Technical Papers

The Breeding Site of *Drosophila lacicola* Patterson[1]

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The *Drosophila virilis* species group has been intensively used for the study of genetics (*1, 2*), cytogenetics (*3, 4*), sexual isolation (*5–7*), and geographical isolation (*8*). Under laboratory conditions the various species have proved to be excellent experimental animals, but even experienced collectors invariably have had trouble in finding the wild specimens. Furthermore, our information to date concerning the breeding sites of the various species has been completely nil, except for *D. virilis* itself, which is a "domestic" species found in produce houses, etc., breeding on rotten fruits. It has been well known, however, that adults can be trapped only in the immediate vicinity of bodies of fresh water.

One species of the group, *D. lacicola* Patterson, is known from Minnesota. During the last week of July 1950, collections made by trapping with rotting bananas disclosed that a large population of this species was concentrated about a small pond on the campus of The University of Minnesota Biological Station at Itasca Park. Such trapping showed (1) that the adults could be taken only in the immediate vicinity of the pond and that traps set 30 ft back from the shore did not capture the flies, and (2) that many of the flies which were caught were young, recently emerged individuals. The latter fact indicated that the species must be breeding in a narrow fringe about the pond.

An intensive search was undertaken, and the breeding site of *D. lacicola* was found to be the rotting phloem of the aspen *Populus tremuloides* Michx. During October and November 1948, several large aspen trees on the edge of the pond had been felled and the trunks cut into cordwood. By 1950 the phloem of the stumps and the pieces of cordwood had decomposed to a dark-brown to black state, although maintaining its original fibrous condition. This material, when exposed by stripping the bark, gave off the rancid, pungent odor characteristic of rotting aspen. Larvae of *D. lacicola* were living in the rotting phloem, and pupae of this species were in the dried, exposed edges of the phloem material, mostly within ½–1 in. of the place where the wood had been sawed. Aspen bark is known to be rich in various sugars, and Clyde Christenson has determined that in these particular pieces of bark yeasts are predominant microorganisms. Apparently the *D. lacicola* larvae feed on these yeasts.

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Adults of *D. lacicola,* but not of the other species which inhabit the region, were attracted to the areas exposed by stripping away the rotting bark, and the eggs of *D. lacicola* were oviposited in considerable numbers upon the inner surface of the bark. Adults of *D. lacicola* were bred from pieces of the bark which were isolated in the laboratory, as well as from individual pupae which were dissected from the bark.

At present the exact breeding sites of the other "wild" species of the *D. virilis* species group are unknown. On the basis of the known distribution of the various species and on the basis of the known distribution of aspen and its close relatives, the cottonwoods, it is suggested that *D. montana* Patterson and Wheeler probably breeds in aspen, and that *D. americana americana* Spencer, *D. americana texana* Patterson, Stone, and Griffen, and *D. novamexicana* Patterson all probably breed in rotting cottonwood phloem.

It is further suggested that *D. virilis,* the domestic species, may have arisen from an ancestral "wild" stock in a semitropical, semiarid area where various fruit-producing plants and cottonwoods grew in close contact in oases or possibly in primitive man's irrigated areas.

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An Improved Micromanipulator for Cellular Micrurgy

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One of the micromanipulators most widely used during the past 25 years is patterned after the instrument originally described by Robert Chambers (*1*). The fine three-way movements of microneedles or micropipettes, produced by manipulating the micrometer screws of this instrument, are curvilinear. Although the paths of the microtips follow arcs of large circles, no difficulties arise for the horizontal movements. The two cross movements in the horizontal plane of the Chambers' micromanipulator are entirely satisfactory, as indicated by the wide usage of this instrument in cellular micrurgy (*2–5*).

