

## Reproduction in the brine shrimp *Artemia*: evolutionary relevance of laboratory cross-fertility tests

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(Accepted 1 December 1999)

### Abstract

The brine shrimp *Artemia*, a typical inhabitant of hypersaline environments and characterized by a highly subdivided population structure, was used as a model to evaluate, under standardized laboratory conditions (at 65 ppt), primary reproductive traits (offspring quality and quantity) along with levels of reproductive isolation and degrees of divergence among populations. Intrapopulation experimental crosses and cross-fertility tests were evaluated in five populations (mostly *A. franciscana*) from coastal and inland environments in Chile, and in reference samples of *A. franciscana* (San Francisco Bay, U.S.A.) and *A. persimilis* (Buenos Aires, Argentina), which are the species likely to be found in Chile. The populations compared displayed significant variability in fecundity (total offspring, brood size) as well as in the ratio encystment/oviviparity. Hybrid offspring, produced abundantly in cross-fertility tests with reference populations, showed a pronounced switch to the encystment mode, particularly in crosses with *A. persimilis*. Exposure to a broad range of ecological conditions seems to have optimized a generalist reproductive strategy in the *Artemia* populations studied that combines variation in both the quantity and quality of zygotes. Laboratory cross-fertility tests evaluated prime reproductive characteristics in individual crosses with fair repeatability, as well as testing barriers to laboratory reproductive isolation. The lack of efficient mechanisms for reproductive isolation in the allopatric *Artemia* populations studied follows a trend often seen in other anostracods. Formerly allopatric populations have not achieved sympatry later as required by the allopatric speciation paradigm, and this is a probable explanation for production of the laboratory hybrids.

**Key words:** *Artemia*, life history, reproductive output, cross-fertility tests, interpopulational differentiation

### INTRODUCTION

Reproductive investment and reproductive isolation are topics of major interest in evolutionary ecology. On the one hand, natural selection compares heritable variants for their effect on reproduction, and so differences between populations from different localities are interpreted as the result of adaptation to different environmental conditions. Individuals with reproductive patterns most suited to maximize fitness in their environment will be favoured by natural selection (Dingle & Hegman, 1982; Loeschke, 1987; De Jong, 1988; Pástor, 1988; Stearns, 1993; Rose & Lauder, 1996). On the other hand, reproductive isolation is a key factor in the definition of species according to the biological species concept (BSC; Mayr, 1969). Hence the process of specia-

tion is considered equivalent to the development of barriers to crossing and, as a corollary of the allopatric mode, geographical or physical barriers between conspecific populations must exist for reproductive isolation to evolve as a by-product of the genetic differences accumulated over time. Hybridization should then be of little evolutionary significance as hybrid offspring are less fit than their parents in a common environment because of a genetic imbalance of divergent genomes, though this view is being challenged (Arnold, 1997).

In this study, the brine shrimp *Artemia* was used as a model organism in evaluating, under laboratory conditions, primary reproductive traits (offspring quantity and quality) of *Artemia* populations of different origins but belonging to the two species likely to be found in Chile. Cross-fertility tests were also performed to evaluate the reproductive output and degrees of divergence among populations. Particular attention was paid to the ability of females to produce dormant

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Table 1. *Artemia* populations used in this study

Country	Locality	Abbreviation	Geographical coordinates	Type of habitat
Chile	Llamará	LLA	21°18'S	Inland
	Yape	YAP	20°40'S	Coastal
	Salar de Atacama	SAT	23°10'S	Inland
	Los Vilos	LVI	31°58'S	Coastal
	Pichilemu	PCH	34°25'S	Coastal, saltwork
Argentina	Buenos Aires	BAI	34°30'S	Inland
U.S.A., CA	San Francisco Bay	SFB	37°28'N	Coastal

(diapause) cysts instead of free-swimming nauplii, which is considered a significant survival strategy under stressful environmental conditions (e.g. freezing temperatures in the winter, drying of a temporary pond or a combination of these with high salinities).

*Artemia* is an anostracan branchiopod with a striking ability to live and reproduce in hypersaline environments. *Artemia* tends to allocate maximum energy to gamete and offspring production (Grosch, 1980). In particular, the quality or type of zygote is significant for the survival of brine shrimp populations since two reproductive modes (encystment and ovoviviparity) exist, and all *Artemia* strains combine both types with the ratio varying widely among them (Browne, 1980; Gajardo & Beardmore, 1989). Dependence on oviparity (cyst production) is commonly seen in populations with a seasonal cycle of either temperature or salinity, though factors such as brood number, hypoxia, photoperiod and availability and type of food can be also of importance. In opposition to this view, Gajardo & Beardmore (1989) suggested that encystment is under genetic control and is associated, at least in part, with the level of heterozygosity (determined electrophoretically) in the mother. This would agree with the observation that encystment seems to be integrated as co-adapted complexes of genes and so affected by hybridization (Browne & Bowen, 1991).

