STUDIES ON THE ECOLOGY AND BEHAVIOUR OF CERTAIN BITING DIPTERA.

A thesis presented for the degree of Doctor of Philosophy at the University of Glasgow.

by

Alec H. Parker

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CONTENTS

FOREWORD.

The investigations described in the first part of this thesis were carried out by the author while he was a member of a team investigating the biology of blood-sucking midges (Culicoides Latreille - Diptera: CERATOPOGONIDAE = HELEIDAE) in Scotland. Midges, at times a source of annoyance in almost all parts of Britain, are, as is well known, particularly troublesome in Scotland, especially in the Highlands. Here, during the summer months, they are a severe source of irritation to both local inhabitants and tourists. Domestic animals are also attacked and may be adversely affected; cattle subjected to heavy attack, for example, are said to suffer a reduced milk yield.

In consequence of its great importance, two research assistants were, in 1946, appointed in the University of Glasgow to investigate the biology of Culicoides in Scotland. The author held one of these appointments until 1948, when he left this field of investigation. New facts have since come to light, and such of these as are relevant will be commented on in the discussions following the sections in which the author's own results are presented.

In Part IA are presented the results of an investigation into the habits of adult Culicoides in the field, the investigation being carried out mainly at the University of Glasgow Insect Field Station, situated at Rossdhu, Loch Lomond. The main object was to study variations in incidence occurring at different times of day and year. Subsidiary investigations were the effect of weather conditions on adult activity, and the relative readiness of different species to attack man.

Part IB is a description of laboratory investigations primarily aimed at obtaining the eggs of as many species of Culicoides as possible, observing them under normal conditions, and, assessing their resistance to adverse conditions of drought and heat. Data on the maintenance of adults in the laboratory for egg-laying purposes are also presented.

Part II is an account of an investigation undertaken while the author was Research Entomologist at the West African Institute for Trypanosomiasis Research, a post which he assumed in 1950. The principal vectors of trypanosomiasis in Africa are, of course, tsetse flies (Glossina Wiedemann - Diptera: MUSCIDAE). Although numerous field observations on the breeding sites of these insects had been recorded, practically nothing was known of the stimuli to which the pregnant female responded in choosing these sites. The investigation described was principally an attempt to determine the nature of these stimuli by laboratory experiments, supplemented, where necessary, by field observations. The species investigated was G. palpalis Robineau-Desvoidy, an important carrier of human sleeping sickness; this species was the one most readily available in the neighbourhood of Kaduna, Northern Nigeria, where the laboratory was situated.

Except where otherwise indicated, all observations and experiments described were the original work of the author.

PART I.

INVESTIGATIONS INTO THE BIOLOGY OF BITING MIDGES (*CULICOIDES* LATREILLE) IN SCOTLAND.

PART IA.

FIELD STUDIES ON ADULT CULICOIDES.

INTRODUCTION.

Britain is by no means unique in suffering from the attacks of *Culicoides*. Vargas (1949) has recently listed all described species, which he finds total 360. The ubiquity of the genus is shown by the fact that, of these, 136 species are Palaearctic (30 British), 30 Nearctic, 68 Neotropical, 60 Ethiopian, 48 Oriental, and 17 Australian. In addition to being a source of irritation and annoyance, they may, like most blood-sucking insects, transmit parasitic organisms. *Filaria ozzardi* Manson, a common human parasite in the West Indies and Central America, and *Filaria perstans* Manson, which has a very high human incidence in the Cameroons and Congo Basin, have both been shown to be transmitted by *Culicoides* (Buckley 1934; Sharp 1928). Members of the genus have likewise been found to transmit blue-tongue of sheep in South Africa (du Toit, 1944) fowl pox in Formosa (Tokunaga, 1937), worm nodules of cattle in Malaya (Buckley, 1938), and nuchal disease of horses in Britain (Steward, 1933). The latter appears to be the only record of Culicoides in the role of a parasite-vector in Britain.

Seasonal Incidence.

It is a generally known fact that in Britain midges, in common with many insect pests, are troublesome only during the warmer months of the year, and this is true of temperate climates in general. In the tropics, Carter, Ingram, and Macfie (1920) in the Gold Coast, and Patel (1921) in India found Culicoides adults to be abundant at all times of year, individual species occurring either all the year round or seasonally. Dove, Hall, and Hull (1932) describe how Culicoides adults occur all the year round in Florida, but are absent in winter in more northerly parts of the United States. In the Caroline Islands Tokunaga and Esaki (1936) have noted short term fluctuations related to the phase of the moon; adults of C. peliouensis Tokunaga appear several days before a new or full moon, and remain numerous for about a week. Dorsey (1947) has since shown that this is because breeding occurs in tidal swamps, and such areas are most favourable when not exposed to direct tidal action. Breeding is therefore maximal when the rise of the tide is minimal, an event linked with the phase of the moon.

To assess seasonal variations, two investigators have made standardised collections at fixed sites throughout the adult Culicoides season. James (1943) operated a light trap every evening at a site in Northern Colorado. This attracted and trapped the midges without requiring the presence of an observer. Hill (1947), conversely, collected the midges alighting on a piece of black cloth; apparently such an object is also attractive, though only to females. Her collections were made between two and one hours before sunset, at intervals varying from 1 to 18 days. The site was in Knowsley Park, Lancashire.

Species occurring in the writer's collections will now be dealt with individually.

C. impunctatus Goetghebuer

This was the most abundant species in Hill's Lancashire collections, and was obtained from early April to early August, with a peak in mid-June. In Scotland, Cameron, Downes, Morison, and Peacock (1946), who collected over many parts of the country, mainly by means of a hand-net, found that C. impunctatus started to appear in late May, occurred in large numbers through June, July, and August, and tailed off in mid-September. As in Hill's collections, it was the most abundant species, often outnumbering all other species together; the majority of the larger collections came from the Highlands where, from the point of view of annoyance to man, it is clearly the most important species. Almost all of the midges captured were females, male C. impunctatus comprising only 1-4% of the total.

C. heliophilus Edwards

This species did not occur in Hill's collections, and Cameron et al. (loc cit.) state only that it is widespread but apparently uncommon. The latter authors suspect it might have appeared more abundant had more collections been made earlier in the year.

C. pallidicornis Kieffer

Hill obtained this species in very small numbers, exclusively between mid-June and mid-July. She found pupae in a nearby plot from early June to mid-August, however, indicating that emergence was taking place over a considerably longer period than the adult collections would suggest; the pupal stage apparently lasted only a few days, so times of pupal incidence may be taken as almost synonymous with those of emergence.

Cameron et al. give no data on variations in the incidence of the species, merely recording it to be fairly common and widely distributed.

C. pulicaris Linnaeus

This species, obtained by Hill in only one collection, made in late September, is described by Cameron *et al*. as widespread but not very common, and yielding a nearly normal 1:1 sex-ratio. Goetghebuer (1919), who investigated the biology of the species in Belgium, found it to be common and widespread in damp and marshy places throughout that country. Adults were obtained from the beginning of spring until autumn, being most abundant from May to August, and Goetghebuer claims that several generations occur per annum.

C. pulicaris, as described in his review of British species of *Culicoides* by Edwards (1939), includes two varieties, *C. pulicaris* Linnaeus *sensu stricto* and *C. pulicaris* var. *punctatus* Meigen, and the above observations may refer to either of these. The two varieties, however, can almost invariably be distinguished on the basis of wing-markings alone, and Downes and Kettle (1952) have recently proposed that they should be elevated to specific rank, the new species thus being *C. pulicaris* Linnaeus, and *C. punctatus* Meigen. This nomenclature will be adopted here.

C. obsoletus Meigen and related forms

This is a group comprising four species, *viz*. *C. obsoletus* Meigen, *C. chiopterus* Meigen, and two species recently named by Downes and Kettle (*loc cit*.)

C. pseudochiopterus and *C. scoticus*. These four species, though readily identifiable by examination of the genitalia of the male, are seldom distinguishable by characters observable in the female. Females of any one of the species will therefore be spoken of as belonging to the *C. obsoletus* group, or as '*C. obsoletus*', and differentiated into species only when males are under consideration.

'*C. obsoletus*' females were almost as abundant as *C. impunctatus* in Hill's collections. The first specimens were obtained in early April, but numbers remained small until May, when they increased rapidly, reaching a peak in mid-June. Numbers then diminished until mid-August, after which they rose again, reaching a second peak in mid-September. The last specimens were captured in late October.

From the double peak in abundance, and other evidence based on the emergence of adults in captivity, Hill considers that '*C. obsoletus*' passes through two complete generations per annum. This had been suspected earlier, for Edwards (1939) states that "though most abundant in June, *C. obsoletus* may be found throughout the Summer, and in a damp Autumn is sometimes very numerous again in September and October, a fact which suggests that it has normally at least two broods in the year."

Cameron *et al*. state of '*C. obsoletus*' in Scotland that it is the second commonest species,"making up 8% of the Glasgow collections and a somewhat larger proportion of those from Edinburgh. It is widely distributed but definitely not a Highland species, and occurs most frequently south of the Forth and Clyde. ...... It was found chiefly in the latter half of the Summer, and extends well into October and is thus later than other species in seasonal occurrence". As with C. impunctatus, only a very small proportion of males - about 1% - were obtained.

'C. obsoletus' also occurs in the United States, and was obtained by James (1943) in his systematic collections in Northern Colorado. This author obtained it from mid-May until late September, the greatest number being captured at the beginning of the latter month; there was no other well defined peak. Thus the seasonal distribution of 'C. obsoletus' as represented in these collections seems to have been similar to that recorded in Britain.

C. fascipennis Staeger

The only relevant information available on this species appears to be the statement of Cameron et al. that it was "fairly common and widely distributed, especially in the Highlands," and the fact that the specimens in the British Museum were mostly captured in July (Edwards, loc cit.).

C. grisescens Edwards

Of this species, Cameron et al. state that though previously regarded as rare, it is fairly common and widespread in the Highlands, to which, however, it is confined. Where it occurred, it usually formed about 3% of the total collection, in places over 50%. It was obtained "slightly later in the season than C. impunctatus, being at its peak in the latter half of August". The sex-ratio was almost normal, about 40% of those captured being males.

C. odibilis Austen

This species is described by Cameron et al. as very local and rare. Hill, while not obtaining it in her adult collections, found pupae in considerable numbers in a nearby breeding site; the emergence period of these pupae was from late June to mid-August, with a peak in late July.

C. cunctans Winnertz

Cameron et al. describe this species as very local and rare. No other relevant data appear to be available.

Daily incidence

Most of the literature on this subject is based on casual observation, and the only investigator to make standardised collections at different times of day with the object of determining variations in activity appears to have been Hill (loc cit.). This author, as in her seasonal collections, captured the midges - exclusively female - alighting on a black cloth.

Of C. impunctatus, to take species individually, Edwards (1939) states that it is especially troublesome during the late afternoon and evening; "it will bite all through the night in sultry weather, but is not much in evidence in the middle of the day, except in sheltered and shady places". A. E. Cameron (1947) records that, in the Trossachs area, C. impunctatus was a nuisance at any time of day when the weather was warm, humid and overcast, and that it continued to be troublesome throughout the night. Goetghebuer (1935) also reports that this species will bite by night as well as by day.

Hill found the numbers of C. impunctatus females attracted to her black cloth over half-hourly periods to be very small or nil up to three hours before sunset. Numbers then increased steeply, reaching a peak between one hour and half an hour before sunset, after which they dropped sharply. As Hill points out, this drop may have resulted from the attractiveness of the black cloth having diminished as daylight waned.

Hill also made one collection in the early morning, the period covered being from sunrise until four hours later. Numbers were smaller, but the pattern of their rise and decline was roughly the inverse of that observed at sunset. Thus less were obtained immediately after sunrise than in the next half-hour, and practically none were captured later than 3½ hours after sunrise.

Of '*C. obsoletus*', Kearns (1942) has stated that adults may attack at any time of day, but most actively in the late afternoon and evening. Hill's results with this species group were essentially similar to those she obtained with *C. impunctatus* but less consistent: very few or none were obtained up to four hours before sunset, after which numbers increased eratically up to the time of maximum yield, which was as before, between one hour and half an hour before sunset. Although made at a time of year at which large numbers of '*C. obsoletus*' were being obtained in the evening collections, Hill's early morning collection failed to yield any representatives of this species group.

'*C. pulicaris*' - that is, *C. pulicaris* or *C. punctatus* - is described by Edwards (1939) as being most active in the afternoon or evening, and Goetghebuer (1919) records that on warm evenings the males may be seen dancing in swarms.

The evidence cited, though inadequate, indicates that the species so far discussed, while they may be obtained at other times of day or night, tend to be most active in the late afternoon and evening, with perhaps another activity peak in the early morning. Similar activity patterns have been recorded for several non-European species. Hinman (1936), found C. mississippiensis Hoffmann in Louisiana to be most active in the early evening; Dorsey (1947), found the heaviest feeding time of C. pelelioiuensis Tokunaga in the Caroline Islands to be late afternoon to midnight, and dawn to mid-morning; Edwards (1922) records the Jamaican species C. loughnani Edwards as biting most actively at sunset; Myers (1935) found that C. furens Poey in the Bahamas bit chiefly in the evening and early morning.

The tendency to be most active in the evening and early morning, however, is evidently not common to all members of the genus. Edwards (1921) observed that C. heliophilus was most active in the middle of the day, biting readily in sunlight, and disappeared before dusk, a habit implicit in the name he gave the species. Similar habits have been claimed for C. nubeculosus Meigen, also a British species, of which Steward (1933) claims that it fed on horses in greatest numbers between 10.30 a.m. and 1.00 p.m., though it might be taken in smaller numbers at any time of day. Salm (1914) observed similar behaviour in C. pungens in the

Sunda Islands, which he says usually attacked man by day, preferably in the sun, and Nieva and Penna (1916) state of C. guttatus Coquillet in Brazil that it is most abundant in the morning, biting in full sunlight, and less so in the evening.

An activity pattern which does not appear to have been recorded for any other species of Culicoides is that claimed for C. austeni Carter by Sharp (1928). Sharp maintains that this species will bite only in darkness, and that none attack on nights when the moon is full.

Influence of Weather Conditions.

Suppositions regarding the effect of weather conditions on activity are implicit in several of the statements cited in the previous section. Also apposite is Steward's (loc cit.) claim that for C. nubeculosus females "warm and humid days are most favourable, and they bite with particular fierceness if rain is threatening". Hill (loc cit.) states that "drizzling rain does not deter the activities of C. impunctatus, although the slightest breeze does so", and observes that a slight breeze has the same effect on 'C. obsoletus'. E. Cameron (1946), speaking of a mixed midge population of which the chief constituent was undoubtedly *C. impunctatus*, says "they bite very severely during slight drizzling rain. Wind disperses them, but if one is working close to the ground ...., they may bite even in a wind." Goetghebuer (1919) states of '*C. pulicaris*' in Belgium that in cold and rainy weather the adults hide in cracks in the bark of trees.

Of non-European species, *C. furens* was observed by Bequaert (1924), working in Honduras, to be most troublesome in calm periods, the lightest breeze driving the adults into hiding in dense vegetation; the draught from an electric fan prevented them from flying inside a room. Painter (1926), also working in Honduras, confirms the pronounced effect of wind on *C. furens*, and goes on to claim that a north-easterly breeze is more favourable than a westerly breeze; he does not, however, describe any differences in the conditions attending winds from these two directions which might suggest the cause of this. Speaking of the same species in the Bahamas, Myers (1935) states that "for full activity *C. furens* needs about 80°F. A much more important inhibitory factor than either strong light or low temperature is wind. During windy weather sandflies " - that is, *Culicoides* - " remain hidden away and do not emerge to bite". Dorsey (1947) claims that the flight of *C. peleliouensis* in the Caroline Islands is discouraged by strong breeze and rain.

The available evidence thus points consistently to the strong inhibitory effect of wind on activity, and suggests that low temperature and perhaps low humidity may have a similar, though less pronounced inhibitory effect. Observations on the effect of rain are contradictory, possibly because of variation in its strength at the time of observation, or because of the simultaneous effect of other weather conditions. None of the statements quoted appear to have been based on carefully standardised observations, and in no case are any figures presented.

Attraction to Man.

Cameron *et al.* (*loc cit.*) point out that their collections indicate that *C. impunctatus* is not only the commonest species in Scotland, but that midge for midge, it bites man more frequently than do the other species obtained at the same time. Thus, in collections made in the Glasgow area, *C. impunctatus* comprised 76% of the total number of midges captured by hand-net, but 90% of those landing on the collectors. In the Dundee collections the corresponding percentages were 45% and nearly 100%, and in the Edinburgh collections approximately 50% and 75%. It must be remembered that the two sets of figures were obtained at different times and often in different localities, and that too much significance must not, therefore, be read into the differences between them. Confirmatory evidence is provided by Hill (*loc cit.*), who found *C. impunctatus* to comprise approximately 60% of her totalled black-cloth collections, the remaining 40% consisting almost entirely of '*C. obsoletus*'; approximately the same proportions were obtained in occasional collections made by sweeping the vegetation with a hand-net. Collections of individuals alighting on the collector, made at similar times and under similar conditions, however, yielded an average of over 90% *C. impunctatus*, indicating that females of this species attack man more readily than do females of the '*C. obsoletus*' group as represented at Knowsley.

Speaking of '*C. obsoletus*' in Scotland, Cameron *et al.* conclude that it is probably second in importance to *C. impunctatus* as a biter of man, but this does not necessarily imply that '*C. obsoletus*' females bite more readily than some of the less common species. Both *C. heliophilus* and the *C. pulicaris* group are described the same authors as of minor but definite importance as man-biters, while *C. pallidicornis* and *C. fascipennis* are described as occasional biters. Apart from single biting records of *C. halophilus* Kieffer and *C. odibilis*, no other Scottish species appear to have been observed to attack man.

It will be apparent that the relative attraction of different species of Culicoides to man was a subject that had not received precise study.

MATERIAL AND METHODS.

To study variations in the adult midge population occurring during the course of the day and year, it was clearly desirable to make standard collections at some fixed site throughout the adult season. Two sites were chosen, both in the vicinity of the University of Glasgow Insect Field Station. This is situated on the west bank of Loch Lomond approximately four miles from its southern tip (National Grid reference: 26/355869). It stands on the edge of a shingle beach onto which borders mixed deciduous woodland. This woodland extends along the shore for more than a mile northwards, but gives place to open farmland only about 100 yards southwards. Inland, the woodland extends approximately ¼ mile before giving place to open fields.

In making standard collections it seemed desirable to use some method which did not depend on an active response by the insect. Hill's (1947) method of utilising the attraction exerted by a black cloth, and James' (1943) light trap method both seemed unsatisfactory in this respect; the black cloth attracted only females and might similarly have selected certain species at the expense of others, and the light trap, though it evidently attracted males as well as females, may also have acted selectively. Furthermore, the efficiency of both methods almost certainly varied with the time of day; it seems reasonable to expect the attractiveness of a black cloth to decrease, and that of a light trap to increase as daylight wanes. Thus neither method can be used with any confidence to assess variations in incidence relative to time of day.

The method of collection adopted was the simple one of making a standard number of strokes with a hand-net. It is, of course, appreciated that this, like every method of collection, must have been in some degree selective; the hand-net collected only the adults flying at the height at which the strokes were made, and may have failed to obtain species or sexes conceivably flying at higher or even lower levels. Its merit was that it did not depend on an active visual response by the insect, and could therefore be used with some degree of confidence to compare incidence at different times of day.

Each collection consisted in the making of 400 strokes with a hand-net along a standard beat approximately 100 yards in length. Obviously, small variations must have occurred in the length of stroke, but by getting into a regular rhythm of movement the author was able to standardise his movements sufficiently to make this source of error unlikely to be of importance. It was clearly desirable to collect along a beat rather than in only one spot, for otherwise all the midges originally in the immediate vicinity would be collected in the first few strokes, and those obtained during the remainder of the collection would be adults subsequently attracted to the collector.

There were two beats. Beat A was along the shingle beach bordering the loch from the field station to a point approximately 100 yards north. The beach was almost devoid of living vegetation, though a certain amount of decaying vegetable debris was cast up from the lock. Its width varied from 20 to 50 yards, and inland it was bordered by woodland consisting predominantly of sparsely placed alder and ash in the tree layer, rhodedendron in the shrub layer, and bracken in the herb layer. Beat B commenced approximately 100 yards inland from the field station, and followed a small stream further inland for about another 100 yards. This beat ran through fairly open woodland, consisting chiefly of oak, ash and beech in the tree layer, birdcherry and rhodedendron in the shrub layer, with bracken again dominant in the herb layer for the greater part of the adult midge season.

Each time a collection was made, the 400 strokes with the hand-net were performed, first along beat A, then along beat B. The midges captured were removed from the net with the help of an aspirator after the first set of strokes, and again after the second set, the two collections being kept separate. Each set of strokes, together with the subsequent removal of midges from the net, took 5 to 10 minutes, depending on the numbers obtained. Collections were for the most part made at hourly intervals.

Ideally, of course, all collections intended to indicate the pattern of daily and seasonal variation would have been made under completely uniform weather conditions. In practice this was impossible, but only those made on warm days when there was little or no wind have been considered in making such assessments. These collections, together with others made under less favourable conditions, have also been utilised in an attempt to obtain further information on the effect of weather conditions on activity. To this end, note was made of temperature, humidity, wind strength, rain strength and light conditions at the time of each collection.

To measure the relative degree to which different species were attracted to man, collections were periodically made of midges alighting on the writer, numbers so obtained being compared with those obtained in standard hand-net collections made in the same locality shortly before and afterwards.

Although a light trap was not employed as a regular method of collection, one was occasionally used as a means of sampling the midge population during the hours of darkness. It consisted essentially of an acetylene lamp standing above a tray of liquid paraffin; the latter ensnared a large proportion of the midges attracted to the light. This trap was found to be highly effective when tested at a time in the late evening when larger numbers of *Culicoides* were in flight.

One other method of collection occasionally employed was to pick off the midges to be found resting on tree-trunks; such collections were made in full daylight when few or no midges were obtainable by netting methods. The difficulty of observing insects as small as *Culicoides* when stationary and often in cracks in the bark made it a laborious method of collection, by the use of which only small numbers were obtained.

SEASONAL VARIATION.

Standardised collections of the type described in the preceding section were made at Rossdhu from the beginning of June to late October, 1947. Culicoides adults were first observed in mid-May, but only in very small numbers; by late October, numbers were once again minute.

It will be shown later that, of the weather conditions measured, only variations in wind strength were unquestionably responsible for important variations in the number of adults captured. In the scale adopted none were ever captured when the wind strength was "strong" or "stiff", and numbers captured when it was "slight" were consistently lower than when there was no wind at all. Collections made on days when the wind strength was at any time strong or stiff have therefore been discounted, as have those in which it was slight for a major part of the time.

As will be shown in the next section, the time of day at which maximum numbers were captured varied with the sex and species. Hence to compare the incidence of different sex-species through the season, it was desirable to consider each at the particular time of day at which greatest numbers were obtained. Even for a particular sex of a particular species, however, this time varied on different days. It has therefore been thought advisable

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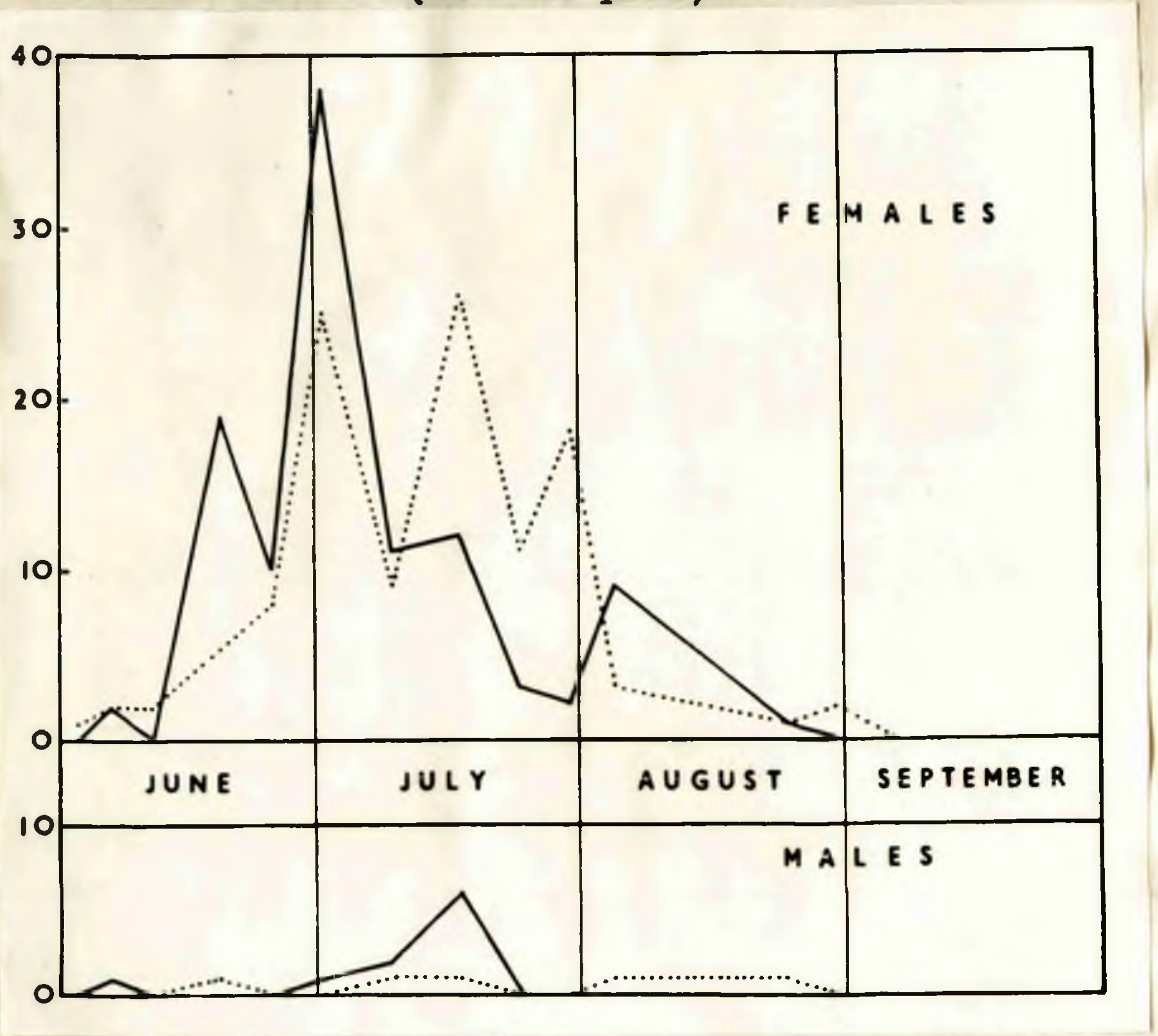

Fig. 1. The seasonal incidence of C. impunctatus. Unbroken lines, beat A; stippled lines, beat B.

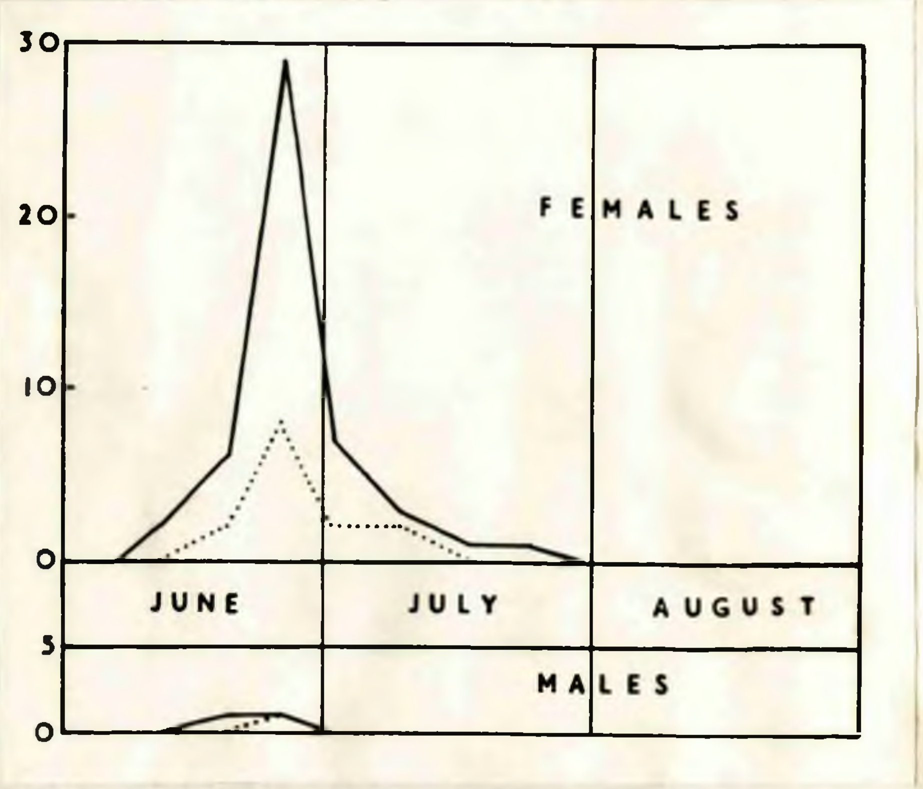

Fig. 2. The seasonal incidence of C. heliophilus. Unbroken and stippled lines as in Fig. 1.

to record, for each sex of each species, the highest number of adults captured in any single collection during the course of any given evening. The resulting figures for the species occurring in moderate or large numbers at Rossdhu are shown graphically in Figs.1-5.

C.impunctatus. (Fig.1)

It will be seen that females of this species were obtained from the beginning of June until the end of August. Along beat A the maximum catch was made in early July; along beat B the greatest number was obtained in mid-July, but the number captured at the beginning of this month was only one less. The general indication is thus that the period of maximal abundance was in the first half of July.

The males captured, in so far as such small numbers are worthy of consideration, also point to a maximum in July.

At Auchterawe, near Fort Augustus, Invernesshire, C.impunctatus adults were observed to be still present at the end of September, indicating that the adult midge season ended, and perhaps started, considerably later in this more northern locality than at Rossdhu.

C.heliophilus. (Fig.2)

This species will be seen to have been obtained from mid-June to late July. Along both beats the female

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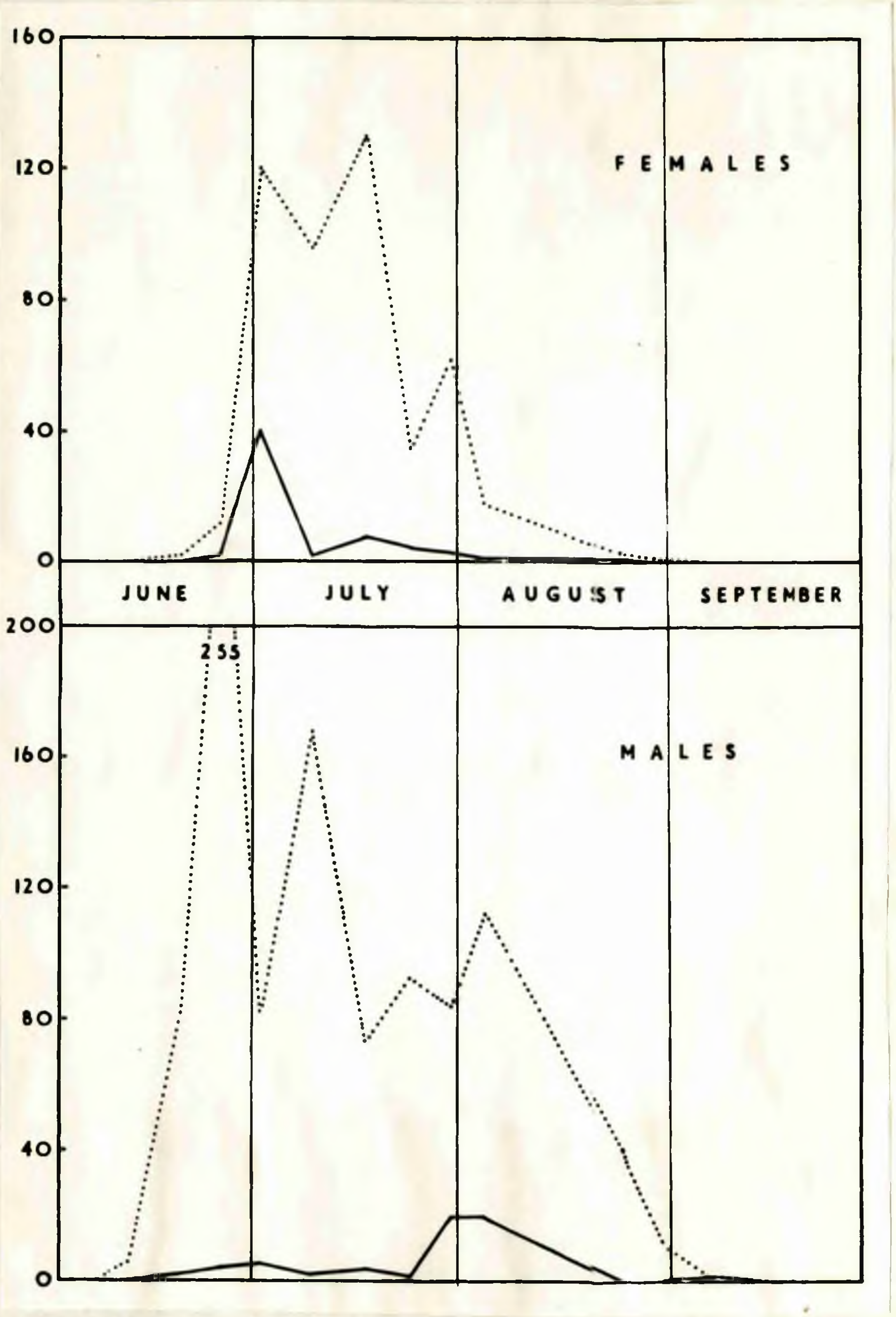

Fig. 3. The seasonal incidence of C. pallidicornis. Unbroken and stippled lines as in Fig. 1.

catch rose to a peak in late June, though the numbers obtained along beat B were perhaps too small to be of any significance. Males, of which more than one was never obtained in any one collection, were obtained only in late June.

C.pallidicornis. (Fig.3)

This species, it should be noted, was obtained in such comparatively large numbers that the scale of the graph has had to be reduced to one quarter that of the previous figures. Females, it will be seen, were obtained from late June to late August, the peak catches being near the beginning and middle of July at beats A and B respectively. Since the size of catches made along the latter beat, where particularly large numbers were obtained, was very high throughout the first half of July, the indication is that this was the period of maximum incidence.

Males were obtained from mid-June to early September. Along beat B, where the higher numbers were obtained, the peak catch was made in late June. Along beat A a very much smaller peak occurred in early August, the number obtained at the end of July being only one less. It is difficult to deduce exactly when the peak period occurred owing to the possibility of error introduced by the habit of male C.pallidicornis of dancing in swarms,

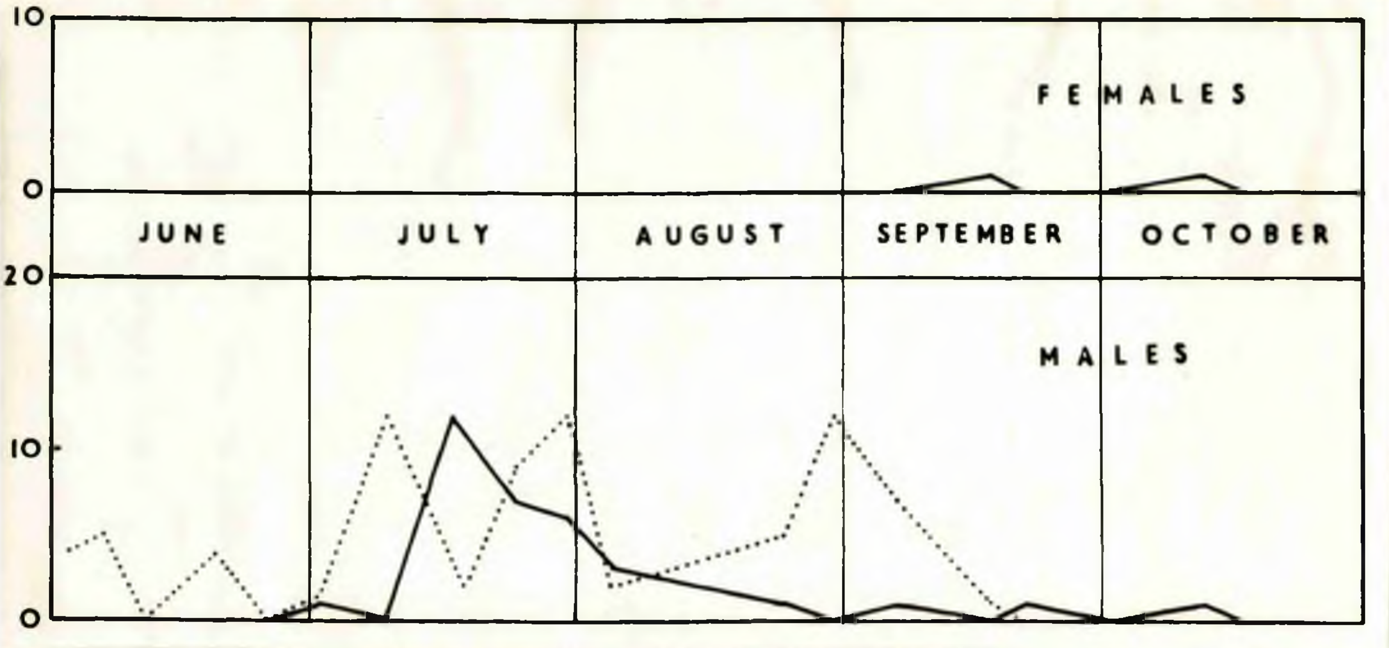

Fig. 4. The seasonal incidence of C.punctatus. Unbroken and stippled lines as in Fig. 1.

a habit which, so far as is known, is not displayed by the females. The size of the male catch must have depended much on how many, and what size of swarms happened to be swept through, and was thus probably more liable to chance variation than that of the supposedly more evenly distributed females. It is therefore not considered justifiable to say more than that the time of maximum incidence of *C.pallidicornis* males almost certainly occurred at some time between late June and early August; in point of fact, there is no reason to doubt the obvious supposition that it occurred at approximately the same time as that of the females.

C.punctatus. (Fig.4)

Males of this species were first observed in mid-May, and continued to be obtained right up to October, although only one specimen was obtained in the latter month. It will be seen that along beat A the peak male catch was obtained in mid-July, while along beat B peaks occurred in early and late July, and again in late August. The disparity in the time of occurrence of these peaks at the two beats, together with their comparatively small size, makes their significance as indicators of periods of maximum abundance dubious; possibly the occurrence of three of the peak catches in July may be taken as indicative

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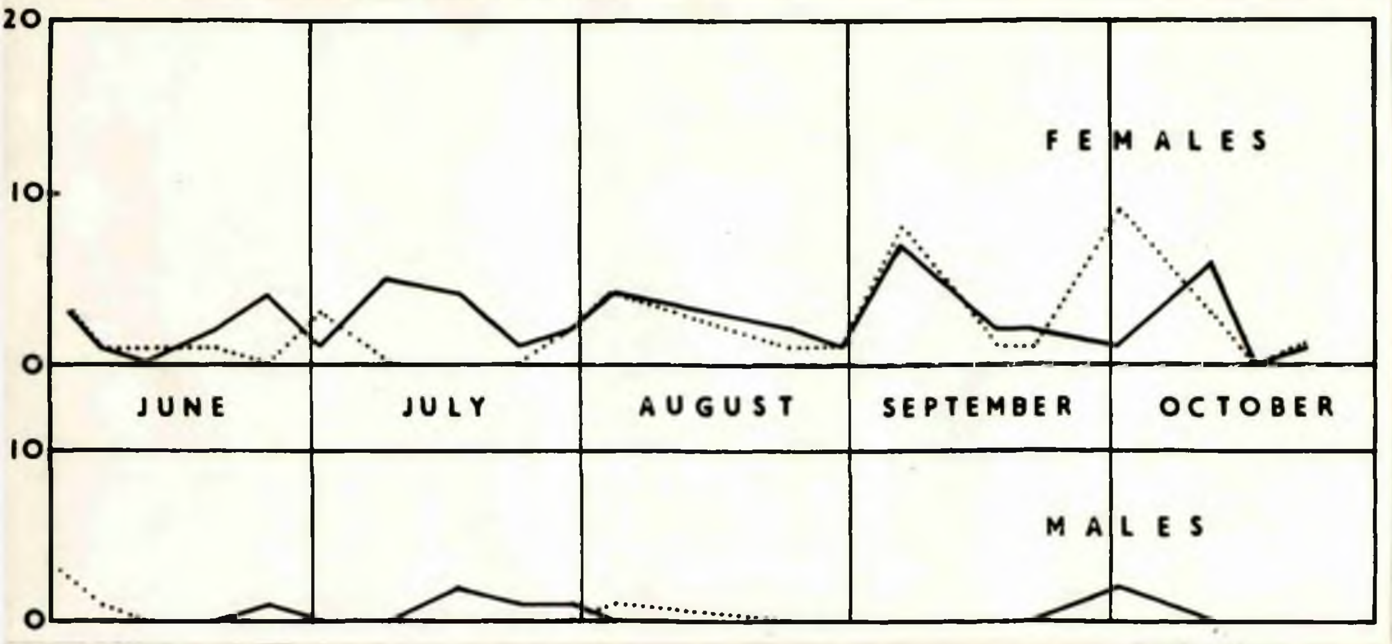

Fig. 5. The seasonal incidence of '<u>C. obsoletus</u>.'
Unbroken and stippled lines as in Fig. 1.

of a larger population at that time.

Females were obtained only in minute numbers, and the fact that they were captured only in September and October cannot be regarded as significant.

C.obsoletus group. (Fig.5)

'C.obsoletus' females were first observed at Rossdhu in mid-May, and as will be seen, were obtained in small numbers throughout the whole collecting period. The variations in incidence do not appear to have followed any consistent trend, except in so far as the highest catches at both sites were made towards the end of the collecting period, and are probably too small to be of significance. Males, it will be noticed, were obtained intermittently over the same period as the females.

