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VARIABILITY OF CHROMOSOME NUMBER IN THE LOBSTERS, *HOMARUS AMERICANUS* AND *HOMARUS GAMMARUS*

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SUMMARY — Examination of 42 testicular cells from *H. americanus* yielded a diploid chromosome number of 110 with a range of 94 to 185. The diploid number in *H. gammarus* was determined to be 95.5 with a range of 90.5 to 134.5 The meiotic chromosome number of *H. americanus* was found to be bimodal at 66.5 and 68. The meiotic chromosome number in *H. gammarus* was 62.5. The range of meiotic chromosome counts in *H. americanus* was 50.5 to 83.5. In *H. gammarus* the range was 45 to 88.

The wide range of chromosome numbers in the spermatocytes of both *H. americanus* and *H. gammarus* indicates a strong probability that supernumerary chromosomes exist in both species. Although the size and overlapping of the chromosomes made counting difficult, it is unlikely that these factors alone would result in such a wide variation in chromosome numbers.

INTRODUCTION

Interest in lobsters is on the increase lately due to their potential for commercial aquaculture. The northern lobster, *Homarus americanus*, supports a valuable traditional fishery of the United States, with current annual landings of 37 million pounds worth $ 75.2 million (Fisheries of the United States, 1980, p. ix). Present objectives of lobster hatcheries often include plans for selective breeding and for genetic studies (HUGHES *et al.* 1972).

In recent years the use of organisms for bioassay for marine contaminants has extended into the realm of crustaceans. One of the more ecologically interesting marine crustaceans that might be of use in such bioassays is the lobster, *H. americanus*, and its European counterpart, *H. gammarus* (THURBERG *et al.* 1977).

Although a considerable amount of work has been done on the chromosomes of Crustacea, detailed knowledge of their chromosome morphology has remained limited (NIIYAMA 1959). Less progress has been made on this class than on others of the phylum Arthropoda, e. g., Insecta. A large portion of the chromosome studies on the Crustacea was performed during the early

years of cytogenetics research with poor results. This was most likely due to technical difficulties and equipment limitations.

Much study has been devoted to the chromosomes of decapod Crustacea (NIIYAMA 1959, 1962, 1966). However, relatively few studies have been concerned with two of the most economically important members of this group, the American and European lobsters. LABBÉ (1904) in a report of his studies on the testicular tissue of the European lobster, *H. gammarus*, described his discovery of 18 tetrads in spermatocytes. ROBERTS (1969), studying testicular tissue from *H. americanus*, reported meiotic cells to yield counts of chromosomes that varied widely with a bimodal expression of 69 and 70. Mitotic counts in ROBERTS' study produced a mode of 138 but also with variable counts.

The present work was undertaken to determine if LABBÉ's early work on *H. gammarus* may have suffered from some of the limitations as poor fixation technique and microscopes of limited capability mentioned by NIIYAMA (1959). Another purpose was to investigate the likelihood of accessory (supernumerary) chromosomes in *H. americanus* as suggested by ROBERTS (1969).

MATERIALS AND METHODS

Twelve mature specimens of *H. americanus* were obtained by trapping in Long Island Sound in the immediate vicinity of Milford, Connecticut. Samples of *H. gammarus* testes were taken from thirty-six specimens procured by personnel of the Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Scotland.

Only testicular tissue was available from the *H. gammarus*. Tissue samples taken from the *H. americanus* included testes and seminal canal systems.

Testicular samples were cut into small pieces, approximately 0.25 to 0.5 cm. The pieces of testes were placed in 20 ml of distilled water for 10 to 20 minutes to cause swelling of the cells and thereby facilitate spreading of the chromosome groups.

In an attempt to obtain early stages of meiotic spermatocytes, entire seminal canal systems, from the testes to the seminal ducts, were dissected from several of the *H. americanus*. Excised seminal systems were treated in the same manner as the testicular tissue.

Some pieces of dissected testicular tissue from *H. americanus* were placed in a 2% solution of colchicine in dilute (15 parts per thousand) sea water for 4 hours.

Tissues were placed in Carnoy's fixative (3 parts ethyl alcohol to 1 part glacial acetic acid). Small portions of tissue were removed from the Carnoy's solution after several hours of fixation. These were placed on standard microscopic slides and stained with a solution of 2% orcein in 45% acetic acid. They were then squashed under a #1 coverslip. Slides were sealed with Kronig Cement (Thomas) and examined under a Zeiss phase-contrast microscope with a 100 × objective.

