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Author's address: Dr. JOHANN-WOLFGANG WÄGELE, Universität Oldenburg, Fachbereich 7, Biologie, P.O.B. 2503, D-2900 Oldenburg, FRG

Centro di Genetica Evoluzionistica del C. N. R., Istituto di Genetica and Istituto di Biologia generale dell' Università di Roma

Nuclear DNA content of Asellus aquaticus and Proasellus coxalis

By F. VALENTINO, A. DEL MARCO, and A. ROCCHI

Received on 21. March 1983

Introduction

In recent years, *Asellus* (*Asellus*) *aquaticus* (L.) Racovitza and *Proasellus coxalis* (Dollfus), two species of isopod crustaceans previously considered allopatric have been found together in the epigeal fresh waters of central and southern Italy; the latter species appears to be slowly occupying biotopes which were formerly exclusive to *Asellus aquaticus*. Until 1970, the two species were considered to belong to the same genus *Asellus* and attributed to two sub-genera, *Asellus* and *Proasellus* (DUDICH 1925).

In 1970, HENRY and MAGNIEZ proposed to make the two sub-genera to full separate genera, because their origin and age appeared very different to them. The genus *Asellus*, in the opinion of these authors, consists of Euro-Asian forms, of which only one has reached Western Europe recently. *Asellus* (*Asellus*) *aquaticus* is the most common species of this genus found in north European epigeal fresh waters. The genus *Proasellus* has colonized Europe south of a line running from the Black Sea to Britain, as also, the Mediterranean Middle East and North Africa. In these regions, as in all those surrounding the Mediterranean Sea, *Proasellus* is represented by the widely distributed polytypical species *Proasellus coxalis*. CHAPPUIS (1949) maintains that the Asellides owe their origin to a single freshwater line, which diverged and spread through the continent in a series of migrations, whereas HENRY and MAGNIEZ (1970) are of the opinion that the group was formed by multiple migrations, which started independently from marine Asellide lines, from widely separate places at different times.

Caryological research has frequently been used to clarify phylogenetic problems. The karyotype of *Asellus aquaticus* (MONTALENTI and ROCCHI 1964 a) consists of $2n = 16$ chromosomes, all metacentric or submetacentric, with the exception of one pair which is subtelocentric. The karyotype of *Proasellus coxalis* consists of $2n = 12$ chromosomes, all metacentric or submetacentric (MONTALENTI and ROCCHI 1964 b) and considerably smaller than those of *Asellus aquaticus*.

U. S. Copyright Clearance Center Code Statement: 0044-3808/83/2104-0262/$ 02.50/0
Z. zool. Syst. Evolut.-forsch. 21 (1983) 262–265

ISSN 0044-3808/InterCode: ZZSEAA

A comparison of the two karyotypes immediately excludes the possibility of evolutionary divergence by means of Robertsonian fusions. Since the chromosomes of the two species do not respond to stain banding techniques other than C-banding (ROCCHI et al 1980) it is impossible to make a direct comparative analysis between the two karyotypes.

In order to get additional information about the divergence of the two genomes we became interested in measuring the DNA content of the two species. In fact, the genome size, obtained by microdensitometer after Feulgen staining, has been widely used for the study of evolution in various animal groups (e. g. BACHMANN et al. 1972; BACHMANN and RHEINSMITH 1973) and constitutes anyway one of the parameters which characterize genome.

Material and method

The specimens of *Asellus (Asellus) aquaticus* used in this experiment had been collected in Sarno river near Naples; the samples of *Proasellus coxalis* had been collected in Tiber river near Rome. The studies of DNA content were made on testicular tissue. The preparations were obtained by squashing the testes after fixation in 45 % acetic acid. The Feulgen staining was carried out simultaneously on the slides of the two species and on slides obtained by the standard method from human leucocytes in culture. All results can thus be converted into comparable relative units of DNA per nucleus; and converted in picogrammes since the quantity of DNA in pg is known for human nuclei (SOBER 1970; REES and JONES 1972). The technique used for the Feulgen staining was as follows: hydrolysis in 1 N HCl at 60°C for ten min; staining with Schiff reagent for 60 min; differentiation in sulphuric acid for 9 min; dehydration and sealing with Euparal.

Measurements of the DNA content of the testicular specimens were made on cells in mitotic porphase or meiotic prophase; those of the human leucocyte specimens were taken on cells in mitotic prophase. 100 nuclei were examined in each species. The absorption profiles were determined by Zeiss microspectrophotometer MPM 0.1 K photometer, apo 100/1.32 objective, optovar 1.25, measuring diaphragm dia = 16 μ, field diaphragm = 20 μ, Apl 0.63 condenser, halogen lamp 12 V 100 w light source. Wavelengths 490 and 560 nm were obtained with a continuous monochromatic filter for application of two-wave cytophotometry and for valuation of distribution error. The simple method of approximation is applied at 560 nm for the total measures of meiotic and mitotic prophases (RASCH and RASCH 1970).

Results and discussion

The results of the microdensitometer measurements are shown in the Table, which also shows the calibration of the relative values in terms of percentage of the value of human DNA.

