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CHROMOSOME NUMBER, STRUCTURE AND AUTOSOMAL POLYMORPHISM IN THE MARINE MUSSELS *MYTILUS EDULIS* AND *MYTILUS CALIFORNIANUS*[1]

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The bay mussel *Mytilus edulis* (Linnaeus) and the California sea mussel *Mytilus californianus* (Conrad), are polymorphic in color, shape and size. They are found intertidally on rocks, stones, or pilings and sometimes in deeper water down to 10 to 20 or 25 fathoms (Soot-Ryen, 1955). *Mytilus edulis* is found in several parts of the world and has been considered by some as a cosmopolite, although this opinion has been debated by Soot-Ryen (1955). *M. edulis* is native to the Pacific coast of North America, but may occur sympatrically with *M. californianus* which is common along the more exposed beaches in crashing waves and, indeed, occurs only where there is surf (Ricketts, Calvin, and Hedgpeth, 1968). Along the exposed beaches, the presence of *M. edulis* appears to be related to the presence of fresh water streams, and at such places individuals of both species may occupy the same rocks. The two species are easy to distinguish except that very small specimens may be difficult to separate from one another.

Mytilus edulis is normally dioecious but rare hermaphroditic individuals can be found (Sagiura, 1962). While reviewing reproduction in molluscs, Fretter and Graham (1964) indicated that individuals of *M. californianus* emitting sperms or eggs are true males and females and that there are no immature gametes of the opposite sex in the gonad. Both species broadcast their gametes and fertilization occurs externally.

On the West coast of the United States, *M. edulis* commences spawning in late April or early May and continues until late August. Young (1942) indicated that in *M. californianus,* beginning in September, spawning gradually increases to reach a maximum in mid-winter and then declines to a minimum from May to August. Occasionally they will spawn in summer, but usually only to a limited extent.

Both species have served as popular research material for embryological and physiological studies. However, nothing is known of their comparative cytogenetics. It is not known whether the forms of the two species are ecophenotypes or

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are genetically different. However, Schlieper (1957) indicated that *M. edulis* populations in the region of the North Sea (30‰) and Western Baltic (15‰) did not belong to genetically or physiologically distinct races since physiological differences disappeared when specimens from the two populations were transposed.

A chromosome study of the two species from several intertidal locations along the Pacific coast of the United States was, therefore, initiated to elucidate the cytogenetic structure.

Material and Methods

Mussels were obtained from several rocky intertidal beaches in Puget Sound and the Pacific coastline of the United States. All observations of chromosome morphology were made from unfertilized eggs (primary oocytes), fertilized eggs, and 2 to 6 cell embryos and morulae. Eggs needed for intraspecific crosses were obtained either by the induction of artificial spawning or by stripping the ripe gonads. When sexually mature, both species spawned after rough handling, shaking in a bucket, by changing of water or by thermal shock. Spawning behavior of the two species is, however, very much unpredictable.

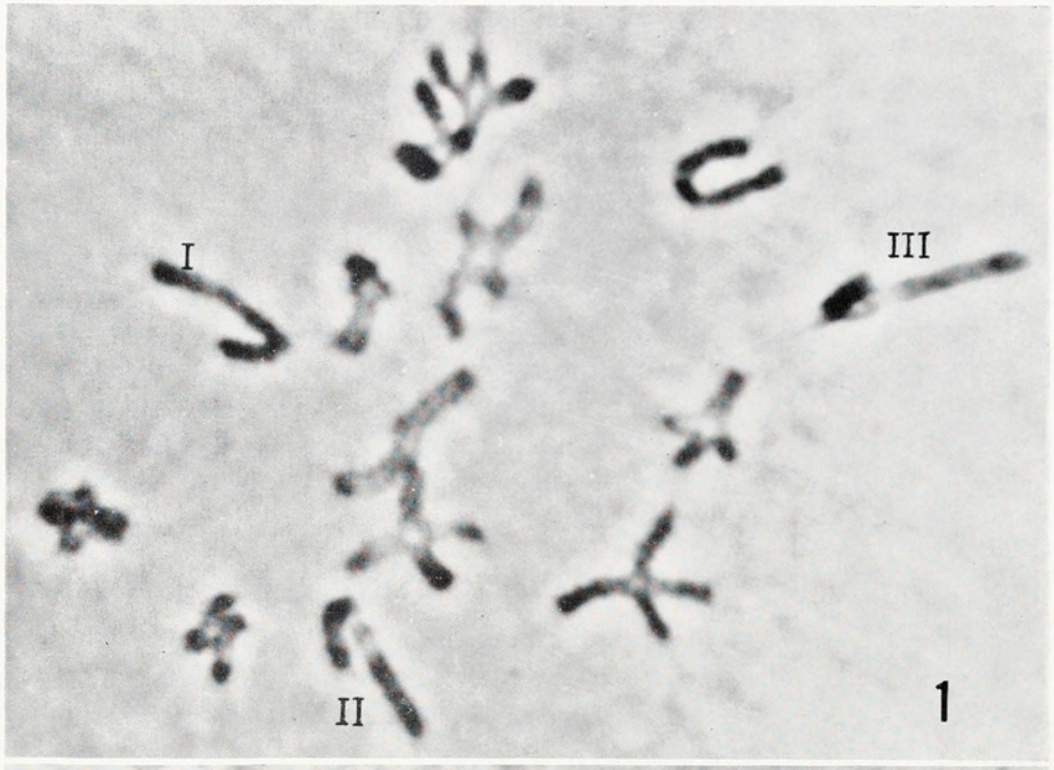
A single *M. edulis* can produce eggs in the number of 10^7 (Fretter and Graham, 1964) and *M. californianus*, probably as many or more. The ripe eggs of the two species obtained by spawning or stripping the gonads measure approximately 60 μ in diameter and are somewhat opaque in *M. edulis* and orange colored in *M. californianus*.

