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THE BIOLOGY AND BEHAVIOUR OF *CULICOIDES BREVITARSIS* KIEFFER (DIPTERA : CERATOPOGONIDAE) WITH PARTICULAR REFERENCE TO THOSE FEATURES ESSENTIAL TO ITS LABORATORY COLONISATION.

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ABSTRACT

Aspects of the behaviour and biology of C.brevitarsis were studied with the aim of establishing a laboratory culture. It was shown that gravid females discover dung pats, the breeding site of the larvae and pupae, visually and probably while flying over the pat. The curvature of the edges of the pat was shown to be important to its recognition, whereas shape in plain view was not. Oviposition occurred throughout the whole day, with the highest rates during the afternoon and early part of the night, and continued throughout the 6 days after the pat was dropped. Larvae and pupae were distributed throughout the pat but with very few in the crust and a tendency for pupae to be concentrated in the wet-caneite zone. Pupation and eclosion were described in detail. Eclosion occurred during the afternoon sometimes extending into the early evening, and was accelerated by exposure to bright light (especially 60,000 lux).

Mature spermatids were present in the vesicula seminalis 24 hours after emergence at which time all of the stored food had been consumed in both males and females held in the laboratory. Mature spermatids were 90 μm long but extremely narrow.

Swarming, presumed to be the time and place of mating, was examined in detail. The most common marker was shown to be the sunward boundary of a zone of very low reflected light, the most usual source in the field being shadows. Cattle grossly altered swarming by reducing the height of and distance between swarms, and increasing the number of males and the likelihood that females will be present in them. The upper limit of wind speed at which swarming would occur was 2.47 ms^{-1}. Swarming occurred during the hour preceding sunset. Females obtained a blood meal from cattle starting ½ an hour before and continuing to as late as 6 hours after sunset. The majority (97%) of females collected from cattle were already mated. Flies fed on the top of the animal near its tail with their density falling very rapidly down the host's side but less rapidly towards its head. Oogenesis was described with particular reference to changes in size, the development of the secondary ovum, and changes during sclerotisation of the primary ovum. The development of small structures, called ansulae, on the surface of the egg was examined, and it was proposed that they formed a plastron layer. The longevity of adults fed on various diets in the laboratory was determined, both sexes living for a mean of 8.9 days when offered sugar solution.

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DECLARATION

The work presented in this thesis is my own, unless otherwise acknowledged in the appropriate place; it has not been previously published or submitted to this or any other University for the award of any degree.

Malcolm M Campbell

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INTRODUCTION

Species within the families Culicidae, Psychodidae, Simulidae, and Ceratopogonidae of the lower Diptera have been incriminated as vectors of pathogens causing both human and animal diseases. Amongst the Ceratopogonidae, species in the genera Culicoides, Leptoconops, and Lasiohelia feed on blood from warm-blooded vertebrates. Many species feed on invertebrates and a wide range of other animals. Any one species feeds from a limited range of hosts, usually one or two animals. In the Ceratopogonidae, as in the rest of the Nematocera, only the female feeds on blood. The males feed on nectar.

The Ceratopogonidae, especially Culicoides, are well known but not well studied. The attack by vast numbers on man in some areas is probably the greatest cause of their notoriety. In some places they have been reported to have retarded the development of tourist resorts (Dove, Hall, & Hull, 1932; Linley & Davies, 1971) Their importance as vectors of pathogens is rapidly becoming appreciated. The adults are very small with wing length rarely exceeding 2mm allowing them to go through screening materials used to exclude mosquitoes and hence in some areas they could be classed as the greater nuisance. Ceratopogonidae have been studied very little probably because of their small size and because they do not transmit a fatal disease to man. The genus Culicoides is not entirely without anthropocentric value: several species are amongst the most important pollinators of the Para rubber tree in tropical America (Warmke, 1951, 1952; Wirth, 1956)

About 800 spp. of Culicoides have been described (Arnaud and Wirth, 1964). They are distributed all around the world with the exception of a few small areas such as New Zealand. The absence of reports of the genus from those areas may be due to inadequate searching rather than their total absence.

Culicoides have been incriminated as the vectors of three groups of pathogens; Nematoda (Filarioidea), Protozoa (Haemosporidiidea), and Viruses. Filarial worms of man were the first pathogens known to be transmitted by Culicoides (Sharp, 1927). The transmission of protozoa was not demonstrated until the work of Fallis and Wood (1957). Du Toit (1944) demonstrated the transmission of a virus. Studies on the transmission of viruses is still proceeding.

Sharp (1927, 1928) reported that Acanthocheilonema perstans was transmitted to man by Culicoides grahami. Henrard and Peel (1949) and Chardome and Peel (1949) suggested that two parasites were involved and subsequent work showed that Culicoides austeni was the likely vector of A perstans (Hopkins & Nicholas, 1952) while C grahami transmitted Dipetalonema (Acanthocheilonema) streptocerca (Duke, 1954, 1956). The microfilariae of A perstans occur in the circulatory system, those of D streptocerca in the skin; neither cause pathogenicity in man.

