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Low among-population genetic differentiation in Chinese bisexual *Artemia* populations

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We studied the population genetic structure of nine bisexual *Artemia sinica* populations from the provinces of Inner Mongolia, Shanxi and Qinghai in China, using variation at nine allozyme loci (cellulose acetate electrophoresis). There is a clear-cut tendency for an increase in genetic variation, as measured by heterozygosity, with increasing habitat size. Although we observe a positive relationship between genetic differentiation and geographical distance, overall F_{ST} values are low: populations separated by approximately 1000 km show average F_{ST} values of 0.05–0.1, whereas populations separated by 100 km show no genetic differentiation at all.

Keywords: *Artemia*, brine shrimp, gene flow, genetic differentiation, genetic variation, habitat size.

Introduction

The brine shrimp *Artemia* (Crustacea: Anostraca) is normally restricted to saline inland lakes and coastal salterns (Vanhaecke *et al*., 1987). In common with other large branchiopods, the brine shrimp *Artemia* is capable of producing diapausing cysts that can withstand adverse conditions such as anoxia, drying, freezing, mechanical disturbance and digestive enzymes, and these cysts are therefore believed to be very suited for passive dispersal by wind, waterfowl or man (Persoone & Sorgeloos, 1980). The presumed good dispersal capacities of the brine shrimp translate into the expectation that, even though genetic differentiation among populations will increase with geographical distance (Slatkin, 1985), overall levels of genetic differentiation will be low. The island-like nature of inland waters may, however, provide opportunities for genetic differentiation, and several studies have indeed emphasized strong among-populational genetic differentiation in various zooplankton taxa, even though many of these species have attributes (e.g. resting eggs) that promote dispersal (Boileau *et al*., 1992; Boileau & Taylor, 1994; De Meester, 1996). Being confronted with these conflicting expectations, we set out to analyse genetic differentiation among Chinese *Artemia* populations inhabiting salt lakes that are separated by up to 1500 km.

Artemia has proven suitable for the study of evolutionary processes such as speciation and genetic or morphometric differentiation (Browne & Bowen, 1991; Pilla & Beardmore, 1994; Triantaphyllidis *et al*., 1997a,b). Allozyme electrophoresis has so far mainly been used for the resolution of taxonomical status, although a number of studies have also considered genetic differentiation among local populations (Bowen & Sterling, 1978; Beardmore & Abreu-Grobois, 1983; Abatzopoulos *et al*., 1993; Gajardo *et al*., 1995). At least five bisexual species and many parthenogenetic species are currently recognized in *Artemia* (Pilla & Beardmore, 1994; Gajardo *et al*., 1995). *Artemia* is distributed in many salt lakes of north-western China, including Inner Mongolia, Xinjiang and Qinghai provinces (Xin *et al*., 1994). Chinese *Artemia* are either bisexual or parthenogenetic. A bisexual strain in Xiechi Lake of Yunchen, Shanxi province, has been characterized as *A. sinica* by Cai (1989), and Hou *et al*. (1993, 1997) found *A. sinica* in another 11 Chinese bisexual populations. Pilla & Beardmore (1994) showed a close genetic relationship between one Inner Mongolian population (unknown lake locality) and the Xiechi population (Yuncheng, Shanxi province). Recently, a new species (named as *A. tibetiana*) has been described from Tibet by Abatzopoulos *et al*. (1998). So far, genetic

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studies on Chinese populations have focused on taxonomic problems and the identification of strains. In the present paper, we provide more detailed information on levels of within-population genetic variation and among-population genetic differentiation in *A. sinica* populations from China, mainly from Inner Mongolia. *Artemia* populations have been cited from 39 salt lakes in this area (Ren *et al*., in press), and these lakes vary widely in size and ecological conditions. In total, we studied nine populations: seven from Inner Mongolia, one from Qinghai and one from Shanxi province.

Materials and methods

Cyst collection and Artemia *culture*

The cysts used to initiate laboratory cultures were collected by staff of the Salt Research Institute (SRI) and the Inner Mongolian Salt company of China, and were stored in the cyst bank of SRI. The localities from which cysts were collected are shown in Fig. 1. More information on the salt lakes sampled is given in Table 1.

