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Brine chemistry matters: Isolation by environment and by distance explain population genetic structure of *Artemia franciscana* in saline lakes

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Abstract

1. The American brine shrimp *Artemia franciscana* is important in aquaculture and has become invasive in other continents, aided by dispersal via waterbirds. However, little is known about processes underlying its genetic diversity and population structure in its natural habitat in North America. These processes, including dispersal and local adaptation, are pivotal drivers of species distribution and community structure, and therefore central to aquatic biodiversity.
2. We studied 15 populations in natural saline lakes of Saskatchewan, Canada to determine the influence of variation in geological history, water chemistry, lake size, and location. We aimed to determine the relative importance of isolation by distance and isolation by environment using the cytochrome *c* oxidase subunit 1 gene (CO1) as a mitochondrial marker and five nuclear microsatellite markers.
3. Geographic patterns for CO1 and microsatellites differed, with lakes clustering in different groups based on genetic distances according to the marker used. CO1 better indicated historical colonisation processes, suggesting potential routes of initial colonisation when lakes were formed after deglaciation 11,000–15,000 years ago.
4. Differentiation between lakes based on nuclear markers was strongly related to variation in hydrochemistry, suggested by distance-based redundancy analysis, but there was no indication of isolation by distance. The ratio between alkalinity and the sum of Ca and Mg concentrations was particularly important, although a lake with a high Cl concentration caused by potash mining also had a unique *Artemia* population.
5. Geochemistry is important in the adaptive radiation of anostracan crustaceans. Our study suggests that it also underlies intraspecific genetic variation between populations, promoting isolation by environment, and making dispersal ineffective when cysts are moved by birds between lakes with different hydrochemistry.

KEYWORDS
brine shrimps, colonisation route, dispersal, hydrogeochemistry, native region

1 | INTRODUCTION

The role of dispersal and environmental filtering in structuring communities and populations is central to research that seeks a better understanding of species distributions and biogeographical patterns (D'Amen et al., 2018; Ovaskainen et al., 2017). Dispersal connects populations across spatial distances, leading to gene flow if followed by successful establishment in the new habitat (Bohonak, 1999). Such successful establishment may be hindered by unsuitable local environmental conditions or by a resident population with a numerical or fitness advantage resulting from priority effects, or local adaptation (De Meester et al. 2016). Several patterns of genetic differentiation are predicted to result from three main processes or their interaction: isolation by distance (IBD; Wright, 1943), isolation by environment (IBE; Wang & Bradburd, 2014) and isolation by adaptation (Nosil et al., 2008). The processes underlying these spatial genetic patterns can be identified by testing relationships between genetic differentiation and geographic and ecological distances (Orsini et al., 2013).

Here, we examine drivers of biogeographic patterns of the brine shrimp *Artemia franciscana* Kellog, 1906 (Branchiopoda: Anostraca) in its native range of North America. This species is highly invasive in other continents, to which it was originally exported from the Great Salt Lake and San Francisco Bay as resistant eggs (cysts) used widely as food in aquaculture (Amat et al., 2005; Horváth et al., 2018). It has become a model species in toxicology (Libralato et al., 2016; Ruebhart et al., 2008). In addition, it is also probably the best known example of an aquatic invertebrate that disperses via migratory waterbirds, which consume *Artemia* and disperse their cysts via gut passage owing to their ability to resist digestion (Muñoz et al., 2013; Reynolds et al., 2015; Sánchez et al., 2012).

We focus our study on saline lakes of the Saskatchewan prairies, within the Great Plains bioregion for anostracans (Rogers, 2014a). This area was covered by an ice sheet during the last glacial period until the onset of deglaciation of southern Saskatchewan about 17,000 years ago, which gradually retreated in a north-east direction (Christiansen, 1979).

The export of cysts from San Francisco Bay and the Great Salt Lake in the U.S.A. has largely determined the genetic structure of *A. franciscana* in Mexico, as well as across continents other than North America (Eimanifar et al., 2014; Horváth et al., 2018). However, populations in central Canada, where the lakes freeze in winter and the hydrochemistry is markedly different, are unaffected by this export (Muñoz et al., 2013). Population genetic studies of this and other *Artemia* species have previously been concentrated in solar salt works or aquaculture ponds (Muñoz et al., ,2008, 2013, 2014), and studies in natural lakes are rare. Naihong et al. (2000) found greater allozyme heterozygosity for *Artemia sinica* in larger lakes. Panmixia has been reported for *A. franciscana* in Great Salt Lake (Eimanifar et al., 2015) and for *Artemia urmiana* in Lake Urmia (Eimanifar & Wink, 2013).

Within North America, the distribution of different species of anostracan crustaceans is strongly related to regional differences in geochemistry, and hence hydrochemistry (Rogers, 2014a). This suggests that variation in geo- and hydrochemistry may also be a strong selective force for local adaptation amongst widely distributed species such as *A. franciscana*, and may potentially result in population genetic patterns that suggest IBE. This is supported by differences observed under laboratory conditions between *A. franciscana* populations in their tolerance of different ionic concentrations (Bowen et al., 1988). Saskatchewan has an estimated 500 saline lakes greater than 1 km^2 in area, mainly dominated by sodium sulfate and magnesium sulfate salts (Hammer, 1993). Beginning in 1918 in Muskiki Lake, sodium sulfate has been industrially extracted from 20 lakes, which supplied about 50% of the North American demand (Last & Ginn, 2005). However, the hydrochemistry varies considerably between different saline lakes in this region (Bowman & Sachs, 2008). Many of these lakes are important for the conservation of migratory waterbirds, including two of our study sites: Chaplin Lake and Old Wives/Frederick Lakes (Important Bird Areas, https://www.ibacanada.ca/).

We would expect that *Artemia* populations colonised the natural saline lakes of Saskatchewan following glacial retreat 11,000–15,000 years ago (Christiansen, 1979), with migratory waterbirds facilitating long-distance dispersal and hence colonisation of newly formed lakes (Muñoz et al., 2013). Contemporary gene flow via bird movements may be expected to increase the similarity between lakes, particularly those that are close together, which may be visited repeatedly by the same individual birds even on a daily basis (Demers et al., 2008; Green et al., 2002), thereby increasing the effect of IBD. Larger lakes are also likely to hold greater abundance and diversity of waterbirds (Bidwell et al., 2014; Sebastián-González & Green, 2014), increasing the arrival and diversifying the origin of cysts brought from other lakes, and hence promoting a correlation between lake size and genetic diversity. By contrast, differences in hydrochemistry and resulting local adaptation may mean that only a subset of lakes provide suitable habitat for a given genotype, promoting IBE and reducing the chances that *Artemia* brought by birds as cysts may survive.

For this study, we apply the mitochondrial cytochrome *c* oxidase subunit 1 gene (CO1) gene and microsatellite markers to study historic and current drivers of *A. franciscana* biogeography in its native distribution. In particular, we test the hypotheses that: (1) genetic diversity is positively correlated with lake area; and (2) spatial genetic structure of populations in the Saskatchewan saline lakes follows a pattern of IBE rather than a pattern of IBD that would result from dispersal limitation (IBD).

