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Preliminary genetic data on some Caribbean *Artemia franciscana* strains based on RAPD's

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Abstract

A total of fourteen *Artemia* samples from Colombia, Venezuela, Curaçao (Netherlands Antilles), Puerto Rico, and reference samples from U.S.A. (San Francisco Bay, SFB) belonging to the superspecies *Artemia franciscana*, and Argentina (*A. persimilis*), were analysed with the RAPD technique in order to demonstrate genetic dissimilarities. Pearson's correlation coefficients between the DNA banding patterns were calculated. They served as input values for the construction of UPGMA dendrograms. The results indicate that, within the collection of Colombian, Venezuelan and the two Netherlands Antilles *Artemia* cyst samples examined, two different groups seem to exist. Geographically, the mountainous area of Sierra Nevada de Santa Marta separates these two groups (lower Caribbean to the South and middle Caribbean to the North). Although the Caribbean, North and South American populations belong to *A. franciscana*, genetic discontinuities are to be expected due to habitat differences and geographic isolation. The Sierra Nevada (with an altitude of about 5800 m) emerges as the barrier very likely to explain the observed RAPD differences. Little genetic variability was present in the Colombian samples from Manaure that were collected almost every ten years, nor in the samples from Galerazamba collected almost two decades apart, although these samples were more likely subjected to different prevailing environmental conditions. The SFB population did not show a very close relation with all Caribbean populations analyzed, including the Puerto Rican. All *A. franciscana* populations analyzed were divergent from *A. persimilis* (Argentina).

Introduction

The crustacean anostracan *Artemia franciscana* is widely distributed along the American continent. This cosmopolitan brine shrimp is reported to be present practically from one extreme of the American continent to the other; from Torres del Paine salt lagoon (50° 29′ S) in Chile (Gajardo et al., 1992; Gajardo & Beardmore, 1993; Gajardo et al., 1998) up to Little Manitou (51° 48′ N) in Eastern Canada (Vanhaecke et al., 1987; Bowen et al., 1988; Triantaphyllidis et al., 1998). Yet their distribution over this area is typically patchy which is a consequence of their natural occurrence being restricted to salt lakes. These salt lakes are characterized by their own physico-chemical characteristics. This could give origin to endemic *Artemia* populations. Despite their isolation by distance and environmental conditions, *Artemia* populations in all these lakes could be linked. It is very likely that birds play an important role in dispersing *Artemia*, principally in natural and artificial coastal saline lagoons. Aquatic migratory birds (i.e. flamingos) have been reported by several authors (Löffler, 1964; Proctor, 1964) to be potential passive vectors for transporting brine shrimp cysts in their digestive tract or attached to their feathers or claws. According to Lincoln & Peterson (1979) birds are the most mobile creatures on Earth, as they travel from the northern USA or even from northern Canada to winter quarters in the West Indies, Central, or South America in a relatively short time. In the Caribbean region, a good active candidate for *Artemia* cyst dispersal could be the Caribbean

flamingo (Rooth, 1965; Sprunt, 1976; Ogilvie & Ogilvie, 1986). So in genetic terms there is potential for gene flux among these isolated populations. Recently some researchers (Lovejoy, 1998; Adams, 1998) have cast some light on the possible geological landscape transformation of the American continent, especially for South America. According to Behling et al. (1998) some parts of the Andes may have been both cooler and moister than at present around 50 000 years ago. A mid-altitude site in the southern Colombian Andes shows this pattern of cooler, and possibly moister conditions.

Genetic differences can be studied by a wide variety of techniques. With respect to decapod crustaceae Nelson & Hedgecock (1980) and Hedgecock et al. (1982) used enzyme polymorphism. Garcia & Benzie (1995) conducted research using RAPD markers in breeding programs of crustaceans (penaeid shrimp). Some of these tools have also served for the characterization of anostracans of the genus *Artemia* using the cysts (Abatzopoulos et al., 1997; Triantaphyllidis et al., 1997). Perez et al. (1994) studied mitochondrial DNA analysis of bisexual and parthenogenetic brine shrimp.

In this paper, we wanted to test the hypothesis that *Artemia* populations in the Caribbean area and the North of South America that are separated by the Sierra Nevada de Santa Marta can be easily distinguished by the RAPD technique.