The cysts of each population were disinfected and hatched according to the methods described by Sorgeloos *et al*. (1986). All populations were cultured at a density of 100 nauplii/L in 3-L glass bottles containing 80 ppt artificial seawater (made up with the commercial sea salt Instant Ocean) under fluorescent light providing a photoperiod of 16:8 light:dark, in a temperature-controlled room (20°C ± 1°C). The *Artemia* were kept under mild aeration and were fed daily with algae (*Dunaliella tertiolecta* Butcher). The brine was renewed weekly.

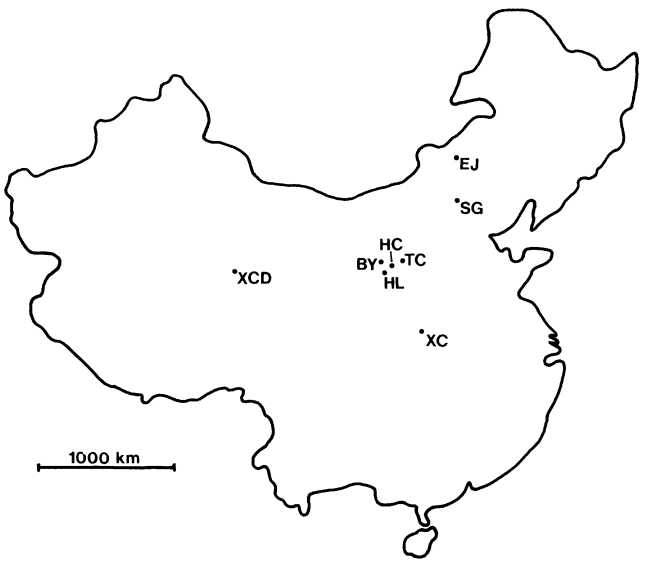

Fig. 1 Location map showing outline of China and indicating the salt lakes that were sampled. For abbreviations, see Table 1.

Table 1 *Artemia sinica* populations studied, showing name of salt lake with abbreviation, location (region, province), surface area, elevation, depth and dominant salt

Lake name		Location		Area km^2	Elevation m	Depth m	Type of lake
1 Taigemiao Chagannor	TC	Yimeng, Inner Mongolia	109°55′E	39°5′N	15	1300	0.1–0.5	Carbonate
2 Haotongyin Chagannor	HC	Yimeng, Inner Mongolia	108°55′E	39°10′N	21.8	1300	0.1–0.5	Carbonate
3 Haolebaoqing	HL	Yimeng, Inner Mongolia	108°30′E	38°54′N	10	1300	0.1–0.5	Carbonate
4 Sanggendalai	SG	Ximeng, Inner Mongolia	115°46′E	42°40′N	3.5	1300	1.5–3	Carbonate
5 Baiyannor	BY	Yimeng, Inner Mogolia	108°00′E	39°23′N	4.9	1359	0.5	Carbonate
6 Ejinor	EJ	Ximeng, Inner Mongolia	116°30′E	45°14′N	26.0	830	0.3–0.5	Sulphate
7 Dahan lake	DH	Ximeng, Inner Mongolia						
8 Xie chi	XC	Yuncheng, Shanxi	111°E	35°N	130	320	0.1–0.5	Carbonate
9 Xiaocaidan	XCD	Qinghai	95°28′E	37°31′N	63.9	3171	0.08–0.69 (0.26)†	Sulphate

†Mean value.

Allozyme electrophoresis

Approximately equal numbers of randomly drawn adult males and females (50–80 from each population) from the stock cultures were stored at −80°C and used for allozyme analysis. Cellulose acetate electrophoresis (following Hebert & Beaton, 1989) was used to screen for allelic variation at nine loci. We initially assessed variation for 19 enzymes: AAT (EC 2.6.1.1), ADH (EC 1.1.1.1), AK (EC 2.7.4.3), AMY (EC 3.2.1.1), AO (EC 1.2.3.1), APK (EC 2.7.3.3), FUM (EC 4.2.1.2), G6PDH (EC 1.1.1.49), HEX (EC 2.7.1.1), IDH (EC 1.1.1.42), LDH (EC 1.1.1.27), MDH (EC 1.1.1.37), ME (EC 1.1.1.40), MPI (EC 5.3.1.8), 6PGDH (EC 1.1.1.44), PGI (EC 5.3.1.9), PGM (EC 5.4.2.2.), SOD (EC 1.15.1.1), XDH (EC 1.1.1.204). Nine loci (*AAT*, *APK*, *IDH-1*, *IDH-2*, *LDH*, *MDH-1*, *MDH-2*, *MPI* and *PGI*) could be reliably stained and proved polymorphic, and they were chosen for our study.

