+ 4.2 and - 2.5. The modifiers of a cross bred may be indicated in some appropriate manner as [+ 4.2, - 2.5].

8. *Linkage* is best represented by the fractional form used by workers on *Drosophila*. The factors are written in the order of linkage, omitting type factors.

COMMITTEE ON GENETIC FORM AND NOMENCLATURE

STANDARDIZED MICROPHOTOGRAPHY

SECOND CONTRIBUTION: THE OBJECT FACTOR

IN my first contribution to the subject of standardized microphotography, published in the *Anatomical Record*, I have pointed out the variables and the methods which I have pursued in treating them. One, or perhaps more correctly a group of variables were, however, left out of consideration quite purposely because of the difficulty in finding for them a standard of permanent value. I have in mind the microscopical section itself or what may be properly called the object factor. The following four elements enter into its composition: (1) the thickness of the section, (2) the light absorption coefficient of the tissue, (3) the relative luminosity of the different stains and (4) the depth or intensity of staining. The second and third of these component elements may be disregarded since experience shows us that exposure is very little influenced by them. There remain, however, the first and fourth, and to determine the influence of these on exposure the following experiments were undertaken.

First of all, to avoid all possible error, slides were chosen of uniform thickness measured with a Ciceri Smiths Patent Micrometer so as to be sure that the distance of the section from the substage condenser should in every case be the same. The cover-glasses were also of uniform thickness. The stomach of a frog preserved in Zenker's fixing fluid was sectioned into series of 5, 10 and 20 micromillimeter thick sections on a Minot microtome and care was taken to have in each case a ribbon of 100 even sections, thus more or less assuring their uniform thickness. The sections were stretched on distilled water heated over a flame and no cement of any kind was used. On one slide three sections of each thickness were placed. On other slides sections only of one kind were placed and their thickness marked in every case by a carborundum pencil.

The slide with all three kinds of sections was stained for 12 hours in alumcarmine, a stain which, as is well known, does not overstain. They were then washed in water and again stained for 12 hours in a weak alcoholic solution of Bleu de Lyon. All sections on this slide received therefore the same treatment and the difference in the depth of stain was entirely due to the thickness of the section stained.

The other slides were treated in a different way. They were stained in Delafield's hæmatoxylin followed by tetrabromfluoresceic acid. This stain was chosen because it is possible at will to control the depth of staining. A set A of three slides, one with 5 micromillimeter sections, one with 10 and one with 20, was treated simultaneously in a Coplin's staining jar. The sections were therefore stained, washed, destained in acid alcohol, treated with ammonia alcohol, stained in a weak solution of tetrabromfluoresceic acid in 95 per cent. alcohol and washed in pure alcohol the same length of time. In regard to depth of stain these slides presented, therefore, the same conditions as the slide stained in alumcarmine and Bleu de Lyon.

Several other slides were treated individually in the same stains. They were all first considerably overstained in Delafield's hæmatoxylin, washed in water and destained in acid alcohol until they had when viewed over a white surface, the same shade of color to the naked eye, regardless of the thickness of the section. If after treatment with ammonia alcohol the blue color was not approximately of the same shade, the darker slide was again transferred to the acid alcohol, until all sections looked approximately alike. They were now stained in the tetrabromfluoresceic acid, the thickest sections remaining a short time in the fluid, the 10 micromillimeter sections somewhat longer and the 5 micromillimeter sections longest, and again compared over a white surface. From several slides thus prepared three were selected which to the naked eye were to all purposes of the same appearance, although their respective thickness were 5, 10 and 20 micromillimeters. Here then we had a Set B of sections in which the depth of stain had nothing to do with the thickness of the section, but was entirely dependent upon the amount of stain absorbed by the tissues.

Fractional exposures on orthonon plates with Cramer ray-filters were made on our standardized microphotographic apparatus and developed by the time and temperature method with

fresh developer for each plate. In the case of the first slide stained in alumcarmine and Bleu de Lyon as in the Set A, the normal exposure for the 10 micromillimeter section was twice that for the 5 one, and the normal exposure for the 20 micromillimeter section was four times that of the 5 one. In the case of the Set B all slides required the same exposure.

In analyzing the results thus obtained we come to the conclusion that the thickness of the section, within the limits given, has no influence whatsoever on the length of exposure; but that the latter stands in a direct ratio to the amount of stain absorbed by the tissue.

For practical purposes, especially for those who are using my table of R-P factors, the results of this investigation may be interpreted as follows: disregard the thickness of the sections and the appearance of the stain under microscope and pay attention only to the intensity as it appears to the naked eye. The table refers to normally well stained sections of medium thickness. Double the exposure for darker appearing and reduce the exposure by half for lighter appearing sections.

It will be observed that in the experiments elements 2 and 3 remained of constant value for the simple reason that the tissue used was not only the same in kind, but actually from the same piece of organ and was stained in the same stain in each set of slides. A comparison of the length of normal exposure in the case of sections stained in alumcarmine and Bleu de Lyon with those stained in hæmatoxylin and tetrabromfluoresceic acid serves to confirm my statement at the beginning of this article, that the relative luminosity of different stains may be entirely disregarded in the matter of exposure.

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SEX RATIOS IN PLATYGASTER

In the *Journal of Heredity* for last year (Vol. X, p. 344) I published data on sex ratios in three species of polyembryonic Hymenoptera. One of these is *Platygaster felti*, which parasitizes the eggs of two species of the Cecidomyiidae, *Walshomyia taxana* and *Rhopalomyia sabinae*. These flies make their galls on the mountain cedar, *Sabina sabinodes*.

Since the above data were published I have had an opportunity to breed out in the laboratory a total of 200 broods of