RESULTS

All meiotic divisions observed in both *H. americanus* and *H. gammarus* were in meiosis I. Several, but not all of the stages of meiosis I were observed (Figs. 1-10). No differences were noted between meiosis of *H. americanus* and that of *H. gammarus*.

At pachytene double chromosome strands are readily visible in the testicular tissue. Numerous heterochromatic regions can be seen at this stage. Pairs cannot be counted as individual chromosome configurations are tangled with one another (Fig. 1).

Contraction of the chromosomes is nearly complete by diakinesis. The chromosomes are evenly distributed throughout the nucleus. Homologous chromosomes remain joined to each other, largely at their terminal ends. Points of attachment between the two members of the chromosome pair represent the vestiges of whatever chiasmata had formed earlier (Fig. 2).

The chromosomes in meiotic metaphase I of *H. americanus* and *H. gammarus* are extremely condensed, measuring only 1 or 2 microns in length. Prometaphase groups (Fig. 4), in which the chromosomes are scattered throughout the cell, are frequently observed. At full metaphase the chromosomes are held together on the metaphase plate in a tight cluster (Figs. 5 and 6). Chromosomes of both species appear quite sticky during metaphase. Metaphase plates comprise by far the largest proportion of division groups observed in dissected testes of both species.

The anaphase groups observed in both *H. americanus* and *H. gammarus* appeared normal in all respects. The tight clustering of chromosomes prevented the estimation of the location of centromeres for possible karyotyping. No pronounced separation of the chromatids was seen. There was no evidence of bridging or laggard chromosomes. In only one cell from *H. americanus* was misdivision of the chromosomes noted, a tripolar spindle. One cell from *H. gammarus* exhibited scattering of the chromosomes.

The chromosomes of both species remain highly contracted and tightly clustered during telophase (Fig. 7). As in anaphase, no bridging or laggard chromosomes were seen. Evidence of a sticky condition of the chromosomes was noted in both species (Fig. 10).

The seminal canals did not yield additional stages of meiosis since they apparently contain only fully matured sperm.

No pre-meiotic mitoses were found in either species. The mitotic divisions that were seen in both species of lobster were found in connective tissue in the gonad and did not deviate from the normal mitotic process.

Examination of fresh testicular tissue treated for 10 to 20 minutes with distilled water prior to fixation and staining yielded the highest number of