The only arthropod in which the microfilariae of Mansonella ozzardi would develop was Culicoides furens (Buckley, 1934). M ozzardi is a non-pathogenic filaria that is parasitic on man in the West Indies. Steward (1933) showed that Culicoides nubeculosus transmitted Onchocerca reticulata (=cervicalis), the cause of fistula of the withers or head (poll-evil) Culicoides species may be the vectors of Onchocerca gibsoni which injures the carcases of cattle in Australia and Malaya by forming hard nodules (Buckley, 1938). He also showed that the microfilariae of O gibsoni developed to the infective stage in about 0.5% of flies. However flies occurred in such large numbers that a cow should be bitten by an infective fly at least once a day thus maintaining a high rate of the parasite in cattle.

An unnamed Culicoides was the only one of a large range of species in which Haemoproteus nettionis would develop (Fallis & Wood, 1957). They transmitted H nettionis to ducks by injecting them with suspensions of Culicoides that were collected in the wild and then ground up. They observed but did not identify sporozoites in the salivary gland of the same Culicoides. Similar evidence was obtained for the transmission of Haemoproteus canachites (Fallis & Bennet, 1960). In both cases it was shown that heavy parasitaemia of ducks followed periods when large numbers of Culicoides were feeding on the ducks (Bennett & Fallis, 1960; Bennett, 1960). Haemoproteus spp are blood parasites.

Leucocytozoon caulleryi, which causes leucocytozoonosis, an economically important disease of poultry in SE Asia, was shown to be transmitted by Culicoides arakawae (Akiba et al,1959; Akiba, 1960). Garnham et al (1961) showed that the malaria-like parasite Hepatocystis (Plasmodium) kochi of Circopithecus monkeys developed within Culicoides adersi.

An allergic dermatitis of horses (Riek, 1954), known as Queensland itch, resulted from the bite of Culicoides brevitarsis (reported as C robertsi). A similar sort of skin reaction, this time called dermatozoonosis, has become a serious problem in some cities of Brazil (Sherlock & Guitton, 1964, 1965). Lesions form on the skin of sensitive people often on their legs, and strong reactions may occur. One clinic treated 213 cases in 1960-1961. The incidence of lesions is greatest when large populations of Culicoides paraensis are present and biting man and on that basis that species has been blamed for the condition which is presumed to be a reaction to its bites.

The organisms discussed above show little or no pathogenicity. Culicoides are now emerging as important vectors of a number of viruses including several that can cause severe economic losses of domestic animals. The first virus thought to be transmitted by Culicoides was Fowlpox (Tokunaga, 1937), but the only evidence seems to have been that some species of the genus bred in fowl pens. Karstad et al (1957) reported the recovery of Eastern Equine Encephalomyelitis (EEE) virus from newly caught adults of Culicoides. Transmission was not proved and to date the vector of EEE remains unknown. Karstad quoted a personal communication from R. Levi-Castillo claiming that Venezuelan equine encephelomyelitis had been isolated from Culicoides.

Three-day sickness, or Emphemeral fever, a virus disease of cattle that periodically occurs in epidemic proportions in Eastern Australia is thought to be transmitted by one or more Arthropods (Seddon, 1938; Mackerras et al 1940). Culicoides brevitarsis is considered to be a likely vector (Standfast & Dyce, 1972a,b).

Buttonwillow virus was isolated from Culicoides variipennis in California (Reeves et al 1970). It infects two mammals, Sylvilagus auduboni (rabbit) and Lepus californicus (black-tailed jack rabbit). The virus responsible for epizootic haemorrhage disease of deer was shown to multiply in several species of Culicoides (Boorman & Gibbs, 1973) which can therefore be presumed capable of transmission.

In 1944 Du Toit incriminated Culicoides as the vector of Bluetongue virus. Suspensions of wild-caught Culicoides that had been ground up produced clinical symptoms, including death, when injected into clean sheep. Sheep that recovered were challenged with BT and resisted infection suggesting that they had developed immunity. Further work confirmed transmission by the bite of Culicoides pallidipennis. Detailed work using clean adults reared

in laboratory colonies has shown that Culicoides variipennis can transmit BT in the USA (Foster, Jones & McCrory, 1963).

BT appears to have originated on the African continent where the endemic ruminants seem to be relatively resistant. It was first recognised as a clinical entity when the Merino and other European breeds of sheep were introduced to Southern Africa (Howell, 1963). It has spread widely around the world, particularly since the 1940's. In 1956 it appeared in Spain and Portugal (Ribeiro et al, 1957). It was present in Spain, Turkey, West Pakistan, Japan, USA, as well as much of Africa by 1960 (Howell, 1963), but is not present in Australia. The first record for the USA was in Texas in 1948, followed by a mild epizootic spanning much of that state in 1952 (Hardy & Price 1952). McGowan (1953) identified it in California in 1952. By 1960 it had spread to 11 states of the USA (USDA, 1960) and could be considered to have become enzootic.

