Notes on the cytology of Rissoacea II. The chromosomes of Assiminea grayana Fleming, 1828 (Gastropoda: Streptoneura)

by

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INTRODUCTION AND ACKNOWLEDGEMENTS

The bisexual snail *Assiminea grayana* Fleming, 1828, is one of the five European representatives of the genus *Assiminea* Fleming, 1828, and the type species of the genus by monotypy. It lives on the bottom amongst the vegetation in brackish areas, along river estuaries and in similar places near the coasts of the southern North Sea: from the English Channel to the West coast of Jutland and along the South-east coast of England. The other European species are *A. cardonae* Paladhile, 1875 (Balearic Islands), *A. eliae* Paladhile, 1875 (Spain and France), *A. ostiorum* Bavay, 1920 (Arcachon, France) and *A. rufostrigata* Hesse, 1916 (Bulgaria and Roumania).

In the present note an account of the karyotype morphology and chromosome behaviour during spermatogenesis is given. The study was carried out in the Institute of Genetics, University of Utrecht. Our thanks are due to Prof. Dr. W. J. BOYES (Institute of Genetics, McGill University, Montreal, Canada) for valuable advice on the karyograms; to Dr. M. R. HONER (Department of Zoology, State Agricultural University, Wageningen), for correcting the English text, and to Mr. D. SMIT and Miss T. VAN WIJNGAARDEN for the technical execution of the illustrations in this paper.

MATERIAL AND METHODS

The work was carried out on 12 males collected at Holwerd (Friesland) 22 VI 1965 and 1 XI 1966, at Nieuwendijk (Zuid-Holland) 12 III 1966 and at Bierum (Friesland) 19 III 1966. The animals from

Bierum were collected by Mr. A. K. SCHUITEMA to whom our thanks are due for his cooperation. Preparations of six females were also inspected but no chromosome counts could be made in the oocytes.

For fixing and staining the lacto-acetic-orcein squash method was used. The technical procedure and equipment were described in the previous paper of this series (BUTOT & KIAUTA, 1966).

One hundred and eighty six microphotographs were taken. The figures in this paper are printed at a magnification × 1500.

KARYOTYPE MORPHOLOGY

The diploid chromosome number in spermatogonial mitosis of *Assiminea grayana* is 24. The following account of the morphological features of the karyotype is based on the spermatogonial karyograms reproduced in Pl. I (figs. 1-3).

The spermatogonial metaphase chromosomes are of decreasing magnitude. The largest chromosome measured was 10.3 μ long, the smallest was 3.1 μ. The chromosomes are constricted either submedianly or subterminally. Two pairs have a median or nearly median constriction. The longest pair of chromosomes is submetacentric, the second pair is nearly metacentric. The fourth, sixth and eighth pairs are subacrocentric. The approximate arm ratio is given in Table I.

Table I. The approximate arm ratio in the spermatogonial metaphase chromosomes of *Assiminea grayana.*

Pair no.	1	2	3	4	5	6	7	8	9	10	11	12
Ratio	1.4	1.1	2.6	3.8	2.8	6	2	3.5	2	1.1	1	could not be measured

The sex determining mechanism is unknown. Whether or not the 12th pair represents the sex chromosomes is not certain. The shape and exact centromere position of these chromosomes are rather unclear in all figures available.

MEIOTIC CHROMOSOME BEHAVIOUR

The primary and secondary spermatocytes can be always readily distinguished at any divisional stage. Apart from the differences in the chromosome morphology, the diameters of the secondary spermatocytes are clearly smaller than those of the cells in the first maturation division.

The resting stage is the period with the longest duration in the meiotic cycle. In the nucleus some minute particles of heterochromatic nature are present at this stage, their number varying in different cells.

At leptotene the chromosomes form a weakly stained, netlike structure in which many minute, well stained chromatin dots can be seen. The identity of the chromosomes cannot be discerned at this stage. Towards the end of the leptotene the chromatin tends to come into the picture gradually.

At zygotene the paired threads become more and more associated. The chromosomes are linearly arranged. The terminations of at least some of them are heterochromatic in the regions where they are attached one to the other.