Since 'C.obsoletus' comprises four species, the question arises as to whether all or only some of these species were present through the whole collecting period. The answer may be sought in the results of collections of the identifiable males. The hand-net collections yielded very few of these, but they can be supplemented by the collections of adults resting on tree-trunks; such collections were made both at Rossdhu and in the grounds of the University of Glasgow.

The combined results, obtained by either method

TABLE I.

The seasonal incidence of *C.obsoletus* group males.

Date	*C. obsoletus*	*C. chiopterus*	*C. scoticus*	*C.pseudo-chiopterus*
May 27	+	-	+	+
June 1	+	-	+	-
.. 2	+	+	-	-
.. 6	-	+	-	-
July 2	-	-	-	+
.. 17	-	+	-	-
.. 24	-	-	+	+
.. 29	-	-	+	-
.. 30	-	-	-	+
Aug. 4	-	-	+	-
.. 24	-	-	+	-
Sept. 8	+	+	+	-
Oct. 1	-	-	-	+

(To face p.30)

of collection, are shown in Table I, from which it will be seen that all four species were present both early (on or before June 2nd) and late (on or after September 8th) in the season. This strongly indicates that all four species were present throughout the adult season of the group as a whole, and that its prolongation was not due to a succession of the different species within the group.

Other species.

The remaining species were obtained only in very small numbers, the maximum number of adults obtained in any one collection being two. Their times of capture are given as being roughly indicative of the seasonal distribution of the species concerned.

C.pulicaris, like C.punctatus was obtained from mid-May to mid-October.

C.fascipennis was obtained at Rossdhu from the beginning of July to the end of August. At Auchterawe, Invernesshire, this species was observed to be present in considerable numbers at the end of September, but, as has already been pointed out, the whole adult Culicoides season appears to have been later there.

C.grisescens occurred in the Rossdhu collections from the end of July until late August. Like C.fascipennis it was obtained at Auchterawe at the end of September, where it appeared to be the most abundant species at that time.

C.odibilis was obtained at Rossdhu from the beginning of July to the end of August.

C.cunctans was obtained only during the second half of July.

Relative abundance.

It will be apparent from the preceding results that both sexes of C.pallidicornis, at the time of their maximum incidence, were more abundant at Rossdhu than either sex of any other species. The order of decreasing abundance of the remaining sex-species, each considered at the time of its maximum incidence, is as follows: C.impunctatus females, C.heliophilus females, C.punctatus males, C.obsoletus group females. The adult season of some species was, of course, more prolonged than that of others, and if the totals over the whole season are considered the order will not be the same; in this case C.heliophilus becomes last on the list, the order remaining otherwise unchanged.

Certain differences in the abundance of individual species along the two beats are also apparent. The most pronounced of these was that both sexes of C.pallidicornis were consistently more numerous along beat B, a result which may have been due to the proximity of the breeding places of this species to this beat, or to the fact that it preferred flying in sheltered woodland to an open beach. C.heliophilus was consistently more numerous along beat A, but in view of the comparatively small numbers of this species obtained confirmation of this result is desirable before any definite conclusion is drawn; the same applies to the less consistent differences between the numbers of C.punctatus males captured at the two sites. C.impunctatus females and C.obsoletus group males were almost equally distributed.

Sex-ratio.

It will have been evident from the preceding results that the relative proportions of the sexes varied greatly among the species obtained. Female percentages, calculated from the totalled maximal catches (i.e. from the figures plotted in Figs.1-5) were, in fact, as follows:

	Number of specimens	% females
C.impunctatus	235	94%
C.heliophilus	66	95%
C.pallidicornis	1604	33%
C.punctatus	111	3%
'C.obsoletus'	102	88%

It will be seen that, except in the case of C.pallidicornis, there was a great preponderance of either one sex or the other. Two of the species listed, namely C.punctatus and 'C.obsoletus', occurred in moderate numbers in collections made from tree-trunks, and it is interesting to note that in both cases the sex-ratio was almost normal; out of a total of 44 C.punctatus, 43% were females, and out of 82 'C.obsoletus' adults 49% were females. The abnormal sex-ratio obtained when a hand-net was used to capture these species thus appears to have been due to some feature of behaviour which made either one sex or the other largely unobtainable, and not to a real difference in their abundance. The same may be true for C.impunctatus and C.heliophilus.

The smaller difference in the numbers of C.pallidicornis males and females obtained could feasibly have resulted from a comparatively slight difference in the habits of the sexes; possibly the habit of dancing in swarms, observed in, and, so far as is known, confined to the males, affected the availability of this sex relative to that of the females.

It may be noted here that males of one other species, namely *C.grisescens*, have also been observed dancing in swarms. The habit had apparently not previously been recorded either in this species or in *C.pallidicornis*.

DAILY VARIATION.

The standardised collections made at Rossdhu were normally terminated about two hours after sunset, by which time all the species obtained had passed their peak. To obtain information on activity during the later hours of the night, the light trap described earlier was on three occasions placed in the woodland near to beat B two hours after sunset and the catch counted the following morning; though it burned most of the night, the jet expired before sunrise.

Surprisingly few *Culicoides* were obtained: on the nights of June 6th/7th and June 11th/12th the total yield was 5, and on June 25th/26th, 10. As reference to the results given in the previous section will show, the

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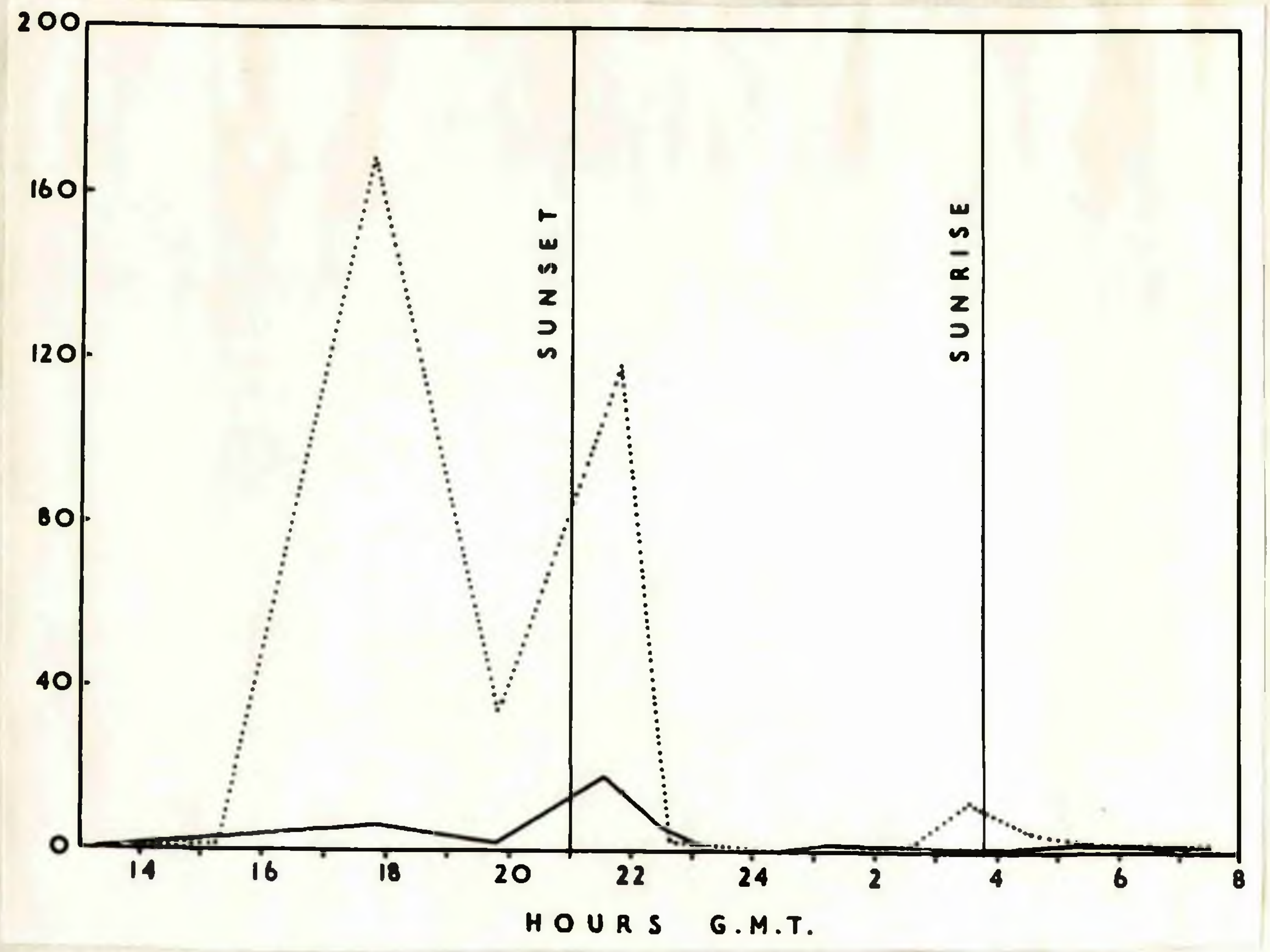

Fig. 6. The incidence of Culicoides adults at Rossdhu, 9th.-10th. July, 1947. Unbroken lines, beat A; stippled lines, beat B.

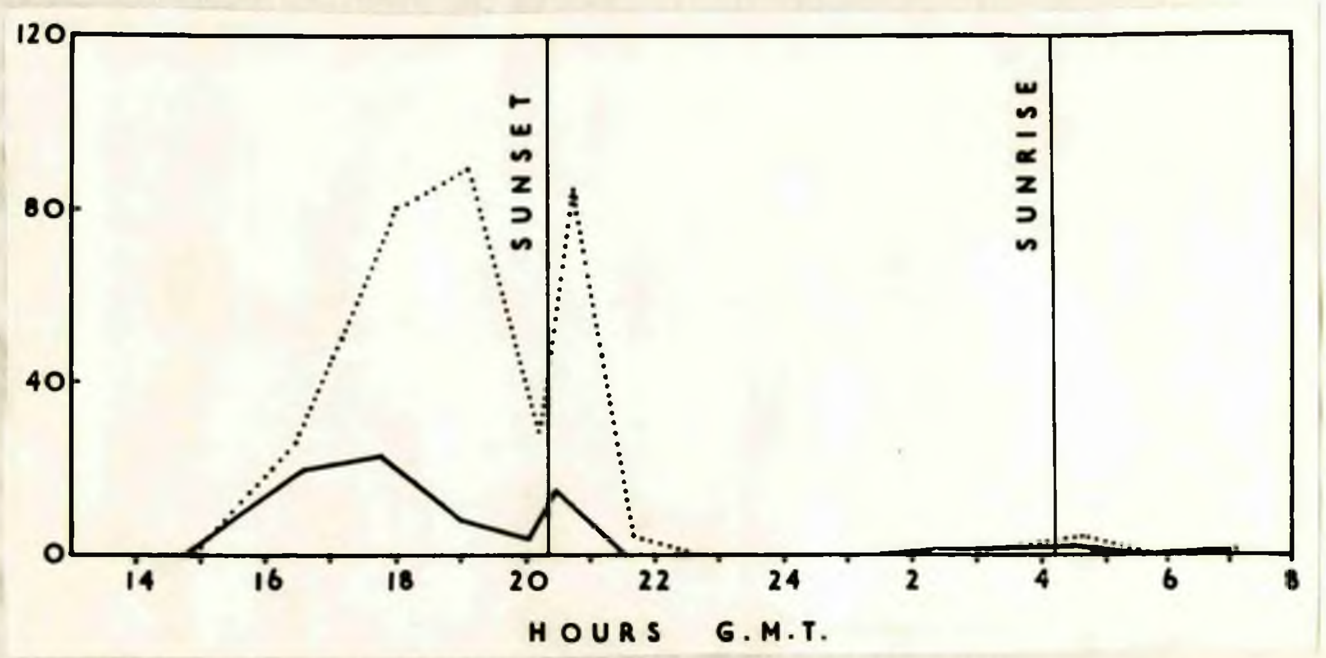

Fig. 7. The incidence of Culicoides adults at Rossdhu, 30th.-31st. July, 1947. Unbroken and stippled lines as in Fig. 6.

latter date was one on which large numbers were obtained earlier in the day, and in all cases the weather was calm and warm. It will be remembered that the trap used had been found highly effective at a time when midges were known to be present in large numbers.

To obtain further information on the nocturnal activity of Culicoides, and also to determine whether, as the literature would indicate, there was an early morning activity peak corresponding with the evening peak, standard hand-net collections were on three occasions made at hourly intervals right through the night, and on up to a few hours after sunrise. The results of two of these collections, together with the results of collections made the previous evenings, are shown in Figs.6 and 7. Those of the third collection have not been figured, as they merely present the same picture with very much smaller numbers.

It will be seen that maximum numbers were always obtained in the late afternoon and evening, there being in each case a double peak, one a few hours before sunset, the other shortly after sunset; the explanation for this will be given later. After the second peak numbers dropped sharply, and practically none were captured during the night. At sunrise there was a small increase, but numbers were very small compared with those obtained the previous evenings. Shortly after sunrise, numbers again diminished almost to vanishing point.

In view of the statements of previous observers, both the minute numbers obtained during the night, and the very small numbers obtained at sunrise, were unexpected, and the possibility that they were due to unfavourable weather conditions cannot be ignored. The following is a summary of the relevant meteorological data obtained between sunset and sunrise on the appropriate nights.

July 9th-10th. Wind: none

Temperature: 51°F at sunset; 49°F at sunrise;
minimum 45°F

Humidity: 82% R.H. (1.7 mm. S.D.) at sunset;
92% R.H. (0.7 mm. S.D.) at sunrise

Rain: none

Moonlight: none

July 30th-31st. Wind: none

Temperature: 60°F at sunset; 56°F at
sunrise; minimum 55°F

Humidity: 85% R.H.(1.9 mm.S.D.) at sunset;
91% R.H.(1.0 mm.S.D.) at sunrise.

Rain: none

Moonlight: from 22.30 to 24.30 hours.

It will be evident that in the first two sets of collections the weather was completely calm all night through. The temperature during the first was fairly low, but the second was made during an exceptionally warm night, when the temperature never dropped below 55°F, a temperature at which, as will be shown in the next section, large numbers were sometimes obtained. The humidity was in both cases at a high level at which, again, large numbers were at other times captured. The presence or absence of moonlight apparently had no effect on numbers obtained. Conditions during the first night collection thus appear to have been mainly favourable, and during the second wholly favourable, to midge activity as indicated by that observed on other occasions. The small numbers obtained in the night and early morning can thus not be attributed to the effect of adverse weather conditions.

The times of maximal incidence of individual species will now be considered, results being shown graphically for each of the more numerous species (Figs.8-12). In each figure is shown the results of the three sets of collections yielding the highest maxima for the sex-species in question.

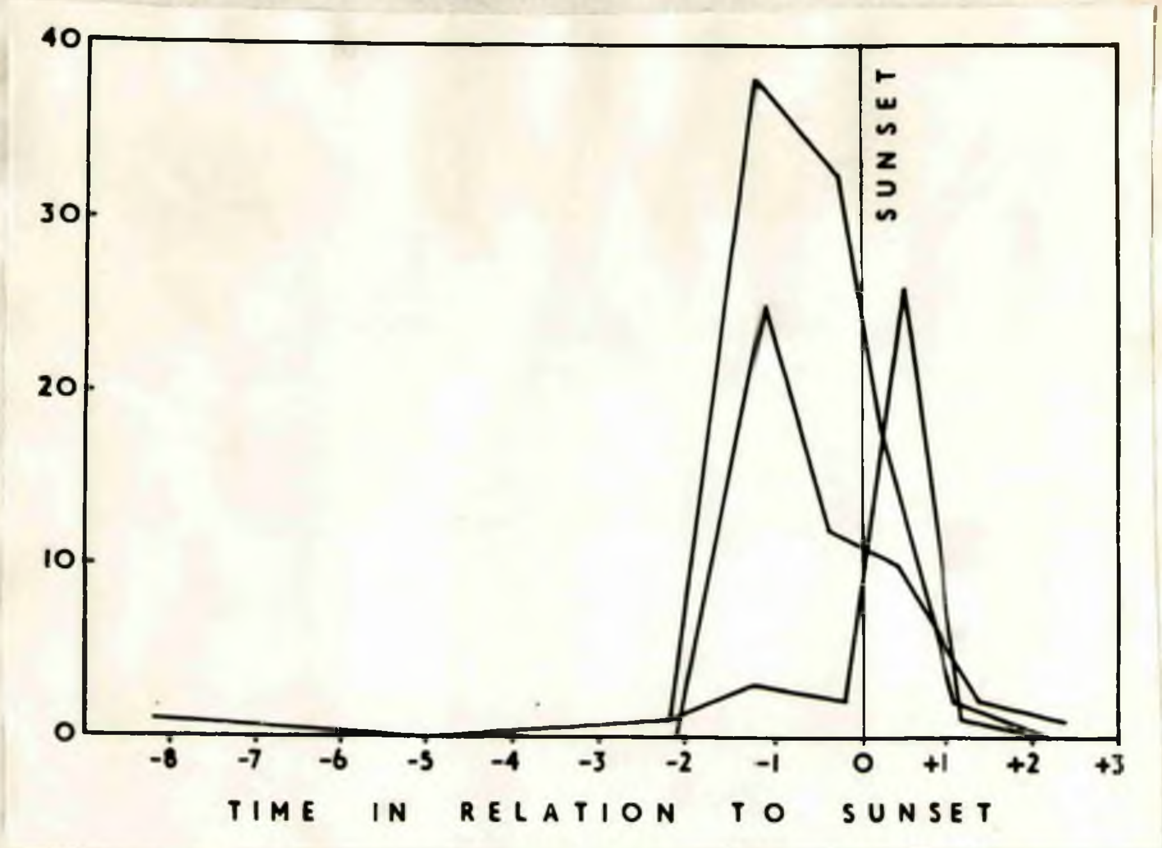

Fig. 8. The incidence of C. impunctatus females at Rossdhu on July 1st. (beats A and B) and July 17th. (beat B only).

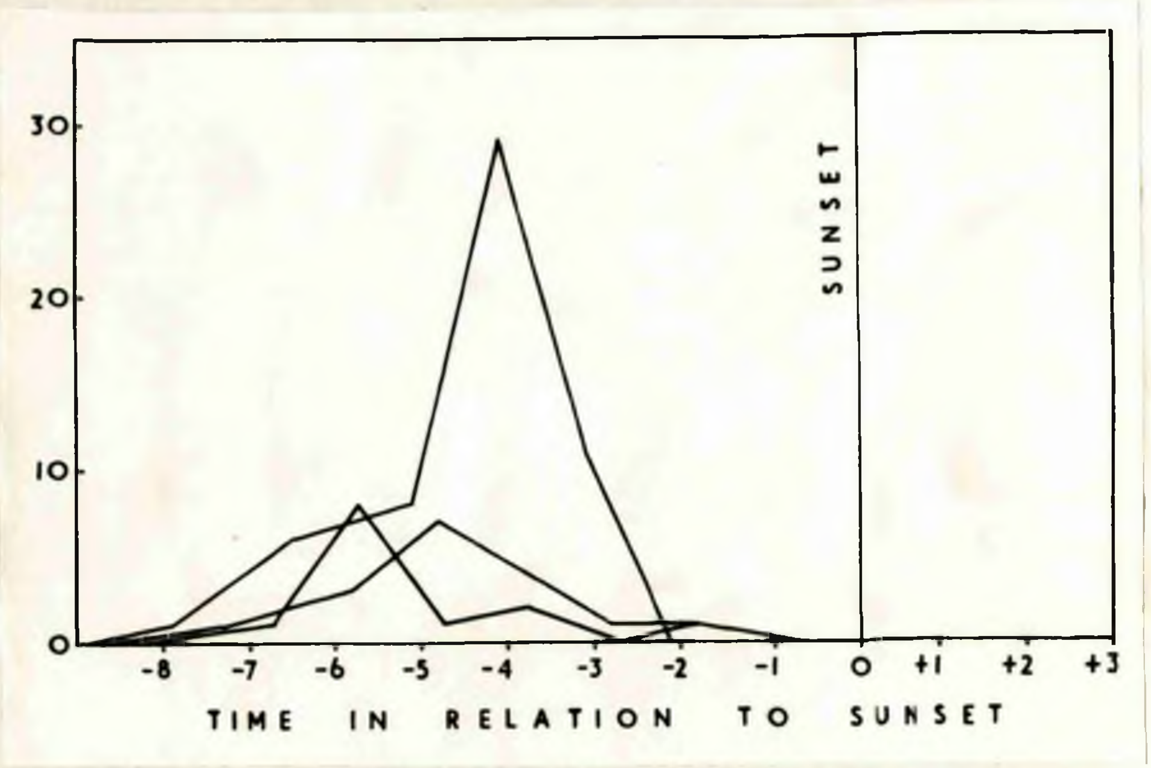

Fig. 9. The incidence of C. heliophilus females at Rossdhu June 25th. (beats A and B) and July 1st. (beat A only).

C. impunctatus.

In Fig.8 variations in the numbers of C. impunctatus females captured at different times of day are shown. It will be seen that in each of the three sets of collections plotted numbers were minute except between two hours before and one hour after sunset. Between these times numbers rose steeply to a peak, the precise time of which varied. Excluding from consideration collections in which only very small numbers were captured, the same activity pattern was consistently obtained: in the 13 collections in which the maximum C. impunctatus female catch comprised at least five individuals, its time of occurrence varied from 1½ hours before (i.e. -1.30 hours) to 1 hour after sunset (+1.00 hours), the mean being 2 minutes after sunset (+0.02 hours).

Males were obtained in such small numbers that their times of capture hardly merit examination. During the afternoon and evening collections they were obtained between -8.30 and +1.30 hours, the collection in which the highest catch of 6 was obtained being made at +1.05 hours. It is interesting to note that of the 13 C. impunctatus obtained either by light-trap or by hand-net during the night hours 9 were males, a sex-ratio very different from that obtained during the evening (see p. 33).

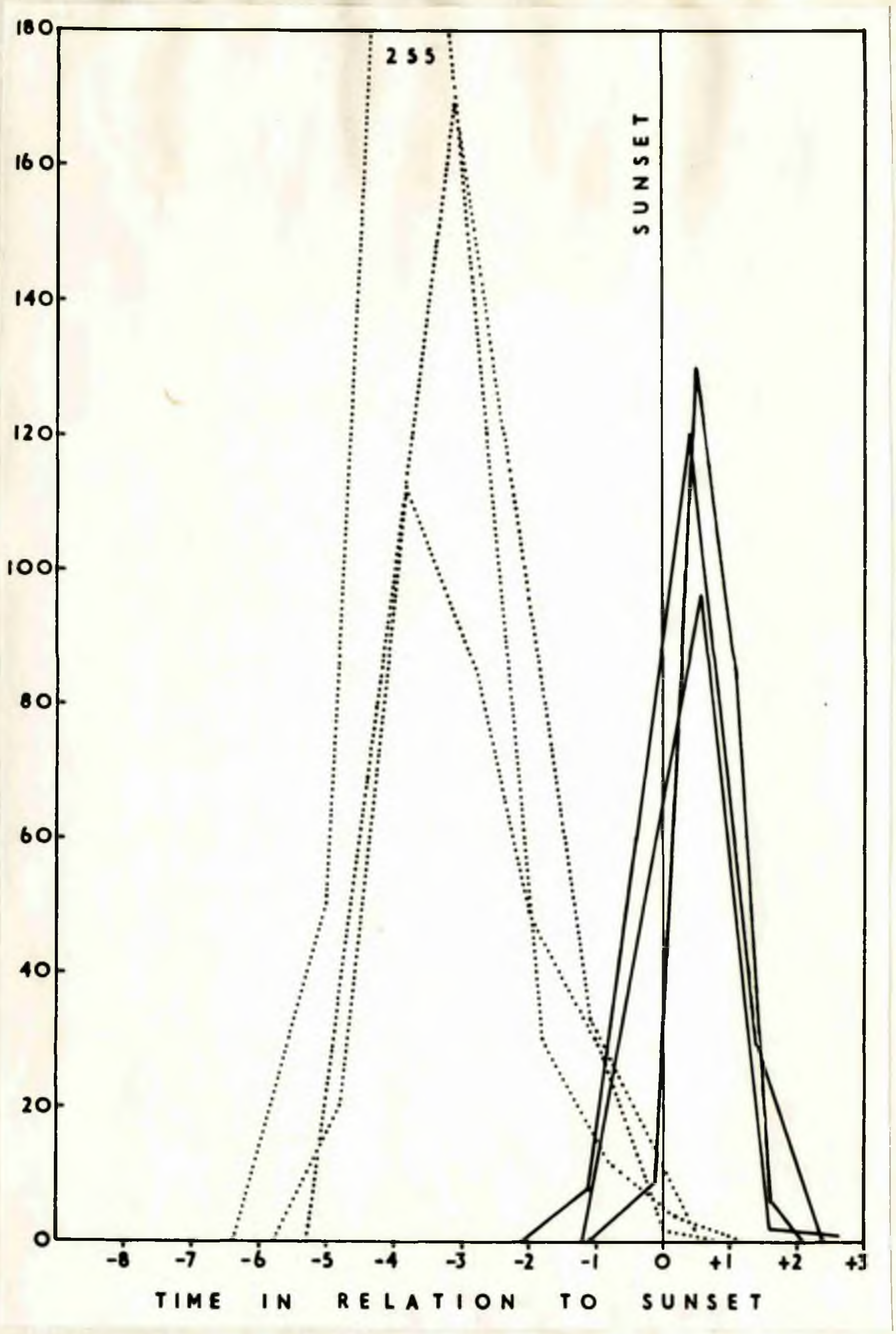

Fig. 10. The incidence of C. pallidicornis at Rossdhu. Stippled lines, males; unbroken lines, females. Males collected on June 25th., July 9th., and Aug. 4th.; females on July 1st., 9th., and 17th. All collections made along beat B.

C.heliophilus.

It will be seen from Fig.9 that his species attained maximum incidence well before sunset, numbers being very small except during the period 7-3 hours before sunset. Only four sets of collections out of the total number made yielded maxima comprising at least five individuals; in these the time at which the maximum occurred varied from -5.40 to -2.00 hours, the mean being -4.10 hours.

Males, of which the maximum number obtained per collection was one, were captured at approximately the same times as the females, namely between -5.40 and -0.40 hours.

C.pallidicornis.

In Fig.10 variations in the daily incidence of both sexes of this species are shown. Males, it will be noticed, reached a maximum several hours before sunset, females shortly after sunset. This striking difference occurred consistently; considering, as before, collections in which the maximum comprised at least five individuals, the maxima of C.pallidicornis males (14 collections) varied from -5.00 to -1.20 hours, with a mean of -2.51 hours, that of the females (9 collections) from 0.00 (i.e. sunset) to +0.40 hours, with a mean of +0.22 hours. There was thus no overlapping between the maxima of the two sexes.

The pronounced double peak noted in Figs.6 and 7

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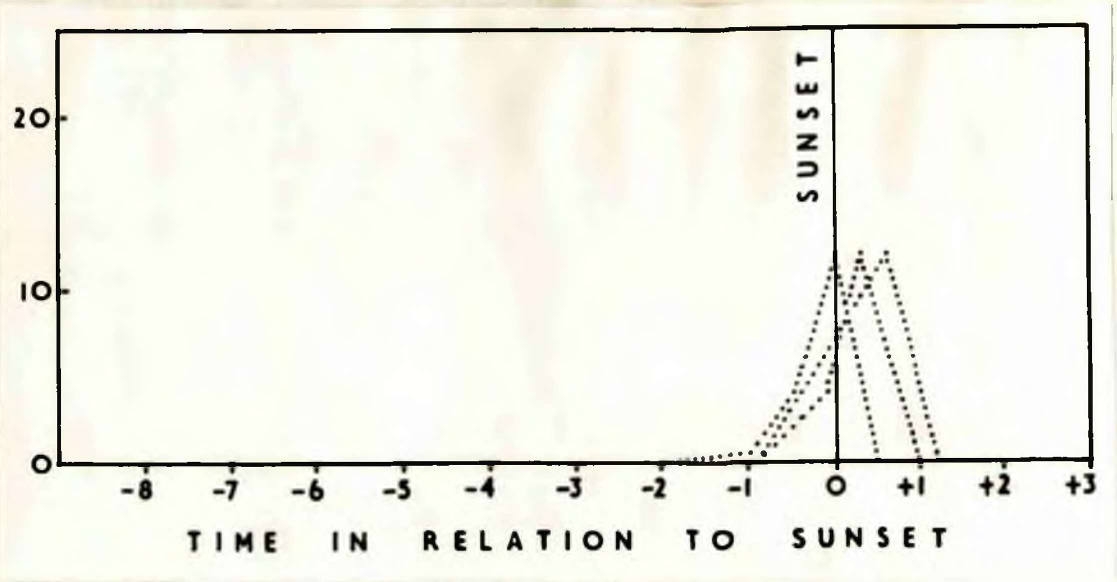

Fig. 11. The incidence of C. punctatus males at Rossdhu on July 9th., July 30th., and Aug. 30th. All collections along beat B.

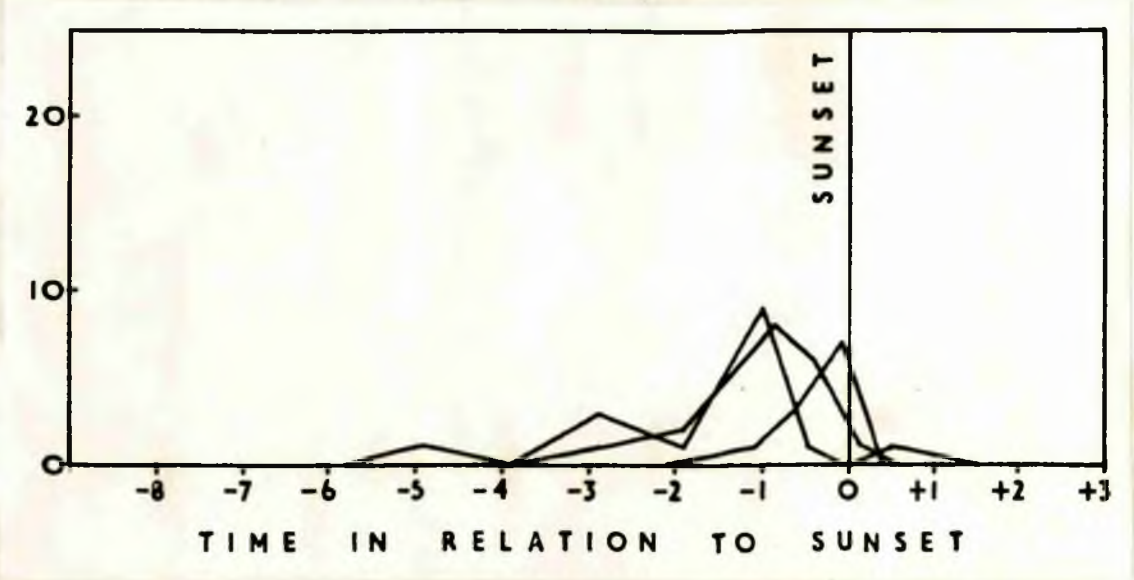

Fig. 12. The incidence of 'C. obsoletus' females at Rossdhu on Sept. 6th. (beats A and B) and Oct. 1st. (beat B).

was due to the preponderance of both sexes of C.pallidicornis in the collections.

C.punctatus.

In the collections plotted in Fig.11 the numbers of C.punctatus males captured will be seen to have risen to small but well-defined peaks between sunset and one hour later. Among the 10 sets of collections yielding maxima of not less than five males, the time of maximum incidence varied from -0.10 to +1.00 hours, the mean being +0.17 hours. Males of this species were also obtained at sunrise, and were largely responsible for the small peak occurring at this time shown in Fig.6.

A total of only three C.punctatus females was obtained, these being captured between -3.00 and 0.00 hours.

C.obsoletus group.

The small maxima attained by females of this group in the collections plotted in Fig.12 will be seen to have occurred between sunset and one hour earlier. Among the six sets of collections yielding maxima of not less than five females, the times of maximum incidence varied from -1.10 to +0.30 hours, with a mean of -0.31 hours.

The few 'C.obsoletus' males captured were obtained at approximately the same times as the females, namely between -2.20 and +0.20 hours.

In view of the very small numbers obtained, the times of capture of the remaining species obtained at Rossdhu - C.pulicaris, C.fascipennis, C.grisescens, C.odibilis, and C.cunctans - will not be considered in detail. In all cases the period over which their times of capture spread included sunset, and no specimens of any were taken earlier than four hours before, or later than one hour after sunset. It has already been mentioned that C.grisescens and C.fascipennis were found to occur in large numbers in another locality, and here females of both species, particularly the former, were much in evidence at dusk, though not at other times of day. The same was true of C.halophilus Kieffer, a maritime species observed flying in large numbers at Arrochar, Dunbartonshire.

In presenting the above results, the assumption has been made that a given activity phase recurs at a time related to that of sunset rather than at a particular time of day independent of this. To test whether this assumption was justified, the total deviation from the mean time of maximum incidence of each sex-species has been calculated (a) from the times expressed in Greenwich Mean Time, and (b) from the times expressed, as before, in relation to the time of sunset.

The resulting figures are shown in Table II, which

TABLE II.

The times of maximum incidence of *Culicoides* adults as measured by Greenwich Mean Time and in relation to sunset.

G.M.T. = Greenwich Mean Time.
S.T. = Time expressed in relation to sunset.

Sex-species	Number of collections	Mean time of maximum incidence		Total deviation from mean (minutes)		P
		G.M.T.	S.T.	G.M.T.	S.T.	
C.impunctatus females	13	20.56	+0.02	467	509	0.2
C.heliophilus females	4	16.55	-4.10	260	260	1.0
C.pallidicornis females	9	21.12	+0.22	189	88	< 0.001
C.pallidicornis males	14	17.44	-2.51	892	828	0.1
C.punctatus males	10	20.35	+0.17	405	180	< 0.001
'*C.obsoletus*' females	5	18.21	-0.31	456	184	< 0.001

(To face p.42)

includes only collections in which the maximum comprised at least five specimens of the sex-species in question. P, the probability, is a measure of the significance of the difference between each pair of total deviations; to estimate it a χ^2 test was employed, as described in the appendix on statistical methods.

It will be seen that the total deviation from the mean time of maximum flight of C. impunctatus females was greater when it was measured in relation to sunset than when it was measured by Greenwich Time. P, however, was only 0.2, or 1 in 5, so this difference cannot be regarded as significant. With the exception of C.heliophilus, in which the two figures were equal, the total deviations of all the remaining sex-species were greater when the time was measured by G.M.T. than when measured in relation to sunset. In the case of C.pallidicornis females, C.punctatus males, and 'C.obsoletus' females P was less than 0.001, so the difference between the deviations may be regarded as highly significant. The difference obtained with C.pallidicornis males was not significant (P = 0.1).

Thus, in the three instances in which significant differences were obtained, it was the total deviation measured in terms of G.M.T. which was the larger. This appears to indicate that the assumption that maximal incidence

TABLE III.

The times of maximum incidence of *Culicoides* adults under dull and bright weather conditions.

t_m = time of maximum incidence.

	C.impunctatus females		*C.pallidicornis* males		*C.pallidicornis* females		*C.punctatus* males	
	Dull	Bright	Dull	Bright	Dull	Bright	Dull	Bright
Number of collections	3	10	5	9	3	6	4	6
Earliest t_m	-1.30	0.00	-5.00	-3.40	0.00	0.00	-1.10	0.00
Latest t_m	-1.10	+1.00	-1.30	-1.20	+0.20	+0.40	0.00	+1.00
Mean t_m	-1.20	+0.25	-3.25	-2.40	+0.10	+0.20	0.00	+0.30
P	<0.001		0.5		0.1		0.03	

(To face p.43)

occurred at a time more or less fixed in relation to sunset rather than at a particular time of day independent of this, was justified.

The relation of the time of maximum incidence to that of sunset indicates that its occurrence was at least to some extent dependent upon some change in external conditions. Of those which occurred, a diminution in light intensity was the most pronounced and regular, and, although no precise light intensity measurements were made, it seemed that further information on the relation of activity to changes in external conditions might be obtained by comparing the times of peak-incidence collections made while the weather was 'bright' with those made while it was 'dull'. In practice, the weather was taken as bright if the sun was visible, or, in the case of collections made after sunset, if the sky was mainly clear. If the sun was obscured by clouds, or, after sunset, the sky was mainly clouded over, the weather was taken as dull.

The results are shown in Table III, in which P is ~~again~~ a measure of the significance of the difference between the two mean figures obtained for each species; it has ~~again~~ been estimated by application of a Student's t test (see appendix). It will be seen that the mean time of maximum incidence was invariably earlier on dull than on bright evenings, but that the difference was of indisputable significance only in the case of C.impunctatus females, in which P was less than 0.001. The value of P obtained with C.punctatus males was 0.03, which is generally regarded as indicating a suspiciously large difference, but the differences obtained with both males and females of C.pallidicornis were clearly insignificant.

The fact that even one of the differences was highly significant seems to indicate that the observed tendency for maximal incidence to occur earlier under dull than under bright weather conditions was not accidental. It must be remembered, however, that the data on which the figures are based are open to error from several sources. One is that on some evenings collections may happen to have been made just at the time of maximal incidence, while on others they may have missed it; another is variation in the weather conditions (other than light intensity) at the time of collection. It is hoped that these sources of error were not sufficiently large to invalidate results, but in the absence of further data it would seem unwise to be dogmatic.

THE EFFECT OF WEATHER CONDITIONS.

As mentioned earlier, a note was made of the temperature, humidity, wind strength, rain strength, and light conditions at the time of each standard collection. In attempting to assess the effect of these conditions on numbers captured, the difficulty, of course, is to distinguish the effect of one condition from that simultaneously exerted by the others. The normal daily and seasonal fluctuations are an additional complication. With the limited data available, these difficulties make it possible to discern only pronounced and clear-cut effects.

To eliminate daily and seasonal variations, it would obviously be desirable to consider only collections made during the periods of peak incidence, both as regards time of day and time of year. The number of such collections, however, is limited, and in practice a compromise has to be made. That adopted here is that with regard to time of year, only collections made between the time when numbers reached 25% of their maximum value for the sex-species in question and the time when they once again dropped below 25% of this value have been included. With regard to daily variation, the only collections included are those which were made between the earliest and latest times at which

TABLE IVB.

The incidence of Culicoides adults in relation to wind strength. Results from beat B.

Sex-species	Wind Strength	Number of collections	Mean catch	Maximum catch
C.impunctatus females	Nil	16	9.5	26
	Very slight	6	1.8	2
	Slight	2	0.9	1
	Stiff	2	0.0	0
	Strong	0	-	-
C.pallidicornis males	Nil	15	58.2	255
	Very slight	7	63.0	169
	Slight	3	6.7	8
	Stiff	2	0.0	0
	Strong	0	-	-
C.pallidicornis females	Nil	6	62.6	130
	Very slight	3	21.0	53
	Slight	1	4.0	4
	Stiff	0	-	-
	Strong	0	-	-
C.punctatus males	Nil	26	3.2	12
	Very slight	7	3.5	7
	Slight	2	0.1	1
	Stiff	3	0.0	0
	Strong	0	-	-
'C.obsoletus' females	Nil	40	1.2	9
	Very slight	10	1.4	3
	Slight	6	1.5	2
	Stiff	3	0.3	1
	Strong	0	-	-

(To face p.46)

TABLE IVA.

The incidence of Culicoides adults in relation to wind strength. Results from beat A.

Sex-species	Wind Strength	Number of collections	Mean catch	Maximum catch
C.impunctatus females	Nil	12	7.4	18
	Very slight	5	12.5	38
	Slight	5	0.4	2
	Stiff	2	0.5	1
	Strong	2	0.0	0
C.pallidicornis males	Nil	7	9.4	21
	Very slight	10	2.0	5
	Slight	5	0.4	2
	Stiff	3	0.0	0
	Strong	2	0.0	0
C.pallidicornis females	Nil	4	13.3	40
	Very slight	2	3.3	8
	Slight	2	0.6	1
	Stiff	1	0.0	0
	Strong	1	0.0	0
C.punctatus males	Nil	18	1.7	12
	Very slight	9	1.9	6
	Slight	6	0.3	2
	Stiff	3	0.0	0
	Strong	2	0.0	0
'C.obsoletus' females	Nil	29	1.4	7
	Very slight	13	1.0	5
	Slight	8	0.1	1
	Stiff	5	0.0	0
	Strong	4	0.0	0

(To face p.46)

the maxima of the sex-species under consideration had at any time been recorded ('maxima' here refers only to maxima comprising at least five specimens of the appropriate sex-species).

In the following tables the numbers of each of the commoner sex-species, other than C. heliophilus, captured under different weather conditions are shown; the data for C. heliophilus females are too meagre to merit inclusion.

Wind-strength.

This was estimated subjectively, four grades, namely, very slight, slight, ~~slight~~, stiff, and strong being recognised. The writer believes that he was able to estimate the grade with reasonable consistency.

Tables IVA and IVB show the sizes of catches obtained at each wind-strength. The reason for separating the data obtained from the two beats is that wind-strength was usually lower at beat B than at the more exposed beat A. With species more abundant along the former beat, the effect of considering data from both beats together would therefore be artificially to inflate the size of catches obtained at lower wind-strengths; with species commoner along beat A the effect would be the reverse.

It will be seen that no midges were ever captured when the wind-strength was strong. On two occasions only - C. impunctatus females at beat A, and 'C. obsoletus' females at beat B - were any captured when the wind was stiff, and on both these occasions only one specimen was obtained. These results are so striking and consistent that, in spite of the relatively small number of collections made at high wind-strengths, there can be little doubt that they indicate that the species considered will not fly when the wind is above a certain strength, however propitious other conditions may be.

A slight wind, in the scale recognised in this investigation, also seems unfavourable for flight, for with one exception - 'C. obsoletus' females at Site B - both the mean and the maximum catch were lower at this wind-strength than at the next lowest grade, usually very considerably so. Numbers captured when the wind was very slight (just perceptible to the writer) were not consistently lower than numbers obtained when no wind at all was perceptible, so it may be inferred that this wind-strength had little or no effect on activity.

Since high wind-strengths evidently have an inhibitory effect on activity which can over-ride the effect of any other condition, it is clear that only collections made at lower wind-speeds should be considered if the effect

TABLE V.