In the early 1940's BT was a rather obscure disease of sheep and considered to be of little importance. It caused only low mortality and was thought to be confined to Africa. In the following 15 - 20 years it has spread widely, highly virulent strains have been recognised and hence BT is assuming a new and emerging importance as a disease of sheep. A particularly virulent epizootic in Cyprus in 1943 was responsible for 60-70% mortality in some flocks (Gambles, 1949). Morbidity may reach 100% (Howell, 1963) but mortality varies widely.

BT is essentially a disease of sheep with the European breeds, particularly the Dorset Horn, being very susceptible (Neitz, 1948). Evidence for the susceptible of cattle is equivocal. BT has been recovered from cattle (Bekker, De Kock and Quinlan, 1934; DeKock, DuToit and Neitz, 1937; and Nevill, 1971). Calves were infected artificially (Spreull, 1905). Classical symptoms of BT have been described in cattle during epizootics (Komarov & Goldschmidt, 1951) but symptoms are rare in enzootic areas (Howell, 1963). Goats show similar susceptibility as cattle (Howell, 1963).

African Horse Sickness is widely distributed in Africa and extends into India. It is a highly fatal disease of equines (Howell, 1963). Its origins appear to be similar to those of BT but it has not yet spread as far. In susceptible equines mortality ranges from 50-95% (Howell, 1963). Du Toit (1944) recovered AHS from wild-caught Culicoides which may be the vector. Schuberg & Kuhn (1912) successfully

demonstrated mechanical transmission by Stomoxys calcitrans. Wetzel et al (1970) found that AHS did not multiply in, and was not transmitted by C pallidipennis, Culicoides milnei, Culicoides distinctipennis, or several mosquitoes. The most plausible explanation of their results is that the insects tested do not transmit AHS. The method of transmission of AHS is not clearly proven. The vectors are unknown.

Tick fever, fowl pox, and possibly Onchocerciasis and Ephemeral fever are some of the only arthropod borne diseases of livestock known to be present in Australia. Murray Valley Encephalitis, Dengue, and epidemic polyarthritis are transmitted to man. Doherty et al (1972) have isolated 24 viruses from arthropods including those responsible for MVE Epidemic polyarthritis (Ross River Virus), and the virus Akabane which can cause extended viraemia in cattle (Standfast & Dyce, 1972 a)

Onchocerciasis occurs in cattle in Australia (Cleland, 1912) but its vector is unknown. Culicoides have been incriminated as vectors of Onchocerca spp in England (Steward, 1933) and in Malaya (Buckley, 1938). It is possible that Culicoides transmit it in Australia. Lee et al (1958) found good evidence that mosquitoes transmit Fowlpox but involvement of Culicoides is still possible. Queensland itch is caused by the bite of C brevitarsis (Riek, 1954).

Three epizootics of Ephemeral fever in Australia (Murray, 1970; Albiston, 1968; Gee et al, 1969) that caused up to 80% morbidity of bovines (Murray, 1970) stimulated extensive work on vectors and arboviruses. Seddon (1934) and Mackerras et al (1940) suggested that Ephemeral fever was arthropod borne. Using evidence from all three epizootics, especially the third (1967-8), Murray showed that a winged arthropod was the only feasible vector and that it was being blown southwards through eastern Australia by the prevailing winds.

Lee et al (1962) summarised previous Australian work that examined the host ranges of various Culicoides by precipitin test. Three species were considered to be prime suspects for vectors to cattle; C brevitarsis, C marksi, and C dycei in descending order of likelihood. Standfast & Dyce (1972 b) collected arthropods from cattle during the third epizootic. They took 28 species; 5 939 mosquitoes (55.5% Culex annulirostris), 18 009 Culicoides (99.7% C brevitarsis). C. brevitarsis comprised 75% of the total collection, C marksi 0.4%, while C dycei was not collected but probably would have been if collections had been made inland. On the basis of occurence C brevitarsis emerges as prime suspect for the transmission of Ephemeral fever. Three different viruses were isolated from the

C brevitarsis collected. Antibodies to all three of the viruses (Akabane, Samford, and D'aguilar were demonstrated in cattle (Doherty et al, 1972). They did not recover the virus of Ephemeral fever from arthropods but that is not good evidence against transmission because the test used is insensitive with unadapted virus. The economic and epidemiological importance of the viruses isolated is not known but D'aguilar has some similarities to BT and AHS. C brevitarsis is capable of and probably does transmit the three viruses to cattle. It's role in the vectoring of Ephemeral fever is unknown but it still remains the prime suspect.

