Observations on some Mechanisms of Host Finding and Host Selection in the Simuliidae and Tabanidae (Diptera)

by

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Scope and Contents:

Observations and experimental data concerning the attraction of simuliids to carbon dioxide and odors from birds and of tabanids to carbon dioxide are presented. The role of these attractants in orientation behavior is discussed. These observations led to the development of an hypothesis of feeding behavior. The relationship between attraction and host preferences is discussed.

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Introduction

For several months of every year, Ontario abounds with large populations of biting Diptera of the families Culicidae, Ceratopogonidae, Simuliidae, Tabanidae, and Muscidae. In many parts of the world, these insects are of immense medical significance as the vectors of a number of serious human diseases but, in Canada, they are of comparatively minor medical importance. Nevertheless, their large numbers and persistent biting habits can make work in the field virtually impossible or unbearable.

The minor medical importance of these flies notwithstanding, they are of notable economic significance in Canada, since they serve as the vectors of a number of pathogens of wildlife and, in particular, of birds. Of paramount significance in this regard are the genera *Leucocytozoon*, *Haemoproteus* (Bennett *et al.* 1965) and *Plasmodium*; these blood parasites can cause severe mortalities in young birds (Bennett and Fallis 1960; Fallis and Bennett 1966). Simuliids serve as the vectors of the filarial worm, *Splendidofilaria fallisensis* Anderson of ducks (Anderson 1956, 1961). Blood-sucking Diptera probably serve as the vectors of the helminths *Setaria tundra* Isaichikov and Raevskaya and *Wehrdikmansia cervipedis* Caballero of the white-tailed deer, *Odocoileus virginianus* (Anderson 1962). The Tabanidae have recently been implicated in the transmission of rickettsias (Amanzhulov *et al.* 1965) and have been suspected of mechanically transmitting the etiological agent of a number of diseases, notably tularemia (Jellison 1950).

The feeding behavior of at least some members of each of these families is unknown, and some of the simuliids are known to show ovarian autogeny (Davies and Peterson 1956; Davies *et al.* 1962). However, most species require a bloodmeal, presumably as a nutritional supplement to facilitate ovarian development. Almost invariably, this bloodmeal is obtained from a homeothermic animal.

The feeding behavior of blood-sucking Diptera may be seen to involve 2 major components, viz:

- the host-finding mechanism;
- the determination of host preferences.

By the term "host-finding mechanism" is understood the actual means by which the fly is able to locate a potential host in space. Any study of host-finding mechanisms must therefore involve, at some stage of the investigation, an inquiry into the sensory information required by the fly to effect orientation to the host and a study of the corresponding behavioral modifications or reactions that these data <sic> produce. The problem of host preferences or host specificity involves a study of the various factors that determine the extent to which a given species will feed on a given host animal.

This behavior associated with the location and selection of a host from which to obtain a bloodmeal presents extraordinarily complex problems of orientation and sensory physiology, and has been the subject of a considerable body of investigations into the biology of biting flies. The Culicidae have received the bulk of attention because of their obvious medico-veterinary importance. However, in spite of the large volume of work done with this family (Clements 1963), there remains considerable dispute concerning the manner in which a mosquito is able to locate the host and no definitive theory of the mechanism of orientation has emerged.

The simuliid and tabanid faunas of Ontario are abundant and taxonomically well documented (Pechuman *et al.* 1961; Davies *et al.* 1962; Wood *et al.* 1963; Wood 1963). Nevertheless, investigations of the mechanisms involved in host finding or in the determination of host preferences in these 2 families are very few, especially insofar as possible distance orientation to the host by olfaction is concerned (Hocking 1960).

In an elegant study of the cephalic sensory organs of the female *Tabanus quinquevittatus* Wiedemann, Scudder (1953) reported the presence, on the antennae, of trichode and coelicone sensory end organs that he presumed to be olfactory in function. In addition, he located ampullary end organs on the antennae that he hypothesized might be involved in the recognition of odors at high concentrations.

The complexity of orientation behavior has already been alluded to; Scudder has demonstrated the presence on the antennae and maxillary palps of the required complex of sensory equipment needed to effect orientation behavior. He has proposed a 2-stage orientation to the host as follows:

1. a long-range location of the host mammal, theoretically involving:
    a. visual perception by the eyes;
    b. chemoreception by 3 types of antennal trichodes, 1 type of palpal trichode, and 1 type of antennal coelicone;
2. near-orientation to the host, theoretically involving:
    a. elements of (1) (above);
    b. thermoreception;
    c. chemoreception by high-odor-density antennal ampullas.

Several workers have commented on the visual element in the attraction of tabanids (Philip 1931; Hansens 1947; Tashiro and Schwardt 1953) and the role of vision in orientation behavior has been thoroughly investigated by Thorsteinson and his students (Bracken *et al.* 1962; Thorsteinson *et al.* 1966). These workers tested the attractiveness of colored spheres to tabanids and found that black and red spheres were highly attractive to all species tested. The attractiveness of gray and white spheres varied inversely with reflectance. These workers also investigated the role of form stimuli and found that 3-d black silhouettes with a convex curvature were most attractive. The experimental arrangement used by these workers was near-orientation to the host, or landing behavior. A distinction between near-orientation and landing behavior is probably arbitrary. In the forested regions of Ontario it is doubtful that vision could serve as a long-range orientation clue as Scudder (1953) indicated, and should probably fall under his stage 2. Vision has been shown to be involved in the landing responses of simuliids (Davies 1951, 1961; Wenk and Schlörer 1963).

Thorsteinson (1958) considered heat as a factor in the near-orientation of tabanids, an idea that later he seemed not anxious to promulgate (Bracken *et al.* 1962). Nevertheless, Scudder (1953) did demonstrate the presence of an epidermal nerve net in *Ta. quinquevittatus* that he thought might be involved in thermoreception.

Thus, while there has been some indication of the sensory data required by simuliids and tabanids in near-orientation and landing behavior, no account exists in the literature concerning a distance-orientation to the host. It would be surprising, indeed, if distance-orientation by olfaction were lacking in these 2 families since olfaction is a most common sensory mode in insects (Hocking 1960) and olfaction is known to be involved in the host orientation of the Culicidae (Willis 1947; Brown 1951; Brown *et al.* 1951; Reeves 1951, 1953; Willis and Roth 1952; Van Thiel and Laarman 1954; Laarman 1955, 1958; Wright 1958, 1964; Brown and Carmichael 1961a, 1961b; Clements 1963; Daykin *et al.* 1965).

One of the most remarkable and intriguing aspects of the biology of blood-sucking Diptera is the phenomenon of host preferences. Fallis (1964) summarized host preferences or host selection in the Simuliidae. The mechanisms governing host preferences or host selection are most profitably viewed in relation to the general host-seeking behavior of the fly. However, the *extent* to which these 2 behavioral processes are functionally integrated is, as yet, a moot point.

The simuliid fauna of Algonquin Park demonstrates the phenomenon of host preferences in several degrees. If considered on the basis of blood-feeding behavior, the Simuliidae fall naturally into 2 major divisions (Davies and Peterson 1956; Davies *et al.* 1962).

1. Non-hematophagous, autogenous species.
    e.g. *Twinnia tibblesi* Stone and Jamnback, *Prosimulium gibsoni* (Twinn), *Cnephia* (*Cnephia*) *dacotensis* (Dyar and Shannon), *Simulium* (*Eusimulium*) *baffinense* Twinn.
2. Hematophagous species.
    This group is the larger of the 2 and contains those forms that take a bloodmeal at least once during the life cycle. Some species may be autogenous or partially autogenous for the 1st cycle of eggs but must take a bloodmeal to either complete the 1st cycle or to initiate a 2nd, or subsequent cycles. Hematophagous Simuliidae feed only on homeothermic animals. The few North American records of feeding on poikilotherms, while interesting, may be regarded as behaviorally aberrant.

This 2nd group may be readily subdivided as follows:

A. Ornithophilic forms.
   Species feeding preferentially or exclusively on birds; e.g. *P. decemarticulatum* (Twinn), *C.* (*Cnephia*) *ornithophilia* Davies, Peterson and Wood, *C.* (*Ectemnia*) *invenusta* (Walker), *S.* (*Byssodon*) *rugglesi* Nicholson and Mickel, and most *Simulium* (*Eusimulium*) spp.

B. Mammalophilic forms.
   Species feeding preferentially or exclusively on mammals; e.g. *P. fuscum* Syme and Davies, *C.* (*Stegopterna*) *mutata* (Malloch), and *Simulium* spp. of the subgenera *Simulium*, *Psilozia*, and *Hagenomyia*.

Within these subgroups, host preferences are evidenced to a greater or lesser degree, depending on the species of fly. Bennett (1960) has examined the phenomenon of host preferences among the Ontario simuliids. He found that *S. rugglesi* fed on ducks in preference to American Crows (*Corvus brachyrhynchos*), Ruffed Grouse (*Bonasa umbellus*), American Robin (*Turdus migratorius*), White-throated Sparrows (*Zonotrichia albicollis*) and a Saw-whet Owl (*Aegolius acadicus*). A similar marked preference for the Anseriformes was shown for *S. anatinum* Wood (as *Simulium* "H"). Moreover, Bennett compared the attractiveness of different species of ducks to *S. rugglesi* and found that the ducks within a single genus differed in their attractiveness. Thus, domestic ducks (*Anas boschas*) seemed relatively more attractive to the fly than either Black Ducks (*A. rubripes*) or Mallards (*Anas platyrhynchos*).

In a similar vein, Bennett examined the host preferences of sylvatic simuliids and found that these, not surprisingly, preferred woodland birds to ducks. He found sylvatic simuliids feeding on grouse, robins, Blue Jays (*Cyanocitta cristata*) and Sharp-shinned Hawks (*Accipiter striatus velox*). Differences in the numerical size and species composition were found in collections of flies from different woodland birds, but Bennett was understandably chary of drawing conclusions concerning host preferences. However, he was of the opinion that *S. aureum* Fries fed on all species tested except crows, that *S. croxtoni* Nicholson and Mickel was common on both grouse and crows, and that *S. latipes* (Meigen) (which probably included *S. quebecense* Twinn) and *C. ornithophilia* (as *Cnephia* "U") were ubiquitous <sic; i.e. found on all tested host species>.

Bennett also made observations of the quantitative differences in the collections, observing, for example, that more flies fed on robins than on woodpeckers.

The Tabanidae are, perhaps, less remarkable in their host preferences than the Simuliidae. Tabanidae are exclusively mammalophilic <no, some species are known to feed on lizards and crocodiles and I myself have a record of tabanids from a snapping turtle!>, notwithstanding a few rare instances of specimens taken from birds (Bennett 1960, 1965). Tabanids from birds are behaviorally analogous to simuliids from poikilothermous animals, and probably represent aberrant behavior. The author has one occasion taken tabanids from a poikilotherm. In addition, the Tabanidae are restricted in their mammalophily, feeding only on the larger forms. Thus, in Algonquin Park, for example, their feeding habits restrict them to only 5 or 6 possible hosts, viz. man, the timber wolf, *Canis lupus lycaeon* Schreiber, the coyote, *C. latrans thamnos* Jackson, the white-tailed deer, *Odocoileus virginianus borealis* (Miller), the moose, *Alces alces americana* (Clinton), and the black bear, *Euarctos americanus americanus* (Pallas). Unfortunately, feeding records for Tabanidae, other than on man or domestic animals, are notoriously uncommon. Observations suggest that the majority of Tabanidae in Algonquin Park obtain their bloodmeals from the Cervidae, but few records exist showing actual feeding on these hosts (Anderson 1962).

Apart from the behavioral relationship between host-seeking behavior and host-selection behavior, the latter, in itself, is a most complex problem. The problem of host-selection behavior is not only of intrinsic interest but has important consequences in the epizootiology of diseases transmitted by blood-sucking Diptera. An *a-priori* examination of the phenomenon of host preferences shows that there are at least 3 groups of factors that may be involved in the determination and medication of the observed preferences. These groups of factors are as follows:

1. Factors essentially “extrinsic” to the actual host-seeking situation.
   e.g. the habitat preferences of the host and/or fly, in terms of both horizontal zonation and vertical stratification; weather conditions.
2. Factors “intrinsic” to the host.
   e.g. population density and availability; behavior; color; movement; shape; size; emanations (e.g. odor, moisture, heat, carbon dioxide).
3. Factors “intrinsic” to the fly.
   e.g. circadian rhythms; physiological state and/or age; nutritional state; olfactory conditioning.

In any preference situation several or many of these factors may be operative in the determination of selection or rejection of a potential host. Habitat preferences of the population of host-seeking flies have often been overlooked in the study of the biology of feeding. Laing (1937) demonstrated that the parasitic wasps *Alysia manducator* Panzer (Braconidae), *Mormoniella vitripennis* Walker (Chalcidae)and *Trichogramma evanescens* Westwood (Chalcidae) select first for habitat and only then select for a host within the selected habitat. Bennett (1960) made preliminary observations of the habitat preferences of the Simuliidae and found a difference between the fauna characterizing lake shores and the sylvatic fauna. Bennett determined habitat preferences by exposing hosts in a variety of habitats. By this method he demonstrated that it is impossible to “draw” a species in large numbers from its characteristic habitat, even by presenting its preferred hosts in the alternative habitat. This is evidence that there are factors determining the rigidity of habitat <selection> over and above those determining host preferences.

However, Bennett’s observations are now open to question on several counts. First, through no fault of the worker, there is obvious taxonomic confusion in his results. Thus, he reported in the lakeshore habitat, *S. rugglesi* and *S. anatinum* (as *Simulium* “H”) to be most abundant, which results agree well with those to be presented below. However, he also reported the presence of *S. excisum* Davies, Peterson and Wood (as *S. subexcisum* of Twinn) and small numbers of *S.* “*latipes*” (which presumably included *S. latipes* and *S. quebecense*) and *S. croxtoni* in the habitat. Data presented below indicate that these species are virtually absent from the lakeshore habitat, under normal circumstances. The author is strongly suspicious that some of Bennett’s records represent misidentifications of *S. anatinum*. *S. croxtoni* is easily confused with *S. euryadminiculum* Davies, a typical lakeshore species that Bennett reported as *S. canonicolum* Dyar and Shannon.

Some of the results reported by Bennett may be due to the fact that living hosts were employed in the assessment of habitat preferences. This immediately creates an unresolvable conflict between host preferences and habitat preferences. Thus, the habitat preferences of *S. rugglesi* were assessed by placing ducklings at varying distances from the lakeshore into the woods, as well as in the forest canopy. The rationale of the experiment is readily appreciated, but there is little or no ecological or behavioral justification for it, since ducks normally do not occur in such habitats. The creation of highly aberrant conditions under which the behavior of a species is assessed may result in the observation of aberrant behavior patterns. The determination of what the species is capable of doing under abnormal conditions is interesting, but difficult to place in an ecological context.

Apart from the above-mentioned studies of the visual components of host-orientation behavior in simuliids and tabanids, little or no attention has been given to other “host-intrinsic” factors. Yet, no factor is so appealing to the investigator, on *a-priori* grounds, as odor in the mediation of host preferences. In addition, Reeves (1951, 1953) considered that the different quantities of carbon dioxide expired by different animals might account for species preferences in the Culicidae. Whitsel and Schoeppner (1965) held similar views on the attraction of midges of the genus *Leptoconops* (Ceratopogonidae) to carbon dioxide. Davis and James (1957) found *S. vittatum* Zetterstedt attracted to meat baits containing much tallow.

Host preferences are characteristically rigid and seem unlikely to vary a great deal from established patterns. However, DeFoliart and Rao (1965) found late-season changes in the biting behavior of *S.* (*Byssodon*) *meridionale* Riley. Typically, *S. meridionale* feeds on Galliformes. It has been noted, however, that late-season specimens may attack man. The cause of this change in the feeding habits is unknown, although it is not unlikely that it is of physiological origin.

A word should be added concerning the use of the word “attractant” in orientation studies. Generally speaking, an attractant is anything that draws (Dethier 1947). However, in a behaviorally complex situation such as is found in blood feeding, in which, presumably, a hierarchy exists (Laarman 1955), such an operational definition of “attractant” is obviously inadequate. In the following sections, therefore, the word is employed in its broadest sense, and when used, does *not* imply an endorsement of any underlying physiological or behavioral mechanism unless so specified.

It is of considerable importance to understand the means by which simuliids and tabanids select and locate their hosts. Both families are of considerable importance as pest species in Ontario, and knowledge of attraction could presumably be put to use in control methods against these insects. The reliance on *Aedes aegypti* as an experimental subject for the investigation of host-orientation mechanisms in the Culicidae is probably unjustified, but extrapolations from the Culicidae to other families are definitely so. The present investigation represents an initial attempt to establish, in part, the nature of the host-seeking and host-selection behavior patterns in simuliids and tabanids. The problem of habitat preferences has been reexamined. Attention has been given to some of the possible factors involved in the determination of host finding and selection, but the objective assessment of this behavior is extraordinarily difficult when attempted under field conditions. A study such as this is complicated by factors of the biology not heretofore appreciated by many workers. Some of these complicating factors have been pointed out in this study.

Materials and Methods

This program of research was carried out during the months of May – September in 1964 and 1965. In addition, some field work relating to the program was done in the summers of 1962 and 1963. All field work was conducted at the Wildlife Research Station of the Ontario Department of Lands and Forests in Algonquin Park.

Algonquin Park lies in the transition zone between the southern deciduous forest biome and the northern boreal forest. Thus, the region is characterized by the number of animals and plants that reach either their northern or southern limits there. Thus, the Park provides for an ideal zoological station. The Research Station is situated in the valley of the Madawaska River and the area utilized for research was within 1 mile of the Station.

The Madawaska River flows through a flat, sandy valley bounded by abrupt hills (Beckel and Atwood 1959). The forest (with the exception of those portions immediately adjacent to bodies of water) consists almost entirely of a deciduous-coniferous mixture, the most common species being white pine (*Pinus strobus*), white birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), and hard maple (*Acer saccharum*).

Within this general habitat, collections of flies have been made in a number of more limited localities, a brief description of each of which follows:

a. Shore of Lake Sasajewun

Lake Sasajewun is a small (40 acres), shallow body of water, formed by the damming of the North Madawaska River at the Research Station. The immediate lakeshore is surrounded on much of its perimeter by speckled alder (*Alnus rugosa*, var. *americana*). This was cleared from an approximately 100-yard section of the shore, and collections were carried out on the grassy shore within 10 feet of the lake.

b. The North Madawaska River

The North Madawaska River is a shallow, swift-flowing stream, not more than 10 yards in width at most places. The entire river course is lined with speckled alder. Collections of Tabanidae were made by placing traps in mid-stream or on small islands in the stream.

c. Cervid Pens

The Ontario Department of Lands and Forests maintains captive moose and a herd of white-tailed deer at the Research Station. The 2 species are kept separately in large, partially forested pens. Collections of flies from deer were not feasible because of the near-wild state of the animals. However, it was often possible to enter the pen with the moose and collect flies. Only those Tabanidae actually landing on the animals were collected. 2 moose were present throughout the fly season in 1965, and both animals were yearling cows. In addition, collections of Tabanidae were made from traps placed near these pens in a small forest clearing.

d. Bat Lake Road

Bat Lake is a small bog lake of approximately 6 acres. Leading through the mixed forest to the lake is an unused roadway approximately 5 yards in width, covered for most of its length by the forest canopy. Collections of Tabanidae were made from traps placed on a slightly more open section of the road. This site was essentially the same as (c) (*above*).

e. The Forest Canopy

Collections of Simuliidae were carried out a several levels in the forest canopy. For the most part, the canopy is 40–45 feet at its highest points. Collections were made from living hosts as well as from traps equipped with a variety of putative attractants. Collecting at various levels was facilitated by the establishment of several hoists and pulley systems in white-spruce trees (Bennett 1960). For the purpose of carrying out simultaneous or comparative collections, several such pulley systems were operated, all within 50 yards or less of one another.

f. The Forest Clearing

As the program of research progressed it became apparent that habitat was influencing the collection of simuliids, particularly in the forest canopy where one must, of necessity, deal with a complex of several species. Thus, in an effort to approximate uniform collecting conditions, some collections in 1965 were made in a large, rectangular forest clearing. On 3 sides the clearing was bordered by the forest, much more dense on 1 side than on the other 2, and the 4th side of the clearing ran along a roadway. 2 taut metal lines were established at an elevation of 14 feet, the 2 lines running at right angles to one another (Fig. 1). Movable pulley systems were set up on each line.

g. Bog.