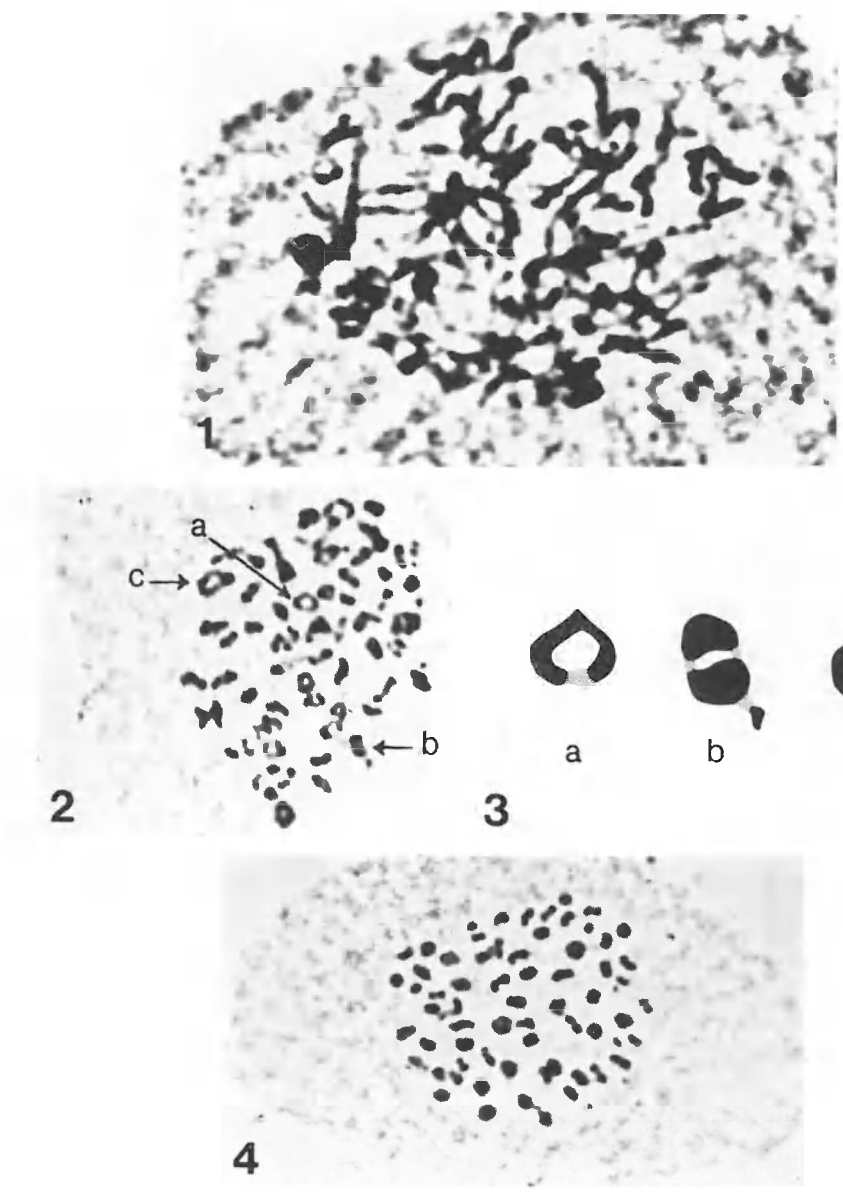
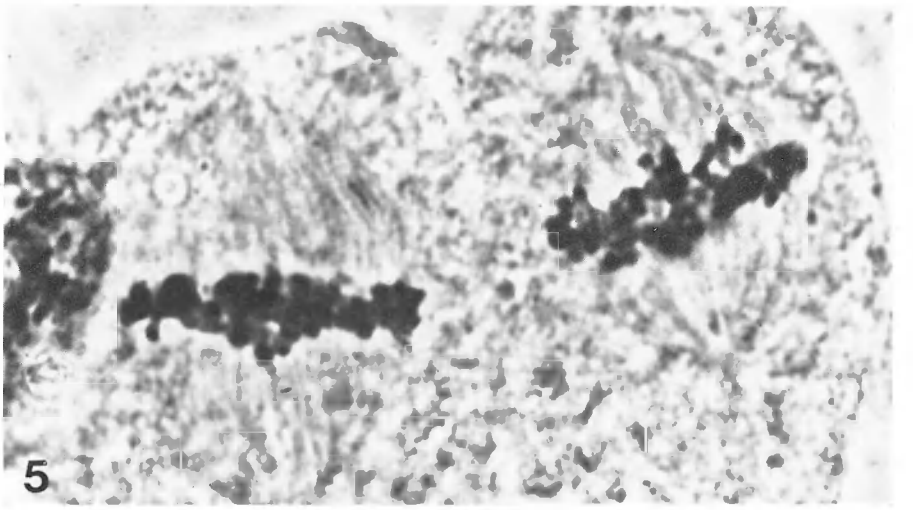
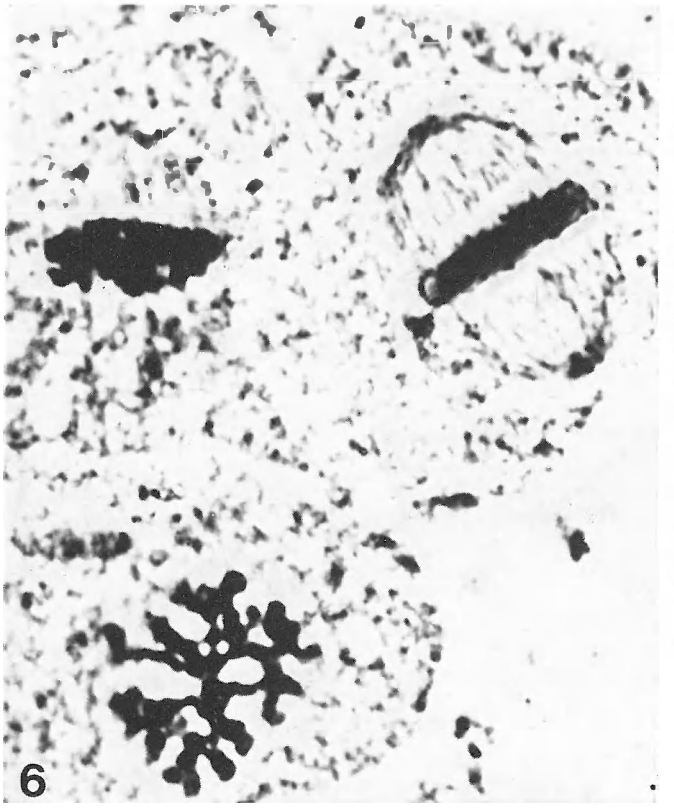


