

Coadaptation: lessons from the brine shrimp *Artemia*, “the aquatic *Drosophila*” (Crustacea; Anostraca)

Coadaptación: lecciones desde el camarón de salmuera *Artemia*,
“la *Drosophila* acuática” (Crustacea; Anostraca)

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ABSTRACT

During the fifties Brncic and the Dobzhansky's school, using the fruit fly *Drosophila* as a test organism, introduced the term integration of the genotype, or genetic coadaptation, which has had great impact on thinking in today's evolutionary biology. In this work we use the brine shrimp *Artemia* – in many respects a sort of aquatic *Drosophila* – as a model organism to evaluate the relationship between population structure, potential for divergence and the degree of morphological and/or genetic change. These aspects, tightly linked with the organization of the genotype, are important to understanding how recombination and adaptive release of genetic and phenotypic variation affect the speciation process in *Artemia*. Analysis of genetic (allozyme, diploid and chromocentre numbers), morphological (Mahalanobis distances) and reproductive data (cross-fertility tests) available for populations of the bisexual, endemic species of the Americas, *Artemia franciscana* and *A. persimilis*, indicate that: (i) *A. franciscana* and *A. persimilis* are morphologically distinct in correspondence with observed genetic differences ($D_{Nei} > 1$; $2n = 42$ and 44 ; 12.5 and 1.5 mean chromocentre numbers, respectively); (ii) populations from Chile and other South American localities (mainly *A. franciscana*) display high levels of genetic variability and a trend to develop large genetic distances between populations; (iii) the plasticity of *Artemia* gene pool is associated, at least in part, with ecological heterogeneity. Hence an adaptive divergence mode is thought to best define the speciation process in *Artemia*; (iv) the successful production of laboratory hybrids in the allopatric *Artemia* populations studied in the Americas, a feature seen in other anostracods, could be explained by the fact that formerly allopatric populations have not achieved later sympatry, as required by the allopatric speciation paradigm.

Key words: *Artemia*, coadaptation, genetic variability, interpopulation differentiation, aquatic *Drosophila*.

RESUMEN

En los años cincuenta Brncic y la escuela de Dobzhansky introdujeron el término integración del genotipo, o coadaptación genética, usando la mosca de la fruta *Drosophila* como organismo de experimentación, el cual ha tenido gran impacto en la biología evolutiva actual. En este trabajo utilizamos al camarón de salmuera *Artemia*, en muchos aspectos una suerte de *Drosophila* acuática, como organismo modelo para evaluar la relación entre estructura poblacional, potencial de divergencia y el grado de cambio morfológico y/o genético. Estos aspectos, estrechamente ligados a la organización del genotipo, son importantes para entender como la recombinación y la liberación adaptativa de variación genética y fenotípica afectan el proceso de especiación en *Artemia*. El análisis de datos genéticos (aloenzimas, número diploide y de cromocentros), morfológicos (distancia de Mahalanobis) y reproductivos (pruebas de fertilidad cruzada) disponibles para las poblaciones bisexuales, endémicas de las Americas, *Artemia franciscana* y *A. persimilis*, indican que: (i) *A. franciscana* y *A. persimilis* son morfológicamente distintas en correspondencia con las diferencias genéticas observadas ($D_{Nei} > 1$; $2n = 42$ y 44 ; $12,5$ y $1,5$ número promedio de cromocentros, respectivamente); (ii) poblaciones de Chile y otras localidades Sudamericanas (principalmente *A. franciscana*) exhiben altos niveles de variabilidad genética y una tendencia a desarrollar grandes distancias genéticas entre poblaciones; (iii) la plasticidad del acervo de genes en *Artemia* está asociado, al menos en parte, con la heterogeneidad ecológica. Por este motivo el modelo de divergencia adaptativa es el que mejor define el proceso de especiación en *Artemia*; (iv) la producción de híbridos de laboratorio entre las poblaciones alopatricas de América estudiadas, un rasgo observado también en otros anostrácodos, podría explicarse por el hecho de que poblaciones originalmente alopatricas no han logrado posterior simpatria, como requiere el paradigma de especiación alopatrico.

Palabras clave: *Artemia*, coadaptación, variabilidad genética, diferenciación interpoblacional, *Drosophila* acuática.