Materials and methods

Most samples were collected directly from each locality (Fig. 1) and were processed avoiding any possible cross contamination. The rest of the samples were obtained from the cyst bank of the Laboratory of Aquaculture & *Artemia* Reference Center (ARC).

DNA extraction from cysts

For each strain (Table 1) 100 mg of decapsulated cysts (Sorgeloos et al., 1986) were resuspended in 1 ml SE buffer (750 mm NaCl; 250 mm Na_2EDTA) to which 10 μl Tris buffer was added (Tris stock 1 M at pH 8). The samples were homogenized at 4 °C with a potter (Braun, Germany) at 1000 rpm during 1 min. To 500 μl of homogenate 20 μl of proteinase K (stock 10 mg/ml), 50 μl of homogenate 20 μl of proteinase K (stock 10 mg/ml) and 50 μl SDS (stock 10%) were added. After 30 min incubation at 65 °C, the sample was extracted once with 500 μl phenol-chloroform (1:1) and once with 500 μl of chloroform. To the supernatant (12 000 rpm, 5 min), 650 μl of water and 1300 μl ice-cold isopropanol was added. The precipitated DNA (1 h at −20 °C) was pelleted at 12 000 rpm during 15 min and resuspended in water. The RNA in the samples was degraded by incubation in the presence of RNAse A (0.2 mg/ml) at 37 °C during 15 min. The DNA samples were stored at −80 °C. Alternatively DNA was extracted from cysts using the Wizard® genomic DNA purification kit (Promega™, mouse tail protocol). The DNA concentration was measured with a spectrofluorophotometer (Shimadzu™ RF-1501). Therefore aliquots of the sample were dissolved in water containing ethidium bromide (final concentration 0.3 μg/ml) and excitated at 325 nm. Light emission was measured at 563 nm. At those light wavelength settings, residual proteins in the samples do not interfere with the measurement. The readings were converted to DNA concentrations with the help of herring sperm DNA (Roche™ Molecular Biochemical) standard series (0–333 pg/μl). The DNA in the sample was adjusted to 50 ng/μl.

RAPD reactions

RADP reactions were performed with two primers (Eurogentec™, Belgium), namely ERIC1R (5' ATGTAAGCTCCTGGGGATTCAC 3′) and ERIC1RA (5′ ATGTAAGCTCCTGGGGATTCAG 3′) in single primer reactions. The PCR (Hybaid PCR express, Labsystems™, Belgium) conditions were as follows: 1 time at 94 °C for 2 min; 5 times at 94 °C for 30 sec, at 40 °C for 2 min, at 68 °C for 8 min; 25 times at 94 °C for 30 sec, at 65 °C for 1 min, at 72 °C for 2 min and a final extension at 72 °C during 5 min. Therefore, 100 ng total DNA and 1U DNA polymerase mixture (Expand™ High Fidelity PCR system, Roche™ Molecular Biochemicals) were mixed into 10 mM Tris HCl, 50 mM KC1, 5 mM $MgC1_2$ containing 20 μM primer and 0.2 mM dNTP's (final reaction volume: 50 μl).

Electrophoresis and gel processing

The generated DNA fragments were separated on an agarose gel (2%) and stained with ethidium bromide (referred to as first experiment, results shown) or on an ExcelGel® system (horizontal polyacrylamide gel electrophoresis) and silver stained (Pharmacia™) (referred to as second experiment, results not shown). A