Fig. 1. — Pachytene division from *H. americanus* testis.
Fig. 2. — Testicular cell from *H. americanus* in diakinesis.
Fig. 3. — Interpretive drawings of chromosomes (a, b and c) as seen in Fig. 2 which show evidence of chiasmata.
Fig. 4. — Prometaphase group in testicular cell taken from *H. americanus*.





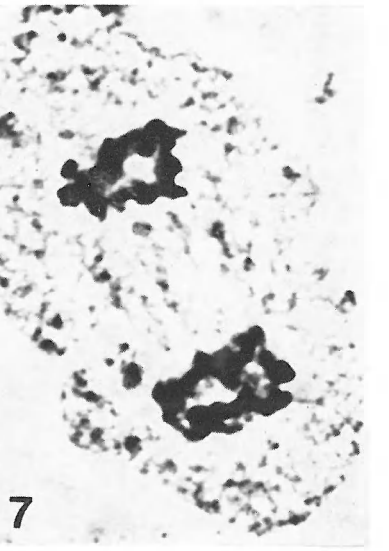
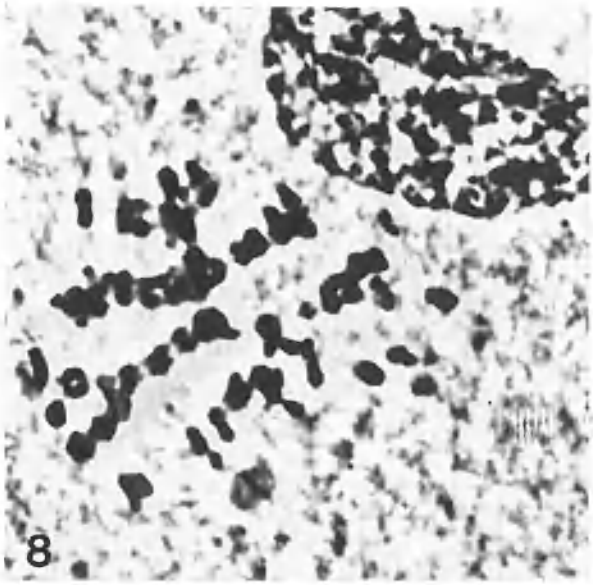
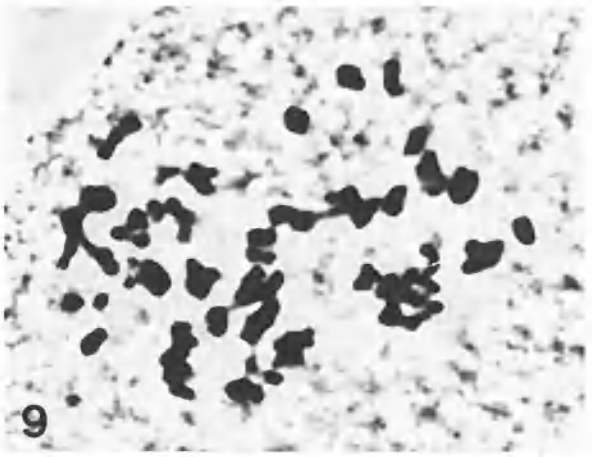


Fig. 5. — Two metaphase I groups in testicular cells from *H. americanus*.
Fig. 6. — Lower cell is at prometaphase showing slightly sticky condition of chromosomes. Cell at upper left is at early metaphase. Cell at upper right is at full metaphase showing contraction of chromosomes into a metaphase « plate » on the spindle.
Fig. 7. — Testicular cell from *H. americanus* at telophase I.