2 | METHODS

2.1 | Study area

From June to August 2011, 50 saline lakes were visited within the Saskatchewan plains and searched for *Artemia*, although many of these lakes were too low in salinity to support *A. franciscana*, and some of them only held other anostracans associated with lower salinities (*Branchinecta* spp.). These lakes were partly selected on the basis of existing literature on saline lakes, as some of them

were included in broader studies of the chemistry and invertebrate communities of saline lakes in Saskatchewan (Bentley et al., 2016; Bowman & Sachs, 2008; Hammer, 1993; Last & Ginn, 2005; Wissel et al., 2011). A minority of our study lakes are well known for their *Artemia* populations, which have been used in previous laboratory studies (e.g. Chaplin and Little Manitou; Bowen et al., 1988). Additional lakes were sampled because they were encountered during ground surveys of Piping Plover (*Charadrius melodus*) and other waterbirds for a separate study. Finally, other lakes were visited because their saline nature was evident from inspection on Google Earth (https://earth.google.com), with distinctive white soils around the water edge indicating crystallisation of saturated salts. Many of our study lakes are previously undocumented, despite previous inventories of the worldwide distribution of *Artemia* (Muñoz & Pacios, 2010). Our sampling was not exhaustive and additional unknown populations of *Artemia* are likely to occur in Saskatchewan. Saline lakes of the Canadian prairies are unaffected by commercial exploitation and export of *A. franciscana* cysts, and the harsh climate makes aquaculture unviable, although adult *Artemia* have been harvested in Chaplin Lake and some other lakes (Hammer, 1986).

2.2 | Sampling

Shorelines were visited during daylight by public access roads and the water column was inspected for brine shrimps, especially on the side where winds concentrated them along the shoreline by wave action. Where present, shrimps were collected with a hand net then placed in alcohol for later confirmation of species identification. At the same time, the shoreline was inspected for concentrations of cysts that form masses at the water's edge or at the high water mark. These cysts were collected in 200-ml plastic bottles, then later washed in distilled water before desiccation for better storage. A water sample was also collected close to the shoreline in 200-ml plastic bottles which were stored in a refrigerator for 1–6 weeks before they were analysed for major ion chemistry. Lake surface area was estimated from Google Earth (2012). Waterbirds were present in all lakes at the time of visit (details available on request). Lakes used in this study were separated from each other by up to 413 km.

2.3 | Water chemistry analysis

Alkalinity (Alk) was determined by sulfuric-acid titration, and concentrations of other major ions were measured by ion-exchange chromatography after diluting brine samples to a suitable concentration range.

Based on these measured parameters, we selected key descriptors of the variation between lakes based on process-based understanding of hydrology and geochemistry of saline lakes in the region (e.g., Bentley et al.,(2016); Last and Ginn (2005)). These descriptors were SO_4 concentration, and the ratios Cl/SO_4, Alk/SO_4, and Alk/(Ca + Mg).

Lake salinity is primarily controlled by the degree of evaporative enrichment of sulfate, which is sourced by the weathering of sulfide minerals (e.g. pyrite) contained in glacial till (Nachshon et al., 2013; Van Stempvoort et al., 1994). Evaporative enrichment is greater in closed-basin lakes with higher water inputs by surface runoff and groundwater discharge (Wood & Sanford, 1990). Sulfate concentration is a useful indicator of enrichment to a point where the saturation of mirabilite ($Na_2SO_4 \cdot 10H_2O$) is reached. Further enrichment increases relative concentration of Mg compared to other cations. If the original composition of water has relatively high alkalinity, the geochemical evolution of water takes a path that is on the alkalinity side of the chemical divide (Hardie & Eugster, 1970). In contrast, originally alkalinity-poor water will take a path on the other side of the chemical divide characterised by high alkaline earth concentrations. Therefore, alkalinity/(Ca + Mg) ratio and alkalinity/SO_4 ratio may be useful for describing the geochemical evolution of saline lakes. Some saline lakes in Saskatchewan (including one in our study, PAT) receive brines from potash mines that contain high concentrations of chloride. Therefore Cl/SO_4 ratio is useful for distinguishing the effects of artificial inputs of chloride.

2.4 | Mitochondrial DNA genotyping

DNA was extracted from cysts sampled from 12 lakes (Table S1) in the study area using the Hotshot protocol (Montero-Pau et al., 2008). Two additional populations from two British Columbia lakes sampled in 2011 belong to a lineage from the other side of the Continental Divide that includes populations of the Great Salt Lake, Utah, and San Francisco Bay (BCO and PBC, Table S1, Figure S1) were included as outgroup in the haplotype network construction. We used *Artemia*-specific primers 1/2CO1_Fol-F and 1/2CO1_Fol-R (Muñoz et al., 2008) to amplify a 709-bp fragment of the mitochondrial CO1. Amplifications were performed in 20 μl total volume containing 1× reaction buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.6 units Taq DNA polymerase (Bioline Corp.), and 0.5 μM of each primer. Polymerase chain reaction conditions were as follows: 94°C for 3 min, followed by 35 cycles of 45 s at 94°C, 60 s at 45°C (60–64°C for 16S locus), and 60 s at 72°C, followed by 5 min at 72°C. Quality of sequences was checked manually and consensus sequences were obtained and aligned in BioEdit v. 7.1.9 (Hall, 1999). The total number of newly obtained sequences varied between 16 and 24 per lake for 12 lakes. All corresponding haplotypes were deposited in NCBI GenBank (Accession numbers: MW799827-MW799878).

2.5 | Nuclear microsatellite (nSSR) genotyping

DNA extractions were carried out on individual cysts (previously rinsed in distilled water) from each population making use of the HotSHOT protocol (Montero-Pau et al., 2008). The cysts were collected from *A. franciscana* populations of 14 lakes in Saskatchewan, Canada (Table 1 and S1). We amplified eight nuclear microsatellite

TABLE 1 Genetic diversity of 14 *Artemia franciscana* populations from Saskatchewan

nSSR				mtDNA			
Pop	AR	A_P	H	*h*	π	h_p	Area (km^2)
AKE	40.9	7	2.876	0.779	0.00290	4	3.282
MAN	40.5	2	3.242	0.270	0.00047	4	15.599
PAT	26.3	4	3.445	0.000	0.00000	0	5.725
WHI	42.0	5	3.240	0.569	0.00129	5	29.618
BEN	50.6	4	3.149	0.380	0.00134	3	0.140
BES	53.8	1	3.442	0.411	0.00075	4	0.707
CHA	49.6	8	3.405	0.641	0.00246	8	23.206
EIN	50.3	4	3.493	0.260	0.00060	2	1.964
FRE	38.9	3	2.873	0.320	0.00122	2	0.890
FRS	54.9	9	3.441	NA	NA	NA	0.590
LYD	51.9	5	3.324	0.537	0.00223	5	2.061
GUL	50.1	4	2.493	NA	NA	NA	0.576
MUS	40.3	0	3.243	0.356	0.00123	7	18.284
MEA	NA	NA	NA	0.253	NA	4	0.900

