first referred to above, which was as follows: The complex previously known as *P. rubigo-vera,* having been found to include frequently two distinct species, it did not become necessary thereby to establish *two* new names where there was only *one* new species. Accepting, therefore, the name *P. glumarum* for the new species, the writer simply retained the old well-known name *P. rubigo-vera* for the common brown rust of wheat and rye, proposing, however, the two names, *P. rubigo-vera tritici* and *P. rubigo-vera secalis,* for the respective biologic forms on these hosts.

In *Botanical Abstracts* there is reference to a paper by Mains[11] on the seed carriage of Euphorbia rust, which recalls very similar experiments of the writer, reported in 1904 in his paper last above cited, pages 28–29. Not having seen the entire article of Mains, it is not known to the writer whether or not his experiments are referred to in that article, but it is true that the writer demonstrated, in careful greenhouse work, the carrying over of rust in the seed of *Euphorbia dentata,* thus proving the existence of a perennial rust in an annual host, the first time in the United States. One other case had at that time been reported from a foreign country.

As already evident from these notes, it seems to the writer a matter of the greatest importance to study the relationship of the rusts of wild grasses, and of these rusts to those on the cereals. Even where the barberry is an important factor between cereal crops, a perennial grass may receive the rust first, from which it will pass on to the cultivated cereal. However, the grass is probably of most importance in over-wintering the cereal rust. It is gratifying to note that already more attention is being given to these grass rusts than previously. Mains[12] has made a good study of Puccinia montanensis and similar species on Elymus and other grasses, and, although heteroecism is the chief subject of discussion, he brings out the important point of over-wintering, apparently unaware of the fact, however, that positive results of observations in that respect had been reported by the writer some time ago in his publication last cited. Mains says:

> The geographic distribution of *P. montanensis,* as indicated by specimens in the herbarium, is British Columbia, Wisconsin, Indiana, southward to New Mexico and southern California, while *Berberis Fendleri* (the aecial host) is limited in its distribution to the mountains of Colorado and New Mexico. Such a difference in distribution, however, would be explained, if this rust is not dependent upon its aecial stage, but is able to overwinter in the uredinial stage. Mr. Bethel has made observations in Colorado which indicate that such an over-wintering may occur there.

In 1898 the writer observed that this rust wintered in the uredo stage on the University Farm at Lincoln, Nebraska. No urediniospores were germinated, but the mycelium was vigorous and spreading rapidly, and the spring had well begun. This subepidermal rust, though not occurring on cereals, is a very interesting one, and, in mode of life, is the most similar of all rusts to *P. glumarum,* in the writer's experience. It had been under the writer's observation for a long time previously in Kansas and Nebraska, and its perennial nature suspected.

MARK ALFRED CARLETON
CUYAMEL, HONDURAS

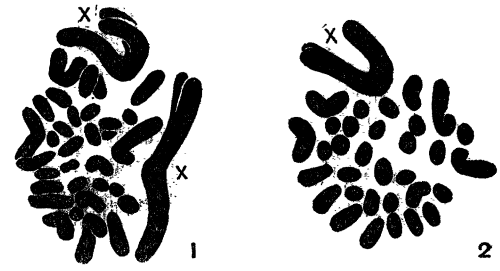
[11] Mains, E. B., "Evidence of the seed carriage of the Euphorbia rusts, *Uromyces proeminens* and *U. dictosperma,*" Proc. Indiana Acad. Sci. *1921*: 137–139, 1922.

[12] Mains, E. B., "The heteroecism of *Puccinia montanensis, P. koeleriae* and *P. apocrypta,*" *Mycologia,* 13, 315–322, No. 6, Nov., 1921.

LABORATORY APPARATUS AND METHODS

MATERIAL FOR DEMONSTRATIONS OF ACCESSORY CHROMOSOMES

IN most male Orthoptera the accessory chromosome may readily be recognized both in spermatogonial and spermatocyte divisions, but in females the paired accessory chromosomes are usually very difficult to identify. In order to find material suitable for demonstrations of the accessory chromosomes in both sexes the cells of many species of long-horned grasshoppers (Tettigoniidae) have been examined. Since such demonstrations seem to be quite widely desired for class use in zoology and for other purposes, it may be of value to report the result of this examination.



