EXTERNAL APPEARANCE AS AN INDICATOR OF OVARIAN DEVELOPMENT IN *CULEX PIPIENS QUINQUEFASCIATUS* SAY.

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Malariologists have devised systems for relating the external appearance of anopheline mosquitoes with ovarian development (Sella, 1920; Beklemishev, 1934; Hocking and MacInnes, 1948; Detinova, 1962). These systems have seldom been applied to culicine species.

The present study was done to determine the reliability of similar techniques when applied to *Culex pipiens quinquefasciatus* Say.

METHODS AND MATERIALS. Nulliparous females from a laboratory strain of *C. p. quinquefasciatus* (Bakersfield colony no. 1) were fed to repletion on human blood and held at a temperature of about 29° C. Samples were withdrawn and examined under a dissecting microscope (10 or 40 diameters magnification) at various intervals after feeding.

The examination procedure consisted of anesthetizing each mosquito individually with chloroform, then placing it on a microslide in a drop of 5 percent saline solution. The external appearance of the abdomen was noted and recorded according to a modification of Sella's (1920) classification, based on the position of the blood meal and the developing ovaries in relation to the abdominal segments. Then the ovaries were taken out with dissecting needles and the stage of ovarian development noted and classed in one of the five stages of Christophers.

The present study used Sella's stage 2 as the initial point, since stage *1* is an unfed condition. The dorsal and ventral appearance of the abdomen was recorded; however, it was found that the use of only the dorsal appearance did not alter the results except in the case of Sella's stage *6*. Stage *3* was found to include two distinct groups and was further subdivided.

The final arrangement of Sella's stages used was: stage *2*, blood in 6 or more segments dorsally, no ovarian development apparent; stage *3a*, blood in 5 segments, ovarian development apparent; stage *3b*, blood in 4 segments; stage *4*, blood in 3 segments; stage *5*, blood in 2 segments; stage *6*, blood in a thin line on the venter of the abdomen, ovaries occupying the entire dorsum of the abdomen; stage *7*, no visible blood, ovaries occupying the entire abdomen. It was found that most often the terminal segments of the abdomen were occupied by spermathecae, Malpighian tubules, etc. and seldom by the

distended midgut or ovaries. An occasional female had blood in part of the seventh segment, but never in the eighth.

RESULTS. Table 1 shows the numbers of mosquitoes found in each of Sella's and Christophers' stages at given times after feeding. Table 2 shows the proportion of mosquitoes in each stage of ovarian development by class of external appearance.

TABLE 1.—Numbers of females held at 29° C. in each stage of ovarian development.

	Sella's stages							Christophers' stages				
Hours	2	3a	3b	4	5	6	7	I	II	III	IV	V
0 & 6	34	..	..	..	..	..	..	34	..	..	..	..
12	17	6	..	..	..	..	..	22	1	..	..	..
18	4	14	3	..	..	..	..	5	16	..	..	..
24	14	16	8	7	4	..	..	9	38	2	..	..
30	4	25	4	..	..	..	..	2	25	6	..	..
36	1	5	21	1	..	..	..	1	14	13	..	..
42	..	1	15	7	2	..	..	1	2	22	..	..
48	..	8	16	14	7	3	..	..	4	41	..	3
54	..	2	15	5	1	..	..	1	1	21	..	..
60	..	..	8	7	3	..	..	..	..	18	..	..
66	..	..	..	1	1	..	2	..	..	1	2	1
72	..	..	1	11	7	19	11	..	..	11	16	22
78	..	..	1	6	4	12	9	..	..	11	6	15
84	..	..	..	..	..	..	29	..	..	..	..	29
90	..	..	..	..	4	13	23	..	..	8	7	25
96	..	..	..	4	5	5	5	..	..	5	6	8
102 +	..	..	..	..	..	..	20	..	..	..	..	20

DISCUSSION. While these techniques were developed using *C. p. quinquefasciatus,* they have been found useful in limited trials with *Culex tarsalis, Culiseta inornata, Aedes nigromaculis, Anopheles freeborni* and *A. franciscanus.* Similar studies done by Beklemishev (1934) on *Anopheles sacharovi, A. pulcherrimus, A. hyrcanus,* and *A. messeae* correspond closely. The greatest difference, that of the composition of Sella's stage 2, could be

TABLE 2.—Proportions of Christophers' stages in each stage of external appearance. Developmental temperature of 29° C.

Abdomen in Sella's stage	Ovaries in Christophers' stage					Number of mosquitoes
	I	II	III	IV	V	
2	0.80	0.20	..	..	..	74
3a	0.18	0.70	0.12	..	..	77
3b	0.02	0.25	0.73	..	..	92
4	..	0.10	0.82	0.06	0.02	63
5	..	0.08	0.68	0.16	0.08	38
6	..	..	0.08	0.50	0.42	52
7	..	..	0.01	0.01	0.98	99

Table 3 shows the class of external appearance by stage of ovarian development. All of the data are based on nulliparous females held at 29° C.

Two points are worth special note. The extraordinary length of stage III, which occurs from 24 to 96 hours and is found in all stages of external appearance except stages 2 and 7, and the relative ephemerality of stage IV, which was seldom found and might be said to coincide with Sella's stage 6.

TABLE 3.—Proportion of Sella's stages in each stage of ovarian development. Developmental temperature of 29° C.

Ovaries in Christophers' stage	Abdomen in Sella's stage							Number of mosquitoes
	2	3a	3b	4	5	6	7	
I	0.80	0.19	0.01	..	..	..	..	75
II	0.15	0.53	0.22	0.07	0.03	..	..	101
III	..	0.06	0.42	0.33	0.16	0.03	..	159
IV	..	..	..	0.11	0.16	0.70	0.03	37
V	..	..	..	0.01	0.02	0.18	0.79	123

explained by species differences or, more likely, by the presence of parous females in Beklemishev's field collections. Zukel (1949) used Rice and Mohan's classification, rather than Sella's for *Anopheles quadrimaculatus* and *A. punctipennis*. Within the limits of the rather rough classification system, these two species seem to agree with other data. Corbet (1964) has correlated Sella's and Christophers' stages in *Mansonia fraseri* and *M. metallica*. His results differ only slightly from those with *C. pipiens*.

Times required for development are similar to those given by other authors for a variety of species. Times for ovarian development (Christophers' stages) are given by Christophers (1911) for *Anopheles subpictus*, by Roy and Majumdar (1939) for *Culex pipiens*, by Hocking and MacInnes (1948) for *Anopheles gambiae* and *A. funestus*, and by Christophers (1960) for *Aedes aegypti*.

Unfortunately, no other studies are available giving the time required to achieve a given external configuration. If Sella's thesis (1920) of gonadotrophic harmony (i.e. the digestion of blood and the maturation of eggs proceed in a highly parallel fashion) is correct, then external appearances should correspond as closely as does ovarian development.

SUMMARY. The data presented show the possibility of utilizing the external appearance of the mosquito abdomen to determine the degree of ovarian development. It is impossible to definitely determine ovarian development without dissection; however, a useful prediction can be made from external appearance only. As would be anticipated, predictability increases with sample size.

All data presented here are based on nulliparous females. Similar studies should be done on parous females.

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