The Chromosome Numbers of certain Barnacles in British Waters

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With two plates (figs. 2 and 3)

Summary

The chromosome numbers of nine species of sessile barnacles have been determined from squashes of young embryos stained by an iron alum aceto-carmine method. All the species of *Balanus* examined, and *Elminius modestus* had a diploid count of 32 chromosomes. *Chthamalus stellatus* and *Verruca stroemia* had each 30 chromosomes. Meiosis occurs after the egg passes into the mantle cavity, and the cytological changes accompanying the extrusion of the two polar bodies are figured.

Introduction

DURING the last 40 years a number of papers have been published dealing with the nuclear cytology and chromosome number of several species of Crustacea, including members of most of the major groups. Very little cytological work, however, has been published on the Cirripedia. This paper gives the chromosome counts and some details of the nucleus of nine species of the commoner barnacles found in British waters.

Chromosome numbers vary considerably among the different groups of Crustacea. In Branchiopoda it is particularly variable, ranging from $2n = 8$ in three species of *Tripos* (Longhurst, 1955) to $2n = 84$ in *Artemia salina* L. (Artom, 1929). The Copepoda generally give rather small diploid counts of 12 to 14 (Stella, 1931; Heberer, 1927). In Amphipoda the counts vary from 26 to 54 (Orian and Callan, 1957); in Isopoda from 16 to 24 (Vandel, 1926). In the Decapoda the chromosome number is very high, four species of Brachyura having 94 to 124 (Niiyama, 1942) and the anomuran *Eupagurus ochotensis* 254 chromosomes (Niiyama, 1951).

In the Cirripedia, if we ignore the approximate haploid count of 4 to 12 made by Groom (1894), chromosome counts have been made only in two species, both belonging to Lepadidae. They are *Lepas anatifera* (L.) which has been reported as having 26 chromosomes (Witschi, 1935), and *Scalpellum scalpellum* L. with 32 chromosomes (Callan, 1941).

Methods

Mitotic and meiotic divisions were studied in alum acetocarmine squash preparations made from developing embryos and oocytes in the egg masses of gravid barnacles.

The success of the preparation was found to depend upon the stage of

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development of the embryo immediately before fixation. The most satisfactory results were obtained from embryos in which the yolk was almost completely covered by blastoderm, but in which limb-bud rudiments had not formed; that is, stages 4–7 in Crisp's (1954) nomenclature. These stages are usually reached a few days after fertilization. In preparations from the later embryonic stages, particularly those which had developed appendages (i.e. stages 9–13), the nuclei were small and rarely in division. It was important to have available embryos of each of the species to be studied in the correct stage of development. We therefore took advantage of concurrent work on temperature conditioning of breeding in Cirripedes (Crisp, 1957) to provide embryos

TABLE I

Sources of material

Species	*Season when young embryos may be collected*	*Collecting ground*
V. stroemia (O. F. Müller)	Nov.–Dec.	Brixham, S. Devon
C. stellatus (Poli)*	June–Sept.	W. Anglesey, Aberffraw
B. balanoides (Linnaeus)	Nov.	Bangor Pier
B. amphitrite var. *denticulata* (Broch)*	May–Sept.	Swansea, Queen's Dock
B. crenatus Bruguière	Jan.–Mar.	Bangor, Penrhyn
B. hameri (Ascanius)	Jan.	Irish Sea
B. perforatus Bruguière	Aug.–Sept.	Gower Peninsula, S. Wales
B. balanus (L.)	Jan.–Feb.	Beaumaris Bay
Elminius modestus Darwin	Mar.–Oct.	Bangor Pier

* Egg masses obtained out of season by maintaining and feeding in warmed sea-water.

from species which were not in the natural breeding condition at the time this work was carried out, viz. November 1957 to January 1958. At this time of year only the embryos of *Balanus balanoides* (L.), *Verruca stroemia* (O. F. Müller), *B. hameri* (Ascanius), and *Elminius modestus* Darwin could be collected in the appropriate stage of development. Egg masses of *B. balanus* (L.) (= *B. porcatus* da Costa) and *B. crenatus* Bruguière had already been fixed during their breeding season early in 1957. The embryos of the southern forms *Chthamalus stellatus* (Poli), *B. amphitrite* var. *denticulata* (Broch), and *B. perforatus* Bruguière were obtained by maintaining the animals at a constant temperature of about 25° C and feeding liberally with planktonic food or *Artemia* larvae. Table I gives the time of year when suitable embryos may be found and the collecting grounds of the species studied.