INTRODUCTION

During the fifties the Dobzhansky school, to whom Danko Brncic was a notable member, were responsible for introduction of the term integration of the genotype, or genetic coadaptation, and made significant contributions thereafter using *Drosophila* as a test organism (Dobzhansky 1950, 1955, Brncic 1954, Wallace 1953, Vetukhiv 1953, 1954, 1956, 1957, Wallace & Vetukhiv 1955, Vetukhiv & Beardmore 1959). It was shown that chromosomal polymorphisms play a major role in keeping the internal and relational balance of polygenes, e.g., blocks of genes adapted to produce superior fitness especially in sub-optimal conditions. Hence hybridization of geographical races would bring about a breakdown of the adapted gene complexes with a consequent loss of superior fitness of the inversion heterozygotes. The role of inversions would be to maintain balanced gene complexes (internal balance) by suppressing recombination. The more populations have diverged genetically (different in the context of polygenes maintained by the inversions) a greater negative effect on fitness should be seen due to recombination or re-shuffling of coadapted complexes.

The concept of genetic coadaptation, has had a significant impact on various aspects of today's evolutionary biology. By integrating experimental data of various kinds on the brine shrimp *Artemia* (a sort of "aquatic *Drosophila*" according to Abreu-Grobois & Beardmore 1982), this study focuses on the relationship between population structure, potential for divergence and the degree of morphological and/or genetic change. These aspects, tightly linked with the organization of the genotype, are important to understanding how recombination and adaptive release of genetic and phenotypic variation affect the speciation process in *Artemia*.

The "aquatic Drosophila": relevance of brine shrimp for evolutionary studies

The brine shrimp *Artemia* is an Anostracan branchiopod with a striking capacity to live and reproduce in hypersaline environments (0.4-3.4 M) that can differ greatly in ionic composition, e.g., chloride, sulphate and carbonate-rich waters (Browne & Bowen 1991, Lenz & Browne 1991). These often scattered and relatively isolated habitats found in all continents with the exception of the Antarctic (Triantaphyllidis et al. 1998) offer suitable conditions for maximal interpopulation differentiation and ecological isolation (Abreu-Grobois 1987, Bowen et al. 1988).

Artemia has been distributed by natural colonisation by waterbirds, wind or by deliberate or accidental spread by man due to its importance for aquaculture (Bengtson et al. 1991).

Complex patterns of speciation linked to a complex set of ecological characteristics of the *Artemia* habitats have led to the differentiation of at least five bisexual species, defined by the criterion of reproductive isolation (Abreu-Grobois 1987), and parthenogenetic species. Endemic to the Old World are the parthenogenetic types designated by Barigozzi (1974) as *A. parthenogenetica*, the bisexuals *A. salina*, Leach 1819, *A. urmiana*, Gunther 1890, *A. sinica*, Cai 1989, *Artemia* sp. from Kazakhstan (Pilla & Beardmore 1994), and *A. tibetiana* (Abatzopoulos et al. 1998). Endemic to the New World are *A. persimilis*, Piccinelli & Prosdocini 1968 and *A. franciscana* Kellogg 1906, the former traditionally thought to be restricted to a few sites in Argentina though this view has been challenged recently (Gajardo et al. 1999). *Artemia franciscana*, the dominant species in South America, is distributed in a series of scattered marine and inland environment occurring in areas of extreme aridity (Triantaphyllidis et al. 1998). Climatic and hydrobiological changes over time, the ecological characteristics of the environments as well as the occurrence of successive cycles of extinction/recolonization have determined that populations are ecologically isolated in natural and artificial water bodies differing in ionic composition of the water, salinity and temperature (Browne & Bowen 1991). Probably because of this, *A. franciscana* strains display considerable genetic heterogeneity and phenotypic variation including environmental tolerances, which ultimately is likely to lead to differences in life history patterns. Under favourable conditions, the brine shrimp female is typically able to reproduce by a larval stage (ovoviviparous reproduction), whereas under unfavourable conditions females typically produce a diapause gastrula (oviparous reproduction) which is highly resistant. The switch to the oviparous mode is therefore considered a significant survival strategy.

In short, *Artemia* is an example of a life form restricted to a limited range of ecological conditions though extremely successful within this range, achieving high population sizes and tolerating large environmental variation (Table 1) (Abreu-Grobois & Beardmore 1982, Abreu-Grobois 1987). *Artemia* is also one of the best known aquatic organisms and, not surprisingly, considered a paradigmatic crustacean that can help to fill gaps in knowledge in evolutionary and comparative biology (Marco et al. 1991).