Australia is free of other major diseases of livestock. The spread of BT and to a lesser extent of AHS around the world suggests that the status quo may alter in the future. The majority of Australia's sheep are derived from the highly susceptible European breeds hence introduction of BT could be disastrous. BT may occur as a benign infection of both cattle and goats and in such form it may already be present throughout S E Asia. Roe (cited by Gambles, 1949) believes that BT was present as a benign infection in Cyprus for about 20 years before the first epizootic was recorded in that area. The virus has been recorded in both West Pakistan and Japan and it is not inconceivable that it could be present in the rest of Asia.

An epizootic of Ephemeral fever was reported from Mindanao in the Phillipines one month before the first diagnosis near Darwin at the beginning of the 1967-8 epizootic in Australia (Murray 1970). The Australian epizootic could have been a southwards movement of the one reported in Mindanao, 4000 km to the north. If BT is present throughout Asia it poses a serious threat to Australia. Both Ephemeral fever and BT have been found under similar epizootiological conditions in Africa (Howell, 1963). Reye (1964) expressed doubt that quarantine could always keep BT out of Australia. The circumstantial evidence given above reinforces his suggestion and indicates that it is possible that BT could be introduced in arthropods blown into northern Australia.

If BT entered Australia it is likely to come in only a restricted number of vectors. Rapid spread could occur only if arthropods already present could act as vectors. In South Africa C pallidipennis, a member of the obsoletus group, is considered to be its vector. However, in the USA C variipennis (nubeculosus group) has been proved the vector. As BT is an introduced virus in USA, C variipennis must be a substitute vector. The same substitution process could occur elsewhere. Australia does not have C variipennis (or any other

member of the nubeculosis group) nor does it have C pallidipennis, which is considered, on morphological grounds, to be conspecific with C brevitarsis (Wirth, W.W.; quoted in Standfast & Dyce, 1972 b) The latter must be considered a potential vector should BT enter Australia.

Speciation can involve changes in all or any of morphology, behaviour, and biology. Therefore a detailed examination of closely related species requires comparisons at the biological and behavioural as well as the morphological levels. Comparison can also be made by breeding in captivity and determining if the progeny of crosses are fully fertile. The full study of a species requires the development of colonies in captivity to allow such precise studies. Such information for C brevitarsis then needs to be compared with similar for C pallidipennis. Ideally the colony of C brevitarsis would be taken overseas for direct comparison with C pallidipennis in perhaps the Ethiopean region. It would then be possible to confirm if the two species are identical and if not to determine the degree of isolation between them. There is a limited amount of work that can be done in the field and therefore I have tried to colonise C brevitarsis. This thesis reports studies of biology and behaviour in the field made as part of the attempt to establish the colony.

The life-cycle of an insect can be divided into a series of stages each of which must occur in captivity before a self sustaining colony is obtained. Figure 0.I shows such a series of stages for the colonisation of C brevitarsis in the form of a critical path plan. The Roman numerals shown on fig. 0.I indicate the part of the thesis appropriate to that section of the path. The information available for C brevitarsis at the beginning of the project of which this thesis forms a part is indicated in the next few paragraphs rather than on the critical path plan.

Cannon & Reye (1966) discovered C brevitarsis breeding in pats of cow dung, but only in small numbers. Dung pats have subsequently proved to be excellent sites for rearing of large numbers, and no other breeding site has been found. No information was available about the time when oviposition occurred or the condition of pats into which eggs were laid.

It was implicitly assumed that development, pupation, and eclosion all occurred in or on the pat. Mr. A.L. Dyce (CSIRO Division of Animal Health) however expressed doubts that the pupa could penetrate