The technique used to demonstrate the chromosomes is essentially that described by Godward (1948). Modifications of this technique, which had been employed successfully in staining the tiny chromosomes of certain members of the Rhodophyceae (Austin, 1956), were equally successful with this animal material. The schedule differed from the basic aceto-carmine technique (Belling, 1926) in that a separate mordant bath of ferric alum was

employed. With barnacle egg masses this procedure has proved superior both to aceto-carmine used alone and also to aceto-orcein.

The aceto-carmine solution was prepared according to Belling (1926) but with the addition of 1 drop of saturated ferric acetate solution per 10 ml of stain (Thomas, 1940). The schedule is summarized below.

1. Fix in 1:3 acetic alcohol, using absolute methyl alcohol. If necessary store fixed material in deep freeze or transfer to 98% alcohol.
2. Wash out fixative by means of several changes of tap water.
3. Steep for 20–30 min in a 1% aqueous solution of ferric ammonium sulphate.
4. Wash in tap water for 20–30 min with frequent changes.
5. Place small pieces of egg mass or single embryos in a drop of aceto-carmine on a microscope slide and heat repeatedly almost to boiling-point. Replenish the carmine and continue heating until the material has turned a purple-black colour; then place a coverslip over the specimen and blot excess stain away. Squash the stained tissue by exerting careful and controlled pressure on the cover glass with a flat-honed ebony instrument.
6. Ring and seal with glycerine jelly. The preparations remain in perfect condition for months and can be made permanent at any time by any of the usual methods (McClintock, 1929; Bradley, 1948).

The above treatment proved uniformly successful when applied to the egg masses of all the nine species of cirripedes investigated. The degree of spreading required to demonstrate the polar body extrusion and meiotic figures in the large yolky eggs of *B. balanoides* needed, however, exceptionally careful control. The chromosome number has been established after examining at least 20 nuclei of each species.

Observations on mitotic divisions

The observations made on the somatic mitosis of the different species of barnacles are summarized in table 2. In most of the species late prophase, prometaphase, and telophase figures were frequent, whilst metaphase and anaphase were less common. The chromosomes could be counted fairly accurately at prophase but were more distinct in prometaphase, becoming somewhat clumped and less suitable again at late metaphase.

From table 2 it can be seen that the chromosome number of all the six species of *Balanus* investigated and of *E. modestus* is $2n = 32$, whilst that of *C. stellatus* and *V. stroemia* is $2n = 30$. Taking into account the earlier counts of 26 chromosomes in *L. anatifera* (Witschi, 1935) and 32 in *S. scalpellum* (Callan, 1941), it seems that the group is as a whole homogeneous and that polyploidy has played no part in its evolution. It is interesting that in its chromosome number *Elminius* resembles *Balanus* and differs from *Chthamalus*, thus supporting Darwin's views as to its inclusion in the Balaninae rather than in the Chthmalinae. Chromosome numbers, however, have only limited value in the taxonomy of cirripedes, though it is possible that a comparative study of chromosome morphology might reveal interesting results.

TABLE 2

Cytological details of nuclei of young cirripede embryos

Species	No. of nuclei examined	Haploid-chromosome number (*n*)	Diploid-chromosome number (2*n*)	Diameter (in μ) of resting nucleus	Prophase: Average size (in μ) of nucleus (diam.)	Prophase: Average size (in μ) of nucleolus (diam.)	Prophase: Average size (in μ) of chromosomes: largest	Prophase: Average size (in μ) of chromosomes: smallest	Prometaphase: Average size (in μ) of nucleus (diam.)	Prometaphase: Average size (in μ) of nucleolus (diam.)	Prometaphase: Average size (in μ) of chromosomes: largest	Prometaphase: Average size (in μ) of chromosomes: smallest
V. stroemia	46	—	30	—	47·1	3·9	5·5	2·8	36·2	—	2·7	1·4
C. stellatus	30	—	30	5·3	28·2	2·5	4·7	2·2	21·6	—	2·4	1·2
B. balanoides	25	16	32	12·7	32·9	2·1	4·9	2·8	29·9	3·4	3·0	1·7
B. amphitrite	25	—	32	7·6	17·0	4·0	3·6	1·8	17·6	2·3	2·4	1·1
B. crenatus	27	—	32	11·7	32·3	4·2	5·6	2·7	26·7	3·8	3·7	2·0
B. hameri	20	—	32	13·0	44·4	4·0	4·3	1·4	38·0	3·7	4·0	1·8
B. perforatus	28	—	32	8·4	26·0	4·3	5·6	3·0	14·8	—	2·1	1·2
B. balanus	30	—	32	10·1	26·1	5·3	4·4	2·4	29·6	3·5	2·4	1·7
E. modestus	34	—	32	9·2	45·0	2·5	8·9	3·1	31·6	—	2·7	1·4