Note: For simple sequence repeat (nSSR) markers (5 loci), rarefied total allelic richness (AR) is indicated as well as the number of private alleles (A_p) and the Shannon-Wiener index for multilocus genotypes (H). SHO is excluded here, as only 8 individuals were genotyped. For mitochondrial (mt) DNA, haplotype diversity (*h*), nucleotide diversity (π) and the number of private haplotypes (h_p) are indicated. Lake size is given as surface area in km^2.

loci (in the following nSSR or simple sequence repeats) using the primer pairs described in Muñoz et al. (2009) (Af_B109, Af_A108, Af_B9, Af_B139, Af_B117, Af_A136, Af_A104). The locus Af_B11 (Muñoz et al., 2009) was initially included but was dropped because its amplification failed in >200 individuals. The results from the genotype accumulation curve computed with the R package *poppr* 2.8.3 (Kamvar et al., 2014) suggest that a full resolution of the multilocus genotypes was already achieved by including only five loci (Figure S2). We thus decided to use a higher number of genotyped individuals with the trade-off of removing locus Af_B11, yielding data from 592 individuals in total. Following tests for Hardy–Weinberg equilibrium (HWE; see below), we excluded two additional loci (Af_B117, Af_A136) which deviated from HWE in all or almost all studied populations (Table S3). Therefore, throughout the paper, we report results for the five nSSR loci Af_B109, Af_A108, Af_B9, Af_B139, Af_A104). All included loci were polymorphic and their allelic richness was between 21 and 48 alleles (Table S4).

2.6 | Statistical analysis

All R packages used for data analyses described in this section were run in R version 3.6.2 (R Core Team, 2019).

2.6.1 | Environmental variables

To quantify and visualise how the environment available to *A. franciscana* differed between individual lakes, principle component analysis (PCA) was performed with R package *vegan* 2.5.6 (Oksanen et al., 2019) and plotted with the autoplot() function of *ggplot2* (Wickham, 2016). The PCA included the following variables: lake surface area, SO_4, and the ratios Cl/SO_4, Alk/SO_4, and Alk/(Ca + Mg).

2.6.2 | Mitochondrial DNA

To analyse mitochondrial DNA (mtDNA), we supplemented the haplotype dataset newly sequenced in this study, with haplotypes that were previously reported from four of our study lakes (MAN, CHA, MEA, and MUS), based on earlier sampling in 2009 (Muñoz et al., 2013). The entire dataset used for the current study therefore included 65 haplotypes in total: 52 haplotypes newly detected in the present study; three haplotypes recorded both in the present study and in Muñoz et al. (2013); and 10 additional haplotypes only reported in Muñoz et al. (2013). Details on sample size and haplotypes can be found in Table S1.

A haplotype network was generated using TCS 1.21 (Clement et al., 2000) at the 95% connection limit. This network includes 12 lake populations from Saskatchewan and two lake populations from British Columbia. Genetic diversity within lakes was characterised by the number of private haplotypes (haplotypes found only in one location), standard diversity indices of haplotype diversity *h* and nucleotide diversity π (Nei, 1987) calculated in Arlequin v. 3.5 (Excoffier & Lischer, 2010) and DnaSP v.5 (Librado & Rozas, 2009). Pairwise Φ_{ST} with Tamura & Nei (TrN) distances and their statistical significance (10,000 permutations) were performed in Arlequin v. 3.5.

An analysis of molecular variance (AMOVA) was conducted to determine the hierarchical structure of CO1 variation (Excoffier

et al., 1992). This analysis was performed with the aforementioned substitution model with 10,000 random permutations in Arlequin and based on the groups of populations that were not significantly genetically differentiated according to pairwise Φ_{ST} (Group 1: BEN, BES, WHI, FRE, CHA; Group 2: AKE; Group 3: EIN, LYD, MAN, MUS, PAT, MEA). We tested three hierarchical levels: between groups, between populations within groups, within populations.

2.6.3 | Microsatellite (nSSR) markers

To estimate null allele frequencies, we used the software FreeNA (Chapuis & Estoup, 2007) that follows the expectation maximization algorithm of Dempster et al. (1977) (detailed in Chapuis & Estoup, 2007). HWE was tested with the function hw.test(), computing an exact test based on 5,000 Monte Carlo permutations of alleles. This function is implemented in the R package *pegas* 0.14 (Paradis, 2010). Results were corrected for multiple comparisons using the method of Holm (1979), a less conservative modification of the Bonferroni test. Total allelic richness per populations was computed by rarefaction implemented in the R package *PopGenReport* 3.0.4 (Adamack & Gruber, 2014). The population with the smallest number of individuals genotyped for nSSRs (SHO, 8 individuals) was excluded from the rarefaction procedures and the second smallest population size (GUL, $n = 21$ individuals) applied as the smallest size for rarefaction. Multilocus genotype diversity H (Shannon–Wiener diversity; Shannon, 1948) was computed with the R package *poppr* 2.8.3 (Kamvar et al., 2014). Locus- and population-specific allele number and number of private alleles per population were calculated with *poppr* 2.8.3 (Kamvar et al., 2014).

For pairwise population genetic distances, we computed Weir's (1996) F_{ST} using FreeNA (Chapuis & Estoup, 2007). This software provides a correction of the bias induced by null alleles following the method described in Chapuis and Estoup (2007). We calculated pairwise population D_{EST} with GenAlEx 6.502 (Peakall & Smouse, 2012) with associated *p*-values based on 999 permutations.

To further explore the population structure suggested by pairwise population distance, we applied discriminant analysis of principal components (DAPC) implemented in the package adegenet 2.1.3 (Jombart, 2008), a model-free analysis that makes no prior assumptions about population genetic processes (such as HWE). We used the function xvalDAPC() to find the optimum number of PCs to be retained prior to their use in the final discriminant analysis, which we performed with the first 40 PCs and the first five discriminant functions. We specified the 14 study populations as *a priori* groups to analyse population membership probabilities for each individual.

2.6.4 | Distance-based redundancy analysis

To test for the importance of IBE versus IBD, we performed distance-based redundancy analysis (dbRDA) with functions implemented in the R package *vegan* 2.5.6 (Oksanen et al., 2019).

We used genetic distance estimators (pairwise D_{EST} for nSSR markers and pairwise Φ_{ST} for mtDNA) as dependent variables. To test the effect of individual environmental variables (the same ones as used in the above PCA) on population differentiation, we built the null model (genetic differentiation ~1) and the full model (genetic differentiation ~Area + SO_4 + Cl/SO_4 + Alk/SO_4 + Alk/Ca + Mg) using the function capscale(). Both the null model and the full model were used as input to the forward selection model choice (on adjusted R^2 and *p*-values) with the function ordiR2step() with 999 permutations. Model choice was repeated 100 times to obtain robust results.

To test the effect of spatial configuration, we computed distance-based Moran's Eigenvector Maps (dbMEMs, previously known as PCNMs) using the R package *adespatial* 0.3.7 (Dray et al., 2019) and chose the dbMEMs with associated Moran's *I* values larger than expected under the null hypothesis of no spatial autocorrelation (here: the first three dbMEMs: MEM1, MEM2, MEM3). For dbRDA, the null model was tested against the full spatial model (genetic differentiation ~MEM1 + MEM2 + MEM3) using the forward selection model choice as described above.