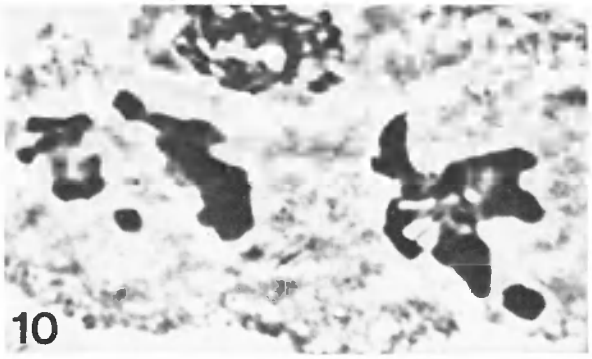


Fig. 8. — Prometaphase division group from *H. americanus* testicular cell which demonstrates stickiness of chromosomes commonly observed in both *H. americanus* and *H. gammarus*.
Fig. 9. — Sticky prometaphase group from *H. gammarus* testis.
Fig. 10. — Extremely sticky groups of chromosomes in such an array as to make it impossible to determine if two metaphases are present, or one telophase. Taken from *H. americanus* testis.

countable chromosome groups. A total of 114 *H. americanus* chromosome groups from 12 animals were examined. Of the 114 *H. americanus* groups, 42 were mitotic and 72 meiotic. In the counts of *H. gammarus* chromosome groups from 36 animals, 35 were mitotic and 96 meiotic. One apparent tetraploid was noted in a sample taken from *H. americanus*, but no determination of chromosome number was made.

The mean of the 42 counts of *H. americanus* mitotic groups is 115.9. The mean of *H. gammarus* mitotic groups is 100.5 for 35 counts (Table 1).

TABLE 1 - *Chromosome counts in mitotic division groups in the testes of « Homarus americanus » and « H. gammarus ».*

	H. americanus	*H. gammarus*
Mean	115.89 (42)	100.53 (35)
Mode	110.0	95.5
Range	94-185	90.5-134.5

Number in parenthesis indicates sample size.

The mode of the mitotic counts in *H. americanus* is 110.0. The mode is 95.5 in *H. gammarus*. Counts of chromosomes in mitotic groups range from 94 to 185 in *H. americanus*. In *H. gammarus* the counts range from 90.5 to 134.5.

Chromosome counts of 72 meiotic groups in *H. americanus* yield a mean of 66.1. In *H. gammarus* the mean is 65.0 for 96 groups (Table 2). Modes

TABLE 2 - *Chromosome counts in mitotic division groups in the testes of « Homarus americanus » and « H. gammarus ».*

	H. americanus	*H. gammarus*
Mean	66.09 (72)	64.98 (96)
Mode	66.5 and 68.0	62.5
Range	50.5-83.5	45-88

Number in parenthesis indicates sample size.

of meiotic chromosome numbers in *H. americanus* are 66.5 and 68.0 (bimodal). In *H. gammarus* the mode is 62.5. The range of chromosome numbers in meiotic groups in *H. americanus* is from 50.5 to 83.5; in *H. gammarus* the range is from 45.0 to 88.0.

Other tissues from *H. americanus* were examined as possible sources of countable chromosome groups. These were antennal gland, digestive gland, blood, and muscle. None of these tissues yielded any countable groups of chromosomes, even after incubation treatment with a 2% solution of colchicine for 40, 60, and 120 minutes, and 24 hours.

DISCUSSION

There is a substantial amount of information concerned with the chromosome complements of the Crustacea (NIIYAMA 1959). The crustaceans whose chromosomes are enumerated include 26 species of decapods, 6 species of isopods, and 19 species of amphipods (NIIYAMA 1959, ORIAN and CALLAN 1957). The chromosome numbers in these species range as high as a haploid number of 188 in the crayfish, *Pacifastacus trowbridgii* (NIIYMA 1962). NIIYMA (1959) reports the chromosome number of the spiny lobster, *Panulirus japonicus*, as N = 70.

In their paper on the chromosome numbers of gammarids, ORIAN and CALLAN (1957) found several species which yielded variable numbers. The number of chromosomes found in oocytes at second meiotic metaphase in *Gammarus pulex* ranges from 52 to 54. In *Marinogammarus marinus* first meiotic anaphase and second meiotic metaphase chromosomes number 50, 51, or 52 in different oocytes. Examination of *M. pirloti* oocytes at second meiotic metaphase and accompanying polar bodies yields chromosome numbers of 59, 61, 62, and 63. ORIAN and CALLAN eliminate the possibility of the existence of a Robertsonian system as the basis of chromosomal polymorphism in the gammarids (since there were no changes in karyotype) and adopt the supposition of the presence of supernumerary chromosomes.