At pachytene the chromosomes are further condensated. They are arranged in a bouquet (Pl. II fig. 2). The terminal sections of some chromosomes are in a few pictures clearly heterochromatic (Pl. II fig. 1).

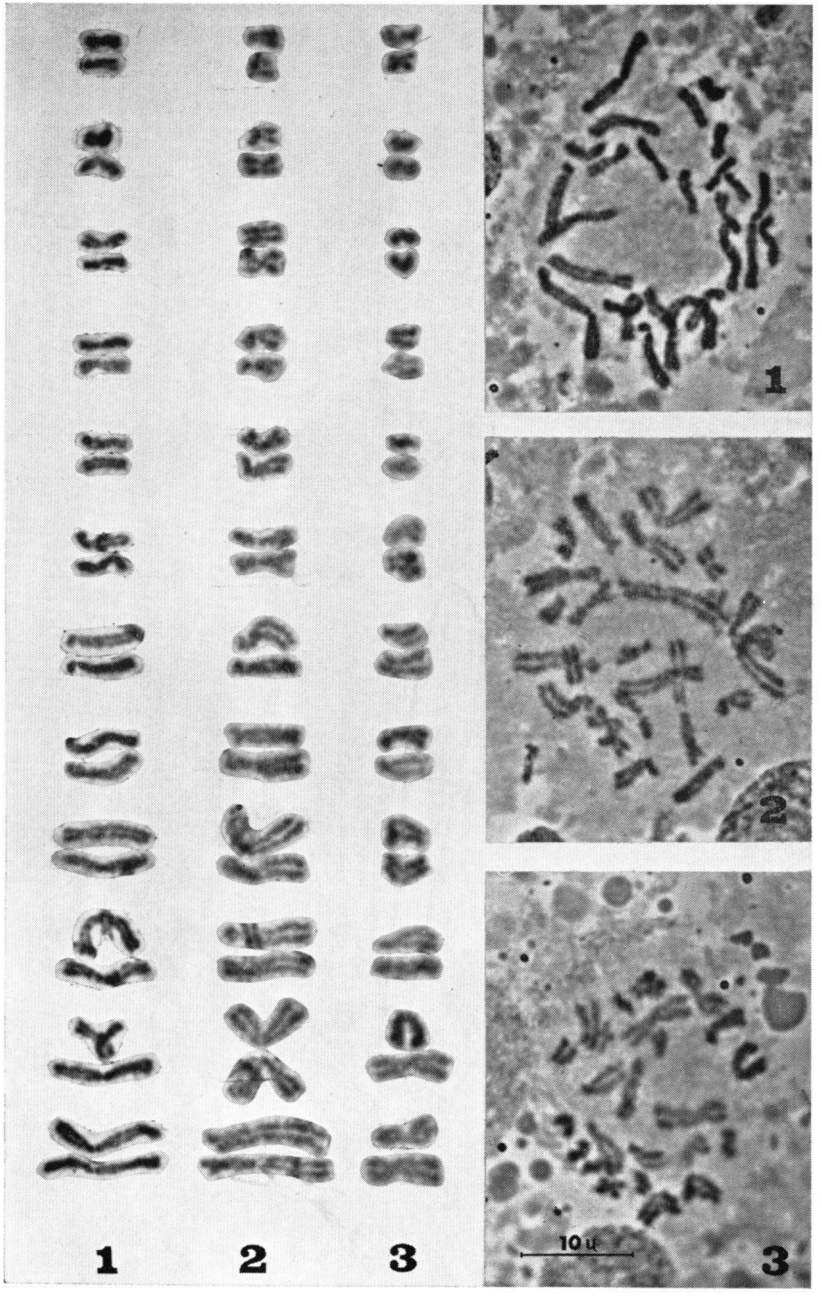
Since diplotene stages are lacking in our material we consider this stage to last only a short time.