Intra-specific crosses were made by adding a few drops of very dilute sperm suspension to thousands of eggs contained in 500 ml of filtered sea water in a beaker. Fertilization and cleavage occurred at room temperature. Samples of eggs and embryos were fixed and preserved in 1:3 acetic alcohol (Glacial acetic acid and absolute ethyl alcohol). All chromosome observations were made from temporary aceto-orcein stained squash preparations of eggs and embryos. Squashes were made as follows: several embryos were placed on a slide and air dried; stained in

Table I

Number of mussels examined from different locations

	Location	Number of animals examined
Mytilus edulis		
State of Washington	Golden Gardens (Seattle)	3
	Alki Point (Seattle)	2
	Bainbridge Island	3
	Coupville	3
State of California	Humboldt Bay	5
Mytilus californianus		
State of Washington	Agate Beach	4
	Clallum Bay	1
	Cattle Point (Friday Harbor)	1
	Port Townsend	2
	Cape Flattery	3
State of Oregon	Cannon Beach	3
State of California	Morro Bay	5



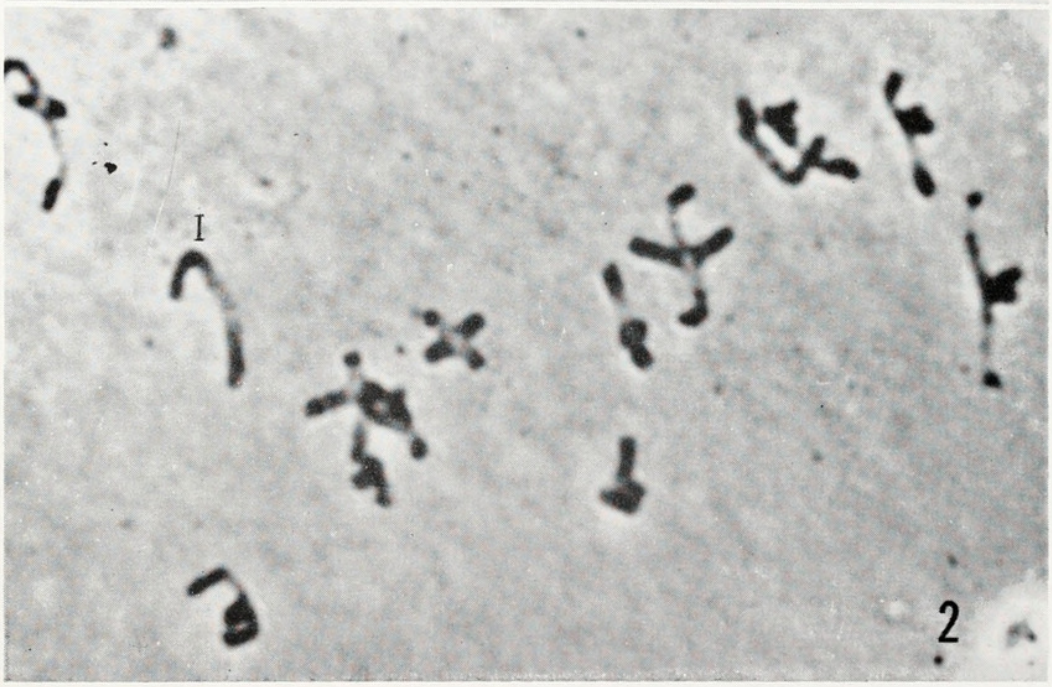


FIGURE 1. Polar view of fourteen metaphase I bivalents of *Mytilus edulis* from Golden Gardens; I, II and III heteromorphic bivalents, × 1512, phase contrast.

FIGURE 2. Equatorial view of fourteen metaphase I bivalents of *Mytilus californianus* from Agate Beach; I heteromorphic bivalent, × 1512, phase contrast.

a drop or two of aceto-orcein for two to several minutes; squashed under a coverslip; extra stain was blotted off and the slide was heated on a flame for a few seconds. This could be examined immediately or sealed off by applying a mixture of lanolin and paraffin wax and kept for later use.

