PRELIMINARY OBSERVATIONS ON PARITY AND NECTAR FEEDING IN THE BLACK FLY, *SIMULIUM JENNINGSI*[1]

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ABSTRACT. Host-seeking females of *Simulium jenningsi* were collected in 7 sequential morning and afternoon samples in the Champlain Valley of New York state. All flies in each collection were dissected to determine 1) parity, 2) the presence of spermatozoa in the spermatheca, and 3) the presence of nectars in the crop. Parous flies were further separated according to the state of the follicular relics.

Chi-square analysis confirmed the presence of a distinct biting cycle in that nullipars predominated in the morning (60%) and parous flies predominated in the afternoon (67%. Parous flies captured in the afternoon most frequently exhibited follicular relics in the sac stage, suggesting that oviposition probably occurs early in the morning or late in the previous evening. All flies were mated and nearly all (94%) had nectar fed. No evidence of autogeny was noted.

The black fly *Simulium (Phosterodoros) jenningsi* Malloch *(sensu lato)* is one of the most widely distributed species in this recently described subgenus (Stone & Snoddy 1969, Stone 1964). It has been observed that *S. jenningsi* naturally feeds on turkeys (Underhill 1944), horses, cattle, mules, and occasionally man (Fallis 1964), making it a possible vector of zoonotic pathogens. Johnson *et al.* (1938) incriminated *S. jenningsi* (=*S. nigroparvum*) as a vector of *Leucocytozoon smithi*, a blood protozoan of turkeys.

Because of its vector potential and annoyance to man, we conducted a preliminary study to determine the parity of host-seeking flies, insemination rates, and prevalence of nectar-positive specimens.

MATERIALS AND METHODS

Females were collected with an aerial net as they swarmed about the head of the collector, who stood in or next to a pasture in Clinton Co., New York, in the Champlain Valley. Cattle were present in the pasture at various times each day. Seven sequential afternoon (13.00–17.25 hr) and morning (08.00–11.00 hr) collections were made from the evening of 05 June 1977 through the evening of 08 June 1977.

Females were identified using phenotypic characters described by Stone (1964) and Stone & Snoddy (1969). Flies were narcotized with CO_2 and dissected in a solution of physiological saline (Ephrussi & Beadle 1936) and glycerine prepared in a ratio of 9:1. The ovaries and spermatheca were removed and examined using phase contrast microscopy at magnifications of 100–400 diameters. Parity was determined using the Polovodova technique (Detinova 1962). The stage of the ultimate follicle was classified using the system of Christophers (1911) and Mer (1936). Follicular relics smaller in size than an ultimate follicle in stage I–II were considered to be in the contracted state while those larger were identified as the sac state. The spermatheca was then crushed and examined for the presence of spermatozoa. After removal of the ovaries, the body of each fly was immediately crushed in a test tube containing 1.0 ml of anthrone reagent to test for fructose in the crop. A change in color of the anthrone reagent from yellow to deep blue within one hour of testing indicated that a female had imbibed nectar (Van Handel 1972). To minimize changes in the ovaries, collections were stored at 5°C. Females from

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each sample were chosen at random and dissected within 3 hr of capture.

RESULTS AND DISCUSSION

Of 125 females dissected, 58 were captured during the morning hours and 67 during afternoons (Table I). Although the total percentage of nulliparous and parous flies engaged in host-seeking did not deviate greatly from 50%, a disparity is evident when flies are grouped by time of collection. Chi-square analysis indicated a significant difference in their distribution, in that nullipars predominated in the morning and pars in the afternoon ($p < 0.005$).

The cyclic host-seeking behavior of some African black flies has been elucidated over the past decade. Several researchers investigating host-seeking by *S. damnosum* Theobald *(sensu lato)* found that patterns of diurnal biting cycles were related to the predominant geographic feature of the region. Duke (1975), using the presence of abdominal fat body to indicate nulliparity, observed a bimodal peak of biting activity in the savanna regions of Cameroon, with nulliparous flies most active in the early morning and late afternoon. Disney (1970) and Duke (1968), using similar techniques, found that black flies in the forested areas of Cameroon exhibited a different biting cycle, with the nulliparous population peaking at 15.00–16.00 hr. Other researchers observed similar trends in Nigeria (Crosskey 1958) and Sierra Leone (Lewis 1956).

Similar patterns have been reported for temperate black flies in North America and Great Britian. In England, Davies (1955) noted that nulliparous *S. ornatum* Mg. (identified by the presence of abdominal fat bodies) accounted for 75% of the host-seeking flies until 18.00 hr at which time the proportion of parous females rose to 50%. Davies (1953) observed nearly identical biting cycles in populations of *S. venustum* Say in Canada.