Collections of tabanids were made in August of 1965 from traps placed at various locations in a small (approximately 15 acres), typical bog. The center of the bog was taken up by a small, shallow pool.

Collection Techniques

At the initiation of this study, no suitable collecting techniques were available. During the course of the investigation, several different collecting methods have been employed. Modifications have been introduced into the collecting methods whenever the result was the obtaining of either larger samples with less labor, or a reduction in the involvement of humans in the collecting area.

Collections of Simuliidae from living hosts have generally been carried out following the methods of Bennett (1960). Tabanidae from living hosts (mostly man or moose) were usually carried out by netting them directly off the host (man and moose) or by netting them from the air surrounding the host (man).

In some of the initial experiments with attractants, a few collections of flies were made by sweeping with an insect net over the putative attractant being tested. Such methods have obvious quantitative limitations and efforts were made to develop automatic sampling traps, especially for simuliids.

In 1964, for the purposes of making collections of flies from carbon-dioxide or bird-extract baits, large gauze collecting cages of the type employed by Bennett (1960) for collecting engorged simuliids, were suspended in the air a few inches above the bait being tested. Flies coming to the attractant flew up into this cage and were thereby trapped. The cage was open only at the bottom. Collecting trapped flies from these cages was done by placing the cage down on a white plywood square and aspirating the flies from the cage through a sleeve at its top. This method for collecting simuliids proved valuable for those species that characteristically frequent the lower strata (e.g. *S. venustum* and *S. rugglesi*). However, the method had very serious limitations when attempts were made to employ it in collecting in the forest canopy. The most serious limitation of the method was the lack of mobility of such large cages. Moreover, the task of aspirating flies from the cage at the termination of the collection is both tedious and time-consuming, especially when host-seeking populations are large. Nevertheless, this method has the very desirable advantage of enabling one to collect hundreds of unengorged, presumably “blood-hungry” simuliids in virtually undamaged condition.

In 1964 an attempt was made to develop mechanized collecting techniques in the form of suction traps. A preliminary test with a small suction trap modeled on the design of the New Jersey light trap indicated that such traps were practical for use with the Simuliidae when equipped with suitable attractants. Such traps have a number of very important advantages, among which are the following:

1. suction traps markedly reduce the amount of labor involved in the actual process of collecting;
2. since the traps are automatic, it is possible to effectively reduce the human element in collecting;
3. suction traps partially eliminate the necessity for the fly to negotiate a landing approach in response to the attractant. Theoretically, therefore, one can more nearly isolate attraction behavior from landing behavior. This aspect applies only to the Simuliidae, which seem to react visually to an object only at very close range.

Initially several suction traps were made from sheet metal; these traps were approximately 18 inches high and 9 inches in diameter. A 12-volt automobile fan was mounted in the cylinder of the trap to provide the suction. To the main body of the trap was attached a cone, to the end of which was attached a small holding cage (a cube, approximately 8 inches on a side) into which the flies were blown. The holding cage was attached temporarily to the suction trap by a cloth sleeve. At the termination of the collection, the holding cage was removed, and the flies could be maintained in it until examination.

These large suction traps were generally successful but suffered from many of the same disadvantages of the gauze collecting cages. That is to say, they proved adequate for the simuliids of the lower strata but were awkward for use in the canopy. Furthermore, the traps required 12-volt wet cells as a power source, and these could be used only in sites that had road access, since the units could only be transported with difficulty.

In 1965 an attempt was made to miniaturize the suction traps and to that end a number of small traps of acrylic plastic were constructed. These traps were modeled after the CDC light trap (Hauscherr's Machine Works, New Jersey), which has been successfully employed as a mosquito trap (Sudia and Chamberlain 1962).

The body of the trap is but 6 inches in length, with an outside diameter of 3.6 inches. The traps were equipped with "Indoperm Elektromotors", type 6v/1000. These small motors can be run on voltages of 3–12 volts. At 6 volts they draw an unloaded current of about 150 mA, with a maximum, when loaded, of about 700 mA. A 3-bladed model-airplane propeller (3 inches diameter) was attached to the shaft of the motor. The body of the trap is strengthened with 3 rings of acrylic plastic just sufficiently large to slip with difficulty over the body (Fig. 2).

The entire unit when assembled weighs <300 g, exclusive of the power source and associated wiring. 6-volt motorcycle batteries were used as the power source. Since the motors equipped with the propeller draw only 300 mA, these batteries will operate the traps for many hours without requiring recharging. As a rule, however, batteries were charged regularly, usually nightly. Whenever 2 such miniature suction traps were employed simultaneously in the same habitat or collection site, the batteries for the 2 traps were connected in parallel to avoid individual differences in the speeds at which the 2 traps were running.

The strengthening ring at the bottom of the trap (Fig. 2) provided the means whereby a collecting cage could be attached to the trap. These collecting cages (Fig. 2) were made from 1-quart food containers of waxed cardboard. The center of the bottom of the container was pushed out, and the remaining rim, which is removable, was used to secure in place a Dacron mesh of a diameter of 4.5 inches, or about 1 inch greater than the diameter of the container. Thus, the bottom gauze could be held in place without any attachment, the rim being sufficiently tight to secure the gauze. The opposite end of the container was equipped with a gauze sleeve about 5 inches in length. This sleeve was of a diameter sufficient to allow it to be slipped with relative ease over the bottom of the suction trap. The sleeve was secured to the container by means of surgical tape. The holding cage thus constructed was attached to the fan trap by passing the sleeve over the bottom of the trap, as high as the first strengthening ring, and securing it in place with a rubber band. At the termination of the collection, the rubber band could be removed with the collecting container and used to secure the sleeve of the cage, thereby transforming the container from a small collecting cage to a holding cage. These small holding cages were of an extremely convenient size. Flies were maintained alive in these cages for varying periods of time (as long as 4 d in some cases) until they could be examined. If collections of flies were to be held for >4 h, the bottom of the holding cage was set in the lid of a plastic Petri dish (9 cm diameter), in which was placed either filter papers or absorbent cotton moistened with distilled water. This provided a source of water for the flies and maintained a relatively high humidity within the small cages. Sucrose in the form of lump sugar was supplied to collections of flies maintained for >24 h (Davies 1953).

Entry to the holding cages was facilitated by the use of 2 windows cut in opposite sides of the container (Fig. 2). A circular hole (0.5 inch) was cut in the side of the container and covered with 2 sheets (about 1.5 inches square) of rubber dam held in place with surgical tape. In the center of the dam, 2 slits were cut, 1 in each sheet, and at right angles to one another. This procedure provided for entry of the tip of an aspirator while preventing the escape of flies.

Large numbers of these holding cages can be constructed in a comparatively short time at minimum <sic; i.e. *low*> expense. They greatly facilitated collecting in the field, particularly in cases in which hourly collections were being attempted. The collecting container could be removed and a new one put in its place and the fan trap be placed back in position, all within 2 or 3 min. Masking-tape labels were placed on the sides of each container so that the date, time, and site of collection could be noted immediately. The tape label was replaced when the collection was examined, and the holding cages could be re-used immediately.

These miniature suction traps are extraordinarily efficient simuliid traps. They provide an easily adaptable, highly portable system adequate for quantitative sampling of all simuliids when equipped with a suitable attractant. A considerable increase in the quantitative data would have ensued had these traps been introduced into the program at an earlier date. The blades of the propeller in these small traps move at a high speed and some flies (perhaps as high as 25% in some instances) are damaged as they pass over the blades. No attempt was made to modify the design of the trap to reduce this damage since, in most cases, only total numbers of flies were of interest, the species composition have been determined either from the non-damaged portion of the collection or from collections made by other methods. It should be pointed out, however, that very few flies are damaged so badly that they cannot be identified to species if necessary.

2 types of trapping methods were employed for the Tabanidae. The suspended-cage method described above for simuliids provides a highly efficient tabanid trap if set close to the ground (i.e. not higher than 3 feet). In addition, “heliothermal” traps as described by Thorsteinson (1958) were used. Thorsteinson thought that these traps functioned by effecting an orientation to heat since the traps are characteristically at much higher temperatures than ambient, through a greenhouse effect. However, orientation to the trap is more probably mediated by visual stimuli. Such traps, when equipped with a suitable attractant, are highly efficient in capturing tabanids.

Attractants Tested

Extracts of the uropygial glands of some birds have been found to attract simuliids or to be involved in orientation. Such extracts are prepared, following surgical excision of the glands from a recently killed bird, by maceration of the gland tissue, and suspension of the macerate in suitable organic solvents. A number of solvents proved successful as extraction solvents, viz. diethyl ether, acetone, and chloroform. However, most extracts were prepared from ether. These extracts were usually stored, in solution, in a refrigerator at 40°F <4.4°C>. For use, filter papers were impregnated with small amounts of the solution and the solvents were evaporated quickly. Usually fresh extracts prepared in this manner were employed in the experimental work. However, if the extracts on paper were to be saved for later use, they were stored in individual envelopes in the refrigerator.

For work with *S. rugglesi*, extracts were prepared from the uropygial glands of domestic ducklings; for *S. euryadminiculum*, extracts were prepared from the glands of the Common Loon (*Gavia immer*). A number of birds were used for the extract preparations for work with the sylvatic simuliids, but most commonly extracts of the glands of Ruffed Grouse, Robin, or Grackle (*Quiscalus quiscula*) were employed. Grackles were most often used because they are a suitable host and were usually abundant in the vicinity of the Station.

Carbon dioxide used in the attraction studies was obtained as the compressed gas in small (10–20 pounds net), easily portable cylinders from Matheson Co., Whitby, ON (1964) or Canadian Liquid Air, Hamilton, ON (1965). The cylinders were equipped with the appropriate gas regulators. For the purposes of measuring flow rates, ball-capillary flow meters were obtained from Matheson Co. The flow meters were equipped with 2 floats, one of fritted glass and the other of stainless steel. This permitted the use of 2 overlapping ranges of flow. These flow meters permitted a fairly accurate dispensation of the gas under field conditions and the flow rates could be maintained with uniformity over considerable periods of time. Usually low rates of flow were employed, especially for the Simuliidae. Rates of flow exceeding 150 ml/min <as “cc/min”> were rarely used for simuliids. Brown (1951) cited 200 ml/min of carbon dioxide as the normal human exhalation rate. Occasionally higher rates of flow were used, especially with work on the Tabanidae, and this necessitated different flow meters. For rates of flow exceeding 900 ml/min, no flow meters were employed; rather, flow rates were calibrated periodically with the aid of a graduated cylinder.

Observations

Habitat Preferences in the Tabanidae

Zonation

Tabanids were collected in a variety of habitats in July and August 1964 and 1965 (Tables 1, 2a, 2b). The habitats in which collections have been made can be divided into 2 major groups, as follows:

1. Sylvatic
2. "Aquatic" with the following subgroups:
   2.1 Bog (1965 only)
   2.2 River
   2.3 Lakeshore

The figures for the 1965 collections have been separated into July and August collections. Collections were not made in the bog habitat in July nor in the river habitat in August. For each month, the figures in Tables 2a and 2b have been standardized so that in each case the collecting effort (in terms of trap hours) is equal for each habitat. In each case, the basis for standardization of the figures has been the trap-site with the greatest colleting effort; all other habitats have been adjusted relative to that site. Thus, the figures for July in Table 2a represent a collecting effort of 197.6 trap-hours, the actual collecting effort in the Bat Lake Road (sylvatic) habitat. The figures for August represent a collecting effort of 78.6 trap-hours, which was the actual collecting effort in the bog habitat. It was felt unnecessary to standardize the August collections relative to those of July. The figures for 1964 presented in Table 1 have not been standardized and are presented only to demonstrate the basis on which the greater collecting effort of 1965 was made.

All collections for the assessment of habitat preferences have been made with carbon-dioxide-baited traps only. Collections from hosts have been excluded from the data in Tables 1, 2a and 2b in an attempt to approach uniform collecting conditions. Carbon-dioxide-baited traps probably provide a nearly unbiased means of sampling host-seeking female tabanids.

No attempt has been made to standardize the figures in the tables relative to the quantities of carbon dioxide that were used at the different collecting sites. The rates of release of the gas employed were not necessarily constant within any one habitat from day to day, nor were they constant from one habitat to another. Thus, for example, all collections in the bog habitat were carried out with carbon dioxide released at 1000 ml/min, whereas the collections in the sylvatic habitat were made with release rates of from 150 to 1000 ml/min. In addition, trapping in the various localities was carried out with *both* types of traps described above. These 2 factors may have introduced errors in terms of the relative abundance of each species noted in the various habitats.

An examination of Tables 1, 2a and 2b will show the degree of habitat preference that exists in the family. Some species have been captured in very small numbers (e.g. *Hy. metabola*, *Hy. zonalis*, and *Ch. sordidus*) and little may be inferred concerning their habitat preferences. However, the data for *At. bicolor* and *Hy. frosti* are probably truly representative of the habitat preferences of these species (Pechuman 1965). It is readily seen that the sylvatic habitat supports the most diverse but not necessarily the most abundant fauna. The fauna of the "aquatic" habitats are specifically more uniform.

The majority of species can be seen to be preferentially (e.g. *Hy. affinis*, *Hy. trepida*) or virtually exclusively (e.g. *Hy. criddlei*, *Ch. carbonarius*, *Ch. cuclux*) sylvatic. A few species appear to be relatively ubiquitous (e.g. *Ch. excitans*, *Hy. epistates*) and these, not surprisingly, perhaps, are the most abundant species.

Table 1. Collections of Tabanidae from carbon-dioxide-baited traps, July and August 1964. Collecting efforts in each habitat have not been standardized.

	Number of flies in each habitat		
Species	**Sylvatic**	**Lakeshore**	**River**
Chrysopsinae			
Ch. cincticornis Walker	6		
Ch. cuclux Whitney	6		
Ch. excitans Walker	173	8	6
Ch. frigidus Osten Sacken	37		9
Ch. indus Osten Sacken	16	4	60
Ch. lateralis Wiedemann	1		
Ch. mitis Osten Sacken	6		
Ch. montanus Osten Sacken	35	1	4
Ch. niger Macquart		1	
Ch. shermani Hine	1		
Ch. univittatus Macquart	9		
Ch. venus Philip	1		2
Tabaninae			
Hy. affinis (Kirby)	1		
Hy. arpadi (Szilády)	2		
Hy. epistates (Osten Sacken)	24	1	6
Hy. illota (Osten Sacken)	6	24	3
Hy. lasiophthalma (Macquart)	1	1	
Hy. nuda (McDunnough)		1	
Hy. trepida (McDunnough)	6	1	1
Hy. typhus (Whitney)	5		
Ta. marginalis Fabricius	27	3	2
Totals	363	45	93

<In the original, the total for “Sylvatic” was incorrectly given as 365.>

Thus, the Tabanidae can be placed in several groups according to their preferred habitat (Table 3). It should be pointed out, however, that the habitat preferences of any species may not be determined by those characteristics that have her been employed to define the habitats. Thus, the terms “aquatic” and “sylvatic” may, in some instances, be misleading, as there are indications that some species (e.g. *Ch. shermani*) prefer open sites, as this species was more abundant in the open sylvatic size (i.e. the cervid pens) than in the relatively more dense site (i.e. Bat Lake Road). *Ch. frigidus* has an extremely interesting distribution, apparently frequenting the more restricted or enclosed habitats, and thus cutting across the habitat boundaries as designated above. The greater number of flies taken at the cervid pens as compared to the Bat Lake Road site may be a reflection of the difference in abundance of flies at the 2 sylvatic sites, or it may reflect an influence of the nearby hosts (deer and moose) at the former.

Table 2a. Collections of Tabanidae in Algonquin Park, July 1965. Carbon-dioxide-baited traps only. Figures represent the number of flies of each species collected in each habitat, based on a collecting effort of 197.6 trap-hours.

	Habitat			
	Sylvatic		**“Aquatic”**	
Species	**Bat Lake**	**Cervid Pens**	**Lakeshore**	**River**
Chrysopsinae				
Ch. carbonarius Walker	8	4		
Ch. cincticornis	13	24	4	3
Ch. cuclux	3	28		
Ch. excitans	129	144	137	58
Ch. frigidus	227	124	11	279
Ch. indus	10	12	4	61
Ch. lateralis	6	32		
Ch. mitis	23	20	11	
Ch. montanus	13	28	7	3
Ch. niger	4	32	4	3
Ch. shermani	1	32	4	
Ch. sordidus Osten Sacken	1			
Ch. univittatus	6	4		
Ch. venus	2	4	4	68
Ch. vittatus Wiedemann	1	20		3
Tabaninae				
Hy. affinis	32	384	30	
Hy. arpadi	8	84	7	
Hy. criddlei (Brooks)	2	40		
Hy. epistates	307	836	262	99
Hy. illota	119	140	500	258
Hy. lasiophthalma	38	20		14
Hy. metabola (McDunnough)[*]	1			
Hy. nuda	19	4		
Hy. trepida	23	128	26	14
Hy. trispila sodalis (Wiedemann)	10	108	52	14
Hy. typhus	13	164	19	10
Hy. zonalis (Kirby)	1			
Ta. marginalis Fabricius	11	48	140	119
Ta. nigripes Wiedemann	5	20	11	
Totals	1036	2484	1233	1006

[*] First record for Nipissing District.

Table 2b. Collections of Tabanidae in Algonquin Park, August 1965. Carbon-dioxide-baited traps only. Figures represent the number of flies of each species collected in each habitat, based on a collecting effort of 78.6 trap-hours.

	Habitat			
	Sylvatic		**“Aquatic”**	
Species	**Bat Lake**	**Cervid Pens**	**Lakeshore**	**River**
Chrysopsinae				
Ch. cincticornis		4		
Ch. excitans	4	14	21	30
Ch. frigidus	23	14	2	7
Ch. indus	4			7
Ch. lateralis	4	18		1
Ch. mitis				3
Ch. montanus	4	16		17
Ch. niger		4		
Ch. shermani	4	13		1
Ch. univittatus	4	34	2	5
Ch. venus			2	17
Ch. vittatus		23		1
Tabaninae				
Hy. affinis		2	4	1
Hy. arpadi				1
Hy. epistates	23	59	25	33
Hy. frosti Pechuman *				1
Hy. illota		4	29	21
Hy. microcephala (Osten Sacken)	4		4	1
Hy. trepida	11	4	2	1
Hy. trispila sodalis	4	23	21	23
Hy. typhus	11	50	33	32
Ta. marginalis	15	23	134	129
Ta. nigripes			6	
Atylotus bicolor (Wiedemann) *				4
Totals	115	305	285	336

* First record for Nipissing District. <Actually there is 1 previous record for *At. bicolor*.>

Table 3. A summary of the habitat preferences of Ontario Tabanidae as indicated by carbon-dioxide collections <sic; i.e. collections in traps baited with carbon dioxide> in a variety of habitats.

1. Essentially ubiquitous species
    - *Ch. excitans*
    - *Ch. frigidus* (absent from lakeshore
    - *Hy. epistates*
2. Exclusively bog species
    - *Hy. frosti*
    - *At. bicolor*
3. Exclusively sylvatic species
    - *Ch. carbonarius*
    - *Ch. cuclux*
    - *Ch. lateralis*
    - *Hy. criddlei*
    - *Hy. nuda*
4. Preferentially sylvatic species
    - *Ch. cincticornis*
    - *Ch. mitis*
    - *Ch. montanus*
    - *Ch. niger*
    - *Ch. shermani*
    - *Ch. univittatus*
    - *Ch. vittatus*
    - *Hy. affinis*
    - *Hy. arpadi*
    - *Hy. lasiophthalma*
    - *Hy. trepida*
    - *Hy. trispila sodalis*
    - *Hy. typhus*
    - *Ta. nigripes*
5. Preferentially "aquatic" species
    - *Ch. indus*
    - *Ch. venus*
    - *Hy. illota*
    - *Ta. marginalis*

With few exceptions, habitat preferences in the Tabanidae are far from rigid. Nevertheless, the data in Tables 1, 2a and 2b indicate that there are great differences in the probability of encountering a given species in different habitats.

Vertical Stratification

The Tabanidae, being mammalophilic, are exclusively characteristic of the lower strata in the habitats in which they are found. Thus, in many scores of collections with carbon dioxide and a variety of trap types placed at various levels in the forest canopy (i.e. >10 feet above the ground) *no* Tabanidae have ever been collected. In other experiments, collections were carried out in the summer of 1965 with cage traps placed at several low heights from the ground; these experiments were conducted in the Bat Lake Road sylvatic habitat. A cage trap placed with its bottom edge at a height of 6 feet above the ground, and equipped with a carbon-dioxide outlet, captured no tabanids, even though simultaneous collections at lower levels showed that flies were abundant.