Chromosome plates were examined and photographed on a Zeiss Photomicro-

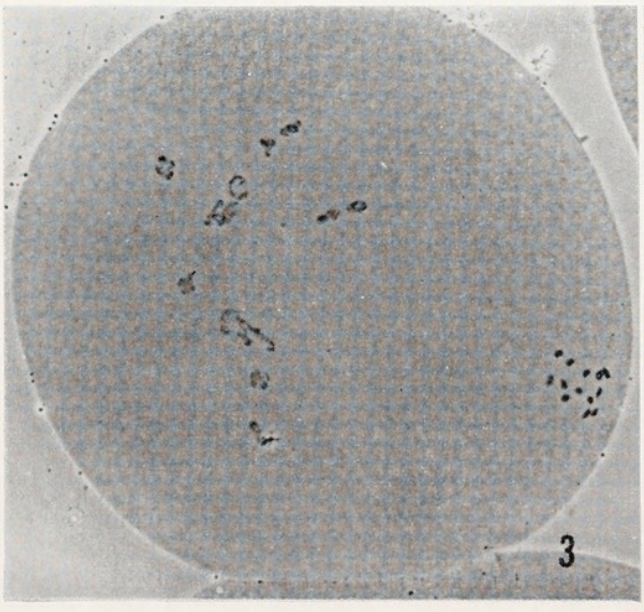
scope employing a green or blue green filter. Camera lucida drawings of chromosomes were made on an American Optical scope using 15 × oculars and 97 × objectives. Extreme care was exercised while making camera lucida drawings so that the proportion of chromosome arms and absolute lengths were not distorted.

Observations

The number of mussels examined cytologically from the respective collection sites is shown in Table I. Suitable material of *M. edulis* was available from Golden Gardens in Seattle, Washington, and Humboldt Bay in California, and for *M. californianus* from Agate Beach and Cape Flattery in the State of Washington. In the material from other localities, counts of meiotic bivalents could be made but a detailed study of their morphology was not possible due to their extreme condensation, crowding and some degree of stickiness.

Meiosis and mitosis

In the two mussels, a natural blocking of meiosis occurs at prometaphase or metaphase I. In the spawned eggs (primary oocytes), the meiotic bivalents were usually observed at prometaphase to metaphase I (Figs. 1 and 2), although diplotene-diakinesis stages were seen also, in evidently immature eggs. Meiosis is completed after the reactivation of the eggs by a fertilizing sperm. The pronucleus of the sperm could often be seen in the egg cytoplasm and sometimes the chromosomes of the male genome were clearly observed (Fig. 3). After fertilization, maturation divisions followed, polar bodies were extruded (Fig. 4) and cleavage occurred. Stages of mitosis were easily seen in cleaving eggs.



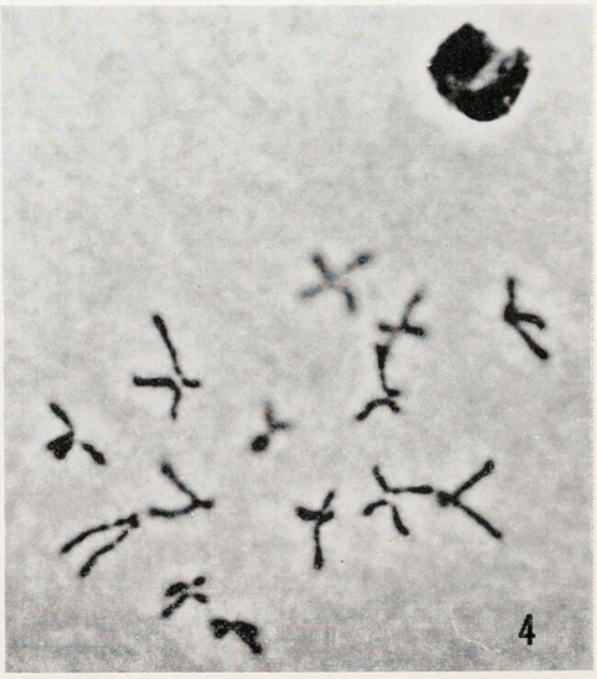


Figure 3. A fertilized oocyte of *M. edulis* showing metaphase I bivalents in the center and 14 chromosomes of the male genome at 4 o'clock position. Two chromosomes may appear as one due to close proximity, × 480, phase contrast.

Figure 4. Fourteen metaphase II chromosomes and polar body extrusion in *M. edulis*, × 1200, phase contrast.

Size and counts of chromosomes

During the meiotic prometaphase the bivalents of the two mussels showed some degree of stretching. The largest bivalent of *M. edulis* stretched in some cases to 11 μ and that of *M. californianus* to 15 μ. At metaphase I the bivalents of the two species measured approximately 8 to 3 μ. The mitotic chromosomes, at mid-metaphase, measured approximately 10 to 3 μ in 4 to 6 cell stage embryos. Both meiotic and mitotic chromosomes showed a slight decrease in size from the largest to the smallest, which made their individual identifications difficult.