The genus *Artemia* is a complex of bisexual and parthenogenetic species, and probably superspecies, defined by the criterion of reproductive isolation (Abreu-Grobois, 1987; Browne & Bowen, 1991). Six bisexual species are currently known and all *Artemia* populations from the New World, with the exception of *A. monica*, and the geographically limited *A. persimilis*, belong to the *A. franciscana* superspecies (Bowen, Buoncristiani & Carl, 1988). Most Chilean *Artemia* populations are *A. franciscana* (Gajardo & Beardmore, 1993; Gajardo, Da Conceicao *et al.*, 1995; Triantaphyllidis, Abatzopoulos & Sorgeloos, 1998), though the recent finding of *A. persimilis* in southern Chile (Gajardo, Colihueque *et al.*, 1998; Gajardo, Mercado *et al.*, 1999; De los Rios & Zuñiga, 2000) shows this species, thought to be found in Argentina only, could be expanding its range of distribution.

*Artemia franciscana*, the dominant and most studied New World species (Vanhaecke, Tackaert & Sorgeloos, 1987; Triantaphyllidis *et al.*, 1998), is distributed in a series of scattered coastal and inland environments that are subject to great variability (i.e. cycles of extinction

and recolonization). The disjunct nature of these hypersaline ponds and lakes promote the genetic differentiation of *Artemia* populations by restricting gene flow, and by offering many different selective regimes. Thus populations may often represent at least an early stage of the geographic speciation process, and so the highly variable gene pool of *Artemia* species is heterogeneously distributed between strains, which is ultimately reflected in quantitative and qualitative differences in the reproductive pattern (Beardmore & Abreu-Grobois, 1993).

## MATERIALS AND METHODS

The *Artemia* populations used are indicated in Table 1. Chilean populations correspond to laboratory-reared brine shrimps descended from wild animals collected in 3 coastal ponds (YAP, LVI, PCH) and 2 inland lakes (LLA, SAT), and kept in aquaria containing 20 l of artificial sea water (at 35 ppt) for 2–8 months (1–3 generations). The salinity of 35 ppt, on which *Artemia* cultures were reared before the breeding tests, was selected for convenience recognizing that *Artemia* strains show high survival over a wide range of salinities (35–110 g/l) (Vanhaecke, Sidall & Sorgeloos, 1984).

Coastal ponds are normally ephemeral with Los Vilos, in contrast to the others, experiencing the effect of rain, which lowers salinity quite significantly. The inland populations are located in the Atacama desert, reputedly one of the driest in the world, exhibiting extreme ecological characteristics and often subject to significant fluctuation in ionic composition, salinity and temperature resulting from the complex hydrological characteristics of the basins (Chong, 1988; Gajardo, Colihueque *et al.*, 1998).

Samples from Buenos Aires (BAI: *A. persimilis*), batch 534 and 0510/74, and San Francisco Bay (SFB: *A. franciscana*), batch 880431, were kindly provided as cysts by the *Artemia* Reference Center, Ghent, and were hatched following standard protocols (Sorgeloos *et al.*, 1986).

### Intrapopulation and cross-fertility tests

The studies of intrapopulation reproductive performance as well as interpopulation crosses, or cross-fertility tests, were carried out at 65 ppt, which is the salinity animals normally encounter in the field. To set

Table 2. Mean reproductive performance ( $\bar{x} \pm \text{SD}$ ) in six characters in seven *Artemia* populations (number of pairs in parenthesis) over 45 days observation

Reproductive characteristic	Population						
	SFB	BAI	LLA	YAP	SAT	LVI	PCH
Total offspring/female	394 $\pm$ 135 (7) <sup>a,b</sup>	505 $\pm$ 123 (8) <sup>b</sup>	163 $\pm$ 54 (8) <sup>a</sup>	435 $\pm$ 357 (11) <sup>a,b</sup>	405 $\pm$ 156 (9) <sup>a,b</sup>	541 $\pm$ 337 (8) <sup>b</sup>	255 $\pm$ 74 (4) <sup>a,b</sup>
Cyst/female	107 $\pm$ 138 <sup>a</sup>	265 $\pm$ 141 <sup>a</sup>	84 $\pm$ 60 <sup>a</sup>	52 $\pm$ 67 <sup>a</sup>	143 $\pm$ 155 <sup>a</sup>	245 $\pm$ 342 <sup>a</sup>	105 $\pm$ 115 <sup>a</sup>
Nauplii/female	287 $\pm$ 177 <sup>a,b</sup>	240 $\pm$ 151 <sup>a,b</sup>	79 $\pm$ 59 <sup>a</sup>	384 $\pm$ 346 <sup>b</sup>	263 $\pm$ 148 <sup>a,b</sup>	295 $\pm$ 262 <sup>a,b</sup>	150 $\pm$ 137 <sup>a,b</sup>
Per cent offspring encysted	27 $\pm$ 34 <sup>a</sup>	54 $\pm$ 26 <sup>a</sup>	51 $\pm$ 29 <sup>a</sup>	47 $\pm$ 30 <sup>a</sup>	31 $\pm$ 30 <sup>a</sup>	32 $\pm$ 37 <sup>a</sup>	47 $\pm$ 50 <sup>a</sup>
Broods/female	3.6 $\pm$ 0.8 <sup>a,b</sup>	6.9 $\pm$ 0.6 <sup>c</sup>	3.0 $\pm$ 0.8 <sup>a</sup>	3.5 $\pm$ 1.9 <sup>a,b</sup>	5.3 $\pm$ 1.0 <sup>b,c</sup>	4.0 $\pm$ 2.1 <sup>a,b</sup>	4.0 $\pm$ 1.2 <sup>a,b</sup>
Days between broods	3.2 $\pm$ 0.4 <sup>a</sup>	2.8 $\pm$ 0.5 <sup>a</sup>	3.4 $\pm$ 0.7 <sup>a</sup>	3.7 $\pm$ 1.9 <sup>a</sup>	3.3 $\pm$ 0.8 <sup>a</sup>	3.1 $\pm$ 1.5 <sup>a</sup>	3.5 $\pm$ 1.0

