LABORATORY COLONIZATION OF *TRICHOPROSOPON DIGITATUM* (RONDANI) (DIPTERA:CULICIDAE) [1]

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Trichoprosopon (T.) digitatum (Rondani) occurs widely in the neotropical region from Mexico to Argentina (Figs. 1 and 2). In the West Indian island of Trinidad, where the present studies were undertaken, this sabethine mosquito is prevalent throughout the wooded areas but most particularly in the cocoa plantations. The immature stages have been found in the floral bracts of *Heliconia,* leaf axils of the aroid *Colocasia* and the bromeliad *Aechmea mertensii,* bamboo stumps, calabash and coconut shells, cocoa pods, and tins. Since the introduction of cocoa into Trinidad, the discarded cocoa pod has become the most important breeding place. During harvesting, as the pods are cut in half and the cocoa beans removed, the empty pericarps accumulate in piles scattered all through the plantations. Rain water then collects in them, and it is in these small receptacles, rich in organic matter, that the *Trichoprosopon* female lays her eggs and the immature stages develop. The larval population in a plantation during the wet season may be very high, but adults, while about, are not noticeably abundant, possibly because they seem not to be readily attracted to man.

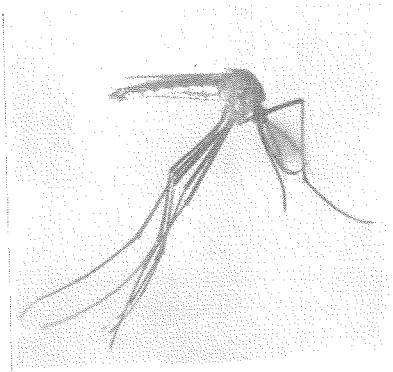

FIG. 1.—Adult male *Trichoprosopon digitatum.* (Photo Drysdale).

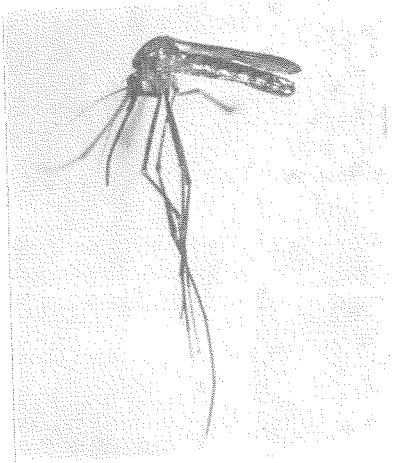

FIG. 2.—Adult female *Trichoprosopon digitatum.* (Photo Drysdale).

The ease with which larvae could be collected and their abundance led to an attempt in 1956 to establish a laboratory colony of *Trichoprosopon digitatum.* It was believed that such a colony would not only yield information on the biology of the species but also prove useful in the

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study of arbovirus transmission cycles. Species of *Trichoprosopon* naturally infected with Triniti, Aruac, and Wyeomyia viruses have been collected in Trinidad (Spence *et al.*, 1964, 1966; Aitken *et al.*, 1968).

The present study represents one of the few instances where a sabethine mosquito has been maintained in colony. Galindo (1958) undertook a similar study of *Sabethes (Sabethoides) chloropterus* (Humboldt) in Panama, and *Wyeomyia (Wyeomyia) smithii* Coquillett has been colonized in the United States (Price, 1958; Wallis and Frempong-Boadu, 1967).

MATERIALS AND METHODS. The insectaries used were out-of-doors screened structures that shielded the mosquitoes from direct sun (and rain) but left them otherwise subject to changes in the external environment. Seasonal air temperatures in the insectary fluctuated from 71° F. (22° C.) to 84° F. (29° C.) but the daily variation was generally between 77° F. (25° C.) and 81° F. (27° C).

Immature stages were periodically collected from discarded pods in cocoa plantations and brought to the laboratory. Larvae were reared in white enamel pans (9 x 15½ x 2½" deep) in approximately 1½" of tap water (initially rain water was used); metal covers were placed over the pans. Larval food consisted of brewer's yeast or laboratory fox chow, pellets of which were added to the rearing-pan water as needed. Pupae, after being washed in a metal sieve, were transferred to water in glass bowls (3⅞" in diameter x 1¾" deep), and the bowls placed in a screened cage, 27" cube (Fig. 3). From

FIG. 3.—*Trichoprosopon* colony cage. Note oilskin hood lifted back to show interior; also antiformica cups for cage legs. (Photo Drysdale).

a pan of water on top of the cage, wet cloth towels were hung down the walls. The increased humidity thus achieved within the cage was maintained by covering the whole with a tailored oilskin hood.