In hundreds of eggs examined from both mussels, a constant count of 14 bivalents was made ($n = 14$) and is, therefore, consistent for 16 specimens of *M. edulis* and 19 specimens of *M. californianus* from different localities (Table I). Fourteen haploid chromosomes of the male genome were seen in fertilized but uncleaved eggs (Fig. 3). The reduced number of 14 chromosomes could be observed at all other stages of maturation divisions and especially at metaphase II of female meiosis (Fig. 4).

Mitotic chromosome counts were made from *M. edulis* material from Golden Gardens and Humboldt Bay and of *M. californianus* from Agate Beach and Cape Flattery. Twenty eight chromosomes were generally counted (Figs. 5 and 6) in mitotic plates of both species ($2n = 28$) but hyperdiploid sets of 29 or 30 and such atypical sets as 27, 26, 25, 24 and others also occurred. A mitotic plate of *M. edulis* with 26 chromosomes is shown in Figure 7. In one 5-celled embryo of this species, one nucleus was at interphase but the other four contained 19 to 20 metaphase chromosomes. In *M. californianus,* aneuploid counts of similar nature were made. Approximately 5–10% of the nuclei contained atypical plates. In both species haploid metaphases with 14 chromosomes were also observed and polyploid sets were also present in less than 1% of the examined mitotic plates.

Chromosome terminology

The following terminology was used in the characterization of chromosome shapes: metacentric (M) two armed, and the relative lengths of the arms approach unity; submetacentric (S) unequal armed, J-shaped chromosome; acrocentric (A) one very small and another very large arm, the centromere is subterminal and interstitial; telocentric (T) one armed, the centromere being strictly terminal. The definitions of metacentric, acrocentric, and telocentric chromosome are borrowed, after slight rewording, from John and Lewis (1968). The distinction between metacentric, submetacentric, and acrocentric chromosome is clearly a relative one. Truly equal armed metacentrics were few, so that slightly unequal armed V-shaped chromosomes were also included in this category. Chromosomes in which the ratio of the small to large arm was approximately 1:2 were considered submetacentric.

Chromosome morphology

No differential heteropycnosity was observed in any of the bivalents or mitotic chromosomes at any observable stage of meiosis or mitosis, so, on this basis, the chromosomes are all autosomal.