TABLE 1

Potential for evolutionary studies of the brine shrimp *Artemia*, the "aquatic *Drosophila*"Potencial para estudios evolutivos de *Artemia*, la "*Drosophila* acuática"

- Differences in habitat and ecological isolation between populations
- Various stages of speciation representing a range in degrees of reproductive isolation
- Easy rearing under laboratory conditions (singly or in large populations) and wide tolerance to environmental parameters
- An increasing database on morphological characters, life history traits, allozymic data and nuclear and mitochondrial DNA sequences is available
- Easy cyst transportation and storage allows interpopulational comparative research on various aspects (reproductive performance, adaptation, etc.)

Genetic variability and divergence within and between Artemia species

Genetic variability is required for evolutionary change, hence critical early stages in speciation depend upon the availability and evolution of genetic differences between populations (Coyne 1992). Allozymes have provided the most extensive data sets of intraspecific diversity, population structure and a way to investigate whether phenotypic population differentiation is underlain by genetic divergence (reviews by Mitton 1997, Møller & Swaddle 1997).

Beardmore and coworkers have extensively screened *Artemia* populations from species around the world. The estimates of genetic variability (based on allozymes) are usually among the highest seen within crustacean species and are comparable to the mean obtained from a variety of invertebrate species (Table 2) (Abreu-Grobois & Beardmore 1982). The plasticity of the *Artemia*

genetic pools, which provides high evolutionary potential, is a typical response of organisms exposed to heterogeneous environments, and this is often the case for *Artemia* habitats (Gajardo et al. 1992). A tendency for an increase in genetic variability, as measured by heterozygosity, with increasing habitat size has been reported in Chinese bisexual *Artemia* populations (Naihong et al. 2000).

Numerous authors have provided convincing evidence of a positive relationship between heterozygosity and fitness traits such as growth rate, viability, fecundity, feed conversion and metabolic efficiency. Hence, the fitness advantage of heterozygotes over homozygotes has been suggested as a mechanism to maintain genetic diversity in natural populations (Mitton 1997). In *Artemia franciscana* heterozygosity has been associated with increased fecundity, and the quality or type of offspring (Gajardo & Beardmore 1989). The brine shrimp produces young as larvae or as

TABLE 2

Genetic variability estimates and F_{st} values for bisexual *Artemia* species from the AmericasEstimadores de variabilidad genética y valores F_{st} para especies bisexuales de *Artemia* de las Américas

Species	Number of populations	Mean P (%) (Range)	Mean observed heterozygosity (Range)	F_{st}	Reference
<i>A. franciscana</i>	21	31 (9 – 50)	9 (2 – 14)	0.240	Abreu-Grobois (1987)
	8	41 (32 – 50)	12.5 (5 – 17)	0.377	Gajardo et al. (1995)
<i>A. persimilis</i> *	7	54.5 (36 – 54.5)	21.7 (18 – 24)	0.453	Gajardo et al. (unpublished observations)

* Preliminary results based on 11 loci. Data for *A. franciscana* considers over 22 loci

encysted embryos resistant to unfavourable conditions. Studies with *A. franciscana* from Great Salt Lake in Utah (Gajardo & Beardmore 1989) show the percentage of zygotes produced as cysts is correlated with the level of heterozygosity (determined electrophoretically) in the mother. More heterozygous females also produce more zygotes, tend to produce more broods and start to reproduce at a younger age than less heterozygous females. The encysted embryos are energetically more expensive to produce than are nauplii, and so the greater reproductive success achieved by more heterozygous individuals may be postulated to be due to their better utilization of energy. Thus, under similar experimental conditions, the more heterozygous females are able to allocate more energy to produce offspring as cysts than are less heterozygous females. Laboratory studies have also shown that male mating success increases with heterozygosity in laboratory populations of *A. franciscana* (Zapata et al. 1990, Mitton 1997). Males heterozygous for three and four loci exhibited significantly higher fitness values (2.99 and 2.55, respectively), in comparison to completely homozygous genotypes (arbitrarily assigned a fitness of 1).

The putative effect of heterozygosity upon life history characteristics (Table 3) is not only of great fundamental biological interest but also has important practical consequences for exploitation, improvement and conservation of the genetic resources in *Artemia*.

Genetic divergence

The disjunct nature of hypersaline environments promotes the genetic differentiation of *Artemia* populations by restricting gene flow, and by offering many selective regimes that tend to select local forms. In addition, stochastic processes (genetic drift) are also likely to promote differentiation of local forms because *Artemia* populations

often experience drastic changes in effective population sizes due to successive cycles of extinction and recolonization, typical of ephemeral or seasonal habitats. It is not surprisingly, therefore, that *Artemia* populations tend to develop large genetic distances (Nei 1978) between populations (Gajardo et al. 1995). Based on these facts, an adaptive divergence mode of speciation is thought to best define the process in *Artemia* (Abreu-Grobois 1987).