In general the size of the nuclei in the barnacle embryo is small, smaller indeed than those of the amphipods recently investigated by Orian and Callan (1957) with modern methods. The alum aceto-carmine technique, however, results in a greater degree of swelling and spreading of the chromosomes than is caused by aceto-orcein (Griggs, 1946).

The smallest nuclei and chromosomes belong to *B. perforatus* (fig. 2, E) and *B. amphitrite*, the latter having particularly small chromosomes (fig. 1, B). *E. modestus* (fig. 2, G), *V. stroemia* (fig. 2, F), *B. balanoides* (fig. 3, H), and particularly *B. hameri* (figs. 2, B; 1, A) have comparatively large nuclei.

FIG. 1. Nuclei in late prometaphase of mitosis in (A) *B. hameri*, and (B) *B. amphitrite*; drawn to the same scale. Both show the nucleolar-organizing or nucleolar-zone chromosomes.

The appearance of the nucleoli and the changes which they undergo are constant and characteristic in each species. A single nucleolus is present in the early prophase stages of all the species studied. *B. balanus* has an unusually large and persistent nucleolus (fig. 2, D). In *V. stroemia* and *E. modestus* the nucleolus is rarely demonstrable. *B. hameri* has a small persistent nucleolus and, like *B. crenatus*, shows the relationship between nucleolar organizing chromosomes and the nucleolus. It appears that there are two or three chromosomes involved in the formation of the nucleolus (figs. 2, B; 1, A). The latter may break up during prophase into fragments of different size. These remain attached to the nucleolar-organizing or nucleolar-zone chromosomes (figs. 1, A, B; 2, B, C). These chromosomes can also be seen in *B. perforatus*, *B. amphitrite*, *E. modestus*, and *C. stellatus*. They persist until the end of prometaphase in *B. amphitrite*, *B. balanoides*, *B. crenatus*, *B. perforatus*, and *B. hameri*, but disappear at the end of prophase in the remaining four species.

It was possible to discern the position of the centromeres in the late prophase and early prometaphase chromosomes of most of the species. They can be seen in *E. modestus* (fig. 2, A), *B. hameri* (fig. 2, B), *B. crenatus* (fig. 2, C), *B. balanus* (fig. 2, D), *V. stroemia* (fig. 2, F), and *B. balanoides* (fig. 3, H). Median and submedian positions of the centromere are prevalent.

The contraction of the chromosomes from prophase to metaphase has also been measured in each species. The greatest degree of contraction was observed in the chromosomes of *E. modestus* where they became reduced by 2·1 to 3·3 times their original early prophase length by the end of prometaphase. This can be compared with 2 to 2·6 times in *B. perforatus* and *V. stroemia* and only 1·3 to 1·5 times in *B. crenatus*. The remaining species are closely similar to the last with a contraction of from 1·5 to 1·9.

Observations on meiotic divisions

These observations were carried out chiefly on the oocyte nuclei of *B. balanoides* and to a lesser extent on those of *B. crenatus*. For studying the meiotic divisions of the nuclei of *B. balanoides*, specimens collected in the first week of November were watched in the laboratory until copulation and insemination occurred. The penis was seen to be inserted in a neighbouring individual and a white stream of semen observed flowing within the semi-transparent tissue of the penis. The egg masses of the inseminated individual were fixed in acetic-alcohol at hourly intervals from one to 36 h after insemination had taken place.