The wide range of chromosome numbers encountered in the examination of spermatocytes of both *H. americanus* and *H. gammarus* in this study indicates a strong probability that supernumerary chromosomes exist in both species. The bimodal nature of meiotic chromosome numbers in *H. americanus* supports this view. Although the small size and overlapping of the chromosomes made counting difficult, it is unlikely that these factors alone would result in such a wide variation in chromosome numbers. ROBERTS (1969), facend with similar data in his study of *H. americanus*, also assumed the presence of supernumeraries.

BATTAGLIA (1964) listed over 160 species of higher plants, belonging to 18 families, as containing supernumerary chromosomes. Mosses and liverworts are also known to possess supernumeraries in many of their species (WIGH 1973).

WHITE (1973) numbers the insect species which have supernumeraries as being near 100. The grasshopper family, Acrididae, is especially endowed with species which contain supernumeraries (MÜNTZING 1974). According to WHITE (1973) only 13 species other than insects have supernumeraries. Those 13 species represent Platyhelmintes (2), Mollusca (2), Crustacea (4), and Vertebrata (5). MÜNTZING (1974) adds to the list a marsupial, *Echymipera kalabu*, a field mouse, *Apoclemus giliacus*, and the Malayan house rat, *Rattus*

rattus diardii. The lobsters, *H. americanus* and *H. gammarus*, on the basis of the present study, should also be added to this list.

Postmeiotic nondisjunction has been shown to be the major cause of numerical increase of supernumeraries in rye and other species of grasses (BOSEMARK 1957). Other mechanisms of numerical increase of chromosomes exist in plants: directed distribution (RUTISHAUSER 1956), somatic nondisjunction (RUTISHAUSER 1960; RÖTHLISBERGER 1971), and endomitotic reduplication (FRÖST 1960). Mitotic nondisjunction (NUR 1969), preferential segregation (NUR 1966), endomitotic reduplication (MELANDER 1950) and asynchronous division coupled with nondisjunction (SHELLHAMMER 1960), all have been shown to exist in various species of animals.

In the lobsters, *H. americanus* and *H. gammarus*, no evidence of micronuclei was seen in any stage meiosis or mitosis. Micronuclei would be indicative of any chromosomes being lost or cast off during division, thus resulting in variation in chromosome number. No other signs of misdivision, such as laggard chromosomes or chromosomes located off the spindle, were seen. This lack of misdivision is surprising when the stickiness of the anaphase groups is taken into consideration. It appears plausible that in both species of lobster a nondisjunction-accumulation mechanism is in action, which probably varies in effectiveness from cell to cell. The inherent difficulties in counting lobster chromosomes, along with the other variation, would make it almost impossible to detect differences in supernumerary numbers among individual lobsters. This, though, might be expected to occur on the basis of the variable chromosome numbers observed in the spermatocytes.

According to WHITE (1973), the genetical properties of supernumeraries must be so slight that individuals possessing several (or none) are viable and differ from individuals with different numbers of supernumeraries in ways which are so subtle as to escape notice. Although the function of supernumerary chromosomes has not been discovered, geneticists are in general agreement that supernumeraries are of adaptive importance since their occurrence and frequency within the species vary between populations of different origin and habitat. LEE (1968) demonstrated that the frequency of supernumeraries is higher in rye plants growing on acidic soils than those growing on basic soils. COATES (1980) found that in the Australian triggerplant of the genus *Stylidium* supernumerary, or B, chromosomes are of two types: macro B chromosomes and micro B chromosomes. The macro type is found over a substantial portion of the species range, while the micro type is found in the more medial regions of the species range.

TRACEY *et al.* (1975) in their study of the biochemical genetics of eight populations of the American lobster, *H. americanus*, found evidence of rather low levels of genetic variability. While supernumerary chromosomes are gen-

erally believed to be inert genetically, it is still probable that they make some contribution to the genetic variability which the lobster does possess. It appears likely that all of the populations of lobster so far examined biochemically and cytogenetically are in states of environmental stress. The presence of supernumerary chromosomes in the karyotype of the lobster may be serving to increase by a small, but significant amount the reservoir of genetic material the animal may utilize to adapt to the constantly changing environment in which it lives.