The basic allopatric model of speciation: inferences from genetic, morphological and reproductive data

Although speciation is formally conceived as an evolutionary splitting of lineages featuring intrinsic genetic and reproductive closure of populations (Mayr 1982), many studies of speciation have initially dealt with morphological evolution, as the primary target of natural selection are phenotypes. However, the underlying phenomena are genetical. Hence the widespread use of biochemical polymorphisms along with the development of technical procedures for cytological comparisons have provided useful tools to study the genetic structure and continuity of populations in a manner independent of morphological variation. Processes like interdemic selection, genetic revolutions, founder-flush or genetic transience address the multiple ways the relationship between genetic and phenotypic similarity and/or differentiation can take during the speciation process in different organisms (reviewed by Carson & Templeton 1984). The major prediction to be tested regarding the allopatric model is that genetic divergence stems partly from adaptation to the prevailing environmental conditions and partly from sampling drift. Under the biological species concept (Mayr 1969: p19), prezygotic and postzygotic reproductive isolation develop between the physically isolated populations as an

TABLE 3

Correlations of important life history characteristics with heterozygosity

Correlaciones con la heterocigosidad de importantes características de la historia de vida

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- Cyst production, total number of zygotes, and number of broods per female positively correlated with heterozygosity (Gajardo & Beardmore 1989)
 - Age at maturation negatively correlated with heterozygosity (Gajardo & Beardmore 1989)
 - Male mating success positively correlated with heterozygosity (Zapata et al. 1990)
 - Weight of *A. sinica* females positively correlated with heterozygosity (Pilla & Beardmore 1994)
-

incidental byproduct of genetic differences accumulated between them.

The relationship between different set of traits has been investigated in several allopatric *Artemia* populations from Chile and South America in an attempt to evaluate their concordance (Gajardo & Beardmore 1993, Gajardo et al. 1995, Colihueque & Gajardo 1996, Gajardo et al. 1998, 1999, 2000). Table 4 (see also Fig. 1), which summarizes these studies, provides morphological (Mahalanobis distances), electrophoretic (Nei's genetic distances) and karyological data (diploid and chromocentre numbers) for populations of *A. franciscana* and *A. persimilis*, the two species found in the continent. In general, there is a good correspondence between the levels of genetic and morphological distance between them. For example, populations displaying genetic distance values characteristically found for congeneric species (higher than one) also differ in diploid ($2n = 42$ in *A. franciscana*, $2n = 44$ in *A. persimilis*), and mean chromocentre numbers (12.5 in *A. franciscana*, 1.5 in *A. persimilis*). The genetic distance values also confirm similarity between populations from the region (Los Vilos, LVI; Macau, MAC; Yape, YAP) and *A. franciscana*, which is believed to be the dominant species. This is in close agreement with morphological similarity established by the multivariate analysis of morphology and both diploid (all $2n =$

42) and chromocentre numbers (range: 10.0-14.8) characteristically seen in *A. franciscana*. Finally, morphological similarity to *A. persimilis* of some Chilean populations (Pichilemu, PCH), also backed by genetic distance ($D > 1$), diploid ($2n = 44$) and chromocentre numbers (mean number of 4.3), raises doubts on the current distributional paradigm according to which *A. franciscana* is the dominant species in the continent. Moreover, the finding of morphologically intermediate populations between *A. persimilis* and *A. franciscana* (see Fig. 1 and Gajardo et al. 1998) deserves more attention as it could suggest that *A. persimilis* is indeed expanding its range of distribution, and perhaps hybridizing with *A. franciscana*

Reproductive isolation

Intrapopulation experimental crosses and cross-fertility tests have been performed in individual crosses of laboratory-reared populations of *A. franciscana* and *A. persimilis* (Gajardo et al. 2000). Populations compared displayed significant variability in fecundity (total offspring, broodsize) and in the ratio encystment/ovoviviparity. Hybrid offspring was abundantly produced in cross-fertility tests and showed a pronounced switch to the encystment mode, particularly in crosses with *A. persimilis*. Although reproductive isolation is