<sup>a,b,c</sup> Mean values with a different superscript are significantly different (Tukey, multiple range analysis) at the  $\alpha$  0.01 level. For identification of populations see Table 1.

up experimental crosses, freshly-hatched nauplii were cultured in 2-l aquaria and, before sexual maturity, randomly-taken pairs were transferred into separate vials containing 50 ml of artificial sea water prepared according to Dietrich & Kalle (1963, in Kinne, 1971) at the desired salinity. On alternate days and just before feeding (1000 cells of microalgae/vial per day), ovoviparous offspring were counted and transferred into separate bottles containing 200 ml of brine, where they were maintained until maturity. Oviparous offspring (cysts) were filtered and counted. Observation lasted 45 days, a time considered sufficient for most of the reproductive span to have elapsed (Gajardo & Beardmore, 1989).

All observations were conducted at constant temperature (25 °C) and the alga *Dunaliella tertiolecta* was used as a food. Overall reproductive differences among populations (including reference populations) from different origins were tested by single classification ANOVA for unequal sample sizes (Sokal & Rohlf, 1981), and significance among means for paired comparisons were tested by the Tukey multiple range analysis using the computer program StatGraph (Statistical Graphics System, version 7.0, Manigistics Inc.).

## RESULTS

### Intra-population variation

Table 2 shows reproductive performance, and significance for mean differences (Tukey test), for six reproductive traits in the *Artemia* populations studied (see also Figs 1 & 2). Large within-population variation is evident in all populations (e.g. none of the populations exhibits a superior trend in fecundity), though ANOVA showed overall significant differences among populations for particular traits such as total number of zygotes per female ( $F_{6,47} = 2.85$ ,  $P < 0.01$ ), and number of broods per female ( $F_{6,47} = 8.70$ ,  $P < 0.05$ ). The Chilean populations, however, exhibited both the smallest (163, LLA) and the largest (541, LVI) total offspring, whereas the percentage of encysted offspring

(see Table 2, Figs 1 & 2) varied widely among strains from a minimum of 27% (SFB) to a maximum of 54% (BAI). Llamara (LLA) and Buenos Aires (BAI) are among those populations switching, in a very significant way, their reproductive mode to cysts, whilst SFB, SAT and LVI tended to have low cyst production. The number of broods per female varied between 3.0 (LLA) and 6.9 (BAI), whilst the inter brood interval (days between broods) was about 2.8–3.7 days (BAI-YAP).

### Cross-fertility tests with reference samples

Reproductive performance and encystment ratios were evaluated in cross-fertility tests with reference samples of *A. franciscana* (SFB) and *A. persimilis* (BAI). Data in Table 3 correspond to reciprocal crosses pooled as most differences between reciprocals were not significant. All crosses were fertile, and fecundity (offspring per female) and offspring quality (cysts or nauplii) results are shown in Figs 1 & 2 (% encysted offspring). Overall differences between the Chilean samples and SFB were significant (i.e. populations cannot be treated as a group) for most traits examined, with the exception of total number of nauplii, whilst comparisons with BAI were significant for total number of nauplii ( $F_{5,95} = 16.0$ ,  $P < 0.001$ ), percentage of encysted brood ( $F_{5,95} = 4.9$ ,  $P < 0.05$ ), broods per female ( $F_{5,95} = 12.5$ ,  $P < 0.05$ ) and days between broods ( $F_{5,95} = 6.4$ ,  $P < 0.05$ ).

For total offspring per female, crosses with SFB varied from 261 (PCH) to 778 (YAP), while those with BAI ranged from 300 (SAT) to 517 (YAP). Although differences are not significant, cross-fertility tests with SFB produce slightly larger progenies than with BAI in almost all comparisons, Yape being in both cases the best performing population. When the Chilean samples were tested against SFB, total offspring per female tended to resemble each intra-strain control, which is less evident when these samples are tested against BAI.

The percentage of offspring encysted was clearly higher in crosses with BAI (range: 71–93%) than with SFB (38–76%), Yape again being the strain with higher performance, producing up to 93% of encysted offspring

