investigations (Dufresne & Hebert 1995; Vergilino, Belzile & Dufresne 2009). Polyploid asexual *D. pulex* complex taxa have also been reported in the *Daphnia pulex* spp. complex outside the Holarctic region, and more specifically in Andean regions of South America (Aguilera *et al.* 2007). What is common to all the asexual polyploid individuals is that they are found under extreme environmental conditions with reduced levels of competition, parasitism and predation (Havel, Hebert & Delorme 1990; Dufresne & Hebert 1995; Aguilera *et al.* 2007).

Table 2. Alpha, beta and gamma diversity partitioning for clonal richness and clonal diversity for the total set of habitats (n = 61) and for subsets determined by habitat type (pond or lake), age class (young and old) and the four categories separately. The mean and one standard deviation (in brackets) for α, β and γ are reported. The last two columns give the data on relative β for both clonal richness and clonal diversity. The raw β diversity values range from 1 to N for N equally weighted samples. β Diversity measures therefore depend on the number of samples in a region analysed. To standardize for this, we here also calculated relative β diversity, which expresses β as a value between 0 and 1 to facilitate comparisons between categories with different numbers of samples. We used the formula for Similarity based on true diversity developed by Jost (2007) $S = [^qD(H\alpha)/^qD(H\gamma)–1/N]/[1–1/N]$ and calculated relative β as $1–S$. A value of 1 means that the clonal composition of the samples are completely distinct, while a value of 0 means that the clonal composition of the samples is identical

		Clonal richness (CR)						Clonal diversity (q = 1)						Relative β	
Category	N	alpha (α)		beta (β) = (γ)/(α)		gamma (γ)		alpha (α)		beta (β) = (γ)/(α)		gamma (γ)		CR	CD
All Populations	61	3·2	(0·058)	13·14	(0·480)	42	(1·301)	1·99	(0·034)	6·83	(0·131)	13·6	(0·271)	0·94	0·87
Young Lake	15	2·4	(0·094)	3·33	(0·225)	8	(0·588)	1·63	(0·050)	2·93	(0·082)	4·79	(0·151)	0·75	0·71
Young Pond	24	2·58	(0·074)	5·42	(0·290)	14	(0·645)	1·85	(0·040)	3·59	(0·084)	6·65	(0·186)	0·85	0·75
Old Lake	6	5·17	(0·276)	5·03	(0·179)	26	(1·539)	2·82	(0·149)	4·48	(0·138)	12·6	(0·939)	0·96	0·93
Old Pond	16	4·13	(0·147)	5·58	(0·320)	23	(0·957)	2·33	(0·082)	5·33	(0·214)	12·4	(0·471)	0·88	0·87
Lake (age pooled)	21	3·19	(0·103)	9·72	(0·408)	31	(0·102)	1·91	(0·063)	5·85	(0·135)	11·2	(0·321)	0·94	0·87
Pond (age pooled)	40	3·2	(0·072)	8·44	(0·335)	27	(0·991)	2·03	(0·041)	5·6	(0·139)	11·4	(0·301)	0·90	0·84
Young (habitat pooled)	39	2·51	(0·057)	6·37	(0·353)	16	(0·803)	1·76	(0·037)	3·78	(0·079)	6·67	(0·154)	0·87	0·76
Old (habitat pooled)	22	4·41	(0·132)	8·39	(0·357)	37	(1·306)	2·45	(0·077)	7·33	(0·228)	18	(0·643)	0·92	0·90

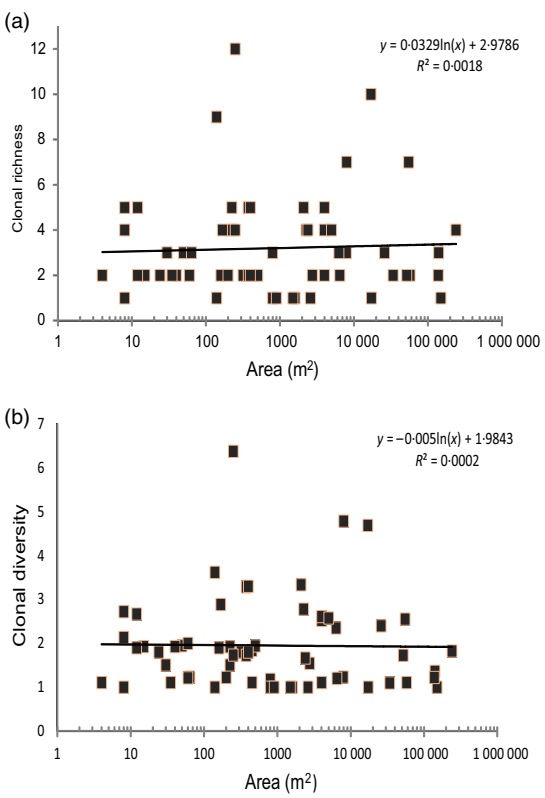

Fig. 5. Relationship between clonal richness (a) and clonal diversity (b) with habitat area (log scale) for all populations. The lines and equations refer to the analysis across all data.