2.6.5 | Network analysis

To visualise the connectivity (as a proxy for gene flow) between populations based on nSSR loci, we constructed a network graph using EDENnetworks version 2.18 (Kivelä et al., 2015) with manual thresholding, based on F_{ST} corrected for null alleles obtained by FreeNA as decribed above. Manual thresholding was performed at different values (0.02, 0.03, and 0.05) for visualisation of possible population clusters under different threshold scenarios. AMOVA for nSSR markers was used to test the significance of the population structure suggested by the lowest threshold (0.02). Three hierarchical levels were tested: (1) between 6 individual populations and two multi-population groups—AKE (group1), WHI (group2), MUS (group3), MAN (group4), PAT (group5), FRE (group6), BEN + BES (group7), and EIN + LYD + CHA + GUL + FRS + SHO (group8); (2) between populations within groups; and (3) within populations. AMOVA was calculated using the R package *ade4* 1.7-13 implemented in *poppr* 2.8.3 (Kamvar et al., 2014). Statistical significance of variance components was tested by permutation tests (999 permutations).

3 | RESULTS

3.1 | Study sites—spatial distribution, lake size, and hydrochemical properties

The 15 Saskatchewan saline lakes included in the genetic analysis spanned over a range of 2.63° in latitude, 3.93° in longitude, and were separated by distances of up to 410 km. Their spatial distribution was represented as Moran eigenvectors for further analysis

(Figure S3). Their surface area ranged from 0.14 to 29.6 km^2 (Table 1). We obtained hydrochemical data for 13 of the lakes, including those for which nSSR data are available (with the exception of FRE and SHO, Table S2). The total dissolved solids of the 13 lake water samples ranged from 13 to 207 g/L, which were roughly proportional to SO_4 concentrations, except for PAT (see below).

A PCA including SO_4 meq, the ratios Cl/SO_4, Alk/SO_4, Alk/(Ca + Mg) and lake area explained 68.9% of the environmental variation between lakes between the first two components (Figure 1). Two lakes were clearly separated from the other sites: PAT was the site of a potash mine and was associated with high values of Cl/SO_4, whereas AKE had particularly high Alk/(Ca + Mg). GUL was also hydrochemically separated from other lakes by a positive association with Alk/SO_4. There was a tendency for greater lake area to be associated with higher SO_4 (Figure 1): MUS and WHI were among the largest lakes, and also had high SO_4.

3.2 | Haplotype and allelic diversity

We identified a total of 52 new haplotypes in the sampled lakes. Of these, seven were unique to the British Columbia locations. Three additional haplotypes were also reported in a previous study that also recorded 10 additional haplotypes not found in our study (Muñoz et al., 2013). To construct the haplotype network and for all other mtDNA analyses below, we included haplotype data from these authors (details in Table S6).

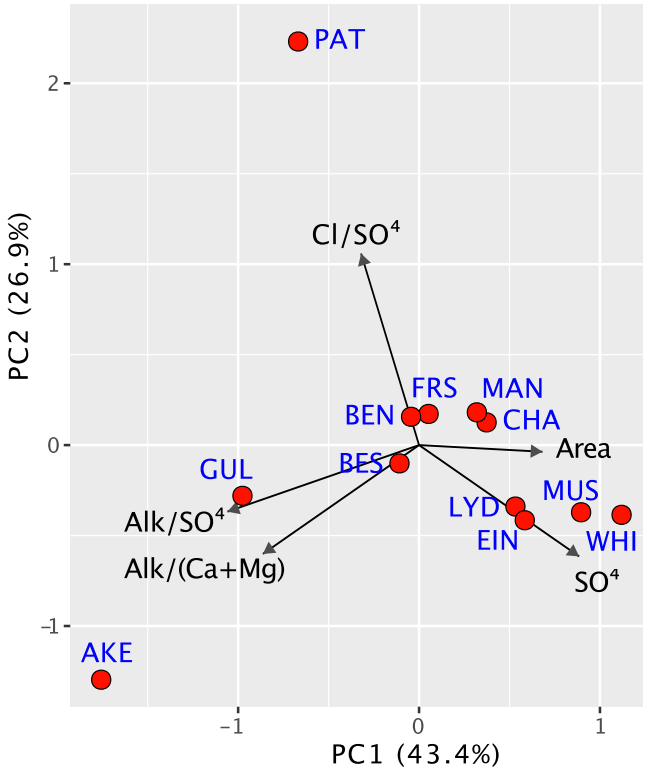

FIGURE 1 Principal component analysis of environmental variables in Saskatchewan saline lakes. The percentage of total variation explained by the first two components is given on the respective axes

Three star-like CO1 haplogroups formed by a total of 65 haplotypes exist in the Saskatchewan area, each with several additional haplotypes of low frequency (Figure 2). These three main groups were centered on two haplotypes also recorded by Muñoz et al. (2013) (Af_35, AF_41, Group A and C in Figure 2) and a newly detected haplotype (Hap_05, Group B). Overall, separation from the British Columbia haplotypes involved about four to eight mutations, while haplotypes within Saskatchewan were not strongly differentiated (the two main haplotypes differed only by one mutation).

Diversity patterns differed between CO1 and nSSR markers (Table 1): CO1 haplotype diversity ranged from 0.000 (PAT) to 0.779 (AKE). For nSSR, rarefied allelic richness was between 26.3 (PAT) and 54.9 (FRS). Diversity of multilocus genotypes H ranged from 2.493 (GUL) to 3.493 (EIN). No linear relationships were detected between allelic or haplotype diversity and lake area (Table S7).

3.3 | Population genetic structure in geographic and environmental space

There was significant structure between groups of populations according to the mitochondrial CO1 gene (Table 2) with two main groups (BEN, BES, FRE, WHI, CHA, and LYD, EIN, MAN, MUS, MEA, PAT), each of which was dominated by one of the two main haplotypes. Differentiation between members of these two groups was strong, with pairwise Φ_{ST} values between 0.548 and 0.891. AKE (with the greatest haplotype diversity) was significantly differentiated from all other populations. An AMOVA of mtDNA based on the above mentioned three groups confirmed that the variation was highest between groups (68%), and much smaller between populations within groups (1.37%). Within population variation was 31% (Table 3a).

While the pattern of differentiation was different from that of mitochondrial markers, we also detected significant genetic differentiation between populations according to pairwise D_{EST} based on nSSR loci (Table 2), ranged from −0.007 (between BEN and BES) and 0.418 (between MUS and AKE).

