the concentrate from peak 2 had electrophoretic mobilities similar to γ- and β-globulin of whole rabbit serum. Most of the material appeared to be γ-globulin, with much lesser amounts of β-globulin. In contrast, electrophoretic patterns obtained from peak 3 concentrate appeared to contain all the protein fractions in whole rabbit serum except the γ-globulin. These data suggest that certain amylase fractions are separated chromatographically with particular serum proteins.

Strips prepared from the electrophoresis of 20 μl samples of the peak concentrates were also incubated on blocks of starch and agar gel for 24 hours at 37°C (6). After removing the paper strips, the blocks were flooded with 0.1N iodine solution. Absence of the starch-iodine reaction indicated areas where starch was hydrolyzed during incubation. When treated in this manner, the material of peak 1 concentrate showed a single pronounced zone of starch hydrolysis on the cathodal side adjacent to the point of application. A similar distinct zone of amylolytic activity on the anodal side was observed with strips containing peak 2 concentrate. The opposite electrophoretic mobilities of amylase activity in peak 1 and 2 concentrates may be attributable to differences in charge of the molecules. These results are consistent with the chromatographic behavior of the fractions.

The starch-hydrolysis pattern yielded by peak 3 concentrate revealed three bands of enzymatic activity. These corresponded respectively to albumin, the zone between α_2- and β-globulin, and γ-globulin. Activity corresponding to the position of γ-globulin was of interest because paper electropherograms obtained from this concentrate failed to show stainable γ-globulin. The principal zones of hydrolysis corresponded to the electrophoretic positions of albumin and γ-globulin, whereas much smaller amounts of starch were split in the region between the α_2- and β-globulin zones. Possibly, the heterogeneity of peak 3 concentrate can be partially attributed to incomplete chromatographic separation.

The amylolytic activities of the three peak concentrates were measured in citrate and phosphate buffers (pH from 5 to 8), with Lintner starch as substrate. The optimum pH for starch hydrolysis of peak concentrates 1, 2, and 3 was 6.2, 6.9, and 6.6, respectively (Fig. 2, top).

Lintner soluble starch in phosphate buffer (0.1M, pH 6.9) was used to estimate Michaelis-Menten constants (Km) for the three peak concentrates at 37°C (Fig. 2, bottom). The enzymatic material in peak 1 concentrate yielded the largest Km (4.4 mg of starch per milliliter). The smallest value for the constant (0.6 mg of starch per milliliter) was observed with peak 2 concentrate. The Km value for peak 3 (0.9 mg of starch per milliliter) was intermediate between the other two constants.

Amylolytic activity of peak 1 concentrate was enhanced at a lower pH and exhibited a larger Km than the other chromatographic fractions. It is possible that the presence of protein in peak 2 and 3 concentrates modified the relationship of enzyme activity to pH and the Km values. Although a number of nonprotein substances have been shown to alter amylolytic activities of hog pancreatic amylase (7), the influence of serum proteins on starch hydrolysis remains to be established.

Thus rabbit serum seems to contain several amylases that can be distinguished chromatographically (8).

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Thermally Induced Genital Appendages on Mosquitoes

Abstract. Temperatures above those normally present in larval sites of certain mosquitoes (for example, Aedes stimulans) cause larvae of potential males to be feminized in all degrees. Certain combinations of temperatures will affect these larvae so that an abnormal pair of genital appendages consistently appears in addition to the usual pair. These appendages appear to be masculine and are presumed to come from imaginal discs that are latent in present-day Diptera.

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In connection with a more inclusive study of the effects of thermal stress on anomalous development of subarctic aedine mosquitoes heretofore unknown, genital appendages have been produced on Aedes stimulans (Fig. 1) and other species. These appendages appear on each side as paired expansions from the caudal part of the eighth abdominal segment (Figs. 1 and 2, bottom left). They develop anterior to and in conjunction with the normal male genitalia and are, in no sense, replacements. This anomalous pair is produced consistently in response to controlled temperatures such as are not encountered normally during larval life. This pair does not appear on the eighth segment when larvae are reared at temperatures that are normal for the species (Fig. 2, top left).

Aedes stimulans is a floodwater mosquito which normally develops through four larval instars only in cold water (5° to 20°C). Under such conditions, the adult population is sexually dimorphic (1). Half of the larval population is limited to becoming females because it is homozygous for female traits. The other half is capable of becoming either sex or admixtures of both sexes because it is heterozygous for sex. The heterozygous component becomes wholly male only when reared at temperatures less than 23°C. It becomes female when reared at temperatures above 28°C. Each dimorphic part (antennae, palpi, genitalia, gonads, and so forth) may be partially or wholly feminized within this range of temperatures (1, 2). When maleness is inhibited thermally, elements for femaleness express themselves as is the case also with at least 12 other subarctic species.

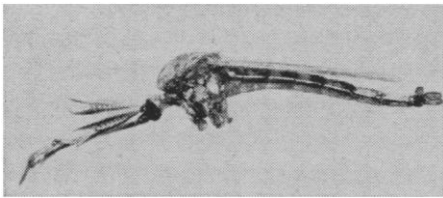When larvae are reared at different combinations of temperatures, wholly

Fig. 1. Lateral view of thermally modified *Aedes stimulans* (legs removed; genital segments rotated) showing adjunct genitalia as lobes immediately anterior to the normal ones. (× 5)

new genital appendages may be developed consistently. Selected temperatures within the range of 18° to 28.4°C (*3*) were used to induce differentiation of the anomalous appendages shown in the figures. Varying the temperatures during larval development caused the greatest differentiation of these appendages. When larvae were exposed to 28.4°C during instars 1, 2 and 3, and to 18°C during the fourth (last) instar, development on the eighth segment was the more extensive. Similar but less extensively developed appendages occurred when larvae in the last instar were exposed to 23.1°C. When larvae of instars 2 and 3 were reared at 28.4°C and those of instars 1 and 4 were exposed to 23.1°C, the append-

ages were always present but were not as well developed. Appendages appeared as small lobes when instars 1 and 3 only were exposed to the high temperature, or when all four instars were reared at 26°C. Aside from possessing appendages on the eighth segment, other external parts of modified individuals were similar to those on normal males. Internally, gonads varied from nearly normal testes to obvious ovaries.

