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Molecular phylogenetics and asexuality in the brine shrimp *Artemia*

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Abstract

Explaining cases of long-term persistence of parthenogenesis has proven an arduous task for evolutionary biologists. Interpreting sexual–asexual interactions though has recently advanced owing to methodological design, increased taxon sampling and choice of model organisms. We inferred the phylogeny of *Artemia*, a halophilic branchiopod genus of sexual and parthenogenetic forms with cosmopolitan distribution, marked geographic patterns and ecological partitioning. Joint analysis of newly derived ITS1 sequences and 16S RFLP markers from global isolates indicates significant interspecific divergence as well as pronounced diversity for parthenogens, matching that of sexual ancestors. Maximum parsimony, maximum likelihood, and Bayesian methods were largely congruent in reconstructing the phylogeny of the genus. Given the current sampling, at least four independent origins of parthenogenesis are deduced. Molecular clock calibrations based on biogeographic landmarks indicate that the lineage leading to *A. persimilis* diverged from the common ancestor of all *Artemia* species between 80 and 90 MYA at the time of separation of Africa from South America, whereas parthenogenesis first appeared at least 3 MYA. Common mitochondrial DNA haplotypes delineate *A. urmiana* and *A. tibetiana* as possible maternal parents of several clonal lineages. A novel topological placement of *A. franciscana* as a sister clade to all Asian *Artemia* and parthenogenetic forms is proposed and also supported by ITS1 length and other existing data.

Keywords: *Artemia*; Molecular phylogeny; Parthenogenesis; Brine shrimp; ITS1 region; Molecular rate; MtDNA; Geological time

1. Introduction

Evolution has solved the problem of reproduction in various ways. Strictly asexual organisms though—those that reproduce without fertilization—are, in the long-term, something of an evolutionary paradox. They are barred not only from the benefits of sexual reproduction (outcrossing and meiotic recombination) but also from the resulting genetic variance needed for evolutionary change. Current theories attempting to explain the dominance of sex (Judson and Normark, 1996; Normark et al., 2003) are largely unsuccessful in accounting for parthenogenetic lineages. Most notably, the inability of such taxa to purge deleterious mutations is thought of as the prime factor driving them to evolutionary degradation (Hurst and Peck, 1996). Nevertheless, parthenogens attain short-term persistence and, occasionally, provocative longevity (Schön et al., 1996; Mark Welch and Meselson, 2000). For this reason, the origin, genetic diversity and phylogenetic history of asexual systems have been atop evolutionary biologists' list of inquiries.

Mutational and ecological models provide more or less specific predictions regarding the genetic and life history architecture of unisexual taxa, the patterns of variation in nuclear and organelle DNA and the timing of consequences due to loss of sexuality (Normark and Moran, 2000; Normark et al., 2003; Simon et al., 2003). In addition, the rate and mode of origin of parthenogenesis may be crucial in determining levels of genetic diversity, the geographic distribution and ecological success of asexuals (Sandoval et al., 1998; Vrijenhoek, 1998; Schön et al., 2000; Law and Crespi, 2002; Paland et al., 2005). Phylogenetic approaches to the evolution and lifespan of asexuality are especially powerful since typical signatures of the loss of sex

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(Delmotte et al., 2001), rates of molecular change (Schön et al., 2003) and hybridization (Delmotte et al., 2003) can be readily detected on reconstructed trees. Methodologically, such investigations are largely dependent on adequate sampling of extant taxa and sufficient screening of genomic regions. The former may seriously influence both the estimated timeframe of asexual lineages and the number of transition events. The latter can help determine whether the observed clonal divergence is mutationally generated or recurrently captured from sexual ancestors (Crease et al., 1989; Chaplin and Hebert, 1997). It is thus obvious that more robust interpretations are made from model systems where the alternative modes of reproduction are confounded within a single organismal lineage and additional biogeographic and ecological data can be related to patterns of divergence. Recent work has emphasized the need for this integration (Simon et al., 2003). It is paradoxical though that, for *Artemia*, admittedly the most accomplished survivor of hypersaline settings and a confusing case of ancient asexuality, similar critical and detailed assessments are overdue.