Network visualisation based on F_{ST} corrected for null alleles (Table S5) revealed a topology with two network components at a threshold of 0.02 (Figure 3a). One included EIN, LYD, GUL, CHA, FRS, and SHO (some of which were well separated in space), while the second included BES and BEN (which were closer together). The other five populations remained disconnected at this threshold. A gradual increase of the threshold led to higher complexity of the networks (Figure 4b,c) with increasing connectivity between the two clusters identified in Figure 4a. However, AKE, WHI, PAT, MAN, and FRE remained separated even at the highest threshold. Using the two network components identified at a threshold of 0.05, we found that genetic diversity (rarefied allelic richness of nSSR loci) differed between connected and disconnected nodes (1-way ANOVA: $F_{(1,11)} = 17.3$, $p = 0.002$; Figure 4d). This analysis suggested that

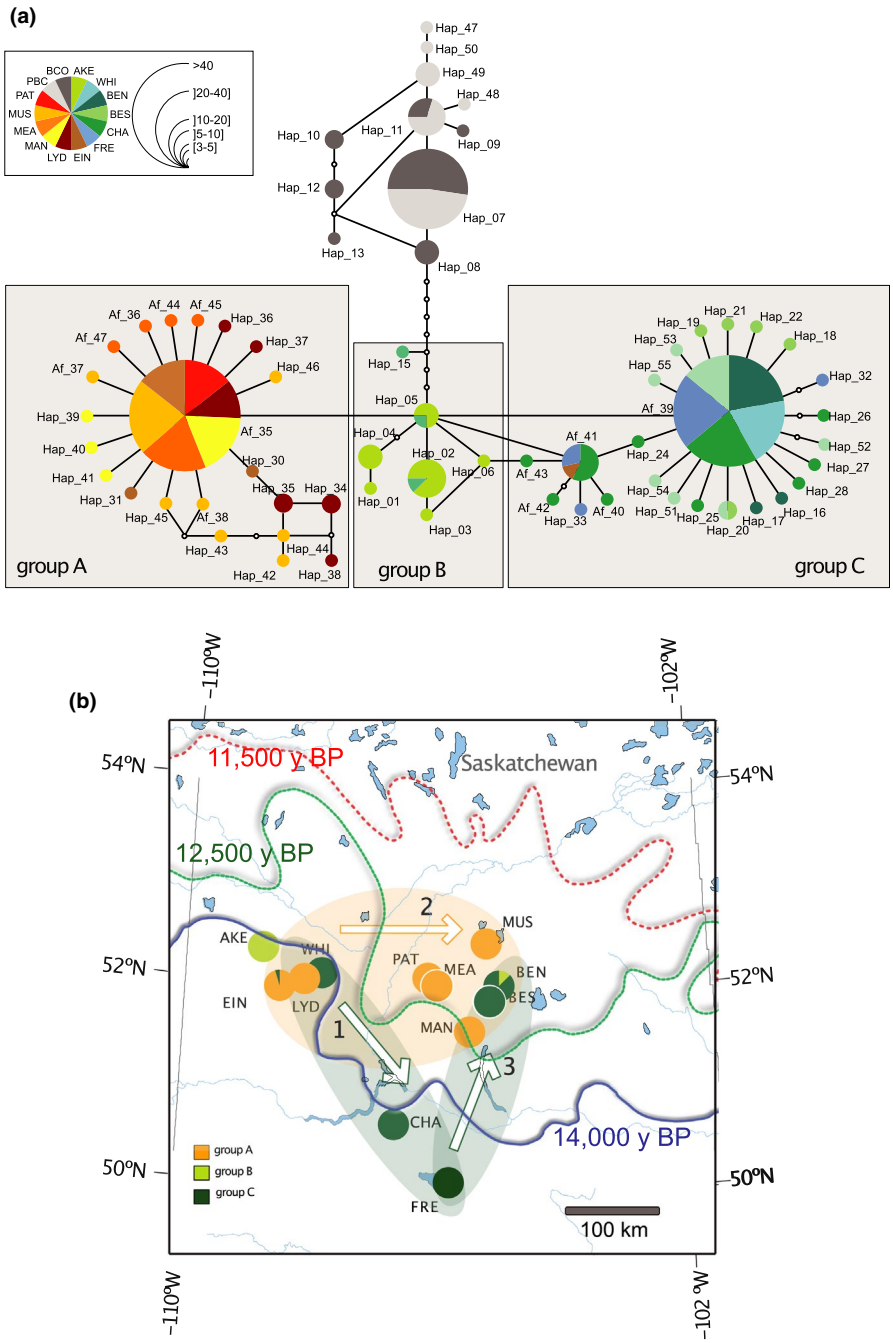

FIGURE 2 (a) Haplotype network (mitochondrial DNA) for Canadian *Artemia franciscana* (includes cytochrome *c* oxidase subunit 1 gene haplotypes from Saskatchewan and British Columbia). Grey squares highlight the four main haplotype groups present in Saskatchewan. The top nodes of the network (grey shades) represent members of two populations (BCO, PBC) in British Columbia *c.* 1,000 km west of the Saskatchewan study area. Haplotype groups A–C are based on genetic differentiation between the populations that hold these haplotypes as identified by pairwise Φ_{ST} and subsequent AMOVA (Tables 2 and 3). (b) Geographic distribution of haplotype groups A–C. Numbered arrows indicate the possible sequence of colonisation after deglaciation (details in the text). Lines indicate the glacial border at 14,000 years ago (blue), 12,500 years ago (green, dotted) and 11,500 years ago (red, hatched; drawn after Christiansen, 1979). Scale is approximate, for exact distances between lakes see Table S8. Note: to avoid confusion between patterns of mitochondrial and nuclear markers, we applied different colour schemes in this figure and Figure 4

populations within a network component were genetically more diverse (median = 50.44, *SD* = 4.41) than separated populations (median = 40.54, *SD* = 6.46).

The population groups identified from the network analysis at a threshold of 0.02 (Figure 3a) were tested by AMOVA (Table 3b). The results were significant for all strata, but by far the highest amount of variation was found within individual populations (83.50%). Variation between groups of populations was 14.09%, suggesting a different pattern than that seen in mtDNA, as also suggested by the differences in group composition (e.g. the nearby BEN and BES group together with the more distant CHA and FRE by CO1, yet they form a separate group according to nSSR). Genetic variation of nSSR was lowest between populations within groups (6.09%).

To analyse environmental conditions and spatial configuration of sample sites that might underlie the observed genetic structure, we performed dbRDA using pairwise genetic distance (Φ_{ST} for the mitochondrial CO1 data, D_{EST} for nSSR loci, Table 2) as independent variables. No significant relationship was detected for mtDNA for either spatial or environmental variables. In contrast, for nSSR markers, the forward selection model choice found strong and significant relationships with the ratio Alk/(Ca + Mg) but not with spatial configuration. Alk/(Ca + Mg) was significant in 87 of 100 repeats of the model choice ($p \leq 0.05$), explaining 30% of the observed genetic variation with a mean *p*-value of 0.04 (Table 4).