The fine vertical movement, however, is unsatis-

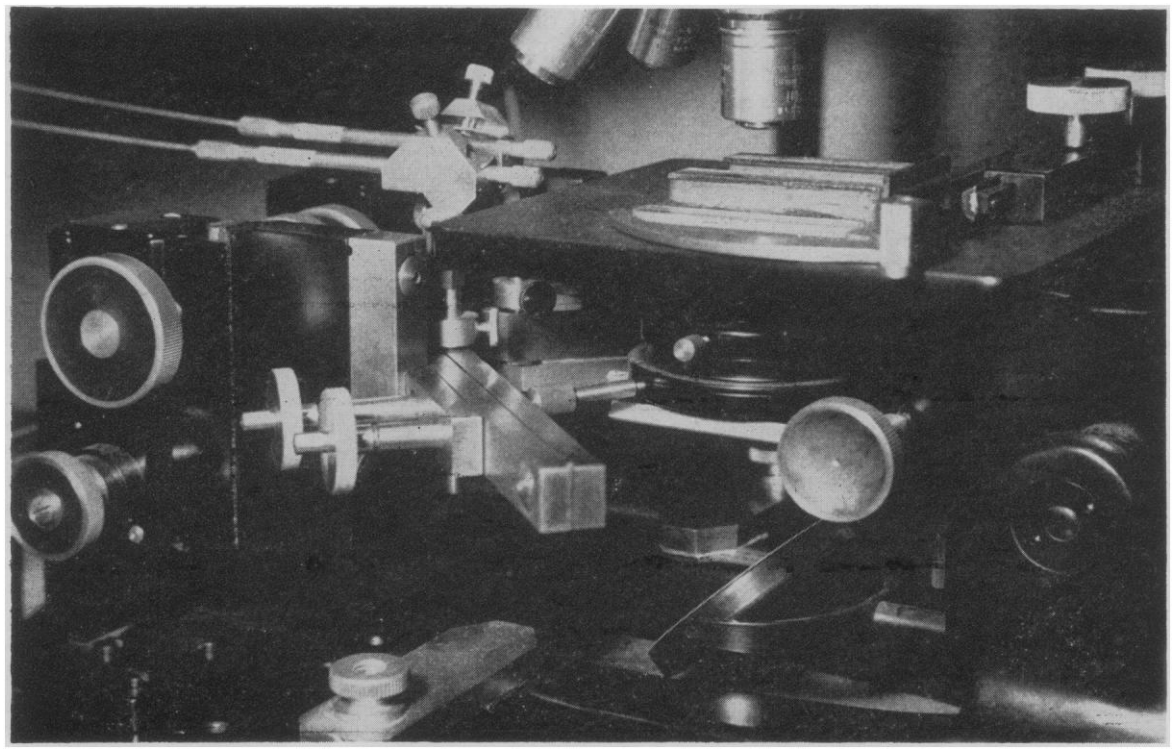

FIG. 1. Constructional features of left micromanipulator and position of right micromanipulator in relation to microscope. Note positions of micrometer screws for the horizontal movements and the coarse and fine controls for the vertical movements.

factory. For example, one of the most useful micrurgical procedures is to set the microtip of a needle or pipette, at first below the cell (suspended in a hanging drop and mounted on a moist chamber (*6*), under a crossline reference point placed in the field of the microscope. After this, the part of the cell to be impaled or injected is maneuvered under the crossline, and then the microneedle or micropipette is inserted into the cell from below by manipulating the vertical micrometer screw. The up-and-down movement of the microneedle with the Chambers' instrument is arc-shaped and produces an appreciable lateral displacement of the microtip along with the vertical motion. As a result, the microtip is no longer under the crossline when it is moved upward, and thus the potential precision of the micro-operation is spoiled.

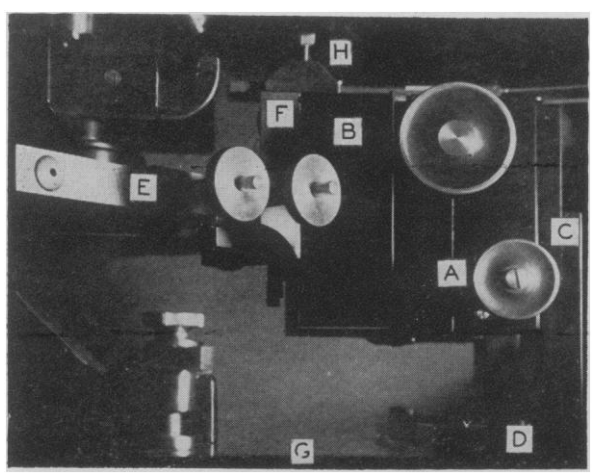

FIG. 2. Side view of right micromanipulator showing how the horizontal movements (*E*), taken from a Chambers' micromanipulator, are mounted on the coarse-fine vertical control mechanism (*A*). Shown also is the supporting bracket (*C*) and the mounting of the brackets to the base plate (*G*).

The Chambers' micromanipulator also has a coarse vertical adjusting mechanism, which unfortunately soon becomes loose from wear; this leads to undesirable vibration of the microneedles or micropipettes in the field of the microscope.

The modifications described in this article were designed to correct the faulty vertical controls and to improve the instrument in other ways. An important consideration is that the parts are standard, and only a minimum amount of skillful machine work is required. The new instrument, together with certain constructional features, is illustrated in Figs. 1 and 2.

