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Environment not dispersal limitation drives clonal composition of Arctic *Daphnia* in a recently deglaciated area

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Abstract

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L.D.M. and E.J. designed the field sampling work. L.D.M., E.J., T.A.D., R.S., M.M., H.A. and K.J. were involved in the field sampling and collected data on lake and pond characteristics and *Daphnia* samples. E.J. coordinated the expedition and the analysis of environmental variables in the laboratory upon return from field sampling. T.H.H., J.M. and L.D.M. designed the molecular work. T.H.H. performed the molecular laboratory work and analysed the data with input from J.M., L.D.M. and L.J.W. T.H.H. wrote the first version of the manuscript with input from J.M., L.D.M. and L.J.W. All co-authors commented on the final version of the manuscript.

Data accessibility

Sampling locations, all raw data of environmental variables, input data and R-scripts used for RDA, pRDA along with microsatellite data used in POLYSAT are available in Dryad[®] digital repository with doi:10.5061/dryad.m874p. DNA Sequence data used for species identification were submitted to GenBank[®]. Accession nos. KX024528–KX024565. Additional analyses are also provided as supporting information.

One of the most prominent manifestations of the ongoing climate warming is the retreat of glaciers and ice sheets around the world. Retreating glaciers result in the formation of new ponds and lakes, which are available for colonization. The gradual appearance of these new habitat patches allows us to determine to what extent the composition of asexual *Daphnia* (water flea) populations is affected by environmental drivers vs. dispersal limitation. Here, we used a landscape genetics approach to assess the processes structuring the clonal composition of species in the *D. pulex* species complex that have colonized periglacial habitats created by ice-sheet retreat in western Greenland. We analysed 61 populations from a young (<50 years) and an old cluster (>150 years) of lakes and ponds. We identified 42 asexual clones that varied widely in spatial distribution. Beta-diversity was higher among older than among younger systems. Lineage sorting by the environment explained 14% of the variation in clonal composition whereas the pure effect of geographical distance was very small and statistically insignificant ($R^2_{\text{adj}}=0.010$, $P=0.085$). Dispersal limitation did not seem important, even among young habitat patches. The observation of several tens of clones colonizing the area combined with environmentally driven clonal composition of populations illustrates that population assembly of asexual species in the Arctic is structured by environmental gradients reflecting differences in the ecology of clones.

Keywords

climate change; colonization; founder effect; isolation-by-colonization; isolation-by-dispersal limitation; landscape genetics

Introduction

Polar and Alpine glaciers are retreating at high rates in different parts of the world due to climate change, thereby opening new habitats for colonization by a variety of terrestrial and aquatic organisms (Sohn *et al.* 1998; Bennike 2000; Barry 2006). This progressive retreat creates, where topology is suitable, new meltwater ponds and lakes at the ice margin and leads to an age gradient of water bodies as the distance from the glacier terminus increases (Sommaruga 2015). One of the most prominent and rapid ice-sheet retreats is seen along the Greenland Ice Sheet (GrIS) in Jakobshavn Isbræ, western Greenland, which has been well documented for >200 years (Weidick *et al.* 1990; Sohn *et al.* 1998; Weider *et al.* 1999; Joughin *et al.* 2004; Luckman & Murray 2005; Rignot & Kanagaratnam 2006). Many of the resulting (relatively young) water bodies have been colonized by the common water flea *Daphnia pulex* sensu lato (s.l.) (Weider *et al.* 1999; Haileselasie *et al.* 2016). Although this species complex contains both sexual (cyclically parthenogenetic) and asexual (obligately parthenogenetic) lineages (the latter often polyploid), polar regions typically harbour only asexual lineages (reviewed in Decaestecker *et al.* 2009). The gradual creation of new habitat patches allows us to determine to what extent the composition of relatively young populations is affected by environmental drivers vs. dispersal limitation.

The processes that structure asexual clonal assemblages and ecological communities are similar (Hebert *et al.* 1988). The study of clonal diversity and composition in an asexual taxon is therefore equivalent, and one can use the same approach as that of community analysis, dissecting the role of environmental and spatial drivers (Legendre *et al.* 2005;

Legendre & Legendre 2012; Orsini *et al.* 2013). Three main hypotheses have been put forward to explain differences in clonal composition: the frozen niche variation (FNV) hypothesis (Vrijenhoek 1979, 1984), the general-purpose genotype (GPG) hypothesis (Lynch 1984) and the neutral theory (Hubbell 2001). Under the FNV hypothesis, banking on differences in ecological performance among clones across habitats, we expect a clonal structure that shows pattern of isolation by environment (Orsini *et al.* 2013), similar to species sorting (Leibold *et al.* 2004). In contrast, under the neutral theory, we only expect clonal composition to reflect dispersal limitation, resulting in a pattern of isolation-by-dispersal limitation (IBDL; Orsini *et al.* 2013). Finally, the GPP hypothesis advocates the general competitive superiority of one or a few super clones, thereby causing clonal composition to be uniform over a large area. Under this hypothesis, we do not expect to find any clear relationship between environmental and spatial gradients. Asexual metapopulations of *Daphnia pulex* s.l. often show evidence of clonal sorting along environmental variables such as salinity, temperature, food availability or predators (Loaring & Hebert 1981; Weider & Hebert 1987; Wilson & Hebert 1992; Pantel *et al.* 2011). The analysis of clonal composition of populations of asexual *Daphnia* species that inhabit recently colonized periglacial ponds and lakes offers an excellent model system to investigate to what extent such patterns of environmental sorting are already evident in metapopulations that developed in recently created habitats, or whether these metapopulations are mainly structured by dispersal limitation or dominance of GPGs (Haileselasie *et al.* 2016).