A discriminant analysis of principal components (DAPC, Figure 4) of nSSR markers revealed a population genetic structure

TABLE 2 Pairwise genetic distances between *Artemia franciscana* populations in Saskatchewan

	AKE	BEN	BES	FRE	FRS	WHI	CHA	LYD	EIN	MEA	MAN	MUS	PAT	SHO	GUL
AKE		0.341	0.321	0.326	0.374	0.316	0.316	0.332	0.296	-	0.323	0.418	0.331	0.322	0.313
BEN	0.476		*−0.007*	0.318	0.150	0.334	0.077	0.093	0.069	-	0.253	0.048	0.204	*0.038*	0.159
BES	0.574	*0.016*		0.294	0.155	0.296	0.075	0.086	0.058	-	0.235	0.070	0.181	*0.044*	0.139
FRE	0.493	*0.000*	*0.038*		0.188	0.140	0.159	0.233	0.213	-	0.108	0.391	0.225	0.260	0.166
FRS	-	-	-	-		0.305	*0.015*	0.072	0.086	-	0.255	0.139	0.326	*0.082*	0.105
WHI	0.521	*0.013*	*−0.006*	*0.025*	-		0.219	0.182	0.159	-	0.100	0.374	0.215	0.293	0.157
CHA	0.386	0.061	0.105	*0.009*	-	0.090		0.029	0.024	-	0.178	0.089	0.220	*0.022*	*0.038*
LYD	0.490	0.636	0.700	0.643	-	0.658	0.548		*0.004*	-	0.213	0.097	0.258	*0.058*	0.075
EIN	0.570	0.736	0.824	0.750	-	0.777	0.593	0.085		-	0.168	0.082	0.192	*0.034*	0.046
MEA	0.622	0.764	0.839	0.777	-	0.801	0.637	0.123	*0.001*		-	-	-	-	-
MAN	0.623	0.769	0.848	0.783	-	0.808	0.637	0.123	*0.001*	*0.000*		0.310	0.149	0.251	0.121
MUS	0.568	0.698	0.761	0.706	-	0.728	0.602	*0.048*	*0.015*	0.029	*0.028*		0.295	0.076	0.152
PAT	0.623	0.794	0.891	0.813	-	0.841	0.632	*0.115*	*−0.007*	*−0.016*	*−0.015*	*0.016*		0.229	0.218
SHO	-	-	-	-	-	-	-	-	-	-	-	-	-		*0.081*

Note: The lower diagonal shows Pairwise Φ_{ST} (Tamura & Nei) based on the mitochondrial DNA marker (cytochrome *c* oxidase subunit 1 gene). The upper diagonal shows D_{EST} computed for five simple sequence repeat markers. Numbers in italics have a non-significant associated *p*-value (>0.01).

TABLE 3 (a, b) Population genetic structure of *Artemia franciscana* in Saskatchewan identified by AMOVA for (a) cytochrome *c* oxidase subunit 1 gene (CO1) haplotypes (mitochondrial DNA) with *p*-values obtained from 10,100 randomisations as implemented in Arlequin v3.5.2.2, and (b) five simple sequence repeat (nSSR) loci, with associated *p*-values obtained by Monte Carlo permutation test with 999 repeats

Source of variation	*df*	Sum of squares	Variance components	% variation	*p*-Value
a) CO1 haplotypes					
Between groups	2	135.42	0.839	68.06	$<10^{-5}$
Between populations within groups	9	7.18	0.017	1.37	$<10^{-5}$
Within populations	281	105.96	0.377	30.57	$<10^{-5}$
Total	292	248.56	1.233		
b) nSSRs					
Between groups	7	176.90	0.321	14.09	0.001
Between populations within groups	6	24.70	0.055	2.41	0.001
Within populations	578	1,100.03	1.903	83.50	0.001
Total	591	1,301.62	2.279		

Note: For (a), population genetic structure was tested for the following groups of populations with low genetic differentiation according to Table 2 (lower diagonal). Group1: AKE; group2: BEN, BES, FRE, WHI, CHA; group3: EIN, LYD, MUS, MAN, PAT, MEA. Groups of populations for (b) were chosen according to connected and unconnected populations resulting from network construction (Figure 3a): AKE (group1), WHI (group2), MUS (group3), MAN (group4), PAT (group5), FRE (group6), group7: BEN, BES, and group8: EIN, LYD, CHA, GUL, FRS, SHO.

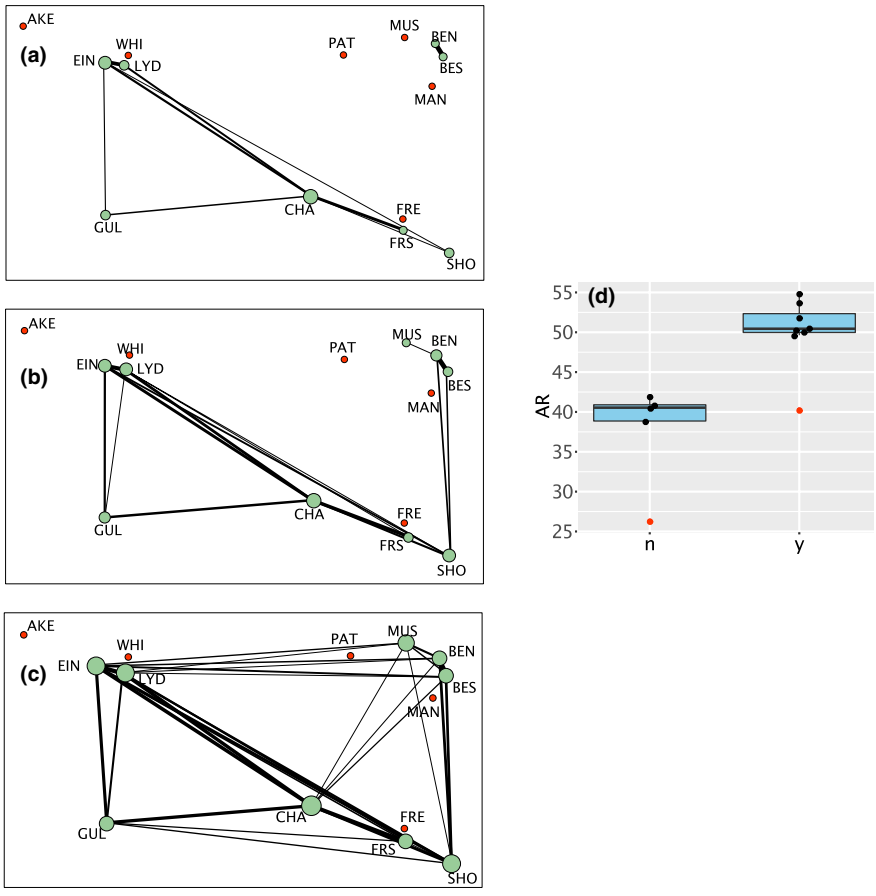

FIGURE 3 EDENetwork for *Artemia franciscana* in Saskatchewan constructed from simple sequence repeat loci. Each population is positioned according to its geographical coordinates. Edges (connections between populations) are shown for three different thresholds at (a) 0.02, (b) 0.03 and (c) 0.05, thus successively allowing weaker connections to connect nodes, adding complexity to the network. The thickness of edges is proportional to the genetic distance between populations, (thicker lines denoting less distance) while the size of nodes is relative to the number of connections between nodes. Green nodes have at least one connection, whereas red nodes are unconnected. (d) box plot with median, comparing the genetic diversity (simple sequence repeat, AR = rarefied allelic richness) of connected (y) and isolated (n) populations (connections as in [a]). Dots represent measurements for each population, with outliers in red

with five comparatively well-defined populations in which the majority of individuals were assigned to their respective population of origin (AKE, WHI, PAT, FRE, and MAN), echoing the unconnected network nodes in Figure 3C. A considerable amount of admixture is suggested for other populations where EIN, LYD, GUL, CHA, and FRS had higher membership probabilities for one of the south-western populations while MUS, BEN, and BES showed a high degree of admixture among themselves.