Emerging adults were provided with sugar water in four bottles equipped with dental wicks; bottles were removed the day before a blood meal was offered. A shaved rabbit, immobilized in a wide-mesh screen cage, constituted the source of blood. Ordinarily a blood meal was offered only once a week, but in periods of colony stress a rabbit was offered nightly (or daily).

Gravid females were provided with a variety of receptacles in which to oviposit: cocoa pods; coconut shells, dry or as green husks; bamboo internodes, green or dry; glass bowls, including those containing pupae; and half-pint Mason jars (glass, $2\frac{3}{8}$" in diameter x 3" deep) that had been painted black on the inside.

COLONIZATION HISTORY. Four colonization attempts were made before a fifth proved successful. Table 1 and Figure 4 carry this history through December 1966.

FIRST TRIAL. The first attempt to colonize *Trichoprosopon digitatum* was made with larvae and pupae collected 22 October 1956 at Santa Barbara Estate, Santa Cruz Valley. After 19 days, the cage contained some 400 adults and the first egg rafts appeared in a dried coconut shell. Only 25 percent of the November rafts were fertile, the fertile eggs coming early in the month when there were still many adults in the cage. Of the resulting immature stages, however, only about 5 percent achieved maturity, so that by early December few adults remained and reinforcements were required.

Throughout 1957, larvae and pupae from "foreign" sources (mainly the Rio Grande Forest, but also Maingot Estate at Vega de Oropouche, Archer's Estate on the Mayaro-Rio Claro Road, and Tucker Valley in the United States Naval Station reservation) were added to the "colony" almost every week. Fertile eggs were

TABLE 1.—History of colonization of *Trichoprosopon digitatum*. Average daily production of pupae from eggs laid in the colony and larvae-pupae introduced from "foreign" sources.

Year and month		Source: Colony	Source: Foreign
		First trial	
1956	Oct.	0	10
	Nov.	1	4
	Dec.	<1	11
1957	Jan.	8	11
	Feb.	9	14
	Mar.	12	12
	Apr.	8	9
	May	2	8
	June	1	4
	July	<1	2
	Aug.	<1	8
	Sept.	0	11
	Oct.	0	5
	Nov.	0	8
	Dec.	0	4
		Second trial	
1958	Jan.	7	28
	Feb.	75	8
	Mar.	49	0
	Apr.	0	6
		Third trial	
	May	0	2
	June	3	24
	July	14	4
	Aug.	3	0
	Sept.	0	8
	Oct.	0	0
		Fourth trial	
	Nov.	0	14
	Dec.	17	36
1959	Jan.	27	21
	Feb.	24	1
	Mar.	35	23
	Apr.	15	0
	May	0	0
		Fifth trial	
	June	0	4
	July	0	4
	Aug.	14	<1
	Sept.	23	9
	Oct.	12	0
	Nov.	4	10
	Dec.	36	27
1960	Jan.	102	11
	Feb.	119	2
	Mar.	55	0
	Apr.	41	0
	May	65	0
	June	6	4
	July	19	2
	Aug.	72	0
	Sept.	81	0
	Oct.	254	0
	Nov.	173	0
	Dec.	208	0

TABLE 1.—Continued.

Year and month		Source: Colony	Source: Foreign
1961	Jan.	165	0
	Feb.	153	0
	Mar.	136	0
	Apr.	107	0
	May	88	..
	June	42	..
	July	106	..
	Aug.	99	..
	Sept.	99	..
	Oct.	97	..
	Nov.	23	..
	Dec.	109	..
1962	Jan.	255	..
	Feb.	358	..
	Mar.	263	..
	Apr.	279	..
	May	306	..
	June	82	..
	July	129	..
	Aug.	175	..
	Sept.	218	..
	Oct.	32	..
	Nov.	44	..
	Dec.	124	..
1963	Jan.	144	..
	Feb.	130	..
	Mar.	103	..
	Apr.	120	..
	May	54	..
	June	45	..
	July	128	..
	Aug.	169	..
	Sept.	155	..
	Oct.	76	..
	Nov.	99	..
	Dec.	163	..
1964	Jan.	198	..
	Feb.	192	..
	Mar.	156	..
	Apr.	107	..
	May	65	..
	June	32	..
	July	95	..
	Aug.	89	..
	Sept.	81	..
	Oct.	138	..
	Nov.	104	..
	Dec.	100	..
1965	Jan.	65	..
	Feb.	120	..
	Mar.	120	..
	Apr.	92	..
	May	141	..
	June	55	..
	July	51	..
	Aug.	111	..
	Sept.	68	..
	Oct.	61	..
	Nov.	92	..
	Dec.	67	..