The above remarks notwithstanding, minor differences in the strata preferences of the 2 subfamilies of Tabanidae are found. Table 4 presents the data from several collections made in July 1965, to demonstrate this strata preference. Cage traps were employed in all instances. The traps were elevated at 2 levels, viz. with the bottom approximately 14 inches from the ground and 34 inches from the ground. The traps were both situated in the center of the roadway and were placed approximately 10 feet apart at their proximal edges. Both traps were baited with carbon dioxide emitted at equal rates, either 90 ml/min (21 July) or 150 ml/min (remainder of experiments).

Table 4. Vertical stratification in the family Tabanidae, analyzed by subfamilies. Increasing the height of the trap above the ground selects noticeably against the Tabaninae; the effect on Chrysopsinae is equivocal.

	21 July		24 July		25 July	
	14"	34"	14"	34"	14"	34"
Chrysopsinae	6	23	15	9	15	9
Tabaninae	17	4	13	6	16	2
Totals	23	27	28	15	31	11

	26 July		27 July		30 July	
	14"	34"	14"	34"	14"	34"
Chrysopsinae	12	4	4	1	1	5
Tabaninae	9	0	7	0	3	0
Totals	21	4	11	1	4	5

Summary

	14"	34'
Chrysopsinae	53	51
Tabaninae	65	12
Totals	118	63

It is clear from these data that the lower traps capture more flies. It is not immediately clear whether this is because more flies initially enter the lower trap or whether the lower trap is more successful in preventing the escape of captured flies. This problem was not subjected to test, although it could easily be done by the use of baffle traps that would prevent any escape of flies. Nevertheless, on no occasion have flies been seen to leave these cage traps unless it be at dusk when all species tend to become ataxic and drop to the ground. During daylight, however the entire collection of flies is invariably situated at the top of the trap, particularly under Plexiglass covers at the edges. This is presumably a phototactic response and serves to prevent downward movements of the captured flies.

Increasing the height of the traps above the ground selects markedly against the Tabaninae. The situation with respect to the Chrysopsinae, however, is equivocal, and will require further testing.

These data corroborate data that have been collected concerning the behavior of tabanids around living hosts. *Chrysops* spp. are often serious pests of man and almost invariably attack the upper portions of the body, particularly the arms, head, and neck. On the other hand, even though man is a manifestly poor host for the Tabaninae, those that are attracted to man invariably attack the lower portions of the body, particularly the leg below the knee. Similar behavior is noted of those Tabanidae that attack cervids. White-tailed deer are excellent hosts for *Chrysops* spp. and deer have been seen severely annoyed by large numbers of these flies above the head of the animals. Collections of tabanids from moose (q.v.) contain large numbers of Tabaninae, almost all of which are taken feeding on the legs, whereas few are taken from the trunk or head. Conversely, those few Chrysopsinae that are taken from moose are almost invariably found to attack the head or snout, with very few indeed landing on the trunk or legs.

Habitat Preferences in the Simuliidae

Zonation

Collections of simuliids using carbon-dioxide-baited traps were carried out in the forest canopy (Table 5), in the forest at ground level (Table 6), and at the lakeshore near water level (Table 7). These data are summarized in Table 8 and it is readily apparent that the habitat preferences of the simuliids are far more rigid than Bennett (1960) indicated. Typical lakeshore species (e.g. *S. rugglesi*, *S. euryadminiculum*) are absent from the sylvatic habitat, and typical canopy simuliids (e.g. *P. decemarticulatum*, *S. aureum*) are not found to any significant extent either at the lakeshore or at ground level in the woods. The presence of large numbers of *Simulium* spp. In the canopy is unexplained. Most of these were *S. venustum*, which is mammalophilic (Davies *et al.* 1962).

Vertical Stratification

A comparison of Tables 5 and 6 will demonstrate that ornithophilic flies of the sylvatic habitat are confined to the upper strata, and are found only rarely at ground level. Bennett (1960), in an investigation of stratification, had found no evidence of a preferred stratum between 5 and 20 feet. However, a few collections with carbon-dioxide-baited traps placed at 3 levels in the forest canopy (Table 9) show the phenomenon of vertical stratification to be far from straightforward and to be in need of a thorough reexamination. The problem of stratification has not been treated as a principal portion of this research program; the data for the 2 d are presented to demonstrate that differences in the strata preferences of the various species apparently do exist. Such differences could not be overlooked in investigations of the host preferences of the canopy simuliids. It is also interesting that the stratification was not the same on both days. Thus, on 15 June, the population of flies appeared to be disposed at a relatively higher level than on the following day.

That strata preferences of the population are not constant from day to day suggests that the stratification patterns observed may vary with the time of day, as a result of movements of the population in response to environmental gradients. The data from the canopy collections of 15 and 16 June have been examined by single collecting periods (Tables 10a, 10b, 10c). *S. venustum* (Table 10a) appears to become relatively more common in the lower strata with the approach of darkness. On the other hand, *P. decemarticulatum* (Table 10b) becomes relatively more common in the upper strata with the approach of nightfall. The results for *S. aureum* and *Eusimulium* spp. are less clear, but the data indicate a relatively constant stratification on the days examined. It is interesting, however, that the preferred strata are higher than those cited by Bennett (1960).

S. rugglesi occupies the lower strata in the lakeshore habitat. Thus, a collection on 10 July 1963 is indicative of the strata preferences of this species:

CO_2, duck extract, 10 feet above water:	7
CO_2, duck extract, 1 foot above water:	372

Both collections were run simultaneously for a duration of 30 min. It is interesting that these data corroborate data presented by Fallis and Bennett (1966) showing that rates of parasitemias of *Leucocytozoon simondi* Mathis and Leger in ducks exposed in the air are very much less than the rates of infection in ducks exposed at water level.

Table 5. Representative collections of Simuliidae from the forest canopy, May – July 1964. Only the results of carbon-dioxide-baited traps are given. Total sample is 6982 specimens <6980 in the original>. *Eusimulium* spp. are *S. latipes* and *S. quebecense*. *Simulium* spp. are mammalophilic flies, mostly *S. venustum*.

Date / Time (EDT)	***aureum***	***croxtoni***	***Eusimulium***	***decemarticulatum***	***Simulium***	**Other**
23 May						
1310–1340					167	
1445–1532	2				235	
30 May						
1210–1240	8		18		9	
31 May						
1833–1900	7	2	27	23	4	
2018–2035	61	1	337	23	2	2
1 June						
1915–1930	10	1	39	11	1	
2 June						
1848–1905	7		1		4	
1910–1925	28		14	1	10	
2008–2025	1					
2028–2045	24		15	3		
2045–2100	5		15			
2100–2115			3			
3 June						
1015–1100	4		1			
1250–1330	19		4	1	10	
1355–1445	39		22	1		
1830–1845	7		2	18	9	
1900–1915	4		12	5	14	
1930–1944	6		6	4	3	
2000–2015	5		17	2	1	
2030–2045	1		7			
2100–2115			2			
5 June						
1315–1330	4	1			11	
1415–1430	11	3			19	
1515–1530	22	5	2		20	1
1615–1630	10	4	2		12	
1800–1815	70	20	21	24	27	
1900–1915	39	29	33	31	28	1
2000–2015	25	5	86	35	12	

6 June						
0830–0845	6	1			67	
0930–0945	4				30	
1030–1045	1				73	1
1130–1145	1				53	
1230–1245	1				63	1
1330–1345	2				62	
1430–1445	3	2			74	2
1530–1545	1		1		115	
1630–1645	3	2			117	1
1745–1800	7			1	118	
1845–1900	2	1	1	7	158	
1945–2000	4		2	10	174	
2045–2100	4	2	34	8	14	
7 June						
1045–1100	2				33	
8 June						
1818–1833	2			2	49	
1900–1915	4		1	4	22	3
2015–2035	3	2	21	1	10	1
2035–2150	3	1	187	1	2	1
9 June						
1705–1807		1	1		767	
1820–1835	3	6	2	3	13	
1920–1935	5	6	12	33	41	
10 June						
1835–1855			5		4	
11 June						
1145–1245		1	1		7	
1300–1345		1			34	
1415–1515		4			5	
1530–1630	15	28	5	8	16	1
1645–1745	3	5	1	2	8	
1800–1905	29	20	30	159	26	
1930–2030	2		69	63	3	
12 June						
1820–1835	2	1	59	43	7	
1920–1935	2	3	123	65	11	1
2020–2035	1	2	89	40	0	
17 June						
1835–1850		5	48	5	1	
1935–1950		4	56	11	5	

24 June						
0845–0945					19	
0955–1055		2	1		34	
1100–1200		5	4		10	
1210–1315		3	12	6	16	
1325–1425		2			4	1
1435–1535		1		1	3	
1545–1650		2	5	1	18	
1700–1800		6	18	5	2	
1800–1900		3	13	1	19	
1930–2030		1	51	11	2	
3 July						
1100–1200		1			8	
1200–1300		2	1		13	
1305–1405		15			34	
1410–1510		6	1		35	
1520–1620		15	3		46	
1630–1800		24	17		70	
1820–1920	4	20	24	2	91	
1930–2030	12	10	127	3	48	
2040–2140	11	6	181	7	7	
4 July						
Unknown	9	1	58	1		
7 July						
1925–2025		2	23		4	
2030–2225			30		1	
8 July						
1805–1905	4	7	31	4	7	
1910–2010		2	8		3	1
2015–2115		2	6		3	
17 July						
1850–2010	29		1		8	
2010–2100	14		4		12	
Totals	617	307	2053	690	3297	18

“Others” includes the following:

Prosimulium sp.	2	(mammalophilic)
C. abdita Peterson	2	
C. mutata	1	
Simulium spp., males	8	
S. vittatum Zetterstedt	1	
Gynandromorph	1	
Undetermined	1	

Table 6. Representative collections of Simuliidae from the forest floor, June – July 1964. Only the results of carbon-dioxide-baited traps are given. Total sample is 5029 specimens. *Eusimulium* spp. are *S. latipes* and *S. quebecense*.

Date / Time (EDT)	*Simulium* spp.	*Eusimulium* spp.	Other
2 June			
1855–1913	17	4	1
1918–1930	43	1	
1932–1945	3		
1948–2005	2		
2028–2045		1	
5 June			
1415–1430	28	1	
1515–1530	6	1	1
1615–1630	49	1	
1800–1815	30	5	3
1910–1925	49		3
2000–2015	4	2	
9 June			
1545–1600	2		
1602–1617	45		
1634–1659	4		
10 June			
1815–1830	107		
11 June			
1145–1245	5		
1300–1400	9		
1415–1515	59		1
1530–1630	68	3	
1645–1745	115	2	
1800–1900	75	1	1
1930–2030	11		
2100–2200	1		
18 June			
1520–1535	36		
1615–1630	11		
1805–1835	268	1	
1837–1907	111		
1910–1940	672		2
1945–2015	279	1	1

Date / Time **(EDT)**	***Simulium* spp.**	***Eusimulium* spp.**	**Other**
24 June			
0845–0945	238		
0955–1055	1004		
1100–1200	695		
1215–1325	45		
1330–1425	279		
1435–1535	134		
1545–1650	189		
1700–1800	162	1	
1815–1915	78	3	
1930–2035	32	6	1
15 July			
1420–1645	4	1	
1700–2100	8	3	
Totals	4977	38	14

Simulium spp. includes the following:

S. tuberosum (Lundström)	6
S. decorum Walker	34
S. vittatum	8
S. parnassum Malloch	1
S. venustum [*]	4928

[*] May include some *S. verecundum* Stone and Jamnback

“Others” includes the following:

Prosimulium sp.	3	(Mammalophilic)
C. abdita	2	
C. mutata	2	
S. rugglesi	6	
S. aureum	1	

Table 7. Representative collections of Simuliidae from the lakeshore, near water level, May – June 1964. All collections were made with baited traps (i.e. carbon dioxide and putative attractants) or control cages. Total sample is 598 specimens. *Simulium* spp. Are mammalophilic, mostly *S. venustum*.

Date / Time (EDT)	***rugglesi***	***anatinum***	***euryadminiculum***	***Simulium***	**Other**
21 May					
1845–1850		2	2		1
1910–1915		2	17		1
23 May					
0940–0950			8		
1008–1018		1	2		
1021–1031			1		
30 May					
1712–1735		1	44		
1920–1945			14	3	
31 May					
1748–1822	2		24	5	2
1850–1918	1	1	22	10	3
2005–2030		2	13		2
8 June					
1805–1840	213	5		13	1
1850–1920	117	5		19	
1930–2000	33	1		5	
Totals	366	20	147	55	10

“Other” includes the following:

S. emarginatum Davies, Peterson and Wood	1
C. mutata	1
C. abdita	1
Eusimulium sp.	5
Undetermined	2

Table 8. Summary of habitat preferences of the Simuliidae.

Habitat	Proportion (%) of the Collection Represented by Each Species								
	No.	*aureum*	*croxtoni*	*Eusimulium*[a]	*decemarticulatum*	*Simulium*[b]	*rugglesi*	*anatinum*	*euryadminiculum*
Canopy	6980	8.8	4.4	29.4	9.9	47.2	0	0	0
Ground	5029	<1	0	<1	0	99.0	<1	0	0
Lakeshore	598	0	0	<1	0	9.2	61.2	3.3	24.6

[a] *S. quebecense* and *S. latipes*, which were not separated.
[b] Mammalophilic species, mostly *S. venustum*.

Table 9. Vertical stratification in the Simuliidae. Collections from the forest habitat, 15[a] and 16[b] June 1965.

		Proportion (%) of Collection Taken at Various Levels (feet)		
Species	Total	5	15	35
15 June				
S. venustum	1026	10.5	31.9	57.7
S. aureum	48	0	6.3	93.7
Eusimulium spp.	122	0	23.8	76.2
P. decemarticulatum	199	2.0	60.7	37.0
16 June				
S. venustum	1753	18.8	45.3	35.9
S. aureum	36	0	58.3	41.7
P. decemarticulatum	87	0	90.7	9.3

[a] 3 collections: 1620–1815; 1830–2050; 2100–2230. All times EDT.
[b] 3 collections: 1430–1630; 1635–1835; 1840–2145. All times EDT.

Table 10a. *Simulium venustum*. Changing vertical stratification with time. Forest habitat, 15 and 16 June 1965. Proportion (%) found at each level during each collecting period.

15 June			
Time, EDT	1620–1815	1830–2050	2100–2230
Total Number of flies	611	414	1
Height in Feet	%	%	%
5	11.3	9.4	0
15	29.5	35.5	0
35	59.2	55.0	100
16 June			
Time, EDT	1430–1630	1635–1835	1840–2145
Total Number of flies	413	791	549
Height in Feet	%	%	%
5	25.1	36.8	42.7
15	47.7	47.7	40.0
35	27.1	15.5	17.3

Table 10b. *Prosimulium decemarticulatum* and *Eusimulium* spp. Vertical stratification with time. Forest habitat, 15 June 1965. Proportion (%) found at each level during each collecting period.

P. decemarticulatum			
Time, EDT	1620–1815	1830–2050	2100–2230
Total Number of flies	42	142	15
Height in Feet	%	%	%
5	9.5	0	0
15	88.0	55.7	33.3
35	2.5	44.3	67.7
Eusimulium spp. (*S. latipes* and *S. quebecense*)			
Time, EDT	1620–1815	1830–2050	2100–2230
Total Number of flies	0	34	108
Height in Feet	%	%	%
5		0	0
15		17.6	21.3
35		82.4	78.7

Table 10c. *Simulium aureum*. Changing vertical stratification with time. Forest habitat, 15 June 1965. Proportion (%) found at each level during each collecting period.

Time, EDT	1620–1815	1830–2050	2100–2230
Total Number of flies	0	30	18
Height in Feet	%	%	%
5		0	0
15		6.7	5.6
35		93.3	94.4

The Role of Host Odor in the Attraction of Simuliidae

I. *Simulium* (*Eusimulium*) *euryadminiculum*

On 15 May 1962 Dr. James Lowther shot a female Common Loon on Lake Sasajewun (Lowther and Wood 1964). The bird had been collected as a zoological specimen and when retrieved, hundreds of simuliids were seen emerging from the feathers of the bird, particularly of the head and neck. A large sample of these flies was collected, but this represented only a fraction of the flies present on the bird. Flies continued to come off the bird for many hours after death, indicating the large numbers that must have been present when shot.

Subsequent identification of these simuliids showed them to be *S. euryadminiculum*, and this constituted the first valid feeding record for the species since its description (Davies 1949).

In the preparation of the bird as a museum specimen, the skin and feathers were washed several times in ordinary household laundry detergent to remove dirt and blood, and then soaked in "Varsol" for several hours in order to remove lipids from the skin. The specimen was then taken to the lakeshore for washing and it was noted that flies were attracted to the Varsol washings that had been spilled on the rocks. A small swarm of flies was oriented over a rock on which these washings had been spilled. A number of these flies were collected by sweeping over the rock with an insect net and they were subsequently identified as *S. euryadminiculum*. On the other hand, pure Varsol spilled purposely on the rocks nearby as a control failed to attract flies. Evidently an attractant principle present in the skin of the loon had been dissolved by the Varsol.

Further work following up this unique observation was not done until 6 June 1962. At that time, the loon (i.e. the same specimen that had been shot on 15 May), which had been stuffed and packed away in a specimen box, was set out on the shore of Lake Sasajewun. The specimen had been stored with large quantities of crystalline *p*-dichlorobenzene to discourage insect pests. Large quantities of the insecticide remained on the specimen, sufficient to enable one to smell the bird (i.e. the dichlorobenzene) at a distance of several feet.

Sweep collections were made at intervals over the bird on the evening of 16 June 1962. A small number of flies was collected in the period 2045–2130 EDT, and 10 of these were identified as *S. euryadminiculum*. The collection appeared to be monospecific.

This procedure was repeated on the evenings of 7 and 8 June 1962, and on the latter night, many hundreds of flies were present about the stuffed skin of the bird. Flies were collected directly from the bird (i.e. only those flies that landed) by aspiration. Nearly 1000 specimens were taken in a collecting period of 80 min, and more could have been collected had it not been for the limiting factor of the speed at which flies can be collected by such means. Large numbers of these flies were later identified as *S. euryadminiculum*. No other species was present in the portions of the collection that were identified.

This unusual collecting method provided a ready means of studying the seasonal distribution of the fly, and specimens of *S. euryadminiculum* were collected as late as 16 July 1962, when 2 specimens were taken. Previously the fly had not been collected later than 21 June (Davies *et al.* 1962).

Thus, it was clear in 1962 that there was an unusually well-defined specificity existing between *S. euryadminiculum* and the Common Loon, and it appeared that the specificity was reciprocal. It was also clear that this specificity and attractiveness persisted long after the death of the host, and that the attractant factor was present in the skin or feathers of the bird, and could be extracted into Varsol. It was reasonable to postulate the existence of a compound, or set of compounds, soluble in Varsol, chemically stable, ecologically specific and of sufficient volatility to serve as an attractant for *S. euryadminiculum*.

Undoubtedly, one of the most remarkable pieces of evidence indicating the potency of attraction that loons have for *S. euryadminiculum* is the extraordinary rapidity with which flies appear following exposure of the carcass. In most instances, loon carcasses, when not in use, were stored in polyethylene plastic bags in a deep freeze at about –25 °F <–32 °C>, or in a refrigerator at 40 °F <4.4 °C>. The carcasses were usually taken to the collecting site wrapped in plastic bags, and swarms of flies would appear about the bird even before it was removed from the storage bag. Hundreds of simuliids would gather about the carcass within minutes, often before one had the opportunity to set the bird down at the lakeshore.

It was of interest to determine the source of the attractant substance. To this end, portions of a loon carcass were extracted with diethyl ether. Extracts of the caudal region had considerable biological activity. This extract was placed on the shore of Lake Sasajewun at 1600 EDT on 3 June 1963. The swarm of flies that quickly formed about the paper on which the extract had been poured was dense enough to enable flies to be aspirated directly from the air. Samples of flies examined from this swarm showed them to be *S. euryadminiculum*.

Subsequent experimentation demonstrated that highly potent extracts could be prepared from the uropygial glands of the bird if these were extracted with suitable organic solvents (e.g. diethyl ether, acetone, chloroform). These extracts were often as successful in attracting large swarms of *S. euryadminiculum* as was the intact carcass. It is significant that extracts did not evoke landing responses in the attracted population, but rather produced only a localization of the swarm in the vicinity of the extracts. There is some evidence that landing responses in simuliids are mediated by visual stimuli, the odor serving only to direct the flies to the immediate vicinity of the host.