Using a landscape genetics approach, we tested the effects of historical factors (colonization dynamics, glaciation) and environmental sorting along an age gradient of contrasting habitats created by ice-sheet retreat in the Jakobshavn Isbræ region of western Greenland. Specifically, the objectives of our study were as follows: (i) to investigate the spatial population genetic structure of asexual *Daphnia* populations along an age gradient of habitats; and (ii) to investigate to what extent environment, space or history (glaciation) are the major determinants of clonal composition in the young metapopulations of *D. pulex* s.l.

Materials and methods

Study area

Greenland (59°45′24″–83°35′54″N, 72°18′09″–22°05′56″W) is the largest island in the world, and 80% of its landmass is capped with ice (Fig. 1). During an expedition in 2012 to the Jakobshavn Isbræ near Ilulissat, water fleas (*Daphnia*) were sampled from 61 populations (see Haileselasie *et al.* 2016 for details). We surveyed the clonal structure of *Daphnia pulex* s.l. in periglacial habitats of contrasting age created by the retreat of the GrIS in the Jakobshavn Isbræ region of western Greenland across 40 ponds and 21 lakes. For each pond and lake, Universal Transverse Mercator (UTM) coordinates, elevation (m), depth (m), water temperature (°C), pH and conductivity (µS/cm) were recorded in the field. In addition, composite samples were collected with a 5-L Schindler sampler at 0.5–3 m interval (depending on the depth of the lakes) from the surface to 1 m above the bottom in the deepest part of the lakes, and then mixed. For chemical analyses, a subsample of 250 mL was frozen, and for chlorophyll *a*, a duplicate 1 L sample was filtered on a GF/C filter

(Whatman) and frozen until analysis. Lake water total phosphorus (TP) was determined as molybdate-reactive phosphorus (Murphy & Riley 1962) following persulphate digestion (Koroleff 1970), and total nitrogen (TN) as nitrite + nitrate after potassium persulphate digestion (Solorzano & Sharp 1980). Chlorophyll *a* was determined spectrophotometrically after ethanol extraction (Jespersen & Christoffersen 1987).

Genotyping

A total of 1420 individuals (~20 individuals per population except for water bodies IP-37, IP-18 and IL-21, see Table S1, Supporting information) was subjected to genetic analysis using nine microsatellite loci described by Colbourne *et al.* (2004), according to protocols in Mergeay (2005). Genomic DNA extraction and PCR amplifications followed the methods stated in Haileselasie *et al.* (2016). Amplified PCR products were separated on an ABI 3130 capillary sequencer (Applied Biosystems®), and allele sizes (fragment lengths) were scored against an internal size standard (Liz500) using the software GENEMAPPER (GeneMapper® v4.0; Applied Biosystems®, Foster City, CA, USA).

Data analysis

For each genotyped individual, we counted the maximum number of alleles per locus that were unambiguously scored by GENEMAPPER (GeneMapper® v4.0; Applied Biosystems®). Allele sizes were automatically scored by GENEMAPPER using panels and bins of Mergeay (2005) and later manually checked. 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 *et al.* (2009). Multilocus genotypes (MLGs) were filtered and determined for the whole data set ($n = 1420$), and individuals with the same genotype at all loci were assumed to belong to the same MLG (to be called 'clone' hereafter).

To assess the genetic relatedness among clones of *D. pulex* s.l., we used a pairwise genetic distance among the clones calculated using Bruvo's distance (Bruvo *et al.* 2004) as implemented in the R PACKAGE POLYSAT (Clark & Jasieniuk 2011). This was followed by a principal coordinate analysis of the distance matrices to graphically summarize the genetic relatedness among clones. Then, the first two axes were used to generate a dendrogram using Ward hierarchical clustering method in R (Murtagh & Legendre 2011; R Development Core 2013). Bruvo's distance method takes into account the genetic relatedness among individual alleles, assuming a stepwise-mutation model (Valdes *et al.* 1993). As a measure of genetic distance between populations, we used the Sørensen index and Jaccard's index (Jaccard 1908; Sørensen 1948). Both distance measures use clonal frequencies as input, but Sørensen index is sensitive to the presence of rare clones.

We applied community ecological data analysis methods to: (i) assess differentiation in clonal composition among populations; and (ii) investigate the relative contribution of local environmental variables and spatial distance to the variation in clonal composition in local populations. We used redundancy analyses (RDAs, a linear constrained ordination technique (Legendre & Legendre 2012), to infer the role of spatial and environmental variables separately and to disentangle the unique contribution of each variable matrix to clonal composition of the studied populations using variation partitioning (Dray *et al.* 2006). In a

multivariate variation partitioning analysis, the contributions of local environmental predictors and spatial predictors, as both the unique and the shared contribution, are tested by generating adjusted redundancy statistics (R^2_{adj}) in a partial RDA. The significance of R^2_{adj} was tested by running 1000 random permutations of clonal composition among sites, and then calculating the proportion of runs where the randomly permuted R^2_{adj} value equalled or exceeded the observed R^2_{adj} as described in Peres-Neto *et al.* (2006). Spatial variables (latitude–longitude coordinates, in UTM readings) were transformed into distance-based Moran Eigenvector Maps (dbMEMs; see Dray *et al.* 2006 and Legendre & Legendre 2012). A significant contribution of space to variation in clonal composition would imply an influence of dispersal limitation and neutral dynamics, resulting in a differentiation by distance pattern (Orsini *et al.* 2013). A significant effect of environment would imply sorting of clones with different traits and niches along environmental variables. Model construction and forward variable selection used the double stop criterion (Blanchet *et al.* 2008). Significant environmental variables ($P < 0.05$) were retained for variation partitioning among spatial and environmental variables. Clonal composition data (abundance per clone per sample) were Hellinger-transformed to reduce the weight of rare clones (Legendre & Gallagher 2001).