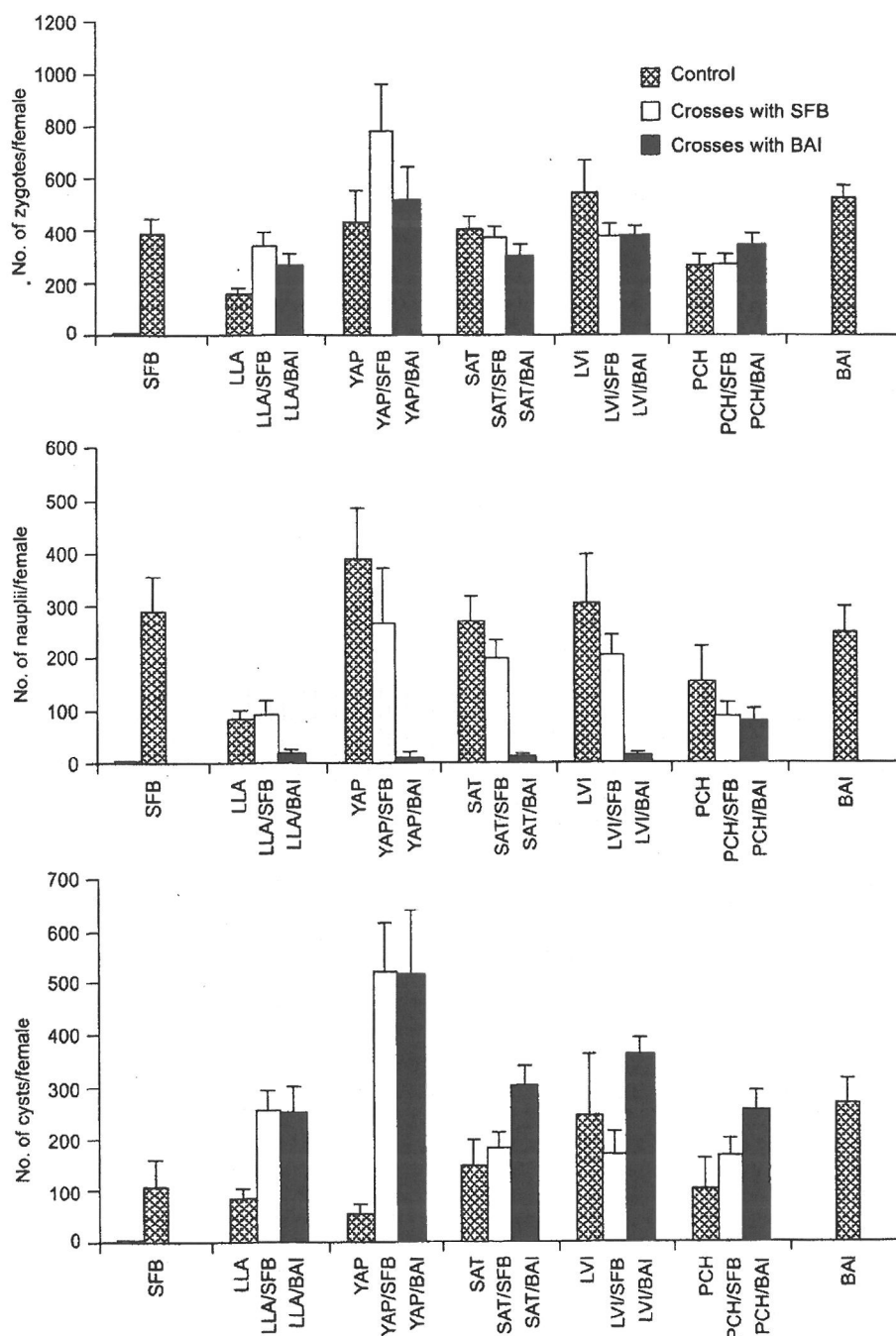


Fig. 1. Reproductive performance of the *Artemia* populations studied (45 days) under laboratory conditions (intra-population crosses = controls), and cross-fertility tests between each population and reference samples of both *A. franciscana* (SFB) and *A. persimilis* (BAI).

when tested against BAI. Broods per female and time between broods were not significant in all comparisons. Finally, although cysts obtained from experimental crosses were difficult to hatch in several hydration/dehydration cycles, those obtained from three original populations (YAP, SAT, LVI,) seem to hatch reasonably well as shown in Table 4.

## DISCUSSION

This study offered an opportunity to evaluate prime reproductive characteristics in individual crosses (and their offspring). In this way, a whole range of genetic types could be monitored under common environmental conditions, a situation difficult to reproduce in wild

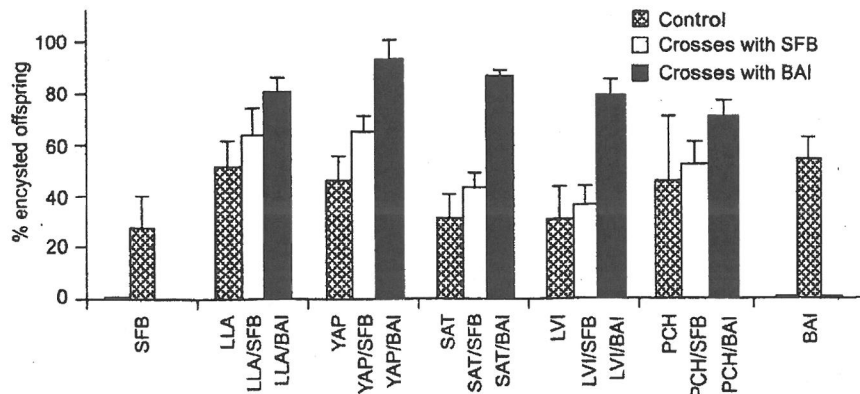


Fig. 2. Percentage of encysted offspring in control groups and cross-fertility tests with SFB and BAI.

populations. Reproductive performance is critical to understanding the evolutionary potential of *Artemia* populations, often exposed to new or different seasonal habitats. This study also sheds light on reproductive output in cross-fertility tests as an indicator of genetic divergence in *Artemia* and related genera.