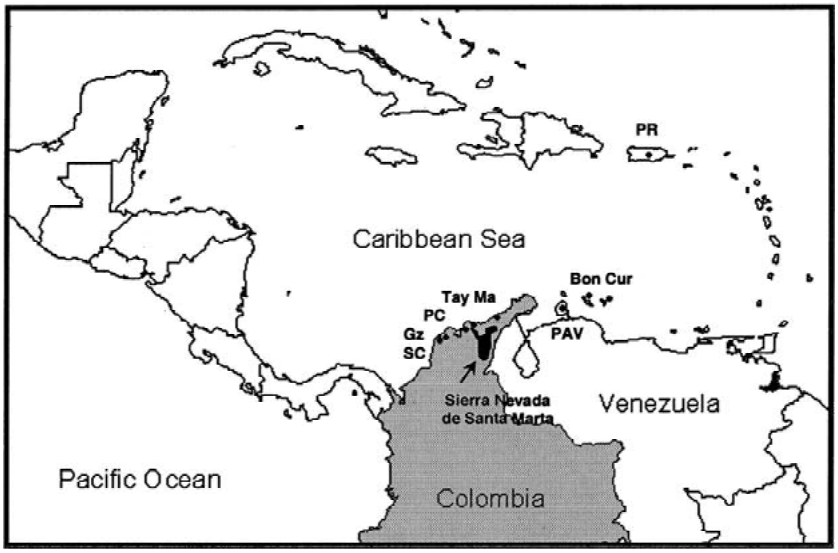

Figure 1. Location of the Caribbean sample collection sites. SC – Salina Cero; Gz – Galerazamba; PC – Pozos Colorados; Tay – Tayrona National Natural Park; Ma – Manaure; PAV – Puerto Araya, Venezuela; Bon – Bonaire, Cur – Curaçao; PR – Puerto Rico.

Table 1. List of samples used in this study, their abbreviations and ARC code numbers

Label	Species /strain	Description
Bon	*A. franciscana*, Bonaire	Netherlands Antilles, ref. ARC28
PR	*A. franciscana*, Bahía Salinas	Puerto Rico, ref. ARC36
Cur	*A. franciscana*, Curaçao Fuik	Netherlands Antilles, ref. ARC502
SFB	*A. franciscana*, SFB	San Francisco Bay, USA, ref. ARC1258
A. persimilis	*A. persimilis*	Argentina, ref. ARC1321
Gz80	*A. franciscana*, Galerazamba	Colombia, 1980 ref. ARC469
Gz98	*A. franciscana*, Galerazamba	Colombia, 1998 ref. ARC
Ma30	*A. franciscana*, Manaure	Colombia, 1977, ref. ARC30
Ma439	*A. franciscana*, Manaure	Colombia, 1980's, ref. ARC439
Ma98	*A. franciscana*, Manaure	Colombia, 1998
PC	*A. franciscana*, Pozos Colorados	Colombia, 1993, ref. ARC1370
PAV	*A. franciscana*, Puerto Araya	Venezuela, 1980's, ref. ARC1006
SC	*A. franciscana*, Salina Cero	Colombia, 1998, ref. ARC1446
Tay	*A. franciscana*, Tayrona	Colombia, 1998, ref. ARC1447

100 bp ladder (Promega™) was loaded as a reference. Agarose gels were photographed with a Polaroid film. Pictures or silver stained gels were scanned and the images were processed with the Gelcompar® software (Applied Math, Kortrijk, Belgium). Composed gels were made using the banding patterns generated by the two different primers. Pearson's correlation coefficient between the patterns was calculated which served as input for an UPGMA (unweighted pair-group method of arithmetic averages) dendrogram.

Results and discussion

In a first experiment, 14 *Artemia* strains, including one *A. persimilis*, were tested (Table 1 and Fig. 2). The RAPD reaction products were separated by agarose eletrophoresis and visualized by ethidium bromide staining. The generated dendrogram contained 5 clusters:

Cluster 1: Ma439, Ma98, PC, Tay, PAV, Ma30, Bonaire and Curaçao.