The incidence of Culicoides adults in relation to temperature.

Sex-species	Temperature (^{o}F)	Number of collections	Mean catch	Maximum catch
C.impunctatus females	45-50	-	-	-
	50-55	9	7.9	17
	55-60	18	8.3	26
	60-65	12	7.9	38
	65-70	-	-	-
C.pallidicornis males	45-50	-	-	-
	50-55	-	-	-
	55-60	12	17.0	83
	60-65	20	38.3	255
	65-70	7	61.5	83
C.pallidicornis females	45-50	-	-	-
	50-55	3	25.9	96
	55-60	8	40.7	120
	60-65	4	24.2	130
	65-70	-	-	-
C.punctatus males	45-50	7	4.3	12
	50-55	6	2.0	4
	55-60	26	2.6	12
	60-65	6	3.3	6
	65-70	15	1.7	12
'C.obsoletus' females	45-50	7	0.4	1
	50-55	27	1.0	6
	55-60	33	1.7	8
	60-65	20	1.3	4
	65-70	5	0.3	1

(To face p.48)

of other conditions is to be assessed. In the following tables, therefore, only collections made when the wind-strength was very slight or nil have been included.

Temperature.

In Table V, the sizes of collections obtained at different 5°F temperature groups are shown. The temperatures encountered will be seen to have ranged from 45/50°F to 65/70°F. Data from both sites have been included.

It will be seen that there was no consistent relationship between the size of the mean catch and the temperature. In the case of C. pallidicornis males the mean catch was greatest at the highest temperature, in C. punctatus males greatest at the lowest; in the remainder it was greatest at intermediate temperatures. The size of the maximum catch similarly showed no consistent trend. This lack of correlation may be due to the limited temperature range over which observations were made, and/or to the many sources of variability liable to influence the data. It is, however, evident that within the range experienced, temperature has no pronounced effect capable of exerting an over-riding effect on activity; the data for C. punctatus males, in particular, show that a single species may be obtained in maximal numbers at both the highest and lowest temperatures

TABLE VI.

The incidence of *Culicoides* adults in relation to saturation deficit.

Sex-species	Saturation deficit (mm.)	Number of collections	Mean catch	Maximum catch
C. impunctatus females	0-1	-	-	-
	1-2	26	7.4	26
	2-3	9	4.4	11
	3-4	4	20.8	38
	4-5	-	-	-
	5-6	-	-	-
C. pallidicornis males	0-1	17	0.9	6
	1-2	40	1.3	9
	2-3	25	1.6	8
	3-4	5	1.8	3
	4-5	3	0.0	0
	5-6	2	0.0	0
C. pallidicornis females	0-1	-	-	-
	1-2	15	33.3	130
	2-3	-	-	-
	3-4	-	-	-
	4-5	-	-	-
	5-6	-	-	-
C. punctatus males	0-1	3	2.3	3
	1-2	33	2.5	12
	2-3	17	2.3	12
	3-4	2	1.0	1
	4-5	4	2.2	3
	5-6	2	6.0	6
'*C. obsoletus*' females	0-1	17	0.9	6
	1-2	40	1.3	9
	2-3	25	1.6	8
	3-4	5	1.8	3
	4-5	3	0.0	0
	5-6	2	0.0	0

(To face p.49)

encountered.

Humidity.

This was measured by means of a whirling psychrometer. Mellanby (1935) has presented evidence which indicates that the evaporation of water from insects is, in general, more closely related to saturation deficit than to other measures of humidity. If therefore, activity is related to humidity, it is more likely to be to the saturation deficit than to the relative humidity. For this reason numbers captured are, in Table VI, shown in relation to the former and not the latter measure.

It will be seen that neither the mean nor the maximum catch bore any consistent relationship to saturation deficit. Admittedly, in two out of four cases the highest mean catch was obtained at the highest saturation deficit, but the remaining figures did not show the same trend; the lowest mean catch, for example, was never obtained at the lowest saturation deficit. The size of the maximum catch similarly showed no consistent trend. It may thus be said that within the range encountered, differences in saturation deficit exert no pronounced over-riding effect on numbers captured.

Grouping of the data in relation to relative humidity, which ranged from 50/60% to 90/100%, similarly failed to reveal any consistent relationship.

Rainfall.

As in the case of wind, this was estimated subjectively, the grades recognised being very soft, soft, moderate, and heavy.

So few collections were made while rain was falling that presentation of the data in tabular form does not seem justified. Adults were captured during very soft, soft, and moderate rain, and while the size of these catches tended to be small, this was not necessarily so, for on one occasion a catch of 12 C.punctatus males - as large as was at any time obtained with this sex-species - was obtained during moderate rainfall. Heavy rain, as recognised by the writer, was never experienced. It is evident from these results that the rain-strengths encountered produced no clear-cut over-riding effect on numbers captured.

Light conditions.

The effect of differences in light conditions on the time of maximal incidence has already been considered. They might also, conceivably, have had some effect on the actual numbers participating in the evening flight. In

TABLE VII.

The incidence of Culicoides in relation to light conditions.

Sex-species	Light conditions	Number of collections	Mean catch	Maximum catch
C.impunctatus females	Bright	15	6.0	18
	Dull	24	9.2	38
C.pallidicornis males	Bright	15	47.8	169
	Dull	24	28.5	255
C.pallidicornis females	Bright	7	25.0	96
	Dull	8	40.6	130
C.punctatus males	Bright	29	2.7	12
	Dull	31	2.5	12
'C.obsoletus' females	Bright	38	1.1	6
	Dull	54	1.4	9

(To face p.51)

Table VII the data have been split up to show the sizes of collections obtained during dull and bright weather, the definition of these terms being that given previously (p. 43).

It will be noticed that while the size of the maximum catch tended to be higher under dull conditions, this tendency was not consistently evident in the sizes of the mean catches; in the case of <u>C. pallidicornis</u> males, the mean catch obtained on bright evenings was considerably the higher of the two. Taking the data as a whole, in fact, they cannot be said to point to any consistent relationship between the brightness or otherwise of the weather and numbers captured.

Of the weather conditions considered, only wind has been shown to have a definite effect on activity. Within the ranges experienced, the remaining conditions produced no conspicuous effects, but may have exerted less obvious differences which were obscured by the variability of the data. No attempt has been made to discern relationships which were not immediately obvious by the use of significance tests, as it is considered that with data subject to variability from so many extraneous sources these might easily be misleading.

TABLE VIII.

The attraction of female *Culicoides* to man.

Species	Number attracted (N_a)	Estimated number per hand-net collection (N_n)	$\frac{N_a}{N_n}$
C.impunctatus	24	11	2.2
	13	4	3.2
	124	16	7.8
	55	8	6.9
	19	0	19.0+
Totals	235	39	6.0
C.heliophilus	1	4	0.3
	16	20	0.8
	4	10	0.4
	1	3	0.3
	0	1	0.0
Totals	22	38	0.6
C.pallidicornis	1	1	1.0
	0	6	0.0
	0	5	0.0
	21	30	0.7
	0	1	0.0
Totals	22	43	0.5
'*C.obsoletus*'	1	3	0.3
	1	3	0.3
	1	3	0.3
	1	4	0.3
	4	5	0.8
Totals	8	18	0.4

(To face p.52)

ATTRACTION TO MAN.

To assess the relative readiness of different species to attack man, the writer periodically sat at some point along either beat A or beat B and captured the adults alighting on him. Using an aspirator in conjunction with a mirror, it was possible to recover midges from most parts of the body, and there is no reason to suppose that the proportion not collected - perhaps very small - varied with different species. The exposures were for 15 minutes, and were made in the intervals between the standard hand-net collections. They were in several cases made at approximately the times of maximum incidence of each sex-species, invariably when the wind-strength was very slight or nil.

In Table VIII the numbers attracted during five exposures are shown for each species of which females were obtained in moderate or large numbers, the exposures selected being those which yielded highest numbers; some species were attracted in less than five exposures, for which reason some zeros appear in the table. In another column are shown estimates of the numbers of each species which would have been expected in standard hand-net collections made at the same time. These estimates are mostly the means of the numbers obtained in the hand-net collections preceding and succeeding the exposures, but where the time of the exposure was not mid-way between that of the hand-net collections allowance has been made for this. As before, times are expressed in relation to sunset. The data refer exclusively to females; the males, of course, are non-bloodsucking, and none ever alighted on the writer during the course of the collections.

It will be seen that the ratio of the number attracted to the estimated number per standard hand-net collection was consistently higher for C. impunctatus than for any of the remaining species. The differences, moreover, are highly significant, for if a χ^2 test is applied to the difference between the totals 235:39 obtained with C. impunctatus and those obtained with any of the remaining species a value of $P = < 0.001$ is obtained. Even allowing for the many sources of error to which the initial data are subject - in particular the difficulty of obtaining an accurate index of the size of the flying population at the time of the exposure - there can be little doubt that such a marked difference means that C. impunctatus females are more strongly attracted to man than are those of the remaining species.

The differences between the results obtained with the remaining species are not significant. There is thus no indication that these species differ among themselves in the readiness with which they are attracted to man.

DISCUSSION.

The recent investigations of Kettle (1949, 1950, 1951) have added much to our knowledge of the bionomics of C. impunctatus. Kettle sampled the adult midge population by covering black metal cylinders with grease-coated celluloid sheets, and placing them on poles stuck vertically into the ground. There were three cylinders per pole at heights of 2, 6, and 10 feet. The celluloid sheets were changed and inspected weekly. The traps were situated in woodland and moorland approximately three miles from the site of the present writer's collections.

Kettle found that in 1948 and 1949 the adult C. impunctatus population was very small at the beginning of May, but reached a considerable size by the end of that month; Hill (1947), it will be remembered, obtained similar results in Lancashire in 1945. The fact that C. impunctatus was still present in only minute numbers at the beginning of June in the writer's collections was thus probably mainly a result of the exceptionally severe and prolonged cold spell of early 1947, when much of Loch Lomond froze over, apparently for the first time for over 50 years. Like the writer, Kettle found numbers to be reduced almost to nil by the end of August.

A point of interest about Kettle's collections is that there were two peaks in abundance, one occurring in late May or early June, the other in late July or August. From other evidence he considers these peaks to be composed of individuals belonging to two different biological races, each passing through only one generation per annum. The writer's collections show only one peak, corresponding to Kettle's first, but as Kettle has pointed out, the second may have taken place between August 4th and 24th, when the writer was unable to make any collections.

The peak obtained with *C. heliophilus* in late June at Rossdhu accords with the statement of Cameron, Downes, Morison, and Peacock (1948) that this species is a vigorous biter at this time. The same authors state that *C. heliophilus* probably passes through two generations per annum, a state of affairs one would expect to be reflected in a fairly prolonged adult season with two peaks in abundance; as neither of these features was observed at Rossdhu it seems possible that the claim is based upon mistaken identifications.

The brief period during which Hill (1947) obtained

C.pallidicornis females in her Lancashire collections, namely, between mid-June and mid-July, overlaps into the period of maximum incidence in the writer's collections, which was the first half of July. Cameron's (1948) finding that maximal emergence of this species occurred at the end of June and the beginning of July is also consistent with the Rossdhu results.

The prolonged adult season of C.punctatus has been confirmed by Cameron (1948), who found the species to emerge from the end of May to September, and by Downes and Kettle (1952), who record it as occurring near Loch Lomond from the beginning of May to early October. One of the sets of collections made by Kettle in 1948 shows a double peak in abundance, one occurring in May and the other in August/September. The 1947 Rossdhu collections gave little indication of a distribution of this type. Admittedly they were not started until June, but as the C.impunctatus season of 1947 occurred approximately one month later than that of 1948, apparently due to the exceptional severity of the preceding winter, one would expect the same to be true of other species. A maximal catch was obtained at one site at the end of August, but three catches just as high were obtained in July, a time when, even allowing a month's delay, numbers, according to Kettle's figures, should have been very small. There seems no obvious explanation which might reconcile these discrepancies; possibly the fluctuations in the writer's collections are too small to be of significance, but those recorded by Kettle are only slightly larger.

C. pulicaris also occurred in Kettle's collections, being, like *C. punctatus*, obtained throughout the whole of the collecting period (May to September); this is consistent with the Rossdhu findings. Whether the double peak obtained by Kettle also occurred at Rossdhu cannot be judged from the minute numbers obtained there.

The protracted nature of the '*C. obsoletus*' adult season at Rossdhu was in accordance with Hill's results. No double peak, such as was observed by the latter author, was indicated, but as numbers captured at Rossdhu were at all times small, this is not necessarily of significance. The tendency for numbers to be higher during the later part of the season was, however, consistent with the statement of Cameron *et al*. (1946) that *C. obsoletus* is found chiefly in the latter half of the summer.

Of the remaining species obtained at Rossdhu, the very small numbers obtained do not seem to merit any comment further than that their times of capture do not in any instance appear to have been inconsistent with those of other investigators, as reviewed earlier.

Judging from the widespread collections of Cameron _et al_. (1946), the predominance of _C. pallidicornis_ at Rossdhu was exceptional; over most of Scotland _C. impunctatus_ appears to be the commonest species. Excluding _C. pallidicornis_ the relative abundance of the species at Rossdhu does not seem to have been very different from their relative abundance over much of Scotland, though _C. punctatus_ was perhaps rather commoner than usual. The fact that '_C. obsoletus_' was obtained only in comparatively small numbers accords with the statement of Cameron _et al_. (_loc. cit._) that it is a lowland species (group) commonest south of the Forth and Clyde, and the minute numbers of _C. grisescens_ and _C. fascipennis_, as opposed to the large numbers of these species obtained in Invernesshire, is in agreement with the statement of the same authors that these are highland species.

From the fact that they occasionally made collections yielding almost equal numbers of male and female _C. impunctatus_, Cameron _et al_. (1946) conclude that the predominance of females usually obtained in their hand-net collections, a state of affairs also experienced at Rossdhu, was due to some unrecognized habit on the part of the males which made them difficult to obtain by this method. This is borne out by the fact that Hill found the two sexes to emerge in approximately equal numbers from pupae obtained in the field. In Kettle's collections too, the sex-ratio was almost normal. The latter result is surprising, for the effectiveness of Kettle's black cylinders as traps presumably depended at least partly on the attraction of Culicoides to black objects, an attraction to which Hill found only females susceptible. His results dispose of any suggestion that the infrequency of males in hand-net collections was due to their reluctance to engage in flight, and he found no pronounced differences in the vertical distribution of the two sexes; in woodland the males were slightly more numerous at 10 feet, and the females more numerous at 2 feet, but the differences were small, and the reverse was true on open moorland. It might be supposed that the males were most active at some time of day at which ~~no~~ collections were not customarily made, but the writer's collections, which, considered collectively, extended over the whole day, gave little indication of this; admittedly the male/female ratio was higher during the night than around sunset, but the actual number of males was still very small. Again, the sight of the collector with his waving net might conceivably have caused dispersal of the males while not affecting the females, but if so it is strange that no similar reaction occurred with C. pallidicornis and C. punctatus, of which, it will be remembered, more males than females were obtained. After consideration of all these possibilities, the writer must confess himself quite unable to find any explanation for the low male percentage which he, and other authors, have obtained.

The explanation of the low male percentage obtained in the Rossdhu collections of C. heliophilus and 'C. obsoletus' is similarly unknown; collections of resting adults of the latter group, it will be remembered, yielded almost equal numbers of the sexes. The same applies to the abnormal sex-ratio obtained with C. punctatus, resting adults of which similarly yielded an almost normal sex-raio, though here it is the females which were in the minority in the flying population.

Observations on the swarming behaviour of male Culicoides have been made by Downes (1950) on C. nubeculosus. Downes obtained only males in the swarms, but found males and females in copula immediately underneath, a fact which he takes to indicate that females sometimes enter the swarm, almost instantaneously to be siezed and borne to the ground; the existence of similar habits in many other Diptera makes this very plausible. If, as therefore seems likely, the male swarms of C. nubeculosus are directed towards the accomplishment of mating, the same is probably true of the C. pallidicornis and C. grisescens swarms observed by the writer, and the phenomenon may indeed occur throughout the genus; Goetghebuer (1919, 1920) has noted its occurrence in males of C. pulicaris and C. fascipennis. As Downes points out, however, swarming is not essential for mating in Culicoides, for C. nubeculosus will mate readily in small tubes, and copulation may even take place while the female is engaged in taking a blood meal (Pomerantzev, 1932).

The occurrence of the female activity peak of most species obtained at Rossdhu around the time of sunset was in accordance with general heresay and the statements of earlier workers. Numbers were similarly expected to fall off during the night, though not to the extent to which they did. It will be remembered that there was nothing apparently unfavourable about the weather conditions which could account for the small numbers obtained, and if it is granted that a collection made by hand-net is a reliable index of activity, it must be accepted that activity, at least of the species encountered at Rossdhu, is, in fact, at a very low ebb during the hours of darkness.

The relatively small size of the early morning peak observed at Rossdhu is consistent with the results obtained by Hill (loc cit.) who, it will be remembered, obtained very small numbers in comparison with her evening catches. It is also consistent with Downes (loc cit.) findings on C. nubeculosus. Downes found the evening peak to be three to four times as great as the morning peak. The morning fliers, and an equivalent number of the evening fliers, had small ovaries with undeveloped oocytes. The remainder of the evening fliers had large ovaries containing ripe eggs ready for laying. Very few females with ovaries in an intermediate condition were obtained. From this Downes concludes that the females with small ovaries, flying both evening and morning, are probably in search of a blood meal; they may consist of a mixture of newly emerged adults and older ones that have recently oviposited. Having fed, he supposes, they do not become active again until the meal has been digested and the eggs have matured; they then become active only in the evening. The writer, not now having access to his collections, is unfortunately unable to add anything further to these interesting results.

The fact that maximal numbers of C. heliophilus females were obtained several hours before sunset was in accordance with previous records of the daytime activity of this species. The occurrence of maximal numbers of C. pallidicornis males at a similar time, however, is puzzling, for the female peak of the same species occurred after sunset. One may, perhaps, conjecture that maximal female incidence is associated with the availability of their hosts, possibly crepuscular mammals. The males, on the other hand, have been observed to be dancing in swarms during their peak period, and if mating is the main purpose of the swarms it may well involve a visual mechanism and be more effectively performed when the light is stronger. On the other hand, Downes records the male swarms of C. nubeculosus as occurring around and after sunset, so the stronger light of the earlier evening is evidently not essential. The separation of the male and female activity peaks in C. pallidicornis thus remains a subject for speculation.

The occurrence of the evening activity peak at a time apparently fixed in relation to that of sunset, rather than at a particular time of day independent of this, together with the tendency for the peak to occur earlier on dull than on bright evenings, strongly suggests that, while some internal rhythym may be partly responsible for variations in activity, an external stimulus also plays an important part. Of the stimuli which might be responsible for the onset of maximal activity, a fall in light intensity was the most regular and consistent. Admittedly, the temperature tended to fall, and the humidity to rise at the same time, but they did so eratically, and their absolute values at the time of maximum incidence varied considerably. It therefore seems probable that the evening activity peak occurs when the light falls to a certain optimum level, while a light intensity both below and above this inhibits activity. The small early morning peak is perhaps initiated by a rise to the same optimum light intensity.

Acceptance of the importance of external stimuli in 'triggering' the onset of a particular activity phase does not, of course, imply that internal conditions are unimportant. Possibly some internal rhythm results in the insect being in a condition especially receptive to the external stimuli at the appropriate time; moreover, Downes' (loc cit.) observation that females in one phase of the life cycle may be active both morning and evening, while in another they are active only in the evening, indicates the extent to which the response to external stimuli may be affected by the internal condition.

It will, perhaps, have been noticed that in the preceding paragraph the word 'activity' has been used when 'incidence as indicated by numbers obtained in the hand-net' would have been more accurate. It is, in fact, a possibility that the adults were active at a level further above the ground than that explored by the net at some of the times of day at which they were apparently scarce. In the absence of any evidence in support of this suggestion there is no reason to lay particular emphasis on it; even if it is true the preceding arguments still hold, with the proviso that it is changes in distribution and not changes in activity that must be considered.

The pronounced inhibitory effect upon activity of stiff or strong wind observed at Rossdhu was fully consistent with previous observations. Had the data been fuller and more rigidly standardised they would probably have revealed relationships between activity and some or all of the other weather conditions considered.

The very much stronger preference of <u>C. impunctatus</u> for man than any other species of which females were obtained in adequate numbers was in accordance with the somewhat less substantiated statements of earlier investigators. The low intensity of attack shown by the remaining species was, of course, only relative, for they have all, at some time, been described as troublesome biters. Other things being equal, the degree of anthropophilism displayed presumably depends on the availability of other hosts.

PART IB.

LABORATORY STUDIES ON THE EGGS OF CULICOIDES.

INTRODUCTION.

The maintenance of adults for egg-laying.

The first investigator to obtain eggs from *Culicoides* females in the laboratory appears to have been Patel (1921) who induced oviposition in the Indian species *C. oxystoma* Kieffer and *C. pattoni* Kieffer. His method was to enclose freshly engorged females in tubes containing dry blotting-paper for which, after 50 - 60 hours, moist was substituted. At least half the females oviposited on the moist blotting paper two to three days later. They were quite healthy afterwards, and readily accepted another blood meal. Some lived up to two weeks in captivity, during which time they were known to take up to five blood meals and lay two batches of eggs.

Lamborn, also working in the tropics, is quoted by Edwards (1922) as having obtained eggs from the Malayan *C. anophelis* Edwards, his method having been to enclose a gravid female in a tube inverted above a bowl of liquid mud. Jobling (1928), in an investigation which he only described fully many years later (Jobling, 1953), obtained eggs from the British C. vexans Staeger by a similar method. Oviposition took place within seven days of receiving a blood meal, which was given at the time of capture, and within another three days all the females were dead; none would accept a blood meal after laying.

Sharp (1928), who worked on C. austeni in the Cameroons, had great difficulty in keeping adults alive in captivity. He found the most satisfactory method was to keep them in a cylindrical cloth cage covered at one end by moist cotton wool which kept the humidity at a high level. Inside the cage was a piece of cotton wool soaked in honey-water for the adults to feed on in between blood meals. A human arm was periodically introduced to provide the latter. Under these conditions adults were kept alive for two weeks. Oviposition occurred after a blood meal, and afterwards another feed was often accepted. Sharp states that two blood meals are probably necessary for the completion of ovulation, but he does not seem to have succeeded in obtaining more than one batch of eggs from any one female.

Steward (1933) kept adults of the British C. nubeculosus in a glass-fronted wooden cage containing damp blotting paper, a piece of moistened apple, and a dish of soil mixed with horse manure. A jar containing privet twigs was also included to supply something approaching a natural resting place. Human blood meals were given at frequent intervals, with the result that eggs were deposited in the dish of manure and on the damp cotton wool.

Buckley (1934) kept adults of the West Indian *C. furens* alive in the laboratory for up to two weeks by a method similar to that of Sharp (*loc cit.*), and also by enclosing them in inverted test-tubes, the open ends of which were closed with muslin, suspended above dishes of water. Later (Buckley, 1938), working in Malaya, he kept various species alive for up to three weeks in lamp-chimneys, the ends of which were closed with gauze, and in which a cut raisin was included. These were kept at room temperature and a high humidity. A blood-meal was supplied by applying the chimneys to the shaved flanks of a cow for one hour, absorption crystals at the distal end of the chimney preventing condensation; under these conditions and using three-day-old flies the proportion feeding was approximately 50%.

Atchley and Hull (1936) obtained eggs from a mixed culture of coastal species occurring in the United States by enclosing the females in lamp-chimneys closed at the upper end with gauze, and resting in dishes containing either moist blotting paper or moist soil. A meal of human blood was given twelve hours after capture, and oviposition usually occurred four to twelve days later; occasionally it occurred after two days, in which case it was assumed that a blood-meal had been taken prior to capture. An increased number of blood-meals had no effect on either length of life or on the number of eggs laid.

Hill (1947), working in Liverpool, maintained <u>C. impunctatus</u> and '<u>C. obscletus</u>' (see definition on p. 10) in the laboratory by a method essentially similar to that of the latter authors. The flies were again confined in lamp-glasses resting on petri-dishes containing moist filter paper. At approximately two-day intervals a blood-meal was offered by applying the lamp-glasses to the shaved ear of a lop-eared rabbit; in cold weather, when the temperature of the ears was sometimes as low as 92°F, Hill found that feeding took place more readily if they were placed on a warm stage which raised them to approximately 100°F. Split raisins or balls of cotton wool soaked in 10% sugar solution were included in the lamp-glasses, and the flies fed readily on these in between blood-meals.

With this arrangement the proportion of C. impunctatus females accepting a blood-meal the day after capture was at least 40%, and sometimes up to 74%. 'C. obsoletus' females were even more ready to feed, up to 96% having been observed to engorge. Subsequent offers of blood-meals were accepted by a progressively smaller proportion of flies as the time of oviposition approached; C. impunctatus would not feed later than two, and 'C. obsoletus' later than one day before laying. Individual flies accepted up to seven blood-meals before oviposition, the quantity consumed becoming progressively smaller. Flies which had fed several times laid no more eggs than those which had received only one blood-meal.

Oviposition occurred up to 15 days after capture with C. impunctatus females, and up to 18 days after capture with 'C. obsoletus' females, the eggs being deposited on the damp filter paper. Hill takes this as an indication that 15 and 18 days are approximately the normal periods required for the development of the eggs in these two species, and that in cases where the eggs were deposited earlier they must have been already developing when the fly was captured. Oviposition was usually complete, but occasionally one or two eggs were retained. It was usually followed by the death of the female in C. impunctatus, and in 'C. obsoletus' invariably so. The few C. impunctatus females that survived readily accepted another blood-meal, but Hill does not seem to have succeeded in obtaining a second egg-batch from any of them.

It will be evident that the maintenance of Culicoides in the laboratory is not without difficulties, for no investigators seem to have succeeded in keeping any adults alive for more than three weeks. It seems generally agreed that a high humidity is required, and that at least one blood-meal is necessary if oviposition is to take place; in between blood-meals the flies will apparently feed on sugar solution in some form or other.

Observations on the eggs.

The first investigator to observe Culicoides eggs appears to have been Lutz (1913), who obtained them in some manner not described from the Brazilian C. marium Lutz. A few years later Carter, Ingram, and Macfie (1920), working in the Gold Coast, obtained the eggs of C. grahami Austen, describing them as sausage-shaped and having an average length of 0.3 mm.

Patel's (1921) work on C. oxystoma and C. pattoni has already been mentioned. He describes the eggs of both species as being elongate, cylindrical, and slightly curved. When freshly laid they were white in colour, but soon changed to dark brown. The chorion of *C. oxystoma* eggs was sculptured, but such markings were absent from *C. pattoni*. *C. oxystoma* eggs were deposited in small groups, the total number laid in one batch by a single female varying up to a maximum of 156. They hatched from three to eleven days later, depending on the season. The larva emerged through a longitudinal aperture in the micropylar end of the egg.

Sharp (1928) found *C. austeni* eggs to be deposited in a long line "like a string of sausages", the average number of eggs per batch being 120. Hatching usually occurred the fourth day after laying, the larva forcing its way out of the egg by a series of expansions and contractions.

Jobling (1928), apparently the first investigator to obtain the eggs of a British species, found that *C. vexans* eggs laid in June and kept in the open did not hatch until the following October or November, a protracted egg-stage markedly different from those described earlier. After over-wintering as larvae they spent a few days in the pupal stage and emerged in February or March.

Hill (1947) found that *C. impunctatus* laid from

30 to 65 eggs per batch, the usual number being about 50. The eggs emerged in a string, their precise configuration when laid varying. Hatching, at temperatures of 16°C to 19°C, occurred from 7 to 20 days after oviposition, the average being 14 days, '<u>C. obsoletus</u>' females laid from 35 to 120 eggs per batch, the average being about 75. Hatching occurred after an interval of from 30 hours to 11 days, the average being 3 days. <u>C. odibilis</u> females laid an average of 80 eggs per batch; the time elapsing before hatching was apparently not noted.

<u>C. impunctatus</u> eggs are described by Hill as being cigar-shaped, but slightly more pointed towards the posterior end. The mean length was 490μ, the mean width 80μ. They were light in colour when first laid, becoming greyish-brown within half an hour. Longitudinal rows of sucker-like structures were present on the chorion, the rows being single dorsally and double ventrally.
'<u>C. obsoletus</u>' eggs were similar in general shape but somewhat shorter, the mean length being 380μ, the mean width 76μ. Sucker-like structures were again present on the chorion, but they were longer-stalked than those of <u>C. impunctatus</u>, their length sometimes being equal to half the width of the egg; they were arranged in ill-defined single longitudinal rows. <u>C. odibilis</u> eggs, similar in

colour and general appearance to those of *C. impunctatus*, had a mean length of 400μ and a mean width of 70μ. Sucker-like structures were present on the chorion in well-defined triple longitudinal rows, the suckers of each central row being larger than those of the two lateral rows; judging from the figure, their size approximated to those of *C. impunctatus* rather than to those of '*C. obsoletus*'.

Of hatching in *C. impunctatus* Hill states that "a small circular cap splits off at the anterior end of the egg, and simultaneously a longitudinal split occurs antero-posteriorly along the dorsal side for approximately a quarter the length of the egg. This longitudinal split turns sharply either to right or to left at its posterior end". In *C. odibilis* the hatching process was practically identical, but '*C. obsoletus*' differed in that the anterior cap was smaller and the longitudinal split longer, sometimes extending the whole length of the egg, and not, apparently, turning laterally.

The viability of the eggs.

Patel (1921) has stated that *C. oxystoma* and *C. pattoni* eggs will not survive in the absence of water, and Winn (1924) that moisture is essential for the development of C. furens eggs. Neither author presents the evidence on which his statement is based, and the writer has been able to find no account of any experimental investigation into this subject.

MATERIAL AND METHODS.

Most of the females used to obtain eggs were captured either in the University grounds, Glasgow, or at Rossdhu. C. grisescens females, however, were obtained from Auchterawe, Invernesshire, and C. halophilus and C. maritimus Kieffer from Arrochar, Dunbartonshire. All were captured either while in flight by means of a hand-net, or while resting on tree trunks; the latter method, though it yielded small numbers, had the advantage that an abnormally large proportion of females so obtained were gravid.

After identification, to effect which light anaesthetisation by ether was when necessary employed, each female was placed singly in a 3 x 1 in. tube. At the bottom of each tube was a layer of Plaster of Paris covered by several layers of filter paper. The filter paper was kept moist by the periodic addition of a few drops of water, which the plaster helped to retain. Each tube was closed by a cork pierced by a large hole, stretched over which was a piece of poplin. Carbohydrate food was provided in the form of a piece of raisin or prune impaled on a pin stuck into the cork. The temperature varied between 13° and 23°C.

Except when obviously gravid all females were offered a blood meal either on the day of capture or the following day. This was done by applying a glass tube containing the female or females to the human arm, or, more usually, by clamping it to the ear of an imprisoned lop-eared rabbit for 15 to 30 minutes. Not all accepted this first offer, and attempts to induce those that did not to feed were made on subsequent days. Once a female had taken a blood meal she was not offered another until after she had oviposited.

All tubes were examined daily, and since eggs were almost invariably laid on the damp filter paper, they could usually be removed simply by withdrawing the top layer of the latter. Occasionally eggs were laid on the sides of the tube, in which case they were sluiced out of it.

After removal, each batch of eggs was usually

TABLE IX.

The length of life of female *Culicoides* in the laboratory, and the proportion which oviposited

Species	Total Number of females	Mean life (days)	Maximum life (days)	Proportion ovipositing
C.impunctatus	50	9	29	10%
C.heliophilus	18	9	18	6%
C.pallidicornis	55	10	34	2%
C.pulicaris	81	10	27	25%
C.punctatus	19	9	21	47%
'*C.obsoletus*'	69	21	107	39%
C.halophilus	7	11	13	29%
C.maritimus	1	3	3	100%
C.fascipennis	2	16	17	50%

(To face p.78)

divided into several groups, each of which was kept on a filter paper in a covered petri dish. One group was always kept in the laboratory at normal room temperatures; these eggs were kept permanently moist by the daily addition of a few drops of water to the filter paper. Another group was sometimes kept in a shaded area out of doors, these eggs also being kept moist. Frequently a third group was subjected to some special treatment, such as drying or heating, which will be described in greater detail later. With the exception of C. grisescens eggs, which, for reasons which will be apparent later, were examined weekly, egg-groups were examined daily, and a note made of any hatchings or other significant changes that had occurred.

THE MAINTENANCE OF ADULTS IN THE LABORATORY.

In Table IX is shown the length of life of Culicoides females of various species captured in the field and kept in tubes in the laboratory as described in the preceding section. The proportion which oviposited is also shown. Of the species of which fairly large numbers were maintained (i.e. all except the last three listed), the mean length of life of all except 'C. obsoletus'

TABLE IXA.

The times between blood-meal and oviposition of female *Culicoides* in the laboratory.

Species	Number of females	Minimum (days)	Maximum (days)	Mean (days)
C.impunctatus	3	6	25	13
C.heliophilus	1	15	15	15
C.pallidicornis	1	12	12	12
C.pulicaris	5	3	22	10
C.punctatus	5	5	19	9
'*C.obsoletus*'	7	8	20	10
C.halophilus	1	12	12	12
C.fascipennis	1	10	10	10

(To face p.79)

will be seen to have varied from nine to ten days. '<u>C. obsoletus</u>' females survived for an average of three weeks, the record being a female which lived for 3½ months. The latter is a very much longer life-span than has previously been recorded for any species of <u>Culicoides</u> in captivity. The female in question was captured in Invernesshire on September 18th, and died in Glasgow on January 3rd. During this period it received only two blood meals, the rest of the time feeding, presumably, on the carbohydrate food provided.

Considering, again, species of which fairly large numbers were maintained, the proportion which oviposited will be seen to have been considerably higher in '<u>C. obsoletus</u>', <u>C. pulicaris</u> and <u>C. punctatus</u> than in the remainder. To a considerable extent this was undoubtedly due to these species having formed the bulk of the population collected from tree-trunks, for an unusually high proportion of females so obtained were visibly gravid, and oviposited within a day or two of capture. The unusual longevity of '<u>C. obsoletus</u>' females also contributed to the high proportion of ovipositions obtained with this species group.

The intervals between acceptance of a blood-meal in the laboratory and oviposition are shown in Table IXA, from which it will be seen that the mean period varied from

9 to 15 days. The maxima varied up to 25 days, and Hill (loc cit.), as already mentioned, has taken similar maximum periods to be indicative of the normal period required for development of the eggs. Until more is known about the circumstances affecting egg-development, however, the word 'normal' can have little meaning in this context. The time taken for the eggs to develop may, for instance, be affected by the number and size of blood-meals taken, and may not be the same in nature as in the comparatively artificial environment of the laboratory. The figures presented in the table may thus be regarded only as a rough indication of the periods which may be expected to elapse between blood-feeding and oviposition when Culicoides females are kept under the conditions described. There are no indications of any specific differences in rates of egg-development, but such differences might be revealed by fuller and more standardised data.

Although the mean post-oviposition life of 'C. obsoletus' females was 5½ weeks - i.e. longer than the mean life of females of this species-group as a whole (see Table IX) - only two of the 27 females from which eggs were obtained could be induced to accept further blood-meals. Those that did so fed 4-6 weeks after laying, and both laid second egg-batches nine days later. Death

TABLE X.

The number of eggs laid per batch by Culicoides females in the laboratory.

Species	Number of egg batches	Minimum	Maximum	Mean
C.impunctatus	5	31	77	49
C.heliophilus	1	38	38	38
C.pallidicornis	1	30	30	30
C.pulicaris	21	63	150	92
C.punctatus	9	70	152	95
'C.obsoletus'	29	28	80	44
C.halophilus	2	51	57	54
C.maritimus	1	55	55	55
C.fascipennis	1	52	52	52
C.grisescens	9	32	69	42

(To face p.81)

followed after two weeks in one, five weeks in the other.

Excepting C. impunctatus females, which always died within a few hours, survival for a few days after oviposition was usual in the remaining species. In spite of all attempts to induce these species to accept further blood-meals, only one C. pulicaris female did so. This female fed two days after laying, laid a second egg-batch eight days later, and died one week afterwards.

OBSERVATIONS ON THE EGGS UNDER NORMAL CONDITIONS.

Eggs were laid mainly on the damp filter paper at the bottom of the tubes, particularly in any fold or crevice that happened to be available. Their precise configuration varied from batch to batch, and showed no obvious specific differences. Generally speaking they were scattered irregularly, sometimes lying singly, sometimes in groups of up to five.

The numbers of eggs laid per batch by females of different species are shown in Table X, from which it will be seen that C. pulicaris and C. punctatus tended to be considerably more productive than the remainder. As a rule, all the eggs in a given batch were laid the same day, but occasionally their extrusion took place

(To face p. 82)

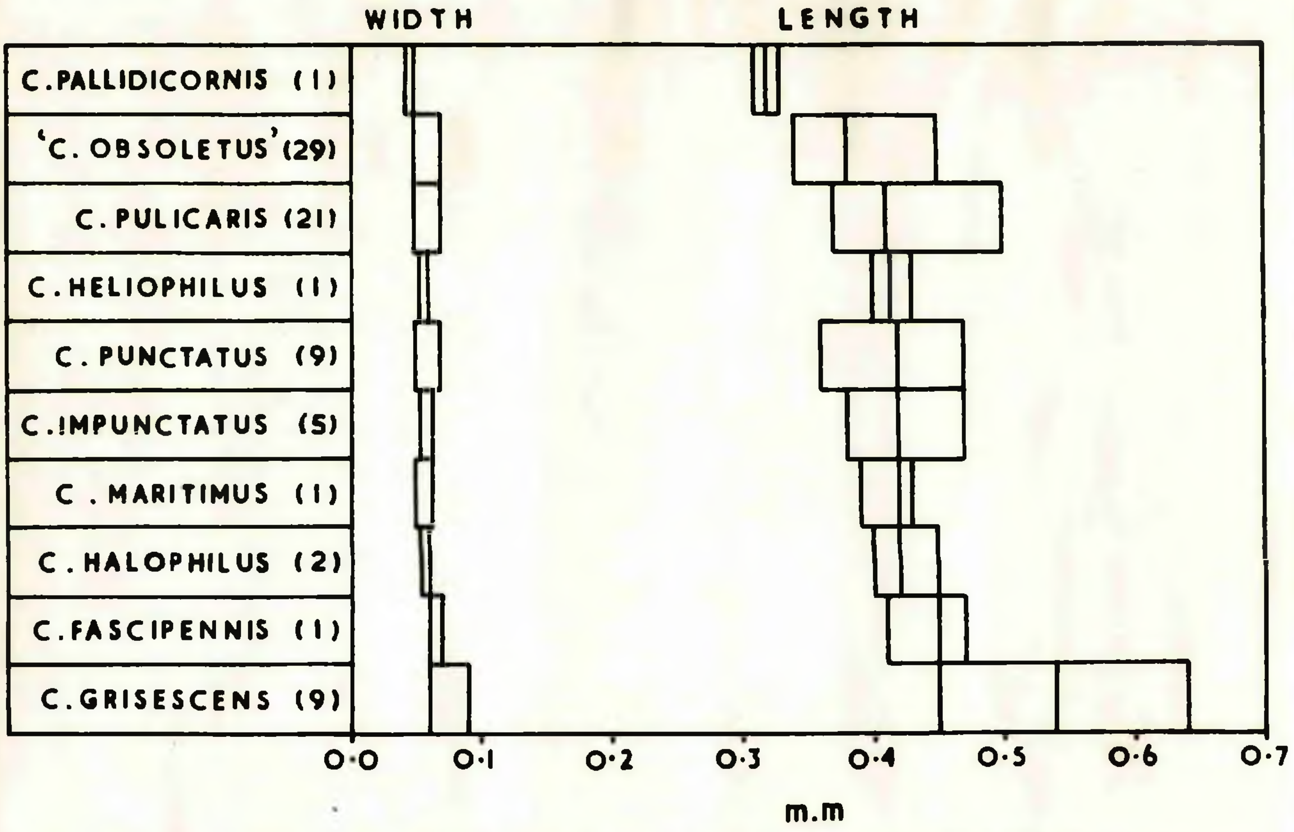

Fig. 13. The dimensions of Culicoides eggs. For explanation see text.

on two or even three consecutive days.

In general appearance the eggs of all species obtained accorded with the descriptions of previous investigators. All were cigar-shaped with a tendency to be more pointed at one end, which proved to be the posterior; a few were crescentic in shape. When newly laid they appeared pale grey in colour, later darkening to some shade of brown. Pale sucker-like structures similar to those described by Hill (loc cit.) for C. impunctatus were observed, though, as will be described shortly, their size and arrangement varied with the species.

The dimensions of the eggs are shown in Fig. 13, which illustrates the range of widths and lengths encountered in each species. The vertical line inside each of the blocks representing length-range gives the mean length for the species. The numbers in brackets after the names of the species are the number of egg-batches obtained; since measurements were taken from 10 eggs taken at random from each batch, these indicate the number of eggs measured. This number, needless to say, must be taken into account in comparing the range of variation in different species.

It will be seen that the smallest eggs were those of C. pallidicornis, the largest those of C. grisescens.

Although only one C. pallidicornis egg-batch was obtained, and fuller data would doubtless reveal a greater range of variation, the divergence between the mean egg-lengths of these two species is sufficiently wide for it to be safely assumed to be a genuine specific difference. The same is probably true of the difference between the mean egg-length of C. pallidicornis and that of any of the remaining species, and similarly of the difference between the mean egg-length of C. grisescens and that of any of the remainder. Other differences are more open to question.

The width measurements, which naturally vary less than do the lengths, will be seen to follow a roughly similar trend: the values for C. pallidicornis are again the smallest, and those for C. grisescens the largest obtained.

The dimensions of the eggs reflect the sizes of the adults from which they were obtained only to a very limited extent. C. grisescens is a large species with an average wing-length of 2.0 mm., while C. pallidicornis, with an average wing-length of 1.5 mm., is considerably smaller. 'C. obsoletus', however, is smaller still, and C. pulicaris and C. punctatus are as large as C. grisescens. A list of the species in order of adult size would thus differ considerably from the one arranged in order of mean egg-lengths.

It will be evident that the range of variation encountered is by no means wholly determined by the number of eggs examined. C. grisescens eggs appear to have been unusually variable, for although based on only nine egg-batches, both their length and width measurements vary more than those of any other species.