Adjunct appendages appear as outgrowths along the caudal margin of the venter of the eighth abdominal segment (Fig. 2, bottom), and are anterior to the normal genitalia of the ninth abdominal segment (Figs. 1 and 2, bottom left). These appendages of the eighth resemble those on the ninth segment (Fig. 2, top) in many details. The prominent portion of each is comparable to the basistyle of the normal genitalia. The slender distal portion of the appendage is similar to the dististyle of the ninth segment. The inner pair of outgrowths, though displaced, is similar to the pair of claspettes of the male genitalia. The mesal, lenticular sclerite is comparable to the phallosome of the following segment. These appendages are not wholly perfected as

they are in the genitalia of the ninth segment; however, they do bear a remarkable resemblance. The similarity is even greater when those of the ninth segment have been suppressed by other thermal means (*1*).

Definitive or imaginal appendages and some organs of insects that have a larval stage develop from the growth of previously latent, undifferentiated groups of cells called imaginal discs. Under given environmental stimuli, differentiation into imaginal parts may be altered unilaterally (*4*), or a disc may cleave to produce a double appendage (*5*). No prior cases are known where bilateral development has been consistently induced on segments which do not normally bear appendages. Consistent bilateral inducement of paired appendages in this instance indicates that normally passive imaginal discs have been stimulated to activity.

The occurrence of appendages on the eighth segment of mosquitoes indicates that they are the first pair of genital appendages. They seem to be masculine appendages, in form at least, but no such pair is known for Diptera or, for that matter, for males of any winged insects. Imaginal discs are present in the eighth segment of females of some winged insects, as is shown by valves of the ovipositor in the case of Hemiptera, Orthoptera and Hymenoptera (*6*). One must look to the primitive, apterygotous genus, *Machilis* (order: Microcoryphia), to find an existing insect with normal genital appendages on the eighth segment of the male (*6*). If the parts shown in the figures really are regenerated masculine ones, then the imaginal discs from which they arise have probably been present but suppressed throughout the evolutionary history of mosquitoes (see *7*).

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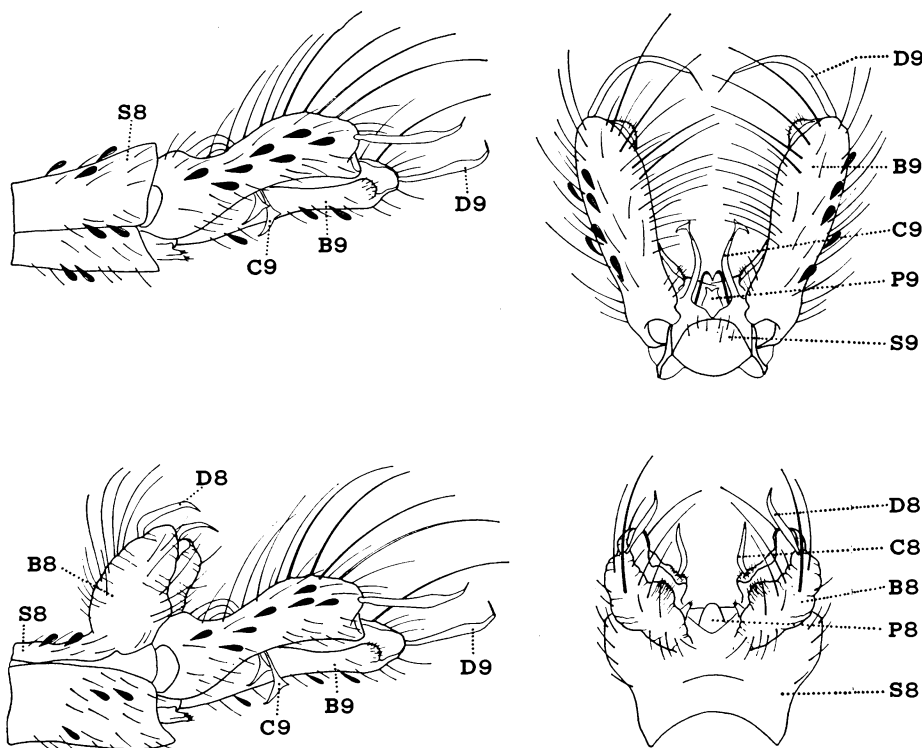University of Illinois, Urbana*

Fig. 2. Genital Segments of *Aedes stimulans* (× 55). Numbers on labels refer to segment 8 or 9. *B*, basistyle; *C*, claspette; *D*, dististyle; *P*, phallosome; *S*, sternite.   (Top left) Lateral view (rotated position) of normal male.   (Bottom left) Lateral view (rotated position) of thermally modified individual.   (Top right) Ventral view of genitalia (segments 9 and 10) of normal male.   (Bottom right) Ventral view of adjunct genitalia on the eighth segment of thermally modified individual.