The formation and loss of the first polar body followed very soon upon oviposition. During the first hour it was possible to see the first meiotic anaphase within the eggs, which were at this time spherical and surrounded by active sperm and sperm tails. The first meiotic anaphase has a well-marked spindle region and a dark cell-plate zone (fig. 3, A). Fig. 3, B shows the extrusion of the first polar body from the still naked egg; this process is figured by Groom (1894) for *B. perforatus*. The first polar body is small, about 10–11 μ in diameter, smaller in fact than the remaining female haploid nucleus, in which the early anaphase of the second meiotic division can be seen. The second division closely follows the first without an interphase (fig. 3, C). The egg then assumes an ovoid shape and the egg membrane separates from the egg itself and forms a conspicuous egg case within which the embryo develops. It seems likely that the sperm nucleus enters the egg before this membrane is fully formed, though this has not been observed.

FIG. 2 (plate). Mitotic division in cirripede nuclei.

A, *E. modestus*: chromosomes in extended prophase state; indistinct nucleolus at top left.

B, *B. hameri*: nucleus at early prometaphase with nucleolus and nucleolar chromosomes clearly shown; $2n = 32$.

C, *B. crenatus*: early prometaphase nucleus showing $2n = 32$ chromosomes and the large but faint nucleolus.

D, *B. balanus*: early prometaphase showing the 32 chromosomes and the large very distinct nucleolus.

E, *B. perforatus*: later prometaphase; $2n = 32$.

F, *V. stroemia*: a well squashed late prometaphase showing the 30 chromosomes.

G, *E. modestus*: late prometaphase to show the 32 chromosomes.

H, *C. stellatus*: very late prometaphase showing the 30 highly contracted chromosomes and a faintly stained interphase nucleus to left.

I, *V. stroemia*: metaphase plate in oblique lateral view; the 30 chromosomes can be easily counted.

J, *V. stroemia*: late anaphase; groups of 30 chromosomes at each pole.