TABLE 1.—Continued.

Year and month		Source: Colony	Source: Foreign
1966	Jan.	92	..
	Feb.	44	..
	Mar.	75	..
	Apr.	66	..
	May	27	..
	June	34	..
	July	59	..
	Aug.	33	..
	Sept.	24	..
	Oct.	55	..
	Nov.	155	..
	Dec.	152	..

obtained regularly, although in small numbers, from January to April; thereafter, fertile egg production fell off markedly, ceasing altogether in September. During this period, dry ice and colored lights (blue and yellow) were used in an attempt to stimulate mating. Likewise, rabbit blood meals were offered nightly several times a week; feeding was poor and largely limited to the nostrils and eyes rather than the shaved back. Green coconut husks, green bamboo internodes, and freshly cut cocoa pods were each tried as oviposition bowls, and the last found to be the best. Also at this time, eggs were transferred from oviposition bowls to rearing pans containing rain water and added brewer's yeast.

During 1956–57 there was an average daily production of 11 pupae from all sources, i.e., eggs laid in the "colony" cage and larvae and pupae introduced from the wild.

SECOND TRIAL. A second and short-lived attempt to establish the colony lasted from January to April 1958. As cocoa pods seemed to be critical for stimulating oviposition and prolonged use caused pods to wear out, fresh ones were obtained, although they were not always readily found. A wooden stand was devised to hold five or six pods upright in the cage, and these were maintained in position constantly.