Anatomical features observed in the eggs of each species will now briefly be described. Many of the observations were made on hatched eggs, the ornamentation of the chorion of which was more readily visible than that of eggs containing a developing larva. In speaking of dorsal and ventral surfaces, it has been assumed that the dorsal side is that on which the longitudinal split made by the hatching larva occurred. This is claimed to be so by Hill for the species from which she obtained eggs, and has been confirmed by the writer for 'C. obsoletus'. With the exception of C. impunctatus and 'C. obsoletus', none of the eggs have previously been described.

C. impunctatus

The anatomy of the egg was very similar to that described by Hill. Pale sucker-like structures (hereafter called suckers for brevity without any implication as to

(To face p. 85)

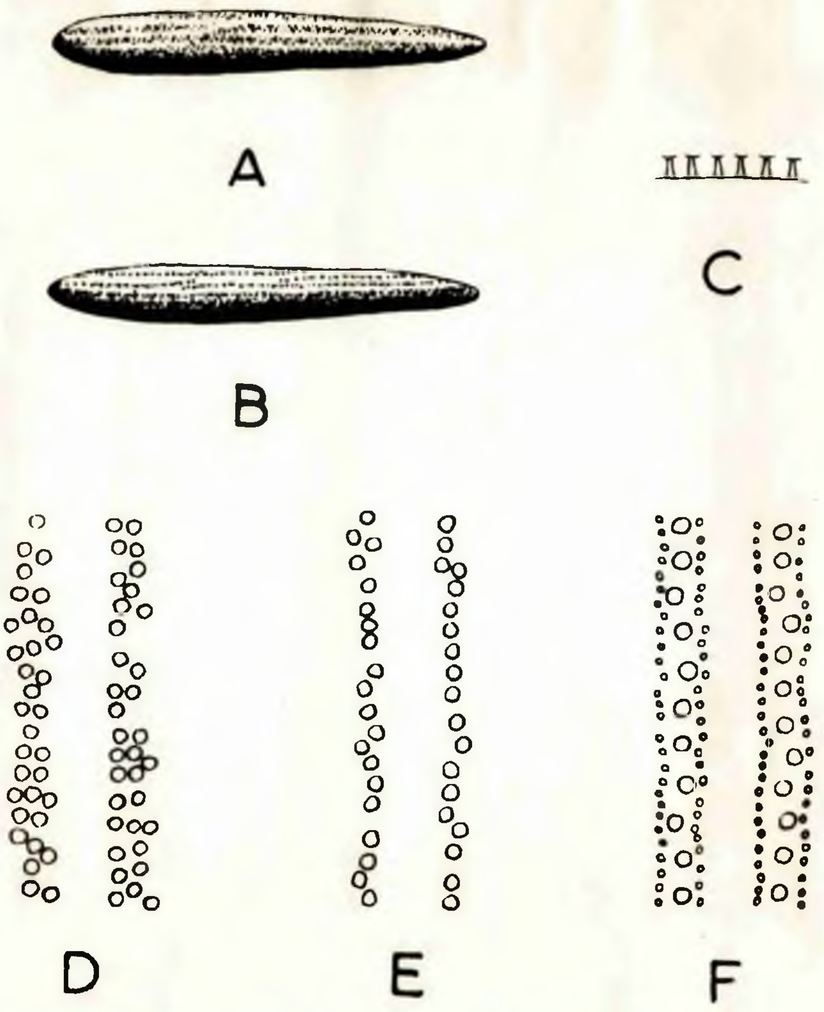

Fig. 14. A-E. Egg of *C. impunctatus*. A. Ventral view x 120. B. Dorsal view x 120. C. Suckers in side view x 1000. D. Surface view of suckers on ventral surface x 1000. E. Ditto on dorsal surface.
F. Surface view of suckers on ventral surface of egg of *C. pallidicornis* x 1200.

their function) projected .002 - .003 mm. from the surface of the egg (Fig.14C). They were arranged in fairly well-defined longitudinal rows, these being single dorsally and double ventrally (Fig.14A-E). (It should be understood that here, and elsewhere, when a row is described as 'double', all that is meant is that this was the predominant arrangement; in places the rows were treble, and in places single, Fig.14D). The size of the suckers was roughly constant over all parts of the egg except that, as in all species observed, those at the extreme anterior end were borne on stalks approximately twice as long as elsewhere.

C. pulicaris

Suckers approximately the size of those of C. impunctatus (hereafter referred to as normal-sized) were arranged in fairly well-defined single rows both dorsally and ventrally.

C. punctatus

Eggs of this species appeared to be identical with those of C. pulicaris.

C. halophilus

Normal-sized suckers were arranged in single rows ventrally. Smaller suckers were arranged in double longitudinal rows dorsally. Both dorsal and ventral rows were fairly well defined.

C. grisescens

The suckers were very small and difficult to see. They appeared to be arranged roughly in single longitudinal rows both dorsally and ventrally. An unusually high proportion of eggs were crescentic in shape, though these were still in the minority.

'C. obsoletus'

The suckers of this species group were either normal-sized or very much smaller, and in some egg-batches appeared to be completely absent; this variation may have been a result of the composite nature of 'C. obsoletus'. Where suckers were visible, they appeared to be present only on the ventral surface, over which they were scattered irregularly or arranged very roughly into multiple longitudinal rows.

C. heliophilus

Normal-sized suckers were arranged in fairly well-defined single longitudinal rows both dorsally and ventrally.

C. pallidicornis

Normal-sized suckers were arranged in well-defined single rows ventrally. Each of these rows, however, was flanked on either side by smaller suckers (Fig. 14F). Similar

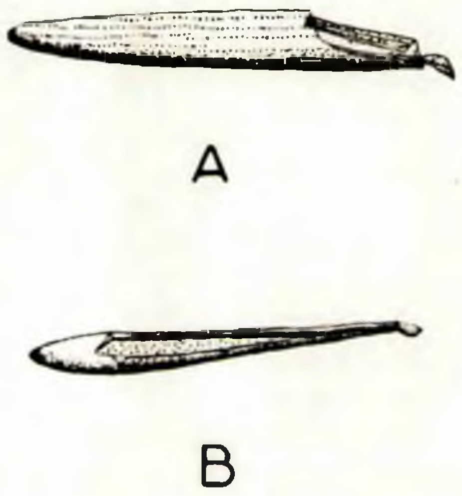

Fig. 15. A. Hatched egg of C. impunctatus x 120. B. Hatched egg of 'C. obsoletus' x 120.

small suckers were arranged in well-defined multiple (mainly triple) rows on the dorsal side.

C. fascipennis

Normal-sized suckers were arranged in double rows ventrally, smaller suckers in double rows dorsally. Both dorsal and ventral rows were fairly well-defined.

C. maritimus

Normal-sized suckers were arranged in fairly well-defined single rows both dorsally and ventrally.

The anatomical features described give little indication of the relationships of the species they represent. The first five species described, for instance, all belong to the C. pulicaris group, but the ornamentation of their chorions varied widely. A few species were indistinguishable by the characters described, but in most cases they could be used as a means of identification.

Hatching. Judjing from the appearance of hatched eggs, the hatching process in C. impunctatus was as described by Hill, for a small circular cap was invariably found separated off at the anterior end of the egg, and from the same end a longitudinal split extended a quarter to a third of the length of the egg. This split

TABLE XI.

The interval between laying and hatching of <u>Culicoides</u> eggs kept in the laboratory.

Species	Date laid	Date hatching first observed	Mean duration of egg stage (days)	Mean laboratory temperature (°C)
<u>C.impunctatus</u>	20.6.48	28.6.48	9	20
	3.8.48	12.8.47	7	21
	8.8.48	15.8.48	8	19
<u>C.pallidicornis</u>	31.7.47	4.8.47	4	20
<u>C.pulicaris</u>	11.5.48	16.5.48	5	19
	18.6.48	23.6.48	6	19
	10.7.48	16.7.48	6	18
	19.9.47	26.9.47	8	16
<u>C.punctatus</u>	2.6.48	7.6.48	6	17
	5.6.48	11.6.48	6	17
	4.7.48	9.7.48	5	18
	5.7.48	10.7.48	5	18
'<u>C.obsoletus</u>'	18.5.47	20.5.47	2	19
	5.6.48	7.6.48	2	19
	18.9.47	19.9.47	2	16
	22.9.47	25.9.47	3	15
<u>C.halophilus</u>	19.7.47	24.7.47	5	19
	25.7.47	1.8.47	6	19
<u>C.fascipennis</u>	8/9.7.48	14.7.48	7	18
<u>C.grisescens</u>	28.9.46	3.5.47	210-217	?
	28.9.46	17.5.47	216-223	?
	29.9.46	10.5.47	205-212	?

(To face p.88)

turned laterally at its posterior end (Fig. 15A).

The appearance of hatched '<u>C. obsoletus</u>' eggs also concurred with Hill's description, these having smaller caps separated off at the anterior end and a longitudinal split extending anything from half to the whole length of the egg, but usually reaching about three quarters of the way along it. With very few exceptions, this split did not turn laterally at its posterior end (Fig. 15B).

Of the remaining species of which hatched eggs were observed (i.e. all species from which eggs were obtained other than <u>C. heliophilus</u> and <u>C. maritimus</u>), all conformed to the type of splitting observed in <u>C. impunctatus</u>. The only variation - a very slight one - was that in <u>C. grisescens</u> and <u>C. pulicaris</u> eggs the anterior end occasionally remained intact instead of splitting off as a small cap; in such cases the larva escaped through the longitudinal split.

In Table XI are shown the intervals between laying and hatching as observed under the laboratory conditions described earlier. Except in the case of <u>C. grisescens</u>, observations, both on the time of hatching and of laying were made once per day, and it is therefore possible for the duration of the egg-stage to have been up to one day greater or less than the period stated. *C. grisescens* eggs were observed only at weekly intervals, the date of laying being, as before, correct to within one day. The duration of the egg stage in this species is therefore given only to within seven days. The mean laboratory temperatures cited are estimated from daily maximum and minimum readings.

Only four of the comparatively large numbers of egg-batches obtained from '*C. obsoletus*', *C. pulicaris*, and *C. punctatus* females have been included in the table, these having been chosen to cover as wide a seasonal range as possible. For other species the data include all egg-batches in which hatching was observed.

It will be seen that eggs of all species other than *C. grisescens* hatched within a few days after laying. Certain differences among them occurred consistently: '*C. obsoletus*' eggs always hatched sooner than those of other species, and, allowing for temperature differences, it is probably true to say that *C. impunctatus* eggs were slowest to develop. Further distinctions do not seem warranted by the data.

C. grisescens eggs, in marked contrast to the remainder, hatched only after approximately seven months;

eggs laid in September hatched in May. A similarly prolonged egg-stage was found in eggs kept in a shaded outdoor position but otherwise under similar conditions to those in the laboratory, though in this case hatching occurred in February, two months earlier than indoors. These findings indicate firstly, that C. grisescens over-winters in the egg-stage, a state of affairs not previously encountered in Culicoides, and secondly that the development of C. grisescens eggs is accelerated by exposure to low temperatures, for during the period September - February mean outdoor temperatures ranged from -2°C to +17°C, while in the artificially heated laboratory they ranged from +6°C to +21°C, and at any given time the laboratory temperature was invariably higher than that out of doors. Indeed, the 1946/7 winter, as remarked earlier, was exceptionally severe, and the moisture of the filter paper on which the eggs kept out of doors were situated was frequently frozen.

The remaining species when kept out of doors hatched one to three days later than their indoor counterparts. Although the indoor - outdoor temperature difference during the summer and autumn months was, of course, less than during the winter, the mean temperature was alwys found to be higher in the laboratory, and the temperature at any given time almost always so. The indication is thus that in this case the effect was the normal one of retardation of development at lower temperatures, and this is borne out by the fact that such retardation could be achieved by keeping eggs at a constant temperature of 4°C in a refrigerator. '<u>C. obsoletus</u>' eggs kept at this temperature for 3 days hatched 6 days after laying, <u>C. pulicaris</u> eggs chilled for 7 days and 21 days hatched after 14 and 26 days respectively; in each case the egg-stage exceeded its normal duration by approximately the period of cold-treatment.

Since there is evidence that during their prolonged adult seasons, '<u>C. obsoletus</u>' and <u>C. pulicaris</u> pass through two generations, attention may be drawn to the fact that the data quoted for these species include those obtained both at the beginning and the end of their adult seasons; there is no suggestion that second-generation eggs of either species behave differently from those produced by the first generation.

The proportion of eggs which hatched in any given batch varied from 0 to 100%, but was usually approximately 75%; there were no obvious specific differences in this respect. Most eggs failing to hatch contained dense embryonic tissue indistinguishable from the contents

(To face p.92)

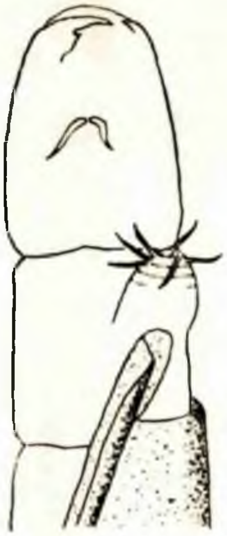

Fig. 16. First-instar larva of '*C. obsoletus*' in the process of hatching x 600.

of normal eggs, and sometimes a fully-developed larva was visible. Among '<u>C. obsoletus</u>' and <u>C. grisescens</u> eggs, however, were some which were extremely pale in colour, and either lacked any visible contents, or contained only what appeared to be oil globules. Such eggs invariably failed to hatch, and may, perhaps, have been unfertilised. Eggs remaining unhatched were sometimes attacked by a fungus which appeared as a dark gelatinous mass attached to one or both ends. Possibly some artificiality in the laboratory environment, acting either directly on the egg or indirectly through the parent, was responsible for the high mortality sometimes experienced.

A note on the anatomy of the larva.

During the course of the above observations, a remarkable feature of larval anatomy was observed. It happened that some '<u>C. obsoletus</u>' eggs were preserved while in the process of hatching; these eggs were unhatched when placed in the mountant (gum chloral), but were probably stimulated to do so by immersion in it. Examination of the emerging larvae showed them to possess a non-segmented protruberance on the ventral side of the first thoracic segment (Fig. 16). This protruberance, which projected for a distance equivalent to approximately half the width of the body bore three pairs of strong setae arranged bilaterally, and as later observations showed that it assisted in locomotion, may be termed a pseudopod. Its presence was entirely unexpected, for in the accounts of previous investigators(Goetghebuer, 1919; Carter, Ingram, and Macfie, 1920; Painter, 1927; Thienemann, 1928; Mayer, 1934; Tokunaga, 1937; Andersen, 1937; Thomsen, 1937; Hill, 1947) Culicoides larvae have invariably been described as apodous.

Living first-instar larvae of 'C. obsoletus' less than one day old were subsequently observed in an aquatic medium, and were seen to pull themselves along the floor of the container by means of the pseudopod, using it in the manner of a Chironomous larva. The typical type of movement in older Culicoides larvae is a rapid serpentine wriggling, by means of which they swim, and occasional movements of this nature, neither so rapid nor so sustained as those made by older larvae, were ~~occasionally~~ made by the first-day larvae. As the larvae got older, swimming movements were performed more frequently and more efficiently, but up to the twelfth day, after which all the larvae unfortunately died, considerable use was still made of the pseudopod. The size of the head-capsule of these twelve-day-old larvae had not changed appreciably since birth, so presumably they were still in the first instar.

To ascertain whether the pseudopod was present throughout larval life, larvae were recovered from '<u>C. obsoletus</u>' larval cultures of various ages; these had been obtained by transferring newly hatched larvae into a medium consisting of wet mud sterilised by heat and leaving for some weeks. A pseudopod was possessed by none of the larvae recovered, application of Dyar's Law to the head-capsule measurements of which showed them to have belonged to the third and fourth instars. It may thus be stated that the prothoracic pseudopod of '<u>C. obsoletus</u>' is present in the first instar, may or may not be present in the second, and is absent in the third and fourth instars.

The presence of a pseudopod in the first instar larva is evidently not a generic feature in <u>Culicoides</u>, for it was absent from <u>C. grisescens</u> and <u>C. halophilus</u>, newly hatched larvae of which were also examined.

EXPERIMENTS ON THE VIABILITY OF THE EGGS

The object of the experiments described here was to determine the survival-capacity of <u>Culicoides</u> eggs under certain conditions they might conceivably encounter in nature. They consisted in exposing eggs to a dry atmosphere, to above-normal temperatures (30°C and 35°C), and to combination of these treatments for various periods. Apart from '<u>C. obsoletus</u>', which had the drawback of including several species, the only eggs available in reasonably large numbers were those of <u>C. pulicaris</u> and <u>C. punctatus</u>. The majority of experiments were therefore performed on these two species.

Egg-batches were subdivided into groups of 20. One group from each batch - the control - was kept moist and at normal laboratory temperatures, while other groups were subjected to the treatments described below.

Exposure to a dry atmosphere

The proceedure adopted was as follows. Each egg-group before treatment was situated on a moist filter paper approximately ½ sq. in. in area. This was placed on a larger dry filter paper for a few minutes to withdraw excess moisture from it, and then transferred to a calcium chloride desiccator. Inside the latter it was placed on another large filter paper, which had been in the desiccator for several days. The duration of the exposure was timed from half an hour after this to the time when the egg-bearing

TABLE XIII.

The survival of *C.punctatus* eggs exposed to a dry atmosphere.

Number of eggs per group = 20.

Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
		Treated (T)	Control (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
12	0	0	18	0	-	5	-
	1	19	18	106	6	5	120
	2	17	18	95	6	5	120
	3	18	18	100	6	5	120
	4	19	18	106	7	5	140
24	0	0	19	0	-	6	-
	1	0 7	19 15	0 47	- 8	6 6	- 133
	2	0 4	19 15	0 27	- 7	6 6	- 116
	3	0 1	19 15	0 7	- 8	6 6	- 133
	4	0 10	19 19	0 53	- 9	6 6	- 150
	5	12 8	19 15	63 53	8 8	6 6	133 133
48	0	0	13	0	-	4	-
	2	0	13	0	-	4	-
	4	0	8	0	-	5	-

(To face p.96)

TABLE XII.

The survival of C.pulicaris eggs exposed to a dry atmosphere.

Number of eggs per group = 20.

Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
		Treated (T)	Control (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
12	0	0	17	0	-	5	-
	1	20	17	117	8	5	160
	2	20	17	117	7	5	140
	3	18	17	106	6	5	120
	4	20 1 18	17 1 19	117 100 95	6 7 8	5 7 6	120 100 133
18	0	0	19	0	-	6	-
	1	0	19	0	-	6	-
	2	0	19	0	-	6	-
	3	0	19	0	-	6	-
	4	9	19	47	7	6	116
24	0	0 9*	17 11	0 (82)	- 7	5 8	- (88)
	1	0 3*	17 11	0 (27)	- 8	5 8	- (100)
	2	0 0 0	17 13 3	0 0 0	- - -	5 6 6	- - -
	3	0 0	13 3	0 0	- -	6 6	- -
	4	1 0 10	20 3 11	5 0 91	10 - 14	6 6 8	167 - 175
48	0	0	16	0	-	5	-
	1	0	16	0	-	5	-
	2	0	5	0	-	7	-
	4	0 0	16 16	0 0	- -	5 5	- -

*Abnormal hatching (see text)

(To face p.96)

filter paper was removed and remoistened.

C. pulicaris and *C. punctatus* eggs at various stages of development were subjected to this treatment for 12, 18, 24, and 48 hours. On removal from the desiccator, they were always greatly flattened, but on remoistening they soon regained their normal turgid appearance.

The results are shown in tables XII and XIII, dealing with *C. pulicaris* and *C. punctatus* respectively. In examining these and subsequent tables, it should be remembered that, as before, the times of laying and of hatching are only correct to within one day, so that the actual duration of the egg stage may have been up to one day greater or less than that shown. The following points may be noted:

(a) Most eggs of both species survived 12 hours, some 18 and 24 hours, but none 48 hours drying. Egg-groups surviving 12 hours drying did not seem to have been adversely affected by the treatment, for the total numbers of eggs which hatched in these groups were actually higher than in the corresponding controls, though the differences were not significant. The reverse was true of groups surviving longer periods of drying, in which the total numbers of eggs of both species hatching were significantly lower than in the corresponding controls ($P = 0.002$ for *C. pulicaris*, P = 0.001 for C. punctatus).

It will be noticed that in two treated C. pulicaris egg-groups hatching was 'abnormal'. In these groups no terminal cap split off, and the longitudinal split was shorter than usual. The larvae nevertheless seem to have succeeded in escaping, for the shells were empty; the word 'hatching' may thus justifiably be employed. Another abnormal type of split occasionally observed among treated eggs was one which extended the whole length of the egg. Living larvae apparently never emerged from such eggs, the contents of which appeared as a gelatinous mass; such eggs have therefore been entered in the tables as unhatched.

(b) There was a tendency for survival to increase with the age of the egg when treated. Thus C. pulicaris eggs survived 12 hours drying only when treated one or more days after having been laid, while 18 and 24 hours treatment was followed by normal hatching only if the eggs were at least four days old. Similarly, C. punctatus eggs survived 12 and 24 hours drying only when treated one or more days after laying.

To test the significance of this tendency a χ^2 test has been applied to the difference between T:C (in this case the number of treated eggs hatching relative to the number hatching in the corresponding control group) for the youngest and oldest eggs subjected to each treatment; when more than one egg-group of a given age was dried for a particular period the values for T and C have been totalled. With C.pulicaris eggs dried for 12 hours, for example, T:C for eggs treated on the day they were laid was 0:17, and for eggs 4 days old 39:37; the value of P obtained for the difference betwen them is 0.003, indicating that, relative to the numbers hatching in the corresponding control groups, significantly more treated eggs hatched in the latter than in the former case. Similar significant differences, with values of P of less than 0.01 are obtained with C.punctatus eggs dried for 12 and 24 hours. With C.pulicaris eggs dried for 18 hours the value of P is 0.02, indicating a suspiciously large difference. With C.pulicaris eggs dried for 24 hours P is 0.01 (significant) if the difference between the numbers of normally hatched eggs is estimated; if the abnormally hatched eggs are included the difference is not significant. Taken as a whole, these results thus indicate that the survival-capacity of C.pulicaris and C.punctatus eggs when desiccated increases significantly with age.

(c) Drying had the effect of delaying hatching;

only once when normal hatching occurred did it take place as early in the treated as in the control group. Considered collectively, moreover, the means of the differences in times of hatching were significant; for C. pulicaris $P = 0.008$, for C. punctatus $P = < 0.001$. The degree to which hatching was delayed did not, however, vary consistently with the duration of the treatment; possibly more precise data would reveal such a relationship.

Exposure to above-normal temperatures

Eggs to be exposed to above-normal temperatures were not removed from the petri dishes inside which they were normally kept on moist filter paper. The whole dish was placed in the upper compartment of a desiccator (not in this case employed as such) which was, and had for some hours been, inside an incubator set at either 30°C or 35°C. The lower compartment of the desiccator contained water, which kept the humidity high, and so prevented the filter papers on which the eggs were situated from drying. The treatment was timed from half an hour after the eggs were placed in the incubator to the time of removal. The temperature of the incubator did not vary more than $\frac{1}{2}$°C above or below that at which it was set.

The results of such treatment for 12, 24, and

TABLE XV.

The survival of C.punctatus eggs exposed to above-normal temperatures.

Number of eggs per group = 20.

Temperature	Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
			Treated (T)	Oontrol (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
30°C	24	0	16	8	200	3	5	60
		2	20	15	133	5	6	83
		4	19	19	100	4	6	67
	48	0	15	19	79	3	5	60
35°C	12	0	19	18	106	4	4	100
		4	20	19	105	5	5	100
	24	0	19	18	106	4	4	100
		2	18	15	120	5	6	83
		4	17	19	89	6	6	100
	48	0	8	20	40	6	5	120
		4	15	19	79	6	5	120

(To face p.100)

TABLE XIV.

The survival of <u>C.pulicaris</u> eggs exposed to above-normal temperatures.

Number of eggs per group = 20.

Temperature	Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
			Treated (T)	Control (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
30°C	12	0	8	15	53	6	5	120
		2	16	18	89	6	6	100
		4	15	15	100	5	5	100
	24	0	12	15	80	6	5	120
		1	19	19	100	6	6	100
		2	18	18	100	5	6	83
		4	20	20	100	6	6	100
	48	0	1	1	100	4	7	57
		3	3	1	300	6	7	86
35°C	12	0	20	18	109	6	5	120
		2	14	18	78	7	6	117
		4	20	18	109	6	5	120
	24	0	0	18	0	-	5	-
		1	1	19	5	9	6	150
		2	18	18	100	8	6	133
		4	19	18	106	7	6	117
	48	0	0	18	0	-	6	-
		4	0	18	0	-	6	-

(To face p.100)

48 hours at various stages of development are shown in Table XIV, dealing with C. pulicaris, and Table XV, dealing with C. punctatus. They bring out the following points:

(a) Exposure to 30°C for 12, 24 and 48 hours, and to 35°C for 12 hours, was always survived by eggs of both species. C. pulicaris eggs usually survived 24, but never 48 hours at 35°C; C. punctatus eggs always survived both 24 and 48 hours at this temperature. Among the egg-groups which survived treatment, the proportion of treated eggs hatching was significantly lower than in the corresponding control groups in only one instance - that of C. pulicaris eggs treated at 35°C for 24 hours one day after laying; the value of P was in this case less than 0.001. The proportion hatching was never significantly higher in the treated than in the control groups.

It will perhaps have been noticed that C. punctatus eggs appear to have been less affected by exposure to 35°C than C. pulicaris eggs. In the case of eggs kept at 35°C for 48 hours, for instance, the totalled values for T and C obtained with C. pulicaris were 0 and 36. With C. punctatus the corresponding figures were 23 and 39. Application of a χ^2 test to the difference between these two pairs of figures gives a value of P of less than 0.001. Similarly, the figures obtained with 24 hours treatment at 35°C were 38:73 for C.pulicaris and 54:52 for C.punctatus, for the difference betwen which P is 0.01. It thus appears that C.pulicaris eggs are significantly less adversely affected by exposure to 35°C than are C.punctatus eggs.

(b) The survival of C.pulicaris eggs tended to increase with their age when treated. Thus 24 hours at 35°C was survived only when the treatment took place at least one day after laying, and with one exception (12 hours treatment at 35°C) the value for T/C was maximal with the oldest, and minimal with the youngest eggs. The differences in the values for T:C, however, were significant only in the case of eggs treated for 24 hours at 35°C; here T:C for the number hatching when treated one day after laying was 1:19, while for eggs treated four days after laying it was 19:18, the value of P for the difference between these being 0.001.

No tendency for the survival-capacity of C.punctatus eggs to increase with age was apparent, except in so far as the proportion hatching when treated at 35°C for 48 hours was higher with eggs treated four days after laying than with those treated on the day of laying; the difference between the two values of T:C, however, was not significant, the value of P being 0.4. The absence of any significant correlation in this species could be explained on the supposition that the conditions to which the eggs were exposed were for the most part well below their limit of endurance.

(c) Exposure to 30°C tended to shorten the duration of the egg-stage, exposure to 35°C to lengthen it. This will be evident from the following figures, which are the average values for the mean duration of the egg-stage of eggs kept at each temperature, and the corresponding control values. The value of P for the difference between each pair of means, obtained by a Student's t test, is also given.

		Treated	Control	P
<u>C. pulicaris</u>	(30°C	5.6 days	5.9 days	0.4
	(35°C	7.2 "	5.8 "	0.007
<u>C. punctatus</u>	(30°C	3.8 "	5.5 "	0.008
	(35°C	5.2 "	5.0 "	0.6

It will be seen that only two of the differences are significant, these being those concerning the retardation of development of <u>C. pulicaris</u> eggs kept at 35°C, and the acceleration of development of C. punctatus eggs at 30°C. The errors to which the initial data are subject must, of course, be borne in mind, but taking these results as they stand, the indications are (i) that exposure to 30°C had no pronounced effect on the speed of development of C. pulicaris eggs, but accelerated that of C. punctatus eggs, and (ii) that exposure to 35°C retarded the development of C. pulicaris eggs but had no pronounced effect upon C. punctatus eggs. That acceleration of development, presumably an indication that the treatment had little or no harmful effect, was displayed to a significant degree only in C. punctatus, and that retardation, presumably indicating an injurious effect, was displayed only in C. pulicaris, is a further indication of the greater resistance to high temperatures of the former species.

As would be expected, there was a tendency in both species for the number of days by which the duration of the egg-stage was increased or diminished by exposure to high temperatures to increase with the duration of treatment, though the differences were not sufficiently large to be significant. There was no consistent relationship between the duration of the egg-stage and the age of the eggs when treated.

TABLE XVII,

The survival of *C.punctatus* eggs exposed to a dry atmosphere together with an above-normal temperature.

Number of eggs per group = 20.

Treatment	Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
			Treated (T)	Control (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
Drying at 30°C	12	0	0	20	0	-	5	-
		4	17*	19	(89)	5	5	(100)
Drying at 35°C	12	0	0	20	0	-	5	-
		4	0	19	0	-	5	-

*Incomplete hatching (see text)

(To face p.104)

TABLE XVI.

The survival of C.pulicaris eggs exposed to a dry atmosphere together with an above-normal temperature.

Number of eggs per group = 20.

Treatment	Duration of treatment (hours)	Time between laying and treatment (days)	Number of eggs hatching			Mean duration of egg stage (days)		
			Treated (T)	Control (C)	$\frac{T}{C}$ 100	Treated (T)	Control (C)	$\frac{T}{C}$ 100
Drying at 30°C	12	0	0	20	0	-	5	-
		4	0	19	0	-	6	-
	24	0	0	16	0	-	5	-
		4	0	20	0	-	6	-
Drying at 35°C	12	0	0	18	0	-	5	-
		4	0	18	0	-	5	-
	24	0	0	18	0	-	5	-
		4	0	18	0	-	5	-

(To face p.104)

Simultaneous exposure to a dry atmosphere and above-normal temperatures.

The proceedure in this case was identical with the drying proceedure except that the desiccator was inside an incubator set at 30°C or 35°C. Under these conditions the temperature of the eggs presumably rose more rapidly than when they were in a petri dish, the temperature of which also had to be raised from room level, and the higher temperature probably also increased the rate of drying. The duration of treatment was therefore timed from only 15 instead of 30 minutes after the eggs had been placed in the warm desiccator.

The results of such treatment for 12 and 24 hours are shown in Tables XVI and XVII dealing with *C. pulicaris* and *C. punctatus* respectively.

It will be seen that all egg-groups, with one exception, failed to hatch. Even when hatching began, it was not completed; larvae emerged half-way from the egg and then died. From a comparison of these results with those presented earlier it is evident that drying and a high temperature in combination are more harmful than is either alone. This is not necessarily an indication that exposure to one condition increased susceptibility to the other; it might be, for instance, that eggs dried at the higher temperature merely lost more water than those dried at room temperatures. Although too much emphasis should not be laid upon the results of a single hatching, it may be noted that these results are consistent with the conclusions reached earlier. Thus the one group which hatched was C. punctatus, and only when treated four days after laying; previous results indicated that C. punctatus was the more resistant species, and that survival-capacity increased with the age of the egg when treated.

The results of the few experiments performed on eggs of other species may be summarised as follows:

C. impunctatus eggs did not survive 24 hours drying when treated 0 and 7 days after laying.

'C. obsoletus' eggs exposed to 24 and 48 hours drying 0, 1 and 2 days after laying survived only once, i.e. when dried for 24 hours on the day they were laid. None survived after a 24 hour exposure to 35°C which commenced 0 and 1 days after laying.

C. grisescens eggs survived all drying treatments to which they were subjected. These were 24 hours drying 8, 10, 13 and 14 days after laying, and 48 hours drying 14 days after laying.

The only discernible difference between these results and those obtained with C. pulicaris and C. punctatus is that C. grisescens eggs, in being able to survive 48 hours drying, were apparently more resistent in this respect than either of the former species. The survival of only the youngest 'C. obsoletus' eggs when dried was not what would be expected from the results quoted earlier, but owing to its composite nature the different egg-groups may not have been the same species. Needless to say, the data are very incomplete, and further experiments would probably reveal further differences.

DISCUSSION.

The writer's experiences with the maintenance of adults in the laboratory were for the most part in conformity with those of previous investigators. One notable discrepancy concerned the duration of the post-oviposition life of 'C. obsoletus' females; while the writer found this to average over a month, Hill (loc cit.), keeping the females under similar conditions, found oviposition invariably to be followed by death. The multispecific nature of 'C. obsoletus', however, makes it possible that

Hill was not dealing with the same species as the writer.

The composite nature of '<u>C. obsoletus</u>' may also account for eggs of this species-group observed by the writer having differed markedly in appearance from the description given by Hill. Whereas Hill found the sucker-like structures to be borne on exceptionally long stalks, the writer, it will be remembered, found them, when present, to be either similar in size to those of <u>C. impunctatus</u>, which Hill also describes, or smaller.

Another discrepancy concerns the structure of <u>C. impunctatus</u> eggs; the mean dimensions given by Hill (length 0.49 mm., width 0.08 mm.) lie right outside the size-range encountered by the writer in this species. This appears too wide a difference to be accounted for by normal variability, but without having had an opportunity to check Hill's measurements, the writer hesitates to emphasize it; some similar differences in larval head measurements discussed by Kettle and Lawson (1952) proved, on re-examination of Hill's material, to have resulted from errors in measurement.

A larval pseudopod similar to that described by the writer in '<u>C. obsoletus</u>' has since been observed by Lawson (1951) and Jobling (1953) in first instar larvae of <u>C. nubeculosus</u> and <u>C. vexans</u>, respectively, so it is evidently not confined to the *C. obsoletus* group. Lawson found the pseudopod to be absent from the second, as well as later larval instars, in *C. nubeculosus*, but the second instar larva of a species belonging to the *C. obsoletus* group has apparently still to be observed. Kettle and Lawson (1952) state that it is absent in *C. halophilus*, and the writer, it will be remembered, found it to be absent from *C. grisescens* also. The comparatively distant relationship between the species in which the pseudopod has been observed, however, leads one to suppose that it will subsequently be found in other species.

In overwintering in the egg stage, *C. grisescens*, so far as is known, is unique among species of *Culicoides*. The only record of an egg-stage of comparable longevity is that of Jobling (1928) for *C. vexans* eggs, but in this case the egg-stage occupied the summer months. *C. grisescens* eggs also differed from eggs of other species investigated in that those kept out of doors, where the temperature was often at or below freezing point, hatched earlier than those kept in the laboratory, where the temperature never dropped below 6°C. This acceleration of development resulting from exposure to low temperatures suggests a diapause comparable to the classical case of the overwintering eggs of the silkworm, *Bombyx mori* (Duclaux, 1869, cited by

Wigglesworth, 1950). Bombyx eggs will complete their development and hatch only if they have been exposed to temperatures near freezing point for a certain period. In C. grisescens, apparently, development was not prevented, but merely delayed if the eggs were exposed to temperatures of +6°C to +21°C, instead of -2°C to +17°C.

The interval of only a few days between laying and hatching found with all species other than C. grisescens indicates that these species overwinter either as larvae or pupae. Admittedly eggs were not in every case obtained at the end of the respective adult seasons, but there is no evidence to suggest that any species produces different types of egg at different times of year. The pupal stage of C. pulicaris, 'C. obsoletus' and C. impunctatus has been observed to be last only a few days, indicating that these species overwinter as larvae. The same may be true of the remaining species, and the fact that a pupal period of a similar duration has been recorded in all species of Culicoides on which the appropriate observations have been made makes this likely. That 'C. obsoletus', C. impunctatus, and C. pallidicornis overwinter as larvae is borne out by Hill's observations on the life history of these species.

If an attempt is made to relate the results of experiments on the viability of Culicoides eggs to conditions in nature, the difficulty at once encountered is our ignorance of the precise microclimatic conditions to which the eggs are exposed. The humidity of the microclimate must depend on the amount of moisture in the vicinity, on local air-movements, and on the temperature; the temperature will depend on the amount of heat absorbed and radiated by the substratum, on its specific heat and conductivity, and on the extent to which complicating conditions such as a high rate of evaporation or bacterial fermentation are operative.

The only available guide to the situations in which the eggs are laid in nature is that provided by the habitats from which larvae have been recovered and adults reared. Even for a single species, these vary considerably. Larvae of C. pulicaris, for instance, were obtained by Kettle and Lawson (loc cit.) in largest numbers from a marshy meadow site in which the water level was not above the surface of the soil, but were also obtained from inundated swampland and from areas of bare mud "which undoubtedly dried up in summer". The writer has bred out adults of the same species from leaf debris, either submerged or just above water level, from damp moss on the banks of a stream, and from submerged mud in a pond receiving the drainings of cattle manure. C. punctatus larvae were found by Kettle and Lawson in the same habitats as those in which they found C. pulicaris, and the writer has bred out the species from damp decaying leaf debris and from the cleanings of a hen-house i.e. damp mud containing a high proportion of fowl droppings.

Almost all of the many C. impunctatus larvae collected by Kettle and Lawson were recovered from boggy moorland where the ground was covered by a layer of Sphagnum moss. Hill obtained considerable numbers from damp peaty soil which was firm enough to walk upon throughout the summer and most of the winter, and the writer bred the species out from damp decaying leaves lying on the surface of firm non-marshy soil.

The larvae of 'C. obsoletus' have been differentiated into species by Kettle and Lawson, who found C. obsoletus sensu stricto mainly in marshland in association with C. pulicaris and C. punctatus, and in Sphagnum bog in association with C. impunctatus. C. chiopterus and C. pseudochiopterus, however, were found in none of these sites, being recovered only from dry cakes of cattle dung lying naturally in the field. "In the later stages" say Kettle and Lawson, "this must be one of the driest habitats occupied by Culicoides larvae". Hill obtained 'C. obsoletus' from soil which was more marshy than that from which she obtained C. impunctatus, and the writer bred adults from farmyard mud containing a high proportion of cattle dung, and from damp decaying leaves or bracken overlying firm non-marshy ground.

C. grisescens larvae have been obtained in nature only by Kettle and Lawson, who found a very small number of them in the mud among semi-aquatic plants at the margin of an area of open water.

Common to all these habitats is the presence of a certain amount of moisture, an obvious necessity for aquatic larvae, but it will be evident that several of them are of a highly impermanent nature. Moreover, the habitats described are ones from which larvae have been recovered or adults reared and it is possible that the eggs are sometimes laid in other situations so unfavourable that the larval stage is never reached. It is therefore feasible that some mortality due to desiccation occurs among the eggs in nature, though it must be remembered that in Britain the atmospheric humidity is never as low as that in a calcium chloride desiccator, and that the eggs are therefore presumably able to survive longer than the maximum periods - 24 hours in the case of C. pulicaris and C. punctatus - for which they survived in the laboratory.

An effect which presumably prolongs the period of exposure of the eggs to dry conditions is the delay in hatching caused by drying observed in the laboratory. Although this must increase the danger to the eggs it is presumably beneficial to the species, for there is little doubt that they are more resistant to drought than the wholly aquatic larvae.

In general, any mortality that occurs, especially among eggs which hatch only a few days after laying, seems likely to be due less frequently to desiccation alone than to the combined effects of desiccation and high temperature; laboratory results showed the latter combination to be the more rapidly fatal condition, and drying out of the breeding sites probably tends to occur mainly during spells of hot dry weather. Air temperatures in Britain, so far as these may be used as a guide to those to which the eggs are exposed, hardly ever rise to 35°C (95°F) and seldom to 30°C (86°F); when they do this temperature is never maintained for more than a few hours. It therefore seems probable that conditions approaching the combinations of heat and drought which caused death within 12 hours in the laboratory are seldom likely to be encountered in nature, and never for prolonged periods. It is, however, quite possible that during hot dry weather, when the eggs are exposed to drought and, intermittently, to heat several days in succession, some mortality due to the combined effects of these two conditions occurs.

C. grisescens eggs, with their prolonged period of development through the winter months, are perhaps more likely than the other species investigated to suffer from the effects of desiccation unaccompanied by those of high temperature, and it will be remembered that there was an indication that this species was more resistant in this respect than the remainder. Diminishing the danger of desiccation, however, is the fact that during the winter the evaporating power of the air is low.

In the mild climate of Britain, the capacity of moist C. pulicaris and C. punctatus eggs to survive 48 hours, and probably longer at 30°C, and 24 or sometimes 48 hours at 35°C, is rarely, if ever, likely to be brought into play; high temperatures probably never cause mortality among eggs of these species unless combined with desiccation.

The preceding discussion is to a regrettable extent hypothetical, and a fuller integration of the laboratory results with conditions in the field can only be made when more is known of the microclimatic conditions to which the eggs are exposed in nature.

PART II.

A STUDY OF THE SELECTION OF THE BREEDING SITE BY THE FEMALE TSETSE FLY *GLOSSINA PALPALIS* ROBINEAU-DESVOIDY.

INTRODUCTION.

As is well known, tsetse flies are viviparous, producing only a single larva per pregnancy. Immediately after extrusion the larva attempts to burrow, and shortly afterwards - it may be several hours if it has not succeeded - becomes inactive and pupates. The pupal period is of several weeks duration and the literature on tsetse breeding habits is therefore based largely on pupal collections.

Observations relevant to the present investigation will now be described.

The breeding habits of G. palpalis.

The first investigator to find the pupae of any species of Glossina in nature appears to have been Bagshawe (1908), who writes of G. palpalis in the neighbourhood of Lake Victoria that "the larvae are dropped in the shade, it may be of shrubs, it may be of bananas, within 45 yards of water". The same year Zupitza (1908), working in the Cameroons, claimed to have found pupae of this species in the damp humus and moss in tree forks, cracks in the bark, and the angles of palm frond sheaths.

Carpenter (1912) found a particularly favoured breeding site to be on the edge of a belt of vegetation on the shore of Lake Victoria, pupae being mostly buried in the coarse sand in the shaded areas under the bushes. The ground beneath fallen trees, especially in places where the trunk made an angle with the ground, was also popular. All pupae were in situations where there was shade for all or a major part of the day. Although close to the water's edge, they were always "in the driest possible situation".