#### Intraspecific comparisons

By comparing reproductive investment and quality of *Artemia* populations from different origins, this work probably reflects the adaptation pattern resulting as a compromise between natural populations and their local habitats, which are diverse in origin, water chemistry, salinity, temperature and stability (Abreu-Grobois, 1987). The comparative or routine method valued for studying adaptation (Reznick & Travis, 1996), has the advantage of simplicity but involves potential disadvantages. For example, differences observed under common experimental conditions could be the result of non-genetic effects such as maternal effects induced by the environment experienced by the female parent. Similarly, the routine environment may not be one in which genetically based differences are manifested, e.g. different genotypes can produce similar phenotypes (phenotypic plasticity), or the artificial environment can induce genetically based phenotypic variation that would not be expressed in any of the natural environments. Finally, laboratory populations are subjected to changes in conditions and selective forces, and hence rapid adaptation to laboratory conditions is likely to occur. Undoubtedly, every possible approach has advantages or disadvantages (see Rose, Nusbaum & Chippindale, 1996 for a detailed account of the pros and cons of evolutionary studies under experimental conditions), but in our study the choice of the routine method benefits from knowledge on the genetic differences gathered for the populations under scrutiny which, in addition, are prone to experience fast genetic changes (Gajardo, Da Conceicao *et al.*, 1995). Indeed, since the introduction in 1977 of *A. franciscana* in South America for commercial purposes (e.g. Macau, Brazil)

(Lenz & Browne, 1991) rapid evolutionary changes seem to have occurred, paralleling some phenotypic differences. To quote two examples, populations at Macau (a coastal saltern) and Salar de Atacama (an inland natural lake) in Chile, where *Artemia* should have been dispersed long before human introduction in other localities of South America, have accumulated novel alleles (as compared electrophoretically against San Francisco Bay, the probable founder population). This is apparently a response to the high degree of environmental differences in their biotopes.

Chilean *Artemia* samples showed important variation in two prime traits: fecundity (total offspring, brood size) and the ratio encystment/ovoviviparity. As explained above, at least a fraction of the variability is heritable and so populations studied reflect a general phenomenon of *Artemia* species, and of *A. franciscana* in particular, which is a trend towards the maintenance of high levels of genetic variability in their gene pools, and great inter-population genetic heterogeneity (Abreu-Grobois, 1987; Gajardo, Da Conceicao *et al.*, 1995). Based on this pronounced tendency for the development of local adaptations and large genetic distances between populations, 'adaptive divergence' is thought to define the process of speciation in *Artemia* best (Abreu-Grobois, 1987).

Besides fecundity, variation in offspring quality (cysts or nauplii) is an important reproductive strategy of *Artemia*, ensuring survival in populations exposed to unstable or stressful conditions. Dependence on oviparity is commonly seen in populations experiencing a seasonal cycle of either temperature or salinity, or living in ephemeral (extreme) habitats (Lenz & Browne, 1991). In seasonal *Artemia* populations experiencing cycles of extinction and recolonization, reseedling occurs by hatching of dormant cysts when water conditions, usually a reduced salinity, become suitable. An evolutionary consequence of shunting offspring into a dormant encysted state is to ensure the survival of the species once favourable conditions return, and this might occur in geographical and temporal conditions different from those experienced by the parents. Cyst production also plays a significant role in the geographical dispersion of any species as cysts are known to be



**Table 3.** Results of reproductive performance ( $\bar{x} \pm \text{SD}$ ) in *Artemia* cross-fertility tests (number of pairs in parenthesis) over 45 days observation

Reproductive characteristic	Population				
	LLA	YAP	SAT	LVI	PCH
Total offspring/female					
SFB	313 $\pm$ 235 (20) <sup>a,b</sup>	778 $\pm$ 309 (20) <sup>c</sup>	374 $\pm$ 158 (19) <sup>a,b</sup>	370 $\pm$ 182 (19) <sup>a,b</sup>	261 $\pm$ 133 (18) <sup>a</sup>
BAI	237 $\pm$ 214 (20) <sup>a</sup>	517 $\pm$ 413 (22) <sup>b</sup>	300 $\pm$ 153 (15) <sup>a,b</sup>	369 $\pm$ 144 (17) <sup>a,b</sup>	328 $\pm$ 187 (20) <sup>a,b</sup>
Cyst/female					
SFB	254 $\pm$ 161 <sup>a</sup>	516 $\pm$ 331 <sup>b</sup>	177 $\pm$ 139 <sup>a</sup>	174 $\pm$ 185 <sup>a</sup>	170 $\pm$ 142 <sup>a</sup>
BAI	249 $\pm$ 220 <sup>a</sup>	511 $\pm$ 422 <sup>b</sup>	293 $\pm$ 154 <sup>a,b</sup>	358 $\pm$ 149 <sup>a,b</sup>	253 $\pm$ 172 <sup>a</sup>
Nauplii/female					
SFB	89 $\pm$ 110 <sup>a,b</sup>	262 $\pm$ 304 <sup>c</sup>	193 $\pm$ 139 <sup>b,c</sup>	196 $\pm$ 159 <sup>b,c</sup>	86 $\pm$ 101 <sup>a</sup>
BAI	15 $\pm$ 34 <sup>a</sup>	8 $\pm$ 25 <sup>a</sup>	8 $\pm$ 20 <sup>a</sup>	12 $\pm$ 37 <sup>a</sup>	74 $\pm$ 112 <sup>a,b</sup>
Per cent offspring encysted					
SFB	64 $\pm$ 16 <sup>b,c,d</sup>	76 $\pm$ 20 <sup>b,c,d</sup>	43 $\pm$ 23 <sup>a,b</sup>	38 $\pm$ 35 <sup>a</sup>	53 $\pm$ 35 <sup>a,b,c</sup>
BAI	80 $\pm$ 26 <sup>c,d</sup>	93 $\pm$ 24 <sup>d</sup>	86 $\pm$ 9 <sup>d</sup>	85 $\pm$ 12 <sup>d</sup>	71 $\pm$ 28 <sup>c,d</sup>
Broods/female					
SFB	3.7 $\pm$ 2.1 <sup>a</sup>	5.6 $\pm$ 1.1 <sup>b</sup>	—	3.5 $\pm$ 1.2 <sup>a</sup>	2.7 $\pm$ 1.6 <sup>a</sup>
BAI	3.1 $\pm$ 1.2 <sup>a</sup>	3.7 $\pm$ 1.4 <sup>a</sup>	3.4 $\pm$ 1.2 <sup>a</sup>	3.7 $\pm$ 0.8 <sup>a</sup>	—
Days between broods					
SFB	3.1 $\pm$ 1.0 <sup>a</sup>	—	3.7 $\pm$ 0.8 <sup>a,b</sup>	3.0 $\pm$ 0.0 <sup>a</sup>	3.4 $\pm$ 1.6 <sup>a,b</sup>
BAI	4.0 $\pm$ 1.6 <sup>a,b</sup>	5.8 $\pm$ 2.7 <sup>c</sup>	3.5 $\pm$ 1.5 <sup>a,b</sup>	—	3.1 $\pm$ 1.9 <sup>a</sup>

