one or two experts in histological technic in order to get their judgment. Their judgment must be based to a considerable extent upon the behavior of the stain in the Flemming triple staining technic, in which it is used together with orange G and gentian violet. In other words, the stain must be of such a shade as to contrast well with both of these two other dyes.

(4) It must be understood that these standards refer to samples to be used for general histological staining. Special standards for safranin to be used for certain special purposes will undoubtedly be necessary. These standards, however, have not yet been determined.

At the present time permission to use the commission's label on the batches of safranin submitted has been given to three companies. Work is still pending on samples submitted by one or two other companies, so that the omission of some concern from this list does not prove that their safranin is necessarily unsatisfactory. The three samples so far approved fulfil the above specifications in every respect except total dye content; but the three samples vary somewhat in the amount of actual dye present. These three samples with their total dye content are as follows:

National Anilin and Chemical Company	90 per cent.
Empire Biochemical Company	87 per cent.
Providence Chemical Laboratories	55 per cent.

That one of those three samples which is below 75 per cent. in total dye content has given perfectly satisfactory results in the hands of the investigators who tested it. Nevertheless, purchasers must take its lower concentration into account. For this reason the commission is requiring that certified samples of safranin be labeled as to their total dye content. This will enable the purchasers to take the matter of concentration into account in making up solutions, and will enable them to make a fair comparison between the products of the different concerns.

Attention of biologists is again called to the fact that these stains do not have to be ordered from the companies listed above. Nearly all the regular dealers in biological supplies are planning to carry the certified stains as rapidly as the certification is extended to cover new products. It is possible, therefore, to order these stains from any laboratory supply house by specifying that certified stains are desired or by even specifying the company whose product is wished for if the purchaser has any preference.

H. J. CONN, *Chairman,*
Commission on Standardization of Biological Stains

GENEVA, N. Y.,
APRIL 1, 1924

STAINING WOODY TISSUES WITH SAFRANIN AND PICRO-ANILIN BLUE

THE use of safranin in combination with picric acid and anilin blue in the staining of woody tissues has been developed at the Forest Products Laboratory, and some excellent results have been obtained with certain kinds of woods. The triple stain herein described calls for safranin as a first stain, followed by a single solution containing picric acid and anilin blue.

Safranin is one of the most important of the coal-tar dye stains because of its selective properties, brilliancy and permanence. Picric acid, which is less well known in microscopy, is a yellow crystalline compound obtained variously, as by the action of nitric acid on phenol. Anilin blue is a basic derivative of the base rosanilin. Best results follow the use of pure chemicals from reliable manufacturers.

Stains are generally divided into two types, general and selective. The former acts on all the elements of a specimen while the latter takes effect on, and makes prominent, only some or parts of them. It is of value to the worker in microscopic anatomy to obtain this differentiation, as he is thereby able to trace and follow structural differences and relationships.

If the material to be sectioned is green, it usually requires no further treatment. If dry wood blocks are used, they should be prepared for the microtome by boiling for about thirty minutes or longer in water. Extremely refractory and hard woods should be immersed in commercial hydrofluoric acid (30–40 per cent.) for a few days and again boiled in several changes of water to wash out the contained acid, previous to cutting sections. The writer secured best results with sections 10–15 micromillimeters in thickness.

The safranin stain is prepared by mixing a saturated water solution of water-soluble safranin, and a saturated alcoholic solution of alcohol-soluble safranin. Equal amounts of these two safranin solutions are mixed. The safranin may be used several times.

The picro-anilin stain is prepared as follows: Make a saturated solution of picric acid and one of anilin blue, each in 95 per cent. alcohol. From these two make a single alcoholic solution containing 78 per cent. of the picric acid and 22 per cent. of the anilin blue solutions.

The staining process is as follows:

(1) Rinse sections with 50 per cent. alcohol.

(2) Flood sections with safranin and leave two hours.

(3) Wash off excess safranin with 50 per cent. alcohol leaving sections light pink in color. If the sections remain red, bleach with 70 per cent. alcohol to which a few drops of acetic acid have been added.

(4) Flood sections with picro-anilin blue and leave two hours.

(5) Draw off excess stain and wash sections for ten seconds in absolute alcohol.