It was impossible to follow up with a series of investigations in 1964 since the population of *S. euryadminiculum* never approached the density that it had attained in 1963. Consequently, quantitative work of any description was essentially impossible.

Nevertheless, several experiments indicated the remarkable stability of the attractant compound. On 12 May 1964, a loon extract was exposed on the shore of Lake Sasajewun. The extract employed was prepared in May 1963 <i.e. extracted into ether> and was still successful in attracting *S. euryadminiculum*. In the interim between 3 June 1963 (the date of preparation of the extract) <i.e. the date on which a portion of the May-1963 extract was poured onto a filter paper> and 12 May 1964, the extract had been stored in the prepared condition — i.e. on the paper and not in solution. Only a few flies appeared around the extract, and it is not known whether this was due to a decrease in the potency of the attractant, or a consequence of the very small population of the fly.

Qualitative observations in 1964 suggested that the addition of small quantities of carbon dioxide, emitted at the exposure site, increased the number of *S. euryadminiculum* coming to the extracts. However, the numbers of flies present die not permit of a quantitative appraisal of this possibility.

In an attempt to determine the nature of the attractant compound involved in the biology of feeding of *S. euryadminiculum*, ether extracts of the uropygial glands of loons were fractionated, and the various fractions were assessed relative to the whole extract to determine whether or not one or more of the fractions retained notable biological activity. The results were discouraging and little progress with this aspect of the work was made in 1964 for reasons outlined above. Subsequently, the author was not involved in this phase of the research program.

II. *Simulium* (*Byssodon*) *rugglesi*

The demonstration that, presumably, olfactory substances originating in the uropygial glands of Common Loons were effective in attracting and effecting the specificity of *S. euryadminiculum*, led to a consideration of the possibility that similar orientation mechanisms might be operative in attracting other species of simuliids.

S. rugglesi has many features in its biology that are similar to those of *S. euryadminiculum*, and therefore make it ideal for such an investigation. That is to say, it is abundant and relatively restricted in its habitat preferences, and, of some importance, is known to feed preferentially, and perhaps exclusively, on the Anseriformes. Consequently, the possibility that the uropygial glands of ducks contain an attractant for *S. rugglesi* was investigated.

It is of considerable significance that dead ducks, unlike dead loons, do *not* attract simuliids. Many opportunities were available for observing dead ducks throughout the entire fly season, as a result of regular and frequent mortalities caused by leucocytozoonosis. The incidence of *S. rugglesi* about dead ducklings is very low compared to the avidity with which it approaches and sucks blood from living ducklings stationed nearby. Most of the observations of dead ducklings were with *A. boschas*; however, similar tests with dead Black Ducks and dead Mallards always demonstrated that dead anseriforms do not attract their specific simuliids in significant numbers. The very few specimens that are occasionally seen to land on dead ducks can probably be accounted for on the basis of visual responses of the population of flies already present in the immediate vicinity, since living ducks were always present in the lakeshore habitat.

To investigate the possibility that a simuliid attractant resided in the uropygial glands of ducks, extracts of these glands were prepared in the manner described above. Usually ether extracts were employed, but occasionally extracts of acetone and chloroform were used as well. The extracts to be tested were poured in small quantities onto 24-cm <diameter> filter papers. Papers on which solvents alone had been poured were used as controls. Usually extracts freshly prepared in this manner were used, although occasionally, the same extract was used for several day in succession.

To test for the attractiveness of these extracts, the papers were placed on the lakeshore on white (24 inch-square) plywood boards. Simultaneous exposure of at least some of the putative attractants was usual, the test materials being placed about 2 m apart. Initially however, no satisfactory collecting method had been developed, and it was not possible to collect from all test materials at the same time. However, qualitative observations were unmistakable; extracts of the uropygial glands of domestic ducklings were manifestly unsuccessful in attracting either *S. rugglesi* or *S. anatinum* (the latter species has similar feeding habits). It should be pointed out that whenever experiments were conducted to assess the possible attractiveness of these extracts, *S. rugglesi* was known to be abundant, active, and biting in the vicinity; this was gauged either by collections from or observations of living, captive ducklings, or from collections with other baits (*see below*).

The results of several tests with extract attractants are listed below. Collecting in each case was carried out by passing an insect net over the sample 6 times. Solvent controls in all cases yielded no flies.

Putative Attractant	*S. rugglesi*	*Simulium* spp.[a]
19 June 1963, 1830 EDT		
Dead duckling	1	3
Acetone extract	1	3
Ether extract	3	0
Varsol extract	0	3
23 June 1963, 1823–1828 EDT		
Dead duckling[b]	0	0
Acetone extract	0	1
Ether extract	0	0
27 June 1963, 1804–1951 EDT[c]		
Filtered ether extract	0	0
Residue, ether extract	0	0
Smear of intact uropygial gland	0	0
Chloroform extract	0	0

[a] Mammalophilic flies, mostly *S. venustum*. Experimenters were almost invariably attacked by large numbers of this species so that the presence of a few *S. venustum* in almost every collection was inevitable. *S. venustum* does not normally feed on ducks.

[b] A living duckling exposed simultaneously (1825 EDT) had many flies attracted to it, most of which landed. 3 of the attracted flies were captured and identified as *S. rugglesi*.

[c] The extract in ether was divided into 2 portions by filtration.

It must here be allowed that to collect flies in this manner (i.e. by sweeping) leaves many of the results open to question since this is generally a poor method for collecting simuliids. The difficulties are increased by the particularly low stratification of the fly; *S. rugglesi* rarely flies more than foot above the ground, and often much less, so that specimens are difficult to capture with a net. Nevertheless, the data given above agree well with the observational data. That is to say, these extracts of uropygial glands fail to attract significant numbers of simuliids.

During July of 1963, use was made of suspended cages to collect simuliids since collections with other attractants had demonstrated that this was an extremely efficient means of collecting the simuliids of the lower strata. Therefore, some of the above experiments were repeated, with the extracts of the uropygial glands of ducks placed beneath a gauze cage suspended about 1 foot from the ground in the lakeshore habitat. The data are given below:

Collections of *S. rugglesi* from ether extracts of the uropygial glands of domestic ducklings, using suspended cages, 1963.

Date (1963)	Time of collection (EDT)	Number of flies
6 July	1700–1730	0
11 July	1845–1900	3
17 July	1535–1835	16

It is clear that the extracts fail to attract *S. rugglesi*. As before, simultaneous collections, either from living ducklings or from other attractants, showed host-seeking *S. rugglesi* to be abundant during the experimental periods when extracts were tested.

III. *The Canopy Simuliids*

In spite of the discouraging results with uropygial-gland extracts of ducks, a similar series of investigations was carried out with the flies normally inhabiting the forest canopy. These flies (*P. decemarticulatum*, *S. croxtoni*, *S. aureum*, *S. quebecense*, and *S. latipes*) will feed on a variety of birds in that habitat (Bennett 1960).

Extracts of the uropygial glands of Ruffed Grouse and Common Grackles were prepared in diethyl ether. These extracts were exposed under cages at a height of 15–20 feet from the forest floor. All exposures were carried out in the mixed forest, from hoists on white-spruce trees. Tests carried out in the evening when fly activity was assessed by other means, showed that the extracts did not attract host-seeking simuliids. Visual observation of the cages showed that no flies approached cages in which extracts were exposed. Occasionally 2 or 3 flies (not necessarily ornithophilic) were taken in these cages, but this was no more than are occasionally taken in cage controls in which no extract is exposed.

Most experiments were carried out using ether extracts of the uropygial glands of grackles since these were abundant in the vicinity of the Research Station; grouse are, perhaps, the better host (Bennett 1960) but these were always difficult to obtain.

The collections made the night of 5 July 1963 are indicative of the results obtained in these experiments. A carbon-dioxide collection made the same night is included for purposes of comparison (Table 11).

Thus, it appeared that ether extracts of birds were unsuccessful in attracting the simuliids frequenting the forest-canopy strata. Experiments carried out in 1964 at a relatively earlier time in the season when the populations were larger confirmed these results (Table 12).

Table 11. Collections of flies from various attractants, forest canopy, 7 July 1963. Ether extracts of the uropygial glands do not attract canopy simuliids. All times are EDT.

Species	Carbon dioxide only 1940–2000	Grouse extract only	Cage only (control) 2015–2030	Carbon dioxide + grouse extract 2030–2050
Simulium spp. [a]	11	0	0	7
S. aureum	2	1	0	1
S. croxtoni	1	0	0	2
Eusimulium spp. [b]	41	1	0	30
P. decemarticulatum	1	0	0	1
Other [c]	0	1	0	0

[a] Mammalophilic species, mostly *S. venustum*
[b] *S. latipes* and *S. quebecense*, which were not separated
[c] *Prosimulium* sp., mammalophilic, probably *P. fuscum*.

Table 12. Collections of flies from various attractants, forest canopy, 1 June 1964. Ether extracts of the uropygial glands do not attract canopy simuliids. All times are EDT.

Species	Carbon dioxide, 30 ml/min, Grackle extract, 1940–1955	Grackle extract only, 2005–202	Carbon dioxide, 30 ml/min, Grackle extract, 2108–2130
Simulium spp. [a]	10	0	1
S. aureum	76	2	9
S. croxtoni	1	0	0
Eusimulium spp. [b]	105	0	31
P. decemarticulatum	31	1	5

[a] Mammalophilic species, mostly *S. venustum*
[b] *S. latipes* and *S. quebecense*, which were not separated

The Role of Carbon Dioxide in the Attraction of Simuliidae

I. *Simulium rugglesi*

Experiments conducted in June of 1963 indicated that carbon dioxide might play a role in the host-finding behavior of simuliids. After having determined that ether extracts of the uropygial glands of ducks did not attract significant numbers of *S. rugglesi*, it was found that releasing a small quantity of carbon dioxide at the site of exposure of the extract dramatically increased the number of flies coming to the extracts. Representative data are shown in Table 13. No flow meters were available in 1963 so gas flow was regulated by passing it first through a small jar containing water; the rate of flow was gauged by the number of bubbles per unit time. The rates were low, however, usually <50 ml/min.

Table 13. Flies collected by 6 sweeps of an insect net over various putative attractants. Experiments were carried out at 1845 EDT, 19 June 1963 in the lakeshore habitat. Carbon dioxide markedly increases the attractiveness of extracts of the uropygial glands of ducklings.

Putative attractant	*S. rugglesi*	*S. venustum*
Carbon dioxide only	2	2
Acetone extract + carbon dioxide	17	0
Ether extract + carbon dioxide	29	1

Although the numbers of flies taken by these methods are small, the data support the unmistakable qualitative observations that whenever carbon dioxide is released by the extract, small swarms of flies gather about the paper on which the extract is poured. Few flies land, however, and those that do land take flight again almost immediately. The small size of the collections is partly due to the fact that the swarms of *S. rugglesi* about the attractant maintain an extremely low stratification and are, therefore, very difficult to capture with an insect net. Qualitative observations suggested that there was no difference between ether and acetone extracts when both were used with carbon dioxide. It will be recalled (*vide ut supra*) that exposures of the duck extracts alone were carried out at 1830 EDT of this same day (19 June 1963) and yielded very few *S. rugglesi*. An exposure of a living duckling in the same habitat was also made from 1500–1800 EDT of 9 June 1963 and yielded 127 *S. rugglesi*, indicating, perhaps, that the artificial attractants were attracting more flies per unit time than the living host.

A similar experiment was conducted on 27 June 1963 when the population of *S. rugglesi* was more abundant. The various putative attractants were exposed for a period of 5 min each, at the end of which period, 6 sweeps of an insect net were made over the site. An interim period of 5 min was allowed to elapse between experiments in order to allow the attracted population to disperse. The procedure was then repeated on another attractant. An ether extract of the glands of several ducklings was filtered by gravity and both filtrate and residue were tested. Larger numbers of *S. rugglesi* was collected (Table 14) but the results are essentially the same, indicating that carbon dioxide greatly enhances the attractiveness of extracts.

These experiments leave little question that carbon dioxide has a pronounced effect on the attractiveness of bird extracts. However, in an effort to obtain still larger samples of flies, use was made of suspended cages to assess the same phenomenon (Table 15). Experiments were restricted to ether extracts of the uropygial glands. The paper on which the extract had been poured and the outlet tube from the carbon-dioxide cylinder were attached to a stake that protruded 6–8 inches above the water level at the lakeshore. A cage of fine Dacron or Terylene mesh was supported at the 4 corners, 8–12 inches above the water, and directly over the above-mentioned stake. Many of the simuliids that were attracted to the extracts and/or carbon dioxide, fly up into the cage when they get beneath it. The results (Table 15) indicate that carbon dioxide alone, when used in the appropriate habitat, can serve as an effective attractant for *S. rugglesi*. Some of the results are suggestive that, although the extract alone will not attract flies, a combination of the extract and carbon dioxide has a synergistic effect, the combination successfully attracting more flies than the carbon dioxide alone. *However*, in view of what is known concerning the temporal variation in biting activity of *S. rugglesi* and other simuliids, and the effect of trap location on the resultant collections, these data can be interpreted in this manner only with extreme caution. Thus, collections on 11 July 1963 (Table 15) were, in the one case, simultaneous, with both traps equipped with carbon dioxide at the same rate. Nevertheless, the collections differ by a factor of >2. Results of experiments carried out on 8 June 1964 (Table 16) demonstrate that carbon dioxide and extract collections are not necessarily more successful than carbon-dioxide collections without the added extract. It is possible that the differences may have bene caused by a decrease in the biting population with time. At any rate, it is clear that the relationship between carbon dioxide and bird odors requires further investigation.

Table 14. Flies collected by 6 sweeps of an insect net over various putative attractants. Lakeshore habitat, 27 June 1937. Carbon dioxide markedly increases the attractiveness of extracts of the uropygial glands of ducklings.

Putative Attractant	Exposure time (EDT)	Number of *S. rugglesi*
Extract filtrate + CO_2	1811–1816	69
CO_2 only	1821–1826	0
Extract residue + CO_2	1836–1843	1
Chloroform extract + CO_2	1952–1957	49
Loon extract (ether) + CO_2	2000–2020	4 *

* 9 *S. euryadminiculum* as well

Table 15. Numbers of *S. rugglesi* attracted to various putative attractants exposed under suspended cages in the lakeshore habitat. The data seem to indicate that carbon dioxide enhances the attractiveness of uropygial glands for *S. rugglesi*, but that the gas itself is also attractive. July 1963.

Day, Time (EDT)	Ether extract	CO_2	Extract + CO_2	No/min
06, 1435–1455			383	19
06, 1600–1630		44		1.5
06, 1700–1730	0			0
06, 1735–1755			274	14
10, 1425–1440		60		4
10, 1450–1505			312	21
11, 1825–1830		257		51
11, 1825–1830		102		20
11, 1845–1900	3			0.2
11, 1915–1920			150	30
16, 1355–1425			29	1

Table 16. Collections of Simuliidae at the lakeshore, 8 June 1964. Carbon dioxide when combined with extracts of the uropygial glands of ducklings is an effective attractant, but not necessarily better than carbon dioxide alone. All times are EDT.

Species	CO_2 only, 1805–1840	CO_2 + duck extract, 1850–1920	CO_2 only, 1930–2000
S. rugglesi	213	117	33
S. anatinum	5	5	1
Simulium spp. [a]	13	19	5

[a] Mammalophilic simuliids, mostly *S. venustum*.

II. *The Canopy Simuliidae*

It has already been shown that extracts of the uropygial glands of suitable host birds (e.g. Robins, Grackles, Ruffed Grouse) are unsuccessful in attracting the canopy simuliids in significant numbers[1]. Having demonstrated, however, the efficacy of carbon dioxide in the attraction of *S. rugglesi*, similar investigations were initiated for the species of simuliids frequenting the forest-canopy habitat. A series of collections was carried out in July of 1963, using suspended cages combined with either carbon dioxide or carbon dioxide and a bird extract. Collections were usually made at 1 tree only, so that the were successive rather than simultaneous. It was customary to collect flies for a period of 15–20 min, and then to change the attractant. Thus, for example, collecting was done with carbon dioxide for 15 min, and then an extract was added, and collecting was done for the next 15 min, followed again by a 15-min collection with carbon dioxide only, etc.

Occasionally, for purposes of comparison, collections were also made from living hosts (usually bantam hens) exposed simultaneously but at another site a short distance from the site of exposure of the artificial attractants. All collections were made at an elevation of 15–20 feet, a level that Bennett (1960) considered to be most favorable for canopy simuliids. Data from several days' experiments (Tables 17, 18, 19, 20) show that carbon dioxide is an effective attractant for canopy simuliids. For each day shown in the tables, the collections from any one type of stimulus-attractant have been summed to give an overall total for the day.

Table 17. Collections of Simuliidae from Bantam Hens in the forest canopy, July 1963 *a*. Compare with Tables 18 and 19.

Species	Day in July					Total	%
	5	21	24	25	26		
Simulium sp. [b]	0	0	0	1	0	1	1
S. aureum	4	27	45	154	66	296	75
S. croxtoni	2	1	3	2	0	8	2
Eusimulium spp. [c]	30	2	24	23	12	91	23

[a] Total collecting effort was 5.8 bird-hours.
[b] Mammalophilic species, probably *S. venustum*.
[c] *S. quebecense* and *S. latipes*, which were not separated.

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[1] Very occasionally, an extract alone seems to work well in the canopy. Thus, a collection from 1948–2005 EDT on 2 June 1964 yielded 52 specimens of *S. aureum* and 6 of *Eusimulium* spp.

Table 18. Collections from the forest canopy, carbon-dioxide baits, July 1963[a].

Species	Day in July									Total	%
	5	18	21	22	23	24	25	26	30		
Simulium spp.[b]	17	1	0	2	5	6	9	3	1	44	11.5
S. aureum	9	12	0	0	2	6	13	7	54	103	26.9
S. croxtoni	4	3	1	0	0	0	0	0	0	8	2.2
Eusimulium spp.[c]	51	62	4	12	8	10	23	12	44	226	59.0
P. decemarticulatum	2	0	0	0	0	0	0	0	0	2	0.5

[a] Total collecting effort, 6.0 trap-hours.
[b] Mammalophilic species, mostly *S. venustum.*
[c] *S. quebecense* and *S. latipes*, which were not separated.

Table 19. Collections from the forest canopy, carbon-dioxide and bird-extract baits[a], July 1963[b].

Species	Day in July									Total	%
	5	18	21	22	23	24	25	26	30		
Simulium spp.[c]	8	2	0	2	2	17	8	7	0	46	9.8
S. aureum	2	43	18	7	6	2	5	20	71	174	37.0
S. croxtoni	3	9	2	0	0	1	1	0	0	16	3.4
Eusimulium spp.[d]	58	39	14	15	4	16	16	12	58	232	49.3
P. decemarticulatum	1	0	0	2	0	0	0	0	0	3	0.6

[a] Bait used on 5 July was from a Ruffed Grouse; all other collections used Grackle extracts.
[b] Total collecting effort, 6.0 trap-hours.
[c] Mammalophilic species, mostly *S. venustum.*
[d] *S. quebecense* and *S. latipes*, which were not separated.

Table 20. A comparison table of collections of canopy simuliids from Bantam Hens and artificial attractants. A summary of the collection, July 1963[a]. The ratio of number of flies in the carbon-dioxide collection to the carbon-dioxide-plus-extract collections is 1.00:1.23.

Species	Bantam Hen only		Carbon Dioxide only		CO_2 + Bird Extracts	
	Number	%	Number	%	Number	%
Simulium spp.[b]	1	0.3	44	11.5	46	9.8
S. aureum	296	74.7	103	26.9	174	37.0
S. croxtoni	8	2.0	8	2.2	16	3.4
Eusimulium spp.[c]	91	23.0	226	59.0	232	49.3
P. decemarticulatum	0	0	2	0.5	3	0.6

[a] Collecting effort for both attractants was 6.0 trap-hours; for the Bantam Hen, 5.8 bird-hours.
[b] Mammalophilic species, mostly *S. venustum*.
[c] *S. quebecense* and *S. latipes*, which were not separated.

In all cases, it is seen that the number of flies coming to the attractants or to a Bantam Hen exposed for purposes of comparison was small. This was a consequence of the lateness of the season when the experiments were carried out. Whether or not the species differences that are observed in the collections are meaningful is a moot point. The carbon-dioxide and carbon-dioxide-*cum*-extract collections both contain many more *Eusimulium* spp. and fewer *S. aureum* as compared to the collections from the Bantam Hen. It is not known whether this is a function of the attractant or simply a difference due to the different collecting sites. At the present time it would be unwise to opt for one or other alternative, at least until more is known concerning the microhabitat preferences of the different species. All attractant collections were carried out at the same site, whereas the collections from the hen were made at another location.