REFERENCES

BATTAGLIA E., 1964. — *Cytogenetics of B-chromosomes.* Caryologia, 17: 245-299.

BOSEMARK N. O., 1957. — *On accessory chromosomes in Festuca pratensis. V. Influence of accessory chromosomes on fertility and vegetative development.* Hereditas, 43: 211-235.

COATES D. J., 1980. — *B chromosomes in Stylidium crossocephalum (Angiospermae: Stylidiaceae).* Chromosoma, 77: 347-358.

FISHERIES OF THE UNITED STATES, 1980. — U. S. Department of Commerce (1981), National Marine Fisheries Service, 132 pp.

FRÖST S., 1960. — *A new mechanism for numerical increase of accessory chromosomes in Crepis pannonica.* Hereditas, 46: 497-503.

HUGHES J. T., SULLIVAN J. J. and SHLESER R., 1972. — *Enhancement of lobster growth.* Science, 177: 1110-1111.

LABBÉ A., 1904. — *Sur la formation des tetrades et les divisions maturatives dans le testicule de l'homard.* C. R. Acad. Sci. Paris, 138: 96-99.

LEE W. J., 1968. — *The frequency and geographical distribution of rye with accessory chromosomes in Korea.* Proc. 12th Int. Congr. Genet., 2: 118.

MELANDER Y., 1950. — *Accessory chromosomes in animals, especially in Polycelis tenuis.* Hereditas, 35: 261-296.

MÜNTZING A., 1974. — *Accessory chromosomes.* In: Annual Review of Genetics, edited by H. L. Roman, A. Campbell and L. M. Sandler, 8: 243-266.

NIIYAMA H., 1959. — *A comparative study of the chromosomes in Decapods, Isopods, and Amphipods, with some remarks on cytotaxonomy and sex determination in the Crustacea.* Mem. Fac. Fish. Hokkaido Univ., 7: 1-60.

—, 1962. — *On the unprecedentedly large number of chromosomes of the crayfish Astacus trowbridgii: Stimpson.* Annot. Zool. Japan, 35: 229-233.

—, 1966. — *The chromosomes of two species of edible crabs (Brachyura, Decapoda, Crustacea).* Bull. Fac. Fish. Hokkaido Univ., 16: 201-205.

NUR U., 1966. — *Harmful supernumerary chromosomes in a mealy bug population.* Genetics, 54: 1225-1238.

—, 1969. — *Mitotic instability leading to an accumulation of B-chromosomes in grasshoppers.* Chromosoma, 27: 1-19.

ORIAN A. J. E. and CALLAN H. G., 1957. — *Chromosome numbers of gammarids.* J. Mar. Biol. Assoc. U. K., 36: 129-142.

ROBERTS F. L., 1969. — *Possible supernumerary chromosomes in the lobster, Homarus americanus.* Crustaceana, 16: 194-196.

RÖTHLISBERGER E., 1971. — *Verteilung der B-chromosomen und blutenentwicklung bei Crepis capillaris.* Ber Schweiz. Bot. Ges., 80: 194-224.

RUTISHAUSER A., 1956. — *Genetics of fragment chromosomes in Trillium grandiflorum.* Heredity, 10: 195-204.

—, 1960. — *Telocentric fragment chromosomes in Trillium grandiflorum.* Heredity, 15: 241-246.

SHELLHAMMER H. S., 1960. — *Supernumerary chromosomes of the harvest mouse, Reithrodontomys megalotis.* Chromosoma, 27: 102-108.

THURBERG F. P., CALABRESE A., GOULD E., GREIG R. A., DAWSON M. A. and TUCKER R. K., 1977. — *Response of the lobster,* Homarus americanus, *to sublethal levels of cadmium and mercury.* In: « Physiological Responses of Marine Biota to Pollutants »; edited by F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg, pp. 185-197. Academic Press, New York.

TRACEY M. L., NELSON K., HEDGECOCK D., SHLESER R. A. and PRESSICK M. L., 1975. — *Biochemical genetics of lobsters: genetic variation and the structure of American lobster* (Homarus americanus) *populations.* J. Fish. Res. Board Can., 32: 2091-2101.

WHITE M. J. D., 1973. — *Animal cytology and evolution.* Cambridge Univ. Press, 3rd ed., 961 pp.

WIGH K., 1973. — *Accessory chromosomes in some mosses.* Hereditas, 74: 211-224.

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