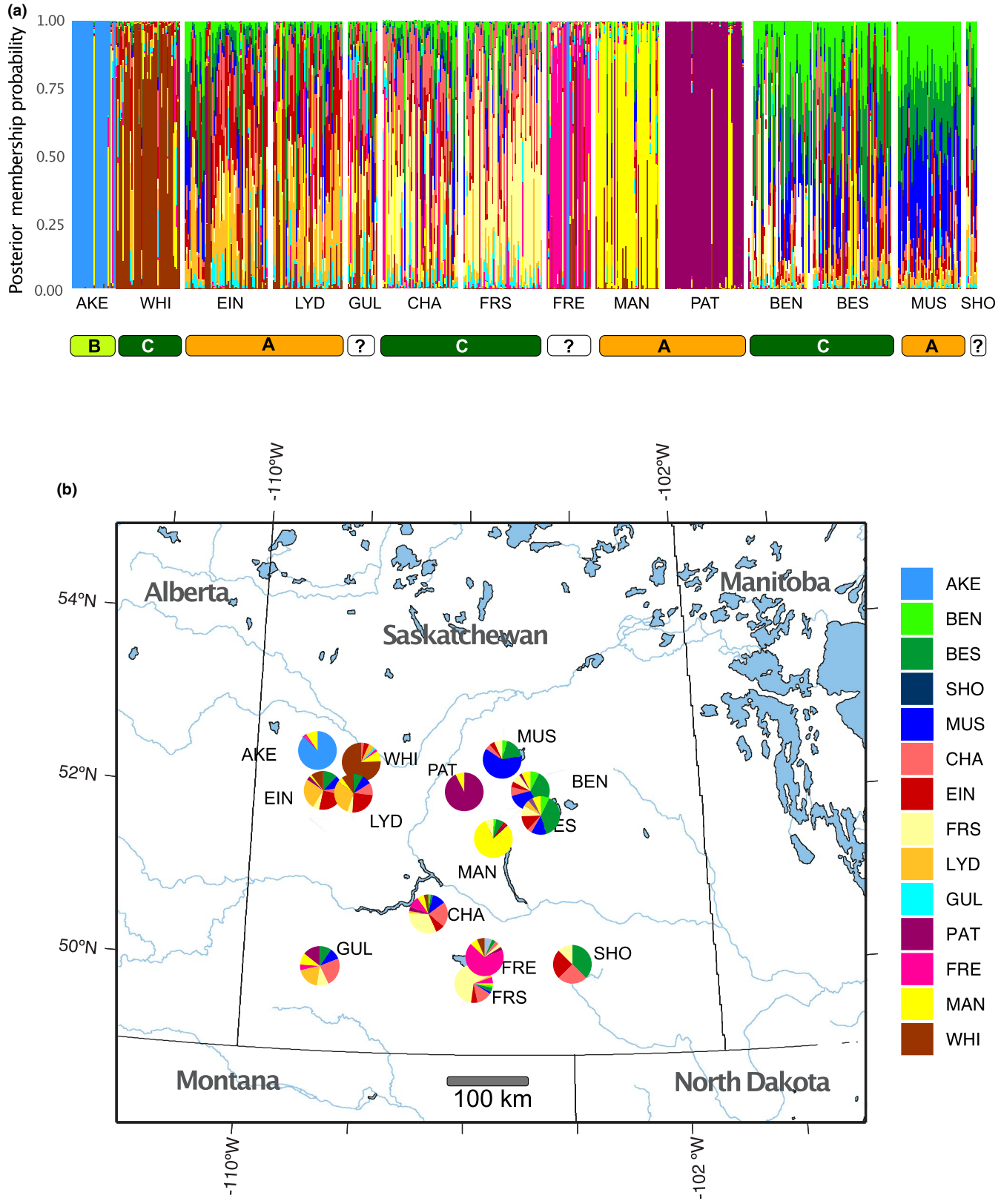

FIGURE 4 (a) Population genetic structure of *Artemia franciscana* according to DAPC based on nuclear markers (simple sequence repeat, see Methods for details). Each individual is represented by a vertical bar, coloured by estimated membership one of the sampled populations. Posterior membership probability <1.0 results in multiple assignments (and thus multi-coloured bars) per individual. Bars below the graph represent the dominant cytochrome c oxidase subunit 1 gene haplotype groups (A, B, and C, from Figure 2) detected in each population (not available for GUL, FRE, SHO, denoted by ?). Note: to avoid confusion between patterns of mitochondrial and nuclear markers, we applied different colour schemes in this figure and Figure 2. (b) Map with sample locations and representation of the percentage of individuals from each lake assigned to each of the 14 populations (by DAPC). Scale is approximate, for exact distances between lakes see Table S8. Colour legend for both figures in b

TABLE 4 Summary of results of distance-based redundancy analysis for simple sequence repeat markers obtained by forward model selection (100 repeats) to test the effect of individual environmental variables (lake area, SO_4, Cl/SO_4, Alk/SO_4, Alk/Ca + Mg) on genetic distance (D_{EST}, for details see Methods)

Statistics	Alk/Ca + Mg
R2.adj	0.30
AIC	−16.3
F	4.49
p.mean	0.04
p.min	0.024
p.max	0.05
p.*SD*	0.007
signif (*n*)	87

Note: Lake area, SO_4, Cl/SO_4, Alk/SO_4 were not selected by forward model selection and therefore were not included in the table. "*signif* (*n*)" = the number of times that results were significant (of 100 repeats).

4 | DISCUSSION

As an important species for aquaculture, *A. franciscana* occurs widely outside its native distribution in North America. It is highly invasive, rapidly outcompeting native *Artemia* species. Invasive populations generally originate from commercially harvested cysts from the Great Salt Lake and San Francisco Bay, or secondary introductions from China (Amat et al., 2005; Horváth et al., 2018). In contrast, this species has rarely been studied in other areas of its native distribution, where it inhabits natural saline lakes with a wide range of hydrochemistries in particular regarding salt composition (Bowman & Sachs, 2008). To identify the roles of IBE and IBD in driving spatial genetic structure of *A. franciscana* in the saline lakes of Central Canada, we applied mitochondrial and nuclear markers to 15 lake populations in the Saskatchewan prairies. We found significant genetic structure between the Saskatchewan populations in both marker systems, and a strong relationship between the divergence pattern observed by nSSR markers and the hydrochemical environment of the lakes.

The mtDNA patterns observed are likely to reflect historical patterns of lake colonisation, especially when lakes were formed after deglaciation, and then became saline enough to provide suitable habitat for *A. franciscana*, a species associated with higher salinities than all other anostracans in North America (Rogers, 2014a, 2014b). In the unique case of Patience (PAT), a lake contaminated with chloride by potash mining, colonisation may have occurred in modern times after mining began. The presence of only one haplotype in PAT, combined with its unique pattern of nuclear markers, suggests strong and rapid local adaptation to these unusual chemical conditions absent from our other study sites, which were lakes in a more natural condition.