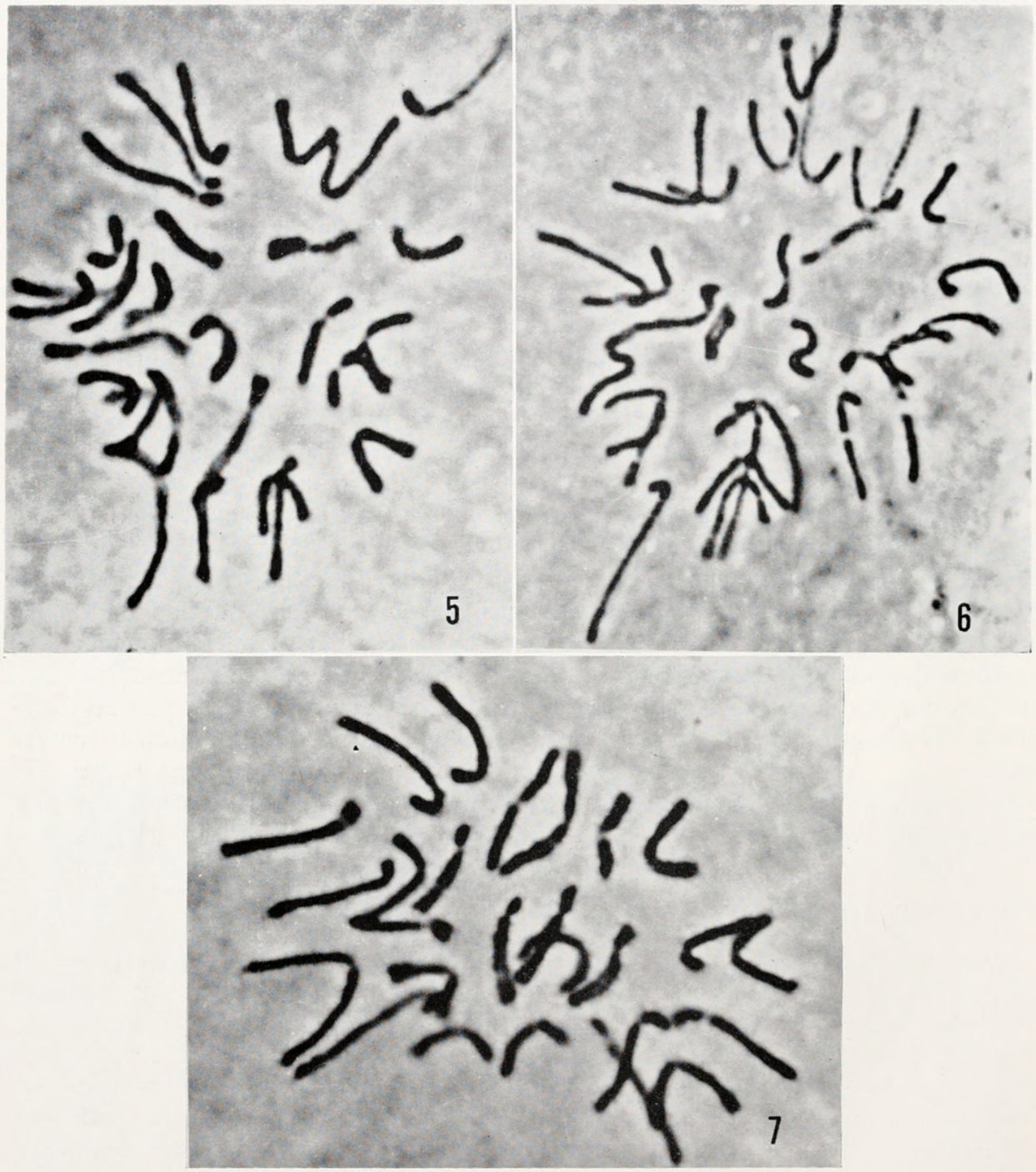


FIGURE 5. Polar view of 28 mitotic metaphase chromosomes of *M. edulis,* × 1512, phase contrast.

FIGURE 6. Polar view of 28 mitotic metaphase chromosomes of *M. californianus,* × 1512, phase contrast.

FIGURE 7. Aneuploid mitotic plate of *M. edulis* with 26 chromosomes, × 1920, phase contrast.

Camera lucida drawings of the meiotic bivalents of *M. edulis* from Humboldt Bay and *M. californianus* from Cape Flattery are shown in Figures 8 and 9, respectively. In these, bivalents were arranged according to the diminishing order of their lengths and the assignment of positions is arbitrary. In the standard meiotic complements of the two mussels (row C, Figs. 8 and 9) six to seven

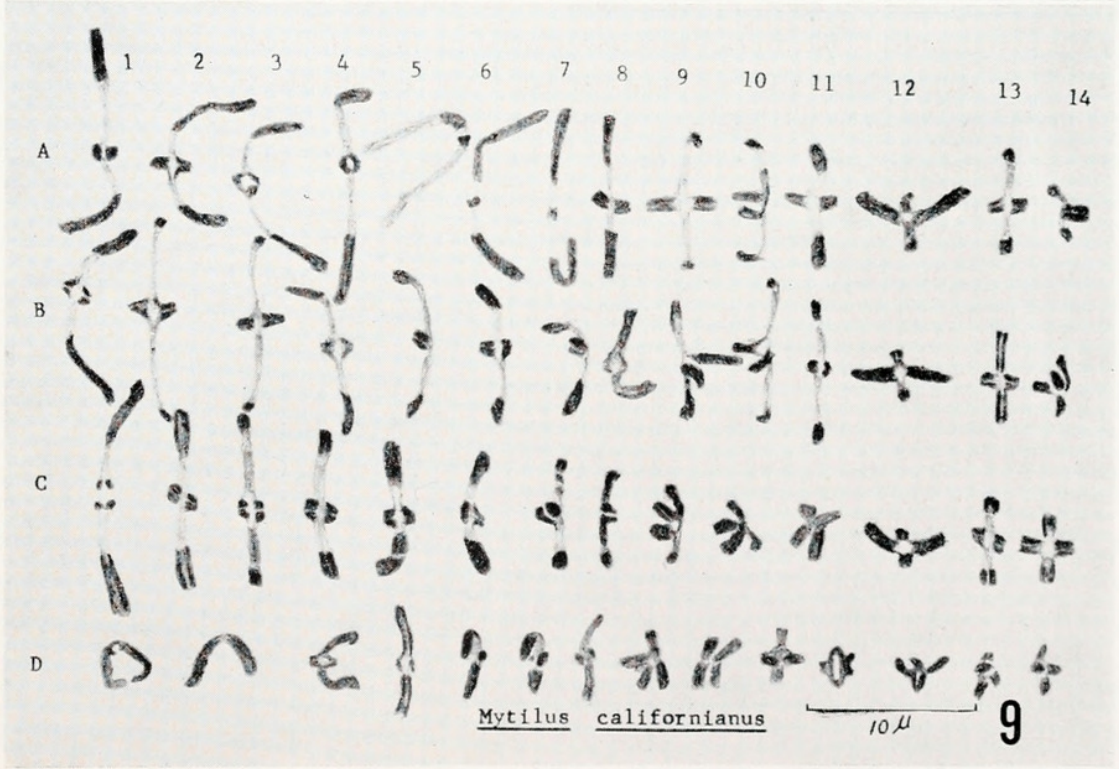


FIGURE 8. Camera lucida drawings of prometaphase and metaphase I bivalents of a single specimen of *M. edulis* from Humboldt Bay.