In a total of 61 sampled populations, we observed 42 different genotypes. Previous studies from the Arctic have reported 15 clones from 28 ponds (Ward *et al*. 1994), 18 clones from 49 ponds (Weider & Hobaek 1994), 21 clones from 20 ponds (Hobaek, Weider & Wolf 1993) and 16 clones from 147 ponds (Weider & Hebert 1987). Compared to these previous results, our estimate of regional clonal diversity of *D. pulicaria*/*middendorffiana* in the Jakobshavn Isbræ region of Greenland is quite high. Further, our estimate of clonal richness and diversity over a relatively small geographic area (approximately 10 km^2) is higher than that reported in previous allozyme-based work from Svalbard, low Arctic sites in Canada (Hobaek, Weider & Wolf 1993), the Ontario region of Canada (Hebert & Crease 1983), Iceland and the Nuuk region in Greenland (Weider *et al*. 1996). We believe the differences in clonal diversity among ours and previous studies are likely to be related to a multitude of factors. One is differences in habitat: our ponds and lakes are tundra ponds unlike rock bluff habitats from Churchill and Scandinavian studies (Weider & Hebert 1987; Ward *et al*. 1994). A second reason may be that we used microsatellite markers whereas previous studies relied on allozyme markers. Microsatellite markers generally allow for a higher resolution. Yet, clones of asexual taxa tend to be genetically divergent and differ in alleles at many loci simultaneously and are therefore relatively easy to detect. In addition, earlier studies used a similar number of markers as used in the present study (Ward *et al*. 1994; Weider & Hobæk 2003). As a result, we are confident that our comparisons are realistic and informative. Whatever the reason, our key observation is that the regional clonal diversity presented here from a region that only recently became ice-free, is not strongly reduced compared to areas that are much older (e.g. 3000–10 000 years for the Churchill and Scandinavian habitats studied by Ward *et al*. (1994). Weider, Beaton & Hebert (1987) used five polymorphic allozyme loci and surveyed clonal diversity in 179 tundra ponds on a small island (Igloolik – approximately

35 km^2) in the high Canadian Arctic at a comparable latitude (69°22′ N, 81°48′ W) to our study region. In that study, which involved habitats generally >1000 years old, a total of 75 unique clones were detected, which is very similar to the regional diversity reported by us in a region with much younger habitats. Overall, our results show that regional genetic diversity of the *Daphnia* metapopulation builds up rapidly, within the first decades to centuries after a region becomes ice-free.

We observed one to 12 *D. pulex*/*middendorffiana* clones to coexist in local populations, with a mean clonal richness of 3·2 clones per population (on average 2·5 clones per population in habitats of the young cluster and 4·4 clones per population in habitats of the older cluster). This is comparable to the results of Weider, Beaton & Hebert (1987) in tundra ponds on Igloolik in the high Canadian Arctic, who observed a range of one to 14 clones per population, and with a mean clonal richness of 4·5 clones per population. Local clonal diversity in that study is very similar to what we observe in our old habitat cluster, even though the age of the ponds in the Igloolik study was estimated to be >1000 years. For a clone/species to occupy a site, it must both arrive at the site by dispersal and maintain a positive population growth in the local environment. Most clones in our survey were only observed in one or a few ponds, but some clones were widespread and common. Partitioning of total diversity into within- and among-population components reveals that most of the genetic variance is at the among-population level.

There are four lines of observations in our data set that lead to the conclusion that dispersal of *Daphnia* in this remote region is high: (i) the high regional clonal diversity, which suggests a fair number of colonization events in the 10 km^2 region during the past decades; (ii) nearly all ponds and lakes are colonized by multiple clones, even the smallest habitats (i.e. 4 m^2); (iii) some clones are widespread, which reflects either rapid spread over a large number of habitats through independent or serial colonization events; and (iv) nearly all habitats in the young cluster (<50 years old) were inhabited by *Daphnia* (exceptions were some temporary puddles of just a few cm deep and the lakes that are directly connected with the glacial melt-water lake, which are known to be inhospitable to *Daphnia* (Sommaruga 2015) Potential vectors of dispersal in this region include Canada goose (*Branta canadensis*), which were regularly seen in low numbers during sampling (L. De Meester pers. obs.).

Ponds and lakes in the older cluster (>150 years old) had higher clonal richness and diversity than habitats in the younger cluster (<50 years old). A clear gradient of clonal richness emerged in relation to the distance of the sampled habitats from the glacier base and the sides of the ice sheet. These distance gradients likely reflect age gradients, as is suggested by the two-directional ice-sheet retreat pattern shown in Fig. 1. A similar pattern of enhanced clonal richness with invasion age has been reported in cattail (Typhaceae) by Travis *et al*. (2011). This increase in clonal richness with age may reflect gradual accumulation of clones because the probability of colonization of a habitat increases with time. Alternatively, habitats may also gradually become more heterogeneous as they age, thus allowing the coexistence of more clones. While young and old habitats in general were similar for most physico-chemical variables that we measured, we did observe some differences with age, notably in total nitrogen concentration and pH (see Appendix S3). The relationship between clonal diversity and age of the ponds may thus also reflect ecological changes that occur in the ponds as they age, and need not necessarily reflect dispersal limitation. Our results differ from those reported by Weider, Beaton & Hebert (1987), who found no significant association between clonal diversity and tundra pond age. In this latter study, age was estimated as pond elevation above mean sea level on 'raised beaches' exposed after glacial retreat and isostatic rebound (see also Järvinen & Ranta 1987) on Igloolik Island. The time scales of this and our study are very different, however, as the majority of the ponds in the latter study had been ice-free and raised from the sea-bed for a much longer time period (i.e. at least several thousand years) than the age gradient, we consider in the present study.