<sup>a,b,c,d</sup> Mean values with different superscript are significantly different (Tukey, multiple range analysis) at the  $\alpha = 0.01$  level.

**Table 4.** Hatching characteristics of three Chilean *Artemia* populations

Population	Hatching percentage	Hatching efficiency <sup>a</sup>	Hatching rate <sup>b</sup>			
			$T_0$	$T_{10}$	$T_{90}$	$T_s$
YAP	65.7	131.00	14.5	14.5	25.5	11
SAT	73.4	146.800	13.5	16.5	25.5	9
LVI	71.7	201.000	12.5	15.5	25.5	10

<sup>a</sup> Number of nauplii/g cysts.

<sup>b</sup> Values refer to time (h) from incubation until appearance of the first nauplii ( $T_0$ ), or the moment by which 10% ( $T_{10}$ ) and 90% ( $T_{90}$ ) of the hatching efficiency has been reached.  $T_s = T_{90} - T_{10}$ .

transported by wind or waterbirds (Vanhaecke, Tackaert *et al.*, 1987), and so this strategy has also a significant effect on population characteristics by counteracting geographical barriers that oppose gene flow. This probably explains why there was no clear difference in reproductive strategy when coastal and inland populations were compared in this and other studies.

The plasticity of the *Artemia* genetic pool plays a relevant role in the maintenance of the diapause strategy over time. For example, females with higher heterozygosity levels exhibit an increased capacity to switch resources into the oviparous mode of reproduction. However, a female producing a given number of cysts has to allocate significantly more energy to this than to the production of the same number of nauplii. Thus the capacity to produce a larger number of cysts indicates unequivocally an ability to provide the higher energy content required, and females that are more heterozygous are evidently better able to do this (Gajardo & Beardmore, 1989).

As well as seasonality, other factors are likely to affect life-history characteristics, such as the continuity, length and predictability of the growing season, and the seasonal variation of food. No such information is

available for the Chilean populations, and may explain why no clear differences in reproductive traits occurred between local populations and why an association between reproductive strategy and habitat differences did not emerge from our analysis.

#### Cross-fertility tests

Cross-fertility tests have been the basis for the diagnosis of *Artemia* species (Browne & Bowen, 1991). Lack of reproductive output (lack of offspring or very few abortive nauplii) has been demonstrated between many populations of various sibling species in the genus, in particular between the New World *A. franciscana* and *A. persimilis* (Browne & Bowen, 1991). However, other standardized laboratory studies within the *A. franciscana* superspecies have shown that many morphologically or genetically divergent allopatric populations exhibit partial or no reproduction (Pilla & Beardmore, 1994). Also fertile  $F_1$  and viable  $F_2$  adults have been obtained in reciprocal crosses between *A. monica* and *A. franciscana* (Bowen, Fogarino *et al.*, 1985). This seems to be a common phenomenon in

other members of the order where morphologically distinct species that have been separated for a long time remain sexually compatible (Wiman, 1979). In *Branchinecta*, Maeda-Martinez, Obregon-Barboza & Dumont (1992) suggested, after obtaining viable interspecific hybrids and on the basis of the existing literature, that the absence of pre-mating mechanisms may be common in *Anostraca*. This might be because these populations have completed only the first stage of the allopatric speciation paradigm (e.g. geographic separation) but not the second stage (regaining sympatry), which is required for the development of pre-mating isolating mechanisms (Mayr, 1969; Coyne, 1992). In *Artemia*, the New World species, *A. franciscana* and *A. persimilis*, are recently diverged (many populations have undergone Holocene radiations according to Browne & Bowen, 1991) and so they would be in the process of developing barriers to gene exchange (Abreu-Grobois, 1987). The observation of incipient sexual selection in *Artemia* by Zapata, Gajardo & Beardmore (1990) supports this idea, though our results tend to confirm doubts raised about the appropriateness of cross-fertility tests for assessing genetic divergence or the evolutionary history of a group (Wiman, 1979; Browne & Bowen, 1991).