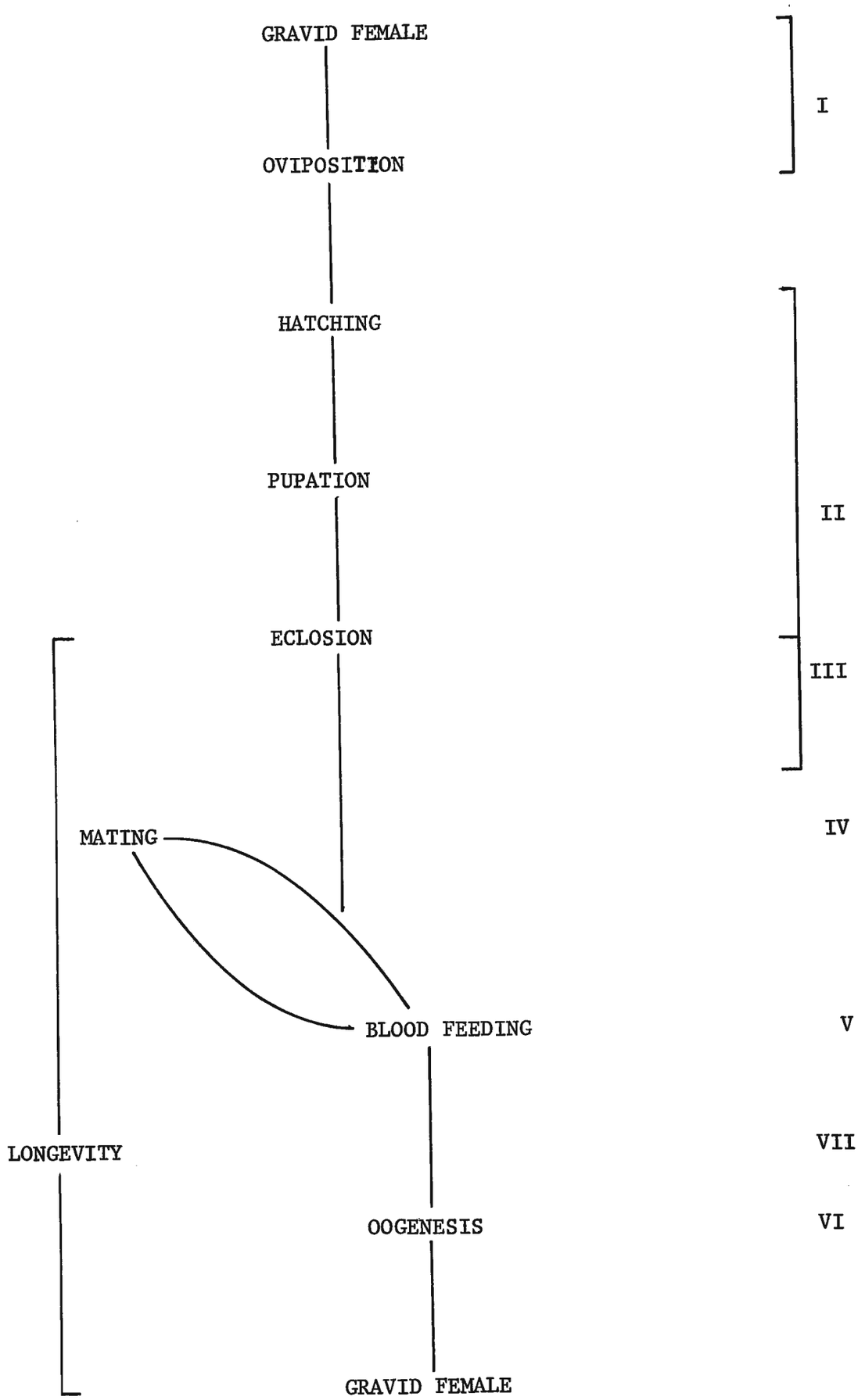


FIGURE O.I Critical Path Plan for Colonisation.

the hard crust of the pat, particularly of pats formed from buffalo dung which dries much harder than cow dung. Nothing was known of the time or place of eclosion.

The time and place of mating were unknown although it was assumed to occur during swarming even though swarming had not been observed. The sequence in which mating and feeding occurred in the field was unknown. The known host range was cattle, horse, sheep (Reye, 1964). Biting was confined to dusk (Standfast and Dyce, 1972 b), with the most intense attack on the back of the animal.

Neither oogenesis nor eggs had been examined. Dyce (1969) had shown that a burgundy red pigment was developed in the abdomen during the first oogenesis cycle. C brevitarsis was assumed to be anautogenous.

The results obtained in this project are presented in this thesis in the sequence shown in the critical path plan. A separate introduction is given to make each part of the thesis self contained. A general description of common methods and materials follows.

MATERIALS AND METHODS

When necessary, adults were sorted on a chill table (Sudia et al, 1965) which was just cold enough to prevent movement. Sex was determined from the antennae which in the male bear many long setae, and if necessary the genitalia.

A 'suction sorting gun' (Dyce et al, 1972) was used to collect adults from cages or the chill table. The adults were stored in cages made from unwaxed cardboard containers of the type used as disposable food containers, capacity 900 ml, closed by a single layer of fine red organza, at 25° and 75-95% humidity. An entry hole in the side of the cage was closed by dental dam.

Adults to be dissected were killed in Clarke insect saline, (Hale, 1958) to which detergent had been added to accelerate the rate of kill. They were dissected at 10-40x magnification by posterior traction in Clarke insect saline (no detergent) using needles of sharpened tungsten wire (Brday, 1965) held in micro-manipulators.

STATISTICAL ANALYSES

Statistical analyses have been based on Steel and Torrie (1960) and Siegel (1956). Examination of the counts of adults (p 17) showed that a logarithmic transformation, $\log_{10}(x+1)$, was necessary before analysis could be used legitimately. For tests of significance means have been presented in the transformed condition M_L (Kettle and Linley, 1967) ($M_L = \frac{\log(x+1)}{N}$ where N equals the number of items summed) and compared using Duncan's New Multiple Range Test (Steel & Torrie, 1960; p 107-9). For discussion the modified geometric mean M_W (coined by Haddow, 1960) has been used (M_W = antilog M_L - 1).