Fresh larvae and pupae were obtained from the wild eight times (3, 10, 17, 22,

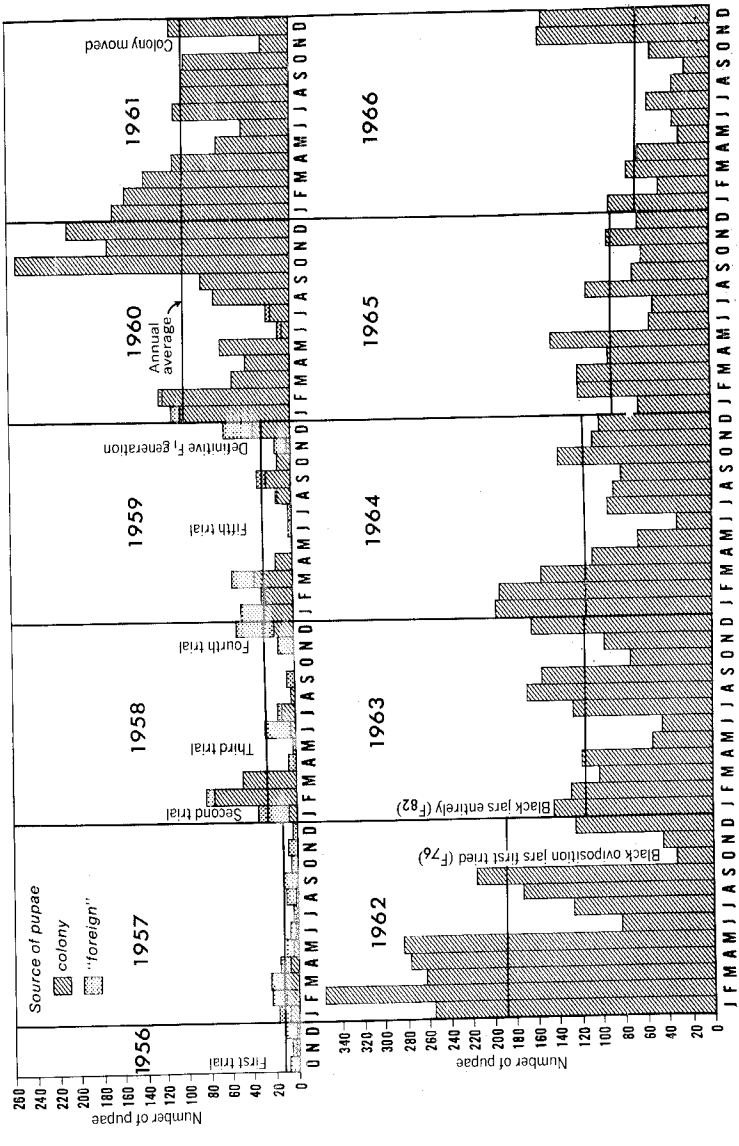

FIG. 4.—Average daily production of *Trichoprosopon digitatum* pupae derived from eggs laid in colony cage and larvae and pupae from "foreign" sources.

29, 31 January; 7 February; and 18 April). Most of this material came from the Rio Grande Forest area near Vega de Oropouche, but some came from Tucker Valley and Mayaro. "Foreign" acquisitions dropped off markedly after January, because in February and March egg laying became heavy (as many as 92 rafts on one day) and high egg fertility suggested that colony establishment was perhaps at hand. Eggs were set out with the water from the cocoa pods in pans to which rain water and yeast were added, and by April eggs were allowed to stay in the pods to hatch. It was not immediately apparent that difficulty was being encountered in rearing the larvae. On 18 February approximately 63 percent of the larvae were reaching the pupal stage; by 19 March, however, this figure had dropped to 7 percent. Brewer's yeast was the food, and apparently overfeeding occurred at times, causing considerable mortality; subsequently, smaller amounts of yeast were used. Gradual depletion in the reserves of adults continued until on 5 May all were dead. In this second attempt, rabbits were introduced into the cage during daylight hours (8:30 a.m.–3:30 p.m.).

During February average daily production of pupae rose to 83 (mainly from "colony" eggs) but dropped to 49 in March; by April, no viable eggs were being deposited.

THIRD TRIAL. The third attempt to establish a breeding colony lasted from mid-May to mid-November 1958. During this period, fresh larval and pupal material from the Rio Grande Forest, Mayaro, and Tucker Valley was introduced into the "colony" on 10 occasions: 13, 23, 31 May; 6, 11, 19, 25 June; 9, 16 July; and 29 September. Oviposition was not heavy. Eggs were laid during 26 days of the period but were fertile on only 11; the greatest number of rafts laid in one 24-hour period was 30. The last viable eggs (23 rafts) were deposited 11 August. As only two additions of "foreign" larvae and pupae were made in July plus one in September, there were insufficient adults in the cage to create proper mating conditions, and the third attempt failed.

FOURTH TRIAL. The fourth trial began on 11 November 1958, when a fresh supply of larvae and pupae and also fresh cocoa pods were brought into the laboratory. During the ensuing 7 months, field mosquitoes were introduced into the "colony" 15 times, as follows: 11, 12, 25, 28 November; 5, 9, 29, 30 December; 5, 16 January; 6, 26, 27 February; and 2, 19 March. These introductions came from the Esperanza and La Fortune Estates, Vega de Oropouche. Eggs appeared gradually, and the first fertile rafts (22) were laid 29 November; thereafter, an abundance of rafts was deposited regularly once each week until the end of March 1959. During this period egg production varied from about 30 to 90 rafts per week. Daily pupal production figures averaged 58 in March, of which 35 represented pupae reared from eggs laid in the cage. In April 110 rafts were laid; a moderate number of eggs were infertile. Altogether, 11 successive generations were achieved by 20 April (Note: a generation was considered to be 14 days). May saw only about 58 rafts laid, all of them sterile. Cocoa pods had now become scarce. By 7 June, only 10 adults remained in the cage and the fourth trial was at an end.

It would appear that, while egg production was adequate from December to March, fertility was not always high; there must also have been considerable larval mortality. In retrospect, apparently little thought was given either to the degree of fertility in individual rafts or to achieving optimal yields of pupae and adults—perhaps out of a sense of complacency engendered by the high yields of apparently fertile rafts. After February, "foreign" introductions fell off and thus, again, June saw insufficient adults to propagate the species.