Carpenter utilised the availibility of a highly favoured breeding site to observe the act of larviposition under natural conditions. During the course of several periods of watching from 0900 to 1315 hours, he saw eight larvipositions, all of which occurred between 1045 and 1300. "Until about 10" he writes, "one sees no pregnant flies; then they begin to appear and rest on the pebbles, and towards mid-day they come in increasing numbers; I have seen eight resting within a square foot. But very often one sees no larvae extruded; presumably certain atmospheric conditions are required to complete the stimuli necessary.

"The actual extrusion is a very quick process; after the hinder extremity of the larva is free on the ground, the female takes a step forward as if to draw out the rest of the larva, and then remains motionless some fifteen minutes before flying away. The larva at once sets to work to bury itself, which it does within a minute or two".

Carpenter also states "Whilst watching flies deposit larvae at favourite spots, I have never seen one do so when in the sun; if it alighted first in the sun it always moved into a shady spot before extruding the larva".

In a second report on G. palpalis in the same region, Carpenter (1913) states that when ideal breeding sites are not available deposition may take place in any nook which offers the requisite shelter. When no loose dry soil was available, for example, pupae were found at the base of grass tussocks where the soil was quite damp.

Yorke and Blacklock (1915), working on a peninsula near Freetown, Sierra Leone, found breeding to be practically confined to the soil within one foot of the base of young oil palms. Long fronds projected out horizontally to a distance of three feet or more from the trunks of these, and frequently curved down to the ground forming an umbrella which afforded favourable breeding conditions. No pupae were found under palms which had lost their fronds. The soil was dry and sandy, with some fine gravel and leaf debris in the upper layers. Breeding was not confined to the vicinity of the water's edge and in places occurred over a mile from the shore.

Duke (1919) and Fiske (1920), working on the shores of Lake Victoria, found G. palpalis pupae in sites similar to those described by Carpenter (1912), and Fiske also found them in fine dry vegetable debris under over-hanging rocks, ferns, and other sources of shade. Places where antelope were in the habit of sunning themselves were also utilised, the pupae being found in large numbers in the shade of nearby dead vines, fallen logs etc.

Working in Northern Nigeria, Johnson and Lloyd (1923) found G. palpalis pupae in various situations in the sand of partly dried stream-beds. There was usually low overhead shade but in a few cases the only shelter was high and scanty. During the rains they were unable to find any pupae, but

Pomeroy and Morris (1932), working in the Gold Coast, claim that breeding is mainly confined to this season, the sites occupied being different from those utilised in the dry season. During the latter, they maintain, the favoured sites are in the shade of the dense vegetation overhanging the partly dried water courses, while in the rains patches of thick bush at considerable distances from the rivers are occupied. Stewart (1937), on the other hand, claims to have found that when the Nabaggo River, also in the Gold Coast, was flooded and the lower part of the riverine vegetation was submerged, breeding took place in cracks and holes in the tree trunks. As details of the results on which these claims are based are not given, it is difficult to appraise them.

According to Swynnerton (1936) some degree of seasonal variation in the sites occupied by G. palpalis occurs during the course of the dry season in East Africa, for he states that at the time of general leaf-fall, breeding, previously relatively widespread, becomes concentrated in thickets which are still leafy.

Symes (1935) thoroughly searched 1200 square yards of ground beside the Kuja River in Kenya. He found that G. palpalis pupae were widely scattered through the forest floor and only a minority were under the numerous fallen logs available. As reported later (Symes and Vane, 1937), most were in places where there was moderate shade at a fairly high level and the soil was light, friable, and covered with vegetable debris.

In a later paper Symes and Southby (1938) point out that recently searched places appeared to become more attractive as breeding sites than they were previously. Working on the shores of Lake Victoria they were able to construct effective artificial breeding sites consisting of branches a few inches in diameter arranged so that various portions were about an inch above an area of well-turned soil. In such a site a female was observed to larviposit from a position on the ground underneath one of the branches.

The recently published results of an extensive study on the ecology of G. palpalis in Northern Nigeria (Nash and Page, 1953) are of particular relevance here as the investigation was carried out in the locality in which most of the writer's observations were made. Standardised monthly collections made around a selected stream throughout the year yielded pupae only during the dry season and early rains, the peak occurring about December, in the middle of the dry season. Nash and Page point out that this must not be taken as an indication that little breeding occurs during the rains, as it may be due to the difficulty of finding the pupae when the soil is wet; that abundant breeding does occur during the rains seems likely from the great increase in the size of the adult population that occurs at this time.

The peak in pupal incidence which occurs in the middle of the dry season is explained by Nash and Page on the grounds that the soil, which at the beginning of the dry season is still damp, becomes progressively easier to search as it dries out. They also maintain the work of the searchers to be facilitated by a shifting inwards of breeding towards the stream-bed, so becoming more and more concentrated in relatively few well protected sites as the dry season advances. The decrease in pupal incidence which occurs in the late dry season on the other hand, is considered to be genuinely related to a decrease in adult density which occurs at that season.

In the above collections the sites searched were of the type which have become known as 'classical' i.e. beneath logs, leaning trees, low foliage and rocks, in hollow trees, on the forest floor and in dried up parts of the stream bed. All were within the fringing forest which flanked the stream on either side and formed the permanent habitat of the tsetse. Since such sites were unproductive during the rains, aerial sites such as rot holes in trees varying from 3 to 15 feet from the ground, and the axils of palm fronds, were searched during this season, but without success. 25 pupae and cases were, however, found just outside the forest, suggesting that the wet season breeding sites should perhaps be looked for in such situations rather than in sites occupied during the dry season. Sites on the edge of, and just outside the forest were subsequently searched one month after the rains ended, and 5 full pupae and 13 cases recovered, one site being under a small log overgrown with grass which was eight yards from the forest. Too much weight should not perhaps be attached to these results as no serious attempt to find whether similar sites were occupied during the dry season appears to have been made. Also, much of the evidence depends on the presence of shells, which could have been there since the previous dry season; Harris (1930) found pupal shells to be still recognisable after two years. The full pupae found a month after the rains, on the other hand, might have been deposited since the rains ended. From indirect evidence Nash and Page conclude that wet season breeding must mainly occur fairly close to the stream, for the fly population of the lower reaches of the stream was found to be more affected by exceptionally heavy rains than the upper. The most obvious difference between the upper and lower reaches was that the latter were more liable to flooding, and this, they suppose, must have destroyed many pupae; it could only have done so if breeding was confined to the immediate vicinity of the forest.

<u>The breeding habits of species other than G. palpalis</u>.

One of the most investigated species of <u>Glossina</u> is <u>G. morsitans</u> Westwood, a species of immense importance in the transmission of animal trypanosomiasis. The breeding sites of this species, which is less confined to the vicinity of water than is <u>G. palpalis</u>, appear to have been first described by Ll. Lloyd (1914), who in Northern Rhodesia found pupae beneath fallen logs and leaning trees, in the hollows in trees, and in animal burrows. All offered relatively dark places where the female could rest concealed before larvipositing, and this seemed to Lloyd to be the primary condition sought by her ~~female~~, for the sites were often places where the larva was unable to burrow into the substratum. More pupae were to be found in breeding sites situated near to native and game paths, fords and drinking places, than elsewhere.

Lamborn (1915), working in Nyasaland, found

G. morsitans breeding in sites similar to those described by Lloyd, but unlike the latter author considered the condition of the soil to be an important factor influencing the female in her choice of site. Few pupae will be found, he claims, unless the soil is dry, has no hard top crust, and contains sand and decaying vegetable debris to make it light.

Seemingly in support of Lloyd's view is Simpson's (1918) observation of a female G. morsitans larvipositing from a position on the underside of a tree trunk about an inch above the ground; unless she had previously explored the latter it would seem unlikely that she was influenced by its nature.

Swynnerton (1923b) describes a case where a large number of G. morsitans pupae were found in completely open ground with no shade of any kind, the site being one where game were in the habit of rolling and playing. This situation, of course, was highly unfavourable for the pupae, a large proportion of which were dead; it was the more surprising in view of the availablity of sheltered breeding sites in the vicinity from which further pupae were recovered. A possible explanation would seem to be that the ingestion of a blood meal precipitated larviposition by certain females already on the verge of this event.

Jack (1919) and, later, Chorley (1929) draw attention to seasonal changes in the breeding sites of G. morsitans in Southern Rhodesia, pointing out that during the late dry season when most trees are leafless, breeding becomes concentrated around the evergreens. At other times of year it occurs mainly in the deciduous woodland.

Nash (1933) constructed artificial breeding sites which proved attractive to G. morsitans in Tanganyika. They consisted of felled logs mounted so that they were a few inches above the ground. The soil beneath was broken up. Such traps became far more effective if the bark was stripped off and laid along the sides, leaving only small apertures which from outside appeared as black windows. As long as the soil underneath the log was loose its type did not seem of importance, for the highly friable humus-rich soil from under an old log appeared to be no more attractive than newly broken soil. When the soil was very hard no pupae were found, but as Nash points out, this may have been because larvae deposited there, being unable to burrow, were destroyed by predators. Where there was uncut grass beneath the log, the soil again being hard, some pupae were found at the bases of the grass stems.

These observations lend support to the view that the type of soil is not of primary importance in determining the choice of larviposition site.

Later, working at Gadau in Northern Nigeria, Nash (1937, 1939) obtained clear evidence of a seasonal shift in the breeding of G. morsitans. Throughout most of the dry season it bred almost exclusively in small isolated thickets, but that towards the end, when the temperature became high, most of the breeding shifted into the forests which existed in the form of strips along the edges of the river and as islands surrounded by the more open woodland. During the rains breeding once again shifted, the sites then occupied being the ground beneath logs and palm fronds in the open woodland.

Measurements of the temperature, evaporation rate, and soil moisture-content in breeding sites of each of the above types throughout the year indicated that conditions in them varied greatly, even when only the most popular site at any one season was considered (Nash, 1939). Admittedly the highest concentrations of pupae occurred in the driest available situations during the heavy rains, when Buxton and Lewis' (1934) results, also obtained at Gadau, indicated there was a danger of pupal mortality from waterlogging, and the highest concentrations occurred in one of the coolest situations at the end of the dry season, when the same author's results indicated there to be a danger of death from high temperatures, but this does not appear necessarily to indicate that these sites were selected because they possessed such properties. It seems that the observed shift in the breeding grounds may, in fact, result from a shift in the adult population not primarily connected with the selection of breeding sites. On this view the microclimatic conditions of the breeding sites may be of little importance, and breeding may take place at any convenient site within the current habitat of the adult population. The question of what conditions or changes in conditions are responsible for seasonal movements of the adult population as a whole is not directly relevant here, but it is interesting to note that Nash (1937) found a correlation between evaporation rate measured four feet above ground level and fly movement; maximum dispersal occurred about a month after the time of minimum evaporation, and maximum concentration about a month after the time of maximum evaporation.

Soil analyses (Nash, 1939) indicated that breeding took place in a wide variety of types. Samples taken from breeding sites at Gadau varied from fairly heavy clay through various types of loam to coarse sand. Organic matter was abundant in some cases, absent in others. Most but not all were slightly alkaline. The water-holding capacity, sticky point, and moisture equivalent all varied widely. It is thus evident that with regard to the conditions assessed, no marked selection of any particular type of soil was made by the depositing females.

Subsequently Nash (1942) attempted to elucidate the cause of a relatively small scale but well-defined shift in breeding from the edge to the centre of a forest island which occurred at the end of the dry season. He came to the conclusion that the most likely explanation lay the negative light reaction demonstrated experimentally by Jack and Williams (1937). These authors found that while G. morsitans was photopositive at medium temperatures, this reaction was reversed above 32°C., the photonegative reaction becoming progressively stronger as the temperature rose. At Gadau, where Nash was working, shade temperatures at the forest edge were above 32°C. for eight to nine hours each day at the end of the dry season, and the photonegative reaction might thus be expected to have caused a retreat into the centremost, and therefore darkest parts of the forest, during these periods. As the temperature remained high until after dark, by which time the flies were inactive, they probably tended to remain in the inner region during the night. This could account for the observed shift in breeding, especially as Potts (1933) found larviposition to occur mainly in the late afternoon and night. This explanation, which implies only a very temporary movement on the part of the adult population, would seem to apply only to seasonal movements of a limited type. It does not appear to explain, for instance, the shift in breeding from small thickets to forest islands which occurred slightly earlier in the dry season, for both of these sites offered dense shade and presumably appeared dark in comparison with the surrounding woodland.

Jack (1939) carried out numerous laboratory experiments on G. morsitans in East Africa, including one which appeared to indicate that females about to larviposit were not especially attracted to shade; when pregnant females were placed in the light section of a box of which half was in the light and half in the shade - presumably at a temperature at which the normal reaction was photopositive - they showed no tendency to enter the dark section before larvipositing.

G. tachinoides Westwood, a species of great importance in the transmission of human sleeping sickness in West Africa, like G. palpalis, is mainly confined to the vicinity of water and the riverine vegetation which accompanies it. Simpson (1918) found pupae of this species most commonly in heavily shaded positions beneath overhanging trees whose foliage was apparently fairly near the ground, but Johnson and Lloyd (1923) found the majority in places where there was no low growth but only high shade. Pomeroy and Morris (1932) claim that although breeding was confined to the vicinity of rivers and streams, pupae might be found almost anywhere within this habitat.

The investigations of Nash (1937, 1939, 1942) at Gadau were carried out on *G. tachinoides* as well as on *G. morsitans*, and in the main the observations quoted and the discussion arising from them apply equally to both species. To avoid reiteration, all that need be said here is that *G. tachinoides* exhibited a seasonal movement similar to that described in *G. morsitans* except for certain differences presumably resulting from its greater dependence on the proximity of water; of these the chief appears to be that at the end of the dry season most of the breeding of *G. tachinoides* shifted from the forest to the willow-shaded margin of the river-bed instead of remaining predominantly at the former site as did that of *G. morsitans*.

Apart from those already mentioned, the commonest species of Glossina in West Africa is probably G. longipalpis Wiedemann. Its breeding habits have nevertheless been little studied. Pomeroy and Morris (1932) found pupae under logs in the heavy vegetation on the banks of the River Volta, and Morris (1934) found breeding occurring around the bases of old palms in an area of low but thick bush near the sea coast.

The East and Central African G. pallidipes Austen is undoubtedly very closely related to G. longipalpis, and possibly ought to be considered a sub-species of the latter (Vanderplank, 1949). Swynnerton (1921) describes the occurrence of small numbers of G. pallidipes pupae under fallen trees in fairly open woodland along with G. morsitans, while Harris (1930) found them in evergreen thickets near streams, game paths, and watering places. Within these thickets the pupae were mostly immediately beneath any branches which were close to the ground. In all sites examined chequered sunlight filtered through to the ground for part of the day. The presence or absence of a leaf carpet seemed of little importance, and Harris expresses the opinion that in selecting a breeding site the pregnant female is affected merely by the shade conditions it affords.

The East African Tsetse and Trypanosomiasis Research and Reclamation Organisation Annual Reports for 1949 and 1952 record a seasonal shift in the breeding of G. pallidipes, which was found to occur under deciduous bushes some distance from the rivers during the early dry season, and to shift to evergreen riverine sites when the weather became hotter. The wet season breeding sites were not discovered.

G. swynnertoni Austen, another important East African species, is described by Swynnerton (1923a, 1936) as breeding under leaning tree trunks, woody scrub and large rocks, at the bases of standing trees, and in tree holes up to six feet from the ground. The substratum was always dry. H. Lloyd (1935) maintains that this species breeds mainly in thickets, about the floor of which pupae are fairly widely scattered. A seasonal shift in breeding is recorded in the E.A.T.T.R.R.O. Annual Report for 1951, pupae being said to be found under logs at the beginning of the dry season, later to concentrate under deciduous bushes, and finally to withdraw to evergreen riverine sites at the end of the dry season.

Burtt (1952) looked for the wet season breeding sites of *G. swynnertoni*, and found pupae in sites similar to those occupied during the earlier part of the dry season viz. under logs and in deciduous thickets, though usually slightly more in the open. The most distinctive feature was that whereas during the dry season pupae were mostly found at a depth of approximately half an inch, they were now on or near the surface; this was understandable from the laboratory finding that the larvae experienced great difficulty in burrowing in wet soil. Burtt also found that the time of larviposition varied according to the circumstances in which the females were placed. Thus the time of maximum deposition when *G. swynnertoni* females were enclosed in glass tubes was 4 to 6 p.m., whereas when they were kept in jars with dry soil in the bottom it was 1 to 2 p.m. Females transferred from the tubes to the jars of soil were several times observed to deposit within a few minutes, but this did not occur when the soil was wet. With one exception all depositions observed in the laboratory took place while the female was resting on the floor of the receptacle. When this was dry soil the female usually remained motionless for several hours before depositing, but when no soil was present the flies were more restless. One deposition was observed in nature, and this also took place on the soil surface after the female had apparently remained in almost the same position for two and a half days.

The breeding habits of the remaining species have been relatively little investigated, and a list of the observations made on them would serve no useful purpose in the present context.

The behaviour of the larva.

Although not primarily the subject of the present investigation, the behaviour of the larva obviously affects the final situation of the pupa, and so should be considered in conjunction with the behaviour of the larvipositing female.

It is a matter of common observation that as soon as it is deposited a tsetse larva attempts to burrow. If it does not succeed it may crawl a distance of several feet, making repeated attempts as it does so. Several authors have observed that failure to burrow results in a postponement of pupation. Thus Potts (1933) found that G. morsitans larvae which had successfully burrowed became rigid and motionless within 15 minutes, while those which had not sometimes remained active for an hour. Even with buried larvae, however, chitinisation of the puparium was not complete until after at least eight hours. Lewis (1934) observed a larva which, though still alive, had not pupated after three days, but as he points out, it may have been deposited prematurely.

A few observations on the reaction of the larva to light have been made. Mellanby (1936) states that G. palpalis larvae did not react to a beam of light, while Burtt (1952) found that G. swynnertoni larvae responded negatively to unidirectional daylight; he did not, however, obtain any reaction to a beam from an electric torch. Further information on this point seemed desirable, for a photonegative reaction could conceivably be of importance in preventing larvae deposited within a shaded area but not immediately able to burrow, from leaving this area.

It was apparent from literature reviewed above that the breeding sites occupied by Glossina varied greatly, even within a species. The presence of a certain amount of shade appeared to be an almost constant feature, though there was no clear indication of whether this was attractive because of its low light intensity relative to unshaded regions, or because of the climatic conditions associated with it. The type of soil was one of the most variable features, and several authors were of the opinion that any selection of breeding site that took place was made from the point of view of its suitability as a resting place for the mother rather than as a habitat for the early stages. In the specific case of G. palpalis in Northern Nigeria, little was known about seasonal changes in breeding habits, and further investigation into the sites occupied during the rains was clearly called for. Further information on the behaviour of the larva - in particular on its reaction to light - was also desirable.

MATERIALS AND METHODS.

All the laboratory experiments to be described involved the use of G. palpalis females in the last stages of pregnancy. These were selected daily from laboratory stocks, females likely to deposit within the next 24 hours being recognisable by their swollen white abdomens, with the black respiratory lobes of the larva showing through the body wall at the hinder end. Such females were offered a final blood-meal and then used in experiments. The following morning any females which had larviposited were collected and returned to the main stock. Those which were still pregnant were left in the experimental chamber unless they showed any inclination to attack the collector, in which case they were given another blood meal and then returned to the experiment, providing, of course, this was still in progress.

A serious handicap to the work was the limited supply of females in the required condition, for larviposition occurred only at approximately ten day intervals, and at least 100 females were needed for most experiments. Single experiments therefore extended at least over several days, and in the early stages, before the fly-breeding unit was properly established, over as long as three weeks.

In most experiments females which had been captured in the field were used, but these were occasionally supplemented by laboratory-bred specimens. The temperature and humidity conditions under which the stocks used were kept were variable, but seldom outside the ranges 24-28°C. and 70-95% R.H. (1-9 mm. S.D.).

Details of the techniques of investigation employed will be given in the sections to which they are relevant.

THE CLIMATE AND VEGETATION OF THE KADUNA AREA.

A clear picture of the climate and vegetation of the locality in which the investigation was carried out is essential to a proper appreciation of the results presented in succeeding sections. Since Kaduna is only about 20 miles south of the northern limit of <u>G. palpalis</u>, the climatic conditions described may presumably be taken as being, in some respects at least, as severe as can be tolerated by this species.

<u>Climate</u>.

This account is largely based on that of Nash and Page (1953), who took meteorological readings at Katabu, near Kaduna, over six successive years. Those utilised in this context were taken in a Stevenson Screen four feet above ground level in open woodland.

<u>The dry season</u>.

The beginning of the dry season: The rains usually terminate about the middle of October, following which the weather tends to be both hot and humid for a few weeks. During this time daily maximum temperatures are in the region of 30-33°C., and minimum temperatures in the region of 19-22°C. The daily maximum relative humidity (actually the relative humidity at the minimum temperature, but the two figures are almost identical) is around 90% and the minimum relative humidity (actually the relative humidity at the maximum temperature) around 45%;

in terms of saturation deficit , the range is from 1-2 mm. to 19 mm.

The early or cold dry season (November to January): During November the south-west monsoon gives place to the Harmattan, a dry dust-laden wind which approaches Nigeria from the Sahara. The humidity then falls, continuing to do so until January, when the maximum and minimum humidities are around 50% and 20% R.H., or 6 mm. and 27 mm. S.D. Maximum temperatures remain at approximately the same level as at the beginning of the dry season except when the Harmattan haze is very thick, in which case they may be as low as 25°C. Minimum temperatures are much lower than previously, being in the region of 12-13°C, and sometimes even as low as 6°C. During the six years of Nash and Page's observations no rain fell during the months of November, December, or January.

The late dry season (February and March): During February the temperature starts to rise, and in March the highest maxima of the year usually occur, a mean monthly maximum of 35.8°C. and an absolute maximum of 39.4°C. having been recorded. March was also the month in which the lowest humidities were recorded, these being sometimes as low as 10%R.H. (37 mm. S.D.). A little rain sometimes fell, but over six years the average rainfall for February was only 0.1 inches, and for March only 0.5 inches.

The wet season.

The early rains (April and May): Day temperatures at first remain high and the nights become very much warmer, resulting in the highest mean monthly temperatures of the year viz. in the region of 27-28°C. The prevailing wind is now the south-west monsoon, and this causes a great rise in humidity, the maximum and minimum values for which are by late May around 90% and 55% R.H. or 2 mm. and 14 mm. S.D. By this time temperatures have also fallen, and the maxima and minima are now in the region of 28-31°C. and 21-23°C. respectively. Rainfall is still fairly light, the average over six years being 2.5 inches for April and 5.0 inches for May.

The heavy rains (June to September): The rainfall increases, and as it does so the humidity continues to rise and the temperature to fall. The heaviest rainfall occurs in August or September, in which months it averaged 11.7 and 9.3 inches respectively. By this time maximum and minimum temperatures are in the region of 25-29°C. and 20-22°C, and the maximum and minimum humidities are of the order of 90-100% and 60-80% R.H., or 0-2 mm. and 5-12 mm. S.D.

The end of the rains: The rains usually cease abruptly during October. Shortly before they do so day temperatures rise, and may sometimes reach 35°C. The humidity remains at a high level but the high day

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Fig. 17. Savannah woodland, photographed near Kaduna at the end of the rains.

temperatures result in correspondingly lower minimum relative humidities and higher saturation deficits.

Vegetation.

This has also been described by Nash and Page (1953), whose account has again been drawn upon.

Kaduna is situated in the more northern part of the Guinea Savannah vegetation zone (Rosevear, 1953), and the characteristic vegetation consists of fairly open savannah woodland with grass growing between the trees. (Fig. 17). The trees are mostly small and of twisted habit, the main species being Isoberlinia doka. The grass attains its maximum growth shortly after the end of the rains, when it is in most places about 4-5 feet in height. During the dry season much of it gets burnt, leaving the ground between the trees an almost bare carpet of ashes until the new shoots appear; this happens within a very short time. Most of the trees shed their leaves during the dry season, and the new leaves, like the new grass shoots, appear in the later part of this season, not, as might be expected, after the commencement of the rains.

Intersecting the woodland are streams fringed by forest. This fringing forest, which forms the permanent habitat of G. palpalis, is characterised by an evergreen

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Fig. 18. Fringing forest, near Kaduna, October.

Fig. 19. Transitional vegetation, near Kaduna, October.

understorey of shrubs and climbers, with emergent trees of savannah affinity above. In many places the overhead canopy is thick enough to suppress almost all undergrowth, and the ground is then bare except for small seedlings and dead leaves. There is no grass (Fig. 18).

The junction between the forest and the woodland may be quite sharp, or there may be a belt of transitional vegetation of considerable width. Transitional vegetation, as the term is used here, is characterised by the presence of large emergents which are more widely spaced than in the forest. In consequence, there is a profusion of shrubby growth with some low grass. There are thus more low shrubs than either the forest or the woodland, while the grass, like the tree canopy, is in an intermediate condition (Fig. 19).

STIMULI INVOLVED IN THE SELECTION OF THE BREEDING SITE

The degree to which various substances or objects were favoured as breeding sites was tested by placing them on the floor of a room into which pregnant females were liberated. Selection in favour of any object was indicated by the presence in or around it of pupae. The room, which was 10'3" by 10'3" and 8'0" high, was designed for work at constant temperatures. Its walls were therefore well insulated, and diurnal fluctuations in temperature and humidity were almost completely eliminated. Over a whole year the temperature varied between 24.0°C. and 28.0°C., and the humidity between 42% and 88%, or 2.8 and 14.6 mm. S.D.

The walls and ceiling of the experimental room were cream-distempered. One of them contained a slightly recessed door of sand-fly netting stretched on a wooden frame. To make it uniform in appearance with the walls this was covered with cream-coloured paper, except for a small observation window in the upper half, and a sleeve in the lower; the latter enabled tsetse to be introduced into the room without opening the door. The floor was of concrete, and also fairly light in colour. Along one side of it was a drain covered over with light brown paper flush with, and stuck down to, the floor.

Illumination was from a 100W tungsten filament bulb, with reflector above, situated in a recess in the centre of the ceiling. Below it, and flush with the ceiling, was a piece of glass which prevented females at large in the room from entering the recess. Lying on the upper surface of the glass was a piece of tracing paper which diffused the light. Except where otherwise stated, the room was illuminated for approximately the same number of hours as there was daylight outside; in practice the light was switched on approximately one hour after sunrise, and off approximately one after after sunset.

A small vestibule was built outside the door of the experimental room so that any one who had been inside the latter could, if necessary de-fly himself before leaving. In the first few experiments a thermo-hygrograph was placed in this vestibule on the assumption that the temperature and humidity conditions it registered would be approximately the same as those inside the experimental room; later, the instrument was placed in the room itself.

Females in the last stages of pregnancy were introduced into the room daily, and each morning those which had deposited, together with still pregant females which showed any inclination to attack the collector, were collected by means of a hand-net, and removed. The latter were returned after they had received a blood meal. As soon as the numbers of pregant females introduced, and spent females collected, indicated that at least 100 depositions had occurred, the experiment was terminated. Pupae lying plainly visible on the floor of the room were collected daily, but those which were hidden were not normally collected until the end of the experiment, to avoid disturbing the test objects. The positions of all pupae were plotted on a scale plan.

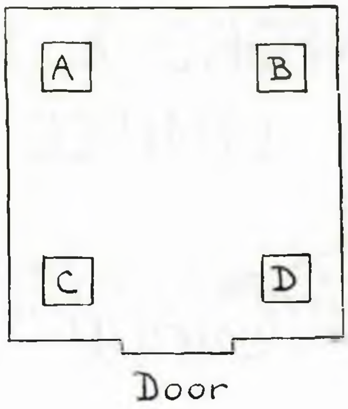
The first substance to which the response was tested was one frequently occurring in natural breeding sites, namely coarse dry river-bed sand. Four wooden trays were constructed, the dimensions of each being 18" by 18" by $3\frac{1}{4}$" externally, and 17" by 17" by 3" internally. Into two of these dry sand (i.e. sand of which the moisture content was in equilibrium with the atmosphere of the experimental room) was placed to a depth of $\frac{1}{2}$", while the other two were left empty. The four trays were placed in corner positions on the floor of the experimental room, the outer corner of each tray being 18" from the appropriate corner of the room. The two sand-containing trays, like the two empty trays, were diagonally opposite each other.

The following result was obtained:-

Total no. of pupae = 104
Pupae in sand-containing tray = 40
" " " " " = 33

Pupae in empty tray = 2
" " " " = 1

Application of a χ^2 test showed the difference between the total number of pupae in the sand-containing trays (73) and the number in the empty trays (3) to have been highly significant (P = < .001). This appeared to indicate a strong preference for the former over the latter, but before any more definite conclusion was drawn, it was thought advisable to check on the possible occurrence of position effects. This was done by placing a $\frac{1}{2}$" layer of sand identical with that used in the first experiment in each of the four trays, which were placed in the same corner positions in the experimental room. These positions will be referred to as A, B, C and D, as indicated in the following diagram:-



The following were the numbers of pupae recovered from trays in these standard positions in two tests; each tray, with the sand it contained, was moved round one place after the first test.

Position.	Test 1.	Test 2.
A	18	17
B	24	13
C	17	15
D	24	18

There was thus no consistent preference for any one position, and in any case none of the differences were significant. It therefore appeared that any significant differences which occurred with objects in the positions indicated could with reasonable safety be attributed to differences in the attractiveness of these objects.

The result of the first experiment could now confidently be taken to indicate that the sand-containing trays were preferred to the empty trays. It could not, however, be assumed that the sand-containing trays were selected as breeding sites, for they might merely have been preferred resting places in which larviposition consequently tended to occur.

To test this possibility, counts of the numbers of females resting inside a sand-containing and an empty tray were made. If the difference in the numbers of pupae recovered from the two trays were merely due to the former being preferred as a resting place, the numbers of females resting in them should differ in similar degree. So that the resting females would be visible, two trays were constructed in which one side of each was ½" instead of 3" in height. These were placed in positions in the experimental room from which they could conveniently be observed from outside, the ½" sides, of course, being towards the observer; the positions were not the same as those in which trays were previously placed, but in this case possible position effects could have no effect on the results. A shallow layer of dry sand was placed in one of the trays, and the number of females resting in each was counted at 10-minute intervals between 9 and 12 a.m. Nigerian time (this is one hour ahead of G.M.T.) each morning for six days.

The totalled results were:-

	Sand-containing tray	Empty tray
No. of females resting	24	21
No. of pupae recovered	34	2

The difference between the first pair of figures is obviously insignificant, and there is thus no suggestion that the sand-containing tray was preferred as a resting place to the empty tray; the number of females resting in either tray was, in fact, very small. On the other hand, the numbers of pupae recovered show that many more depositions took place in the sand-containing than in the empty tray. These results seem explicable only on the grounds that the former was selected primarily, and not merely incidentally, as a breeding site.

The observed response to sand-containing trays raised the question as to what stimulus or stimuli were responsible for this reaction. There was no evidence that empty trays possessed any attractive property, for in the first experiment the number of pupae recovered from these was 3, while the number in the remainder of the room (excluding the sand-containing trays) was 28. From the relative areas of the trays and the remainder of the room the distribution expected would have been 1.4 in the two empty trays and 29.6 on the floor of the room, which a χ^2 test shows to have been insignificantly different from that observed ($P = > 0.1$).

One possible explanation of the attractiveness of the sand-containing trays was that they were slightly darker in appearance than the floor of the room: the following were readings obtained from a Weston exposure-meter held 12" above the centres of the trays in a standard position in relation to the light source, chosen so that the meter cast no shadow on the trays.

Tray containing sand	0.1 candles per square foot
" " "	0.1 " " " "
Empty tray	0.4 " " " "
" "	0.3 " " " "
Floor of room	0.2 " " " "

Since the sensitivity of an exposure meter varies in different parts of the spectrum these readings can presumably only be taken to indicate approximately the light-reflecting values of the trays as compared with the floor of the room.

To obtain some indication of the importance or otherwise of visual stimuli, the first experiment was repeated in complete darkness. Disappearance of the response under these conditions would not necessarily indicate that the response was wholly visual, for darkness might inhibit the general activity of the females. If, however, it did not disappear, there would be a clear indication that, under these conditions at least, some other stimulus was operative.

The result was as follows:-

Total no. of pupae = 102

No. of pupae in sand-containing tray = 8
" " " " " " " = 5

No. of pupae in empty tray = 2
" " " " " " = 0

The difference between the total numbers in the sand-containing and empty trays is significant, P being 0.01. There was thus apparently still some response to the sand-containing trays when visual stimuli were completely eliminated, but it was on a very much smaller scale than when the room was illuminated; the ratio of the number of pupae in the sand-containing trays to the number in the remainder of the room was now only 13:89, when before it was 73:31, and for the difference between these distributions $P = < 0.001$.

The residual response obtained in complete darkness might have been due to an olfactory stimulus acting either at long or at short range - though the sand had no smell perceptible to the investigator - or it might have been tactile, acting upon females which alighted by chance on the sand, or a combination of these.

As the above experiment did not eliminate the possibility that visual stimuli played an important part in the response to sand-containing trays when these were illuminated, an attempt was now made to determine whether shaded trays were more attractive than unshaded. Two sand-containing trays were shaded from the light by pieces of plywood, 2'0" by 3'6" in size, standing vertically on the floor adjacent to them. The two remaining trays, which also contained sand, were illuminated by the overhead light as usual. All trays were in the usual corner positions. The results were:-

Total no. of pupae = 137

No. in shaded tray = 26
" " " " 20

No. in unshaded tray = 24
" " " " = 18

There was thus no consistent preference for the shaded over the unshaded trays. This did not necessarily indicate that no preference of this nature existed, for the lack of response might have been due to there having been insufficient contrast between the shaded and unshaded regions in the comparitively dim light of the experimental chamber. Another circumstance that may have detracted from the attractiveness of the shaded trays is that they were not visible from all directions; the pieces of plywood which shaded them inevitably concealed them when viewed from the direction of the light source.

In the last experiment the thermohygrograph which had previously stood in the vestibule was inside the experimental room, where it stood on the floor in the centre of the wall opposite the door. At the end of the experiment it was noticed that an unduly large number of pupae were on the floor in its vicinity, and a few even inside the cabinet of the instrument. The thermohygrograph was of the wet and dry coil type, and it seemed that the attraction which it apparently exerted on larvipositing

TABLE XVIII

The response to thermohygrographs with and without water in their wells.

a = pupae on the floor of the tray.

b = pupae on or in some part of the instrument other than the well.

c = pupae in the well.

Test no.	Wells containing water				Dry wells				Empty trays	Total no. of pupae
	a	b	c	Total	a	b	c	Total		
(1)	8	1	2	11	5	0	7	12	0	100
	6	2	2	10	8	0	7	15	0	
(2)	7	1	1	9	11	1	7	19	0	115
	11	0	4	15	6	1	9	16	0	
Totals	32	4	9	45	30	2	30	62	0	216

(To face p. 154)

females might have been due to its black colour, which made it a conspicuous object against the light concrete floor, the nooks and crannies in it, or the water in the well, which must have raised the humidity locally.

To confirm the apparent attraction, and to test the latter possibility, two experiments were carried out in which four black wet and dry coil thermohygrographs, two with water in their wells, and two with dry wells, were placed in the usual corner positions in the experimental room; in the second experiment wells which were previously dry were filled with water, and vice versa. Each thermohygrograph stood inside a brown paper tray, 1'1" by 8¼", with sides ½" high; these retained any larvae deposited in, on, or near the instruments. Two empty trays indentical with the remainder were placed midway between positions A and C and positions B and D (see p. 147) as a check on any possible response to the trays themselves.

The numbers of pupae recovered are shown in Table XVIII. It will be seen, firstly, that there was undoubtedly a strong response to the thermohygrographs whether they contained water in the wells or not. In the first experiment, for instance, the total number of pupae found in association with the four thermohygrographs was 48, while the number in the remainder of the room was 52.

On the basis of the relative areas of the trays containing the thermohygrographs and of the rest of the room only 2.6 of these 100 pupae would be expected in the trays, and 97.4 in the remainder of the room. The difference between the observed and expect distributions is highly significant ($P = < 0.001$), indicating a strong preference for the trays containing the instruments over the other parts of the room. The same result is obtained if any one of the instruments is considered individually.

It is thus evident that the thermohygrographs and/or their immediate vicinities were highly favoured larviposition sites. Their attractiveness does not in any way appear to have depended on the trays they stood in, for no pupae were deposited in empty trays.

It will be seen that the response to thermohygrographs with water in their wells was invariably slightly lower than to those with dry wells, the totalled numbers of pupae obtained being 45 and 62, respectively. This difference was not significant ($P = > 0.1$), so cannot be assumed to reflect a real preference for the dry instruments. The excess was in any case entirely due to the difference in the numbers of pupae in the wells, there being a total of 30 in the dry wells, and only 9 in those containing water. If these are excluded, the total number of pupae found in or around the 'wet' and 'dry' thermohygrographs becomes 36 and 32, respectively.

The relatively small numbers of pupae found in wells containing water was understandable, for these were fairly full, and so offered a relatively small area of resting space for the female. The site was certainly not one favourable for the larva, for almost all 'pupae' recovered from wells containing water were actually dead larvae which had never succeeded in pupating.

That a considerable number of pupae were found in the dry wells was in itself interesting because the wells were mainly covered over, and their interiors dark and cave-like. Unless all pupae found in the wells were dropped there from above, which, in view of the results obtained with 'wet' thermohygrographs seems unlikely, some females must have entered into these. Similarly, the presence of pupae in the cabinet meant that females must have passed through a small aperture to reach there. These observations suggested that females about to larviposit had some preference for relatively confined situations.

The nooks and crannies afforded by the thermohygrographs may thus have enhanced their attractiveness at close quarters. At a greater range the only property they possessed which could feasibly have been responsible for the

(To face p. 157)

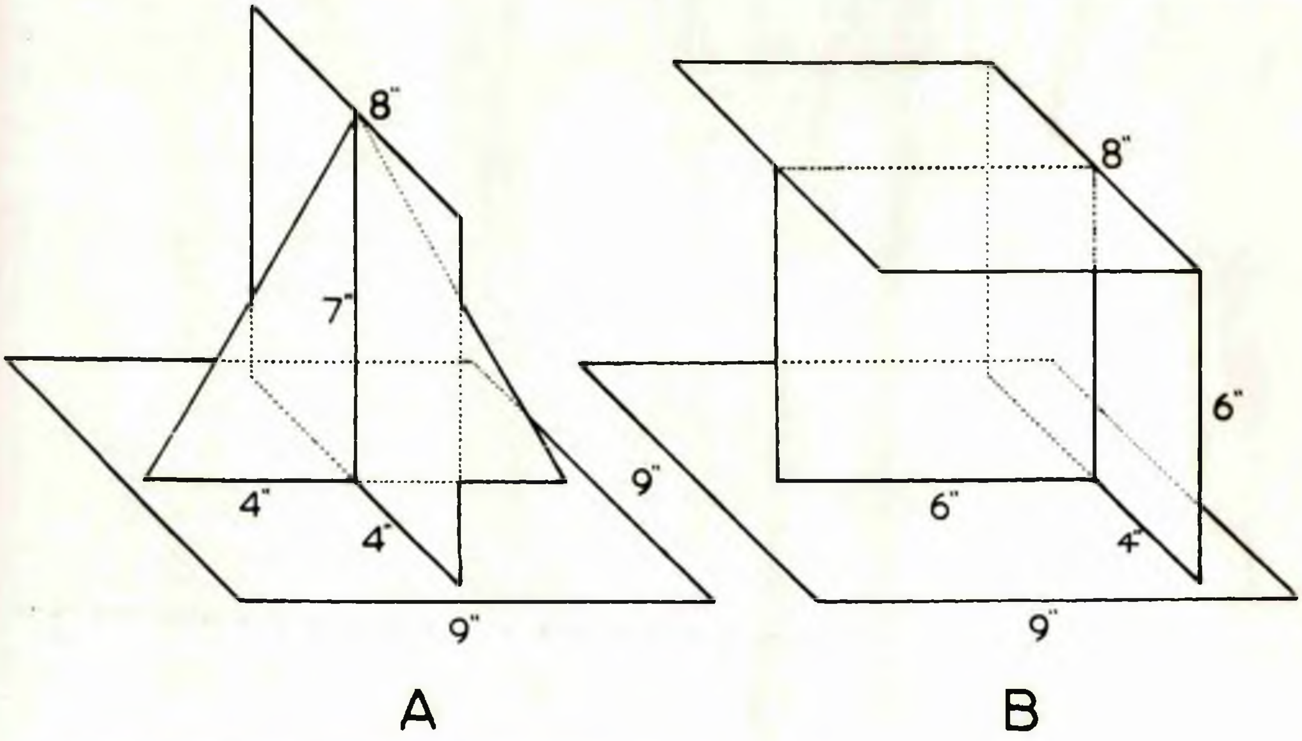


Fig. 20. Showing the arrangement of black surfaces described in the text.

attraction exerted was their dark appearance. It therefore seemed that the response must have been at least partly, and possibly wholly, visual.

A thermohygrograph presented a relatively complicated stimulus-situation, and an attempt was now made to determine which were the more important ingredients by testing the response to dark objects of somewhat simpler shape. So that it would not deflect the response of the females, the thermohygrograph used to record climatic conditions was now enclosed in a white-painted wooden box, one side of which was of white sandfly netting. Four objects made from non-shiny black paper pasted on to cardboard foundations were tested. Each stood in, and was stuck down to, a tray of light brown paper, 15" by 15", with sides ½" high. The black objects were as follows:-

(a) A plain black square 9" by 9", lying flat in the centre of the tray.

(b) A similar black square on the floor of the tray, with another horizontal black square, 7" by 7", 3" above it. This was supported by two thin metal rods prjecting from a light-coloured burette stand which stood outside the tray.

(c) Two vertical black surfaces at right angles to each other standing on a black square as shown in Fig. 20A. The object stood in the centre of a light brown paper tray as before.

(d) A combination of vertical and horizontal black surfaces standing on a black square (Fig. 20B) in the centre of a brown paper tray.

Object (a) thus presented only a horizontal black surface with no shade on it. (b) offered the same as (a) plus a horizontal black surface above which cast shade and offered a resting place on its underside. (c) offered the same as (a) plus vertical resting surfaces which cast shade. (d) offered all the ingredients enumerated for (a), (b) and (c).

