

International study on *Artemia*. LXII. Genomic relationships between *Artemia franciscana* and *A. persimilis*, inferred from chromocentre numbers

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Chromocentres, i.e. heavily stainable heterochromatic areas with highly repetitive DNA (a 130-bp repeat in the order of 6×10^5 copies per haploid genome) observed in the resting nucleus, are a reliable taxonomic trait and a good marker for speciation in *Artemia*. This chromosome marker was evaluated in populations of two New World sibling species: *A. franciscana*, from North, Central and South America, and *A. persimilis* from Argentina. *Artemia persimilis* showed the characteristically low average chromocentre numbers (< 3) of the ancestral Mediterranean species, whilst higher numbers (> 5) were seen in *A. franciscana*. The increase in chromocentre numbers from *A. persimilis* to *A. franciscana* occurred in a rather steady, continuous geographical pattern with an overlapping zone between both species. A north–south steady latitudinal decline of chromocentres was observed towards the equator in *A. franciscana* populations from the northern hemisphere, whilst the trait increased from this point towards southern latitudes with a population in Chilean Patagonia (below 52°S) exhibiting the highest chromocentre frequency. Since chromocentres are positively correlated with repetitive DNA content, differentiation between the species through the amplification of heterochromatin or an increased DNA content in *A. franciscana* (although this species has a lower diploid chromosome number), might nevertheless reflect chromosomal reorganizations between the species. Chromocentres, together with other traits, reveal a probable hybrid zone between the two species in Chilean Patagonia.

Keywords: *Artemia*, chromocentre, genomic relationship, heterochromatin.

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) represents a highly favourable system for evolutionary studies, as key elements driving speciation in other organisms can be observed in *Artemia*; for example, ecological isolation, adaptive switches in reproductive mode dependent on environmental conditions (oviparity vs. ovoviviparity), highly subdivided allopatric populations resulting from the development of local adaptations and large genetic distances between populations. (Abreu-Grobois & Beardmore, 1982; Abreu-Grobois, 1987; Browne & Bowen, 1991; Beardmore & Abreu-Grobois, 1983). Additionally, the easy transportation of cysts and the cultivation of populations under laboratory conditions have permitted broad interpopulation

comparisons. Nevertheless, little is as yet known of the genomic changes underlying speciation in *Artemia*, particularly in the New World species.

The genus *Artemia* (Crustacea, Anostraca), distributed in hypersaline environments in all continents except the Antarctic (Triantaphyllidis *et al.*, 1998), is a complex of eight bisexual species and many parthenogenetic types (Browne & Bowen, 1991). Although in the Old World both bisexual and parthenogenetic populations exist, only two bisexual species have been described for the New World, namely *Artemia persimilis* (Piccinelli and Prosdocimi) and *A. franciscana* (Kellogg). The latter is widely distributed in North, Central and South America and represents the most intensively studied species, whereas *A. persimilis* is often referred to as a narrowly distributed species, restricted to Argentina (Abreu-Grobois & Beardmore, 1982; Abreu-Grobois, 1987; Gajardo & Beardmore, 1993; Cohen *et al.*, 1999).

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Nevertheless, this view has been challenged recently as the species has been found in Chilean Patagonia (Gajardo *et al.*, 1998; Gajardo *et al.*, 1999; De los Rios & Zuñiga, 2000).

Artemia persimilis is thought to be closely related to the original group of species which evolved in the Mediterranean area, from which *A. franciscana* is also derived (Abreu-Grobois, 1987; Badaracco *et al.*, 1987; Browne & Bowen, 1991). Following a colonization event by a small founder population, *A. persimilis* would have evolved in the New World in geographical isolation from *A. franciscana* (Abreu-Grobois, 1987). The two species differ by one pair of chromosomes ($2n = 44$ and 42 for *A. persimilis* and *A. franciscana*, respectively), by a higher number of chromocentres with highly repetitive DNA in *A. franciscana* and by a genetic distance value (Nei, 1978) ($D > 0.5$) that is higher than the mean described for conspecific comparisons ($D = 0.109$) (Abreu-Grobois, 1987; Gajardo *et al.*, 1999). Despite these genetic differences, the two species are described as being morphologically very similar (Browne & Bowen, 1991), though this is not always the case (Gajardo *et al.*, 1998).