Given that both the lake and pond habitats investigated belonged to two age clusters (>150 years vs. <50 years old), we also conducted an additional pRDA of clonal composition using dbMEMs, environmental predictors and age as independent variables. We introduced age using dummy variables for ‘young’ (<50 years) and ‘old’ (>150 years old) habitats because ponds and lakes occurred in two clusters of different age. We also used distance of each habitat from the ice sheet as an additional proxy for the age of each habitat, which yielded a more continuous gradient of age. The contribution of local environmental predictors, spatial predictors, and age as both unique and shared contributions were tested by generating adjusted redundancy statistics (R^2_{adj}). Finally, we tested for the effect of habitat type (lakes and ponds) on clonal composition in a pRDA using dbMEMs, environmental predictors, age and habitat type as independent variables.

We used the VEGAN package in R (Oksanen *et al.* 2013; R Development Core 2013) for running RDA and pRDA variation partitioning, and the package PACKFOR in R (Dray *et al.* 2009) to run the forward selection procedure in RDA (Blanchet *et al.* 2008). We used the package PCNM in R (Legendre *et al.* 2012) to generate dbMEM.

We applied an indicator species analysis using the ‘indicspecies’ module in R (Cáceres & Legendre 2009) to measure the association between the abundance values of specific clones and environmental characteristics of the sampled populations grouped by the age of the habitat (>150 years vs. <50 years old). The statistical significance of this relationship was tested using a permutation test (see De Cáceres *et al.* 2010; Dufrêne & Legendre 1997 for details on the methodology).

Results

We detected a total of 42 clones with pairwise genetic distances between clones ranging from 0.01 to 0.62 (Table S2, Supporting information). A principal coordinate analysis of genetic distances between clones and a dendrogram on the basis of Bruvo's genetic distance revealed four clusters (Fig. 2A, B). Cluster 1 included 11 clones that are putative triploids or tetraploids. Cluster 2 was composed of 20 clones, which included putative diploid and triploid clones, whereas cluster 3 consisted entirely of triploid clones. Cluster 4 was composed of clones that were triploid, and all clones in this cluster except M21 were only found in old (>150 years) ponds and lakes (Figs 2 and 3).

Generally, beta-diversities in the study area were high, with the mean Sørensen index being 0.88 ± 0.20 (Table 1). Differentiation in clonal composition among 'old' populations (mean Sørensen index 0.92) was higher than among 'young' populations, but still differentiation among the latter populations was high (mean Sørensen index: 0.79; Table 1).

The majority of ponds were inhabited by *Daphnia pulicaria* except for 10 ponds, where *D. pulicaria* and *D. middendorffiana* coexisted (for species identification see Haileselasie *et al.* 2016). IP-31 was the only pond that was solely inhabited by *D. middendorffiana*. None of the lakes contained *D. middendorffiana*. Sixteen clones were detected in both lakes and ponds. M1 and M3 were detected from young lakes and both young and old ponds but were the most dominant clones in ponds. The most dominant clone in lakes was M5 ($N_L = 9$) followed by M2 ($N_L = 7$). Clone M5 was nearly restricted to young lakes, but also occurred at very low frequencies in a few young and old ponds. Clones M2 and M4 occurred in both young and old ponds, and in very low frequencies also in old lakes. Nine clones (M6, M8, M16, M17, M20, M23, M26, M29 and M32) were restricted to old lake and old pond systems. Fourteen of the 15 clones that were restricted to lakes only occurred in old lakes (Fig. 3A). Indicator species analysis, quantifying the association of a clone's abundance to habitat type and age, indicated that M1, M3 and M5 were strongly and significantly associated with young systems (Pearson's phi coefficient of association $r_\phi = 0.650, 0.628$ and 0.547 , respectively, all $P < 0.05$), whereas clones M6, M8 and M32 were highly significantly associated with old systems ($r_\phi = 0.603, 0.564$ and 0.477 , respectively, all $P < 0.01$) (Table S3, Supporting information).

There was a clear association between the occurrence of specific clones and several of the measured environmental variables. For example, clones M2, M4, M9 and M13 were positively associated with high nutrient concentrations and chlorophyll *a* (Fig. 4). Clones M1 and M3 were associated with ponds with high conductivity, whereas clones M6 and M8 were associated with habitats with low conductivity (Fig. 4). Clones M5 and M11 were strongly associated with depth, with M11 being detected only from three deep lakes: IL-22, IL-21 and IL-25. Lakes IL-22 and IL-21 represent two of the three sites where fish (Arctic Charr, *Salvelinus alpinus*) were detected by gill-netting during the sampling campaign.

The RDA model indicates that a significant portion of the variation in clonal composition is explained by both spatial variables and environmental variables (Table 2). If we only include environmental and spatial variables in the RDA, environmental variation explained 18% of

the variation in clonal composition ($R^2_{\text{adj}}=0.183$, $P < 0.001$), whereas the global model of spatial processes explained about 11% of the variation in clonal composition ($R^2_{\text{adj}}=0.110$, $P < 0.001$; Table 2). Among the environmental variables, conductivity, depth, TP and TN contributed significantly to explaining the variation in clonal composition in the studied lakes and ponds. Among the spatial variables, dbMEM1 and dbMEM3 were found to contribute significantly to the variation in clonal composition (Table 2). Given that these are low number MEMs, they reflect large-scale patterns of spatial scale. In a partial RDA, the complete model (ENV U SPACE) accounted for 21% of the variation in clonal composition ($R^2_{\text{adj}}=0.21$, $P < 0.001$; Table 2) with environmental variables ($R^2_{\text{adj}}=0.14$, $P < 0.001$) being most important. A small, but significant portion of the variation in clonal composition was also explained by pure spatial descriptors ($R^2_{\text{adj}}=0.04$, $P < 0.01$), while shared environmental and spatial processes contributed 3% (Table 2 and Fig. S1, Supporting information). A large part of the variation in clonal composition, however, remained unexplained ($R^2_{\text{adj}}=0.78$; Table 2).