An initial experiment in which these four objects were tested gave the following results:-

Total no. of pupae = 125
No. of pupae in tray (a) = 3
" " " " " (b) = 3
" " " " " (c) = 15
" " " " " (d) = 20

The following points may be noted:-

(1) The number of pupae in tray (a) was not significantly higher than would have been expected from a random distribution. Thus there were 3 pupae in the tray and 84 in the remainder of the room excluding the remaining trays, while on the basis of the areas involved the distribution

expected would have been 1.5:85.5; the difference between the observed and expected distributions gives $P = > 0.1$. This result was also obtained in two subsequent tests, but a black square of which the sides were 15" long yielded 6 pupae out of a total of 110, which proved to be significantly non-random ($P = < 0.001$). It therefore seemed that a black surface lying flat on the ground did exert some attraction if it was large enough.

(2) There was no evidence that tray (b) exerted any attraction, and this was also borne out in two subsequent tests. When the height of the suspended square was lowered to $\frac{3}{4}$", however, a significantly non-random response of 6 out of 121 pupae was obtained ($P = < 0.001$). Under these circumstances object (b) did, therefore, seem to have some attraction.

(3) There was a well-defined positive response to (c) which was significantly greater than that to (a) or (b) in any form. This may have been because the vertical surfaces of (c) made it more conspicuous from certain angles of approach and/or to a preference at close quarters for this arrangement of the black surfaces; it seemed that the latter might not have been a visual reaction.

(4) There was a well-defined positive response to (d). This was not significantly greater than the response to (c), a result which was borne out in a subsequent test. This again

implied that an overhead horizontal surface was relatively unimportant in inducing larviposition, but since (c) and (d) also differed in other respects, it was thought unwise to lay too much emphasis on a comparison of this nature.

The triangular 'buttresses' of (c) were parallel to the direction of the light, an arrangement which resulted in one half of this object being completely in the shade and the other completely illuminated. To test whether the shaded side was preferred to the illuminated, a wall ½" high was built transversely across the tray so that larvae deposited on the two sides remained separate. In two experiments the following results were obtained;-

	Shaded side	Illuminated side
Experiment (1)	16	16
" (2)	16	18

There was clearly no marked preference for either side, and thus no indication that, under the conditions of the experiment, shaded black surfaces were preferred to unshaded.

There was no indication that objects of the type described above were in any way attractive if they were not dark in colour. Thus, a tray containing an object identical with (d) except that all the surfaces were covered with light brown instead of black paper yielded only 1 out of a total of 120 pupae in one test, and 1 out of 114 pupae in another. Both these yields were slightly, though not significantly, lower than expectation on the basis of random distribution.

Further information about the tendency to deposit in confined spaces of which some indication was obtained in the experiments with thermohygrographs was sought by testing the response to small boxes. Both the insides and outsides of these were covered with black paper, and they were mounted on a 9" black square which was in the centre of a light brown paper tray, 15" by 15" as before. The boxes were 1" cubes with one side open except for a wall rising 0.4" from the floor which retained larvae deposited inside them. Two such trays were constructed, each containing three boxes, and two identical with them except that no boxes were present (control trays). The results of an experiment with these four trays were as follows, the figures in brackets being the numbers of pupae <u>inside</u> the boxes:-

Total no. of pupae = 114
No. of pupae in tray containing boxes = 20 (14)
" " " " " " " = 28 (20)
No. in control tray = 3
" " " " = 3

It will be seen that the response to the trays which contained boxes was higher than that to the control trays, the difference between the totals being highly significant ($P = < 0.001$). Also, there were always more pupae inside the boxes than in the remainder of the trays that contained them, the totalled figures again differing significantly ($P = 0.006$). The interiors of the boxes were thus preferred to other parts of the same trays in spite of the total area of black surface having been much greater in the latter. This preference may have been simply a visual one for the darkest situation available, or an expression of a preference for confined nooks whether they were darker or not, or a combination of these.

To determine whether there was any special preference for the interiors of very small boxes, tests were carried out with trays containing boxes of three different sizes, namely 1", 2" and 3" cubes. As before they were black both outside and in, and one side was left open to allow the females to enter. The numbers of pupae inside the boxes in two tests were:-

	1" cube	2" cube	3" cube
Test (1)	1	5	15
Test (2)	0	2	10
Totals	1	7	25

There was thus no preference for the smaller boxes, and the distribution 1:7:25 did not differ significantly from what would have been predicted from the sizes of the openings of the boxes; for the number of pupae obtained this would have been 1.9:9.1:22.0.

An attempt was now made to analyse the response to dark nooks i.e. dark situations in which the fly was enclosed on several sides, by separating the 'dark' (visual) and 'nook' (possibly non-visual) elements. For this purpose both trays containing boxes, and trays containing objects of type (c) (p. 157) were used. The boxes were 1", 2" and 3" cubes as in the last experiment. In one tray only the interiors of the boxes were black, the exteriors, and all the remainder of the tray being of the light brown paper previously used in constructing trays; this appeared from previous experiments to have no marked attractive or repulsive effects on the pregnant females. In another tray only the exteriors of the boxes were black, the interiors, and the remainder of the tray again being of light brown paper. Two tests with these trays were carried out, the results being as follows:-

	Test (1)	Test (2)	Totals
Dark interiors, light exteriors			
Pupae inside boxes	7	5	12
Pupae outside boxes	1	1	2
Light interiors, dark exteriors			
Pupae inside boxes	2	0	2
Pupae outside boxes	1	2	3
Total number of pupae deposited	112	107	

(To face p.164)

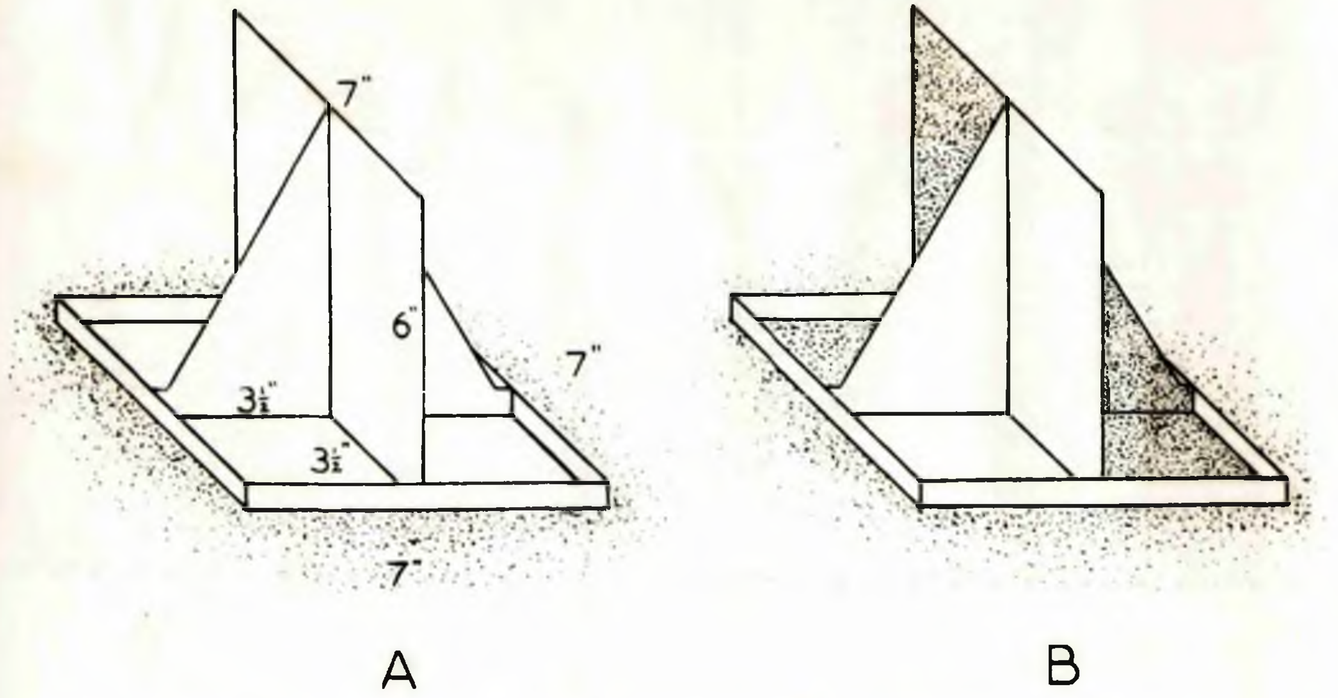


Fig. 21. Showing the arrangement of dark and light surfaces described in the text.

As was expected from previous results, there were significantly more pupae inside than outside the boxes when the interiors were dark (P = 0.01). This preference was lost when the interiors were light, but the exteriors, though dark, were still not actually preferred. It was tempting to interpret this as meaning that both a visual response to dark surfaces and a non-visual response to 'nooks' were operating, and that in one case they were acting in unison, and in the other in conflict. Such a conclusion seemed somewhat speculative, however, especially since the number of pupae both inside and outside the boxes with light interiors was very small.

Information on this point was also sought by the use of modified objects of type (c) which were tested simultaneously with the boxes described above. As will be seen from Fig. 21, the vertical walls of (c) were slightly smaller than before, and now stood in a 7" square which was bounded by walls ½" in height; this square was divided into quadrants by the vertical walls of (c), and a larva deposited in any one of the quadrants was unable to escape from it. The 7" square stood inside a paper tray, the sides of which were 15" by 15" as usual.

In one tray (Fig. 21A) all surfaces of (c) were light brown, as was the floor of the 7" square it stood in; this will be referred to as tray A. In the other tray

TABLE XIX

The response to dark and light-coloured objects of type (c).

Test no.	Tray A		Tray B			Total no. of pupae.
	Inner square	Peripheral region	Inner square		Peripheral region	
			Dark sectors	Light sectors		
(1)	0	8	12	0	4	112
(2)	0	15	12	1	5	107
Totals	0	23	24	1	9	219

(To face p. 165)

(Fig. 21B) half the vertical surfaces of (c), and half the floor area of the square that (c) stood in, were black, and half light brown. The central region of the tray was thus divided into two black and two light brown sectors, similarly coloured sectors being diagonally opposite each other; this will be referred to as tray B. In both trays the region between the inner square containing (c) and the outer edge of the tray was black; this will be referred to as the peripheral region. All the ½" high walls which served as barriers to larval movement were of light brown paper.

Two tests with these trays were carried out, the results being shown in Table XIX. It will be seen, firstly, that of the black surfaces available in tray B, those in the inner region, which included vertical components, were preferred to those in the periphery; the totals of 24 and 9 gave P = 0.01. If the numbers of pupae per unit area of black surface were taken the difference was much more marked, for the total area of dark surface available within the inner square was only 45 sq. in. while there was 178 sq. in. in the periphery. The inner regions evidently lost their attractiveness, however, if they were not black, for in tray B only 1 pupa was recovered from the light segments as opposed to 24 from the dark, and in tray A there were 23 pupae in the dark peripheral region to 0 in the centre; the latter was a complete reversal of the state of affairs in tray B.

This result implied that the visual attraction exerted by dark surfaces over-rode any possible non-visual attraction that relatively enclosed situations may have possessed. The observed preference for confined over more open situations observed when both were dark could also it seemed, have been primarily visual, for a greater part of the visual field of a female would have been occupied by dark surfaces when she was in the former than in the latter type of situation. This question, which is an aspect of the problem of whether the breeding site 'furniture' is of any importance apart from its effect on light conditions, will be reconsidered later.

The types of object so far found to be attractive as larviposition sites - namely dark objects and sand - were now brought together, and the response to both measured simultaneously. The trays containing modified objects of type (c) made for the last experiment were again used, a layer of dry river-bed sand ¼" deep being placed in two of the four sectors of the inner region of both tray A and tray B; in the case of the latter sand was placed in one of the black and in one of the light-coloured sectors. The remaining sectors were left empty as before. In both trays one of the sand-containing sectors was illuminated, and one shaded; in case there should be any preference for shaded

TABLE XX

The response to dry sand in combination with dark and light surfaces.

Test no.	Tray A			Tray B					Total no. of pupae
	Inner square		Peripheral region	Inner square				Peripheral region	
	Contain-ing sand	Empty		Dark, with sand	Dark, no sand	Light, with sand	Light, no sand		
(1)	3	0	6	28	7	4	0	2	102
(2)	5	0	5	22	8	4	0	3	101
Totals	8	0	11	50	15	8	0	5	203

(To face p.167)

sides - though it will be remembered that none had so far been found - each tray was each morning turned through 180^{0}, so that both sides spent the same period in equivalently illuminated positions.

The results of two tests are shown in Table XX. From this it will be seen that both the sand and the dark surfaces were attractive, and that a maximal response was obtained when both were present together; indeed, in tray B the total response to the dark sector containing sand (50 pupae) was significantly greater than that to the dark sector empty of sand (15 pupae) plus that to the light sector containing sand (8 pupae), P being 0.002. In tray A the dual attraction exerted by the sand in the light-coloured centre and the dark periphery resulted in an almost equal distribution of pupae between these regions, instead of all the pupae being in the periphery, as was the case in the previous experiments with this tray when no sand was present.

The clearly-defined preference for a dark sector containing sand over an empty dark sector was a result of considerable significance, for the sand was much lighter in colour than the black floor it covered. The attraction exerted by the sand was thus not due to its being darker than its surroundings, and as all evidence so far obtained had indicated purely visual responses to make for a selection of the darkest regions available, the implication seemed to be that the response to the sand was in this case non-visual. Possibly the flies were attracted to the dark trays from a distance by a visual reaction, and to the sand at short range - perhaps only when actually in contact with it - by a non-visual mechanism.

At this point the question of the attraction to the wooden sand-containing trays used in the initial experiments was reconsidered. With a response of the magnitude of that observed, it seemed that these must have exerted an attraction at some distance, as well as at close quarters, and it has already been pointed out that the former could have been visual, depending on the comparitively dark appearance of the tray and the sand inside it in contrast to the light concrete floor. If this were true it seemed that the response should be largely eliminated if the tray were covered over with a piece of light-coloured plywood. Legs were accordingly placed in the corners of each of two trays, each extending 2½" vertically above the rims of these trays. On these legs were rested pieces of plywood, 19" square (the outside dimensions of the trays, it will be remembered, were 18" by 18") which thus concealed the contents of each tray from view; the plywood was lighter in appearance than the floor of the room, comparitive exposure-meter readings being 0.7 and 0.5 candles per sq. foot.

A $\frac{1}{2}$" layer of dry sand was placed in one of the covered trays, the other being left empty. An uncovered tray containing sand, and an uncovered empty tray were also placed in the experimental room. The following results were obtained:-

	Covered	Uncovered
Pupae in trays containing sand	45	13
Pupae in empty trays	7	0
Total no. of pupae deposited	107	

Covering the tray, far from diminishing the response to the sand, thus significantly increased it ($P = < 0.001$), from which it seemed that the strip of shade between the rim of the tray and its cover was attractive, perhaps from some distance, and/or that conditions inside the covered tray were preferred to those in the uncovered. In the same way an empty covered tray yielded more pupae than an uncovered one, the difference being suspiciously large ($P = 0.02$). This was the first indication obtained in these experiments that shade as such could be attractive in the same way as could a black object. The experiment thus confirmed the importance of the visual response, though not in the way expected.

On the other hand, there was no apparent difference in the appearance of the two covered trays, and since the shade cast in both was so dense, it seemed unlikely that the difference in response to them could have been due to the sand having been slightly darker in appearance than the floor of the empty tray. A more feasible explanation was that this difference, which was highly significant ($P = < 0.001$), was due to a non-visual response operating after the female had entered the trays.

The response to a strip of shade described above raised the question whether such a response played any part in the response to the sand-containing trays observed in the earlier experiments. These trays, it will be remembered, were 3" deep, but contained a layer of sand only approximately ½" deep. This meant that two of the sides of each tray cast strips of shade 1" wide at their bases on to the sand adjacent to these sides.

To test whether these strips were important, two trays were filled to the brim with sand so that no shade was cast in them, the other two having a ½" layer in the bottom as usual. To retain larvae which might fall out of the former a small plasticine wall was built on the floor around, and 1" away from the sides of these trays; similar walls were built around the remaining two trays so that conditions would be exactly equivalent. In the following results the pupae found between these plasticine walls and the trays are shown in brackets after the numbers in the corresponding trays, and are included in the totals:-

	Trays full to brim	Trays with $\frac{1}{2}$" sand
	4 (1)	13 (1)
	6 (0)	22 (1)
Totals	11	37
Total no. of pupae deposited	111	

The difference between these totals was highly significant ($P = < 0.001$), indicating that the presence of the strip of shade was of considerable importance in the response. There was no suggestion that a strip of shade without the sand was attractive, for the maximum number of depositions occurring in the region between the outer sides of the tray and the plasticine wall, part of which was also shaded, was one, and it will be remembered that in earlier experiments an empty tray was not found to exert any attraction. On the other hand, sand in the complete absence of shade does seem to have exerted some attraction, for the observed distribution of 10 pupae in these trays and 63 in the remainder of the room (excepting the other trays) was significantly non-random ($P = < 0.001$).

Before pursuing the trend of the above experiments further, another material from natural breeding sites was tested alongside the river-bed sand so far employed. This was dry loose soil collected from a known breeding site in the fringing forest. The soil, which was filtered through a 0.1" mesh sieve to remove large particles, was slightly darker in appearance than the sand; comparitive exposure-meter readings were 0.5 and 0.8 candles per square foot.

Two trays containing sand, and two containing forest soil were placed in the experimental room; one of each contained only a ½" layer in the floor of the tray, and the other was full to the brim as in the last experiment. Small plasticine walls were present around the trays as previously, and the numbers of pupae found between these and the trays are in the following results shown in brackets as before:-

	Soil	Sand
Trays with ½" sand	43 (0)	6 (0)
Trays full to brim	6 (0)	0 (1)
Totals	49	7
Total no. of pupae deposited	117	

These results, in addition to confirming those of the previous experiment, clearly showed that the soil was very significantly more attractive than the sand ($P = < 0.001$); this could have been due to its darker appearance, its consistency, or even its scent, if it had any perceptible to tsetse.

In this experiment , the contents of the trays, instead of being poured bodily through a sieve which would retain the pupae, as formerly, were taken out a teaspoonfull at a time so that the precise position of the pupae could be observed; if the strips of shade which were evidently of such importance exerted a short-range attraction it would be expected that pupae would be concentrated in the parts of the tray on which they fell.

This was found to be so. In the tray containing a ½" layer of soil, 37 out of a total of 43 pupae were in the shaded strips along the two sides of the tray. There were thus significantly more in the shaded region than in the remainder of the tray ($P = < 0.001$) although its area was only approximately one ninth of the total. There was no indication of any special attraction to the sides of the tray as such, for no pupae were found in the areas within one inch of the two unshaded sides. A similar result was obtained with the tray containing a shallow layer of sand, all 6 of the pupae this yielded being within the shaded area. In the tray which was filled to the brim with soil, on the other hand, no pupae at all were found in the marginal regions, none of which, of course, were shaded.

A further point is that the number of pupae found at the junction of the two shaded sides was significantly higher than would have been expected had the pupae been evenly distributed throughout the shaded region. Thus in the tray containing a shallow layer of soil there were 15 pupae in the region extending 2" in either direction from this junction, and 22 along the remainder of the shaded sides, as opposed to the expected values of 3.4 and 33.6; this difference gave $P = < 0.001$. Similarly, in the tray containing a shallow layer of sand, 4 out of 6 pupae were in the corner region. This concentration could have been due to selection by the females and/or by the larvae. The females might have had some preference, possibly non-visual, for the relatively enclosed nature of the corner, or they could have been attracted by its dark appearance relative to other shaded regions; the latter resulted largely from some of the glue used to secure the corner joints having seeped out leaving a dark stain. On the other hand, the concentration might have been due to the larvae having been able to burrow into the soil more easily at the corner than elsewhere along the sides. That this was not wholly responsible for the result obtained was suggested by the concentration having occurred only in the corner at the junction of the two strips of shade; the two corners at the opposite ends of these strips yielded no pupae. Yet another possibility was that the larvae behaved photonegatively; such a reaction could quite conceivably have resulted in the distribution observed.

The question of whether selection was made by the parent females or by the larvae applied not only to the concentration in corners but to concentration in all shaded parts of the tray. To determine whether larvae deposited in the centre would move to the shaded part of the periphery, a small cage of pregnant females was suspended above the centre of a tray of soil standing in one of the usual positions in the experimental room. The cage was of large mesh mosquito netting, through which tsetse larvae were capable of passing, stretched on a wire frame. The shadow cast by the cage fell outside the tray.

All larvae were thus known to be deposited within an area in the centre of the tray equivalent to that of the floor of the cage, i.e. 5½" by 1½". At the end of the experiment 12 out of a total of 23 pupae were found in this area. Only one was in the shaded strip along the two sides.

The distribution was thus completely different from that obtained when the females were at large in the room, and there was no indication that any selection of the shaded areas by the larvae took place. It therefore seemed likely that at least part of the selection of such areas observed in previous experiments was made by the parent females.

The finding that the attractiveness of a tray of sand or soil depended so much on the strips of shade cast by the sides led to experiments in which the response to trays with sides only $\frac{3}{8}$" high, full to the brim with dry forest soil, was compared with that to similar soil-containing trays with which vertical and horizontal shade-casting objects were associated. The latter were pieces of wood,9" square, the surfaces of which were purposely left rough and unplaned so that females could rest on them without difficulty. In the centre of one tray of soil, one of these pieces of wood was mounted vertically at right angles to the incident light, so that it cast shade on one side; a small plasticine wall continued out from it to the sides of the tray isolated the shade-containing and unshaded halves from each other as far as larval movement was concerned. Two inches above another tray of soil a similar piece of wood was suspended horizontally by thin wires connected to burette stands outside the tray. A tray containing soil but no shade-casting object, and a tray containing a vertical piece of unplaned wood but no soil, were also included. Small plasticine walls were built around the outsides of the trays as before, the pupae found between these and the trays being, in the following results, again shown in brackets:-

Tray with soil and no shade	2 (2) pupae
Tray with vertical surface and no soil	1 (2) "
Tray with soil and vertical surface	8 (0) "
Tray with soil and horizontal surface	53 (0) "
Total no. of pupae	108

The numbers of pupae in the two trays which contained both soil and shade-containing objects were significantly non-random (in each case $P = < 0.001$), indicating that both these combinations were attractive. The number associated with the tray containing a vertical surface and no soil was not significantly non-random ($P = > 0.1$), but the 4 pupae associated with the tray containing soil alone was suspiciously larger than expectation on a random ~~this~~ basis ($P = 0.03$).

As in the previous experiment, the soil was removed spoonful by spoonful, and it was found that in the tray containing soil and a vertical surface 6 of the 8 pupae were on the shaded side, and 5 of these actually within the shaded area. In the tray in which there was a horizontal piece of wood above the soil, 35 of the 53 pupae were underneath this, a significantly non-random distribution ($P = < 0.001$). If, instead of considering the area directly beneath the horizontal surface, the area on which it cast shade was considered - as the light source was not overhead the two areas only partly coincided - a similar result was obtained, the number of pupae in the shaded region being 34.

The greater response to the tray containing soil beneath a horizontal surface than to the tray containing soil and a vertical surface (for the difference between them, $P = < 0.001$) might appear at first sight to be at variance with previous results obtained with black objects; these, it will be remembered, indicated situations which included vertical surfaces to be preferred to those containing only horizontal ones. In fact, direct comparison between the results is not justifiable, for in the one case a visual attraction was exerted by the object itself rather than by the shade it cast, while in the other the reverse was true. The latter state of affairs, which was that pertaining in the experiment described above, was presumably the closer approximation to conditions encountered in nature.

In a further experiment the response to a shallow tray containing soil with a horizontal piece of wood 2" above it, as in the previous experiment, was compared with that to its constituent parts i.e. to a tray containing soil alone, and to a tray with the horizontal surface alone but no soil. The following result was obtained:-

Tray with soil alone	5 (2)	pupae
Tray with horizontal surface alone	2 (5)	"
Tray with soil and horizontal surface	32 (4)	"
Total no. of pupae	102	

The total response to the tray containing both soil and a horizontal surface (36) was thus greater than that to the tray containing soil alone plus that to the tray containing the horizontal surface alone (14), the difference being significant ($P = 0.003$). This indicated that the pronounced response obtained with a tray of soil below a horizontal surface resulted from these two ingredients being present in combination, and was not due merely to the sum of the effects exerted by each ingredient separately.

An attempt was now made to determine whether the horizontal surface was of importance merely because of the shade it cast on the soil beneath, or whether it was also important as a resting place for the larvipositing females. To this end, use was made of the observed inability of the females to rest on the underside of a horizontal sheet of clean glass. Nine-inch squares of glass were fixed by small brackets on to the undersides of two horizontal pieces of wood of the type used previously, these being suspended 1" and 4" above shallow trays full to the brim with forest soil. Suspended the same distance above two similar trays, also full of soil, were identical pieces of wood in which the rough undersurfaces were uncovered. The shaded resting places afforded by the rough undersurfaces in the latter two trays were thus eliminated in the former, except to some extent at the edges, where a foothold could still be obtained. The usual plasticine walls were present outside the trays.

	Glass undersides	Rough undersides
Horizontal surface 1" above soil	15 (0) pupae	15 (0) pupae
" " 4" " "	16 (0) "	6 (1) "
Total no. of pupae	101	

There was thus not the slightest indication that the presence of the glass diminished the response, and in the case of horizontal surfaces 4" above the soil the response was actually higher when it was present, though not significantly so (P = 0.09). Furthermore, the distribution of the pupae in the soil was apparently unaffected by the presence of the glass. In the case of the trays in which the horizontal surface was 1" above, 12 out of 15 were in the area beneath the latter when no glass was present, and 10 out of 15 when there was glass underneath; these two distributions were not significantly different ($P = > 0.1$). When the horizontal surfaces were 4" above the soil the proportion under the latter when glass was present (11 out of 16) was actually higher than when no glass was there (3 out of 6) though the difference was again insignificant ($P = > 0.1$).

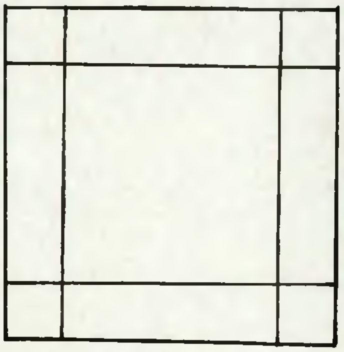
If deposition occurred while the females were resting on the underside of the horizontal surfaces, it would be expected to be concentrated around the peripheral region of the area below the latter when glass was present, as in this case it was only at the edges that the females were able to obtain a foothold. The numbers of pupae within the strip one inch either side of the outline of the area immediately below the glass-covered horizontal surface, and in the remainder of the area beneath the horizontal surface were as follows:-

	Peripheral region (72 sq. in.)	Inner region (49 sq. in.)
Horizontal surface 1" above	2	9
" " 4" "	2	11
Totals	4	20

The number of pupae in the region below the periphery of the horizontal surfaces was thus significantly lower (P= 0.002) than the number in the inner regions below these surfaces even though the former area was the greater. There was thus no suggestion of the concentration that would have been expected if deposition had occurred while the females were resting on the only part of the horizontal surface on which they could in this case obtain a foothold. The inference is that deposition took place mainly or wholly on the soil surface, and that the horizontal pieces of wood were of little importance except because of the shade they cast.

A final attempt was now made to discover whether any preferential response to 'nooks' as such was displayed i.e. whether relatively enclosed situations were preferred to more open regions even when they were no darker than the latter. Several of the results obtained previously could have been produced by an effect of this nature, but were always also open to other interpretations.

Four wooden trays, 18" square and 3" deep as in many previous experiments, were employed. The inner faces of their walls were covered with the light brown paper which previous experiments had indicated to lack any marked attractive or repulsive properties. The floor of each tray was covered with a layer of loose forest soil $\frac{1}{4}$" deep, and this was partitioned by four plasticine walls approximately $\frac{1}{2}$" high which formed barriers to larval movement. The arrangement of these walls was as shown in the following plan, the distance of each from the adjacent side of the tray being one sixth of the total width of the tray.



There were thus four corner regions, four other outer regions, and a central region. The area of each corner region was one quarter that of each of the remaining outer regions, and the area of each of the latter was one quarter that of the central region. The trays were in the usual places in the experimental room but turned through 45° relative to their former positions, so that only one side now cast a shadow on the soil inside; this fell in a strip 1¼" wide at the base.

The above arrangement was so designed that any preference for the corner regions over the remaining outer regions, and any preference for the remaining outer regions over the central region, could be discerned. As already noted, the preference for corners observed previously could have resulted from selection by the larvae, or from the corner region having been selected by the parent female because it was slightly darker in appearance than the remaining shaded areas. In the present experiment the first possibility was eliminated by the presence of plasticine walls which prevented the movement of larvae from other regions, and there were now no dark stains in any of the corners to make them darker in appearance than the remainder of the sides. It therefore seemed that any concentration in the corner regions which might be obtained could be assumed to be an expression of a genuine preference for a relatively confined situation independent of any response to dark regions. Any preference for the remaining outer regions

TABLE XXI

The numbers of pupae recovered from different regions of subdivided trays.

Total no. of pupae = 115

Tray no.	Central region	Shaded outer region*	Illuminated outer region*	Shaded corners	Illuminated corners
(1)	0	10	1	6	0
(2)	1	14	3	5	0
(3)	0	10	0	6	0
(4)	3	10	4	4	0
Totals	4	44	8	21	0

* excluding the corner regions

(To face p. 184)

adjacent to the illuminated sides over the central region would be a further indication of such a tendency.

The results of an experiment in which four trays of the type described were simultaneously tested are shown in Table XXI. It will be seen that they confirm the conclusion drawn earlier that the female selects a shaded region for larviposition, for the total number of pupae in the shaded outer region was significantly greater than the number in the illuminated outer regions ($P = < 0.001$) even though the area of the latter was three times that of the former.

Comparing the pupae in the corners with those in the remaining outer regions, firstly in the shaded areas, it will be seen that there was a total of 21 in the corners to 44 along the rest of the shaded side. From the relative areas one would expect half as many in the former as in the latter; the number in the shaded corners was thus slightly below expectation relative to the remainder of the shaded region. There were no pupae in the illuminated corners and 8 along the illuminated sides; the former was thus again below the number expected from the relative areas, which were in this case in the ratio 1:6.

Comparing the number of pupae in the illuminated outer regions other than the corners (i.e. 8) with the number in the central area (4) it will be seen that the former was above expectation, for the areas were in the ratio 3:4. The observed

TABLE XXII

The numbers of pupae recovered from different regions of subdivided trays.

Total no. of pupae = 109

Tray no.	Central region	Illuminated outer region*	Illuminated corners	Remainder of tray
(1)	10	6	0	1
(2)	10	10	1	0
(3)	12	10	0	3
(4)	13	8	1	0
Totals	45	34	2	4

* excluding corner regions

(To face p. 185)

and expected distributions did not, however, differ significantly ($P = >0.1$).

A further experiment was performed to determine whether the number of pupae in the illuminated outer regions would be significantly greater than expectation as compared with the central region if the number of pupae involved was larger. In the last experiment the majority of the pupae were in the shaded area, and to deflect some of these to other parts of the tray this area was now left bare of soil.

This pro-cedure was successful, almost all the depositions now taking place in the unshaded, but soil-containing regions. As will be seen from Table XXII there were now 45 pupae in the central region and 34 in the illuminated outer regions other than the corners. This was almost exactly the distribution expected from the 4:3 ratio of the areas, so that there was no suggestion of any preference for the outer regions.

It may be noted that the distribution of pupae between the corners and the remaining outer regions confirmed those of the previous experiment; as the areas were in the ratio 1:6, more than the 2 pupae observed in the former would have been expected from the 34 pupae in the latter.

The above experiments thus gave no indication that the proximity of one or more vertical surface was in itself a feature which enhanced the attractiveness of a larviposition site.

It was seen earlier that the presence of loose sand greatly increased the attractiveness of a black object as a larviposition site even though it made it lighter in appearance (p.167), and the non-visual attractive properties of dry loose soil or sand were now investigated. As a preliminary experiment the response to a tray containing dry loose forest soil, as in previous experiments, was compared with that to a tray containing soil which, while otherwise similar, had been wetted and then allowed to dry into a hard compact mass. Another tray contained loose soil in one half and hard soil in the other, the two halves being separated by a small paper wall which served as a barrier to larval movement. The walls of the trays were 3" high, and the soil in them ½" deep. The following numbers of pupae were obtained:-

	Loose soil	Hard soil
In different trays	9	1
In the same tray	14	2
Total no. of pupae	96	

The difference between the first pair of figures was suspiciously large (P = 0.03), while that between the second pair was significant (P = 0.006), indicating a definite preference for the loose soil.

The relative response to soil particles of different sizes was now tested. It will be remembered that the loose soil so far used contained particles of all sizes below 0.1"; these were now separated by sieving into the following fractions:-

Large particles	0.1 - 0.07 inches diameter
Medium particles	0.04- 0.02 " "
Small particles	0.01 and less" "

Soil particles of these sizes were placed to a depth of ½" in three trays of the type used in the last experiment. Each of these trays thus contained particles of only one size-range. A fourth tray was subdivided into three equal-sized and equally illuminated sections by small paper walls, and soil particles of one size-range put in each section. The following result was obtained:-

	Large	Medium	Small
In different trays	16	4	1
In the same tray	5	1	0
Total no. of pupae		108	

The number of pupae in the tray containing only large particles was significantly higher than the numbers in the trays containing only medium-sized or only small particles ($P = 0.01$, $P = < 0.001$, respectively), indicating that the former was preferred to either of the latter particle-sizes. There was no significant discrimination between the medium-sized and small particles. Results obtained with different-sized particles in the same tray showed the same trend, but the numbers were too small for significant differences to be obtained.

Here then were two apparent preferences, one for loose over hard soil and the other for large over small particles. Before any conclusions could be drawn, however, account had to be taken of some types of soil having been darker in appearance than others. The following are exposure-meter readings of the light reflected from trays containing loose and hardened soil taken in a standard position in relation to a 100 W tungsten filament lamp.

Loose soil	0.8 candles per square foot
Hard soil	1.2 " " " "

Similar comparative readings from trays containing soils of each of the three particle sizes were:-

Large particles	0.6 candles per square foot
Medium particles	0.7 " " " "
Small particles	1.0 " " " "

Thus in each case the more attractive type of soil was also slightly the darker. In view of the importance previous experiments had shown visual stimuli to possess the possibility of these differences having been responsible for the observed preferences could not be ignored.

An attempt to determine whether this was so was made by comparing the response to hard soil and to soil consisting only of small particles with that to the river-bed sand used previously. The sand particles, which had been sieved so that none were less than 0.04" in diameter, were much larger than those of the fine soil, and unlike the hard soil, they were loose. At the same time the sand was lighter in appearance than either the hard or the fine soil, as was shown by the following comparative exposure-meter readings:-

Sand	1.4 candles per square foot
Fine soil	1.0 " " " "
Hard soil	1.2 " " " "

Unless the sand had any special attraction over the soil unconnected with its consistency - and nothing in previous experiments suggested that this was so - any preference for it over the soils which was exhibited must have been a preference for either its larger particles or loose consistency. Half-inch deep layers of the appropriate materials were placed in separate trays, the sides of which were 3" high, as previously.

Tray containing sand	14 pupae
Tray containing fine soil	2 "
Tray containing hard soil	9 "
Total no. of pupae	105 "

The number of pupae in the sand-containing tray was significantly greater than the number in the tray containing fine soil ($P = 0.006$). This was a reversal of the result obtained when the response to sand was compared with that to soil of mixed particle size, although the latter was in both cases the darker. This strongly suggested a preference for large over small particles independent of any difference in their appearance.

The difference between the response to the sand and that to the hard soil was not significant ($P = > 0.1$), so no similar inference could be drawn in this case.

In the above experiment, one tray not so far mentioned was also included. This was one in which sand particles otherwise identical with those previously used were stuck down with gum. This was done by covering the floor of the tray with a thin layer of Stephen's gum mucilage, and sprinkling a complete layer of sand particles over this while it was still wet. When the gum had dried, particles which had not stuck to the floor were removed by inverting the tray. The difference between the stuck-down and loose sand was similar to that between hard and loose soil except that the surface of the hard soil was also smoother in texture than that of the loose. Also, the light reflecting value was slightly higher with the loose than with the immobilised sand, comparative exposure-meter readings being 1.4 and 1.2 candles per square foot.

The number of pupae recovered from the tray containing immobilised sand particles was 6. This was not significantly lower than the 14 pupae in the tray containing loose sand (P = 0.1), so it could not be concluded that there was any preference for mobile over immobile particles. The result might, however, have been influenced by the slightly darker appearance of the immobilised sand; on the other hand the glue could have had some repellent effect, though when dry it had no odour perceptible to the writer.

To eliminate the effect of variations in the degree of darkness or lightness of different types of soil or sand, resort was once again made to the use of an object of type C (Fig. 21A). The vertical surfaces, which were of the same dimensions as shown in the figure, were now of plywood covered with black paper, and stood in a wooden tray just large enough to contain them; there was now no peripheral region. The vertical surfaces thus divided the floor of the tray into four equal sectors, each of which was isolated from every other so far as larval movement was concerned. The sides of the tray were ½" high. Trays of this type, as previously, were placed in the experimental room with the triangular buttresses parallel to the direction of the incident light so that half of each tray was completely in the shade, and the other half directly illuminated. When one type of soil was placed in the illuminated half and another in the shaded, the latter was much the darker in appearance whatever the light-reflecting values under equal illumination. It therefore seemed that useful results might be obtained by the use of pairs of such trays, the type of soil that was shaded in one being illuminated in the other and vice-versa.

In the first experiment large and small particles of soil, and loose and immobilised sand were again compared; the evidence previously obtained for a preference for large over small particles independent of the darker appearance of the former was indirect, and needed confirmation, and objections to the previous experiment with loose and immobilised sand have already been mentioned. To equalise any possible effects of the gum used to stick down the particles this was now poured into both sides of the tray in liquid form. A thin layer of sand was then sprinkled on to one side before the gum set, and an equivalent layer on to the other side after the gum had hardened; while gum was present on both sides, the particles on one side thus became stuck down, while on the other they remained free.

The following comparative exposure-meter readings taken in the experimental room showed the shaded soils to have been very much the darker in appearance whatever their consistency:-

TABLE XXIII

The response to mobile and immobile sand particles, and to large and small soil particles.

S = pupae on shaded sides

I = pupae on illuminated sides.

Figures linked by dashes were recovered from different sides of the same tray.

Total no. of pupae = 118

Soil particles			Sand particles		
Large		Small	Mobile		Immobile
18 (I)	—	9 (S)	6 (I)	—	6 (S)
18 (S)	—	3 (I)	3 (S)	—	10 (I)
36		12	9		16

(To face p. 193)

	Illuminated	Shaded
Loose sand	0.20	0.00
Immobilised sand	0.20	0.00
Small soil particles	0.15	0.00
Large soil particles	0.10	0.00

The results obtained are shown in Table XXIII, from which it will be seen that the large soil particles attracted more pupae than small whether they were shaded or not, the totals differing significantly (P = 0.001). This was a clear indication that the former were the more attractive independent of their darker appearance, a result which confirmed those obtained previously.

The loose sand yielded the same number of pupae as the stuck-down particles when it was illuminated, and less when it was shaded; the totals did not differ significantly ($P = >0.1$). There was thus no suggestion that the mobility of the particles of a sandy surface was a feature selected by larvipositing tsetse.

It will be noticed that there was no consistent preference for the shaded side. In the trays containing soil the total number on the shaded sides was 27 while the number on the illuminated sides was 21, whereas in the sand-containing trays the corresponding figures were 9 and 16. None of these differences were significant.

The absence of any preference for shaded regions under the above circumstances, which was also observed in earlier experiments with black objects of this shape unaccompanied by soil or sand, was surprising in view of the preference for shaded areas observed under other conditions. It was, however, very convenient for the present purposes, for it offered a means of masking differences in the appearance of different types of soil without introducing another element in itself attractive to the pregnant female.

The lack of discrimination between loose and stuck-down sand raised the question whether the preference for loose over hardened soil observed earlier was due to the smoother surface of the latter rather than to its immobility; there was also its lighter appearance, the possible effect of which had not so far been eliminated.

To test this possibility the black objects used in the previous experiment were again employed, the types of soil compared being:-

(a) Hardened soil with a smooth surface and hardened soil with a rough surface. The former was prepared by wetting soil consisting only of small particles and allowing it to dry. The latter was made in the same way except that large particles were sprinkled on to the surface of the soil while it was still wet; most of these adhered to the surface when it had dried, and those which had not were sucked off.

TABLE XXIV

The response to hardened soil with rough and smooth surfaces, and to loose and hardened soil with a rough surface.

Symbols have same meaning as in preceding table.

Total no. of pupae = 103

Hardened soil		Soil with rough surface	
Rough surface	Smooth surface	Mobile particles	Immobile particles
10 (I) —	0 (S)	14 (I) —	6 (S)
5 (S) —	o (I)	11 (S) —	4 (I)
15	0	25	10

(To face p. 195)

(b) Hardened soil with a rough surface, prepared as above, and loose soil of mixed particle size, which had a surface of similar texture.

The experimental arrangement was exactly as in the previous experiment. From Table XXIV it will be seen that more pupae were recovered from the hardened soil with a rough surface than from that with a smooth surface, the difference between the totals being significant ($P = < 0.001$). This indicated that a rough surface was preferred to a smooth one even when both were immobile. That the same was true when both surfaces were mobile seemed likely from the preference for large over small loose particles observed earlier.

The difference between the total numbers of pupae obtained in the loose and hardened soils with rough surfaces was suspiciously large ($P = 0.02$). There was thus a suggestion that a mobile surface was more attractive than an immobile one even when both were rough in texture. The difference, if a real one, could conceivably have been due to slight differences in the degree of roughness of the two surfaces which it was not found possible entirely to eliminate; some evening out of the irregularities of the surface layer was inevitable if the soil particles in the surface layer were to adhere firmly to the underlying particles. It would be rash to postulate any selection of surfaces on the basis of their mobility from the evidence presented, especially in view of the result obtained with loose and stuck-down sand.

To measure the relative importance of unevenness of surface texture and mobility, loose soil consisting only of small particles, the surface of which was therefore smooth but mobile, was compared was compared with hardened, and therefore immobile soil with a rough surface. Soil of each kind was placed in each of four trays of the kind used in the previous two experiments.