Artemia is a genus (Crustacea, Anostraca) of sexual and parthenogenetic forms with a global distribution in inland salt lakes and coastal lagoons (Triantaphyllidis et al., 1998; Van Stappen, 2002). The organism has featured in the literature extensively, by virtue of its importance in aquaculture and as a model system for varied research. From an evolutionary perspective in particular, different adaptations, distinctive genetic features and marked biogeographic patterns are all found in the genus, thus offering unique opportunities for studies on phylogeny and the interaction between sexuality and parthenogenesis (Abatzopoulos et al., 2002a). Currently, six bisexual species (Abatzopoulos et al., 2002b) and a heterogeneous group of obligate parthenogens are recognized, which are either apomictic or automictic. A sharp geographic boundary separates the New World bisexuals (*A. franciscana*, *A. persimilis*) from their Old World relatives (*A. salina*, *A. urmiana*, *A. sinica*, and *A. tibetiana*). Similarly, parthenogenetic populations are restricted to the Old World, where they comprise the majority. Bisexuals are diploid with $2n = 42$ (except *A. persimilis* where $2n = 44$), while parthenogens range in ploidy from $2n$ to $5n$ (Abatzopoulos et al., 1986). Morphometric and/or morphological, life history and genetic divergences are widely partitioned both within and between the different reproductive modes (for review see Browne, 1992). Allopatric divergence and ecological specialization are believed to have shaped *Artemia* evolution, while the influence of dispersal on contemporary regional distributions has only recently been explored (Green et al., 2005).

A historical aspect of *Artemia* phylogeny has been mainly obtained through allozyme studies. Beardmore and Abreu-Grobois (1983) outlined the series of phylogenetic events in the genus. Evaluation of the degree and patterns of interspecific divergence have indicated that the primal evolutionary event has been the separation of New and Old World bisexual lineages. This was followed by the separation of *A. franciscana* and *A. persimilis* in the New World and the divergence of *A. salina* and *A. urmiana* lines in the Old World (see tree in Fig. 1). Based on an allozymi-

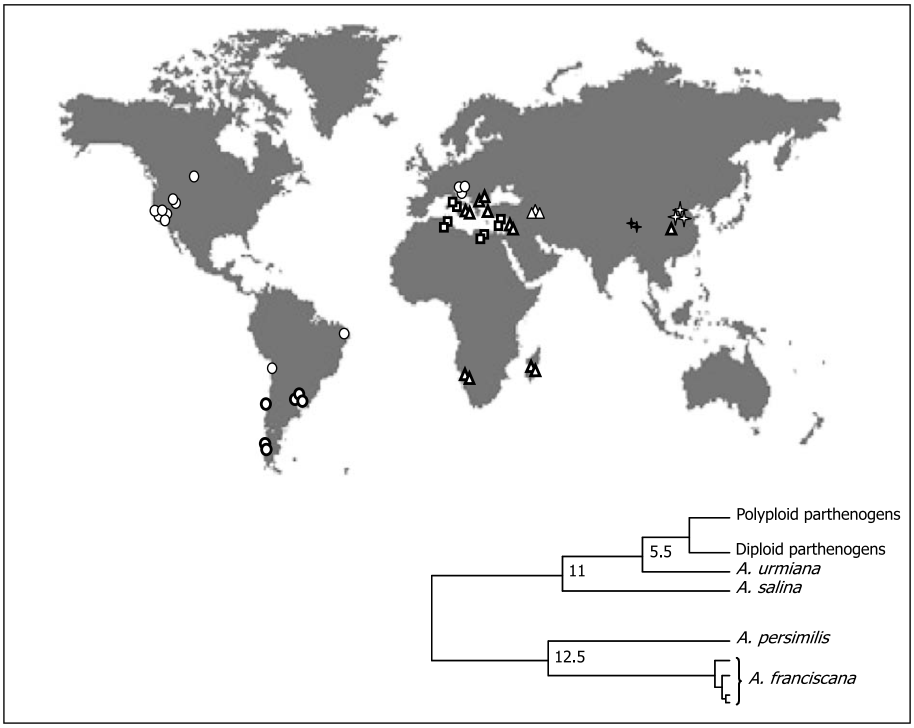

Fig. 1. The 46 *Artemia* individuals (sequenced for ITS1 region) as distributed in the world map. ○, *A. franciscana*; **o**, *A. persimilis*; ▣, *A. salina*; △, *A. urmiana*; +, *A. tibetiana*; ✦, *A. sinica*; and ▲, parthenogenetic *Artemia* strains. Inserted tree shows currently accepted phylogenetic relationships in the genus as determined by allozyme markers (see also Beardmore and Abreu-Grobois, 1983). Numbers at nodes are divergence times in million years.