Data analysis

All statistical analyses of allozyme data were performed with TFPGA (Tools for Population Genetic Analyses) version 1.3 (Miller, 1997) and with GENEPOP 1.2 (Raymond & Rousset, 1995). The percentage of polymorphic loci (0.99 criterion) was calculated for each population. Two estimates of heterozygosity were calculated: direct count and expected heterozygosities under Hardy–Weinberg equilibrium. Tests for deviations from H.–W. equilibrium and for linkage disequilibrium were carried out using exact tests (GENEPOP). Genetic structuring within and between populations was estimated using *F*-statistics. The significance of the among-population genetic differentiation over all loci was calculated by the exact test. To test for a correlation between genetic differentiation and geographical distance, we calculated pairwise F_{ST} values for all possible pairs of the eight populations that remained after excluding the one Inner Mongolian population of unknown locality. The correlation of this measure of genetic differentiation with the logarithm of geographical distance between the populations was analysed using a Mantel test for dependent variables (Sokal & Rohlf, 1995). The same analysis was also performed separately for the six populations from Inner Mongolia.

Results

The percentage of polymorphic loci and the mean heterozygosity in the nine populations studied are shown in Table 2. Twelve out of 59 tests for deviations cf from H.–W. equilibrium yielded *P*-values <0.05, the deviation being significant at the 0.05 level in three cases after sequential Bonferroni correction for table-wide errors. We found no evidence for linkage disequilibrium (nine tests out of 324 yielded *P*-values <0.05; none significant at the 0.05 level after sequential Bonferroni correction for table-wide errors). The mean heterozygosity (expected) ranged from 0.110 to 0.161. Haolebaoqing (HL) and Xiechi (XC) showed the highest percentage of polymorphic loci, and Xiechi (XC) displayed the highest value of heterozygosity. When we plot expected heterozygosity for each population against the surface area of the habitat, there is a clear tendency for higher heterozygosities with larger surface area (Fig. 2; the regression is significant, $P = 0.04$).

Table 2 Summary of measures of genetic variability in nine *Artemia sinica* populations

Population	Percentage polymorphic loci (0.99 criterion)	Mean heterozygosity	
		Direct count	Expected under H.–W.
TC	66.67	0.1125	0.1383
HC	55.56	0.1167	0.1340
HL	77.78	0.1226	0.1396
SG	55.56	0.1026	0.1228
BY	55.56	0.1204	0.1241
EJ	44.44	0.1120	0.1147
DH	55.56	0.1019	0.1097
XC	77.78	0.1537	0.1613
XCD	55.56	0.1423	0.1562

Figure 3 shows the UPGMA dendrogram constructed from a matrix of pairwise values of Nei's (1972) genetic distance for all nine populations studied. The Xiechi population (XC) from Shanxi province is clearly separated from all Inner Mongolia populations. Within the group of populations from Inner Mongolia, the populations from Yimeng cluster together and are genetically very similar. The only odd feature in the dendrogram is the fact that the Xiaocaidan population (XCD) from Qinghai province clusters among the Ximeng populations (Inner Mongolia). The average distance between the Xiaocaidan and the Inner Mongolia populations is approximately 1200 km.

Single-locus and average values of F_{IS}, F_{IT} and F_{ST} for all nine populations are shown in Table 3. The mean value of F_{ST} over all nine loci and all populations is 0.083, indicating moderate genetic differentiation. Allele frequencies differ significantly among populations by an exact test (all nine loci combined, $P < 0.0001$).

A significant correlation ($P < 0.05$) was found between pairwise F_{ST} values and the logarithm of geographical distance for all eight *A. sinica* populations for which the exact location is known (Fig. 4a) and for the six Inner Mongolia populations (Fig. 4b) using a