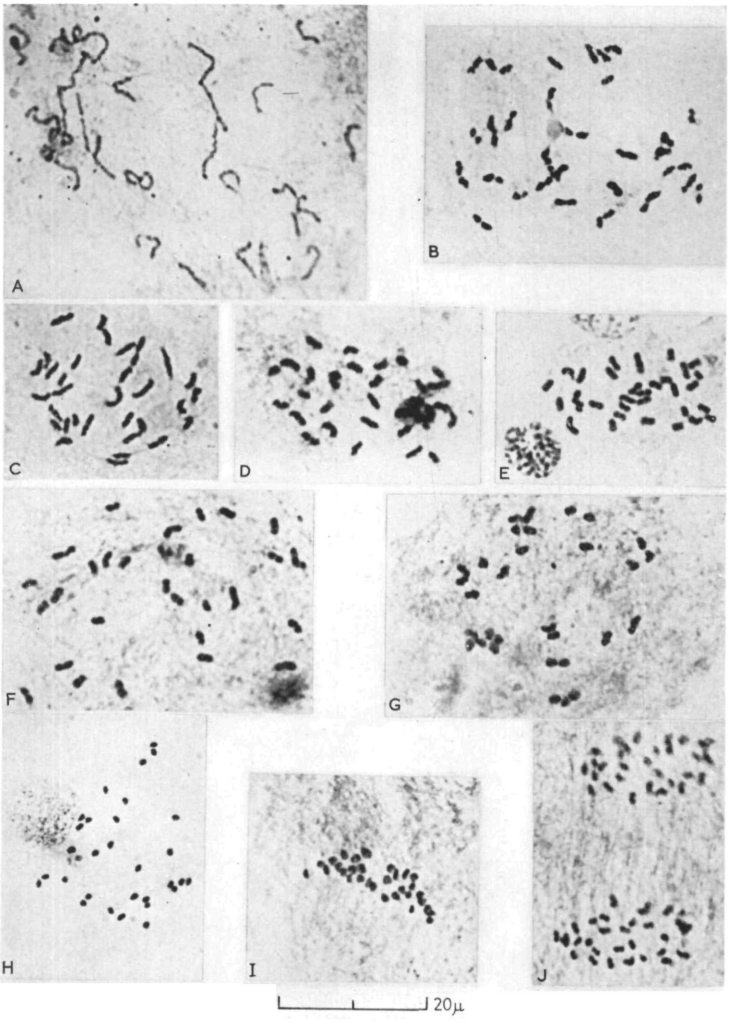

FIG. 2

A. P. AUSTIN, D. J. CRISP, *and* A. M. PATIL

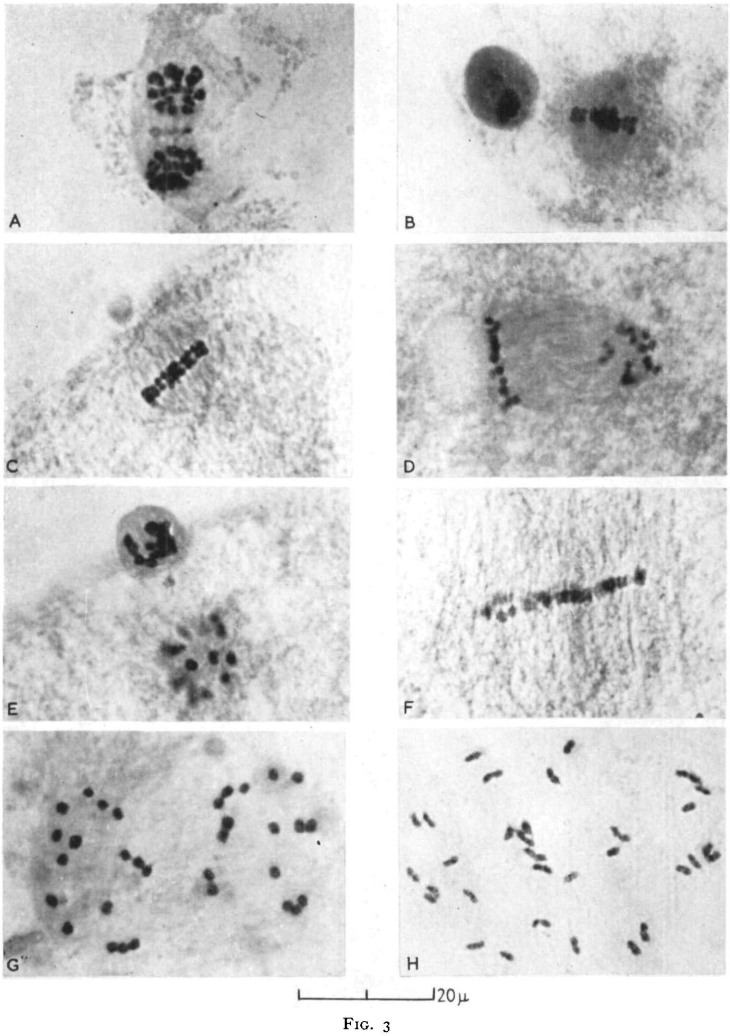

FIG. 3

A. P. AUSTIN, D. J. CRISP, *and* A. M. PATIL

The second polar body (fig. 3, D, E) is formed and extruded inside the fully formed egg case, as described by Groom, where it persists for up to 20 h after insemination. The second polar body has not been seen to divide into two; indeed, the clumping of its chromosomes at the time it is extruded suggests that a further division may not occur. At 14–24 h after insemination the poles of the egg become progressively more conspicuous and at this time the nucleus enters the first cleavage division (fig. 3, F). The first cleavage of the egg usually begins about 20–30 h after insemination, and is closely followed by further divisions. Chromosome counts were made on the female nucleus of *B. balanoides* at late anaphase of the second meiotic division (fig. 3, G). In more than a dozen oocytes the haploid number was established as $n = 16$. This confirms the diploid count of $2n = 32$ in the somatic chromosomes of young embryos of the same species.

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FIG. 3 (plate). A–F, sequence of changes during meiosis in *B. balanoides*.

A, first meiotic telophase. The large chromosomes are closely grouped, the spindle region is distinct and the cell-plate zone rather dark.

B, extrusion of the 1st polar body, in which the chromosomes appear to have become clumped. The remaining female haploid nucleus is entering anaphase of the 2nd meiotic division.

C, 2nd meiotic anaphase after loss of 1st polar body.

D, formation of the 2nd polar body. The spindle region and the vesicle which will separate as the polar body can be clearly seen.

E, extrusion of the 2nd polar body. The chromosomes become clumped together as soon as the polar body separates from the egg.

F, 1st cleavage division. Lateral view of the metaphase plate of the 1st division of the diploid (zygotic) nucleus.

G–H, chromosomes of *B. balanoides*.

G, a well squashed 2nd meiotic anaphase, to show the two groups of 16 chromosomes.

H, a well squashed mitotic prometaphase in *B. balanoides* to show the $2n = 32$ number of chromosomes.

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