Regression analysis and analysis of variance have been combined in some experiments. The slope of the regression (b) and its error (s_b) are given. Variances were compared by the variance ratio (F) test. Students t-test has been used with paired and unpaired data and when the variances of the two populations were different t' was computed as for t, but the value of t' associated with the required level of probability was calculated (Steel & Torrie, 1960; p 81). The ss column for ss has been deleted from tables of analysis of variance because that allowed tables to be fitted onto one page.

The test used for determining dependence in 2x2 contingency tables was chosen according to the following set of rules (Siegel, 1956; p 110).

1. when N 40, used χ^2 corrected for continuity,
2. when N is between 20 and 40 use χ^2 if all expected frequencies are 5 or more,
3. when N is less than 20 use the Fisher test;

where N is the total number of observations.

The median test (Siegel, 1956; pp 111-6) was commonly used on data comprising counts. It determines if the scores above and below the median depend on the treatment being tested. Observations in each treatment are cast into the group above or below the combined median to form a 2x2 contingency table. Dependence within the table was tested by the appropriate test (last paragraph).

The original data for each part is given in the appendix of the same number. Appendix III is not included because the data was mainly descriptive.

DISCOVERY OF DUNG BY GRAVID FEMALES

INTRODUCTION

Culicoides larvae breed in many different habitats; included are small and discrete sites such as the dung pats of bovines (C brevitarsis; C chiopterus & C dewulfi, Kettle 1962); the ecotone between soil and water, treeholes, and rotting banana stumps (Carter, Ingram, & Macfie, 1920); rotting cactus (Ryckman & Ames, 1953); and mud fouled by animals (Jones, 1958) Any one species breeds in a very restricted range of habitats. Kettle and Lawson (1952) described six habitats that were part of the ecotone between soil and water, and determined which were preferred by eleven species of Culicoides. Battle and Turner (1972) related the species composition, again for species in the ecotone, to the nutrient content of the soil. The size and species composition of populations in three habitats appeared to be influenced by the concentration of the sodium ion, and to a lesser extent by pH and the content of organic matter (Kardatze and Rowley, 1971).

Breeding sites have been studied in some detail and therefore are reasonably well understood. However the reasons for the restrictions of species to specific sites are not known. Larvae of C furens survived equally well when the salt content of the water ranged from tap water to 300% sea water (Kettle, 1960) but in nature they occur only in salt water suggesting that breeding sites are not necessarily defined by the conditions in which larvae can survive. Similar observations have been made with mosquitoes (Clements, 1963) It is likely therefore that the restriction to specific breeding sites is at least partially a function of the behaviour of the ovipositing female, behaviour which has not been examined in Culicoides.

C brevitarsis offered an ideal opportunity to investigate behaviour preceding oviposition: it breeds in a discrete, easily defined medium in large numbers and has a short life cycle (11 days in mid-Summer) An understanding of the time of day when oviposition occurs, the site of oviposition, the condition of pats at oviposition, and the methods by which the gravid females 'discover' them could help to establish a colony. Experiments were carried out to examine the influence of the pat on the behaviour of ovipositing females. A specific introduction will be given with each experiment.

To avoid unnecessary circumlocution and verbosity terms such as find, recognise, discover, and search will be applied to the behaviour

of the gravid female without implying that their behaviour is self directed.

Many pats (0-3 d old) were searched (visually) through 24h at Highvale in December 1972. C brevitarsis were not observed but the same pats, when examined later in the laboratory, contained larvae. The apparent absence of adults from the pats may be due to one or more of a number of factors. It precluded the determination of the time of oviposition by direct observation.

The time of day of oviposition was difficult to determine because of technical problems (pp 38). Therefore its determination was delayed until after other experiments which, it was hoped, would indicate the time of oviposition.

MATERIALS & METHODS

Materials and methods common to the series of experiments are given here. All were carried out at Collard (p 62), in an area of approximately 0.5 hectares, adjacent to a yard in which cows were milked (fig. 4.II). Cattle were present at both 800h and 1600h daily for a period ranging from 1 to 4h and sometimes longer. Many pats, of various ages, were present in addition to those used in experiments.

The sward was composed of a range of pasture and weed species varying from 20 to 120mm deep. A car track gave rise to two bare strips, approximately 400mm wide, across the area. All experiments were located in areas where the sward was homogeneous and 20 to 50mm deep but areas near the cattle yard or the car tracks were avoided.