Spermatogonial cells from the testis and follicular cells of the ovary of *Orchelimum concinnum* and *Orchelimum vulgare* were found to be especially favorable. Although the number of chromosomes is rather difficult to determine, there are probably 33 in the male (Fig. 2) and 34 in the female (Fig. 1). The accessory chromosomes are V-shaped and three or four times the size of the largest autosome, as can be seen in the figures (X). Other undetermined species

of the same genus were examined and found to show very similar conditions. The genus has a very extensive distribution and may be obtained practically throughout the United States, being "very common in summer and early autumn in damp meadows and along the margins of streams and ponds."

Keys for determining material may be found in Blatchley's "Orthoptera of Northeastern America" and in Rehn and Hebard's "Synopsis of the Species of the Genus Orchelimum," Transactions American Entomological Society XLI.

In order to obtain many fine spermatogonial divisions, immature males should be killed with a few drops of xylol, the testes dissected out and fixed for three hours or longer in the following fixative, which must be freshly made up:

75 cc saturated aqueous solution picric acid, 15 cc formalin, 10 cc glacial acetic acid, 0.5 gram urea and 1.0 gram chromic acid crystals.

Wash in 70 per cent. alcohol until no longer yellow. Dehydrate clear, infiltrate with paraffin and section 7–8 μ thick. Stain in iron haematoxylin.

Ovaries should be dissected from adult females and treated as above. Since the larger eggs usually crumble when sectioned they should be removed before dehydration. Slides should be differentiated to show polar views of metaphases of dividing follicle cells.

The material is also very suitable for the demonstration of the distinctive differential behavior of the accessory chromosome in spermatogenesis. Since this element is so extraordinarily large its peculiarities in the prophases are particularly striking.

ROBERT L. KING

ZOOLOGICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA

SPECIAL ARTICLES

THE RELATIVE REACTION OF LIVING MAMMALIAN TISSUES

THE methods which have led to a comprehension of acid-base conditions in the blood are for the most part inapplicable to the study of the reaction of individual tissues in the living mammal. This field of inquiry has remained, not closed indeed, but well nigh unentered. The observations which follow indicate that the tissues in general are less alkaline than the blood, and that in some of the organs, on occasion at least, a notable acidity prevails.

Rats and mice can be vitally stained with litmus by repeated injection of it in the purified blue state. The coloration persists for weeks without manifest detriment to health. At first the whole creature becomes blue owing to the amount of indicator present in the body fluids; but soon a striking color differentiation develops. The hairless surfaces now appear violet because of a red-stained connective tissue overlain by a blue-stained epithelium. Long after the indicator has practically disappeared from the body fluids the surface epithelium, the bones, osteoid tissue, cartilage, tendons, aorta and heart valves are still diffusely blue, whereas other tissues, notably the connective tissue everywhere, the liver, pancreas and kidney are of a pronounced rosy red. The red color is almost wholly the consequence of a segregation of litmus in intracellular granules acid in character; and the amount of granular matter may become so great that the predominant reaction in the tissue when crushed is likewise acid to the indicator. In uninjured connective tissue a blue ground-work can be made out between cells laden with the red granules, whereas the parenchyma of certain of the viscera containing macrophages filled with such granules has a ruddy sheen, difficult to discriminate in the presence of these latter, which might be taken to indicate an acid state.[1] Cells with red granules dying here and there in the body are for a brief period colored diffusely blue; and local derangements within elements yet alive are signalized by a change in the tint of individual granules. The acidity of some at least of these granules in which litmus is stored is so considerable that when brom cresol green instead is deposited in them they are rendered yellow, a hue which, under controlled circumstances in the test tube, would indicate an acidity at least as great as pH 4.0.

The sodium salts of the phenol indicators stain living tissues far more rapidly, deeply and diffusely than does litmus; and several are well tolerated, as the event has shown. Mice given thymol blue, which is yellow at pH 8.0 and blue at pH 9.4, become yellow practically throughout, a fact which sufficiently indicates that the range of the indicator is too far to the alkaline side for it to be useful in the present connection. After cresol red (yellow at pH 7.2; purplish red at pH 8.4), the hairless body surfaces are reddish yellow, whereas the tissues exposed when the anesthetized animal is laid open have a clear yellow hue. The plasma of blood removed from the right ventricle into paraffined containers and under

[1] In 1913 (*J. Exp. Med.*, xviii, 183) the writer recorded the fact that some tissues grow well in plasma rendered acid to litmus through their metabolic activities; and Lewis and Felton, Fischer and Mendeléeff have since demonstrated that some survive at a pH as low as 5.5. Stieglitz (*Arch. Int. Med.*, 1924, xxxiii, 483) reports that the kidney cortex of dogs receiving azolitmin intravenously becomes red with the indicator in the course of a few minutes.