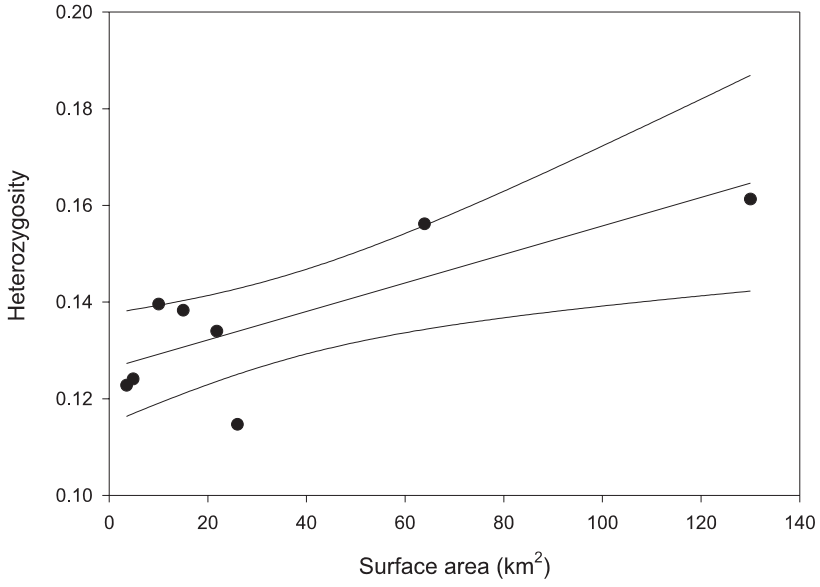

Fig. 2 The relationship between expected heterozygosity and salt lake surface area for nine *Artemia sinica* populations as revealed by linear regression analysis. Regression line: $y = 0.003x + 0.1262$, $R^2 = 0.62$, $P = 0.04$.

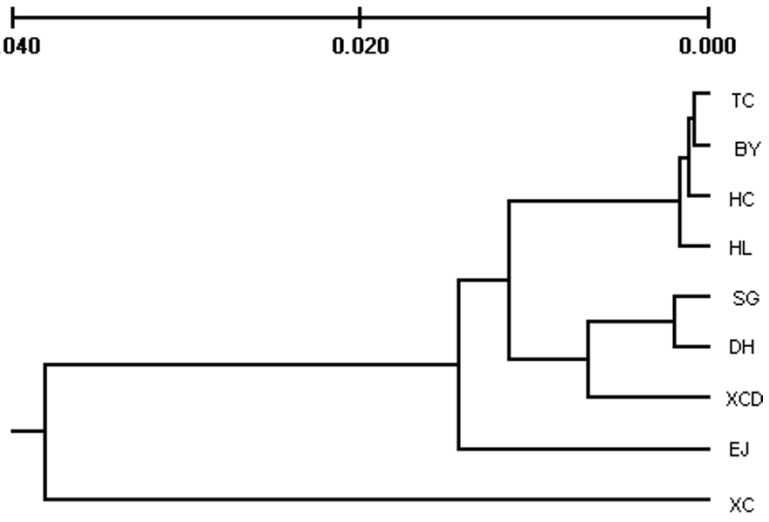

Fig. 3 UPGMA dendrogram of Nei's (1972) genetic distance for nine *Artemia sinica* populations sampled from different provinces in China. For abbreviations of lake names, see Table 1.

Mantel test. There is a clear tendency for an increase in genetic differentiation with increased geographical distance. The value of the correlation coefficient (r) was increased to 0.83 when performing the analysis on the six Inner Mongolia populations only. The most striking feature of Fig. 4, however, is that populations that are separated by as much as 100 km are not genetically differentiated at all ($F_{ST} \approx 0$).

Discussion

Average values of heterozygosity and the percentage of polymorphic loci observed in our study are comparable with values from several other studies on bisexual *Artemia* (Abreu-Grobois, 1987; Pilla & Beardmore, 1994; Gajardo *et al*., 1995). The mean F_{ST} value (0.083) obtained for the nine *A. sinica* populations studied by us is, however, smaller than the F_{ST} values that have been calculated for several conspecific *A. franciscana* ($F_{ST} = 0.24$–0.38) and *A. salina* ($F_{ST} = 0.12$) populations (Abreu-Grobis & Beardmore, 1982; Abreu-Grobois, 1987; Gajardo *et al*. unpubl. data). As these other studies did not span larger geographical distances than ours (>1500 km), we can conclude that genetic differentiation among the *A. sinica* populations studied by us is rather low. Figure 4 shows that populations that are separated by 100 km show an average F_{ST} value approximating 0, whereas populations separated by as much as 1200 km have F_{ST} values ranging from as low as 0.02 to 0.25. This low level of