FIGURE 9. Camera lucida drawings of prometaphase and metaphase I bivalents of a single specimen of *M. californianus* from Cape Flattery.

bivalents resembled crosses of equal or unequal arms and possessed interstitial chiasmata (see also Figs. 1 and 2) and three to four bivalents were rod like. Each of the 14 bivalents of the two mussels possessed, generally, a single chiasma at prometaphase or metaphase I. Based on the analysis of the female metaphase I bivalents the chiasma frequency in the two mussels amounts to 1.0 and the recombination index 28. This was, however, exceeded in some plates due to the presence of a single ring bivalent which was observed in approximately 5% of the examined plates of *M. edulis* and even fewer plates of *M. californianus.*

Morphology of mitotic chromosomes was critically examined in camera lucida drawings of 10 complements of *M. edulis* and 8 of *M. californianus,* although numerous other plates were observed for this purpose. The centromeric positions of the chromosomes were clearly seen so that their characterization as metacentric, submetacentric, and acrocentric was relatively easy. In both species, usually three pairs of chromosomes were acrocentric and the rest metacentric or submetacentric. Telocentric chromosomes were either absent or only an occasional one was observed. Secondary constrictions were generally not seen, although they may be present; for instance on chromosome 1 in row A (Fig. 11).

Chromosome polymorphism

Structural chromosome polymorphism in both meiotic and mitotic chromosomes was observed and was attributed to the presence of pericentric inversions or centric-shifts (centromere shifts) in autosomes.

In approximately 5% of the meiotic plates of both mussels, along with cross and rod like bivalents, J-shaped or inverted J-shaped bivalents were observed or an occasional asymmetrical bivalent was seen. These were clearly heteromorphic bivalents, that is, the centromeres of the chromosomes composing them existed at different positions. For example, the three bivalents of *M. edulis* marked I, II, and III (Fig. 1) are heteromorphic and so is bivalent I of *M. californianus* (Fig. 2). Similarly, bivalent 4 of *M. edulis* (row A, Fig. 8) and bivalent 6 and 7 of *M. californianus* (row A, Fig. 9) are also heteromorphic. These latter two bivalents of *M. californianus* are composed of metacentric and acrocentric chromosomes and resemble the bivalents heterozygous for "elastic constrictions" reported by White (1957) in morabine grasshoppers. The frequency of such bivalents remains to be determined but they did not appear to be present in more than 5% of the meiotic plates examined from a single specimen of *M. californianus* from Cape Flattery. Meiotic complements shown in row A of Figures 8 and 9 are inversion or centric-shift heterozygotes, since they contain hetermorphic bivalents.

Another aspect of polymorphism in meiotic bivalents involved the substitution of metacentric or ring bivalents in place of acrocentric or telocentric rod bivalents and vice versa. In *M. edulis,* bivalent 8 (row B and D, Fig. 8) is a ring bivalent and may be an alternate form of the acrocentric bivalents at the same position in row A and C of this figure. Bivalent 2 and 3 of *M. californianus* (row B, Fig. 9) are acrocentric bivalents, but bivalents 2 and 3 in row A of the same figure are composed of metacentric chromosomes. Bivalent 11 of this species (row D, Fig. 9) is a ring bivalent although others at this position in rows A to C are rod or cross like bivalents.