Our samples encompassed only 4 days and therefore may not accurately reflect the host-seeking behavior of *S. jenningsi* over the entire season. However, since differences in parity, suggesting a host-seeking cycle, were statistically significant,

Table 1. Reproductive characteristics of *Simulium (Phosterodoros) jenningsi* collected from 05 June through 08 June 1977 in the Champlain Valley, New York.[1]

Time of Collection	No. per age class[2,3] N	No. per age class[2,3] P	No. per stage of follicular relic sac	No. per stage of follicular relic contracted	No. per stage of ultimate follicle N	No. per stage of ultimate follicle I	No. per stage of ultimate follicle I-II	No. nectar fed / No. examined	No. mated / No. examined
AM (08.00–11.00 hr)	35 (60)	23 (40)	5 (22)	18 (78)	0	5 (15)	30 (85)	30/34 (88)	35/35 (100)
					0	3 (13)	20 (87)	23/23 (100)	23/23 (100)
PM (13.25–17.25 hr)	22 (33)	45 (67)	32 (71)	13 (29)	0	3 (14)	19 (86)	21/22 (95)	22/22 (100)
					1 (2)	7 (16)	37 (82)	43/45 (96)	44/44 (100)
Total[4]	57 (46)	68 (54)	37 (30)	31 (25)	1 (1)	18 (14)	106 (85)	117/124 (94)	124/124 (100)

[1] Numerals in parentheses reflect percentage of total number examined per age class unless noted otherwise.

[2] N = nulliparous; P = parous.

[3] Numerals in parentheses reflect percentage of total number examined per time of collection.

[4] Percentages computed on total number of females dissected.

further assessment of the reproductive bionomics is warranted.

The follicular relics also suggested a cyclic pattern of oviposition by *S. jenningsi*. Parous flies were further separated according to the state of the relics. Females collected in the morning exhibited relics predominantly in the contracted state and those captured in the afternoon primarily had sac-stage follicular tubes (Table I). Chi-square analysis confirmed significance of these data ($p < 0.005$). In Guatemala, Garms (1975) used follicular relics to show a diurnal cycle of oviposition in *S. ochraceum* Walker, hypothesizing that egg deposition occurred in the early afternoon. Cupp and Collins (1979), also working in Guatemala, demonstrated that the sac stage in *S. ochraceum* coalesces to the contracted state within 4–12 hours, lending support to Garms' hypothesis. Assuming similar rates of contraction, this finding supports our conclusion that *S. jenningsi*, captured in the afternoon with sac-stage follicles, had oviposited during the late morning or early afternoon of the same day.

Examination of the developing follicles in host-seeking black flies can reveal the presence of autogeny or anautogeny (Davies 1961). Magnarelli & Cupp (1977) found no follicles past stage II in blood-seeking, nulliparous *S. tuberosum* (Lundström) and *S. venustum*, and considered both species anautogenous. Similar results are reported for *S. ochraceum* in Guatemala by Cupp & Collins (1979). Of the 57 nulliparous *S. jenningsi* examined in the Champlain Valley, none exhibited follicular development beyond stage I–II; 85% were in this stage. Therefore, we conclude that this population of *S. jenningsi* is chiefly anautogenous with ovariole stage I–II being the resting stage. The fact that all females were inseminated also suggests that mating occurs soon after emergence and may serve to stimulate host-seeking behavior.

Nectar-feeding was prevalent among both nullipars and pars (Table I). Of the 124 females tested, 117 (94%) reacted to the anthrone reagent, indicating the presence of fructose or sucrose in the crop. There were no significant differences in the incidence of nectar feeding between morning and afternoon collections or nulliparous and parous flies. This relatively high percentage of nectar-positive females parallels results on Australian, African, and Central American simuliids. Hunter (1977) found that "almost all adults" of *Austrosimulium pestilens* Mack. & Mack. captured in the field were fructose positive. In Ghana, Lewis & Domoney (1966) found sugars in each of 89 specimens of *Simulium* spp. Cupp and Collins (1979) found 68% of 334 females of *S. ochraceum* females nectar-positive.

Magnarelli (1977) hypothesized that some salt marsh *Aedes* mosquitoes, known to fly great distances, might convert carbohydrates to stored energy reserves (glycogen and triglycerides) and use the remaining sugars for flight energy. Therefore, a single feeding of nectars may be entirely metabolized in a prolonged flight from the site of emergence, and would not be detected using anthrone. *S. jenningsi* and other members of this subgenus have been reported to fly up to 48 km from known breeding sites (see Stone and Jamnback 1955). In the Champlain Valley, the nearest known larval habitat was 13 km from our collecting site. Consequently, the high order of nectar imbibition determined in this study suggests that members of this subgenus 1) metabolize sugars slowly and are highly efficient in using nectars as sources of energy for flight, or 2) imbide nectars frequently.

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