FIFTH TRIAL. The fifth and finally successful attempt to establish the colony was initiated on 23 June 1959 with the receipt of fresh cocoa pods as well as larvae and pupae collected in Esperanza Estate, Vega

de Oropouche. Eleven additional introductions of larvae and pupae were made during the ensuing 12-month period, as follows: 7 July; 29 August; 4, 21 September; 20, 27 November; 4 December; 13, 14, 25 January; and 27 June 1960. All came from Esperanza except that of 14 January, which came from the adjoining La Fortune Estate.

The first fertile egg rafts (25) were obtained 27 July, and thereafter approximately similar numbers of eggs were obtained weekly. A temporary setback occurred in October and November when few eggs were laid, most of them sterile. Fortunately, however, well over 800 pupae had been produced from September eggs, and these, together with timely introductions from "foreign" sources in September, November, and early December, helped to tide the colony over the unfavorable period. In retrospect, the date establishing the F_1 generation of eggs was selected as 9 November 1959. The daily average production of pupae rose to 113 in January 1960, and to 121 in February. Although it then dropped off considerably, reaching a low of 10 in June, it quickly climbed again to a high of 254 in October. By this time the colony was fully established and on its own. A high point in production came in February 1962, when a daily average of 358 pupae was achieved. These high production figures were not considered necessary, and in subsequent years the colony was maintained at lower levels. Average daily production of pupae by year may be summarized as follows:

1956–57 (15 mos.)	11
1958	25
1959	24
1960	100
1961	100
1962	189
1963	116
1964	113
1965	87
1966	68

On 22 November 1961 the laboratory took up new quarters at Federation Park in another part of Port-of-Spain, but this move apparently had no ill effect on the colony, then in its 52nd generation.

On 27 October 1962, cocoa pods being unavailable, two half-pint Mason jars (painted black inside) and two glass bowls containing rain water were introduced into the cage as oviposition receptacles; this was 3 years after the colony was started. Eggs were laid only in the black jars. By 18 November two cocoa pods had been procured, and during the ensuing 4 weeks pods and jars were compared and found to be about equally acceptable to ovipositing females; jars, however, were slightly preferred. Accordingly, when pods again gave out on 19 January 1963, they were not replaced, and black jars, which had then been in use for 2½ months, became the standard oviposition receptacle; the colony was now in its 82nd generation. Although in previous years it had been very rare to find an egg raft in a pupation bowl, during 1963 such observations became increasingly frequent until eventually the bowls were almost equal to the jars in attractiveness to ovipositing females. The jars continue to be used, however, because of their convenience for manipulating eggs. Rafts appearing in the bowls, unless required, are discarded following eclosion of the adults.

COLONY MAINTENANCE SCHEDULE. The schedule presently (1966) used for maintaining the colony is as follows:

Monday:	Set out eggs in pan of water and add 1 pellet of yeast or ½ pellet of fox chow; feed larvae and pick pupae; remove sugar-water bottles from cage at 4 p.m.
Tuesday:	Introduce rabbit into cage 8:30 a.m.–3:30 p.m.; replace sugar-water bottles after blood meal.
Wednesday:	Pick pupae.
Thursday:	Feed larvae.
Friday:	Pick pupae.
Saturday:	Pick pupae; place 2 black oviposition jars in cage.

Note: The larvae have now become so adapted to colony life that they can tolerate large quantities of food; overfeeding does not appear to be a problem, and whole pellets of fox chow are added as needed. Perhaps this situation is a reflection of the conditions formerly tolerated by larvae in the organically rich cocoa pods.

By the end of 1966, the colony had passed through 185 generations.

BIOLOGICAL NOTES. *Trichoprosopon digitatum* is a large mosquito, and its white larva and pupa are similarly conspicuous. The larva is noteworthy for being capable of rapidly fluttering its prominent anal gills, as was observed by Raymond Shannon many years ago. The reason for this operation is not clear, unless possibly it is an aeration device to increase oxygen intake from the organically rich larval medium. Both larvae and pupae are negatively phototropic.