Chromocentres are heavily stained heterochromatic areas in interphase nuclei containing a highly repetitive DNA family of the type *Alu I* (also named satellite I DNA), i.e. a 113-bp sequence repeated in the order of 6×10^5 copies per haploid genome of *A. franciscana* (Barigozzi *et al.*, 1987). This genomic trait varies between and within species, and is correlated to some extent with the genetic differentiation measured by Nei's distances using allozyme data (Abreu-Grobois & Beardmore, 1982; Colihueque & Gajardo, 1996), as well as with the amount of repetitive DNA and chromocentre numbers. For example, *A. franciscana* shows the highest concentration of repetitive DNA and also shows the highest chromocentre frequency (Badaracco *et al.*, 1987).

This work compares chromocentre numbers in samples of *A. franciscana* from North, Central and South America, and samples of *A. persimilis* from Argentina. A comprehensive population survey over a considerable latitudinal range is expected to shed light on the relevance of this chromosome marker to the understanding of intraspecific genetic differentiation and of divergence between sibling *Artemia* species.

Materials and methods

The *Artemia* samples used for the study are from various locations in North, Central and South America (Table 1). Most were obtained from the cyst bank of the *Artemia* Reference Centre, others were kindly provided as cysts by individuals, as acknowledged in Table 1, while the Chilean populations are from laboratory cultures founded by live wild animals. Cytogenetic

data for the Chilean populations (using a non-fluorescent dye, as in this paper) and a detailed protocol for the squash method, have been published elsewhere (Colihueque & Gajardo, 1996). Briefly, the methodology uses 12–24 h nauplii (25°C) obtained either from newly hatched cysts or from cultures maintained in the laboratory. Initial colchicine treatment in sea water for 45 min (0.1%), was followed by thermal shock (42°C), hypotonic treatment in warm distilled water (38°C) for 90 min and fixation in acetic acid (50%) for 15 min. After squashing, nauplii were subjected to a Hoesch (pH 5.5) fluorescent stain for 10 min under darkness, then washed with McIlvain buffer (pH 5.5) and dried at room temperature. Plates were observed under a fluorescent microscope with a UV filter. Photographs were taken using a Tmax Kodak film. The chromocentre numbers were determined by checking over 200 resting nuclei per sample ($n = 100$ individuals). The chromocentre numbers of F_1 *A. franciscana*/*A. persimilis* laboratory hybrids, produced by the procedure described in Gajardo *et al.* (2001), were also determined.

Basic statistics, as well as the degree of clustering of the populations, were computed using the STATISTICA package (Statsoft, version 1998). The cluster analysis is based on Euclidean distances, i.e. the actual geometric distances or dissimilarities between chromocentre numbers in a multidimensional space.

Results

Figure 1 depicts the mean chromocentre numbers in samples of *A. persimilis* ($2n = 44$) and *A. franciscana* ($2n = 42$, unless otherwise indicated). Mean chromocentre number ranged widely from 0 to a maximum of 17.7, and in many comparisons the range of the variation overlaps considerably between populations. On the whole, variation among samples is, however, greater than within samples (ANOVA, $F_{(19, 580)} 191$, $P < 0.001$) indicating heterogeneity among populations. Nonetheless, the previously assumed ascription of populations to either *A. persimilis* or *A. franciscana* is confirmed by a Tukey multiple range analysis. Both species form relatively homogeneous groups at opposite ends of Fig. 1, *A. persimilis* showing very low (< 3) average chromocentre numbers whilst higher numbers (> 5) are characteristically seen in *A. franciscana*. In between, an array of *A. franciscana* populations (some of which fall significantly outside the previously established lower range for the species), account for a sort of steady transition from lower to higher chromocentre numbers. Interestingly, the laboratory-produced hybrid offspring between *A. franciscana* and *A. persimilis*, SFB/BAI (preliminary results) exhibits chromocentre numbers (Fig. 1) intermediate between those of the parental species. Moreover,

Country	Location	Abbreviation or Batch no.†
1 USA, CA	San Francisco Bay	1258
2 Bahamas	Long Island	271
3 Neth. Antilles	Curacao	502
4 Venezuela	Tucacas	218
5 Ecuador	Pacoa	472
	San Pedro (salt operation)	SPE
	Unknown	ECU
6 Peru	Piura	381
	Unknown	PER
	Chilca	CHI
	Casma	CAS
7 Brazil	Macau	202
	Cabo Frio	1096
	Río Grande do Norte	322
8 Chile	Pichilemu	PCH
	Salar de Atacama	SAT
	Los Vilos	LVI
	Convento	CON
	Llamara	LLA
	Torres del Paine	TPA
9 Argentina	Hidalgo	HID
	Colorada chica	1256
	Buenos Aires province	1430
	Buenos Aires	1321
	Buenos Aires	1393
	Río Negro	1150

†ARC classification; populations from Laguna Hidalgo (HID), Argentina, and Casma (CAS), Peru, were kindly provided by Prof. F. Amat and Mr M. Luján, respectively.

populations from the northern hemisphere and northern South America (Ecuador, Venezuela, Peru) show a (north–south) cline of chromocentre number towards the equator (see inset in Fig. 1), and the chromocentres are less fluorescent than those typically seen in *A. franciscana*. This pattern is then reversed towards southern latitudes, and notably, the highest chromocentre frequency is observed in the Chilean population from Torres del Paine (located below 52°S).