Similarly, although the collections with carbon dioxide and carbon dioxide with extract are different in some cases, it would be premature, on the basis of these few data, to ascribe the differences to the presence of the extract.

In 1964, the problem of attractants for canopy simuliids was reinvestigated. Of particular interest was the possibility, apparent in 1963, that bird odors might influence either the absolute size or the species composition of collections, or both. Thus, a series of experiments was conducted in the canopy using again both carbon dioxide and carbon dioxide combined with either extracts of the uropygial glands of birds. These experiments were done in June to take advantage of the larger populations than are present in July. It was during the course of these experiments that the problem of position effect and temporal variation (*q.v.*) Became acutely apparent. It is important to note that these 2 sources of error have made it extremely difficult to assess the effects of bird odors with any degree of certainty or satisfaction.

Nevertheless, the 1964 data do parallel the 1963 data in showing that extracts seem to increase the proportion and absolute number of ornithophilic flies in collections, as compared to collections made with only carbon dioxide (Tables 21, 22, 23, 24). It is possible to make a partial correction for temporal and spatial differences in biting activity of the populations by summing a number of collections made over a period of time and correcting for trapping effort, if necessary (Table 24). Collections in 1964 (Tables 21, 22, 23) employed both suspended cages and large suction traps.

Table 21. Collections of Simuliidae from Bantam Hens exposed in the forest canopy, May – June 1964 [a]. Compare with Tables 22 and 23.

Species	Date of Collection			Total
	30 May	11 June	24 June	
Simulium spp. [b]	0	67	111	178
S. aureum	23	17	28	68
S. croxtoni	1	73	31	105
Eusimulium spp. [c]	19	104	345	468
P. decemarticulatum	0	129	23	152

[a] Actual collecting effort was 18.5 bird-hours.

[b] Mammalophilic species, mostly *S. venustum*.

[c] *S. quebecense* and *S. latipes*, which were not separated; the former species was more abundant than the latter.

Table 22. Collections of simuliids from the forest canopy, 1964[a]. Carbon-dioxide baits only.

Species	Date of Collection									Total
	23 V	30-V	31-V	1-VI	2-VI	8-VI	9-VI	11-VI	24-VI	
Simulium sp.[b]	602	9	7	1	14	83	54	100	129	999
S. aureum	2	8	68	10	64	12	8	52	0	224
S. croxtoni	0	0	3	1	0	3	12	59	25	103
Eusimulium spp.[c]	0	18	364	39	49	209	14	129	104	926
P. decemarticulatum	0	0	46	11	4	8	36	234	25	364

[a] Total collecting effort, 24.8 trap-hours.
[b] Mammalophilic species, mostly *S. venustum*.
[c] *S. quebecense* and *S. latipes*, which were not separated. The former species more was more abundant than the latter.

Table 23. Collections from the forest canopy, carbon dioxide and bird-extract baits, 1964[a].

Species	30-V	31-V	1-VI	2-VI	8-VI	9-VI	11-VI	24-VI	Total
Simulium sp.[b]	0	12	11	4	5	155	140	33	360
S. aureum	101	169	85	78	1	16	85	23	558
S. croxtoni	4	10	1	0	0	27	71	40	153
Eusimulium spp.[c]	54	482	136	58	2	6	215	351	1304
P. decemarticulatum	18	69	36	8	5	25	243	32	436

[a] Total collecting effort, 20.7 trap-hours.
[b] Mammalophilic species, mostly *S. venustum*.
[c] *S. quebecense* and *S. latipes*, which were not separated. The former species more was more abundant than the latter.

Table 24. A comparison table of collections of canopy simuliids form Bantam Hens and artificial attractants. A summary of the collections, summer 1964. Numbers of simuliids given in the table have been normalized for a collecting effort of 24.8 trap- or bird-hours. The ratio of number of flies in the carbon-dioxide collection to the carbon-dioxide-plus-extract collections is 1.00:1.29.

Species	Bantam Hen only		Carbon Dioxide only		CO_2 + Bird Extracts	
	Number	%	Number	%	Number	%
Simulium spp.[b]	238	18.3	999	38.2	432	12.8
S. aureum	91	7.0	224	8.6	670	19.7
S. croxtoni	141	10.7	103	3.9	184	5.5
Eusimulium spp.[c]	627	48.2	926	35.4	1565	46.4
P. decemarticulatum	204	15.7	364	13.9	523	15.4
Totals	1301		2616		3374	

[a] Mammalophilic species, mostly *S. venustum*.
[b] *S. quebecense* and *S. latipes*, which were not separated.

III. Discrimination of carbon dioxide by blackflies

By 1965 there was little doubt that carbon dioxide served as an extremely efficient <sic; i.e. *effective*> attractant for simuliids. However, it was decided to carry out several carefully controlled experiments to obtain quantitative data of unquestionable significance. To this end, miniature plastic suction traps were employed in the canopy, and gauze suspended cages on the ground. 1 trap was equipped with carbon dioxide and the other served as a control (Table 25). In all cases, collections were run simultaneously. Only the total results are shown, but there is absolutely no doubt that blackflies can orientate to a source of carbon dioxide in the field.

Table 25. 3 experiments to demonstrate attraction of simuliids to carbon dioxide. Details of each are given under the individual sections.

9 June 1965

2 miniature suction traps in the forest canopy; collecting effort was 7.0 trap-hours for each trap. Carbon dioxide was supplied to 1 trap at 90 ml/min.

Species	Number of flies	
	Carbon-dioxide Trap	Control
All species, mostly ornithophilic	2361	10

9 June 1965

2 suspended gauze cages, 1 foot above ground in the forest habitat. Cages situated approximately 10 feet apart. Carbon dioxide supplied to 1 cage at 90 ml/min.

Species	Number of flies	
	Carbon-dioxide Trap	Control
All species, mostly *S. venustum*	802	34

14 June 1965

2 miniature suction traps in the forest canopy. Collecting effort was 9.0 trap-hours for each trap. Carbon dioxide was supplied to 1 trap at 90 ml/min.

Species	Number of flies	
	Carbon-dioxide Trap	Control
Simulium spp., mammalophilic	86	0
S. aureum	3	0
Eusimulium spp.	8	0
P. decemarticulatum	206	0

If carbon dioxide attracts simuliids, then, without advocating any underlying mechanism of action of the gas, it is reasonable to postulate a relationship between the quantity of gas supplied at the trap site and the size of the resultant collection. It is reasonable to suppose that larger quantities of the gas should trap larger numbers of flies.

Several attempts were made to test this hypothesis in 1964. These experiments were generally unsuccessful, as judged by inconsistencies in the data. Undoubtedly, care was not taken to insure an absolute uniformity of habitat (i.e. with respect to light, surrounding vegetation, relationship to the wind, etc.). An experiment carried out on the forest floor on 18 June 1964 (Table 26) is representative of the results obtained. Use was made of large suction traps deployed approximately 10 feet apart along a forest roadway. The variability of the results, in an unexpected fashion, is immediately apparent.

Table 26. Collections of Simuliidae from suction traps with different release rates of carbon dioxide. Forest habitat, 18 June 1964 [a].

Rate of CO_2 release, mm/min	Number of flies at each collection time (EDT)			
	1805–1835	1837–1907	1910–1940	1945–2015
5	NC [b]	NC	174	220
10	78	56	18	10
30	191	55	482	35

[a] All specimens, except 5, were *S. venustum*.

[b] No collection.

The data presented in Table 26 are a good example of the manner in which position effect can influence the results of experiments with the Simuliidae. As long as work is carried out in habitats in which such a position effect can be demonstrated, all quantitative data will be suspect.

In an attempt to circumvent this problem, experimentation in 1965 was conducted in a large forest clearing (Fig. 1), a description of which has already been given.

Preliminary collections demonstrated that ornithophilic flies were virtually absent from this open habitat, in spite of the fact that sites where they were normally abundant were <100 yards away. It is not known whether this absence of ornithophilic flies represents a habitat selection on the part of the population, or a peculiarity of the season, since most ornithophilic populations were very small or nonexistent in 1965. However, there was a fairly large population of *S. venustum* in the clearing, with a few (<5%) other mammalophilic species, and a very small number of ornithophilic flies.

The crucial test for uniformity of habitat would be that 2 traps with identical attractants capture equal numbers of flies. Thus, an experiment was set up on 21 June 1965, on that line in the clearing seeming to show the best possibility of being uniform along its length (Table 27). Miniature suction traps were set on the line, approximately 25 feet apart, and gas was released from each trap at 90 ml/min. The results demonstrate the remarkable equivalence of the 2 traps.

Table 27. Avoidance of the position effect by a judicious selection of experimental sites. 2 traps with equal quantities of carbon dioxide (90 ml/min), 25 feet apart in a forest clearing yield equal numbers of simuliids.

Time of collection (EDT)	Number of Simuliidae Captured	
	West Trap	East Trap
1800–1945	796	728
2000–2015	650	688
2115–2200	32	34
Totals	1478	1450

This demonstration that equivalent traps capture equivalent numbers of flies opened the way to further experimentation. Unfortunately, the fly season ended rather quickly and there was time for only a few experiments.

The first task associated with carbon-dioxide attraction of simuliids was the demonstration that the flies were capable of discriminating between 2 traps on the basis of the gas alone. Thus, 2 traps were set up in the forest clearing, approximately 25 feet apart. 1 trap released carbon dioxide at 10 ml/min and the other at 50 ml/min. In spite of the demonstration of the uniformity of the habitat (Table 27), precaution against a possible position effect was taken by alternating the positions of the 2 traps at the termination of each collecting period. It is seen (Table 28) that simuliids (in this case mostly *S. venustum*) select the trap with the higher release rate of carbon dioxide.

Table 28. Discrimination of carbon dioxide by *Simulium venustum*. 2 traps placed 25 feet apart in a forest clearing and given different rates of release of carbon dioxide. The relative position of the traps was reversed at the termination of each collecting period. The flies consistently select the trap with the greater quantity of carbon dioxide.

Time of collection (EDT)	Number of flies and relative position of trap	
	10 ml CO_2/min	50 ml CO_2/min
1330–1430	17 (E)	108 (W)
1430–1530	128 (W)	187 (E)
1530–1630	211 (E)	384 (W)
1630–1800	421 (W)	839 (E)
1800–1905	485 (E)	620 (W)
1905–2010	131 (W)	645 (E)
2010–2115	55 (E)	835 (W)
2115–2215	4 (W)	21 (E)
Totals	1452	3639

Discrimination ratio = 1452:3639 = 1.0:2.5.

Having demonstrated the ability of simuliids to effectively discriminate between 2 traps with different quantities of carbon dioxide (i.e. the 2 traps had different release rates) it was decided to investigate the same phenomenon as a function of the distance between the 2 carbon-dioxide sources. The ratio of carbon-dioxide release rates was increased to 1:10. The experimental procedure again involved a regular oscillation of the relative positions of the traps, in order to avoid a possible position effect. The initial collection was done with the 2 miniature suction traps 27 feet apart, and at each subsequent collection the traps were moved closer together.

It is seen that the discrimination coefficient decreases with decreasing distance between the 2 traps (Table 29). However, the population was still selecting the higher-release-rate trap when the traps were only 1 foot (center to center) apart and the selection becomes more definite with increasing distance between traps.

Table 29. Discrimination of carbon dioxide by *S. venustum* as a function of the distance between sources. The net discrimination coefficient = 714:2406 = 1.00:3.36. The relative position of the trap is given as a superscript (E or W) following the number of flies captured).

	Time of collection										
	0930–1030	1045–1145	1150–1250	1255–1355	1400–1500	1510–1610	1645–1745	1745–1845	1850–1950	1950–2050	2055–2155
Distance between traps (feet)	27	20	15	10	8	6	4	2	1	2	4
No. flies, CO_2 @ 10 ml/min	6^{E}	15^{W}	0^{E}	10^{W}	9^{E}	25^{W}	69^{E}	129^{W}	287^{E}	159^{W}	5^{E}
No. flies, CO_2 @ 100 ml/min	193^{W}	67^{E}	51^{W}	88^{E}	158^{W}	264^{E}	507^{W}	320^{E}	463^{W}	280^{E}	15^{W}
Discrimination Coefficient[a]	32.2	4.5	51.0	8.8	17.5	10.1	7.3	2.5	1.6	1.8	3.0

[a] (Number of flies in trap at 100 ml/min CO_2) ÷ (Number of flies in trap at 10 ml/min CO_2). Catches of 0 were arbitrarily set equal to 1 for purposes of computation.

The Role of Carbon Dioxide in the Attraction of Tabanidae

The attractiveness of carbon dioxide to tabanids was investigated by the use of carbon-dioxide-baited gauze traps. These traps were set out on stands, approximately 14 inches from the ground. Control cages were placed in the same habitat, some distance from the carbon-dioxide trap (i.e. usually >10 but <20 feet). The impression was that if the traps were closer together than 10 feet, the flies would rely on their well-developed vision and the presence of absence of carbon dioxide would not mean anything to the fly.

Most of the experiments to test for carbon-dioxide attraction in the Tabanidae were carried out in the sylvatic habitat, as this afforded the optimal qualities of diversity and abundance of the fauna, as well as ease of accessibility. A few preliminary experiments carried out in 1964 (Table 31) indicated the probability of an orientation to carbon dioxide by tabanids. On this basis, a more extensive collecting effort was made in 1965 (Table 32), which confirms the attraction to carbon dioxide. Rates of carbon-dioxide release varied, but were usually 1000 ml/min. Table 32 also contains the results of a few collections made in the river habitat in 1965. In *all* cases, only those collections are reported for which there was always a simultaneous control.

The data of 1964 and 1965 are summarized in Table 30.

Table 30. Attraction of Tabanidae to carbon dioxide. Summary of collections made in 1964 and 1965. The discrimination ratio between baited and control traps is 1555:180 = 8.6:1.0.

Collection Site	Year	Number of Tabanidae	
		Carbon Dioxide Bait	Control
Bat Lake Road (sylvatic)	1964	295	70
Bat Lake Road (sylvatic)	1965	1000	65
North Madawaska River (Aquatic)	1965	260	45
Totals		1555	180

Table 31. Carbon-dioxide attraction of Tabanidae, 1964. All collections were done in the Bat Lake Road sylvatic habitat, with carbon dioxide usually released at 150 ml/min.

Species	CO_2 Cage	Control
Chrysopsinae		
Ch. cincticornis	5	1
Ch. cuclux	7	1
Ch. excitans	142	31
Ch. frigidus	34	3
Ch. indus	15	1
Ch. lateralis	1	0
Ch. mitis	6	0
Ch. montanus	24	11
Ch. shermani	1	0
Ch. univittatus	9	0
Ch. venus	1	0
Tabaninae		
Hy. affinis	1	0
Hy. arpadi	2	0
Hy. epistates	15	9
Hy. illota	4	2
Hy. lasiophthalma	1	0
Hy. trepida	6	0
Hy. typhus	4	1
Ta. marginalis	17	10
Totals	295	70

Table 32. Collections of Tabanidae from carbon-dioxide traps, 1964. Rates of release were usually at 1000 ml/min.

Species	Bat Lake Road (sylvatic)		North Madawaska River	
	CO_2	Control	CO_2	Control
Chrysopsinae				
Ch. carbonarius	5	0	0	0
Ch. cincticornis	11	2	1	0
Ch. cuclux	4	0	0	0
Ch. excitans	125	5	17	0
Ch. frigidus	231	2	82	1
Ch. indus	10	1	16	2
Ch. lateralis	8	0	0	0
Ch. mitis	23	0	0	0
Ch. montanus	14	0	0	1
Ch. niger	3	1	1	0
Ch. shermani	2	0	0	0
Ch. sordidus	1	0	0	0
Ch. univittatus	8	0	1	0
Ch. venus	2	0	17	4
Ch. vittatus	1	0	0	0
Tabaninae				
Hy. affinis	28	4	0	0
Hy. arpadi	6	2	0	0
Hy. criddlei	2	0	0	0
Hy. epistates	320	11	29	4
Hy. illota	95	22	59	17
Hy. lasiophthalma	36	4	4	0
Hy. metabola	1	0	0	0
Hy. microcephala	1	0	0	0
Hy. nuda	14	5	0	0
Hy. trepida	26	0	3	1
Hy. trispila sodalis	10	1	5	0
Hy. typhus	15	0	4	0
Hy. zonalis	1	0	0	0
Ta. marginalis	11	4	21	15
Ta. nigripes	4	1	0	0
Totals	1018.[*]	65	260	45

<[*] Reported as 1000 in the thesis..>

Some Remarks on Host Preferences in the Simuliidae and Tabanidae

This program of research has not been primarily concerned with the problem of host preferences. Yet, it is easily appreciated that the mechanisms of host location might be very intimately associated with the mechanisms by which host preferences are determined. The problem of this relationship is dealt with in a theoretical manner below.

Notwithstanding the above remarks, a few observations should be included on host preferences in the 2 families.

Simuliidae

S. euryadminiculum, so far as is known, has the most remarkable and specific association with its host of any simuliid in Ontario. In many series of collections from both loons and loon extracts, few specimens of other species have been taken, as compared with literally thousands of specimens of *S. euryadminiculum*. The converse also applied, and is as interesting. That is to say, *S. euryadminiculum* appears to be not only the only simuliid to feed on loons, but also appears to feed *only* on loons. Thus, the specificity appears to be a dual one. There are few records of *S. euryadminiculum* reported from any other species. It has been reported from ducklings (Davies and Peterson 1956) but the author is extremely suspicious that these records represent misidentifications of *S. anatinum*.

The author has, however, taken specimens of *S. euryadminiculum* from ducks, *but only when loons were exposed in the immediate vicinity*. A few experiments carried out in 1963 are pertinent here. On 25 May 1963, a loon carcass was exposed on the shore of Lake Sasajewun. Ducklings (living) were also exposed in the immediate vicinity. 46 specimens of *S. anatinum* were collected from ducklings, of which 37 were engorged. In addition, 25 specimens of *S. euryadminiculum* were taken from the same ducklings. It is highly significant that none of the *S. euryadminiculum* was fed.

Tabanidae

Collections of tabanids were made in 1964 and 1965 from man and in 1965 from moose. It has not been possible to standardize the collections insofar as collecting effort is concerned (Table 33) but the data show a very clear difference in the host preferences of the 2 subfamilies of tabanids. The Chrysopsinae, with 1 or 2 exceptions, do not feed to any extent on moose, but many species are serious pests of man. The Tabaninae, on the other hand, feed preferentially on moose, and are relatively unimportant pests of man. The absence of feeding records of some species on moose may be due to habitat preferences, since the moose were always maintained in the sylvatic habitat.

Table 33. Collections of Tabanidae in Algonquin Park, 1964 and 1965. Totals shown for each year are the sums for all traps and all hosts in all localities and are included for reference purposes. Collections from moose were made in a sylvatic habitat from captive, female, yearling animals.

Species	Totals		From Man		Moose
	1964	1965	1964	1965	1965
Chrysopsinae					
Ch. carbonarius	4	15	0	0	0
Ch. cincticornis	12	27	1	4	1
Ch. cuclux	28	42	0	6	9
Ch. excitans	236	378	5	63	7
Ch. frigidus	59	389	2	2	7
Ch. indus	85	79	3	35	0
Ch. lateralis	8	35	0	5	0
Ch. mitis	16	66	0	8	7
Ch. montanus	116	168	15	99	0
Ch. niger	12	78	2	9	22
Ch. shermani	11	83	6	54	0
Ch. sordidus	1	1	0	0	0
Ch. univittatus	47	120	19	69	0
Ch. venus	5	49	0	0	0
Ch. vittatus	52	146	46	125	0
Total Chrysopsinae	692	1676	99	479	53
Tabaninae					
Hy. affinis	2	298	0	3	128
Hy. arpadi	2	88	0	0	46
Hy. criddlei	0	17	0	0	3
Hy. epistates	46	795	0	2	25
Hy. frosti *	0	1	0	0	0
Hy. illota	39	464	0	14	7
Hy. lasiophthalma	4	109	0	1	49
Hy. metabola *	1	5	0	0	2
Hy. microcephala	3	4	0	0	0
Hy. nuda	6	70	0	3	31
Hy. trepida	18	111	1	10	14
Hy. trispila sodalis	3	111	0	1	0
Hy. typhus, form “B”	14	150	0	0	0
Hy. zonalis	1	5	0	0	4
Ta. marginalis	39	352	1	4	0
Ta. nigripes	5	21	0	0	2
Ta. reinwardtii Wiedemann	1	1	0	0	0
At. bicolor	0	4	0	0	0
Total Tabaninae	184	2606	2	38	311

* First record for Nipissing District.