Ovipositing females characteristically stand guard for about 24 hours over their clutch or raft of eggs, as if they were incubating them. The raft is held between the mesothoracic pair of legs, and it is only with considerable difficulty that the female can be dislodged. The eggs are laid singly and become stuck together in a vertical position, forming polygonal rosettes (Fig. 5). From above, the bluntly rounded egg is dark gray with tiny whitish, bract-like excrescences that give it a frosted look (Fig. 6). The portion of the egg lying below the water surface is pointed, angled to one side (Figs. 7 and 8), and shiny black with a reticulated pattern; this dark area extends about ⅔ up the side of the egg in the form of three, evenly spaced loops and indicates where the egg is glued to its neighbors. Because rafts are so fragile, it is not always easy to determine their size; as many as 85 eggs have been counted, but the usual number is about 60 eggs. Although females may oviposit during the day as well as at

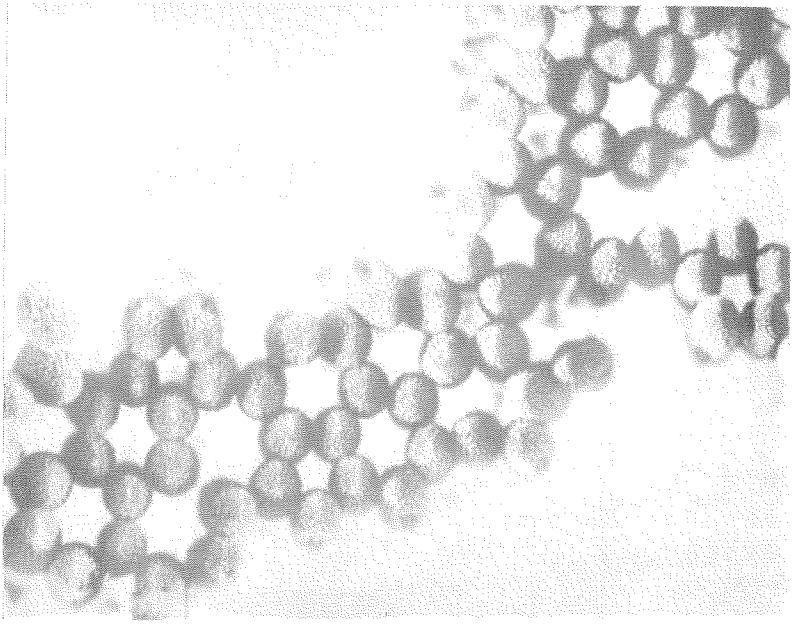

FIG. 5.—*Trichoprosopon digitatum* egg rafts viewed from above. (Photo Drysdale).

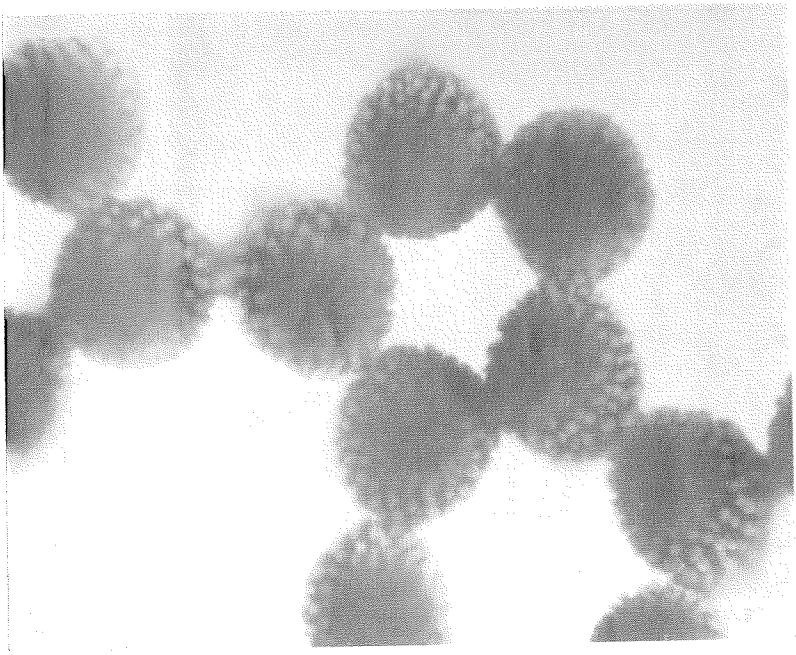

FIG. 6.—Close-up (dorsal view) showing details of *Trichoprosopon* egg sculpturing. (Photo Drysdale).

night, most eggs appear to be deposited at night.