This laboratory-based work made it possible to evaluate variability and divergence of populations for complex traits such as encystment, which is known to be affected by hybridization in *Artemia* (Browne & Bowen, 1991). Such complex traits, with high additive genetic variance, are normally integrated as co-adapted complexes of genes. Break-up of these co-adapted complexes in crosses can explain why hybrid offspring show lower levels of fertility and/or viability. Thus, the outcome is maladaptive as individuals have a reduced reproductive output, particularly after  $F_1$ . In *Artemia* in particular, Browne and co-workers (see Browne & Bowen, 1991) were able to support this argument after demonstrating a decline in reproductive output of 20% and 80% ( $F_1$  and  $F_2$ , respectively) when independent laboratory lines originating from reciprocal crosses were mated.

In our work, encystment/ovoviviparity ratios increased in cross-fertility tests, particularly between the Chilean populations and the Argentinean population (*A. persimilis*), the more taxonomically distant were the species (Gajardo, Da Conceicao *et al.*, 1995; Gajardo, Colihueque *et al.*, 1998). The causal relationship between genetic reorganization, fitness and switch to encystment in the hybrids needs to be further addressed. It may be related, however, to the role of cysts in ensuring that species/population continuity can occur even after the death of their parents. Encystment in *A. franciscana* is associated, at least in part, with the level of heterozygosity determined electrophoretically in the mother (Gajardo & Beardmore, 1989). Hence, when cyst production is high in any population, the more heterozygous females may be more resistant to adversity than less heterozygous ones and more able to produce cysts for longer and with a wider range of genotypes. Novel genotypes could result in adaptive evolution

allowing colonization of novel habitats or the displacement of parental species by hybrid offspring that are more fit in a particular environment (Arnold, 1997).

Finally, the reproductive behaviour observed in the *Artemia* populations studied may be a response to the very broad range of ecological variables typically found in *Artemia* habitats. Under these circumstances a single reproductive phenotype (or reproductive mode) would be less favoured.

### Acknowledgements

This work is paper LXI of the International Interdisciplinary Study on *Artemia* populations co-ordinated by the Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, Belgium and has been funded in part by grants from FONDECYT (1940812), Chile, the International Foundation for Science (A/14404-2) and the Ministry of the Flemish Community (Grant 174B0199). Two reviewers made constructive comments on the original version of the manuscript.

### REFERENCES

- Abreu-Grobois, F. A. (1987). A review of the genetics of *Artemia*. In *Artemia research and its applications* 1: 61–99. Sorgeloos, P., Bengtson, D. A., Decleir, W. & Jaspers, E. (Eds). Wetteren, Belgium: Universa Press.
- Arnold, M. L. (1997). *Natural hybridization and evolution*. Oxford: Oxford University Press.
- Beardmore, J. A. & Abreu-Grobois, F. A. (1993). Taxonomy and evolution in the brine shrimp *Artemia*. In *Protein polymorphism: adaptive and taxonomic significance*: 153–164. Oxford, G. S. & Rollinson, D. (Eds). Systematics Association. London: Academic Press.
- Bowen, S. T., Buoncristiani, M. R. & Carl, J. R. (1988). *Artemia* habitats: ion concentrations tolerated by one superspecies. *Hydrobiologia* 158: 201–214.
- Bowen, S. T., Fogarino, E. A., Hitcher, K. N., Dana, G. L., Chow, H. S., Buoncristiani, M. R. & Carl, J. R. (1985). Ecological isolation in *Artemia*: population differences in tolerance of anion concentrations. *J. Crustacean Biol.* 5: 106–129.
- Browne, R. A. (1980). Reproductive pattern and mode in the brine shrimp. *Ecology* 61: 466–470.
- Browne, R. A. & Bowen, S. T. (1991). Taxonomy and population genetics of *Artemia*. In *Artemia biology*: 221–235. Browne, R. A., Sorgeloos, P. & Trotman, C. N. A. (Eds). Boca Raton, FL: CRC.
- Chong, A. (1988). The Cenozoic saline deposit of the Chilean Andes between 10°00' and 27°00' South latitude. In *Lecture notes in earth sciences* 17: 137–151. Balburg, H., Bretkrenz, Ch. & Giese, P. (Eds). Berlin: Springer-Verlag.
- Coyne, J. A. (1992). Genetics and speciation. *Nature (Lond.)* 355: 511–515.
- De Jong, G. (1988). *Populations genetics and evolution*. Berlin: Springer-Verlag.
- De los Rios, P. & Zuñiga, O. (2000). Biometric comparison of the frontal knob in American populations of *Artemia* (Anostraca, Artemiidae). *Rev. Chil. Hist. Nat.* 73: 31–38.
- Dingle, H. & Hegmann, J. (1982). *Evolution and genetics of life histories*. Berlin: Springer-Verlag.
- Gajardo, G. M. & Beardmore, J. A. (1989). Ability to switch reproductive mode in *Artemia* is related to maternal heterozygosity. *Mar. Ecol. Prog. Ser.* 55: 191–195.