(6) Transfer sections to clove oil and mount in Canada balsam.

The above method of differential staining is one of substitution, whereby the alcoholic solution of picro-anilin blue is made to wash out the safranin from all but so-called structures, for which the latter stain has a great affinity.

Criticism might be aimed at the short period of time for step five above in dehydrating, but results secured seem to justify the means used, a longer period causing excessive loss of color.

Using white oak wood as an example, the middle lamella is stained red, crystal forms a bright blue, and cell walls from light-yellow to blue or greenish-blue. Furthermore, in oak, many wood fibers whose lumen was constricted to a wavy line or "lazy S" in shape were found to have the entire thickened inner or tertiary wall stained a bright blue, while the secondary layer (in these fibers comparatively narrow) was stained yellow or olive green like the entire wall exclusive of the middle lamella of some other fibers.

An identification of these fibers with blue inner walls links them closely with mucilaginous cells. According to Jeffrey,[1] the presence of these cells reduces the swelling and shrinking of wood.

In addition to the differentiation obtained, this combination of stains has the advantage that the general yellow-green or apple-green hue of the sections is not tiring to the eye.

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[1] Jeffrey, E. C. "The Anatomy of Woody Plants," p. 35.

SPECIAL ARTICLES

THE COINCIDENT PRODUCTION OF DEXTRAL AND SINISTRAL YOUNG IN THE LAND-GASTEROPOD PARTULA

AMONG the many problems of gasteropod asymmetry, none is more interesting than that which is concerned with the nature of the factors by which the direction of the coil is controlled. According to general experience, the prevailing mode is the dextral or clockwise from foot to apex when viewed from in front; the opposite sinistral form of coil is displayed by occasional examples of some dextral species. Again, certain species are uniformly sinistral, while others are sinistral in the main with sporadic dextral individuals. It is justifiable to denote the direction of the coil, a hereditary quality, on the same grounds that any other resemblances between offspring and parents are called hereditary, even though the parental characters are not always repeated faithfully in the progeny.

Boycott and Diver[1] have recently recorded the results of their studies upon Limnaea in which they have employed the usual dextral and the unusual sinistral kinds of snails. They regard their findings as evidence that dextrality is a Mendelian dominant with reference to the reversed mode of coil. Sturtevant[2] discusses the results of Boycott and Diver, and ingeniously interprets them in terms of maternal inheritance under earlier chromosomal control. Morgan[3] reviews the phenomena of spiral cleavage in relation to the dextral and sinistral modes of coil, and accepts Sturtevant's interpretation.

Mayor[4] and the present writer[5] have studied the Partulae living in Tahiti, where certain colonies of species, such as *Partula otaheitana,* comprise both dextral and sinistral snails. We found no exceptions to the rule that the young produced at any one time by a given adult were *all* of the *same* mode of coil, whether or not this agreed with the parental form of asymmetry. An adult of either type might bear young of its own mode exclusively or a series of offspring which all displayed the opposite direction of twist. Boycott and Diver observed the same relations in most of the offspring broods of Limnaea, but mixed broods also occurred in their material.

Exceptional instances have now been found in a species of Partula where dextral and sinistral young occurred simultaneously in the parental brood-pouch. The species in question is *Partula suturalis,* which dwells in the island of Moorea, a member of the Society Islands, situated about 20 miles from Tahiti. This species now ranges over almost all Moorea, and some of its colonies are made up of both dextral and sinistral snails. When the embryonic young were extracted from the parent animals taken in the valley of Faamaariri in the Vaiare region, five cases were found where two young were present in the brood-pouch, one of which was sinistral, while the other was dextral. In four of these instances the parent was dextral and in the fifth case the adult was sinistral.

It is particularly interesting that the exceptions herein recorded were found in *only one* association of mixed character. In this Faamaariri series, the noteworthy instances number five out of 148 where two or more young were present in the brood chamber; the

[1] Proc. Roy. Soc., 95 B, 1923.

[2] SCIENCE, LVIII, No. 1501, 1923.

[3] *Scientific Monthly,* XVIII, No. 3, 1924.

[4] Mem. Mus. Comp. Zool., XXVI, No. 2, 1902.

[5] Carnegie Inst. Pub. No. 228, 1917.