Table 3 Wright's *F*-statistics for nine *Artemia sinica* populations

Locus	F_{IS}	F_{IT}	F_{ST}
APK	0.1914	0.2214	0.0371
MDH-1	−0.0075	−0.0050	0.0024
MDH-2	0.1756	0.1743	−0.0016
MPI	0.2037	0.3070	0.1297
LDH	0.6635	0.6654	0.0056
IDH-1	0.0610	0.1216	0.0646
IDH-2	−0.0323	0.0723	0.1013
PGI	0.0310	0.0379	0.0072
AAT	0.0546	0.0743	0.0209
All loci	0.1062	0.1805	0.0831

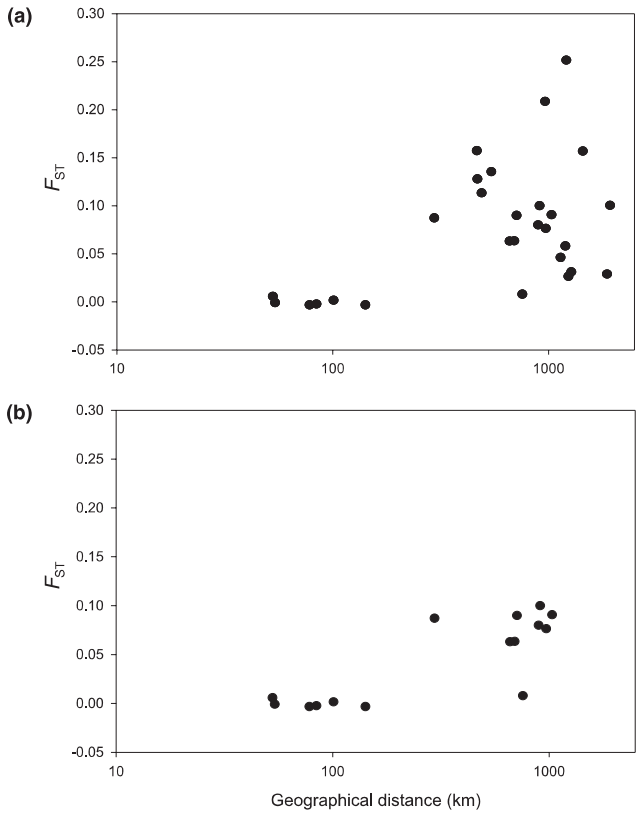

Fig. 4 Correlation between pairwise values of genetic differentiation among populations (F_{ST}) and the logarithm of geographical distance between the habitats from which these populations were sampled: (a) for all eight *Artemia sinica* populations from which the exact locality is known ($r = 0.51$, $P = 0.048$); (b) for all six Inner Mongolia populations ($r = 0.83$, $P = 0.035$).

genetic differentiation may reflect the good dispersal capacities of the resting stages (cysts) and transport by migratory waterfowl and wind. The F_{ST} values obtained here are lower than the values reported for other Crustacea, such as *Daphnia* (Lynch & Spitze, 1994). However, one should recall that because of linkage effects, average F_{ST} values in cyclically parthenogenetic organisms such as *Daphnia* are expected to be higher than in obligately sexual organisms (see Vanoverbeke & De Meester, 1997). But our values of interpopulational genetic differentiation are also low when compared to obligately bisexual zooplankton (Boileau & Taylor, 1994) and freshwater anostracans (Riddoch *et al*., 1994; Brendonck *et al*., in press; these studies observed significant interpopulational genetic differentiation in populations separated by a few tens of metres only). It remains speculative to try to explain this discrepancy, but it is likely that the size of the habitats plays a role. All populations studied by us inhabit rather large lakes (3.5–64 km^2; estimated volumes $2.4–39 \times 10^6$ m^3) and are estimated to consist of $> 10^9$ individuals, which may have considerably reduced the impact of two random processes. First, large populations reduce the speed with which populations differentiate from each other through genetic drift. Secondly, the impact of founder events may have been reduced because 'effective' propagule sizes colonizing the habitat may have been large, thus effectively sampling regional diversity. We hypothesize that in large lakes, the 'effective' propagule size is large because colonization success of immigrants remains relatively high for a long time. The latter follows from the fact that it takes longer before a colonizing population approaches carrying capacity in a large than in a small habitat. This argument is based on the idea that effective gene flow is often much lower than dispersal, and that the discrepancy between the two increases with time, as the establishment success of immigrants reduces in habitats in which competition with resident conspecifics is severe (De Meester, 1996).

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