The Position Effect in Simuliidae Collections

A program of regular collecting of Simuliidae will often demonstrate irregularities in the collections that cannot be accounted for on the basis of known general habitat preferences, nor on the basis of the nature of the attractants being studied. Rather, the quantitative features of the collections are found to be a function of the site at which they were made. Thus, collections made as few as 50 yards apart in the same habitat will often yield collections of entirely different orders of magnitude. On occasion, position effects of this nature can be observed when traps are only a few feet apart.

To illustrate, the data in Table 34a have been taken from a series of experiments carried out in the forest canopy on 11 June 1964. A series of collections was made throughout the daylight and twilight hours, using 2 attractants, viz. carbon dioxide and carbon dioxide combined with the extract of the uropygial glands of a Common Grackle. The 2 attractants were exposed simultaneously in 2 trees, and the relative positions were reversed at the termination of each collecting period. Carbon dioxide emanated at the same rate from both traps.

Table 34a. Position effect in simuliid collections. Collections of flies from 2 trees, both sites with carbon dioxide. A superscript ‘x’ indicates the presence of a grackle extract at the tree in question. Tree #1 invariably shows a greater population of simuliids.

Time (EDT)	Tree #1	Tree #2
1145–1245	11^{x}	9
1300–1400	35	18^{x}
1415–1515	75^{x}	9
1530–1630	73	57^{x}
1645–1745	68^{x}	19
1800–1900	264	181^{x}
1930–2030	325^{x}	137
2100–2200	29	22^{x}
Total	880	452

It is immediately apparent that the 1st tree yielded larger collections than the 2nd, regardless of the stimulus-attractant present at that tree. It is significant that the differences in collections were constant for the day shown. This is typical. However, the differences are not necessarily repeatable or constant on subsequent days. Thus, Table 34b presents a case in which position effects were changing throughout the day. Moreover, it will be noted that the same 2 trees have been selected for both Tables 34a and 34b and that the overall results as shown in the totals are reversed in the 2 tables.

The only way in which these position effects can be avoided is to exercise stringent selection methods for experimental sites. These sites need not be the same from day to day. The crucial test for uniformity of habitat is equivalent catches in equivalent traps. It will be recalled (*vide ut supra*) that uniformity of this nature was approached in the forest clearing. However, care must be taken in the selection of sites even under what are apparently homogenous conditions. Thus, a series of collections was made in this forest clearing, using the line that has an end abutting against the forest edge. It is interesting that the totals for the 2 traps (Table 35) are not markedly different, but that there are large differences in the individual hourly collections. These data are a good example of the difficulty one experiences in selecting experimental sites in the field.

Table 34b. Position effect in simuliid collections. Experiment carried out on the same 2 trees as in Table 34a, under similar conditions, but on a different day, shows that position effect is not constant.

Time (EDT)	Tree #1	Tree #2
0845–0945	19	0
0955–1055	37	9
1100–1200	33	19
1210–1315	37	46
1325–1425	12	9
1435–1535	5	8
1545–1650	26	16
1700–1800	31	61
1815–1915	36	189
1930–2030	65	106
Total	301	463

Table 35. Position effect in simuliid collections. Collections of flies from 2 miniature suction traps each with carbon dioxide at 50 ml/min. Both traps in a ‘uniform’ habitat, viz. a forest clearing, but 1 trap closer to a wooded area than the other. 25 June 1965 with the last collection spilling over to the morning of 25 June.

Time (EDT)	Number of flies capture	
	Trap near woods	Trap in clearing
0745–0845	13	32
0845–0945	288	324
0945–1045	168	211
1045–1145	124	247
1145–1245	101	111
1245–1345	103	61
1345–1445	122	75
1445–1545	120	140
1545–1645	116	118
1645–1745	231	161
1745–1845	221	87
1845–1945	266	179
1945–2045	303	136
2045–2145	6	4
2145–0800	2	3
Total	2184	1889

The significance of these results is immediately apparent. Position effects have been noted with the canopy simuliids, with *S. rugglesi*, and *S. venustum*. In those instances in which one is dealing with a single species, the problems of position effect are serious but not necessarily insurmountable. However, in work with a group of flies, such as are found in the forest canopy, it is virtually impossible to assess such important features of their biology as relative attractiveness of various stimuli, host preferences, etc. Thus, from the data presented in Table 34a, it is difficult to determine whether or not the presence of the extract is having any influence on the composition or size of the collection, since its effects (if any) are being partially masked by a position difference.

Work that, in the future, will concern itself with such issues as hos preferences of the canopy simuliids must deal first with the problem of position effect. It can be overcome in the manner mentioned above, by moving to a site of uniform habitat. This is a difficult undertaking in the forest canopy. The only other alternative is to study the problem of position effect *in situ*, so that appropriate correction factors can be applied to experimental data.

It would seem, in view of the inconstant nature of the position effect, that the most likely factor responsible is wind. A reexamination of the data given in Table 34a will demonstrate that the discrimination between the 2 trees was not uniform, but rather was cyclic. The ratios are shown below (the asterisk indicates the presence of the extract at tree #1:

Time (EDT)	Discrimination Ratio
1145–1245	1.2 *
1300–1400	1.9
1415–1515	8.3 *
1530–1630	1.3
1645–1745	3.6 *
1800–1900	1.6
1930–2030	2.4 *
2100–2200	1.3

In the original, the ratio for the 6th collecting period (1800–1900) was erroneously reported as 1.5

Thus, the population demonstrated better discrimination between the 2 attractants whenever the extract was in tree #1. The average discrimination coefficient for the extract in this position is 3.9 <erroneously reported as 4.1>; the average discrimination coefficient for the extract in position at tree #2 is 1.5. This is, perhaps, circumstantial evidence that the extract *is* influencing the collection. Judicious recording of local wind effects might lead to an hypothesis concerning the possibility of upwind orientation in the Simuliidae. A fluctuation in discrimination ratios might be expected if one trapping site were downwind or upwind from the other, whereas uniformity would be expected if both traps were disposed along a line at right angles to the wind.

No earnest attempt has been made in this study to investigate the problem of position effect. Rather, an attempt was made to minimize its effect by the utilization of experimental sites that had fairly uniform habitat, as defined by the absence of this error.

Temporal Variation in the Biting Activity of the Simuliidae

The various species of the Simuliidae can be shown to have a characteristic biting-activity cycle (Table 36). The data shown are representative data only. The significant fact here is not that blackflies demonstrate peaking activity in their biting cycle, which fact is well known already (Bennett 1960; Wolfe and Peterson 1960), but rather that the time of peak activity appears to be species specific. Thus, the population of canopy simuliids various heterogeneously with time.

This aspect of the biology of feeding has not been carefully investigated, but its significance in experimentation cannot be underestimated. Thus, comparisons of different attractants are of different hosts cannot but be biased if they are done at different times of the day. The problem is further complicated by the lack of a suitable means of obtaining an index of the biting population that is relatively unbiased.

Table 36. Temporal variation in the biting activity of simuliids. Unless otherwise noted, all collections are from the forest canopy, 11 June 1964. <This table was misnumbered as "26" in the thesis.>

	Number of flies collected at specific times (EDT)							
Species	1145–1245	1300–1400	1415–1515	1530–1630	1645–1745	1800–1900	1930–2030	2100–2200
Simulium spp.[a], ground level	7	9	60	68	117	77	11	1
Simulium spp.[a]	50	66	64	53	56	57	18	4
S. aureum	0	0	12	26	46	45	23	5
S. croxtoni	3	4	45	62	21	66	6	4
Eusimulium spp.[b]	2	0	4	23	4	90	266	70
P. decemarticulatum	0	0	0	19	6	315	255	19

[a] Mammalophilic species, mostly *S. venustum*.
[b] *S. quebecense* and *S. latipes*, which were not separated.

There are perhaps 2 solutions to the problem posed by temporal variation:

1. Insure that all collections that are done for comparative purposes are simultaneous, bearing in mind the position effect.
2. For every collection, insure that a non-biased sample of the population is taken. At present, there is no adequate means of assessing the population in such an unbiased fashion.

Discussion

The field experiments herein reported leave little doubt that both simuliids and tabanids have a marked ability to orient to and locate a source of carbon dioxide. It is not unlikely, therefore, that carbon dioxide plays a role in the normal host-finding mechanisms of each family. However, the actual means by which the insect makes use of carbon dioxide are much less clear, and field experiments of the nature described here do not readily lend themselves to an interpretation of the mechanisms involved.

A considerable body of experimental investigation of the host-finding mechanisms of mosquitoes has been done under laboratory conditions. In spite of the very considerable difficulty in extrapolating from laboratory conditions to actual field conditions, much of this work with mosquitoes is pertinent.

Brown *et al.* (1951) studied the reactions of *Ae. aegypti* to carbon dioxide. The criterion of attraction employed in their olfactometer was approach of the insect to within 0.5 inch of the experimental port releasing carbon dioxide. 10% carbon dioxide added to dry air more than doubled the attractiveness of the air stream relative to the control stream. However, carbon dioxide, when added to a moist air stream, did not significantly increase its attractiveness. Pure carbon dioxide was found to be 30% less attractive than a dry air stream with 10% added carbon dioxide. When the pure gas was used, the mosquitoes would not approach the experimental port within the distance established as the criterion of attraction. Laarman (1955, 1958) demonstrated that *Anopheles labranchiae atroparvus* was attracted to an air stream containing human breath, but that when carbon dioxide was added to the control air stream, the superiority of the test stream (containing human breath) disappeared. Crumb (1922), by way of contrast, reported that carbon dioxide did not increase the attractiveness of an air stream to *Culex pipiens*. Van Thiel (1947) investigated the responses of *An. maculipennis* to carbon dioxide, employing a small olfactometer. He found that all concentrations of the gas, from 0.25% to 10% were repellent. However, by changing the design of the olfactometer, he was able to demonstrate that carbon dioxide was attractive to *An. maculipennis* at all concentrations from 0.25% to 10%. Similarly, Willis (1947) reported that carbon dioxide was not attractive to mosquitoes in the olfactometer that he employed. However, Willis and Roth (1952) demonstrated that in a small olfactometer, carbon dioxide was repellent at all concentrations tested, but in a large olfactometer, the gas was very significantly attractive. It is significant that in the small olfactometers employed by these workers, by virtue of the design of the experiment, *all* mosquitoes took part in the experiment, whereas in the large olfactometer, only a small portion (usually <10%) of the available population participated in any one experiment. Brown *et al.* (1951) had concluded that the larger the space in which mosquitoes were confined, the more carbon dioxide could act as an effective attractant.

Several workers have demonstrated that carbon dioxide, when released in the field, proves a highly effective attractant for mosquitoes. Thus, Reeves (1951) found that carbon dioxide, employed as the solid, increased the catches of mosquitoes in stable traps. Headlee (1941) demonstrated in field trials, that carbon dioxide increased the catches of mosquitoes in the New Jersey light trap, and a combination of the light with carbon dioxide was most effective. Essentially similar findings were reported by Huffaker and Back (1943). Brown (1951) reported that the emission of carbon dioxide at a concentration of 10% from the head of a dummy, greatly increased the attractiveness (as measured by the landing responses) of the dummy for a number of northern species of *Aedes*.

Several workers have indicated that carbon dioxide plays a role of an "activator" and have attributed attractiveness to this effect. Rudolfs (1922) reported that carbon dioxide had an activating effect on *Ae. sollicitans* and *Ae. cantator*. The gas caused a general increase in the activity of caged mosquitoes. However, it is difficult to assess Rudolfs' work inasmuch as he employed non-objective criteria in his assessment of responses (e.g. restlessness, fear, pleasure, etc.). Rudolfs also reported that carbon dioxide, released at a slow but unspecified rate, was unattractive to mosquitoes under field conditions. Burgess (1959) found that, in order to make *Ae. aegypti* respond to heat in the form of convection currents, it was necessary to release a very small quantity of carbon dioxide (15–20 ml) into the cage, whereupon the mosquitoes would demonstrate strong probing responses to the convection current. Willis (1947) also reported that carbon dioxide in small quantities had an activating effect on caged mosquitoes. Laarman (1955) found that mosquitoes normally were relatively inactive, but that when carbon dioxide was allowed to flow through the cage at a slow rate such as to give a concentration of approximately 4%, the mosquitoes started to fly about immediately, and gradually accumulated under the orifice at which carbon dioxide was emanating. Similarly, Laarman (1958) showed that *An. maculipennis* demonstrated a marked orientation to moisture and heat. Laarman separated the responses of the population into alighting responses and hovering responses. Moisture resulted in alighting responses. However, in the presence of carbon dioxide at the experimental port, the predominant reaction was hovering, even at high humidities. Further, mosquitoes did not respond to heat alone, but in the presence of carbon dioxide, demonstrated a very marked preference for the experimental port at the higher temperature. These findings are essentially the same as those reported by Burgess (1959) for *Ae. aegypti*. Daykin *et al.* (1965) found that *Ae. aegypti* had a spontaneous fly-off rate, and that this was markedly increased in the presence of carbon dioxide. A comparison of the experimental data with theoretical considerations led these authors to postulate that the effect of carbon dioxide was not due to a gradual build-up of the gas, but rather was mediated by receptors responding specifically to carbon dioxide. Willis and Roth (1952) demonstrated the presence of sensilla responding to carbon dioxide on the antenna of female *Ae. aegypti*. Daykin *et al.* (1965) considered that the first step in host finding by *Ae. aegypti* was an activation by carbon dioxide.

Several aspects of this work with the Culicidae deserve a critical assessment. First, in spite of the fact that much experimental work has been carried out, there is, as yet, no definitive, completely satisfactory theory explaining the physiological and ethological role of carbon dioxide in the host-finding mechanism. There is little doubt that carbon dioxide can serve as a most effective ‘activator’ for caged mosquitoes, causing them to take flight almost immediately. In some cases, it is apparent that the presence of carbon dioxide renders the population more reactive to other ‘host’ stimuli, and thus serves the role of a true activator. Simuliids react similarly to carbon dioxide, as do, indeed, most insects. Blackflies clustered about a carbon-dioxide source in the field are often vicious biters, as compared to the unattracted population situated at a distance.

However, that carbon dioxide, under laboratory and, perhaps, field conditions, can serve as an activator, says little of the physiological mechanisms involved, and does not treat of the possibility that the gas might be involved in mediating true orientation responses. Certainly, the attraction of biting flies under field conditions to carbon-dioxide sources, argues strongly in favor of an involvement in orientation. It is unfortunate that much of the work with the culicids under laboratory conditions does not lend itself to a clear interpretation. There is little doubt that host-seeking behavior in mosquitoes (Laarman 1958) and other biting Diptera is effected through a behavioral hierarchy, and it has been a consistent failure of the published work with biting flies to take this into consideration. Consequently, much of the work on attraction leaves considerable doubt as to what behavioral level in the hierarchy was under investigation. Different workers have employed a variety of criteria as indices of attraction, most commonly approach to, landing on, or probing at, the experimental port. Laarman (1958) has pointed out that the 2 aspects of attraction, viz. landing and approach, involve quite different aspects of host-seeking behavior, and are probably mediate by different stimuli.

It is clear, therefore, that the word “attraction” has ceased to have a meaningful use in studies of host-orientation behavior. The word has been used by most workers without a proper regard for the behavioral complexities that it involves. New terminology is required.

It is of considerable interest and significance that the actual experimental design employed in the laboratory assessment of attraction behavior has a considerable influence on the observed results. Thus several workers have reported that carbon dioxide is repellent when tested in confined spaces, but is found to be highly attractive in other circumstances. Willis and Roth (1952) found that, in a large olfactometer, only a portion of the population responded during any one experiment. It is clear that mosquitoes are not uniformly responsive to host odors at all times. Thus, care must be taken to insure that the test population has a “choice” of either responding or not responding. Willis’ (1947) observation that carbon dioxide is repellent probably resulted from the fact that the very design of his olfactometer necessitated the entire population being considered as the test population. It is likely that uniformity in results will not obtain until some degree of uniformity in test conditions is adopted.

Little has emerged from the experimental work with mosquitoes that would indicate whether carbon dioxide serves as an activator at great distances from the host or only in proximity to the host. Furthermore, it is not at all clear whether carbon dioxide is employed by mosquitoes as a means of orientation to the host (Clements 1963). Certainly the situation with the Simuliidae and Tabanidae is even less enlightening.

The mode of action of carbon dioxide has not been studied, and can be treated only in a theoretical sense. In its role as an activator, it is conceivable that carbon dioxide could act in one of 2 ways, or both. It is known that carbon dioxide stimulates flight activity in resting insects, and it is possible that flying insects are more responsive to host odor and other host-orienting factors that could subserve orientation functions. On the other hand, carbon dioxide might have a physiological function, resulting in a lowering of the thresholds to other host stimuli, thereby rendering the insect more amenable to stimulation by the host.

Work with mosquitoes under field conditions, and the present reported work with simuliids and tabanids, make it clear that these insects can orientate to and locate a source of carbon dioxide. It would be difficult to ascribe this action to "activation" and to do so would result in a rather sterile semantic dispute as to what is meant by attraction or activation. To be useful, the concept of activation should not include an element of orientation. It may well be that in the initial stages of attraction to the host, carbon dioxide serves as an activator, as in caged mosquitoes, causing the insect to take flight. However, this is speculation, since most of the information concerning the flight activity of females of the Culicidae, Simuliidae, and Tabanidae, deals with attraction to the host or to host-substitutes, and little is known about behavior patterns prior to this. It is not known if attraction behavior is preceded by a nonspecific, appetitive element, or whether the insect must be stimulated to take flight by various host factors. Colless' (1956, 1957) findings that the catches of biting insects correspond well with theoretical depletion curves in which the rate of catch is proportional to the number remaining uncaught, support the concept of random wandering in a closed domain. Colless proposed that attraction of biting insects to the host operated only at relatively close range. Laarman (1965), on the other hand, considered it unlikely that mosquitoes start their search for a host without an extrinsic chemical stimulus. Thus, the problem of whether the feeding drive is characterized by an initial period of appetitive behavior in the form of random searching requires further investigation.

Insofar as orientation mechanisms are concerned, the direct orientation to odor by a flying insect is not readily conceivable in a theoretical sense. Several workers have been careful to point this out. Kennedy (1939), in an elegant study of the visual responses of *Ae. aegypti*, demonstrated that flying mosquitoes would not tolerate images passing over the compound eyes from back to front, the visual equivalent of being swept downwind. Rather, the insect always oriented into the wind by a compensation for lateral movement of images across the eye, and flew at such a speed as to maintain a constant relation with the background or slightly faster, so that images passed over the eye from front to back. Kennedy was able to demonstrate that the insect modified its air speed when in wind in order to maintain or attempt to maintain, a constant rate of movement of images across the eye, and for low wind speeds, this was equivalent to a ground speed of about 17 cm/s in the chamber that he used for his studies. Winds in excess of 150 cm/s rendered such compensation impossible, and the insect would immediately land. Kennedy further demonstrated that mosquitoes could not orient upwind in complete darkness. He concluded: "It is maintained here that flying orientation to a wind-borne scent is not, in a direct sense, easily conceivable. But the *activating* effect of scent, combined with visual orientation upwind, would serve as an effective host-finding mechanism for mosquitoes emerging downwind of human habitations and flying low" (p. 241).

Kennedy thought that mosquitoes would necessarily fly low to the ground, because the apparent rate of movement of the background across the eyes would be a function of the distance of the insect above this background. Visually compensated, upwind orientation was subsequently emphasized by Bässler (1958) and Kalmus and Hocking (1960). Kalmus and Hocking and Laarman (1955) observed mosquitoes to fly low, and to approach hosts from downwind.

Daykin *et al.* (1965) showed that *Ae. aegypti* tended to fly in straight paths when in an attractive-odor cloud, but that immediately upon leaving the odor cloud, the insect would undertake an immediate turn, the effect of which in many cases was to bring the insect back into the odor.

A combination of the responses described by Daykin *et al.* (1965) with visually compensated upwind orientation, could account for orientation of biting insects to their hosts. It is conceivable that carbon dioxide serves as the releaser for this behavior. The maintenance of upwind flight may depend on a continuous activation by carbon dioxide.