As can be seen from the Table, *Proasellus coxalis* contains a quantity of DNA which is more than three times less (3.69) than that of *Asellus aquaticus*. The DNA content per

Table

Average amounts of DNA (Feulgen) per nucleus, given in arbitrary units (a. u.) and as percentage of human DNA

Species	Feulgen-DNA a. u.	SE	Relative units
Proasellus coxalis	14.68	±0.28	18.11
Asellus aquaticus	54.18	±0.44	66.87
Man	81.02	±0.39	100

nucleus of these two species can be expressed in picogrammes, on the basis of the DNA content in the control specimens. If we consider the human haploid DNA amount as being 3 pg (SOBER 1970; REES and JONES 1972), then *Proasellus coxalis* has a value of 0.54 pg per haploid set and *Asellus aquaticus* a value of 2 pg. BACHMANN and RHEINSMITH (1973) have measured the total DNA content per genome in several groups of Crustacea and have observed that, in spite of a wide range of nuclear DNA values, a DNA amount between 2 pg and 3 pg per haploid set represents the crustacean norm.

Asellus aquaticus therefore fits perfectly into this category whereas *Proasellus coxalis* is considerably removed from it. The same authors (BACHMANN and RHEINSMITH 1973) have also noted a trend towards small genomes in advanced and specialized groups.

Observations of this kind have also been made on many other plant and animal groups (e. g. REES and HAZARIKA 1969; BACHMANN et al. 1972) showing that the divergence and the evolution of species in some groups appear to be accompanied by a decrease in the nuclear DNA. Some researchers maintain that various characteristics, whether cellular or organismic, as size or developmental time, are controlled by the quantity of nuclear DNA (nucleotype) which, apart from its informational content, expresses its own type of control (BENNET 1972, 1982). At least in higher plants, a strong relationship has been observed between nucleotype and cell size, cell cycle, generation time and length of life cycle.

Regarding the two species which we examined here, the observations made for the cellular parameters and for the biological life cycles does agree well with the above concept. In fact, autoradiographic studies of the duration of spermatogenesis of *Asellus aquaticus* following injection of ^{3}H-thymidine (ROCCHI BRASIELLO and VITAGLIANO TADINI 1969) and of *Proasellus coxalis* (ROCCHI BRASIELLO 1967), demonstrate that the period necessary for the maturation of primary spermatocytes into mature sperm is 19 days for the former species and 14 days for the latter. Longevity of *Asellus aquaticus* is notably higher (almost double) than of *Proasellus coxalis*. The time necessary for its sexual differentiation is longer by almost one third and it produces a progeny of almost double number. In intraspecific competition however, it is certainly inferior (FANO et al. 1977).

We do not know by what fraction of DNA (repetitive or non-repetitive) *Asellus aquaticus* and *Proasellus coxalis* differ, nor in what proportion. Yet, the study of heterochromatic regions by means of C-banding and fluorochromes has shown that they appear in *Asellus aquaticus* exclusively at telomeric positions, and almost exclusively in association with the nucleolar organizers; moreover, their fluorescence is brilliant when stained with chromomycine A_3 whereas they remain dark when stained with quinacrine or with Hoechst 33258. This permits us to suppose that these regions of DNA are rich on GC (ROCCHI et al. 1980). A preliminary observation shows exactly the same localization of heterochromatin in *Proasellus coxalis* and the same staining quality as in *Asellus aquaticus*, but its quantity is reduced (DI CASTRO et al. 1982).

The available data do not yet permit us to describe the phyletic origin of the two species, but do not exclude definitely the hypothesis of their common origin (CHAPPUIS 1943).

Summary

Asellus aquaticus and *Proasellus coxalis*, two crustacean isopod species, were considered for many years to belong to the same genus *Asellus*. More recently, however, they were put into two different genera. We considered it useful to add the measurements of the DNA content of two genomes to the caryological data already known. *Asellus aquaticus* contains 3.7 times more DNA than *Proasellus coxalis* (2 pg per haploid set for *Asellus aquaticus* and 0.54 pg for *Proasellus coxalis*). The possible significance of this observation for species formation and ecological adaptations is discussed.

Zusammenfassung

DNA-Inhalt des Zellkerns von Asellus aquaticus und Proasellus coxalis

Die Wasserasseln *Asellus aquaticus* und *Proasellus coxalis* (Crustacea, Isopoda) wurden für lange Zeit demselben Genus, *Asellus*, zugeschrieben. Auf Grund neuerer Untersuchungen wurden diese Arten aber in zwei getrennte Genera eingeordnet. Es erschien uns daher sinnvoll, den bekannten karyologischen Daten die Messung des DNA-Gehaltes der zwei Genome hinzuzufügen. *Asellus aquaticus* enthält 3,7mal soviel DNA wie *Proasellus coxalis* (*Asellus aquaticus* 2 pg pro haploides Genom und *Proasellus coxalis* 0,54 pg). Die mögliche Bedeutung dieser Beobachtungen für die Artbildung und für die ökologische Anpassung der Arten wird diskutiert.

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Authors' addresses: F. VALENTINO, Centro di Genetica Evoluzionistica, Istituto di Genetica, Fac. di Sc. Mat. Fis. Nat., A. DEL MARCO, Istituto di Biologia generale dell'Universitá di Roma, and A. ROCCHI, Istituto di Genetica dell'Università di Roma, Città Universitaria, I-00185 Roma