- Gajardo, G. M. & Beardmore, J. A. (1993). Electrophoretic evidence suggests that the *Artemia* found in the Salar de Atacama, Chile, is *A. franciscana* Kellogg. *Hydrobiologia* 257: 65–71.
- Gajardo, G., Colihueque, N., Parraguez, M. & Sorgeloos, P. (1998). International study on *Artemia*. LVIII. Morphologic differentiation and reproductive isolation of *Artemia* populations from South America. *Int. J. Salt Lake Res.* 7(2): 133–151.
- Gajardo, G. M., Da Conceicao, M., Weber L. & Beardmore, J. A. (1995). Genetic variability and interpopulational differences in *Artemia* strains from South America. *Hydrobiologia* 302: 21–29.
- Gajardo, G., Mercado, C., Beardmore, J. A. & Sorgeloos, P. (1999). International study on *Artemia*. LX. allozyme data suggest that a new *Artemia* population in southern Chile (50° 29' S; 73° 45' W) is *A. persimilis*. *Hydrobiologia* 405: 1–7.
- Gajardo, G. M., Wilson, R. & Zúñiga, O. (1992). Report on the occurrence of *Artemia* in a saline deposit of the Chilean Andes. *Crustaceana* 62: 169–174.
- Grosch, D. S. (1980). Alterations to the reproductive performance of *Artemia* caused by antifouling paints, algicides, and aquatic herbicides. In *The brine shrimp Artemia* 1. *Morphology, genetics, radiobiology, toxicology*: 201–211. Persoone, G., Sorgeloos, P., Roels, O. A. & Jaspers, E. (Eds). Wetteren: Universa Press.
- Kinne, O. (1971). Salinity: 4. 3 Animals, 4. 31 Invertebrates. In *Marine ecology. A comprehensive, integrated treatise on life in oceans and coastal waters* I. *Environmental factors*. Part 2: 821–995. Kinne, O. (Ed.). New York: Wiley Interscience.
- Lenz P. H & Browne, R. A. (1991). Ecology of *Artemia*. In *Artemia biology*: 237–253. Browne, R. A., Sorgeloos, P. & Trotman, C. N. A. (Eds). Boca Raton, FL: CRC.
- Loeschcke, V. (1987). *Genetic constraints on adaptive evolution*. Berlin: Springer-Verlag.
- Maeda-Martinez, A. M., Obregon-Barboza, H. & Dumont, H. J. (1992). *Branchinecta belki* n. sp. (Branchiopoda: Anostraca), a new fairy shrimp from Mexico, hybridizing with *B. packardii* Pearse under laboratory conditions. *Hydrobiologia* 239: 151–162.
- Mayr, E. (1969). *Principles of systematic zoology*. New York: McGraw-Hill.
- Pàztor, E. (1988). Unexploited dimensions of optimization in life history theory. In *Population genetics and evolution*: 19–32. De Jong, G. (Ed.). Berlin: Springer-Verlag.
- Pilla, E. J. S. & Beardmore, J. A. (1994). Genetic and morphometric differentiation in Old World bisexual species of the brine shrimp *Artemia*. *Heredity* 73: 47–56.
- Reznick, D. & Travis, J. (1996). The empirical study of adaptation in natural populations. In *Adaptation*: 243–289. Rose, M. R. & Lauder, G. V. (Eds). New York: Academic Press.
- Rose, M. R. & Lauder, G. V. (1996). Post-spandrel adaptionism. In *Adaptation*: 1–8. Rose, M. R. & Lauder, G. V. (Eds). New York: Academic Press.
- Rose, M. R., Nusbaum, T. J. & Chippindale, A. K. (1996). Laboratory evolution: the experimental wonderland and the Cheshire cat syndrome. In *Adaptation*: 221–241. Rose, M. R. & Lauder, G. V. (Eds). New York: Academic Press.
- Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. New York: W. H. Freeman.
- Sorgeloos, P., Lavens, P., Leger, Ph., Tackaert, W. & Versichele, D. (1986). *Manual for the culture and use of brine shrimp Artemia in aquaculture*. Belgium: State University of Ghent.
- Stearns, S. C. (1993). *The evolution of life histories*. Oxford: Oxford University Press.
- Triantaphyllidis, G. V., Abatzopoulos, T. J. & Sorgeloos, P. (1998). Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). *J. Biogeogr.* 25: 213–226.
- Vanhaecke, P. W., Sidall, S. E. & Sorgeloos, P. (1984). International study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographic origin. *J. Exp. Mar. Biol. Ecol.* 80: 259–275.
- Vanhaecke, P., Tackaert, W. & Sorgeloos, P. (1987). The biogeography of *Artemia*: an updated review. In *Artemia research and its applications* 2: 129–155. Sorgeloos, P., Bengtson, D. A., Decler, W. & Jaspers, E. (Eds). Wetteren, Belgium: Universa Press.
- Wiman, F. H. (1979). Mating patterns and speciation in the fairy shrimp genus *Streptocephalus*. *Evolution* 33(1): 172–181.
- Zapata, C., Gajardo, G. & Beardmore, J. A. (1990). Multilocus heterozygosity and sexual selection in the brine shrimp *Artemia franciscana*. *Mar. Ecol. Prog. Ser.* 62: 211–217.



