GENOMIC DNA HYBRIDIZATION AS AN ATTEMPT TO EVALUATE PHYLOGENETIC RELATIONSHIPS OF OSTRACODA*)

BY

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ABSTRACT

Molecular distances obtained by measuring similarity of large DNA fragments using DNA hybridization procedures are an alternative to distances based on sequences of particular genes or their products. However, when studying small animals, the low amount of DNA that can be isolated from a single individual may be a serious problem. Here we present a modified DNA hybridization method, applicable when only a small amount of DNA is available. We use the hybridization data to reconstruct the phylogeny of five species representing three extant superfamilies of Ostracoda, consistent with the traditional frame of classification. We compare three methods of internal branch testing (the bootstrap, the Dopazo test, and the Weighted Least Squares Likelihood Ratio Test) to assess the statistical significance of the results. Our analysis indicates that the total DNA hybridization technique may be useful in preliminary studies of taxonomic relationships between species of small animals.

RÉSUMÉ

Les distances moléculaires obtenues par la mesure de la similarité de l’ADN par hybridation de grands fragments d’ADN constituent une alternative aux distances évaluées par les séquences de gènes particuliers ou de leurs produits. Cependant, si l’étude concerne des animaux de petite taille, la faible quantité d’ADN isolable à partir d’un seul individu peut constituer un handicap. Nous exposons ici une méthode modifiée d’hybridation de l’ADN, applicable lorsque seule une faible quantité d’ADN est disponible. Nous utilisons les données d’hybridation pour reconstruire la phylogénie de cinq espèces représentant trois vastes super-familles d’Ostracodes, en accord avec le cadre traditionnel de la classification. Nous comparons trois méthodes d’essai de validation des branchements (le “bootstrap”, le test Dopazo et le test de vraisemblance des moindres carrés) pour

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évaluer la significativité des résultats. Notre analyse montre que la technique d'hybridation totale de l'ADN peut être utile pour des études préliminaires de relations taxonomiques entre espèces de petits animaux.

INTRODUCTION

Ostracodes are small (0.5-3.0 mm long in the adult stage) and inhabit a wide variety of aquatic and semiaquatic habitats. Their body is completely enclosed in a bivalved carapace, which in most groups is calcified and easily preserved in sediments. This is the reason why ostracodes have the richest fossil record among crustaceans, spreading over about 500 Mya (Hou et al., 1996). Specifically shaped carapaces of extinct and living ostracodes play an important part in palaeontological and neontological studies. Until recently, comparisons of the morphological characters were the only source of information about the pattern of ostracode taxonomy.

DNA sequence analysis methods have been used for intrapopulation studies on some ostracode species (e.g., Schön et al., 2000; Yamaguchi, 2000) or even for comparison between species representing different orders and superfamilies (e.g., Schön et al., 2003; Yamaguchi & Endo, 2003). However, this method has some disadvantages in systematic studies above the species level. This may be caused by the difficulties in aligning sequences obtained from distant taxa (such difficulties were reported for the ostracode ITS1 sequences by Schön et al., 2003), or saturation at the neutral sites (such as observed for ostracode COI sequences, cf. Schön et al., 2003). While some ostracode species show extremely high variability due to the spin-off of asexual lineages from sexual populations (e.g., in *Eucypris virens* (Jurine, 1820), cf. Schön et al., 1998, 2000), ancient asexuals, i.e., darwinulids, show very low diversity. This poses a problem in choosing a gene suitable for phylogenetic analysis. The comparison of the DNA sequence of one or several particular gene(s) may be a basis for proposals of relationships between taxa, although we can not be sure that an analysis of other genes would yield the same results.

An alternative approach is to use the technique of total DNA hybridization (see Hillis et al., 1996, for a review) in order to obtain a measure of sequence similarity between taxa. This technique was described in the early 1960s (Doty et al., 1960; Marmur & Doty, 1961) and soon used successfully in studies of viruses (Schildkraut et al., 1962) and bacteria (McCarthy & Bolton, 1963). In the case of higher organisms, DNA was often compared after removing the repetitive fraction. That research focused mostly on systematic studies (at the level of species and families). The published work on interspecies comparisons concerns, for example,