Dung was collected within 10 min of dropping and was covered until used (30 min). Dung from one cow was very wet; it was not used. Artificial pats were formed by upending one container of dung (2.1 litres) onto the ground

Each pat was subsampled taking one core, 62mm in diameter, from its centre to its full depth using the blade of a 'hole saw' (an attachment for electric drills) fastened in one end of a cylinder. The depth of the core was not measured because of compression during coring.

Initially the number of C brevitarsis in each core was determined in two ways, but after comparing the two methods (pp 16) counting of larvae recovered was abandoned in favour of counting the adults which emerged.

I.1 EXAMINATION OF TECHNIQUES

A. Comparison of techniques to determine the number in the core.

INTRODUCTION

A method was required to determine the number of C brevitarsis in the core. Washing the core through a series of sieves, immersing the residue on the finest in saturated magnesium sulphate, and then collecting and counting the larvae floating on the surface is a technique commonly used (e.g. Kettle & Lawson, 1952). Alternatively adults which emerged from the core could be counted. The latter required less time.

MATERIALS & METHODS

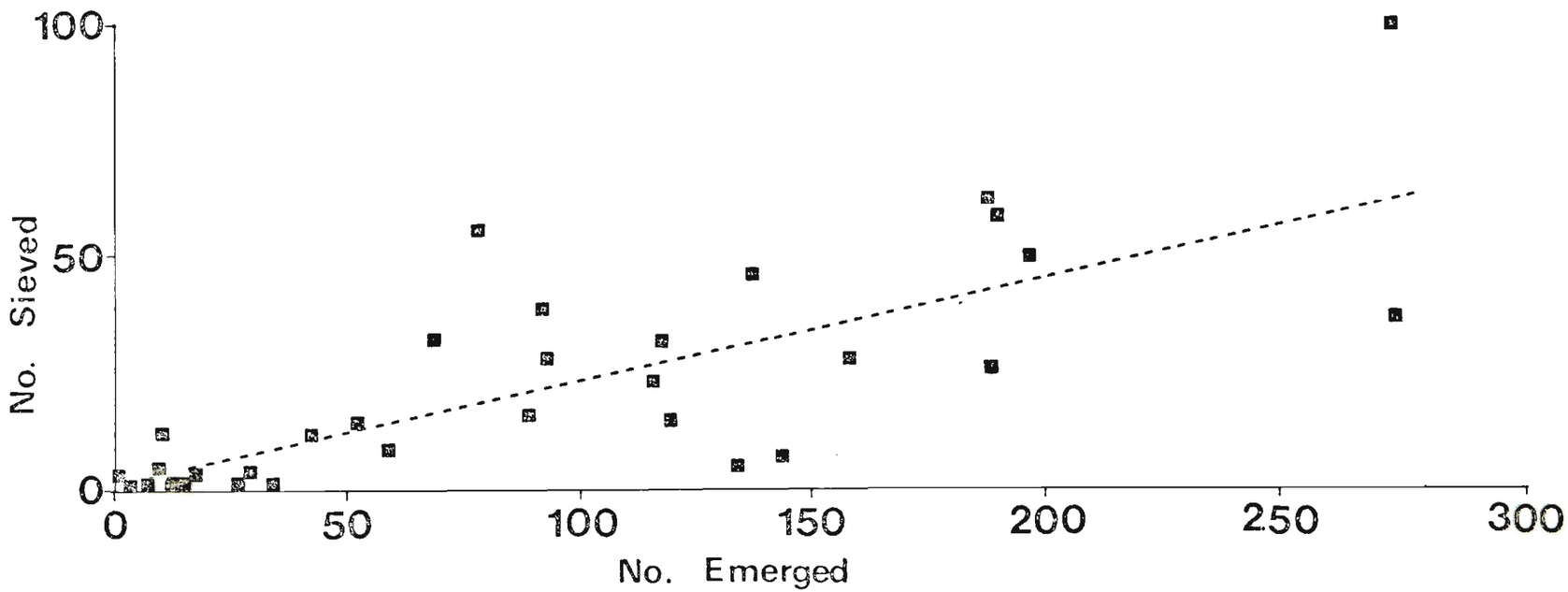
Two cores were removed from the centre of each of 36 pats. One was held for the emergence of adults, the other was washed. For emergence each core was held at 25° in a plastic cup covered with a translucent funnel the inner surface of which was coated with castor oil. Trapped adults were counted and removed daily. To ensure that all were counted, each core was retained for nine days after the last adult emerged. Alternatively, cores were washed through three sieves (20, 40, and 100 meshes per inch) with 9 litres of water. The residue on the 100 mesh (Aperture size 150 μm) was collected and the larvae floated out in saturated magnesium sulphate. When that was shown to be ineffective, washing was increased to three successive lots of 9 litres. The larvae in the residue on the 40 (aperture= 400 μm), 100, and 120 (125 μm) mesh sieves were counted after each lot of washing.