Including age as an independent variable in the partial RDA did not change the percentage of variation explained by environmental variables ($R^2_{\text{adj}}=0.14$, $P < 0.001$; Fig. 5). It did, however, strongly reduce the variation explained by spatial descriptors, and the pure effect of space became statistically insignificant ($R^2_{\text{adj}}=0.020$, $P = 0.52$; Fig. 5 and Table S4, Supporting information). The combined effect of environmental variables, age and space ($R^2_{\text{adj}}=0.004$) ; was higher than that of the pure effect of space (Fig. 5 and Table S4, Supporting information). The pure effect of age was significant and slightly higher than space ($R^2_{\text{adj}}=0.03$, $P = 0.03$; Fig. 5) and its effect was also confounded with space effects (Fig. 5; see also Table S4, Supporting information). Only a small fraction of the variation was shared among spatial descriptors, environmental heterogeneity and age of habitats. Visualizing the effect of the two significant spatial variables indeed showed that they reflected an age gradient (Fig. 6). dbMEM3 split the habitats in the old and young clusters of ponds and lakes, along the northeast to southwest gradient, whereas dbMEM1 divided the habitats along a southeast to northwest gradient. The former relates to the distance to the tongue of the glacier, whereas the latter reflects the distance to the margin of the ice sheet.

Incorporating habitat as an independent variable in the pRDA did not improve the model ($R^2_{\text{adj}}=0.215$) ; habitat type (pond vs. lake) did not have a significant unique effect ($R^2_{\text{adj}}= - 0.008$, $P = 0.895$; Fig. S2 and Table S5, Supporting information) on clonal composition.

Discussion

Using a metacommunity ecology analysis approach in a landscape genetics context, we assessed the contribution of local environmental variables and spatial descriptors to the observed pattern of clonal composition of relatively young, obligately asexual *Daphnia pulex* metapopulations in the Jakobshavn Isbræ region of western Greenland. We found that

among-population genetic differentiation (beta-diversity) in the metapopulations was high, reflecting pronounced differences in the relative abundances of MLGs in even neighbouring populations, and that clonal distributions were nonrandom and strongly structured along environmental variables. The latter observation suggests that even in the relatively young metapopulation (the young habitats are estimated to be <50 years old), dispersal limitation is not a key structuring factor, while niche differences among clones are.

Most studies on population genetic analyses assume dispersal limitation to be a major structuring factor, and therefore, these studies often investigate patterns of isolation by distance (Bohonak 2002; Maes & Volckaert 2002; Planes & Fauvelot 2002; Broquet *et al.* 2006; Orsini *et al.* 2013). In a multivariate context, this translates to a significant effect of space in a variation partitioning analysis (Borcard & Legendre 2002). Space accounted for a small, but significant effect on the clonal composition of local *D. pulex* populations in the studied metapopulations of this asexual species complex. However, when age was introduced as an independent variable in the partial RDA, it became clear that the spatial signal actually reflected a significant effect of age, and no pure spatial effect on clonal composition remained (see Table 2). When we visualized the patterns generated by the significant MEM variables, it was also clear that indeed, both spatial variables reflected age gradients (see Fig. 6 and Table 2). For IBDL to generate a pattern of isolation by distance (Bohonak 1999), the populations need to be in gene flow–drift equilibrium (Orsini *et al.* 2013). In our study, the young age of the studied ponds and lakes (<50 years for the young metapopulation) implies that they are likely still far from gene flow–drift equilibrium (Weider *et al.* 1999). The lack of evidence for dispersal limitation indicates that the spatial distance among sites did not limit the distribution of clones in the Jakobshavn Isbræ region of Greenland, irrespective of the young age of a large part of the habitats.

The nonrandom distribution of clones and their affinity with age categories (old vs. young ponds and lakes) in our data set can be explained by two processes: either ponds and lakes are colonized by an increasing number of clones as they age, or older systems differ in environmental conditions (e.g. because of the colonization by fish or macrophytes, or of changes in physicochemical characteristics). The observation that a partial RDA revealed neither a significant effect of space, nor of age, but a somewhat higher shared space $\times$ age category, indicated that age of the studied habitats was spatially structured. The shared environment $\times$ space $\times$ age component in Fig. 5 reflects that a considerable part of the overall effect of age is associated with environmental gradients. Given the absence of a strong pure spatial signal and a highly significant effect of environment, the most logical explanation for the age $\times$ space $\times$ environment effect is that age reflects changes in the environment (Engstrom *et al.* 2000). If true, then the clonal composition of the *D. pulex* metapopulations from this region of western Greenland is almost uniquely driven by environmental gradients in these young habitats.

We have detected a clear pattern of correspondence of clonal composition to some of the measured environmental variables, most notably with conductivity and nutrient levels (see Fig. 4). This suggests that the MLGs are ecologically different and represent distinct physiological ecotypes that are distributed nonrandomly along environmental gradients. The existence of such physiological ecotypes has been reported before from Arctic Canada

(Weider & Hebert 1987). Our results are indeed in agreement with previous studies that have provided evidence for fitness differences among clones and for associations between the occurrences of specific clones and environmental conditions, for example, along strong salinity gradients (Weider & Hebert 1987; Wilson & Hebert 1992). Wilson & Hebert (1993) showed that there was an association between the occurrence of specific *Daphnia* clones and the presence of the large copepod predator *Hesperodiaptomus arcticus*. In the present study, we have shown an association between the relative abundances of clones and environmental variables along natural abiotic gradients (with TP concentrations ranging from 0.001 to 0.026 mg/L and the conductivity ranging from 23.5 to 1197 $\mu\text{S}/\text{cm}$). In addition, we have shown that this clonal sorting along environmental gradients already occurs in very young habitats (<50 years old). Our observation of sorting along environmental gradients in young metapopulations adds to the evidence of a lack of dispersal limitation, because dispersal rates must be high enough for the ‘right’ clones to reach the ‘right’ habitats (see Table 2, Fig. 5, Fig. S1 and Table S5, Supporting information). We note that evolution-mediated priority effects, the key feature of the monopolization hypothesis (De Meester *et al.* 2002, 2016), are expected to be less important in asexual taxa than in similar sexual taxa, due to a reduced ability for rapid local adaptation in the asexuals. As a consequence, it is indeed expected, provided that dispersal is not limiting, that fitness differences among dispersed clones will lead to a match between environmental gradients and landscape genetic structure in asexual taxa, similar to species sorting in communities (Leibold *et al.* 2004; De Bie *et al.* 2012).