	Loose soil, smooth surface	Hard soil, rough surface
Tray (1)	1	13
" (2)	2	15
" (3)	2	12
" (4)	2	9
Totals	7	59

Total no. of pupae 119

The very much greater response to the soil with the rough surface (for the difference between the totals, $P = < 0.001$) indicated that any preference for a mobile over an immobile surface that might exist was of very minor importance compared with the preference for a rough over a smooth surface which was exerted independent of the mobility of the particles.

Attention was now turned to some of the features associated with natural breeding sites not so far considered which might possibly have offered stimuli attractive to the pregnant female. One possibility was that soil or sand had some attractive odour, though there was none perceptible to the writer. To test this a ½" deep layer of dry forest soil from a natural breeding site was placed in the floor of each of two wooden trays, the external dimensions of which were 18" by 18" by 3" as in many earlier experiments. Dead leaves found on the soil surface in the breeding site were scattered over the soil in one tray. Two similar control trays were empty, their floors being covered with black paper. One inch below the rim of each tray a platform of sand-fly netting which had been dyed black was built, this being sealed to the edges with adhesive tape. The object of this platform was to prevent the females from coming in contact with the contents of the trays, while not preventing them from being stimulated by any possible odour there may have been. In appearance the control trays, with their black floors, were slightly darker than the trays which contained soil.

The result was as follows:-

Tray containing soil and leaves	6	pupae
Tray containing soil alone	0	"
Control tray	6	"
" "	3	"
Total no. of pupae	102	"

There was clearly no preference for the trays containing soil, and therefore no suggestion that either the soil or the leaves had any odour attractive to the pregnant females. In view of this completely negative result further experiments along these lines did not seem justified.

There was in existence no satisfactory evidence of any preference for obviously damp soil or sand over a drier substratum for breeding purposes, and pupae had rarely been found in such situations in nature. This might conceivably have been due to the greater difficulty of searching in damp than in dry substrata, and the response to damp soils was accordingly tested in the laboratory. Wooden trays 18" square and 3" deep were again used, a ¼"-deep layer of loose forest soil of mixed particle-size from a natural breeding site being placed in each. Each tray was supported by four columns which stood in zinc trays of water. The floors of two of the trays were pierced by numerous small holes into each of which a strip of cotton fabric was plugged; these strips hung down into the water, which ascended them by capillarity and so wetted the soil in the tray above.

The heights of the perforated trays above the water were such that in one the soil surface was 1" above water-level, and in the other 2¼" above; there was therefore a difference in the moisture-content of the soil in these two trays. The remaining two trays (controls) contained 'dry' soil i.e. approximately in equilibrium with the atmosphere of the room at large; there were no perforations in the floors of these and no cotton strips hanging from them, but, like the test trays, they stood in trays of water, one so that the soil-surface was 1", and one so that it was 2¼", above water-level. Each tray of damp soil thus had a corresponding control tray virtually identical with it except for the moisture-content of the soil inside it.

Moisture-contents were estimated by taking samples of soil from each tray after equilibrium had been reached (i.e. a few days after the experiment was set up), weighing them, and drying at 105°C for 24 hours. The dry weight was taken after the soil had cooled down to room temperature in a calcium chloride desiccator. The results of two such sets of analyses were as follows, the moisture contents being expressed as percentages of the dry weight:-

	(1)	(2)
Very damp soil	59.5%	56.7%
Moderately damp soil	27.6%	25.2%
Dry soil	3.4%	3.6%
Dry soil	3.1%	3.4%

The damp soils were considerably darker in appearance than the dry, comparitive exposure-meter readings being:-

TABLE XXV

The response to damp and dry soils compared.

Experiment no.	Very damp soil		Moderately damp soil		Total no. of pupae
	Test	Control	Test	Control	
(1)	3	21	5	16	119
(2)	4	20	2	19	111
Totals	7	41	7	35	230

(To face p. 200)

Very damp soil	0.3	candles	per	square	foot
Moderately damp soil	0.2	"	"	"	"
Dry soil	0.8	"	"	"	"
Dry soil	0.7	"	"	"	"

The results of two experiments with these trays are shown in Table XXV, from which it will be seen that both the very damp and the moderately damp soil trays yielded less pupae than their corresponding controls; in both cases the differences gave $P = < 0.001$. This result, obtained in spite of the darker appearance of the damp soils, was a clear indication that under the conditions of the experiment, the latter were avoided relative to soils approximately in equilibrium with the ambient atmosphere. It will be noted that there was no apparent difference in the attractiveness of the moderately damp and very damp soils.

One possible objection to the above experiments was that they were carried out during the heavy rains when the thermohygrograph in the experimental room showed the relative humidity to have varied from 75% to 84%, and the saturation deficit from 3.8 mm. to 6.0 mm. It remained possible that damp soil would be found attractive if the response were tested at a drier time of year. An experiment similar to the above was therefore carried out at the end of November when the Harmattan was already thick, and the humidity in the experimental room varied from 45% to 53% R.H. (11.0 mm. to 13.2 mm. S.D.).

One tray contained a ¼"-deep layer of soil of which the moisture-content was approximately the same as that of the moderately damp soil of the last experiment; to attain this degree of dampness in the now drier atmosphere of the room it was found necessary to lower the soil surface to 1½" above the level of the water. As before, there was a corresponding control tray containing 'dry' soil.

The remaining test tray contained soil with a moisture-content only slightly above that of the 'dry' soil. To achieve this the wooden floor of one tray was replaced by a tightly stretched piece of cotton fabric supported by thin copper wires. The whole tray rested on an enamel tray of almost the same size which contained water. The level of the latter was ¼" below the floor of the soil-containing tray, so that moisture vapour could diffuse into the ¼" deep layer of soil above. The corresponding control tray rested on a similar enamel tray which did not contain water.

The following were the results of two sets of moisture-content analyses, obtained as previously:-

	(1)	(2)
Moderately damp soil	29.4%	31.2%
Slightly damp soil	2.4%	2.3%
Dry soil	2.0%	1.9%
Dry soil	1.9%	1.7%

From these figures there appeared to be little difference between the dry and the slightly damp soil. Direct readings of relative humidity taken with an Edney paper hygrometer lying on the soil surface, however, indicated that there was an appreciable difference in the humidities of the air immediately above the soil. Edney hygrometers are not, of course, accurate over a wide range, but a series of tests in which readings were checked with those from a whirling hygrometer indicated that over a range of about 20% R.H., and providing the instrument was left for 30 minutes for equilibrium to be reached, readings accurate to within 2% were obtained. Calibrated readings, each of which may thus have been 2% greater or less than the true values, were as follows:-

Moderately damp soil	82% R.H.	(3.9 mm. S.D.)
Slightly damp soil	57% R.H.	(10.2mm. S.D.)
Dry soil	49% R.H.	(12.2mm. S.D.)
Dry soil	48% R.H.	(12.4mm. S.D.)

The relative humidity at the actual surface of the moderately damp soil was presumably 100%, but the hygroscopic element of the hygrometer was about ½cm. above this, and the gradient was presumably fairly steep.

The numbers of pupae recovered were as follows:-

Moderately damp soil	4
Control	23
Slightly damp soil	11
Control	18
Total no. of pupae	103

The difference between the first pair of figures was highly significant ($P = < 0.001$) indicating that the moderately damp soil was relatively avoided in the dry as well as in the wet season. The difference between the second pair was not significant ($P = > 0.1$), so there was no evidence of any discrimination between soils of these moisture-contents.

The above experiments thus failed to demonstrate any reaction to moisture other than one of avoidance, and this line of investigation was not pursued further.

Many natural breeding sites, because of their sheltered situation, are cooler than much of the surrounding habitat, and the response to artificially cooled soil was therefore now tested. Temperatures taken at the soil surface in the last experiment gave readings 0.5°C. below room temperature with the slightly damp soil, and 1.5°C. below for the moderately damp, but here there was the complication of the high moisture-content.

Wooden trays 18" by 18" by 3" were again used, a length of thin convoluted copper tubing being fixed to the floor of two of them. This tubing was cooled by the passage through it of cold water. The latter was water from the mains which had passed through coils of metal tubing inside two refrigerators outside the experimental room, and thence by rubber tubing to the coils in the trays. After leaving these, the water was conveyed to a drain in the floor; the entire system was enclosed. The tubing in the trays was covered by a thin layer of soil, as was the remainder of the floor of the tray; care was taken to arrange the tubing so that there was a length immediately beneath the soil in the shaded region, which previous results had shown to be the site of the majority of depositions. The temperature of the water could be regulated by adjustment of its rate of flow, and since it passed first through one tray, then through the other, the temperature of the soil in the former was slightly lower than in the latter. Two control trays contained equivalent amounts of soil at room temperature; to equalise any possible effects on the females produced by the tubing which conveyed water to and from the cold trays, similar lengths were placed on the floor in equivalent positions in relation to the control trays. Dry loose forest soil of mixed particle size from a natural breeding site was used in all trays.

The ambient temperature was 24.5 - 25.5°C; this was within the normal range of daytime temperatures encountered by G. palpalis in its natural habitat. Readings taken at the soil surface in the shaded parts of the trays showed that in the first cooled tray the surface temperature varied from 16.5°C. to 19.0°C, and in the second from 20.0°C. to 21.0°C., the variation being due mainly to fluctuations in the initial temperature of the tap water.

The numbers of pupae recovered were as follows:-

Soil at 16.5 - 19.0°C.	11
Control	35
Soil at 20.0 - 21.0°C.	3
Control	26
Total no. of pupae	103

The differences between both the first and the second pairs of figures gave $P = < 0.001$, indicating that soils 3.5 - 5.5°C. or 5.5 - 9.0°C. below an ambient temperature of 24.5 - 25.5°C. were not only not preferred to soil at the latter temperature, but were avoided relative to it.

In a second experiment the temperature of the cooled soil was slightly higher than previously. The ambient temperature was now 24.0 - 25.0°C. and the temperature at the surface of the cooled soils 23.0 - 24.0°C. and 21.5 - 23.0°C.

The following numbers of pupae were recovered:-

Soil at 21.5 - 23.0°C.	11
Control	25
Soil at 23.0 - 24.0°C.	16
Control	17
Total no. of pupae	94

The difference between the first pair of figures was suspiciously large (P = 0.03), but these results provided no clear indication of any discrimination between soils at an ambient temperature of approximately 24.5°C. and those up to 3.5°C. below it.

Since lowering the temperature automatically raised the humidity, the possibility that the apparent avoidance of low temperatures was partly or even wholly due to differences in humidity had to be considered. Assuming the actual amount of moisture in the air to remain constant, reference to Glaisher's hygrometrical tables showed that if the relative humidity at 25°C. was 47% - this was the mean ambient value during the first experiment - it would be 57-61% at 20.0 - 21.0°C. and 65-77% at 16.5 - 19.0°C. The corresponding saturation deficits were 12.6 mm. at 25°C., 6.8 - 8.1 mm. at 20.0 - 21.0°C., and 3.2 - 5.8 mm. at 16.5 - 19.0°C. When expressed in terms of saturation deficit the differences were thus more marked than when expressed in terms of relative humidity, and the humidity of the soil at 16.5°C. was apparently as low as the value derived from the hygrometer reading taken above the relatively avoided 'moderately damp' soil.

Although the hygrometer readings were measures of the humidity of the air approximately 0.5 cm. above the soil, whereas the figures derived from Glaisher's tables were estimates of the humidity at the actual soil surface, the possibility that the apparent avoidance of low temperatures was, in fact, an avoidance of the high humidities associated with them thus remained one that, on the evidence available, could not be dismissed. Conversely, the below-ambient temperatures of the damp soils could have played some part in making them unattractive, though the results of the last experiment did not suggest that the drop of 1.5°C. apparently produced by evaporation at the surface of 'moderately damp' soil would be liekly to exert a pronounced repulsive effect. Elaborate experiments, for which the present investigation did not appear to call, would have been needed to disentangle these temperature and humidity reactions from each other.

That the temperatures and humidities tested in the above experiments in many cases corresponded with conditions in nature was shown by climatic readings taken in moderately enclosed breeding sites, and at the more open ground immediately adjacent to them.

Seven sites were chosen, and were as follows:-

Fig. 22.

Fig. 25.

Fig. 23.

Fig. 24.

A. Beneath a procumbent liane trunk.within the fringing forest. The trunk was approximately 6" thick, and, at the place where the readings were taken, 5" above the ground (Fig. 22).

B. At the base of a liane trunk approximately 12" thick which turned horizontally about 10" above the ground. This site was thus protected on one side as well as from above. Like A, it was within the fringing forest (Fig. 23).

C. At the base of a Phoenix palm. Small fronds sprouting from the base of the tree arched over to form an umbrella. Readings were taken inside this, the fronds which formed the roof being at this point approximately 6" above. The site was in the fringing forest (Fig. 24).

D. Inside the hollow base of a standing tree. The hollow was approximately 9" in diameter and at least several feet in height. There were two entrances to it, one some 5" high and 12" wide and the other about 6" by 9". The tree stood at the junction - here fairly sharp - between the forest and the woodland(Fig. 25).

E. Beneath a fallen log approximately 18" thick and 3" above the ground, situated on the edge of the forest at a place where the forest/woodland junction was fairly sharp (Fig. 26).

F. Beneath the fronds of a Borassus palm, which formed an umbrella approximately 18" above the soil surface. The palm

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Fig. 26.

Fig. 27.

Fig. 28.

stood in a belt of transitional woodland (Fig. 27).

G. Beneath a fallen log in the savannah woodland. The log, which was approximately 5" in diameter and 4" above the ground at the place where the readings were taken, was about 8 yards from the edge of the forest. Unlike any of the other sites, tall grass grew around and over it (Fig. 28).

With the exception of D, all the above sites were known to have been used as breeding sites.

Readings were taken on the ground in each of the above situations and on the more open ground adjacent to them. In both cases care was taken to ensure that the instruments were in the shade, which, when readings were taken outside the breeding sites, was usually provided by the overhead canopy of trees or high shrubs. Outside site G, shade was provided by the tall grass, as well as by the tree canopy, but neither of these was thick enough to provide more than a light sun-dappled shade.

Temperature was measured by ordinary thermometers, and humidity by Edney hygrometers calibrated for the range over which they were to be used. The latter were left in the atmosphere of which the humidity was to be measured for 30 minutes to allow equilibrium to be reached. As mentioned earlier, it was found that under these conditions the reading obtained was not more than 2% greater or less than that indicated by a whirling hygrometer. Differences greater than 4% could therefore with reasonable safety be assumed to be real ones.

Exposure-meter readings of the light reflected from the 'interior' i.e. densely shaded region of the above breeding sites, and from the adjacent more open ground were also made, the instrument being in either case held 10" from the ground. It will be obvious that these readings could give only a very approximate indication of the degree of darkness of the breeding sites, partly because of the difficulty of standardising the position of the exposure-meter in relation to breeding sites of different shapes and sizes and positions in relation to the sun, and also because of variations in the sensitivity of the instrument in different parts of the spectrum.

As the object of the readings was to determine what conditions natural breeding sites presented to larvipositing females it was clearly desirable to take them at the time of maximum deposition. To ascertain this, females were enclosed in wide-mesh netting cages which were placed out-of-doors in the shade of a broad-leaved tree, and the number of depositions which had occurred counted at hourly intervals. At this juncture the writer read Burtt's (1952) paper on G. swynnertoni, which contained the observation that the time of maximum deposition of this species was advanced several hours if dry soil was present. Clearly the latter was the more natural state of affairs, at least during the dry season, and

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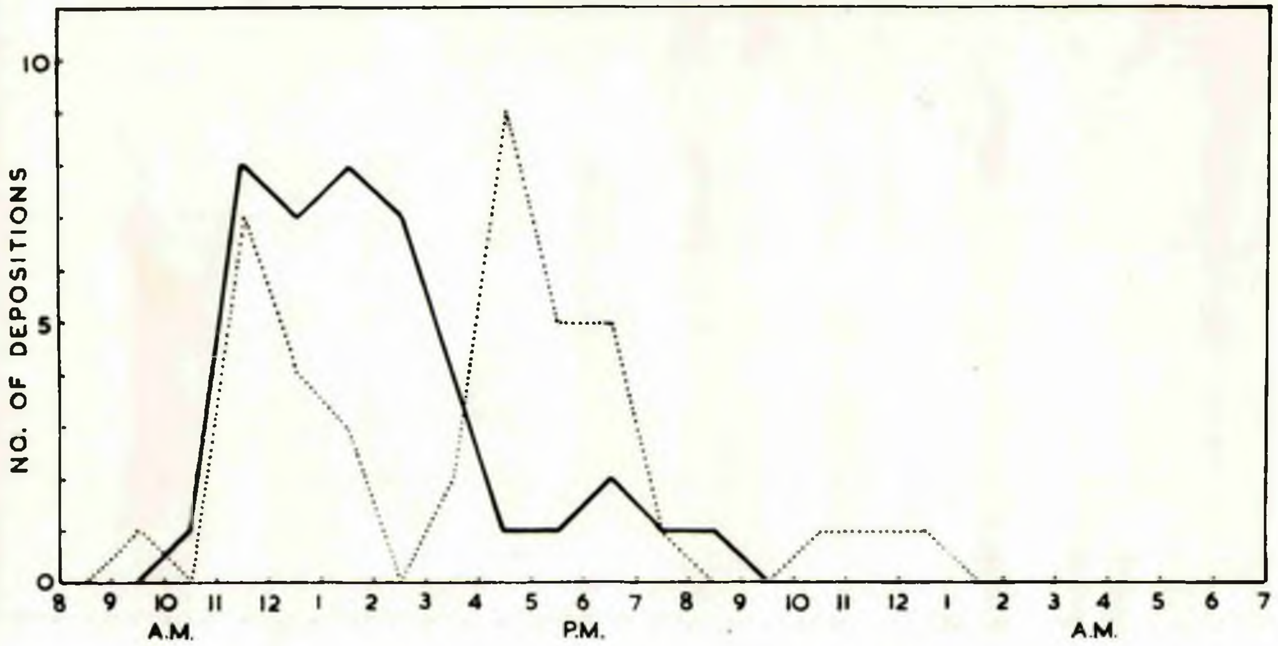

Fig. 29. Times of larviposition of G. palpalis in a shaded outdoor area (Nigerian time). Unbroken line, soil present; stippled line, soil absent.

the writer therefore repeated his observations with the females enclosed in cages in the bottom of which was dry soil; although the larvae buried in the soil the occurrence of larviposition could be detected from the appearance of the females.

The results of the two sets of observations are shown in Fig. 29, from which it will be seen that when no soil was present there were two peaks, one at 11 - 12 am., and the other at 4 - 5 pm. Nigerian time (this was one hour ahead of G.M.T.). Since the earlier peak occurred immediately after the time at which a blood-meal was each day offered to all females, it seemed likely that it was due to depositions which the latter precipitated from females already in the last stages of pregnancy. That this was so was substantiated by the occurrence of a similar peak at the same time when soil was present.

It was therefore the second peaks which were presumed to bear some relation to the time of day independent of the time of the last meal. These were in accordance with Burtt's findings, for in the absence of soil the peak occurred between 4 and 5 p.m., and in its presence between 1 and 2 p.m. A thermohygrograph situated beside the cages indicated that the latter was usually the hottest time of day.

Climatic readings at the seven stations described were therefore taken between 12.00 and 3.00 p.m.; the simultaneous use of four hygrometers made it possible to

TABLE XXVI

Climatic readings taken in G. palpalis breeding sites, and at the more open ground adjacent to them.
Temperatures are expressed in degrees centigrade.
Humidities are expressed in percentage relative humidity, with the saturation deficit, in millimetres, in brackets.
Light intensities are expressed in candles per square foot (cf.).

Date	Site A		Site B		Site C		Site D		Site E		Site F		Site G	
	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
Aug. 10 (Heavy rains)	23.75°C 90%(2.1) 0.6 cf.	24.25°C 85%(3.3) 4.8 cf.	24.0°C 86%(3.1) 0.4 cf.	24.5°C 84%(3.7) 4.8cf.	24.0°C 89%(2.3) 0.2 cf.	24.75°C 87%(3.0) 3.4cf.	23.5°C 90%(2.1) 0.0cf.	24.75°C 87%(3.0) 4.6 cf.	24.0°C 90%(2.2) 2.4cf.	25.25°C 81%(4.5) 6.4 cf.	25.0°C 87%(3.0) 1.8 cf.	26.0°C 87%(3.2) 6.0 cf.	26.5°C 90%(2.5) 6.5 cf.	28.0°C 83%(4.8) 12.0cf.
Sept.19 (Heavy rains)	23.5°C 90%(2.1) 1.2 cf.	24.0°C 87%(2.8) 3.4 cf.	23.0°C 91%(1.9) 1.6 cf.	23.5°C 87%(2.8) 6.0cf.	21.0°C 91%(1.6) 0.2 cf.	21.5°C 88%(2.2) 6.0cf.	22.0°C 90%(2.0) 0.0cf.	23.5°C 84%(3.5) 6.5 cf.	22.5°C 93%(1.4) 2.4cf.	23.5°C 82%(3.9) 7.0 cf.	23.5°C 87%(2.8) 2.4 cf.	24.5°C 88%(2.7) 9.7 cf.	24.0°C 90%(2.2) 4.8 cf.	25.5°C 86%(3.4) 9.8cf.
Oct. 16 (end of rains)	26.0°C 78%(5.5) 1.0 cf.	26.5°C 77%(5.9) 3.2 cf.	25.5°C 81%(4.6) 0.8 cf.	26.5°C 76%(6.2) 3.2 cf.	25.0°C 80%(4.7) 0.2 cf.	27.5°C 75%(6.8) 1.4cf.	25.5°C 80%(4.9) 1.4cf.	28.5°C 72%(8.1) 4.8 cf.	29.5°C 77%(7.0) 0.8cf.	29.0°C 72%(8.3) 2.4 cf.	28.0°C 76%(6.8) 1.6 cf.	29.5°C 71%(8.9) 6.5 cf.	27.5°C 81%(5.1) 6.5 cf.	29.5°C 72%(8.6) 10.0cf.
Oct. 26 (Start of dry season)	27.5°C 71%(7.9) 0.8 cf.	27.5°C 72%(7.6) 4.0 cf.	26.0°C 77%(5.7) 0.2 cf.	27.0°C 74%(6.9) 1.9 cf.	25.5°C 79%(5.1) 0.4 cf.	26.5°C 76%(6.2) 3.2cf.	25.0°C 78%(5.1) 0.1cf.	28.0°C 70%(8.4) 9.0 cf.	26.25°C 76%(6.0) 0.8cf.	27.5°C 75%(6.8) 3.4 cf.	28.5°C 74%(7.5) 3.2 cf.	29.0°C 71%(8.6) 6.5 cf.	29.5°C 75%(7.7) 6.5 cf.	32.0°C 59%(14.5) 16.0cf.
Nov. 2 (Start of dry season)	27.5°C 51%(13.4) 0.8 cf.	27.5°C 52%(13.1) 1.6 cf.	28.5°C 38%(18.0) 0.2 cf.	28.5°C 38%(18.0) 1.6 cf.	25.5°C 60%(9.8) 0.6 cf.	27.0°C 57%(11.4) 2.0 cf.	25.0°C 55%(10.3) 0.0cf.	28.5°C 38%(18.0) 3.3 cf.	24.5°C 59%(9.4) 0.1cf.	27.5°C 46%(14.8) 1.6 cf.	28.0°C 57%(12.1) 3.0 cf.	27.5°C 60%(10.9) 3.6 cf.	28.0°C 63%(10.4) 4.8 cf.	29.0°C 51%(14.6) 13.0cf.
Dec. 15 (Cold dry season)	23.5°C 34%(14.3) 1.6 cf.	23.5°C 33%(14.5) 4.8 cf.	23.5°C 29%(15.4) 1.8 cf.	24.5°C 28%(16.6) 5.0 cf.	23.75°C 33%(14.7) 1.0 cf.	26.5°C 31%(17.8) 5.5 cf.	24.5°C 29%(16.3) 0.6cf.	27.5°C 20%(21.9) 5.8 cf.	22.0°C 37%(12.4) 0.8cf.	25.5°C 27%(17.8) 3.5 cf.	24.5°C 39%(14.0) 1.6 cf.	25.5°C 34%(16.1) 6.5 cf.	30.75°C 24%(25.1) 12.0cf.	32.5°C 26%(27.0) 17.0cf.
Dec. 20 (Cold dry season)	23.0°C 31%(14.3) 1.6 cf.	24.0°C 28%(16.0) 4.0 cf.	24.25°C 32%(15.4) 1.6 cf.	25.0°C 28%(17.0) 4.8 cf.	24.75°C 35%(15.1) 0.8 cf.	25.5°C 31%(16.8) 5.0 cf.	25.5°C 31%(16.8) 0.6cf.	28.0°C 21%(22.3) 6.0 cf.	23.25°C 33%(14.2) 0.7cf.	25.75°C 23%(19.0) 3.2 cf.	26.25°C 35%(16.5) 5.6 cf.	27.25°C 31%(18.6) 6.6 cf.	32.0°C 18%(29.1) 6.8 cf.	32.5°C 21%(28.9) 9.0cf.

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complete the readings in this period. Two sets were taken during the heavy rains, one just before the rains ended; two during the hot dry weather at the beginning of the dry season; and two during the cold dry season.

The results are shown in Table XXVI, from which it will be seen that the temperature in the breeding site was almost invariably slightly lower than in the adjacent more open ground, and that the differences were in all cases within the range of those tested in the laboratory. Whenever the difference in relative humidity was greater than 4%, and could therefore with reasonable safety be considered a real one, it was the reading taken inside the breeding site which was the higher. The differences, again, were within the range tested in the laboratory. It must be noted, however, that in some cases the ambient temperatures and humidities were considerably different from those at which the experiments were carried out; these differences may have affected the temperature and humidity reactions of the females.

Two features associated with all the above sites which laboratory experiments had shown to be highly attractive to pregnant females, were, of course, their dark appearance in contrast to their surroundings, resulting largely from the low shade cast - this is indicated, if only approximately, by the light intensity readings - and the presence of soil with an uneven surface.

Breeding did not, however, invariably occur in sites of the kind described above. Much took place in relatively open ground within the forest where practically the only low shade available was that cast by the fallen leaves with which the ground was almost always at least partly covered (see Fig. 18). The question therefore arose as to whether the small amount of shade cast by a leaf was sufficient to make the ground beneath it an acceptable breeding site. This could, of course, only apply to leaves of which part stood far enough above the ground for females to rest underneath them, but since they tended to curl when dry, this was true of a large proportion.

To test the above supposition four wooden trays, each 18" by 18" by $\frac{3}{8}$" were filled to the brim with dry loose forest soil of mixed particle-size. Dry leaves collected from a part of the forest floor known to be a breeding site were scattered over the soil in two of the trays. All four were placed in the usual positions in the experimental room, and in addition a further tray similar to the others except that it contained a small log resting on otherwise bare soil, was placed midway between positions A and B (p.147). The log was approximately 15" long and 2$\frac{1}{2}$" in diameter. Being slightly bow-shaped, it touched the ground at either end, and rose to 2" above it in the centre. It was included so that any attraction exerted by the leaf-covered surfaces could be compared with that exerted by this more familiar type of breeding site.

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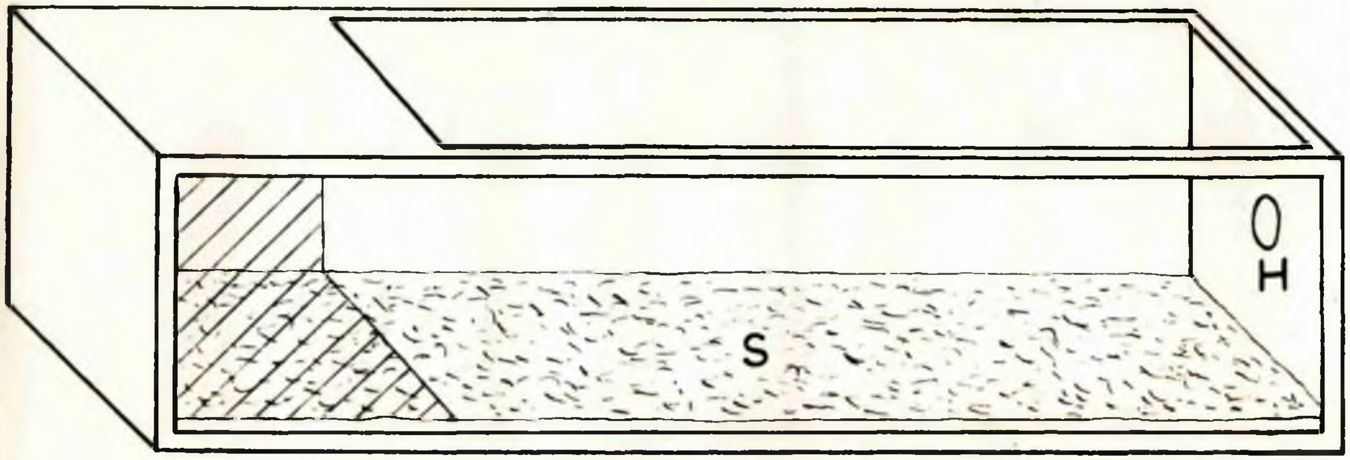

Fig. 30. Box for observing larviposition. S, soil covering floor; H, hole allowing admission of females; the glass roof was also movable. Hatched area shaded, the remainder illuminated.

The following numbers of pupae were recovered:-

In soil alone	2
" " "	1
In soil with leaves on surface	25
" " " " " "	21
In soil with log on surface	47
Total no. of pupae	115

It was thus evident, firstly, that a partial covering of leaves on the surface greatly increased the attractiveness of the soil (for the difference between 3 and 46, $P = < 0.001$). Secondly, soil with a log on the surface elicited a response which was still higher than that to the leaf-covered soil, the difference being significant (for the difference between 47 and 25 - the higher figure obtained with the leaf-covered soil - $P = 0.01$). Although no previous experiments had been carried out with trays in the position of the one containing the log, no tendency for a high concentration of pupae to occur in this part of the room had at any time been noted, and there was no reason to suppose that this difference was not genuine.

It was felt that the conclusions so far reached should now be checked by direct observations of larviposition. For this purpose, a coffin-shaped box, 6" by 6" by 24" (Fig.30) was constructed. One of the long sides was of glass, for observation purposes, and the remaining sides of unplaned mahogany offering an easy foothold to the females. The latter were introduced into the box through a small hole at one end which was corked when not in use. One quarter of the roof was of the same unplaned wood as the sides, and the rest of glass; as the source of light was a 100W bulb approximately five feet above this meant that one quarter of the interior of the box was shaded, and three quarters directly illuminated, the line of demarcation being fairly sharp. The floor was covered with a layer of dry loose forest soil of mixed particle-size approximately ½" deep.

Pregnant females were introduced into the box and watched continuously from 12 a.m. to 3 p.m., this period being chosen as the one in which the maximum number of depositions was likely to occur. Each time a female larviposited, its position, and any other noteworthy observation, was recorded. In addition, at five minute intervals the number of pregnant and spent females resting on the soil, the vertical sides and the roof in the shaded part, and the corresponding numbers in the illuminated part, were counted and recorded. The following morning all females were collected, offered a blood meal, and any which had deposited returned to the breeding stock. If further observations were to be made, females which were still pregnant were returned to the observation box together with newly selected pregnant females.

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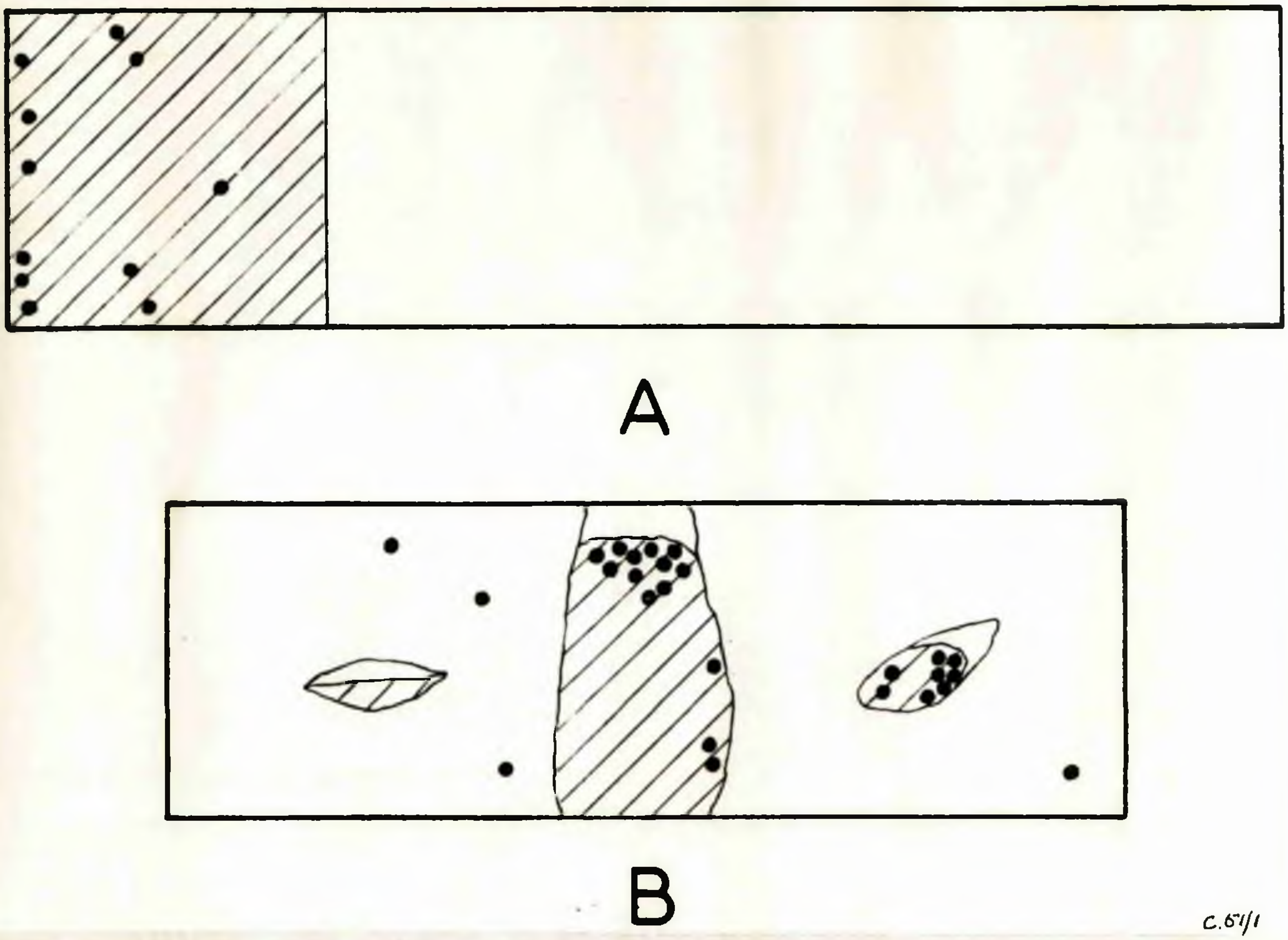

Fig. 31. The sites of larviposition in the observation box. Shaded areas indicated by hatching.

During three days of such obervations, a total of 11 depositions was observed. All these took place while the pregnant females were resting on the soil surface in the shaded region, their actual positions being shown in Fig. 31A. It will be seen that 6 of the 11 depositions occurred adjacent to one of the walls; as this was the one furthest from the illuminated region it seemed likely that this distribution, which was significantly non-random ($P = 0.003$), expressed a tendency to select the darkest situation available. No other preferences for any particular part of the shaded area were apparent.

It will be remebered that an earlier experiment indicated that the breeding site was selected as such, and not as a preferred resting place in which deposition consequently tended to occur. Confirmation of this was now sought. The depositions observed in the shaded and unshaded regions were in the ratio 11:0, and if this was due to the females having preferred to rest in the former, the totalled counts of the numbers of pregnant females resting in these regions should have borne approximately the same relation to each other. In fact the totals were 866 in the shaded area, and 1398 in the unshaded. The distribution of 11 pupae in these proportions would have been 4.2:6.8, which differed significantly from the observed 11:0 ($P = < 0.001$).

It may be noted that, apart from the depositing females, there seemed to be a preference for resting on the sides and roof of the observation box rather than on the soil. Taking only the illuminated part, the total number of both pregnant and spent females counted on the soil surface was 208, while the number on the sides - the roof, being of glass did not afford a resting place - was 1270. The relative areas were in the ratio 3:7 so the expected distribution of the 1478 females would have been 443:1035. The difference between this and the observed distribution was highly significant ($P = < 0.001$) indicating that the sides of the box were preferred as a resting place to the soil surface. It therefore appeared that the preference for a position on the soil surface displayed by the females about to larviposit, like the preference for dense shade, was not a feature of behaviour exhibited at all stages of adult life.

Another series of direct observations was now made in which the shade was cast by natural breeding site furniture i.e. by two dead leaves and a strip of bark. The same observation box was used, the shaded section now being partitioned off and inaccessible to the females. The leaves and the bark, which are shown in plan in Fig. 31B, were placed in such positions on the surface of the soil that females resting in the shade they cast were visible to the observer. The strip of bark was a substantial one, and its deeply furrowed outer surface faced downwards. One end rested on the soil surface, and the other against the glass side of the observation box, 4" above the soil. One of the leaves projected up to $\frac{3}{4}$", and the other up to 1" above the soil surface.

As before, pregnant females were introduced into the box and observed continuously from 12 a.m. to 3 p.m. The position of any female at the time of larviposition was again recorded, and where possible note was also made of how long the female had been in this situation, and of how long she remained there after larvipositing.

During four days of observation, a total of 26 depositions was observed, the distribution of which is shown in Fig. 31B. It will be seen that 14 took place in the shade of the bark, 8 in the shade of the leaves, and 4 in the illuminated region. The female was in all cases on the soil surface. Most of the depositions that occurred under the bark were near to the place where it rested on the ground, the average height of the bark above the female as estimated by inspection being $1\frac{1}{4}$". This, like the concentration adjacent to one of the sides in the last experiment, may have been because the point where the bark touched the ground was the most densely shaded position available.

Of the 26 females observed to larviposit, the writer was able to determine with certainty the time of arrival and departure of 13. The period these 13 females spent in the shade before larvipositing varied from 3 to 128 minutes, the mean being 45 minutes. After having deposited they remained in the shade for 5 to 57 minutes, mean 18 minutes. With the exception of one female, which rested on the underside of a leaf for 55 minutes before dropping to the soil and depositing 15 minutes later, the pre-deposition period in a shaded area was invariably spent on the soil surface, the female seldom moving at all after arrival. The female usually also remained almost motionless during the post-larviposition period in the shade, but in three instances spent females flew on to the shaded undersurface of the bark before flying into the illuminated region.

These figures give some indication of the period during which the pregnant female may be expected to inhabit the dense shade of a breeding site. No suggestion is made that this is the only period during which the female inhabits shade of any sort, for the light intensity in the illuminated region of the observation box approximated more to that in the shade of a natural G. palpalis habitat than to direct sunshine, and it is well known (e.g. Macfie, 1912) that the latter is rapidly fatal to tsetse.

THE BEHAVIOUR OF THE LARVA.

Since it was found that soil with an uneven surface was more attractive as a larviposition site than soil with a smooth surface, it was thought it would be of interest to know if the larvae were more readily able to burrow into the former. This was certainly not so when the particles were immobile, for both types of soil were then equally impenetrable, but it could have been true when the particles were mobile.

To test this, wide-mesh netting cages of pregnant females were suspended at equal heights above three identical wooden boxes each of which contained a 2" deep layer of dry loose forest soil. In one box this consisted only of large particles, in one only of small particles, and in one of particles of all sizes up to 0.1"; the sizes of the large and small particles were as given previously (p.187). The surface of the first type of soil was thus very much more uneven than the second, and the third intermediate between them. Larvae were able to escape from the cages in which they were deposited by the pregnant females, and so drop into the soil below.

The numbers of pupae buried and on the surface were as follows:-

	Buried	On surface
Large particles	15	0
Mixed particles	14	6
Small particles	4	10

The difference between the distribution 15:0 and 4:10 is highly significant ($P = < 0.001$) indicating that the

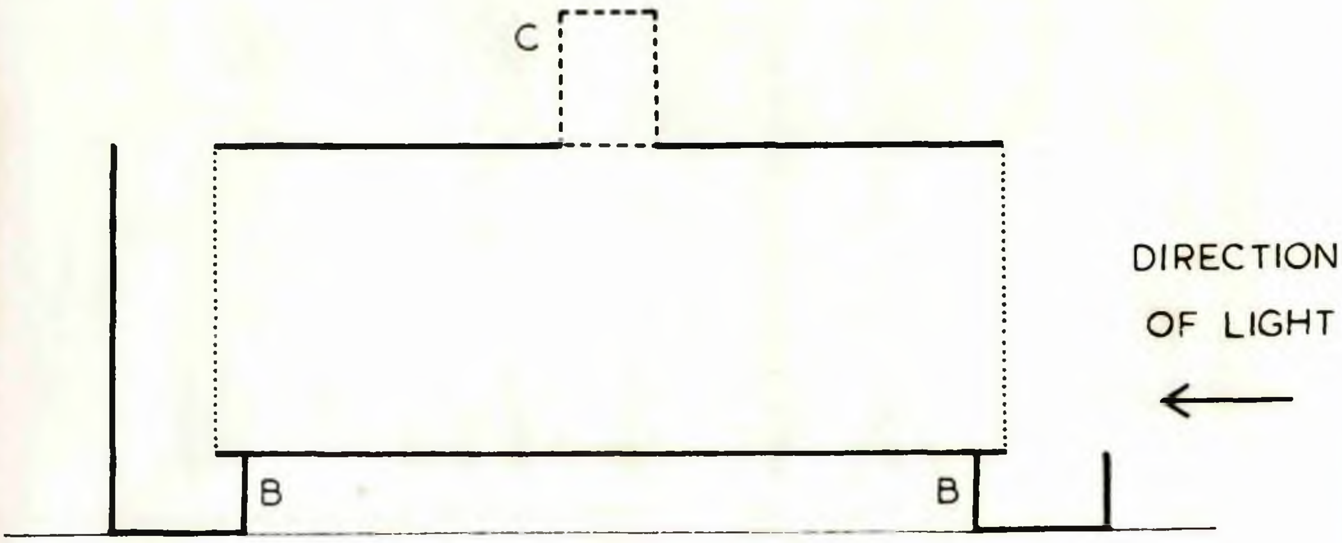

Fig. 32. Apparatus for demonstrating the reaction of G. palpalis larvae to light. C, cage containing pregnant females; B, boxes to retain larvae which fall from the end of the trough. Thick lines indicate blackened surfaces.

soil with the rougher surface was the more readily penetrable. The remaining differences were not indisputably significant, though that between the soil of mixed particle size and the small particles was suspiciously large (P = 0.04).