Adults are generally inactive for the first 2 days following eclosion, spending most of this time clinging to the screen walls of the cage and only occasionally flying briefly. Females will feed on the second day after emergence, but it is not known how soon mating takes place; maturation of the ovaries requires about 48 hours. Females feed during the day and at night. When the colony was established, with use of the rabbit as the blood meal host, *Trichoprosopon* females were initially very fastidious about the feeding site, concentrating on the nostrils and to a lesser extent the eyelids and ignoring the shaved back; eventually, however, the last became the principal feeding site. At times, chicks and the toad *Bufo marinus* were immobilized in the cage, but no feeding on them was observed. Although difficulties were encountered, some success was obtained in feeding females on day-old mice. Females will settle and commence to engorge if one's hand is placed inside the cage.

Both sexes, particularly the males, are active in the cage all day long as well as at night, but their activity increases greatly at dusk (from about 16:15 hours). Likewise, mating activity may be diurnal or nocturnal but occurs mainly at dusk. The males are then actively swarming in the center of the cage; females fly into the swarm and are grappled by the males. It would seem that the long, upturned white-tipped hind legs of the female are used by the male to facilitate grasping and holding while effecting copulation, which occurs momentarily in flight. As the falling pair hits the floor of the cage, the genitalia have already become disengaged; the grasping legs then release their hold, and the two insects separate and fly off. Experience suggests that, for successful establishment of a colony, there should be several hundred adults in the cage to stimulate swarm-

ing and thus produce a favorable environment for mating and oviposition.

The life cycle of *T. digitatum*, as eventually worked out in the laboratory, is approximately as follows:

Stage	Duration at water temperature of 79–84° F. (26–29° C.)
Egg	1 day (26–30 hours)
1st larval instar	1 day
2nd larval instar	1 day (24–36 hours)
3rd larval instar	2 days (or less)
4th larval instar	3½ to 4 days
Pupa	3½ to 4 days

Approximately 20 percent of the larvae have pupated 1 week after egg hatching and the remaining 80 percent by 8 days. Thus, the cycle from egg to adult is roughly 13 to 14 days. Duration of the adult stage is unknown, but under laboratory conditions it is considered to be from 4 to 6 weeks.

SUMMARY. Five attempts were made before successful establishment of a laboratory colony of *Trichoprosopon digitatum*. History of the various trials is presented. The definitive colonization stems from immature stages first collected in Vega de Oropouche, Trinidad, on 23 June 1959, almost 3 years after initiation of the study. By the end of 1966, the colony had passed through 185 generations. Biological characteristics of the various life stages are presented and the life cycle described.

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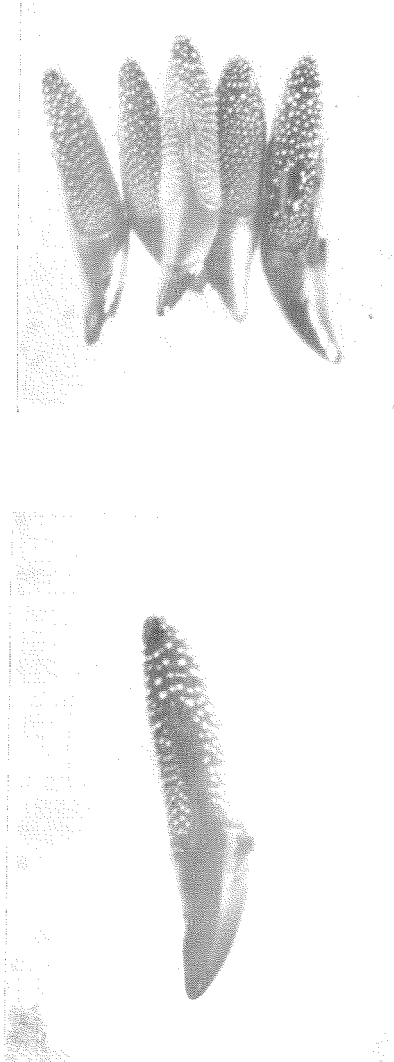

FIGS. 7, 8.—Lateral views of *Trichoprosopon* eggs. Note shiny, pointed ventral (subsurface) portion, including the dorsally extended "loops," and the dorsal (above water) sculptured portion. (Photos Drysdale).