Our results are in agreement with predictions of the FNV Hypothesis (Vrijenhoek 1984). This hypothesis predicts that ecological differences among clones of asexual species result in differential clonal composition across heterogeneous landscapes. Previous studies similarly have reported sorting of MLGs along environmental gradients such as salinity (Hobaek *et al.* 1993), productivity (Pantel *et al.* 2011) and resource gradients (Aguilera *et al.* 2007). Given that clones of asexual *Daphnia* species originate through crosses of sexual ancestors with clones that carry a meiosis suppressor gene (Hebert 1987; Lynch *et al.* 2008; Xu *et al.* 2015), the ecological differences among clones may reflect ecological differences among genotypes in the sexual ancestors, where these ecological differences are fixed (i.e. ‘frozen’) in the asexual clones. Numerous studies (Spitze 1992; Beckerman *et al.* 2007; Dennis *et al.* 2010) have shown that cyclic parthenogenetic species of the *D. pulex* species complex harbour extensive genetic variation for ecologically relevant traits. This offers much scope for generating ecologically differentiated asexual clones, which then sort along environmental gradients. Strikingly, these clones are sufficiently numerous at a regional scale and dispersal is sufficiently common that this results in a clear signal of match between clonal composition and local environmental conditions (‘clonal sorting’ by the environment). This pattern was apparent even in a young metapopulation such as the cluster of ponds and lakes that originated <50 years ago by icesheet retreat, as detailed in this present study (Fig. 6).

Our results do not match the predictions of the GPP hypothesis (Lynch 1984), where clonal composition should be relatively uniform over a large area and metapopulations are expected to be dominated by a limited number of widespread, competitively superior genotypes. In contrast, we observed a quite high local and regional clonal diversity (Haileselasie *et al.*

2016), which is comparable or higher to that found in previous studies from Arctic sites in Canada (Hobaek *et al.* 1993), Iceland and the Godhavn (Disko Island) region in Greenland (Weider *et al.* 1996). Our results also do not fit the predictions of the neutral theory, as clonal composition in local habitats was structured by environmental gradients rather than by spatial drivers (see Table 2, Fig. 5 and Table S4, Supporting information). As a matter of fact, nearly all signal of space was confounded with either environmental variation or age, where the latter might also reflect unmeasured environmental variation through habitat succession. Of course, it remains that 78% of the variation could not be explained by environmental or spatial gradients and the amount of variation in clonal composition that could be explained by pure environmental factors was only 14% (see Table 2). Yet, the assessment of environmental drivers is likely to be an underestimation. First, we only measured a subset of potentially important environmental variables. For instance, we did not take potentially important biotic variables into consideration such as the presence of specific invertebrate predators (Wilson & Hebert 1993), parasites and composition of the phytoplankton or important invertebrate competitors. Second, the redundancy analysis only takes linear relationships into consideration.

Most clones in the young (<50 years old) ponds and lakes in the Jakobshavn Isbræ region also occurred in the older lake and pond cluster, suggesting that the populations inhabiting older lakes and ponds may be the source of colonizers for new habitats. Yet, there are a few locally abundant clones that only occurred in young ponds and lakes, which may have colonized from outside the sampling area and may perhaps be unique to young systems. From our study, it is at least clear that they are more abundant in the younger systems.