One must deal with the additional problem of the ability of simuliids to discriminate between traps emitting carbon dioxide at different rates. As shown above, *S. venustum* demonstrates a remarkable ability to choose the trap emitting the larger quantities of the gas. The flies are still capable of selecting the higher-rate trap even when the 2 traps are situated very close together. This behavior implies an assessment by the fly of carbon dioxide density, and therefore, a true attraction to the gas. The mechanism by which this could take place is not clear, but it is conceivable that, by an extension of Daykin *et al.* (1965), the flies tend to turn back from regions of lower carbon-dioxide density into regions of higher density, a behavioral pattern that would tend to lead them up the gradient. To propose a system of direct monitoring of odor density introduces the additional problem of sensory adaptation. However, Wright (1958) proposed that flying insects could detect a gradient by virtue of the nonuniform structure of odor clouds. Odors tend to be dispersed in a lamellar or filamentous fashion, and Wright considered it theoretically possible for an insect to sense the gradient by virtue of the frequency with which it encountered the filaments of the odor cloud. An increasing frequency of encounter would indicate movement up the gradient, whereas reduced frequencies would indicate movement down the gradient. No experimental justification for or demonstration of such a mechanism has been given, and it is unlikely that such a mechanism could be operative at appreciable distances from the host. Indeed, any function of carbon dioxide at a distance, other than its role as an activator or releaser, is difficult to conceive. However, it is possible that, at close range, the insect might be capable of detecting differences in concentration, sensory data that would indicate the nature and distance of the gradient. This sensory capability, coupled with a behavioral selection of regions of higher density, would tend to lead the insect up the gradient, and hence to the host or trap.

No direct evidence exists to show that either simuliids or tabanids in fact fly upwind to the host in their orientation behavior. There are a few observations of *S. euryadminiculum* in which the flies were seen to approach the attractant from downwind, an to maintain a downwind orientation as they hovered near the attractant.

In addition, some indirect evidence exists to demonstrate an apparent effect of wind on the ability of blackflies to locate a source of carbon dioxide. An examination of the data in Table 29 will demonstrate that wind is having an effect on the discrimination of carbon-dioxide sources by *S. venustum*. It is seen that discrimination, at least initially, was much better whenever the trap with the more carbon dioxide was situated west of the other trap. The prevailing wind on the day in question was from the west. Thus, the discrimination coefficients fluctuate between high and low values. The regular decrease in the discrimination coefficients when the traps were closer together than 8 feet is puzzling, but may indicate that wind does not play such an important role when the fly gets close to the source, or that the wind velocity had decreased by that time. That some discrimination was evident at 2 feet and perhaps at 1 foot, indicates that the flies were capable of detecting regions of high odor density from regions of low odor density, even when these were very close together.

Brouwer (1960) demonstrated that the attractiveness of the human arm for *An. stephensi* was not due to carbon dioxide excreted by the skin, suggesting that other odors are involved in the attraction of mosquitoes. Many workers have shown in olfactometer experiments that a variety of naturally occurring substances are attractive to mosquitoes. Brown and Carmichael (1961a, 1961b) demonstrated that the amino acids lysine and alanine were attractive to *Ae. aegypti*. Lipsitz and Brown (1964) showed that arginine was also attractive, although to a lesser extent as compared to lysine and alanine. They considered that the attractiveness of lysine may be due to its ability to act as a carrier of carbon dioxide. Laarman (1955) demonstrated that the vapor obtained by passing air through citrated rabbit's blood was more than twice as attractive to *An. maculipennis atroparvus* than a control of air passed through physiological saline. This vapor from the rabbit was attractive even after remove of carbon dioxide, and when humidity was equal in the 2 air streams. Burgess and Brown (1957) demonstrated that beef blood, if kept from clotting, is attractive to *Ae. aegypti*. It is interesting that Rudolfs (1922) reported that blood was nonattractive, but he may have allowed it to clot. Burgess and Brown (1957) showed that if carbon dioxide was removed from the air stream that had been passed through blood, the attractiveness was reduced, indicating that carbon dioxide was also important. However, they cautioned that treatments to remove carbon dioxide may remove other components as well. Thus, blood treated to remove the carbon dioxide was no more attractive than washed blood corpuscles, and the addition of carbon dioxide to the blood increased its attractiveness, relative to the washed corpuscles, by about 30%. It is significant, however, that the addition of carbon dioxide to whole blood increased its attractiveness, relative to washed corpuscles, by about 130%, again indicating that factors other than carbon dioxide are involved. Burgess and Brown concluded that carbon dioxide was involved in the attraction of mosquitoes but that it was not the most important factor.

Roessler (1963) reported that blood and urine were attractive to *Ae. aegypti*. Both blood and urine contain various steroids, and Roessler investigated the attractiveness of estrone, estradiol, estriol, and androstandion in an olfactometer. He concluded that estriol stimulated the mosquitoes to fly eagerly into the wind, but that it was unable to direct them in an inclining gradient. In studying the attraction of females <human females> to mosquitoes, he found that attraction was cyclic, and this could be correlated with the menstrual cycle. Schaerffenberg and Kupka (1951) reported the presence in blood of a substance, "Blutdoftstoff", that was attractive to *Cx. pipiens* and *An. maculipennis* when exposed in open dishes and compared with controls of pure water. However, individual blood proteins, lipoids, and hematin were found to be unattractive.

It is evident, then, that odor plays a role in the attraction of mosquitoes to their hosts. However, many of the objections raised to the olfactory role of carbon dioxide, as determined by olfactometer experiments, also apply to the experimentation with other odors. As yet, there is no clear indication of the manner in which various odors are involved in the attraction of mosquitoes. What is true in the laboratory may not be true in the field (Pitman *et al.* 1966) and it is quite possible that many compounds or substances found attractive to mosquitoes under laboratory conditions may be only "mimic" substances, and may play only a negligible role in the attraction under natural conditions. Laarman (1955, 1958) was of the opinion that odor served only as an activator, therefore differing in no way from the role of carbon dioxide. Indeed, there is probably little justification for considering carbon dioxide separately from other odors, apart from the fact that it has little potential for specificity and is produced by animals in relatively large amounts. We may conclude that the function of odors in the attraction of mosquitoes remains undetermined.

There is little doubt that odor is involved in the attraction of *S. euryadminiculum* to its host, and the odor in question seems to originate in the uropygial glands of the bird. It is necessary to postulate an activator-releaser effect for this odor, with subsequent upwind orientation by the fly. *S. euryadminiculum* has been observed to approach extracts from downwind. The flies appear capable of locating the source of the odor with considerable accuracy, small swarms of flies being found hovering or flying directly over the attractant at a height of a few inches, or slightly to leeward. The odor serves only to mediate this initial attraction to the locale; *S. euryadminiculum* will rarely land on papers impregnated with the extract, and those that do land take flight almost immediately. Whenever extracts are exposed in the lakeshore habitat, swarms of flies are seen to react to a variety of objects that break the contour of the site. Thus, flies have been seen to swarm around and occasionally land on small logs, paper models in the form of an inverted 'V', and a 3-l brown bottle. Placing a bottle in the midst of a swarm of *S. euryadminiculum* causes the swarm to instantaneously coalesce about the bottle top (which is black) and to occasionally land. It is probable, therefore, that vision is of considerable importance in the near-orientation of simuliids, but the reluctance of many flies to land suggests that the visual requirements are specific, or that other factors may mediate landing responses. It is significant that on landing on various objects used as host substitutes, few flies crawl or show probing responses, most taking flight again immediately.

The nature of the odor involved in the attraction of *S. euryadminiculum* remains unknown. It seems evident, however, that the source of the odor is the uropygial gland of the host. This is a gland of the sebaceous type, of a bilobed structure, lying at the base of the tail. The gland has a central cavity that serves to collect the oily secretion. The secretion can be discharged through a dorsal, nipple-like pore. Species differences exist in the secretions of various birds (Marshall 1960), thereby providing at least a functional framework for specificity. Approximately half of the secretion of the gland is ether soluble, and this lipoidal fraction contains both saponifiable and nonsaponifiable compounds and lecithin. Paris (1914) (as reported in Marshall 1960) reports that the secretion of the gland in many birds is odorous during the breeding season. He considers the uropygial gland to be homologous to the scent gland of reptiles, thereby suggesting that the function of the gland may differ considerably from its commonly supposed function of providing oil for the grooming of the plumage. Marshall (1960) reports that no specific, indispensable function has been ascribed to the gland.

The extent to which odor is involved in the attraction of other simuliids remains equivocal. It has been pointed out that extracts of the uropygial glands of various host birds are unsuccessful in attracting either *S. rugglesi* or the canopy simuliids. It would appear that the attraction of *S. euryadminiculum* to an odor from the host is unique. *S. rugglesi* and the canopy simuliids, however, can orient well to a source of carbon dioxide placed in the appropriate habitat.

It is suspected that the combination of bird extracts with carbon dioxide increases the number of flies coming to the baits as compared to the number coming to carbon dioxide alone. *However*, this study has made it clear that there are a large number of variables that make the interpretation of the degree of attraction extraordinarily difficult under field conditions. It has been shown that the very position in which a collection is made can influence the results, and until the problems associated with position effect have been adequately solved, quantitative comparisons of various attractants will be difficult. It is conceivable that appropriate statistical analyses of large samples might demonstrate an attraction to bird odors. It is interesting that Brown and his coworkers (Lipsitz and Brown 1964) proposed a mechanism for the attractiveness of lysine involving its ability to act as a carrier for carbon dioxide.

There are indications that the presence of bird odors does influence the collections of simuliids in the forest canopy (*see* page 103 <i.e. original page 103>). The attraction of simuliids to carbon dioxide was compared to the attraction to carbon dioxide and a bird extract. It is seen that the discrimination ratios obtained are a function of the relative position of the 2 attractants. It is possible, however, that the same fluctuation in discrimination ratios might have been obtained without the added extract, but this possibility was not tested.

Work with the Tabanidae, at least until the present time, has indicated only the importance of vision in the attraction of these insects (Bracken *et al.* 1962; Thorsteinson *et al.* 1966). The author has pointed out that vision most probably serves as an orientation clue in the landing responses of the fly. No data exist on the extent to which tabanids make use of vision in their attraction behavior, nor on the distance over which vision, when unobstructed, can serve as an orientation clue. This quantitative approach to the study of the elements of the behavioral hierarchy is unequivocally required.

It is reasonable to suppose that, given variable conditions of habitats, accessibility and exposure of the host, size, color and movement, some degree of behavioral flexibility will exist in the approach and landing behavior of tabanids and simuliids. There is a need for quantitative estimates of the distance from the host when vision and not olfaction serves as the primary orientation clue. A study of the relationships between the various factors involved in orientation might facilitate a description of the hierarchy, for steps in the hierarchy could then be defined in terms of the sensory mode by which they are mediated. Such an approach might bring considerable uniformity to studies of attraction behavior of insects.

That simuliids discriminate between traps situated very close together and differing only in their carbon-dioxide-emission rates indicates that these insects probably do not rely on vision until they are very close to the host. However, it must be borne in mind that the miniature traps employed do not present a very striking silhouette, and there exists the possibility that variously colored traps might effect a dissolution of the discrimination when the traps are separated by distances >2 feet. The Tabanidae, on the other hand, have a well-developed visual capacity, and probably rely on vision to effect orientation at much greater distances from the host. Tabanids have been observed to make rapid, circular flights around a trap at a distance of 8 or 10 feet, and it seems likely that they are reacting to the trap visually. The success of the Thorsteinson “heliothermal” trap (Thorsteinson 1958) also indicates that vision must be effective at a greater distance in the tabanids than in the simuliids.

On the basis of the observations and considerations, a theoretical hierarchy can be constructed to relate the various elements in the feeding behavior of simuliids and tabanids.

Stage 1. Appetitive Behavior

As has been pointed out already, it is not known whether this phase of the behavior is characterized by a random search by the insect, or whether the insect rests and awaits stimulation by host factors. Few data exist on the basis of which a choice between these 2 possibilities could be made. It is possible, of course, that both elements are involved. Thus, the insect may initiate spontaneous exploratory behavior of a random sort, in response to the feeding drive, but it may also be stimulated to flight from the resting condition by various host factors. The feeding drive in mosquitoes, and undoubtedly in other biting flies, appears to involve a circadian rhythm (Clements 1963) that can be extensively modified by prevailing weather conditions (Edmund 1952; Clements 1963).

In addition, however, it is here proposed that an essential element in the initial stages of the attraction behavior of both simuliids and tabanids is a selection of habitat by the insect. It has been demonstrated that these insects prefer certain habitats to others, depending on the species, and it seems that it is difficult to “draw” a species in large numbers from its preferred habitat (Bennett 1960). Thus, it appears that stage 1 of attraction behavior takes place only within the preferred habitat of the species involved. This statement is the more applicable the more rigid are the habitat preferences. That habitat may influence the receptivity of the insect to stimulation by the host probably accounts, in part, for the failure to get many species of biting insects to suck blood under laboratory conditions.

Stage 2. Upwind Orientation

Whatever may be the nature of the appetitive element of stage 1, it is here proposed that directed responses to the host involve a releaser. The most likely releasers involved in distance orientation are olfactory stimuli from the host. It seems probably that carbon dioxide can release an upwind-oriented behavior in all simuliids and tabanids, but that odor may also function as a releaser, as in *S. euryadminiculum*. It is conceded that direct upwind orientation to an odor cloud or carbon dioxide is not possible, and it is proposed that the insect makes use of visual clues in upwind orientation. It is interesting, in this regard, to note that both simuliids and tabanids are strictly diurnal insects.

Stage 3. Near-Orientation

It is clear that simuliids, and perhaps tabanids, can select a trap on the basis of differences in the carbon-dioxide concentration about the trap. It is here proposed that directed orientation to odor gradients be called “near-orientation”. The quantitative aspects of this phenomenon remain unstudied, especially for the Tabanidae, and the mechanisms proposed have not been demonstrated experimentally. It is of interest to recall Scudder’s (1953) hypothesis concerning the function of antennal ampullas (pages 3–4), which he considered might be involved in the monitoring of odors at high density. It is possible that other factors, such as heat and moisture gradients, are involved in near-orientation.

Stage 4. Landing Behavior

It is likely for both simuliids and tabanids that the final orientation to the host, while the insect is still on the wing, is mediated by visual stimuli. It is proposed here that the terminal stages of in-flight orientation that are mediated by visual stimuli be classed under the term “landing behavior”. It is possible that other factors are involved in landing behavior, since, for example, moisture is known to release landing behavior in the Culicidae (Laarman 1955).

Stage 5. Crawling or Burrowing

Few biting flies will suck blood immediately on landing. Mosquitoes are seen to land low on the host and then to crawl up (Kalmus and Hocking 1960). Blackflies are often observed to crawl over the surface of the host, actively palpating the surface with the fore tarsi or, in the case of ornithophilic flies, to burrow through the feathers. The insect appears to effect an exploratory-behavior pattern on the surface of the host, and the selection of a suitable feeding site may involve contact chemoreception, thermoreception, or tactoreception.

Stage 6. Probing and Feeding

This level of the hierarchy involves the insertion of the mouthparts and the sucking of blood. It is probably consummatory.

One of the most vexing of all problems associated with the biology of biting flies is that of host preferences or host specificities. Fallis (1964) reviewed the feeding records for the Simuliidae and some accounts have been given of host preferences in the Culicidae (Dow *et al.* 1957). It is customary to classify mosquitoes as either zoophilic or anthropophilic (Clements 1963) but this separation probably represents only a bias on the part of workers engaged in studies of the transmission of *Plasmodium* spp. by *Anopheles* spp. On the other hand, it has been found convenient to use the terms *mammalophilic* and *ornithophilic*, especially for the Simuliidae. Such a classification can be readily justified from field records of feeding, and has the additional advantage that it parallels, at least in some instances, the taxonomic divisions of the family. Thus, for example, the genus *Simulium* can be subdivided into mammalophilic and ornithophilic forms, and the latter are mostly restricted to the subgenus *Eusimulium*.

Generally speaking, host preferences are exceedingly difficult to determine. Most authors have failed to recognize that the word “preference” is quantitative. It is not sufficient, therefore, to assess host preferences on the basis of feeding records alone, as most authors have done. One can adequately assess preferences only in a choice situation in which the number choosing a given host can be known as a function of the number available to make a choice. This situation cannot be realized under field conditions. A technique of much promise has been the identification of bloodmeals by the precipitin test (Tempelis and Reeves 1964), but, as Weitz (1960) has pointed out, it is exceedingly difficult to obtain a random sample of the population on which precipitin tests can be made.

Bennett (1960) attempted to assess the host preferences of the species of Simuliidae frequenting the forest canopy. It is now very clear that given the associated problems of position effect, temporal variation in the size of populations, and changes in vertical stratification with time, as well as the possibility of species differences in the strata preferences, determinations of host preferences involving several species of flies and potential hosts must be carried out with extreme caution.

This program of research has not dealt specifically with the problem of host preferences. However, a study of the hierarchy involved in the feeding behavior of simuliids and tabanids will reveal the mechanisms of host selection. The selection of a particular host by a biting insect implies a “rejection” of a large number of other hosts. Thus, the problem of selection can be viewed in relationship to the hierarchy outlined above. Thus, rejection of a potential host will involve a blockage of the hierarchy at a particular point. It is clear, therefore, that selection is a multi-leveled hierarchy closely associated with host-finding mechanisms.

Habitat selection by biting insects implies an immediate rejection of a large number of potential hosts. It has been shown that habitat selection is found, in varying degrees, in both the simuliids and tabanids. It seems likely, therefore, that the initial step in host selection is the selection of a particular habitat in which the host will be sought. This does *not* imply that the presence of a host determines the habitat in which the fly is found, for carbon-dioxide collections in the absence of hosts demonstrate the presence of flies in the habitat, and further, that a fly cannot be drawn from its habitat by its preferred host indicates that factors other than host factors determine the habitat preferences.

The host specificity demonstrated by *S. euryadminiculum* appears to be mediated by the attractant odor. No other species are taken in collections with loon extracts. Thus, it is reasonable to postulate that the odor involved acts as a specific activator, leading to upwind flight only by *S. euryadminiculum*. Carbon dioxide, being found in the exhalations of all animals, offers little potential for specificity, although some authors (Reeves 1953; Whitsel and Schoeppner 1965) have considered that host selection may involve a selection or rejection of certain densities of carbon dioxide. It is difficult to conceive a mechanism for selection involving so general a stimulant as carbon dioxide. On the other hand, biting flies might reject animals if the carbon-dioxide concentration about the animal were too high. However, odors offer the best possibilities for specificity, and odors from the uropygial glands of birds may account for the specificities observed.

It is conceivable that selection could operate at the level in the hierarchy concerned with landing behavior. This might account for the preference of the Tabanidae for moose and of the Culicinae for the smaller mammals.

It has been shown that *S. euryadminiculum* can be induced to land on ducklings, but that it will not feed. It is clear that selection is operative at this level as well, and, in the final analysis, engorgement may be the only effective means of measuring selection of hosts by biting flies.

It may be concluded that host preferences in biting flies are mediated through the behavioral hierarchy involved in feeding behaviors. Selection or rejection of a host may occur at any or all levels in the hierarchy and future studies of host preferences should take the existence of this hierarchy into consideration.

Summary

1. Habitat preferences are demonstrated for the Tabanidae and confirmed for the Simuliidae. The Tabanidae are found to select for either sylvatic or “aquatic” habitats, and a few species prefer bogs. The Simuliidae select sylvatic or lakeshore habitats. In addition, habitat selection is presented as a function of vertical stratification. Stratification is a dominant feature in the biology of the Simuliidae, but of relatively minor significance in the Tabanidae.
2. *Simulium euryadminiculum* is attracted to an extract of the uropygial glands of the Common Loon. The role of odor in the attraction of other species of simuliids is investigated, and the results are equivocal.
3. Both the Simuliidae and the Tabanidae can orientate to and locate a source of carbon dioxide in the field. The ability of simuliids to discriminate between sources of carbon dioxide is investigated.
4. Position effects, temporal variation of populations, and specific strata preferences are briefly introduced to illustrate major sources of error often overlooked in the study of feeding behavior.
5. The role of odor and carbon dioxide is discussed in relation to similar investigations with the Culicidae. It is postulated that carbon dioxide, in addition to its activator effect, releases visually compensated upwind orientation. It is further postulated that odors act in a similar, but more specific fashion. Direct orientation to carbon-dioxide gradients is discussed.
6. A theory of feeding behavior, involving a hierarchy, is presented. It is postulated that each phase of the hierarchy is governed by different host stimuli.
7. It is postulated that host selection is, itself, a multicomponent function, and operates through the hierarchy of feeding behavior by the imposition of sensory specificities at each level. Habitat preferences are viewed as the first step in host selection. Selection of a host involves rejection of other potential hosts by means of interruptions of the hierarchy.

