COMPARATIVE CYTOGENETIC ANALYSIS OF THREE STYLOMMATOPHORAN SLUGS (MOLLUSCA, PULMONATA)

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INTRODUCTION

The Stylommatophora include 20,000 to 30,000 extant species of land snails and slugs, belonging to 70–90 families. Their classification system is still controversial because phylogeny and systematic relationships at the family level are poorly understood. Besides morphological studies, ribosomal RNA genes and the H3/H4 histone gene cluster (Ambruster et al., 2005; Wade et al., 2006) have also been used to resolve the relationships within this order. Recently, by comparison of primary sequence of mitochondrial and nuclear genes, Grande et al. (2004) resolved the Stylommatophora as the early split monophyletic sister group of all the other gastropod taxa.

Available data on the cytogenetics of Stylommatophora are extremely poor (reviewed by Patterson, 1969, and Thiriot-Quièvreux, 2003) and mostly concern the haploid (n) and/or diploid (2n) chromosome numbers and karyotype morphology. Vinogradov (2000) determined genome size (GS) and genome base composition in 15 pulmonate species, with five being stylommatophorans. In the last few years, only two thorough cytogenetic investigations have been carried out on Milax nigricans, Cantareus aspersus, and C. mazzullii (Vitturi et al., 2004, 2005), and their results proved to be useful to examine: (1) genomic organization at molecular level using fluorescent in situ hybridization (FISH) with repetitive DNA sequences as probes; (2) reciprocal position of ribosomal (18S–28S and 5S rDNA) and telomeric (TTAGGG)n multigene families using simultaneous double-colour FISH; and 3) evolution of genome by comparison of genome size (C-value) and base-pair composition.

In the present paper, we describe a cytogenetic comparative analysis of Limacus flavus (Linnaeus, 1758) (Limacidae), Tandonia sowerbyi (Férussac, 1823), and Deroceras panormitanum (Lessona & Pollonera, 1882) (Agriolimacidae), carried out by standard (i.e., Giemsa and fluorochrome staining), and molecular (in situ hybridization) methods. Percentage of adenine-thymine DNA (AT-DNA) and genome size (GS) were also investigated for the three species and, in addition, for Milax nigricans (Philippi, 1836) (Milacidae), still unexplored on these parameters. This study was carried out with the aim of contributing to the karyological knowledge of the order Stylommatophora, hitherto broadly neglected from this point of view. In addition, we wanted to test whether cytogenetic analysis could reveal any inter-specific genomic differences that may be considered as informative characters in the phylogenetic tree construction.

MATERIAL AND METHODS

The specimens of Limacus flavus (Linnaeus, 1758) (N = 80), Tandonia sowerbyi (Férussac, 1823) (N = 50), Deroceras panormitanum (Lessona & Pollonera, 1882) (N = 55), and Milax nigricans (Philippi, 1836) (N = 30) were collected in the lawns around Polizzi Generosa, northwestern Sicily, Palermo Province, during 2004, 2005 and 2006. Sexually mature individuals occurred only on September–October (L. flavus, T. sowerbyi, and M. nigricans) or October–November (D. panormitanum) of each year. Specimens were identified according to the guidelines of Giusti (1973). Systematics follows the scheme proposed by Bodon et al. (1995). Vouchers of all investigated species were deposited in the laboratory of cytogenetics and molecular genetics (University of Urbino, Via Maggetti 22).

Chromosomes were obtained by air-drying from sexually mature testicular lobes after in vivo colchicine treatment (0.5 mg/ml for 1 h at room temperature), observed with a Leica microscope, and photographed with a Kodak Ektacolor 800 ASA film. Chromosome classifi-

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