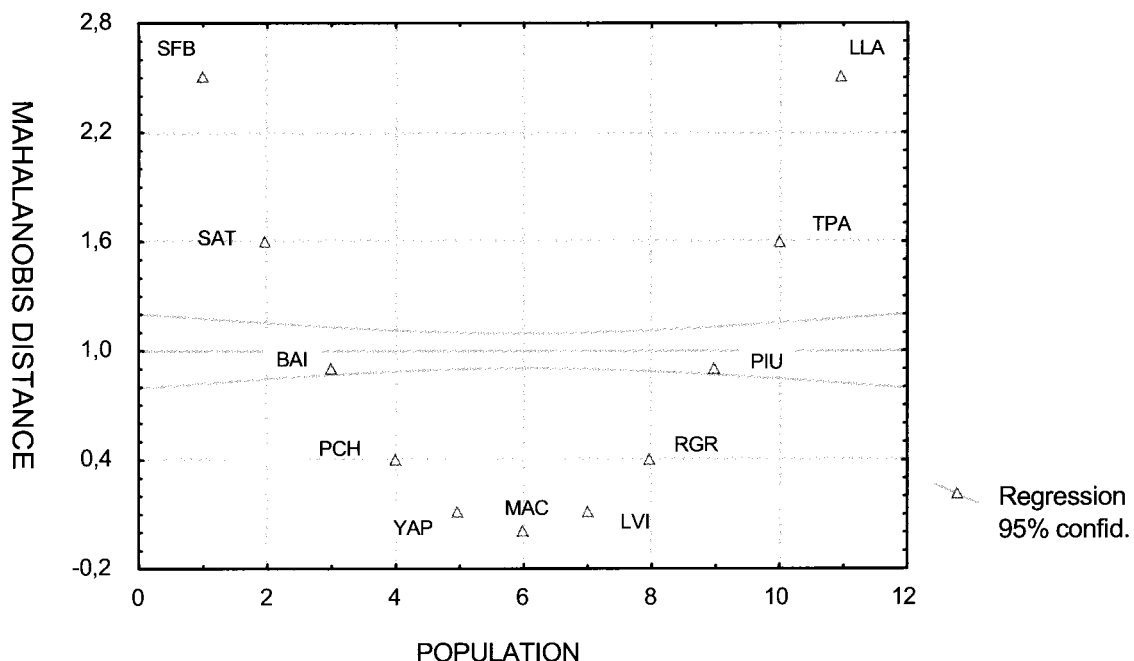


Fig. 1. Mahalanobis distance for *Artemia* populations from South America (SFB = San Francisco Bay; SAT = Salar de Atacama; BAI = Buenos Aires; PCH = Pichilemu; YAP = Yape; MAC = Macau; LVI = Los Vilos; RGR = Río Grande; PIU = Piura; TPA = Torres del Paine; LLA = Llamara).

Distancia de mahalanobis en poblaciones Sudamericanas de *Artemia*.

TABLE 4

Comparison of morphological and genetic information in samples of *A. franciscana* and *A. persimilis* from South America

Comparación de información morfológica y genética en muestras de *A. franciscana* y *A. persimilis* de Sudamérica

Population compared ^a (Abbreviation)	Morphological ^b divergence (Mahalanobis distance)	Genetic divergence ^c		
		Genetic distance value	2n	Mean number of chromocentre
SFB - BAI	< 1.60	> 1.00	42/44	2.5/1.5
SFB - LVI	2.30	0.217	42	10.0
SFB - MAC	2.49	0.024	42	13.4
SFB - YAP	2.39	0.354	42	14.8
PCH - BAI	0.49	> 1.00	44/44	0.0
SFB - SAT - BAI	0.88 - 1.59	< 0.144	44	4.3
PIU - BAT	1.00	< 0.800	42	7.8

^a SFB = San Francisco Bay, USA; BAI = Buenos Aires, Argentina; LVI = Los Vilos, Chile; MAC = Macau, Brasil; YAP = Yape, Iquique, Chile; PCH = Pichilemu, Chile; SAT = Salar de Atacama, Chile; PIU = Piura, Perú

^b Gajardo, unpublished results

^c Gajardo et al. (1995), Colihueque & Gajardo (1996)

regarded as a key trait for species recognition, the production of laboratory hybrids between morphologically or genetically divergent allopatric populations appears to be a common phenomenon in some *Artemia* populations (Bowen et al. 1985, Pilla & Beardmore 1994), and other members of the Order. Indeed, there are examples showing that morphologically distinct species that have been separated for long period of time are sexually compatible (Winan 1979, Maeda-Martinez et al. 1992). This might be related with the fact that these populations have only completed the first stage of the allopatric speciation process (geographical separation) but have not completed the second stage (regaining sympatry) required for the development of pre-mating isolating mechanisms (Mayr 1969, Coyne 1992). In addition, *A. franciscana* and *A. persimilis* would have diverged recently according to Browne & Bowen (1991), and so populations would be in the process of developing barriers to gene exchange (Gajardo et al. 2000).