The vertical control movement (*A*) was made from a Bausch & Lomb microscope body equipped with coarse and fine adjustments (No. 29–31–03–01). The body tube was removed from the rack-and-pinion assembly and replaced by a block of Dural metal (*B*), measuring $1.3 \times 3.0 \times 7.7$ .cm. This metal block (*B*) was fastened to the rack with 3 machine screws.

The two horizontal control movements (*E*) were separated as a unit from the vertical supporting pillar

of the standard Chambers' micromanipulator at the junction of the pin hinge. The original coarse vertical adjusting mechanism was also removed. The upright piece (F) of the horizontal movements was attached to the free edge of the Dural block (B) with 2 machine screws.

The horizontal movements (E) mounted on the vertical control mechanisms (A) are supported by right-angle, reinforced brackets (C), 2.2 cm wide and 11.0 cm high, made from brass 0.6 cm thick. A bracket 11.0 cm high, with the vertical control mechanism (A) attached as shown in Fig. 2, is ideal for microscope stages approximately 12.5 cm high. For microscopes with lower or higher stages, the height of the supporting bracket should equal the height of the stage minus 1.5 cm. The bases (D) of the supporting brackets, measuring 4.0×4.5 cm, were attached with machine screws to the micromanipulator base plate (G) in front of the microscope.

The microneedle holder clamps (H) are securely held by the micromanipulator with a single set screw (not shown in the figures).

The finished instrument is neat, stable, and correctly adapted to the microscope. There are several advantages provided by this new instrument, which has now been in service more than 2 years. *First,* the movements of the microtips in the vertical axis are rectilinear, and thus the crossline reference technique may be properly used. *Second,* the rack-and-pinion coarse movement is convenient for adjusting the preliminary height of the microneedle or micropipette over a range of several centimeters. It is essential that micromanipulators be equipped with mechanically controlled coarse vertical adjustments so that the microneedles or micropipettes may be quickly moved up or down (*6*). Mechanically controlled, coarse adjustments for the horizontal controls are unnecessary, since the preliminary settings of the microinstruments may be accomplished adequately by freehand under low magnification. *Third,* the fine adjustment of the vertical controls permits the movement of microinstruments into or out of operating position with considerable precision. The range of this movement, sufficient for any occasion, is about 0.3 cm. *Fourth,* micromanipulators equipped with this type of vertical control may be adapted equally well either to conventional microscopes or to the inverted microscope (*6*).

The instrument described in this article was made by the Gamma Scientific Company, from whom the standard Chambers' micromanipulator may also be obtained.

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Constitutional Factors in Resistance to Infection: The Effect of Cortisone on the Pathogenesis of Tuberculosis[1]

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In an endeavor to identify the constitutional factors responsible for the genetic resistance and susceptibility to tuberculosis of highly inbred rabbit races, it was found that the administration of estrogen to susceptible rabbits materially increases their resistance, whereas the periodic exposure of resistant rabbits to chorionic gonadotropin enhances their susceptibility to the disease (*1, 2*). It was noted in this report that tuberculosis in rabbits is accompanied by a marked hypertrophy of the adrenal cortex. In later studies it was observed that the degree of hypertrophy of the adrenal cortex of natively resistant rabbits affected by tuberculosis is much greater than that of susceptible rabbits similarly infected; therefore, investigations on the role of the adrenal cortex in resistance to the disease were begun. The general plan of the undertaking is to determine whether by increasing the adrenal function resistance can be increased and, conversely, whether by lowering this function the native resistance can be diminished.

Using cortisone, which is one of the important hormones of the adrenal cortex in synthetic form, the following experiment was performed. Twenty littermates of the genetically uniform and highly susceptible strain, FC, were divided into two groups of 10 each. They were placed in a room at a constant temperature of $21 \pm 2°$ C. Their urine was collected for analysis of the contained steroids. Total and differential counts of blood cells and fasting blood sugar were determined. At the same time the spread of India ink and of rabbit hemoglobin in the skin was measured 4 hr after injection. The inflammation at the site of injection of these substances in the skin was ascertained on the next day. After these base lines were established, 10 of the rabbits received 2 mg cortisone acetate per kg, intramuscularly, on alternate days. The 10 control littermates received the same volumes of the suspending medium without the cortisone by the same route, at the same intervals. Three days after the beginning of cortisone treatment, when the absolute number of circulating lymphocytes in the blood of the experimental animals had been markedly depressed, and when the fasting blood sugar of the same animals had increased by comparison with the essentially unchanged levels of these items in the control animals, both groups were simultaneously exposed to the quantitative inhalation of known numbers of viable, viru-

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