We earlier reported evidence that clonal richness continues to increase as ponds and lakes age (Haileselasie *et al.* 2016). The present analysis reveals that this is possibly due to a change in environmental conditions, likely including an increase in habitat heterogeneity (see Engstrom *et al.* 2000), 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. Our results suggest that these will be rapidly colonized by planktonic aquatic organisms (Sommaruga 2015; Peter & Sommaruga 2016) such as *Daphnia* and that the resulting metapopulations will be genetically diverse and sorted according to ecological niche differences. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Aguilera X, Mergeay J, Wollebrants A, Declerck S, De Meester L. Asexuality and polyploidy in *Daphnia* from the tropical Andes. *Limnology and Oceanography*. 2007; 52:2079.
- Barry RG. The status of research on glaciers and global glacier recession: a review. *Progress in Physical Geography*. 2006; 30:285–306.
- Beckerman AP, Wieski K, Baird DJ. Behavioural versus physiological mediation of life history under predation risk. *Oecologia*. 2007; 152:335–343. [PubMed: 17219130]
- Bennike O. Palaeoecological studies of Holocene lake sediments from west Greenland. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 2000; 155:285–304.
- Blanchet FG, Legendre P, Borcard D. Forward selection of explanatory variables. *Ecology*. 2008; 89:2623–2632. [PubMed: 18831183]
- Bohonak AJ. Dispersal, gene flow, and population structure. *Quarterly Review of Biology*. 1999; 74:21–45. [PubMed: 10081813]
- Bohonak A. IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity*. 2002; 93:153–154. [PubMed: 12140277]
- Borcard D, Legendre P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*. 2002; 153:51–68.
- Broquet T, Ray N, Petit E, Fryxell JM, Burel F. Genetic isolation by distance and landscape connectivity in the American marten (*Martes americana*). *Landscape Ecology*. 2006; 21:877–889.
- Bruvo R, Michiels NK, D'Souza TG, Schulenburg H. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology*. 2004; 13:2101–2106. [PubMed: 15189230]
- Cáceres MD, Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology*. 2009; 90:3566–3574. [PubMed: 20120823]
- Clark LV, Jasieniuk M. POLYSAT: an R package for polyploid microsatellite analysis. *Molecular Ecology Resources*. 2011; 11:562–566. [PubMed: 21481215]
- Colbourne JK, Robison B, Bogart K, Lynch M. Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using *Daphnia*. *Molecular Ecology Notes*. 2004; 4:485–490.
- Csatho B, Schenk T, Van Der Veen CJ, Krabill WB. Intermittent thinning of Jakobshavn Isbrae, West Greenland, since the little ice age. *Journal of Glaciology*. 2008; 54:131–144.
- De Bie T, De Meester L, Brendonck L, et al. Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters*. 2012; 15:740–747. [PubMed: 22583795]
- De Cáceres M, Legendre P, Moretti M. Improving indicator species analysis by combining groups of sites. *Oikos*. 2010; 119:1674–1684.
- De Meester L, Gomez A, Okamura B, Schwenk K. The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica-International Journal of Ecology*. 2002; 23:121–135.
- De Meester L, Vanoverbeke J, Kilsdonk LJ, Urban MC. Evolving perspectives on monopolization and priority effects. *Trends in Ecology & Evolution*. 2016; 31:136–146. [PubMed: 26778169]
- Decaestecker, E., De Meester, L., Mergeay, J. Cyclical parthenogenesis in *Daphnia*: sexual versus asexual reproduction. *Lost Sex*. Isa, SchönKoen, Martens, Peter, van Dijk, editors. Springer Netherlands; Dordrecht, the Netherlands: 2009. p. 295–316.
- Dennis S, Carter MJ, Hentley W, Beckerman A. Phenotypic convergence along a gradient of predation risk. *Proceedings of the Royal Society of London B: Biological Sciences*. 2010; 278:1687–1696.

- Dray S, Legendre P, Peres-Neto PR. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*. 2006; 196:483–493.
- Dray, S., Legendre, P., Blanchet, G. packfor: Forward Selection with Permutation (Canoco p. 46). The R Project for Statistical Computing; Vienna, Austria: 2009. R package version 0.0-7/r58
- Dufrène M, Legendre P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*. 1997; 67:345–366.
- Engstrom DR, Fritz SC, Almendinger JE, Juggins S. Chemical and biological trends during lake evolution in recently deglaciated terrain. *Nature*. 2000; 408:161–166. [PubMed: 11089963]
- Haileselasie TH, Mergeay J, Weider LJ, Jeppesen E, De Meester L. Colonization history and clonal richness of asexual *Daphnia* in periglacial habitats of contrasting age in West Greenland. *Journal of Animal Ecology*. 2016; 85:1108–1117. [PubMed: 27279332]
- Hebert, PDN. Genotypic characteristics of cyclic parthenogens and their obligately asexual derivatives. *The Evolution of Sex and Its Consequences*. Stearns, SC., editor. Birkhäuser Basel; Basel, Switzerland: 1987. p. 175–195.
- Hebert PDN, Ward RD, Weider LJ. Clonal-diversity patterns and breeding-system variation in *Daphnia pulex* asexual-sexual complex. *Evolution*. 1988; 42:147–159. [PubMed: 28563844]
- Hobaek A, Weider LJ, Wolf HG. Ecological genetics of Norwegian *Daphnia* III. Clonal richness in an Arctic apomictic complex. *Heredity*. 1993; 71:323–330.
- Hubbell, SP. *The Unified Neutral Theory of Biodiversity and Biogeography* (MPB-32). Princeton University Press; Princeton, NJ USA: 2001.
- Jaccard P. Nouvelles recherches sur la distribution florale. *Bulletin de la Société vaudoise des sciences naturelles*. 1908; 44:223–270.
- Jespersen A-M, Christoffersen K. Measurements of chlorophyll – a from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie*. 1987; 109:445–454.
- Joughin I, Abdalati W, Fahnestock M. Large fluctuations in speed on Greenland's Jakobshavn Isbrae glacier. *Nature*. 2004; 432:608–610. [PubMed: 15577906]
- Koroleff F. Determination of total phosphorus in natural waters by means of persulphate oxidation. *Interlab Report*. 1970; 3:19–22.
- Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species data. *Oecologia*. 2001; 129:271–280. [PubMed: 28547606]
- Legendre, P., Legendre, L. *Numerical Ecology*. Elsevier Science; Amsterdam, the Netherlands: 2012.
- Legendre P, Borcard D, Peres-Neto PR. Analyzing beta diversity: partitioning the spatial variation of community composition data. *Ecological Monographs*. 2005; 75:435–450.
- Legendre, P., Borcard, D., Blanchet, F., Dray, S. PCNM: MEM Spatial Eigenfunction and Principal Coordinate Analyses. The R Project for Statistical Computing; Vienna, Austria: 2012. R package version 2
- Leibold MA, Holyoak M, Mouquet N, et al. The meta-community concept: a framework for multi-scale community ecology. *Ecology Letters*. 2004; 7:601–613.
- Loaring JM, Hebert PDN. Ecological differences among clones of *Daphnia pulex* Leydig. *Oecologia*. 1981; 51:162–168. [PubMed: 28310076]
- Luckman A, Murray T. Seasonal variation in velocity before retreat of Jakobshavn Isbrae, Greenland. *Geophysical Research Letters*. 2005; 32:L08501.
- Lynch M. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *Quarterly Review of Biology*. 1984; 59:257–290.
- Lynch M, Seyfert A, Eads B, Williams E. Localization of the genetic determinants of meiosis suppression in *Daphnia pulex*. *Genetics*. 2008; 180:317–327. [PubMed: 18689898]
- Maes G, Volckaert F. Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). *Biological Journal of the Linnean Society*. 2002; 77:509–521.
- Mergeay, J. *Paleoecology and paleogenetics in the tropics: the Daphnia of Lake Naivasha, Kenya*. PhD Thesis, Katholieke Universiteit Leuven; Leuven, Belgium: 2005.
- Mergeay J, Aguilera X, Declerck S, et al. The genetic legacy of polyploid Bolivian *Daphnia*: the tropical Andes as a source for the North and South American *D. pulicaria* complex. *Molecular Ecology*. 2008; 17:1789–1800. [PubMed: 18284570]

- Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*. 1962; 27:31–36.
- Murtagh F, Legendre P. Ward's hierarchical clustering method: clustering criterion and agglomerative algorithm. *Stat*. 2011; 1050:11.
- Oksanen, J., Blanchet, FG., Kindt, R., et al. [accessed 10 April 2014] *vegan*: Community Ecology Package. 2013. R package version 2.0-10., Available at: <http://cran.r-project.org/>
- Orsini L, Vanoverbeke J, Swillen I, Mergeay J, De Meester L. Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*. 2013; 22:5983–5999. [PubMed: 24128305]
- Pantel JH, Juenger TE, Leibold MA. Environmental gradients structure *Daphnia pulex* × *pulex* clonal distribution. *Journal of Evolutionary Biology*. 2011; 24:723–732. [PubMed: 21288271]
- Peres-Neto PR, Legendre P, Dray S, Borcard D. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology*. 2006; 87:2614–2625. [PubMed: 17089669]
- Peter H, Sommaruga R. Shifts in diversity and function of lake bacterial communities upon glacier retreat. *The ISME Journal*. 2016; 10:1545–1554. [PubMed: 26771929]
- Planes S, Fauvelot C. Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution*. 2002; 56:378–399. [PubMed: 11926506]
- R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; Vienna, Austria: 2013. ISBN 3-900051-07-0
- Rignot E, Kanagaratnam P. Changes in the velocity structure of the Greenland Ice Sheet. *Science*. 2006; 311:986–990. [PubMed: 16484490]
- Sohn H-G, Jezek KC, van der Veen CJ. Jakobshavn Glacier, west Greenland: 30 years of spaceborne observations. *Geophysical Research Letters*. 1998; 25:2699–2702.
- Solorzano L, Sharp JH. Determination of total dissolved nitrogen in natural waters. *Limnology and Oceanography*. 1980; 25:751–754.
- Sommaruga R. When glaciers and ice sheets melt: consequences for planktonic organisms. *Journal of Plankton Research*. 2015; 37:509–518. [PubMed: 26869738]
- Sørensen T. A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Biologiske Skrifter*. 1948; 5:1–34.
- Spitze K. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. *American Naturalist*. 1992; 139:229–247.
- Valdes AM, Slatkin M, Freimer N. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. *Genetics*. 1993; 133:737–749. [PubMed: 8454213]
- Vergilino R, Belzile C, Dufresne F. Genome size evolution and polyploidy in the *Daphnia pulex* complex (Cladocera: *Daphniidae*). *Biological Journal of the Linnean Society*. 2009; 97:68–79.
- Vrijenhoek RC. Factors affecting clonal diversity and coexistence. *American Zoologist*. 1979; 19:787–797.
- Vrijenhoek, R. Ecological differentiation among clones: the frozen niche variation model. *Population Biology and Evolution*. Wöhrmann, K., Loeschcke, V., editors. Springer-Verlag; Heidelberg, Germany: 1984. p. 217–231.
- Weider LJ, Hebert PDN. Ecological and physiological differentiation among Low-Arctic clones of *Daphnia Pulex*. *Ecology*. 1987; 68:188–198.
- Weider LJ, HobaK A, Crease TJ, Stibor H. Molecular characterization of clonal population structure and biogeography of arctic apomictic *Daphnia* from Greenland and Iceland. *Molecular Ecology*. 1996; 5:107–118.
- Weider LJ, Hobak A, Hebert PDN, Crease TJ. Holarctic phylogeography of an asexual species complex – II. Allozymic variation and clonal structure in Arctic *Daphnia*. *Molecular Ecology*. 1999; 8:1–13.
- Weidick A, Oerter H, Reeh N, Thomsen HH, Thorning L. The recession of the Inland Ice margin during the Holocene climatic optimum in the Jakobshavn Isfjord area of West Greenland. *Palaeogeography, Palaeoclimatology, Palaeoecology*. 1990; 82:389–399.

- Wilson CC, Hebert PDN. The maintenance of taxon diversity in an asexual assemblage: an experimental analysis. *Ecology*. 1992; 73:1462–1472.
- Wilson CC, Hebert PDN. Impact of copepod predation on distribution patterns of *Daphnia pulex* clones. *Limnology and Oceanography*. 1993; 38:1304–1310.
- Xu S, Spitze K, Ackerman MS, et al. Hybridization and the origin of contagious asexuality in *Daphnia pulex*. *Molecular Biology and Evolution*. 2015; 32:3215–3225. [PubMed: 26351296]

Fig. 1.

Map of Greenland with our study site indicated by inset map to the right. The ponds and lakes belonging to the ‘young cluster’ are situated between the 1850 and current ice-sheet margin and are thus estimated to be less than approximately 50 years old); the ponds and lakes of the ‘old cluster’ are in front of the 1850 line, so more than 150 years old. Position of lines adapted from Weidick *et al.* (1990) and Csatho *et al.* (2008). The background map is taken from Landsat image[©]2015 digital globe. White dots indicate lakes while black dots are ponds.