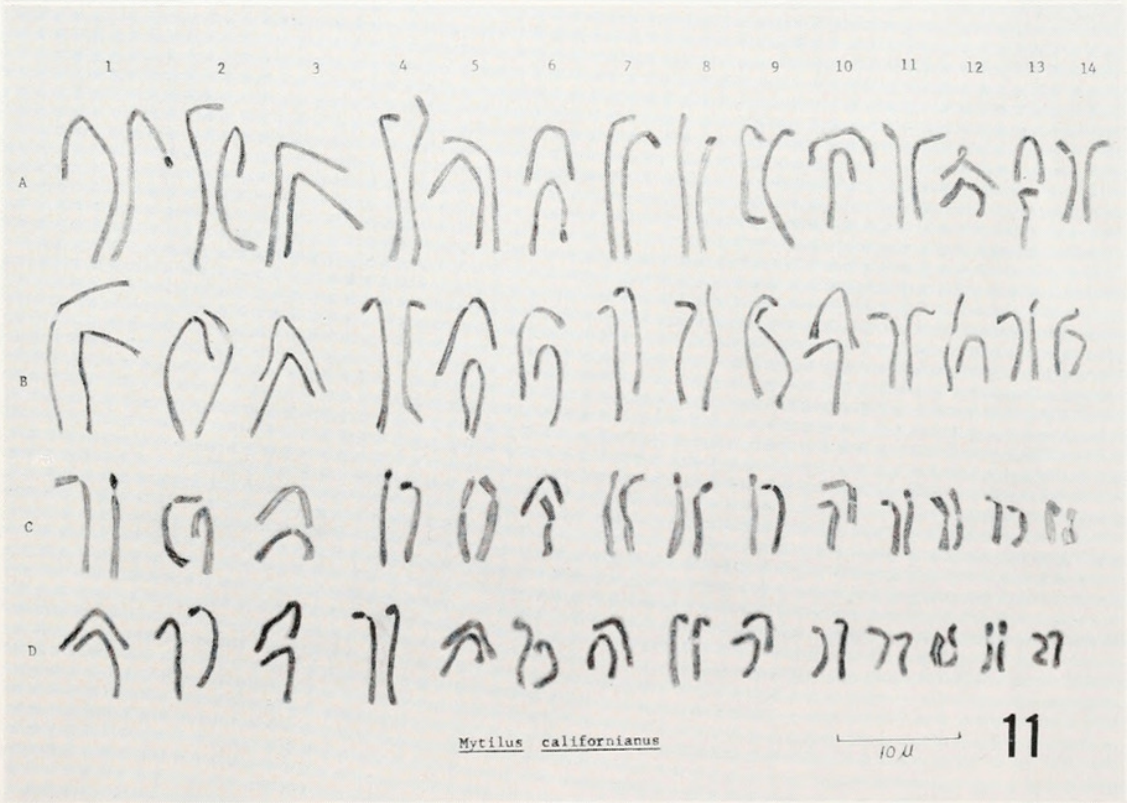


FIGURE 10. Karyotypes of *M. edulis* from Golden Gardens based on camera lucida drawings of 4 complements of a single specimen.

FIGURE 11. Karyotypes of *M. californianus* from Cape Flattery (row A and B) and Agate Beach (row C and D) based on camera lucida drawings of 2 complements of 1 specimen each from the two localities.

A distinct autosomal polymorphism was observed in mitotic karyotypes also. Camera lucida drawings of 4 mitotic complements of a single specimen of Golden Gardens *M. edulis* are shown in Figure 10 and of one specimen each of *M. californianus* from Cape Flattery (row A and B) and from Agate Beach (row C and D) in Figure 11. In these sets, homologues of the 14 chromosome pairs were established on the basis of centromere positions and chromosome lengths. In some cases metacentric and acrocentric (MA), metacentric and submetacentric (MS), submetacentric and acrocentric (SA), and telocentric and acrocentric (TA) chromosomes had to be placed together so that heteromorphic pairs resulted. Examples of heteromorphic homologues present in Figures 10 and 11 will be mentioned below:

In Figure 10, pair 1 in row B and C of *M. edulis* is clearly heteromorphic, being composed of acrocentric and metacentric (AM) and metacentric and acrocentric (MA) chromosomes respectively; pair 2 in row B is composed of submeta- and acrocentric (SA) elements and pair 14 in this row, of submetacentric and metacentric (SM) elements. In Figure 11, pair 8 in row A and B of *M. californianus* is composed of telocentric and acrocentric (TA) and submetacentric and telocentric (ST) elements; pair 1 in row C and D is heteromorphic, being composed of submetacentric and acrocentric (SA) and metacentric and submetacentric (MS) elements, respectively; pair 2 in row D is heteromorphic for submetacentric and acrocentric (SA) elements.

Along with heteromorphic chromosome pairs MS, MA and SA *etc.*, metacentric (MM), submetacentric (SS), and acrocentric (AA) pairs seemed to replace each other at the same positions in different complements of the two species. This may be due to pericentric inversions or centric-shifts in both chromosomes of a pair. In *M. edulis,* the arrangement of chromosome pairs in rows A to D in Figure 10 on position 7 is: MM, MM, AA and AA; on 9: MM, SS, MM, MM; on 11: MM, AA, SS, and AA; and on 12: SS, AA, AA, and SS, respectively. In *M. californianus* the arrangement in rows A to D in Figure 11 on position 7: SS, AA, AA, and MM; on 9: SA, SS, AA, and MM; on 10: MM, MM, MM, and AA; on 11: SS, SS, AA, and MM; and on 13: MM, SS, AA, and SS, respectively. Slight variation of centromeric positions in the corresponding arms of some other homologues were present but will not be emphasized here. These may be due to small inversions, duplication deficiencies or merely differential contraction of arms.

The occurrence of hetermorphic chromosome pairs and pairs carrying homozygous inversions or centric-shifts introduces a variation in the number of different kinds of chromosomes in mitotic complements. In both mussels, 2 to 6 pairs of acrocentric chromosomes were present in different complements. In the face of so much variation in chromosome morphology, it was difficult to denote any one complement as standard. The four complements of the single specimen of *M. edulis* from Golden Gardens shown in Figure 10 differ from each other morphologically to a slight or greater extent. This is also true for the four sets belonging to two specimens of *M. californianus* (Fig. 11) from Cape Flattery and Agate Beach. Complement D of *M. edulis* (Fig. 10) is a completely homozygous set, whether inversion or standard. Complement B of *M. californianus* (Fig. 11) would have been homozygous except for a heteromorphic pair on position 8.

Discussion

This paper confirms the chromosome counts of 2n = 28 (n = 14) reported by us earlier (Ahmed and Sparks, 1967b) for both *M. edulis* and *M. californianus*. Menzel's recent paper (1968) assigns the number 2n = 24 (n = 12) to *M. edulis* from the East coast of the United States. It is possible that intraspecific chromosome number variations might exist between the East and West coast forms of *M. edulis*, yet there were no numerical variations in our counts of meiotic bivalents from the hundreds of eggs from each of the 16 specimens of *M. edulis* and 19 specimens of *M. californianus*. Some atypical counts were made during mitosis and 2n = 24 in our material was considered aneuploid and existed in only a few cases.