Bibliography

Papers marked with an asterisk have been seen as abstracts only.

<Numbers in angle brackets following a citation are accession numbers in my (SMS) library.>

* Amanzhulov, S.A., Amosenkova, N.I. and Postricheva, O.V. 1965. Spontaneous transmission of *Rickettsia burneti* in horseflies, *Tabanus staegeri*. *Med. Parazitol. Parazitar. Bolezni*, **34**(5): 612–614. (In Russian; English summary)

Anderson, R.C. 1956. The life cycle and seasonal transmission of *Ornithofilaria fallisensis* Anderson, a parasite of domestic and wild ducks. *Can. J. Zool.*, **34**: 485–525. <abstract #7311>

Anderson, R.C. 1961. *Splendidofilaria wehri* n. sp., with a revision of *Splendidofilaria* and related genera. *Can. J. Zool.*, **39**: 201–207.

Anderson, R.C. 1962. The helminth and arthropod parasites of the white-tailed deer (*Odocoileus virginianus*): a general review. *Trans. R. Can. Inst.*, no. 70, 34: 57–92. <#505>

Bässler, U. 1958. Versuche zur Orientierung der Stechmücken: die Schwarmbildung und die Bedeutung des Johnstonschen Organs. *Z. Vergl. Physiol.*, **41**: 300–330.

Beckel, W.E. and Atwood, H.L. 1959. A contribution to the bionomics of the mosquitoes of Algonquin Park. *Can. J. Zool.*, **37**: 763–770. <#8456>

Bennett, G.F. 1960. On some ornithophilic blood-sucking Diptera in Algonquin Park, Ontario, Canada. *Can. J. Zool.*, **38**: 377–389. <#6115>

Bennett, G.F. 1965. Personal communication.

Bennett, G.F. and Fallis, A.M. 1960. Blood parasites of birds in Algonquin Park, Canada, and a discussion of their transmission. *Can. J. Zool.*, **38**: 261–273. <abstract #1254>

Bennett, G.F., Garnham, P.C.C. and Fallis, A.M. 1965. On the status of the genera *Leucocytozoon* Ziemann, 1898 and *Haemoproteus* Kruse, 1890 (Haemosporidiida: Leucocytozoidae and Haemoproteidae). *Can. J. Zool.*, **43**: 927–932.

Bracken, G.K., Hanec, W. and Thorsteinson, A.J. 1962. The orientation of horseflies and deerflies (Diptera: Tabanidae). II. The role of some visual factors in the attractiveness of decoy silhouettes. *Can. J. Zool.*, **40**: 685–695. <#458>

Brouwer, R. 1960. The attraction of carbon dioxide excreted by the skin of the arm for malaria mosquitoes. *Trop. Geogr. Med.*, **12**: 62–66. <abstract #3792>

Brown, A.W.A. 1951. Studies of the responses of the female *Aedes* mosquito. Part IV. Field experiments on Canadian species. *Bull. Entomol. Res.*, **42**: 575–582.

Brown, A.W.A. and Carmichael, A.G. 1961a. Lysine and alanine as mosquito attractants. *J. Econ. Entomol.*, **54**: 317–324.

Brown, A.W.A. and Carmichael, A.G. 1961b. Lysine as a mosquito attractant. *Nature*, **189**: 508–509.

Brown, A.W.A., Sarkaria, D.S. and Thompson, R.P. 1951. Studies of the responses of the female *Aedes* mosquito. Part I. The search for attractant vapors. *Bull. Entomol. Res.*, **42**: 105–114.

Burgess, L. 1959. Probing behaviors of *Aedes aegypti* (L.) in response to heat and moisture. *Nature*, **184**: 1968–1969.

Burgess, L. and Brown, A.W.A. 1957. Studies on the responses of the female *Aedes* mosquito. Part VIII. The attractiveness of beef blood to *Aedes aegypti* (L.). *Bull. Entomol. Res.*, **48**: 783–793.

Clements, A.N. 1963. *The physiology of mosquitoes.* The Macmillan Company, New York. <I have the book>

Colless, D.H. 1956. The *Anopheles leucosphyrus* group. *Trans. R. Entomol. Soc. London*, **108**: 37–116.

Colless, D.H. 1957. Components of the catch curve of *Culex annulus* in Singapore. *Nature*, **180**: 1496–1497.

Crumb, S.E. 1922. A mosquito attractant. *Science*, **55**: 446–447.

Davies, D.M. 1949. Description of *Simulium euryadminiculum*, a new species of blackfly (Diptera: Simuliidae). *Can. Entomol.*, **81**: 45–49.

Davies, D.M. 1951. Some observations of the number of blackflies (Diptera: Simuliidae) landing on colored cloths. *Can. J. Zool.*, **29**: 65–70.

Davies, D.M. 1953. Longevity of blackflies in captivity. *Can. J. Zool.*, **31**: 304–312. <abstract #2217>

Davies, D.M. 1961. Color affects the landing of blood-sucking blackflies (Diptera: Simuliidae) on their hosts. *Proc. Entomol. Soc. Ont.*, **91**(1960): 267–268. <#6104>

Davies, D.M. and Peterson, B.V. 1956. Observations on the mating, feeding, ovarian development, and oviposition of adult blackflies (Diptera: Simuliidae). *Can. J. Zool.*, **34**: 615–655. <#2222>

Davies, D.M., Peterson, B.V. and Wood, D.M. 1962. The blackflies (Diptera: Simuliidae) of Ontario. Part I. Adult identification and distribution with descriptions of six new species. *Proc. Entomol. Soc. Ont.*, **92**(1961): 70–154. <#1253>

Davis, H.G. and James, M.T. 1957. Blackflies attracted to meat bait. *Proc. Entomol. Soc. Wash.*, **59**: 243–244.

Daykin, P.N., Kellogg, F.E. and Wright, R.H. 1965. Host finding and repulsion of *Aedes aegypti*. *Can. Entomol.*, **97**: 239–263. <abstract #4670>

DeFoliart, G.R. and Rao, M.R. 1965. The ornithophilic blackfly *Simulium meriodionale* Riley (Diptera: Simuliidae) feeding on man during autumn. *J. Med. Entomol.*, **2**: 84–85. <abstract #1514>

Dethier, V.G. 1947. *Chemical insect attractants and repellents.* The Blakiston Co., Philadelphia.

Dow, R.P., Reeves, W.C. and Bellamy, R.E. 1957. Field tests of avian host preference of *Culex tarsalis* Coq. *Am. J. Trop. Med. Hyg.*, **6**: 294–303.

Edmund, A.G. 1952. The relation between blackfly activity and meteorological conditions. M.A. Thesis, University of Toronto. vii, 1–72 pp.

Fallis, A.M. 1964. Feeding and related behavior of female Simuliidae (Diptera). *Exp. Parasitol.*, **15**: 439–470. <#6111>

Fallis, A.M. and Bennett, G.F. 1966. On the epizootiology of infections caused by *Leucocytozoon simondi* in Algonquin Park, Canada. *Can. J. Zool.*, **44**: 101–112. <abstract #1779>

* Hansens, E.J. 1947. Greenhead flies like dark colors. *New Jersey Agric.*, **29**(4): 3–4. <#1462>

Headlee, T.J. 1941. New Jersey mosquito problems. *Proc. New Jersey Mosquito Exterm. Assoc.*, **28**: 7–12.

Hocking, B. 1960. Smell in insects. A bibliography with abstracts (to December 1958). Defence Research Board, Canada, EP Tech. Report No. 8. 266 pp.

Huffaker, C.B. and Back, R.C. 1943. A study of methods of sampling mosquito populations. *J. Econ. Entomol.*, **36**: 561–569.

* Jellison, W.L. 1950. Tularemia. Geographical distribution of “deerfly fever” and the biting fly, *Chrysops discalis* Williston. *Public Health Reports*, **65**: 1321–1329. <#13353>

Kalmus, H. and Hocking, B. 1960. Behavior of *Aedes* mosquitoes in relation to blood feeding and repellents. *Entomol. Exp. Appl.*, **3**: 1–26.

Kennedy, J.S. 1939. The visual responses of flying mosquitoes. *Proc. Zool. Soc. London*, A, **109**: 221–242. <#573>

Laarman, J.J. 1955. The host-seeking behavior of the malaria mosquito, *Anopheles maculipennis atroparvus*. *Acta Leidensia*, **25**: 1–144.

Laarman, J.J. 1958. The host-seeking behavior of anopheline mosquitoes. *Trop. Geogr. Med.*, **10**: 293–305. <abstract #6459>

Laarman, J.J. 1964–1965. The plasticity of response patterns in host-seeking mosquitoes. *Acta Leidensia*, **33–34**: 136–138.

Laing, J. 1937. Host-finding by insect parasites. 1. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *J. Anim. Ecol.*, **6**: 298–317.

Lipsitz, E.Y. and Brown, A.W.A. 1964. Studies on the responses of the female *Aedes* mosquito. IX. The mode of attractiveness of lysine and other amino acids. *Bull. Entomol. Res.*, **54**: 675–687.

Lowther, J.K. and Wood, D.M. 1964. Specificity of a blackfly, *Simulium euryadminiculum* Davies, toward its host, the common loon. *Can. Entomol.*, **96**: 911–913. <#6096>

Marshall, A.J. ed. 1960. *Biology and comparative physiology of birds.* Vol. 1. Academic Press, New York.

* Paris, P. 1914. Recherches sur la glande uropygienne des oiseaux. *Arch. Zool. Exp. Gen.*, **53**: 139–276.

Pechuman, L.L. 1965. Personal communication.

Pechuman, L.L., Teskey, H.J. and Davies, D.M. 1961. The Tabanidae (Diptera) of Ontario. *Proc. Entomol. Soc. Ont.*, **91**(1960): 77–121. <#1297>

* Philip, C.B. 1931. The Tabanidae (horseflies) of Minnesota with special reference to their biologies and taxonomy. *Minn. Agric. Exp. Station, Tech. Bull*. no. 80. 132 pp. <#2634>

Pitman, G.B., Vite, J.P. and Renwick, J.A.A. 1966. Variation in olfactory behavior of *Ips confusus* (LeC.) (Coleoptera: Scolytidae) between laboratory and field bioassays. *Naturwissenschaften*, **53**: 46–47.

Reeves, W.C. 1951. Field studies on carbon dioxide as a possible host stimulant to mosquitoes. *Proc. Soc. Exp. Biol. Med.*, **77**: 64–66.

Reeves, W.C. 1953. Quantitative field studies on a carbon-dioxide chemotropism of mosquitoes. *Am. J. Trop. Med. Hyg.*, **2**: 325–331. <abstract #4665>

Roessler, P. 1963. The attractiveness of steroids and amino acids to female *Aedes aegypti*. *Proc. New Jersey Mosquito Exterm. Assoc.*, **50**: 250–255. <abstract #205>

Rudolfs, W. 1922. Chemotropism of mosquitoes. *Bull. New Jersey Agric. Exp. Station*, 367. pp. 1–23. <abstract #5016>

* Schaerffenberg, B. and Kupka, H. 1951. Untersuchungen über die geruchliche Orientierung blutsaugender Insekten. I. Über die Wirkung eines Blutduftstoffes auf *Stomoxys* und *Culex*. *Österreich. Zool. Zeitschr.*, **3**: 410–424.

Scudder, H.I. 1953. Cephalic sensory organs of the female horsefly, *Tabanus quinquevittatus* Wiedemann (Diptera: Tabanidae). Ph.D. Thesis, Cornell University, Ithaca, New York. 86 pp. 48 figs. <#3756>

* Sudia, W.D. and Chamberlain, R.W. 1962. Battery-operated light trap, an improved model. *Mosquito News*, **22**: 126–129.

Tashiro, H. and Schwardt, H.H. 1953. Biological studies of horseflies in New York. *J. Econ. Entomol.*, **46**: 813–822. <#3294>

Tempelis, C.H. and Reeves, W.C. 1964. Feeding habits of one anopheline and three culicine mosquitoes by the precipitin test. *J. Med. Entomol.*, **1**: 148–151.

Thorsteinson, A.J. 1958. The orientation of horseflies and deerflies (Diptera: Tabanidae): I. The attractance of heat to tabanids. *Entomol. Exp. Appl.*, **1**: 191–196. <#13429>

Thorsteinson, A.J., Bracken, G.K. and Tostowaryk, W. 1966. The orientation behavior of horseflies and deerflies (Diptera: Tabanidae). V. The influence of the number and inclination of reflecting surfaces on attractiveness to tabanids of glossy-black polyhedra. *Can. J. Zool.*, **44**: 275–279. <#1954>

Van Thiel, P.H. 1947. Attraction exercée sur *Anopheles maculipennis atroparvus* par l'acide carbonique dans un olfactomètre. *Acta Trop.*, **4**: 10–20. <abstract #4353; originally cited as "Thiel, P.H. van">

Van Thiel, P.H. and Laarman, J.J. 1954. What are the reactions by which the female Anopheles finds its blood supplier? *Netherlands Soc. Trop. Med.*, **6**: 156–161. <originally cited as "Thiel, P.H. van">

* Weitz, B. 1960. Feeding habits of bloodsucking arthropods. *Exp. Parasitol.*, **9**: 63–82.

Wenk, P. and Schlörer, G. 1963. Wirtsorientierung und Kopulation bei blutsaugenden Simuliiden (Diptera). *Z. Tropenmed. Parasitol.*, **14**: 177–191.

Whitsel, R.H. and Schoeppner, R.F. 1965. The attractiveness of carbon dioxide to female *Leptoconops torrens* Tns. and *L. kerteszi* Kieff. *Mosquito News*, **25**: 403–410.

Willis, E.R. 1947. The olfactory responses of female mosquitoes. *J. Econ. Entomol.*, **40**: 769–778.

Willis, E.R. and Roth, L.M. 1952. Reactions of *Aedes aegypti* (L.) to carbon dioxide. *J. Exp. Zool.*, **121**: 149–179.

Wolfe, L.S. and Peterson, D.G. 1960. Diurnal behavior and biting habits of blackflies (Diptera: Simuliidae) in the forests of Québec. *Can. J. Zool.*, **38**: 489–497. <#6478>

Wood, D.M. 1963. Two new species of Ontario blackflies (Diptera: Simuliidae). *Proc. Entomol. Soc. Ont.*, **93**(1962): 94–98. <#3778>

Wood, D.M., Peterson, B.V., Davies, D.M. and Györkös, H. 1963. The blackflies (Diptera: Simuliidae) of Ontario. Part II. Larval identification, with descriptions and illustrations. *Proc. Entomol. Soc. Ont.*, **93**: 99–129. <#1252>

Wright, R.H. 1958. The olfactory guidance of flying insects. *Can. Entomol.*, **90**: 81–89.
Wright, R.H. 1964. *The science of smell*. George Allen & Unwin Ltd., London. pp. xii + 1–164.

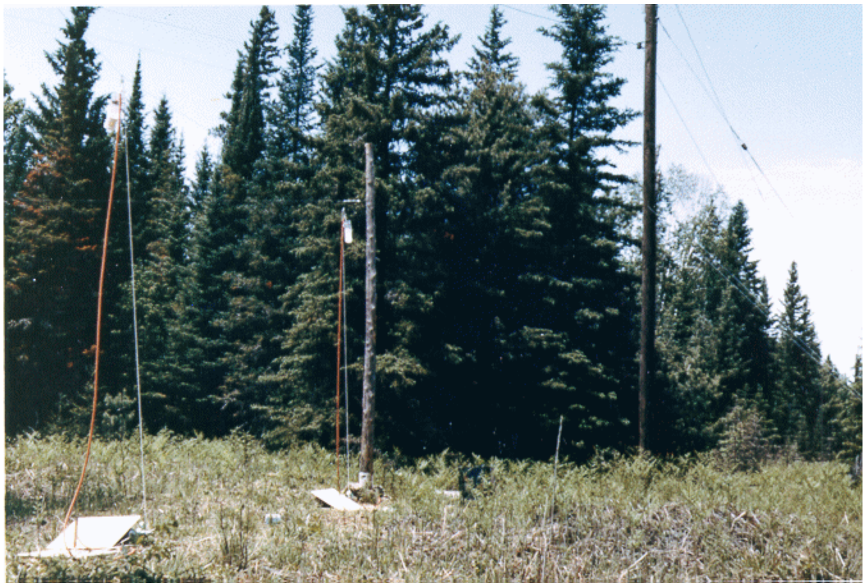

Fig. 1a. A view of the forest clearing with 2 miniature suction traps in operation.
<In the original thesis, this was Fig. 1>

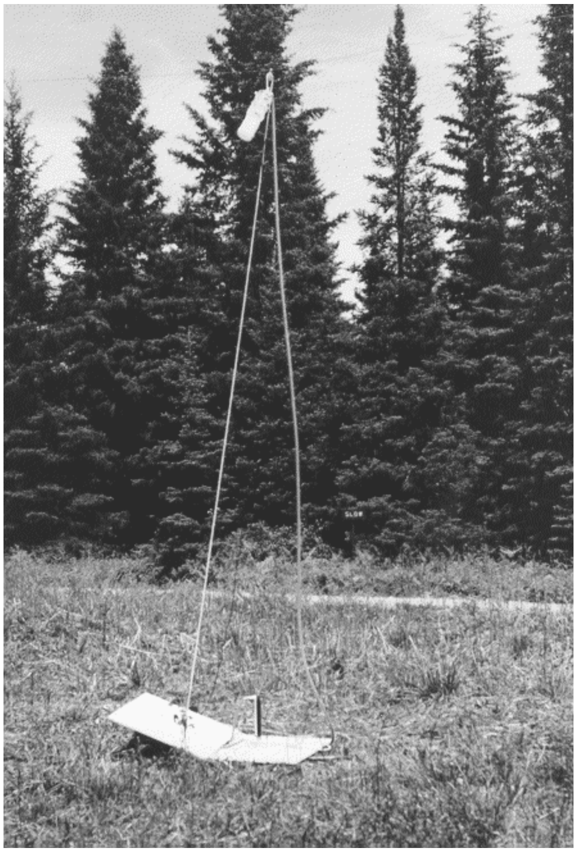

Fig. 1b. A view of the forest clearing with 1 miniature suction trap in operation. <Not included in the original thesis.>

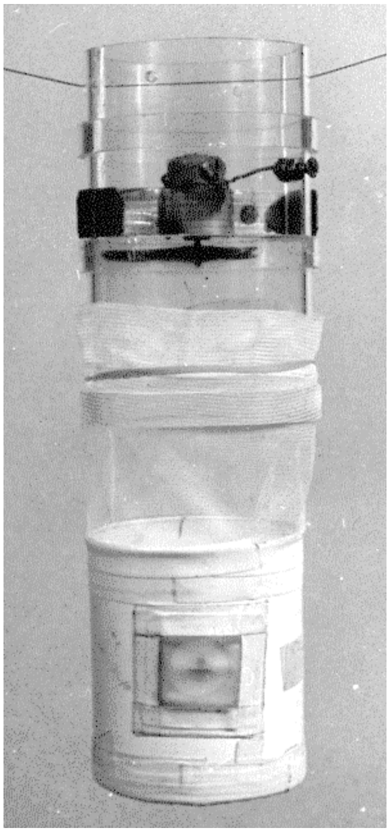

Fig. 2a. Close-up side-view of the miniature suction trap, showing attached collecting apparatus.

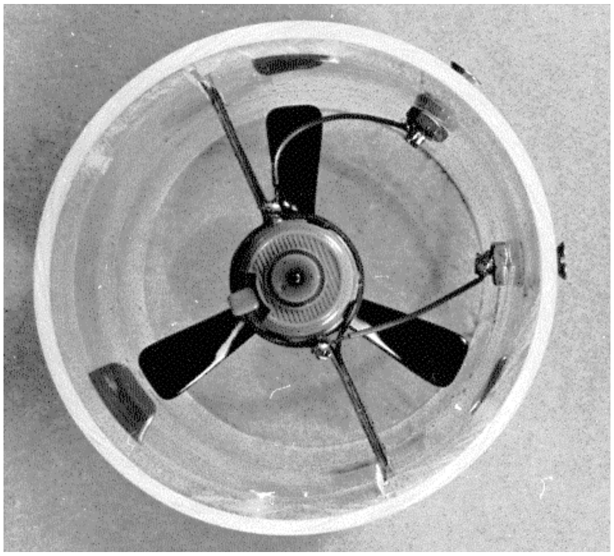

Fig. 2b. Top-view of the miniature suction trap.