Fig. 2. Genetic relatedness of *Daphnia* clones based on Bruvo's genetic distance (Bruvo *et al.* 2004), visually represented by the first two principal coordinate analysis axes (A) and dendrogram (B). Clusters are shown as numbers; the position of each clone in the tree is determined by the first two axes of a PCA as shown in panel A. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig. 3.

Clonal composition in lakes (A) and ponds (B). The frequency of a given clone in a particular habitat is proportional to the area of the pie covered by its coloured symbol. Each pie represents one lake (A) or pond (B) in its geographical position (longitude and latitude).

Fig. 4.

A visual representation of the first two RDA axes summarizing the variation in environmental variables and the position of each water body relative to these variables (A) and an RDA plot of the first two axes regressing clonal composition on environmental data (B). Lakes and ponds in Panel A are indicated by their number as listed in Table S1 (Supporting information). Panel B shows the position of clones when all environmental variables are considered. Arrows correspond to the contribution of each environmental variable to clonal composition. The clones are represented by 'M' followed by a number (i.e. M1 = the mean position of multilocus genotype M1). [Colour figure can be viewed at wileyonlinelibrary.com]

Fig. 5.

Venn diagram illustrating the results of variation partitioning from the partial RDA of the clonal composition of populations of the water flea *Daphnia pulex* in recently colonized ponds and lakes in Jakobshavn Isbrae, western Greenland. The partial redundancy analysis (pRDA) shows the unique and shared contribution of spatial descriptors (SPACE), environmental heterogeneity (ENV) and approximate age of population (AGE) to clonal composition. We used distance of the population from the ice-sheet terminus and a dummy variable for the young and old cluster as proxies of age. The numbers in the Venn diagram represent the fraction of variation in clonal composition that is explained by each independent variable.

Fig. 6.

Values of the two significant MEMs retained in the forward selection of a RDA, representing linearly independent spatial population structure for the whole data set on clonal frequencies in lakes and ponds in Jakobshavn Isbrae (A: MEM1; B: MEM3). Positive values are depicted by filled symbols, while negative values are shown as open symbols. The size of the symbols illustrates their absolute values. The position of symbols refers to the geographical position of the habitats occupied by the populations. The eigenvalues of the two significant MEMs each subdivide the populations into two sets corresponding to two geographical areas. MEM1 subdivides the area along a northeast to southwest gradient and shows that the primary spatial pattern is along the primary axis of glacial retreat (>150 years old vs. <50 years old ponds and lakes), whereas MEM2 subdivides the area into a northeast to southwest gradient and shows a secondary spatial pattern along the second glacial retreat axis orthogonal to the margin of the ice sheet and likely represents a second axis of age differences within each cluster.

Table 1

Population genetic differentiation (expressed by distance-based dissimilarities using the Sørensen and Jaccard indices) between predefined population groups based on age (old vs. young), type (lake vs. pond) and all populations from the Jakobshavn Isbræ area of western Greenland. *N* = number of populations in the category; SD = standard deviation

Category	<i>N</i>	Dissimilarity index	
		Sørensen Mean ± SD	Jaccard Mean ± SD
Overall (all populations)	61	0.88 ± 0.20	0.86 ± 0.22
Lakes <50 years old	15	0.88 ± 0.22	0.77 ± 0.24
Ponds <50 years old	24	0.74 ± 0.32	0.72 ± 0.33
Lakes >150 years old	6	0.93 ± 0.14	0.92 ± 0.17
Ponds >150 years old	16	0.90 ± 0.13	0.88 ± 0.15
Lakes (all)	21	0.89 ± 0.20	0.88 ± 0.21
Ponds (all)	40	0.85 ± 0.24	0.84 ± 0.25
Habitats <50 years old	39	0.79 ± 0.26	0.77 ± 0.28
Habitats >150 years old	22	0.92 ± 0.12	0.92 ± 0.14

Table 2

Results of the redundancy analysis (RDA) and variation partitioning following partial RDA, quantifying the unique and shared contributions of spatial (SPACE) and environmental variables (ENV) to the overall variation in clonal composition of populations of the water flea *Daphnia pulex* s.l. in recently colonized ponds and lakes in Jakobshavn Isbrae, western Greenland

RDA model [†]	<i>R</i> ²	<i>R</i> ² _{adj}	<i>P</i> value
Global RDA (only spatial distance)			
Global model (SPACE)	0.220	0.110	0.001 ***
FS _{total}	0.102	0.070	0.001 ***
dbMEM 1	0.068		0.001 ***
dbMEM 3	0.033		0.022 *
Global RDA (only environmental variables)			
Global model (ENV)	0.308	0.183	0.001 ***
FS _{total}	0.224	0.168	0.001 ***
Conductivity	0.099		0.001 **
Depth	0.046		0.002 **
Total phosphorus	0.038		0.012 *
Total nitrogen	0.041		0.006 **
pRDA (both SPACE and ENV) [‡]			
TOTAL	0.287	0.210	0.001 ***
SPACE ENV		0.04	0.005 **
ENV SPACE		0.14	0.001 ***
ENV∩SPACE		0.03	
Unexplained		0.778	

[†]The analysis first shows the overall model performance (global model) for either space or environmental variables and next the sum of the performance of the forward selected variables (FS_{total}) and the contribution of each selected variable to the model (dbMEM stands for distance-based Moran Eigenvector Map – MEM variable, with the number indicating MEM variables that detect increasingly small-scale spatial patterns).

[‡]This portion of the table shows the results of the variation analysis: TOTAL summarizes the contribution of all variables, which can be differentiated into the unique contribution of space (SPACE|ENV), the unique contribution of environmental variables (ENV|SPACE) and the shared ENV∩SPACE component. The latter component refers to the shared explanation that cannot be attributed uniquely to SPACE or ENV and likely reflects spatially structured environmental variation.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ and ns : non-significant.