Aneuploid counts ranging from 19 to 27 and of 29 or 30 chromosomes were made in approximately 5–10% of the mitotic plates examined. These might have been due to any or all of such processes as non-disjunction or abnormal segregation, centric fusions and centric fissions. The exact cause or causes of aneuploidy in both species remain to be determined but some decreased counts in mitotic plates might be due to centric fusions. A centric fusion involves the fusion of two non-homologous acrocentrics or rod like chromosomes to form a metacentric or V-shaped chromosome, thereby decreasing the chromosome number. White (1954) emphasized that centric fusions will occur in animal species with more than one pair of acrocentric chromosomes. In the two mussels way is open for centric fusions to occur in the presence of 2 to 6 pairs of acrocentrics. Hyperdiploid counts of 29 or 30 might have been, also, due to the presence of supernumeraries.

The 14 meiotic bivalents of the two mussels were, with few exceptions, homomorphic, and isopynotic at all observable stages of meiosis. They were, therefore, all autosomal. None of the bivalents displayed any other characteristic of a sex-bivalent. There is no evidence for the existence of sex-chromosomes in any of the marine pelecypods which have been investigated cytologically. The bivalents of *Mercenaria mercenaria, M. campechiensis* and their hybrids (Menzel and Menzel, 1965), and those of the oysters *Ostrea lurida* and *Crassostrea gigas* (Ahmed and Sparks, 1967a) and *Crassostrea virginica* (Longwell, Stiles and Smith, 1967) are all homomorphic and isopycnotic. The sex-determining mechanism of *C. virginica*, the American oyster, may be along the lines of the multifactorial hypothesis or a system of polygenes (Montalenti and Bacci, 1951). Here, various combinations of 4 pairs of genes responsible for sex-determination may produce long or short, or equally long male and female phases. True males and females occur when crossing over and recombination gives rise to more extreme forms. This latter condition might prevail in *M. edulis* and *M. californianus* since both species reproduce, generally, as true males and females and sex-reversal usually does not occur.

Evidence of structural rearrangements exists in both mussels. Heteromorphic bivalents were observed in less than 5% of meiotic plates of both species. One or two such bivalents of *M. californianus* resembled the bivalents heterozygous for "elastic constrictions" reported by White (1957) in the grasshopper, *Keyacris* (*Moraba*) *scurra* populations. Such bivalents were considered by White to constitute a "minute" structural change.

The presence of heteromorphic pairs in meiosis as well as mitosis, the substitution of rod bivalents in place of metacentric or ring bivalents and vice versa, indicates that pericentric inversions or centric-shifts are involved. A centric-shift affecting one of a pair of chromosomes may give rise to a heteromorphic bivalent in meiosis and heteromorphic homologues in mitosis. A homologous centric-shift may change a rod shaped acrocentric or telocentric bivalent into a metacentric or ring shaped bivalent. Pericentric inversions or centric-shifts in autosomes are known to occur in several animal species and the pertinent literature has recently been reviewed by John and Lewis (1968).

The presence of heterozygous pericentric inversions or centric-shifts in homologous autosomes will ordinarily lead, due to free crossing over, to the production of genetically unbalanced gametes. The mussels must have evolved a mechanism to escape the consequences of such a phenomenon. Both mussels, however, could support a large wastage of gametes since a vast number of them is produced. *M. edulis* and *californianus* are very successful species and the autosomal polymorphism does not seem to have hindered their fitness and, indeed, it may even be of adaptive significance. The visible polymorphism (polyphenism) in mussel populations may be due to floating genetic differences. The population cytology of the two mussels needs further investigation in the light of this preliminary evidence of autosomal polymorphism.

We wish to express our gratitude to Dr. H. L. Roman, Chairman of the Department of Genetics and Dr. W. S. Hsu of the Department of Zoology of the University of Washington for their help in the interpretation of some observations. Thanks are due to Dr. D. W. Hagen, of the College of Fisheries, for comments and criticism of the manuscript and for several suggestions and discussions.

Summary

The bay mussel *Mytilus edulis* (Linnaeus) and the California sea mussel *Mytilus californianus* (Conrad) have a common diploid chromosome number 28 (n = 14) that is, there were 14 bivalents during meiosis and 28 chromosomes during mitosis. A total of 35 specimens of the two species were examined from several intertidal locations in Puget Sound and the Northwest Pacific coast of the United States. The number of meiotic bivalents in stripped or spawned eggs was always constant but aneuploid counts of mitotic chromosomes were made in cleaving eggs and embryos. Heteromorphic bivalents in meiotic plates and heteromorphic homologues in mitotic karyotypes were observed. The number of metacentric, submetacentric and acrocentric chromosomes was variable. There were 2 to 6 pairs of acrocentrics in different complements. This polymorphism indicates the existence of pericentric inversions or centric-shifts in both meiotic and mitotic chromosomes.

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