Surprisingly, while we observed an increase in clonal richness with age, we observed no differences in clonal richness between small ponds and larger and deeper lakes. This is in contrast to the aforementioned Igloolik study (Weider, Beaton & Hebert 1987) which found a significant positive relationship between clonal diversity and tundra pond surface area. Such a relationship between diversity and surface area is generally expected from the theory of island biogeography (MacArthur & Wilson 1967; Losos & Ricklefs 2009). Clones of asexual species can be considered as independent units that colonize local habitats from the regional genotype pool, comparable to species. Assuming that population size scales with habitat size, we would both expect a higher probability of colonization as well as a higher probability of coexistence in larger compared to smaller habitats (MacArthur & Wilson 1967; Losos & Ricklefs 2009). If clonal richness in the ponds and lakes of the Jakobshavn Isbræ is solely determined by colonization dynamics, one would expect higher richness in habitats with larger surface areas because the probability of arriving in a larger habitat by random processes increases with surface area. However, if dispersal vectors (like Canada geese Figuerola, Green & Michot 2005) have higher affinity for small ponds than for large lakes, this may counteract the expected diversity–area relationship. Our results also indicate that small habitats do not impede clones to coexist over longer time spans, which is reflected in an increased clonal diversity with age also in the pond systems. Conversely, large habitats do not necessarily translate into an accumulation of greater clonal diversity than smaller ponds. The latter may be caused by persistent founder effects, which may buffer the interaction between dispersal propensity and habitat size (Boileau, Hebert & Schwartz 1992; Ventura *et al*. 2014).

In summary, we observed that the water flea *Daphnia* has been able to colonize ponds and lakes that have only recently been deglaciated as a consequence of glacial retreat and that high regional clonal diversity occurs in this relatively young terrain. Our survey suggests that within a few decades, all suitable habitats are colonized, often by multiple genotypes, suggesting rapid build-up of populations as new habitats become available. We also found evidence that clonal richness continues to increase as ponds and lakes age. From our current survey, it is not possible to conclude whether this reflects dispersal limitation or an increase in habitat heterogeneity, favouring the coexistence of genotypes. With climate warming, larger expanses of polar and alpine regions will become ice-free and numerous new ponds and lakes will be created, and our results suggest that these will be rapidly colonized by aquatic organisms like *Daphnia* and that the resulting populations are genetically diverse. Given the importance of Daphnia as a keystone grazer of algae and bacteria, an important nutrient recycler, as well as serving as a critical prey item for a variety of (in)vertebrate predators, its ability to rapidly colonize recently deglaciated habitats in the face of climate change could have far-reaching impacts in aquatic ecosystems on a global scale.

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Data accessibility

DNA sequences are available under the GenBank Accession numbers KX024528-KX024565.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Location of sampling sites in two age clusters.

Appendix S2. Geographic coordinates, number of individuals genotyped (N), number of clones detected (CR) and various population genetic diversity measures: clonal richness (R; corrected for sample size expressed as proportion of clones to total individuals genotyped), clonal diversity (H1: Shannon diversity; CD: Simpson index), age class (young/old), size (ha), distance to the ice sheet terminus (km), and morph type of the *Daphnia* (translucent, melanized, or both) found in the lake/pond investigated.

Appendix S3. Box plot comparison of physico-chemical parameters measured: area (ha), pH, temperature (°C), altitude (m), total phosphorus (mg/l), total nitrogen (mg/l), chlorophyll-a (μg/l) and depth (m).

Appendix S4. Additive partitioning of clonal diversity of *Daphnia pulicaria/middendorffiana* in the total set of lakes and ponds sampled in the Jakobshavn Isbræ region: (A) using a hierarchical structure with age (left stacked bar: β1 = among populations within habitat type within age groups; β2 = among habitat types within age groups; β3 = among age groups) or habitat type (right stacked bar: β1 = among populations within habitat type within age groups; β2 = among age groups within habitat types; β3 = among habitat types) as the highest hierarchical level; (B) using the four different habitat types x age clusters as focal clusters and comparing them with the rest of the populations (β1 = among populations within the cluster; β2 = among populations across clusters).

Appendix S5. Correlation between clonal richness and clonal diversity with habitat surface area (in ha) for all populations and for all ponds, all lakes, all young and all old systems separately.