

primarily to supply a stock of mosquitoes known to be free from naturally acquired malaria infection. The insectary employed differs radically in several respects from the one previously used. The new structure has not as yet been described, although the essential features of its operation are discussed in a paper by Boyd and Cain entitled "On Large-scale Rearing of *Anopheles quadrimaculatus* in Captivity," to appear in an early number of the *American Journal of Hygiene*."

The operation of the new insectary resulted in the production of five successive lineal generations of *A. quadrimaculatus* between February 18 and July 17, 1932; and multiplication in each generation occurred at an increasing rate. The periods over which the different stages of each generation were current are listed below. In several instances they were artificially curtailed to meet the exigencies of insectary operation incident to the production of stock for infection

Five Successive Generations of *A. quadrimaculatus* Produced between February 18 and July 17, 1932.

I. Ova	From insectary females, deposited prior to February 18, 1932
Larvae	February 18 to March 30
Imagines	February 22 to April 1
II. Ova	March 20 to April 19
Larvae	March 30 to April 26
Imagines	April 14 to May 14
III. Ova	May 6 to May 22
Larvae	May 6 to June 10
Imagines	May 19 to July 8
IV. Ova	May 24 to July 7
Larvae	May 26 to present (July 22)
Imagines	June 15 to present (July 22)
V. Ova	July 17 to present (July 22)
Larvae	July 17 to present (July 22)

and to our desire to avoid the mixing of adult females of different generations.

Over the three months' period from November, 1931, to February 18, 1932, the insectary was operated solely from the standpoint of furnishing stock for infection purposes. This stock was reared from the ova of wild females, and we have reason to believe that during this period, when no effort was made to keep the generations separate, there actually occurred three further generations. When it became apparent that abundant propagation was taking place in captivity, the insectary operation was modified in order to disclose each generation and to avoid the mixing of females of two generations. However, since the operation of the insectary prior to February 18 did not permit us to distinguish generations clearly, the possibilities of that period are ignored in this chronology.

The production of fertile ova is sufficient proof of the occurrence of copulation. As many larvae as our facilities permit are reared according to the methods described in the paper by Boyd and Cain. The imagines produced compare favorably in size with wild individuals. Males are fed on raisins, while the females feed readily on the person of the attendant, their only blood supply.

While the hazards of the future can not be forecast, there is nothing in the present outlook to indicate any limitations to the indefinite propagation of this strain. Needless to say, the possession of this strain is of the greatest advantage in the transmission of naturally induced malaria and offers research opportunities hitherto unavailable.

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SPECIAL ARTICLES

STIMULATION OF SPAWNING AND CROSS-FERTILIZATION BETWEEN AMERICAN AND JAPANESE OYSTERS

It has been shown previously by one of the authors that spawning of male and female American oysters, *Ostrea virginica*, can be induced by the sexual products of the opposite sex. The reaction appeared to be specific in the sense that sperm of other molluscs (*Mytilus*, *Mya*) had no effect on oysters. Negative results were obtained also in attempting to stimulate the ripe males of *O. virginica*, grown in Honolulu, by the eggs of *O. cucullata*, a species introduced from Australia.

This summer the authors have experimented with a Japanese oyster, *O. gigas*, which was imported as seed from Japan, grown for about two years in Puget

Sound and shipped in April, 1932, to Woods Hole, Mass.

Both *Ostrea virginica* and *O. gigas* can be stimulated by the sperm or eggs of either species. Thus, the spawning reaction of female *O. virginica* can be provoked by adding sperm of *O. gigas*, and similarly a female *O. gigas* responds to the presence of sperm of *O. virginica*. Spawning of the males is equally well provoked by the addition of eggs or egg water of either species.

Table 1 shows that in females the latent periods of spawning reactions vary from 8 to 26 minutes, while in the males the variation is from 4 to 6 seconds. In both cases the latency is within the limits previously observed in spawning *O. virginica* and apparently independent of the kind of eggs or sperm used

TABLE 1
LATENT PERIODS OF SPAWNING REACTIONS OF FEMALE
OYSTERS

Female	Male	Temp. °C.	Lat. per. mins.
<i>O. virginica</i> × <i>O. virginica</i>		23	9
"	"	23	20
"	"	23	21
"	"	23	24
<i>O. virginica</i> × <i>O. gigas</i>		22	15
"	"	22.5	17
"	"	23	26
<i>O. gigas</i> × <i>O. gigas</i>		27.5	15
<i>O. gigas</i> × <i>O. virginica</i>		27	9.5
"	"	27	8.0

for stimulation. This may indicate that the sexual products of both species possess similar active principles which induce the spawning reactions.

Eggs of both species were easily fertilized by either sperm, the ensuing development resulting in apparently normal straight hinge larvae. Comparison with controls showed no increased mortality among the hybrids.

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THE STRUCTURE OF CHROMOSOMES OF ZEA MAYS AS REVEALED BY THE FEULGEN REACTION

DURING the progress of a series of studies on the microchemistry of the nucleolus, the Feulgen reaction for thymonucleic acid¹ was applied to root-tips of *Zea mays* fixed according to Zirkle² in a solution of sulfuric acid 2 cc, formalin 10 cc, distilled water 90 cc, with a pH of < 1.0.

Staining with haematoxylin after the above fixation, Zirkle found the chromatin reticulum and periphery of the later spireme and of the chromosomes stained blue, while the nucleoli and "core" of the spireme and of the chromosomes remained colorless. He also describes a physical connection between the nucleolus and the spireme.

Feulgen staining bears out the haematoxylin demonstrated morphology to a startling degree. In the resting cell and early prophase, chromatin granules, chromatin reticulum and spireme are stained a solid, intense reddish-violet; as mitosis progresses and the diameter of the spireme increases, a minute canal appears within the spireme and extends its full length. The appearance of this canal or "core" is accom-

¹ R. Feulgen, "Die Nuclealfärbung," Handbuch der biologischen Arbeitsmethoden, 213: 1055-1073. 1926.

² C. Zirkle, "Nucleolus in Root Tip Mitosis in *Zea Mays*," Botanical Gazette, 86: 402-418. 1928.

panied by a decrease in the size of the nucleolus; as the spireme increases in cross-section, there is a corresponding increase in the size of its "core" and a decrease in the amount of nucleolar material visible.

The late prophasic chromosomes have the appearance of tubules of almost uniform bore; the nucleolus may disappear entirely or vestiges may remain to follow the chromosomes to the equatorial plate and again to the poles.

Using monochromatic and polarized light at the highest magnifications available, the writer was unable to observe a physical connection between the nucleolus and the spireme, or to ascertain definitely the existence of nucleolar or other material within the latter. Neither was he able to find mitotic stages which revealed the behavior of the chromosomal "core" during the splitting of the chromosomes at the equatorial plate and during reconstruction, although at the equatorial plate the chromosomes show distinctly their "tubular" structure.

The external diameters of the spiremes and chromosomes, after this fixation, are, taking measured averages, no greater than the external diameters of the spiremes and chromosomes of similarly situated cells, in the same phases, following other fixations. The outlines of the cells are smooth, the surfaces of the spiremes and chromosomes show no undue irregularity; in short, there is no evidence of an artifact greater than that resulting from the fixing of the cells by other methods.

While "tubulated" chromosomes have been described many times previously, they were almost invariably demonstrated by the cruder stains whose action is dependent upon mordanting, length of time of staining and destaining, and various other purely physical factors. Their description after a method which stains chromatin with chemical selectivity may aid in an interpretation of their true structure.

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