The clustering of populations resulting from similarities and/or dissimilarities between chromocentre numbers, shown in Fig. 2, confirms the differences between the two species but also intraspecific heterogeneity. As expected, most populations group as *A. franciscana*, though in different clusters. Three populations, two from northern Chile (SAT, PCH) and one from Ecuador (SPE, salt operation) fall within the *A. persimilis* group.

Discussion

Despite the fact that studies on *Artemia* speciation have considered a wide range of traits, the phylogeny of the genus is far from being established (Badaracco *et al.*,

Table 1 *Artemia* populations used in this study. Populations 1–4 are from the north, and populations 5–9 are from the south of the equator

1995), and in particular very little is known with regard to the relationship between closely related species. Studies have focused on morphology (Amat, 1980; Gajardo *et al.*, 1998), cytogenetics (Barigozzi, 1974, 1980; Barigozzi *et al.*, 1984; Abatzopoulos *et al.*, 1987; Colihueque & Gajardo, 1996); protein polymorphism (Abreu-Grobois & Beardmore, 1982; Gajardo & Beardmore, 1989, 1993; Pilla & Beardmore, 1994; Gajardo *et al.*, 1995, 1999), mitochondrial (Perez *et al.*, 1994) and nuclear DNA traits (Badaracco *et al.*, 1987, 1995; Sun *et al.*, 1999).

This population-orientated study of two New World sibling species has important implications for *Artemia* evolutionary biology. Firstly, it confirms the genetic differentiation between *A. persimilis* and *A. franciscana* (Fig. 2), validating chromocentre numbers as a reliable indicator of speciation. Secondly, it supports the current distribution hypothesis stating that *A. persimilis* is a geographically restricted species (only found in Argentina). The very low chromocentre numbers characteristic of this species are shared by Old World species such as *A. salina* (Barigozzi, 1980), hence confirming the primitive status of the species, whereas *A. franciscana*

even directly adaptive to certain types of habitats and ecological niches (White, 1978).

The north–south cline in chromocentre number seen in *A. franciscana* (inset, Fig. 1) and its actual wider distribution, as compared to other *Artemia* species, might be an indication of this adaptive relevance. In addition to the chromosomal reorganization brought about by the amplification of certain repetitive sequences in *Artemia*, differences in overall genome size are also likely to be a target of selection (Hartl, 2000).

The seemingly puzzling situation with the Chilean population from Torres del Paine is not, however, inexplicable, as the two sets of data do not necessarily provide congruent evolutionary information, since they are likely to reflect different characteristics of genome organization. Individuals of mixed ancestry (sharing *A. persimilis* and *A. franciscana* characteristics in a single genome), or hybrid types, are likely to be found in novel habitats, unlike the parental ones. Indeed, the ‘Hybrid superiority’ model put forward by Arnold (1997), which could be applied to this *Artemia* population, belongs to the environment-dependent or selection-gradient concept, and predicts that hybrid zones may often be found in transitional regions. The area of Torres del Paine is ecologically transitional in many respects. It is located below latitude 58°S in Chilean Patagonia in a semiarid and humid area exhibiting high environmental variation. These are unusual conditions compared to those described for *Artemia* in general and for *A. franciscana* in particular (see Gajardo *et al.*, 1992, 1999). Although quite geographically separated from other *Artemia* sites in Chile, the chances for gene flow across the Andes (the natural barrier between Chile and Argentina) are greater at this latitude, where the Andes are lower. Under such circumstances it would not be surprising to find a hybrid *Artemia* population exhibiting characteristics of both parental species. If *A. persimilis* is expanding its range of distribution, introgression might be possible, particularly considering the current rate of either intentional or natural dissemination of cysts. The ongoing evaluation of chromocentre frequency in laboratory-produced back-crosses will enable us to assess the likelihood of hybridization in nature.

Acknowledgements

This work is paper LXII of the International Interdisciplinary Study on *Artemia* populations co-ordinated by the laboratory of Aquaculture & Artemia Reference Centre, University of Ghent, Belgium. We gratefully acknowledge the technical help of Margarita Parraguéz. Financial support was provided by the Ministry of the Flemish Community, Science Division (Grant No. BIL98/03), and Fondecyt (1940812), Chile.

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