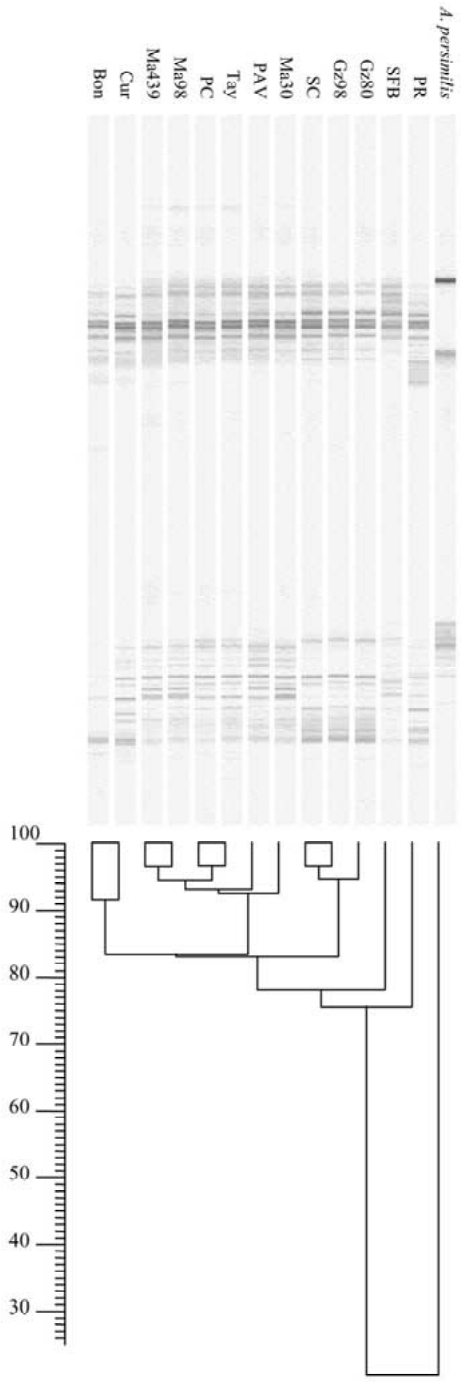

Figure 2. Dendrogram 1 based on Low Melting Agarose gel (Software optimization 0.5%) SC – Salina Cero; Gz – Galerazamba; PC – Pozos Colorados; Tay – Tayrona National Natural Park; Ma – Manaure, PAV – Puerto Araya, Venezuela; Bon – Bonaire; Cur – Curaçao; SFB – San Francisco Bay; PR – Puerto Rico.

Cluster 2: Gz80, Gz98 and SC
Cluster 3: SFB
Cluster 4: Puerto Rico
Cluster 5: *A. persimilis*

For convenience the first cluster is called middle Caribbean and the second lower Caribbean. In a second, identical and independent experiment (in which the same RAPD markers were separated by horizontal PAGE and stained by silver) restricted to the strains of cluster 1 and 2, these two clusters were produced again, each of them containing the same strains (except for Bonaire and Curaçao which were not included in this experiment). This second experiment demonstrated that the results were reproducible (results not shown).

The clustering pattern obtained in the first experiment, corroborated by the clustering pattern obtained in the second dendrogram, suggests that the populations in these two clusters are not identical and that some genetic dissimilarities between them might exist. The Pearson coefficients in the Colombian samples from Manaure that were collected in 1977 (Ma30), mid 1980s (Ma439) and 1998 (Ma98) were very high, as well as in the samples from Galerazamba collected in 1980 (Gz80) and 1998 (Gz98), although these samples were collected during different years and more likely subjected to different prevailing environmental conditions. This suggests high genetic similarities between these samples.

The splitting of the populations from Colombia, Venezuela, Bonaire and Curaçao into two clusters: (1) Middle Caribbean: from the North of the Sierra Nevada to Bonaire and Curaçao; and (2) Lower Caribbean: from the South of the Sierra Nevada to Salina Cero, coincides with the existence of a geographical barrier in Colombia named the Sierra Nevada de Santa Marta. This important mountainous mass is a remote derivation of the Andes Mountain range, which gradually plunges from its 5775 m highest peak into the sea. The Sierra Nevada might constitute a barrier to shorebirds and hence *Artemia* cyst dispersal. The dendrogram also illustrates the relationship of the five Colombian, the Puerto Araya (Venezuela), Bonaire and Curaçao populations to the other populations included in the first experiment. The Puerto Rican and San Francisco Bay populations were demonstrated to be further apart than the five Colombian, the Venezuelan and the other Caribbean (Bonaire and Curaçao) populations. Yet all these populations, considered to be *A. franciscana*, were very distinct from *A. persimilis*.

Further biogeographical research on *Artemia franciscana* should include more American populations from as far as Canada (Chaplin Lake) up to Chile and more genetic markers, in an attempt to solve the puzzle on the phylogeography of this species on the American continent. This study should be assisted by parallel studies on shorebird routes along existing aquatic hypersaline environments at different latitudes of the continent.

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