Some experiments on the ability of the larva to burrow in wet soil were also carried out; these will be described in the next section, to which they are relevant.

Another larval reaction investigated was that to light, the apparatus employed being shown in Fig. 32. It consisted of a wooden trough, 15" long, 5" wide, and 6" deep, open at either end. Its inner surfaces were covered with dull black paper. A wide-mesh netting cage containing pregnant females was suspended above the centre of the trough, so that any larvae deposited dropped into the centre of the latter. If larvae crawled to the end of the trough and fell out of it, they were caught and retained by a small box, one of which was placed at either end.

The trough was placed in a darkened room and levelled by means of a spirit level. A 100W bulb, the filament of which was on approximately the same level as the floor of the trough, was placed two feet away from one of the open ends. Two battery jars full of water were placed between the source of light and the end of the trough to absorb the heat emitted, with the result that the temperatures registered by a mercury thermometer at either end of the trough were identical.

A piece of sheet cork painted with Indian ink - this gave a very absorbent black surface - was placed a few inches beyond the non-illuminated end of the trough so that this end presented as dark an appearance as possible. The roof, except for the cage containing the pregnant females, was also covered with blackened sheets of cork.

The distribution of the pupae recovered was as follows:-

In the trough	0 pupae
In the box at the light end	0 pupae
In the box at the dark end	13 pupae

The number of pupae in the box at the dark end was very significantly higher than the number at the light end ($P = 0.001$), but as it was impossible to be certain that all parts of the trough were really level, and the larvae displayed such a strong tendency to burrow downwards, the experiment was repeated with the trough sloping slightly upwards towards the dark end; the latter was now approximately ¼" higher than the light end. If there was still an aggregation under these conditions it could only be due to a photonegative reaction.

The following result was obtained:-

In the trough	0 pupae
In the box at the light end	0 pupae
In the box at the dark end	16 pupae

The difference between the numbers in the boxes at the dark and light ends was highly significant ($P = < 0.001$), showing that under the conditions of the experiment <u>G. palpalis</u> larvae were undoubtedly strongly photonegative.

Since a photonegative reaction occurred, it seemed possible that the burrowing reaction might at least partly depend upon it, and might thus occur less readily or even not at all in complete darkness.

This supposition was tested by suspending a wide-mesh netting cage containing pregnant females two inches above a wooden box containing dry loose soil consisting only of large particles. The soil was illuminated by a 100W bulb a few feet away, and the number of pupae subsequently found buried or on the surface of the soil counted. Both the box of soil and the cage of females were then placed inside a larger light-proof box - they were now therefore in complete darkness- and the numbers of pupae buried and on the surface again subsequently counted.

The following were the results:-

	On surface	Buried
Illuminated	0	15
In complete darkness	0	13

Thus in both cases all pupae were buried, and there was no suggestion that the photonegative response played any part in the burrowing reaction.

BREEDING IN RELATION TO SEASON.

It was pointed out in the introduction that little was known of the breeding habits of G. palpalis during the rains. In the laboratory, the experiments already described with black objects and different types of soil were carried out both during the rains and during the dry season, and no marked differences in the magnitude of the response were noted. Similarly, as pointed out in the previous section, the reaction to moist soil was similar in both seasons.

The latter experiments gave a clear indication that soil with a moisture content of 2-4% was strongly preferred as a breeding site to that with a content of 25% upwards, but there was no apparent discrimination between the latter and soils with moisture-contents of 56-60%. Analyses of samples of the top ½" of soil taken from a variety of sites in the field during the heavy rains indicated that at this season the moisture-content of all soils was above the level at which laboratory results indicated discrimination was made, for the values obtained varied from 47.5% to 28.3%. These samples were taken under logs and palm fronds, in tree holes, and in more open ground in fringing forest, transitional vegetation and woodland. It therefore seemed unlikely that the moisture-

(To face p. 225)

Fig. 33. Shelter offering dry soil during the rains.

content of the soil was a factor strongly influencing the selection of the breeding site during the heavy rains, though it might do so near the beginning and end of the wet season; another set of soil samples collected shortly after the end of the rains gave values varying from 4.6% to 19.4%. There was no consistent tendency, either in the samples collected during the heavy rains, or in those collected after the rains had ceased, for the soil to be moister in the more protected or the more exposed situations; this varied from site to site.

In view of the preference for dry soil observed in the laboratory, it was thought likely that an artificially constructed breeding site offering shade and dry loose soil throughout the rains would probably be a highly favoured breeding place. Accordingly, two small shelters were constructed. These consisted of thatched straw roofs supported by small wooden posts, there being a gap of approximately 12" between the lower edge of the thatch and the ground (Fig.33). Inside each shelter was a zinc tray with sides $\frac{3}{4}$" high, full to the brim with dry loose soil collected from the adjacent ground. The shelters were erected at the beginning of the rains, and the soil in the trays sieved for pupae at fortnightly intervals until they ended; the soil remained dry throughout.

During the first part of the wet season, one shelter was situated in a belt of fringing forest only a few yards wide and the other in the woodland three yards from the edge of the forest, but no pupae were obtained here. It was thought that this might be due to the fly population in the vicinity having been smaller than expected, and in August the shelters were therefore moved to a situation where flies were more numerous. Here, one shelter was placed in a relatively thick belt of dense forest, and the other in a nearby belt of transitional vegetation. Even here no pupae were obtained, although breeding was found to be occurring in the immediate vicinity.

The writer has no adequate explanation to offer for this result. All the conditions found to be attractive to larvipositing females were apparently satisfied, and the only reasonable assumption seemed to be that some element(s) in the situation presented exerted a counteractive repulsive effect; in the absence of further investigation, any suggestion as to what this might be would be purely speculative.

Pupa-searching in natural breeding sites was also carried out during the rains, fresh impetus being given to this formerly largely unproductive search by Burtt's (loc. cit.) finding *G. swynnertoni* pupae in considerable numbers during the rains in the same sites as during the dry season. As described earlier, Burtt found that during the rains the pupae were nearer the surface than in the dry season, this, apparently, being because the larvae were unable to burrow to a depth of more than $\frac{1}{8}$" in damp soil.

TABLE XXVII

The burrowing capacity of G. palpalis larvae into dry and damp soils.

Type of soil	Pupae on surface	Buried pupae	Depth of pupae		
			Min.	Max.	Mean
Dry	0	20	0.3"	1.0"	0.53"
Moderately damp	8	12	0.0"	0.3"	0.09"
Very damp	13	7	0.0"	0.3"	0.07"

(To face p.227)

It was clearly desirable to know whether G. palpalis larvae also behaved in this way. Pregnant females were therefore enclosed in glass cylinders, each of which contained a 1"-deep layer of either dry, moderately damp, or very damp forest soil, the particles being of all sizes up to 0.1". The moisture-contents of the damp soils were approximately the same as those of similarly described soils in earlier experiments (p.199), and were similarly obtained by varying the height of the soil surface above a water table.

Table XXVII gives the positions of 20 pupae in each type of soil. It will be seen that the results conformed in essence with those of Burtt, for the mean depth of the pupae in the dry soil differed very considerably from the mean depth in both the very damp and the moderately damp soil, and the distributions did not overlap. There was no consistent difference in the depth of the pupae in the two damp soils, and the difference between the means was not significant.

In searching for pupae during the rains, Burtt's technique of first examining closely the surface and then scraping away a shallow layer was adopted. As pointed out by this author, the pupae are extremely difficult to see, especially as they tend to become covered by mud splashings, and searching is extremely slow and laborious. The sites searched were those which previous experience had shown to be occupied during the dry season - these were in the fringing forest and transitional vegetation - and sites in the adjacent woodland.

Any full pupae found were kept in the laboratory to determine the time of hatching, and so, approximately, that of deposition. Most of the pupae recovered, however, were empty shells which could have been deposited the previous dry season; Harris (1930) found pupal shells of G. pallidipes in a natural breeding site still to be recognizable after 26 months. Even when they were on the surface of the soil the pupae could not be assumed to have been deposited when the soil was damp, for it was often impossible to be certain that the soil was loose in any particular situation during the dry season, and if not the larvae would have been unable to penetrate. Moreover, laboratory observations showed that, in emerging, the flies usually pulled the pupal cases a considerable way towards the surface with them. This was demonstrated by covering 50 pupae with a 1"-deep layer of dry sand, and another 50 with a similar layer of dry loose forest soil. When all had hatched shells were found at the following depths:-

Depth	Dry sand	Dry soil
0.0" - 0.2"	3	0
0.2" - 0.4"	45	49
0.4" - 1.0"	2	1

Thus by far the highest concentration of shells was in each case between 0.2" and 0.4"; all these must have been pulled up through at least 0.6" of sand or soil by the emerging flies.

Only full live pupae could therefore confidently be assumed to indicate the sites in which breeding occurred during the rains. During over 200 man-hours searching by the writer and trained assistants in the neighbourhood of Kaduna, only 5 of these were found. They were in the following situations:

R.1. Under a fallen log in a belt of transitional vegetation. There was no canopy directly above the log but nearby bushes undoubtedly shaded it for much of the day. The pupa was under a fairly complete leaf-carpet but on the surface of the soil. It emerged on August 20th.

R.2. Under the small fronds sprouting from the base of a Phoenix palm in the forest (Climatic reading station C, p.208). There was practically no leaf debris, and the pupa was on the surface of the soil. It was damaged during collection and so did not emerge.

R.3. Under a low bough of a deciduous tree just inside the forest. The pupa was under a fairly complete leaf-carpet but on the surface of the soil. It emerged on September 10th.

R.4. Under a small log which was covered with grass. The vegetation was transitional but more open than usual, there being only a thin overhead canopy. The pupa was just under the surface of the soil, which was not covered by leaves. It did not emerge.

R.5. In open ground near to R.2, where it was on the surface of the soil but under fallen leaf debris. It emerged on October 21st.

These sites were again searched during the cold dry season, and live pupae found in R.1, R.2, and R.5. There appeared little doubt that R.3 was also suitable for dry season breeding, but it seemed possible that the more exposed R.4 was occupied only during the rains.

A total of 49 pupal shells was found during the rains. Of these, 34 were under fallen logs or low-lying near-horizontal boughs of trees in transitional vegetation, 17 being on the soil surface, though usually beneath leaf debris, and 17 slightly buried. Also in transitional vegetation were 3 shells beneath dead _Raphia_ palm fronds, 1 beneath the fronds of a seedling _Borassus_ palm, and 1 at the base of a large deciduous tree; these were on the soil surface, though beneath fallen leaves. 10 shells were found in forest sites, 2 being at the base of a large _Phoenix_ palm, and 8 on a pathway near a ford used by man and cattle; these were also on the soil surface, some being beneath fallen leaves. No shells were found in any woodland site.

Collections made in late December showed 8 of the 14 situations in which shells were found during the rains to be occupied by live pupae during the dry season, those unoccupied being apparently no more exposed than the remainder. On the other hand breeding was also occurring in certain situations which could not conceivably have been utilised during the rains, namely stream-bed sites which were submerged during the latter season.

Except for the latter observation, there was thus no positive evidence that the sites occupied during the rains differed from those occupied during the dry season. Further evidence on this point was sought in a comparison of collections made at different times during the dry season. It seemed unlikely that any shift in breeding there might be would occur instantaneously at the end of the rains, and if not, it would be expected to result in a difference in the distribution of the sites occupied at the beginning, and later in the dry season.

Collections were made in the neighbourhood of Kaduna in early November, when the hot, humid conditions characteristic of the beginning of the dry season still prevailed, and in late December, when cold dry season conditions had been prevalent for over a month. As it was desirable to consider only live pupae, use was made of Potts' (1933) flotation test with methylated spirit (S.G.0.82); since Potts found that 97% of the pupae which sank in this were alive it appeared that no serious error would be introduced by assuming this to be true of all pupae which behaved in this way.

In early November, live pupae were found in 22 sites, 14 of which were within three yards of a stream-bed. Searching these, and some additional sites, in late December resulted in the recovery of pupae from 24 sites, 18 of which were within three yards of a stream-bed; two of the latter were under water in early November. In terms of vegetation, 14 out of the 22 November sites were in fringing forest, while this was true of 20 out of the 24 December sites; in each case the remainder were in transitional vegetation. In both instances the proportion nearer a stream was thus slightly higher in the later collections, but neither difference in distribution was significant.

If the numbers of pupae were taken instead of the numbers of sites the difference appeared greater, for while only 28 out of 52 pupae were within three yards of a stream-bed in early November, 171 out of 207 were in a similar situation in late December. The latter result, however, was almost entirely due to one site close to a stream-bed, which yielded 158 pupae; if these were omitted the result was reversed. A similar result was obtained if the distribution of the pupae was expressed in terms of vegetation-types.

These results, like those formerly presented, thus gave little indication of a seasonal shift in the breeding of

G. palpalis. More positive evidence might have been obtained had it been possible to make more extensive collections, covering the later as well as the early dry season.

DISCUSSION.

It has been shown that shortly before larvipositing, pregnant G. palpalis females display certain preferences which lead to the selection of a breeding site; there has been no suggestion, either from direct or indirect evidence, that the latter is merely a favoured resting place in which larviposition consequently tends to occur. The observation that deposition occurs while the female is resting on the soil surface is in conformity with the field observations of Carpenter (1912) and Symes and Southby (1938) on G. palpalis, and of Burtt (1952) on G. swynnertoni. Simpson's (1918) single observation of a G. morsitans female larvipositing from the under-side of a tree trunk indicates the need for further investigation on this species.

Experiments to determine the responses involved in the selection of the breeding site have shown that females about to deposit are strongly attracted to black objects or patches of deep shade. Reactions of this nature were previously by no mean unknown in G. palpalis and other species of tsetse. Thus Fiske (1920) found a dark umbrella to be attractive to

G. palpalis, while Symes (1935) found that this species arrived in large numbers at a newly created patch of deep shade.

Fiske notes that both males and 'food-hunting' females were attracted to his dark umbrella, so a positive response to dark objects is evidently not confined to females about to larviposit. The relationship of these visual reactions to each other, and their occurrence under different circumstances, requires elucidation, for until comparitive experiments have been carried out under controlled conditions it cannot be assumed that the responses involved are in each case identical. It remains possible that the female seeks deeper shade just before larviposition than at any other phase of adult life.

It appears that the visual responses displayed by pregnant females can to a considerable extent account for the distribution of G. palpalis pupae in nature. As the writer's and previous results have shown, they are usually found in situations densely shaded by some nearby or overhead object, whether this is a fallen log, the trunk or branch of a living tree, a spray of foliage, or a rock. These situations are often themselves within fringing forest, the overhead canopy of which usually casts a somewhat lighter shade over much of the whole area surrounding the low-shaded situation.

Sometimes pupae are found in more open ground within the forest, but even here they are often beneath fallen leaves, the shade cast by which has been shown to be attractive in the same way as that cast by larger objects. At first it was thought there might also be some special, possibly non-visual response to confined situations, but later experiments failed to show they possessed any which could not be attributed to the particularly dark appearance often presented.

Also influencing the female in choice of site is the type of substratum she encounters on arrival; it has been shown that the presence of coarse dry sand makes a dark situation still more attractive, and the same must be true of dry forest soil with an uneven surface, the response to which was higher than to sand. This preference for situations containing sand or soil even though they may be lighter in appearance than others lacking such a substratum is almost certainly a tactile response, for there has been no suggestion that olfactory stimuli are of any importance. Also, the observed discrimination between soils differing only in the texture of their surfaces does not seem explicable on any other basis.

The preference for a rough over a smooth dry soil surface probably tends to result in the selection of loose over hard soil, for it seems likely that the former will tend to be associated with more uneven surfaces than the latter.

In addition there may be an actual preference for mobile over immobile particles, though this response, if it occurs at all, would appear to be weak. Any selection in favour of looser soil that does take place is clearly advantageous to the larva during the dry season, which, as a result, is more likely to be able to burrow and so reach a protected situation. It has been shown that where both smooth and rough-surfaced dry soils are loose, the latter is the more readily penetrable, so in this case also selection of the more uneven surface presumably tends to be beneficial.

It will be obvious that while the above responses may be expected to result in the deposition of larvae in situations in which they are capable of burrowing, they will by no means always do so, especially as suitable substrata may sometimes be rare and / or the ground may be completely covered with fallen leaves. To suppose, as certain authors who investigated species other than <u>G. palpalis</u> have done, that because pupae are sometimes found lying on the soil surface, no selection of a breeding site takes place, seems hardly justified.

During the dry season it appears that a position below the soil surface will benefit the pupa both by protecting it from predators, and climatically; the temperature evidently tends to be lower, and the humidity higher, than on the surface (Nash, 1942), and the work of Buxton and

Lewis (1934) indicates that, within the range normally encountered in nature, both differences are likely to be beneficial. Buxton and Lewis found a higher proportion of G. morsitans and G. tachinoides pupae to survive at 24°C than at 30°C, and there is no reason to suppose that G. palpalis would be dissimilar in this respect, especially as its distribution indicates it to be the least resistant species of the three to heat and drought. They also found the pupal mortality of G. tachinoides to decrease with rising humidity up to 98% R.H., and a similar result was obtained with G. palpalis by Mellanby (1936), who found survival to be maximal at saturation point as long as no mould developed; the latter did not occur with buried pupae, and may be rare in nature.

The fact that most breeding sites are low-shaded situations presumably results in further climatic advantages for the pupa in the dry season, for it has been shown that the temperature in such situations is almost invariably slightly lower, and the humidity slightly higher, than in adjacent more open ground. Laboratory experiments have given no evidence of any discrimination between differences of the magnitude observed in the field, but ambient conditions in the latter were sometimes considerably different from those under which experiments were performed. Nevertheless, it seems extremely unlikely that positive temperature and humidity responses are normally important in the selection of the breeding site, especially as the visual response to dark places is apparently by itself sufficient to guide the female to densely shaded situations.

When the temperature of the soil was more than 3.5°C below the ambient (24.5 - 25.5°C.) it was avoided relative to soil at the latter, and if differences of this magnitude are reached in nature - the greatest recorded by the writer was 3.5°C. - a low temperature avoidance-reaction may sometimes counteract the positive visual response to a dark situation. This, again, is doubtless affected by the value of the ambient temperature. As to the function of this avoidance, one can only guess that it may be of advantage in that it probably tends to prevent deposition in situations in which the development of the pupa would be unduly slow. The longer the pupal stage, the greater its liability to predation and other hazards must be, and possibly unusually low temperatures have some directly unfavourable effect.

Any very large humidity differences tested in the laboratory were associated with obviously damp soil, and these, it will be remembered, were avoided relative to dry soil. When the latter is available, this reaction may be advantageous in tending to prevent deposition in damp soils into which the larva is largely incapable of burrowing. There may also be some danger from water-logging in very wet situations.

During the heavy rains even the driest soil obtainable in the field had a moisture content as high as that of the 'moderately damp' soil tested in the laboratory, and at this season almost all pupae must be on or very near the soil surface. Although this presumably increases their to liability to certain hazards, it is undoubtedly preferable to their being deeply buried in water-logged soil, for the work of several investigators has indicated that in such a situation they would fail to survive; Carpenter (1912) found that no G. palpalis pupae survived a 120-hour submersion in water, and that shorter periods reduced emergence, and Buxton and Lewis (1934), working at a rather high temperature (30°C) found that the maximum period of burial in water-logged soil survived by recently deposited G. morsitans and G. tachinoides pupae was between 24 and 48, and 12 and 24 hours respectively. Carpenter found that floating pupae survived longer than those completely submerged, and it seems likely that the deleterious effects of submersion are largely or even wholly due to the blocking of the spiracles; if so it may be expected that pupae on, or just below the soil surface will tend to survive as long as the spiracles are in contact with air. The fact that the larva burrows head foremost means that the spiracles, being posterior, are the part of the body most likely to project above the surface.

The exposed situation of the pupa during the rains means that it is more likely to be subject to unfavourable temperatures than if it were more deeply buried, but except at the beginning and end of this season - and during the former, sites containing a fairly dry substratum into which the larva is capable of burrowing are still likely to be available - maximum temperatures are fairly low. The ambient humidity, exposure to which would be dangerous during the dry season, is similarly at a high level unlikely to be harmful to the pupa.

The implications of the ability of the larva to burrow in different types of soil have already been discussed. Its negative reaction to light seems likely to be of advantage in that it probably tends to prevent a larva deposited in a shaded area in which it is not immediately able to burrow from leaving this area. Admittedly the writer did not observe any such tendency when making direct observations of larviposition in the laboratory, but this may have been because the light intensity of the illuminated areas was too low to induce an avoiding reaction; that the larvae will not react photonegatively unless the light intensity is fairly high is indicated by Burtt's (1952) finding that the light from an electric torch failed to produce this response. A light intensity below the threshold value may also account for Mellanby's (1936) failure to observe a photonegative response in G. palpalis larvae.

The photonegative response of the larva may also normally play some part in the initiation of burrowing - since the photosensitive anterior end of the body is the first to be buried it is not likely to be involved in subsequent stages - but the fact that this reaction readily occurs in complete darkness clearly shows that light responses are not essential to its performance.

It was pointed out in the introduction that the evidence for a seasonal shift in the breeding of G. palpalis in West Africa was not altogether satisfactory, and the author's results have done little to substantiate it. In so far as some dry season sites are in situations which are flooded during the rains, however, it may be taken for granted that there is some difference in the sites occupied during these two seasons. Also, on general grounds one would expect that under the arid conditions of the dry season, breeding would be more concentrated in the moist densely vegetated regions in the vicinity of the streams than during the rains. Nash (1939) obtained clear evidence that a seasonal shift of a similar nature took place in G. tachinoides and G. morsitans in Northern Nigeria, and it would be surprising if no such tendency occurred in G. palpalis in the northern part of its range, though it is almost certainly a less well-defined one.

SUMMARY

PART I

IA. Field studies on adult *Culicoides.*

1. Previous observations relevant to the subjects investigated are reviewed.

2. Methods of making standardised collections of adult *Culicoides* are discussed, and the hand-net technique adopted is described.

3. The seasonal incidence of *C.impunctatus*, *C.heliophilus*, *C.pallidicornis*, *C.punctatus*, and the *C.obsoletus* group at a site on the shore of Loch Lomond is described. The sex-ratios of these species were markedly unequal - *C.pallidicornis* less so than the remainder - but collections of resting adults of *C.punctatus* and the *C.obsoletus* group yielded almost equal numbers of males and females.

4. Maximal numbers of all species were obtained during the evening. Apart from a very much smaller peak at sunrise practically no adults were obtained at other times of day or night. The evening peak of most species was around sunset, but that of *C.heliophilus* females and *C.pallidicornis* males was several hours earlier.

5. The time of maximum incidence appeared to be more closely related to that of sunset than to any fixed time of day. There was a tendency for the peak to occur earlier on dull than on bright days.

6. The number of adults captured was very much affected by wind strength, but the variations in temperature, humidity, and rainfall encountered had no discernible effects.

7. A series of exposures showed C. impunctatus females to be very much more strongly attracted to man than females of C. heliophilus, C. pallidicornis, and the C. obsoletus group.

8. The results are discussed in the light of previous, and subsequent work.

IB. Laboratory studies on the eggs of Culicoides.

1. Earlier work is reviewed.

2. The technique of maintaining Culicoides females in the laboratory is described, as is their longevity, the readiness of different species to oviposit, and the duration of pregnacy under these conditions.

3. The eggs of ten species are described, eight for the first time.

4. Eggs of most species hatched a few days after laying, but *C.grisescens* eggs laid in September hatched in February or later.

5. A non-segmented pseudopod on the first thoracic segment of the first-instar larva of ~~o~~ the *C.obsoletus* group is described.

6. *C.pulicaris* and *C.punctatus* eggs usually survived 12 hours, some 18 and 24 hours, but none 48 hours exposure to a dry atmosphere at room temperature. Survival tended to increase with the age of the egg. Hatching tended to be delayed by treatment.

7. Moist *C.pulicaris* and *C.punctatus* eggs survived 12,24, and 48 hours at 30°C, and 12 hours at 35°C. 24 and 48 hours at 35°C were sometimes survived, the former species being more susceptible than the latter. Survival again tended to increase with the age of the egg. Exposure to 30°C tended to shorten the duration of the egg stage, exposure to 35°C to lengthen it.

8. *C.pulicaris* and *C.punctatus* eggs exposed to a dry atmosphere at 30°C or 35°C for 12 or 24 hours never survived.

9. The few experiments carried out on the eggs of *C.impunctatus*, *C.grisescens*, and the *C.obsoletus* group gave no indication of any differences in survival-capacity from the above results except that *C.grisescens* eggs survived 48 hours drying.

10. The results are discussed in relation to those of other investigators, and to conditions in nature.

PART II

1. Previous observations on the breeding habits of <u>G.palpalis</u> and other species of tsetse are reviewed.

2. The climate and vegetation in the neighbourhood of Kaduna, Northern Nigeria, where the investigation was carried out, are described.

3. In the laboratory, <u>G.palpalis</u> females selected trays containing dry sand as larviposition sites; the response largely, but not wholly, disappeared in darkness.

4. Black thermohygrographs were attractive as larviposition sites, as were a variety of black objects of simpler shape. Deposition readily occurred in the interiors of small black boxes.

5. Several of the preceeding results had suggested there might be some preference for confined situations possibly independant of that for ~~the~~ dark regions, but experiments with partly dark and partly light objects failed to bear this out. Later experiments in which the response to soil in confined and more open siutations was compared gave a similar result.

6. Dry sand combined with a black object was more attractive than either alone. The attractiveness of sand

was similarly increased when dense shade fell on it.

6. Dry loose soil was more attractive than sand, and a very high response was obtained when it was associated with a horizontal object which cast shade on it. The latter object was apparently of no importance in providing a resting place for the pregnant females.

7. Experiments in which the complicating effect of differences in appearance were eliminated showed that soil with a rough surface was preferred to that with a smooth, this being true whether or not the particles were mobile. There was no clear evidence of any discrimination between mobile and immobile particles independant of surface-texture.

8. No evidence was obtained that soil from a natural breeding site had any olfactory property attractive to pregnant <u>G.Palpalis</u>.

9. Visibly damp soils were avoided relative to soils approximately in equilibrium with an ambient humidity of 50% or 80% R.H.

10. Soil from 3.5°C to 9.0°C below an ambient temperature of approximately 25.0°C was avoided relative to soil at the latter temperature.

11. Climatic measurements taken at natural breeding sites at the time of maximum larviposition showed the temperature to be almost invariably slightly lower, and the humidity higher, in the breeding site itself than at adjacent more open ground.

The differences were not as large as those which produced definite avoidance in the laboratory.

12. Dead leaves scattered over the surface of dry soil in the laboratory increased its attractiveness in the same way as did larger shade-casting objects.

13. In 37 direct observations of larviposition, this act always occurred on the soil surface, usually in the shade. The average time spent in the latter situation was 45 minutes before deposition and 18 minutes afterwards.

14. G.palpalis larvae burrowed more readily into rough than into smooth-surfaced soil, and more readily into dry than into wet soil. They were photonegative, but this reaction was evidently not essential for burrowing, which occured in complete darkness.

15. Attempts to find the wet season breeding sites of G.palpalis met with little success, only five full pupae being found. Only one of these was in a site unlikely to be occupied during the dry season. Similarly, the sites in which pupal shells were found during the rains were mostly occupied during the dry season, though during the latter season breeding also occured in sites submerged during the rains. Laboratory experiments gave no indication that the responses of the pregnant females varied with the season.

16. The significance of the results in nature is discussed.

REFERENCES

PART I

ANDERSON F.S. (1937) Meddelelser om Gronland, 116, 1.

ATCHLEY F.O. and HULL J.B.(1936). J. Parasit., 22, 514.

BEQUEART J. (1924). Rept. med.Dept. Un. Fruit Co., 13, 193.

BUCKLEY, J.J.C. (1934). J.Helminth., 12, 99

______ (1938). Ibid., 16, 121.

CAMERON, A.E. (1947). Trans. Highland Agric. Soc. Scotland,1947.

______ (1948). Ibid., 1948.

CAMERON, A.E., DOWNES J.A., MORRISON G.D. and PEACOCK A.D.(1946). In Control of midges. Edin.: H.M.S.O.

______ (1948). Second report on control of midges. Edin.: H.M.S.O.

CAMERON, E. (1946). In Control of midges. Edin: H.M.S.O.

CARTER H.F., INGRAM A., and MACFIE J.W.S. (1920). Ann. trop. Med. Parasit., 14, 187.

DORSEY C.K. (1947). J. econ. Ent., 40. 805.

DOWNES J.A. (1950). Nature, 166, 510

______ and KETTLE D.S. (1952). Proc. R. ent. Soc. Lond.(B) 21, 61.

DOVE W.E., HALL D.G., and HULL J.B. (1932). Ann. ent. Soc. AMER., 25, 505.

EDWARDS F.W. (1921). Scot. Nat., 111 - 112, 59.

______ (1922). Bull. ent. Res., 13, 161.

EDWARDS F.W. (1939). *In* British blood-sucking flies. Lond.: British Museum (Nat. Hist.).

GOETGHEBUER M. (1919). Bull.Ann.Soc.ent.Belg., *59*, 25

______ (1920). Mem. Mus. Hist. nat. Belg., *8*, 1.

______ (1935). Bull. Ann. Soc. ent. Belg., *75*, 309.

HILL M.A. (1947). Ann. trop. Med. Parasit., *41*, 55.

HINMAN E.H. (1936). Amer. J. Hyg., *15*, 773.

HOFFMANN W.A. (1925). *Ibid.*, *5*, 274.

JAMES M.T. (1943). Pan-Pac. Ent., *19*, 148.

JOBLING B. (1928). Trans. R. Soc. trop. Med. Hyg., *22*, 304.

______ (1953). Parasit., *43*, 148.

KEARNS H.G.H.(1942). Ann. appl. Biol., *29*, 310

KETTLE D.S. (1949). Ann. trop. Med. Parasit., *43*, 284.

______ (1950). Trans. R. ent. Soc. Lond., *101*, 125.

______ (1951). Bull. ent. Res., *42*, 239

______ and LAWSON J.W.H. (1952). Bull. ent. Res., *43*, 421.

LAWSON J.W.H. (1951). Trans. R. ent. Soc. Lond., *102*, 511.

LUTZ A. (1913). Mem. Inst. oswaldo Cruz, *5*, 45.

MALLOCH J.R. (1915). Bull. Ill. Lab. nat. Hist., *10*, 275.

MAYER K. VON (1934). Arch. Naturg. N.F., *3*, 205.

MELLANBY K (1935). Biol. Rev., *10*, 317

MYERS J.G. (1935). Trop. Agric. Trinidad, *12*, 71.

NEIVA A, and PENNA B. (1916). Men. Inst. Oswaldo Cruz, *8*, 74.

PAINTER R.H. (1926.) Rept. med. Dept. Un. Fruit Co., *15*, 245.

PATEL P.G. (1921). Proc. 4th. ent. Mtg. Pusa, 272.

POMERANTZEV B.I. (1932). Mag. Parasit. Leningrad, 3, 183

SALM A.J. (1914). Arch.Parasit. Paris, 16, 404.

SHARP N.A.D. (1928), Trans.R. Soc. trop. Med. Hyg., 21, 371.

STEWARD J.S. (1933). Rept. Univ. Camb. Inst. Anim. Path., 3, 272.

THIENEMANN A.(1928). Arch. Hydrobiol. Plankt., 19, 585.

THOMSEN L.C. (1937). In Aquatic Diptera. Cornell Univ. Agric. Expt. Sta. Memoirs.

TOIT R.N. du (1944). Onderstepoort J. vet. Sci., 19, 7.

TOKUNAGA M. (1937). Tenthredo, Kyoto, 1, 233.

______ and ESAKI T. (1936). Mashi, 9, 55.

VARGAS L. (1949). Rev. Soc. Mexicana Hist. nat., 10, 191.

WIGGLESWORTH V.B. (1950). Principles of insect physiology. 4th ed. Lond.

WINN C.M. (1924). Rept. med. Dept. Un. fruit Co., 13, 206.

PART II

BAGSHAWE A.G.(1908). Rept. S.S. Comm. R. Soc., 4, 48.

BEQUAERT J.C. (1946). Amer. J. trop. Med., 26, Suppl. 5, 57.

BURTT E. (1952). Acta tropica, 9, 304.

BUXTON P.A. and LEWIS D.J. (1934). Philos. Trans. B, 224, 175

CARPENTER G.D.H. (1912). Rept. S.S. Comm. R. Soc. 12, 79.

______ (1913). Ibid., 14, 1.

CHORLEY J.K. (1929). Bull. ent. Res., 20, 279

DUKE L.H. (1919) Ibid., 9, 263.

East African Tsetse and Trypanosomiasis Research and Reclamation Organisation: Annual Reports1949-1952. Nairobi.

HARRIS R.H.T.P. (1930). Report on the bionomics of the tsetse fly *Glossina pallidipes* Austen. The Natal Witness, Pietermaritzburg.

JACK R.W. (1919). Bull. ent. Res., *10*, 71.

______ (1939). Mem. Dept. Agric. S. Rhodesia., *1*.

______ and WILLIAMS W.L. (1937). Bull. ent. Res., *28*, 499.

JOHNSON W.B. and LLOYD Ll. (1923). *Ibid.*, *13*, 373.

LAMBORN W.A. (1915). *Ibid.*, *6*, 249.

LEWIS D.J. (1934). *Ibid.*, *25*, 195.

LEWIS E.A. (1939). *Ibid.*, *30*, 345.

______ (1942). *Ibid.* *32*, 303.

LLOYD H.M. (1935). *Ibid.*, *26*, 439.

LLOYD Ll. (1914). *Ibid.*, *5*, 49.

MACFIE J.W.S. (1912). *Ibid.*, *3*, 61.

MELLANBY K. (1936). *Ibid.*, *27*, 611.

MORRIS K.R.S. (1934). *Ibid.*, *25*, 309.

NASH T.A.M. (1933). *Ibid.*, *24*, 107.

______ (1937). *Ibid.*, *28*, 75.

______ (1939). *Ibid.*, *30* 259.

______ (1942). *Ibid.*, *32*, 327

______ and PAGE W.A. (1953). Trans. R. ent. Soc. Lond., *104*, 71.

POMEROY A.W. and MORRIS K.R.S. (1932). Bull. ent. Res., *23*, 501.

POTTS W.H. (1933). *Ibid.*, *24*, 293.

ROSEVEAR D.R. (1953). Vegetation *in* The Nigeria Handbook. Lond.: Crown Agents for the Colonies.

SIMPSON J.J. (1911). Bull. ent. Res., *2*, 187.

______ (1918). *Ibid.*, 8, 193.

STEWART J.L. (1937). Report on the eradication of tsetse flies of the _G.palpalis_ group .... Accra: Govnt. Printer.

SWYNNERTON C.F.N. (1921). Bull. ent. Res., _11_, 315.

______ (1923a). _Ibid._, _13_, 317

______ (1923b). _Ibid._, _14_, 119.

______ (1936). Trans. R. ent. Soc. Lond., _84_, 1.

SYMES C.B. (1935). E. Afric. med. J., Dec., 1935.

______ and SOUTHBY R. (1938). The reduction of _G.palpalis_ in a lake shore area by the 'block' method. Nairobi: Govnt. Printer.

______ and VANE R.T. (1937) The eradication of _G.palpalis_ from river areas by the 'block' method. Nairobi: Govnt. Printer.

VANDERPLANK F.L. (1949). Proc. R. ent. Soc. Lond. (B), _18_, 65.

YORKE W. and BLACKLOCK B. (1915). Ann. Trop. Med. and Parasit., _9_, 349.

ZUPITZA A. (1908) Arch f. Schiffs. -u. Trop. Hyg., _12_, 25.

APPENDIX ON STATISTICAL METHODS

P has been estimated by the use of Student's t or, more frequently, χ^2, the formulae employed being taken mainly from Brownlee (1948).

Student's t was used when the significance of the difference between the means of two sets of figures was required. If x_1, x_2, x_3..... were the values of one set of figures, and y_1, y_2, y_3..... the values of the other set, and if n_x was the number of figures if the first set and n_y the number in the second set, σ was obtained from the formula

$$\sigma = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n_x} + \Sigma y^2 - \frac{(\Sigma y)^2}{n_y}}{n_x + n_y - 2}}$$

If $\bar{x}$ and $\bar{y}$ were the means of the values of x and y respectively, then

$$t = \frac{\bar{x} - \bar{y}}{\sigma} \sqrt{\frac{n_x \cdot n_y}{n_x + n_y}}$$

The number of degrees of freedom was $n_x + n_y - 2$ and P was obtained by looking up t in the appropriate tables or graph.

A χ^2 test was employed whenever the significance of the difference between two figures or two distributions was required. The general formula is

$$\chi^2 = \Sigma \left[\frac{(O - E)^2}{E}\right]$$

O being the observed values and E the expected. When the difference between a single pair of figures was to be evaluated, E became the mean between these figures and the formula was thus

$$\chi^2 = \frac{(x_1 - \bar{x})^2}{\bar{x}} + \frac{(x_2 - \bar{x})^2}{\bar{x}}$$

x_1 and x_2 being the two observed figures, and $\bar{x}$ their mean. A correction for discontinuity of distribution was also made, which consisted of adding 0.5 to the value of x below expectation and subtracting 0.5 from the other. P was then obtained by reference to the appropriate tables or graph, the number of degrees of freedom being one.

Where the significance of the difference between an observed distribution a:b and an expected distribution of c:d was required, and a + b equalled c + d, the formula needed little modification:

$$\chi^2 = \frac{(a - c)^2}{c} + \frac{(b - d)^2}{d}$$

a correction for discontinuity again being made. The number of degrees of freedom, being one less than the number of observations, was, in the above case, again one.

When the significance of the difference between two distributions a:b and c:d in which a + b did not equal c + d was required, χ^2 was obtained from the formula

$$\chi^2 = \frac{(a+b+c+d)(ad-bc)^2}{(a+b)(b+d)(c+d)(a+c)}$$

A correction for discontinuity was again made, and the number of degrees of freedom was again one.

Reference.

Brownlee K.A.(1948). Industrial experimentation. 3rd. ed.
Lond.: H.M.S.O.

STUDIES ON THE ECOLOGY AND BEHAVIOUR OF CERTAIN BITING DIPTERA

Biting midges (*Culicoides* Latreille) are familiar pests in Scotland. Standardised collections were made at a site near the University of Glasgow Insect Field Station, Rossdhu, Loch Lomond, to obtain information on the habits of some of the more important species. Different species were found to have different seasonal distributions, and while most were obtained in largest numbers around sunset, the peak was in two cases several hours earlier. Apart from a very much smaller peak at sunrise, practically no adults were obtained at other times of day or night.

Numbers captured were very much influenced by wind, but other weather conditions measured had no discernible effects. *C. impunctatus* was much more strongly attracted to man than other species present.

Gravid *Culicoides* females were kept in the laboratory, and eggs obtained from ten species. They were cigar-shaped and covered with small sucker-like protuberances, the size and arrangement of which varied with the species, as did the size of the entire egg. Hatching usually occurred within a few days, but *C. grisescens* eggs laid in September hatched in February or later; evidently this species spends much of the colder part of the year in the egg stage, whereas the available evidence on other species of *Culicoides* indicates that this season is normally spent in the larval stage. A chance observation revealed the presence of a non-segmented appendage on the first-instar larva of a member of the *C. obsoletus* group; *Culicoides* larvae had always previously been described as apodous.

C. pulicaris and *C. punctatus* eggs sometimes survived 24, but never 48 hours exposure to a dry atmosphere at room temperature. Moist eggs of the same species always survived

48 hours at 30°C and sometimes the same period at 35°C. Exposure to a dry atmosphere at 30°C or 35°C was never survived even for 12 hours. Limited data indicated that other species had similar survival-capacities, though C. grisescens survived 48 hours drying. From the sites in which larvae are found, desiccation and above-normal temperatures seem unlikely to be frequent cases of high oval mortality in nature.

The second part of the work was mainly a study of the behaviour of the pregnant tsetse fly, Glossina palpalis Robineau-Desvoidy, carried out in Northern Nigeria. Although various investigators had recorded the breeding sites of this and several other species of tsetse as being typically low-shaded situations such as fallen logs, low bushes and overhanging rocks, the conditions actually attractive to the depositing females remained largely unknown. Thus, the observed sites could have been selected because of their relatively dark appearance, the resting places or climatic conditions they offered, and/or because of some property of their substrata.

Laboratory experiments showed pregnant G. palpalis females to be receptive to two types of stimulus: (a) visual, which resulted in their selection of various black objects, and in the enhanced attractiveness of sand or soil when shaded, and (b) tactile, which resulted in a preference for soil with a rough surface over that with a smooth. Resting places were apparently of importance only by virtue of the shade they cast, and no evidence that soil possessed any attractive olfactory property, or that the temperature and humidity conditions associated with natural breeding sites were attractive, was obtained. It seems likely that the observed visual and tactile reactions are largely responsible for the distribution of *G.palpalis* pupae in nature, though avoidance reactions such as those demonstrated towards damp or unusually cool soil presumably also sometimes affect the choice of site.

G.palpalis larvae which, as soon as deposited, attempt to burrow, were found to be able to do so more readily in soil with a rough than with a smooth surface, and more readily in dry than in damp soil. They were photonegative, a reaction which in nature probably tends to keep them within shaded areas.

Since only the dry season breeding sites of *G.palpalis* were well known, a further attempt to locate those occupied during the rains was made. It met with little success, and there was no clear indication that the wet season sites differed materially from those occupied during the dry season, except in so far as some sites occupied during the latter were flooded and so uninhabitable during the rains. This was in accordance with laboratory results, which gave no indication that the behaviour of the pregnant females varied with the season.