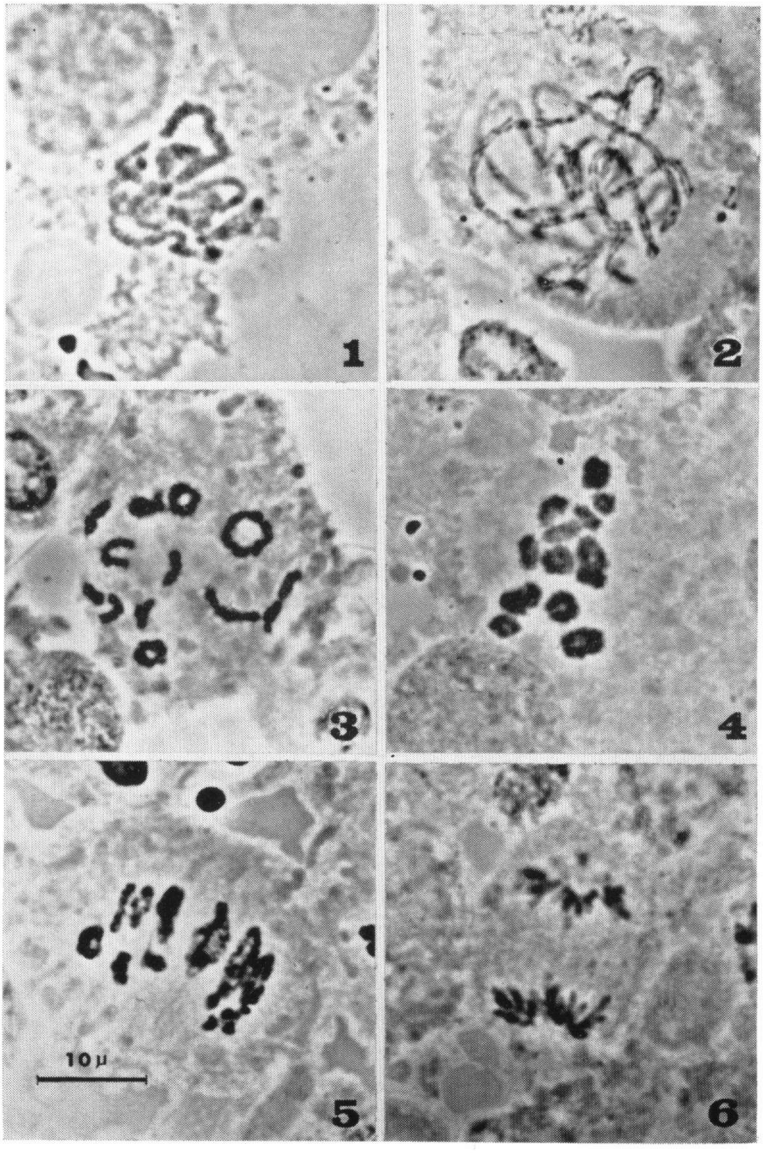
In diakinesis 12 bivalents could be counted in all figures. At early diakinesis they appear as rings, crosses and rods of varying sizes. The two longest bivalents show two chiasmata in almost all of our figures. In some of them two chiasmata appear in two of the smaller bivalents as well. All other bivalents have presumably one chiasma only (Pl. II fig. 3). At the beginning of diakinesis the chiasma is usually still in the place where it was originally formed. The one-chiasma bivalents are therefore asymmetrical with regard to the longitudinal axis of the chromosome. With the advancement of the stage the chiasmata become terminalized and some bivalents appear as more or less symmetrical crosses.

Approaching metaphase I the chromosomes contract further and the rings and crosses gradually disappear, or almost disappear. The chromosomes take on this stage a more or less elliptical or oval shape. An unstained space in the middle region of the majority of the bivalents is not uncommon (Pl. II fig. 4).

In the only available figure of the lateral view of early anaphase not all chromosomes are linked up strictly in the equatorial plane. At least two bivalents are placed in a more forward position towards the same pole. It is likely that this situation is merely due to the squash technique employed. It is therefore necessary to delay any consideration of the figure until more material will become available (Pl. II fig. 5).

Plate I. *Assiminea grayana* Fleming, 1828. Nieuwendijk (Zuid-Holland), spermatogonial mitoses.