4.1 | What mitochondrial markers reveal

For lakes other than PAT, our results suggest a close relationship of colonisation by *Artemia* and deglaciation after the last glacial period, which first opened up habitat alongside the southwestern border of the ice sheet (14,000 years ago). Under such a scenario, the area may first have been colonised in the western area by individuals originating from ice-free saline lake populations further to the west or south, such as Alberta or the U.S.A. From the four western-most Saskatchewan populations (AKE, EIN, WHI, LYD), which hold all three haplotype groups identified in this study, dispersal may have proceeded towards the newly opened south-eastern habitats, subsequently colonised by one of the main haplotype groups (group C). Later, as the glacier retreated north-eastwards and aquatic habitat opened towards the east in Saskatchewan, these northern lakes were largely colonised by the second main haplotype group (group A). Initial colonisation by the two main haplotypes was followed by haplotype diversification, consistent with the observed star-like network pattern. After the area was ice-free in the next 1,000 years (Christiansen, 1979), additional dispersal from the southern and western areas may have allowed colonisation of these postglacial lakes. While this proposed sequence for colonisation is consistent with the haplotype pattern observed in this study, it would require further study by including more *A. franciscana* populations from additional lakes in Saskatchewan and to the west in Alberta. Future sampling should also include populations further south in the Great Plains bioregion of the U.S.A. (Rogers, 2014b) to test the possibility that dispersal towards the northern areas may have followed the retreating ice sheet, and originated from regions south of Saskatchewan.

4.2 | What nuclear markers reveal

In contrast, nSSR markers showed significant patterns of IBE, explaining *c*. 30% of the variation in the genetic structure, but unrelated to geographic configuration of the sampled lakes. The observed patterns are indicative of local adaptation to the environment according to the criteria detailed in Orsini et al. (2013). Since *Artemia* produces vast egg banks in established populations, ecological and genetic priority effects occurring soon after initial colonisation and subsequent monopolisation effects (De Meester et al., 2002, 2016) may have reinforced such a pattern, including the lack of a spatial signal that could be related to such priority effects. Our evidence for IBE suggests that bird-mediated dispersal of cysts between lakes differing markedly in hydrochemistry may not be effective (i.e. will not lead to colonisation and establishment in the new location). Laboratory tests show that nauplii larvae fail to reach adulthood when placed in brine of unsuitable ionic composition (Bowen et al., 1988), and that cyst hatching rates are sensitive to metal concentrations (Brix et al., 2006).

In several cases, strong differentiation is highly consistent with local adaptation to extreme hydrochemical conditions, notably in PAT and AKE, but also in WHI, which had the highest SO_4 concentrations in our study. In contrast to the mitochondrial data, nSSR markers revealed that PAT was a highly divergent population, strongly related to an elevated Cl/SO_4 ratio resulting from potash mining. Similarly, in AKE, this was related to a particularly high Alk/(Ca + Mg) ratio. Adaptation of different *A. franciscana* populations to specific ion concentrations was demonstrated in the laboratory by Bowen et al. (1988), leading to ecological isolation of individual populations and an inability to colonise other lakes with divergent chemistry. This is also consistent with the apparent influence of geochemistry on the evolution and distribution of different anostracan taxa in North America (Rogers, 2014a). Since the nSSR markers are essentially neutral, to the extent that lakes have similar genetic diversity due to similar hydrochemistry, this cannot simply reflect convergent selection on genes for osmoregulation or other key physiological processes. Instead, the genetic structure is likely to reflect contemporary gene flow, which is inhibited by divergent chemistry but promoted by a close spatial relationship between lakes.

Network analysis revealed a high connectivity between many populations, reflecting their genetic similarity for nuclear markers. Together with PAT and AKE, a strongly isolated population was FRE, which is surprising since this population is adjacent to FRS and only isolated from it by a dam which also serves as a road. This creates two of several separate sub-basins in Frederick Lake used for the extraction of sodium sulfate via evaporation. The first basin (FRE) discharges water to FRS, which is a closed basin with no surface water outlet. As a result, the water in FRE is expected to be less saline compared to the concentrated brine in FRS, although we have no chemistry data for FRE. Elsewhere, BES and BEN were also separated by a road, but were not differentiated genetically, and had rather similar hydrochemistry, as they were parts of a larger lake connected by culverts under the road.

Other lakes that were relatively isolated according to network analysis were WHI (which was characterised by the highest SO_4 concentrations) and MAN. It is possible that connectivity (i.e. genetic similarity) is influenced by the extent of interchange of migratory birds between lakes. The most important site for waterbirds is CHA, which is protected as a Ramsar site in recognition of the major concentrations of shorebirds it holds. CHA was a central node in the network, connected with more populations in the network than any other lake. By contrast, adequate data on bird movements between our study lakes do not exist, but our bird counts (A.J.G., unpublished data) would suggest that WHI and MAN should be reasonably well-connected via waterbirds with other lakes. WHI held thousands of Wilson's phalaropes *Phalaropus tricolor*, which were feeding on *Artemia* and are likely to be excellent vectors for their dispersal. Hatching of nauplii from faeces collected at Frederick Lake from the American avocet *Recurvirostra americana* during the course of this study confirmed the internal transport of viable *A. franciscana* cysts in our study area (A.J.G., unpublished data). We did not find evidence that lake area significantly affected genetic diversity. However, network analysis showed that there is greater genetic diversity in highly connected populations than in the less connected populations, and future research could test whether this reflects movement patterns of birds between lakes. Outside of migratory periods, waterbirds will generally provide higher connectivity for sites that are close together, and hence coincide within the home range of more individual birds (Green et al., 2002).

5 | CONCLUSIONS

Saline lakes of Saskatchewan provide a fascinating insight into the population genetics of *Artemia* under natural conditions. The importance of IBE in this keystone crustacean was only apparent as a consequence of our unusual application of hydrochemical analysis. Studies of the population or invasion genetics of aquatic invertebrates in inland waters typically limit their analyses of water quality to conductivity or salinity, and few if any have gone into as much detail about ionic composition as in our study. More frequent collaborations between hydrogeologists and molecular ecologists are recommended. Although many of the sites occupied by other *Artemia* species in different continents are coastal lagoons and salt ponds that are likely to have little diversity in salt composition, many others are inland salt lakes (Muñoz & Pacios, 2010) that may be hotspots of genetic diversity associated with local adaptation to unique chemical conditions. Furthermore, the *Artemia* populations in such inland lakes (e.g. *A. sinica* and *Artemia tibetiana* populations, Lin et al., 2017) may be resistant to invasion by *A. franciscana* populations originating from aquaculture, owing to contrasting hydrochemistry.

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AUTHOR CONTRIBUTION

A.J.G. conceived the study and collected samples. M.T.B. collected samples. C.L. and J.S.F. generated the molecular data. M.H. generated and analysed the hydrochemical data. D.F. analysed the molecular data with C.L. and wrote the paper with A.J.G. and input from all other authors.

DATA AVAILABILITY STATEMENT

Sequence data that support the findings of this study are available in NCBI GenBank, accession numbers MW799827-MW799878.

Hydrochemical data are made available at https://digital.csic.es/handle/10261/241491.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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