COMMENTS AND CONCLUSIONS

The idea that particular combinations of genes or phenotypic features are coadapted, or integrated, to produce a superior fitness, was approached in *Artemia* (a sort of "aquatic *Drosophila*"). Both the amount and relevance of genetic variation, the

intraspecific genetic architecture and degree of morphological and genetic change were investigated in the New World *Artemia* species, *A. franciscana* and *A. persimilis*. In very few studies morphological differences have been confronted to genetic differences such as those revealed from allozyme and karyotype analysis. Hence the integration of both sets of data provides a biologically more realistic approach to understanding fitness and intraspecific evolution in *Artemia* because organisms are indeed complex and highly integrated entities characterized by intricate interactions among parts (Rollo 1995).

Despite the fact that *A. franciscana* and *A. persimilis* are sibling species and have undergone recent radiation, so that little morphological differences should be expected (Lenz & Browne 1991), multivariate analysis of the morphology suggest significant differences between them. Morphological and genetic data (genetic distance values, diploid and chromocenter numbers) were correlated in most comparisons. Due to differences in habitat (especially water chemistry), ecological isolation has occurred between some *A. franciscana* populations leading to various stages of speciation, up to and including complete reproductive isolation, despite relatively small genetic distances. The high degree of environmental differences and other very drastic factors affecting populations in nature, e.g., changes in effective population size, and the effect of ge-

netic drift, provide the ideal conditions for rapid changes in the genetic structure which can impact other levels of the biological organization. In *A. franciscana*, for example, there is a strong tendency towards interpopulational differentiation since both climatic and hydrobiological changes over time as well as successive cycles of extinction/recolonization have caused a pattern of scattered and relatively isolated habitats. These conditions provide room for rapid populational changes and, not surprisingly, laboratory studies indicate that *Artemia* strains vary significantly with respect to their life history, presumably as a result of selective pressures in their native habitats (Lenz & Browne 1991).

The most effective way to spread new adaptations is through the establishment of new local populations. Thus if extinctions and recolonization are frequent, group selection can lead to fixation of genes in populations even if those genes are opposed by natural selection, or rapid genetic evolution can result from the combination of genetic drift and the new selection regime offered to the new population. Bottleneck effects, likely to occur very often in *Artemia* increase endogamy and under these circumstances the effect of a handful of genes could be expressed (Carson & Templeton 1984).

One of the ultimate questions for both the ecology and evolution of *Artemia* relates to the understanding of how such a variety of life history patterns can be produced. While ecologists focus on the integrated phenotype and its interface with the environment, geneticists focus on the association of genes that interact to affect fitness. As theory suggest, in constant environments a single specialized phenotype may be deployed, whilst in variable environments a single generalized phenotype may be favoured, or alternatively, a genetic or phenotypically plastic polymorphism might evolve (see reviews by Rollo 1995, Møller & Swaddle 1997). Clearly, the question of specialist vs. generalist organisms remains a central issue in understanding the causes of *Artemia* adaptation. Recombination may be one feature that contributes to such variance in fitness among individuals. In such case a positive association of key fitness components may emerge across genotypes (some individuals are better in most aspects of Darwinian fitness) (see Gajardo & Beardmore 1989, Zapata et al. 1990). In this way the link between genetic variability and the coadapted genome becomes clearer, and explain how heterozygote advantage would maintain genetic variability in sexual *Artemia* species.

ACKNOWLEDGMENTS

The authors acknowledge the kind invitation of the Sociedad de Genética de Chile and the editors to contribute in this special issue in honor of Professor Danko Brncic. Gonzalo Gajardo was formerly a graduate student in Professor's Brncic laboratory and a British Council postdoctoral fellow in Swansea, Wales, under Professor Beardmore's supervision. John A. Beardmore was a postdoctoral fellow in Dobzhansky's laboratory in New York at the time Professor Brncic was there. The work reviewed here was partially funded by FONDECYT 1940812, the European Union (contract CT1-CT91-0945) and the International Foundation for Science (A/14404-2).

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