1
2
3
1
2
10 µ
3


1
2
3
4
10 µ
5
6

The few figures of telophase I are unclear. It is perhaps peculiar that in some of them one element could be seen in a forward position at the pole (Pl. II fig. 6).

Interkinetic cells are considerably smaller than in the resting stage of the first maturation division, and they remain so throughout the second meiotic cycle. The same applies to the secondary spermatocyte chromosomes.

The second division appears to proceed very rapidly to metaphase II and no early stages were ever observed in our material.

Judging from the frequency of metaphase II figures in our preparations, the duration of this stage must be considerably shorter than it is in the first meiotic division. In none of our figures of this stage could the chromosomes be counted with certainty.

DISCUSSION

To date five species of the genus *Assiminea* have been examined cytologically. The chromosome numbers are shown in Table II.

Table II. Haploid chromosome numbers in the genus *Assiminea*.

Assiminea	n	Locality	Authority
(*Angustassiminea*)			
castanea Westerlund, 1883	15	Japan	Patterson, 1967
yoshidayukioi Kuroda, 1959	15	Japan	Patterson, 1967
(*Assiminea*)			
grayana Fleming, 1828	12 ♂	Netherlands	Butot & Kiauta, 1966; present study
japonica Martens, 1877	12	Japan	Patterson, 1967
parasitologica Kuroda, 1958	12	Japan	Patterson, 1967

Plate II. *Assiminea grayana* Fleming, 1828. Fig. 1: Holwerd (Friesland), pachytene; fig. 2: Nieuwendijk (Zuid-Holland), pachytene; fig. 3: Bierum (Friesland), diakinesis; fig. 4: Nieuwendijk (Zuid-Holland), metaphase I; fig. 5: Bierum (Friesland), lateral view of early anaphase I; fig. 6: Nieuwendijk (Zuid-Holland), telophase I.

The total length of chromosomes of each of the Japanese species examined could not be determined on the base of the figures available, no mitotic figures or any other details being published. A single figure of the polar view of meiotic metaphase I of *A. japonica* could be compared with our figures of this stage. It shows at least four bivalents with presumably two chiasmata (rings). This situation is essentially similar to that in *A. grayana.* Judging from the figures of meiotic metaphase I of other Japanese species, published by PATTERSON (1967), *A. parasitologica* may join *A. japonica* and *A. grayana* in one group and not only because of its chromosome number. The other group of *A. castanea* and *A. yoshidayukioi* (n = 15) have smaller chromosomes among which apparently only one element appears with two chiasmata as a ring. Chromosome morphology, according to the figures published, seems essentially different in the two groups.

THIELE (1927) and HABE (1942) have tried to divide the genus *Assiminea* in subgeneric taxa on morphological characters of radula, shell and operculum. ABBOTT (1958) is convinced that, with regard to radular characters, independent lines of development have occurred within the genus. This is also true to a more or less extent for shell characters as ABBOTT believes (l.c. p. 231). With some doubt he sorted out the "*nitidula*-complex", a world wide group of small, translucent, nut brown shells, with a subsutural thread. The name *Angustassiminea* Habe, 1943 is available for ABBOTT's "*nitidula*-complex", type by original designation *Assiminea castanea* Westerlund, 1883. The chromosome number of this species is n = 15 (PATTERSON, 1967). The only other known species having 15 pairs of chromosomes is *Assiminea yoshidayukioi* Kuroda, 1959, which was not referred to one of the "sections" so far described. KURODA (1959) mentioned some features in which the species differs from *A. castanea.* It has, however, the radular formula and other features in common with *A. castanea* and could easily be referred to *Angustassiminea* Habe. Cytological evidence is, as yet, in favour of the acceptance of *Angustassiminea* Habe, 1943, as a valid subgenus in *Assiminea,* the only possibility left open by ABBOTT (1958) for a useful and natural subdivision of the genus.

As to the chromosome numbers the genus *Assiminea* occupies quite an outstanding position among the rissoacean snails. Leaving aside the unreliable record for *Hydrobia aponensis* v. Martens (n = 8-9, RANZOLI, 1950) the chromosome numbers of *Assiminea* are the lowest among the so far cytologically examined Rissoacea.

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