B. Variability between cores from the same pat

MATERIALS & METHODS

Cores were removed from positions as close as possible to each other and the centre of the pat; four cores from each of a series of 14 pats and six from each of another series of six pats. The order in which the cores were taken was recorded and used as the basis for allocating a position to each core; i.e. cores at position 1 were the first removed from the pat etc. The adults which emerged from each core were counted.

Fig. 1.I Relationship between assessment of larval populations by two methods.


100
50
0
No. Sieved
0
50
100
150
200
250
300
No. Emerged

RESULTS

A Comparison of techniques

The numbers of C brevitarsis determined by the two techniques were compared using the 'paired-comparison' t-test. The relationship was also compared by regression analysis. The mean number which emerged was four times greater than was washed out (table 1.1). The slope of the regression relating counts by the two techniques (fig 1.I) was 0.23 indicating that the number of larvae recovered was only a quarter of the number of adults.

TABLE 1.1 Mean counts of larvae and adults from cores of dung.

	mean	
by flotation	19.8	larvae
by emergence	82.2	adults
n	36	

$t = 5.95$; $P < 0.001$
$y = 0.69 + 0.23x$; x = no. adults, y = no. larvae
$b = 0.23$ $\quad S_b = 0.03$

Many larvae were retained on the 40 mesh sieve (table 1.2). and some passed through the 100 mesh to be retained on the 120 mesh. Many were still in the dung that had been washed by only one lot of water and as a result only a low percentage (28.7 in one and 13.6 in the other core) of larvae were collected on the 100 mesh sieve after one washing,explaining the discrepancy between numbers of larvae recorded and adults emerged.

TABLE 1.2 Number of larvae recovered by repeated washing

Sieve size	sample 1				sample 2			
	washing				washing			
	1	2	3	total	1	2	3	total
40	121	108	5	234	398	37	6	441
100	129	56	12	197	81	52	20	153
120	15	3	0	18	3	0	0	3
total	265	167	17	449	482	89	26	597

B Variability between cores from the same pat

The counts from cores within the same pat were not significantly different in either series (tables 1.3 and 1.4). In one series (table 1.3) significant differences were recorded between pats.

DISCUSSION

A. Comparison of techniques

The estimate based on washing was 23% of that based on counting adults (b = 0.23). The efficiency of the washing technique could be increased but the cost, in time, would be great. First and second instar larvae of C brevitarsis are difficult to see and rarely recovered by flotation, therefore even if the washing technique were made more efficient many larvae in the early instars would not be seen and counted.

TABLE 1.3 Mean number of adults from 4 cores from each of 14 pats.

Source	DF	MS	VR	P
within pats	3	0.022	0.44	NS
between pats	13	0.846	16.70	< 0.001
residual	39	0.051		

Means

within pats	M_W	M_L
1	92.5	1.93
2	82.8	1.92
3	92.0	1.97
4	73.6	1.87

TABLE 1.4 Mean number of adults from 6 cores from each of 6 pats

Source	DF	MS	VR	P
within pats	5	0.075	1.13	NS
between pats	5	0.127	1.91	NS
residual	25	0.067		

Means

within pats	M_W	M_L
1	103.4	2.02
2	114.7	2.06
3	92.0	1.97
4	63.5	1.81
5	60.6	1.79
6	76.6	1.89

In all subsequent experiments the adults that emerged from a core were counted.

The counts obtained were for a core of dung with fixed surface area; therefore they are equivalent to an estimate of the density of C brevitarsis per a unit area.

B. Variability

The insignificant variation between cores taken from the centre of the pat allowed one core to be used to estimate the number of C brevitarsis in each treatment in most subsequent experiments. Variation between pats was expected therefore a single pat was used as the experimental unit. Pats were put out in randomised blocks, a complete set of treatments at the one time. In most experiments replicates were put out on successive days, thus confounding day to day variation with replicate variance.

I.2 THE AGE OF THE PAT AT OVIPOSITION

INTRODUCTION

The age of the pat, measured in either minutes or days after dropping, influences the insect fauna on it. (Campbell, in preparation; Parker, 1972a,b). Both the time of day and the age of the pat could influence its discovery by gravid females. This section deals with the age of the pat at oviposition. Direct observation of C brevitarsis on pats was not possible (p 13), therefore less direct methods of investigation were required.

MATERIALS & METHODS

Two complementary experiments were carried out; one examined oviposition in the seven days after dropping (part 2A), the other in pats 6 to 30 days old (part 2B). Insufficient dung and emergence traps forced the separation into two parts.

Part 2A

Seven pats were formed on ten successive days (= 10 replicates). One from each replicate was sampled 24h after dropping and then daily to the seventh day.

Part 2B

Nine pats were formed daily for seven days (= 7 replicates), but one replicate was later discarded because it had been damaged by a horse. One pat from each replicate was sampled on the sixth and thereafter each third day to the thirtieth.

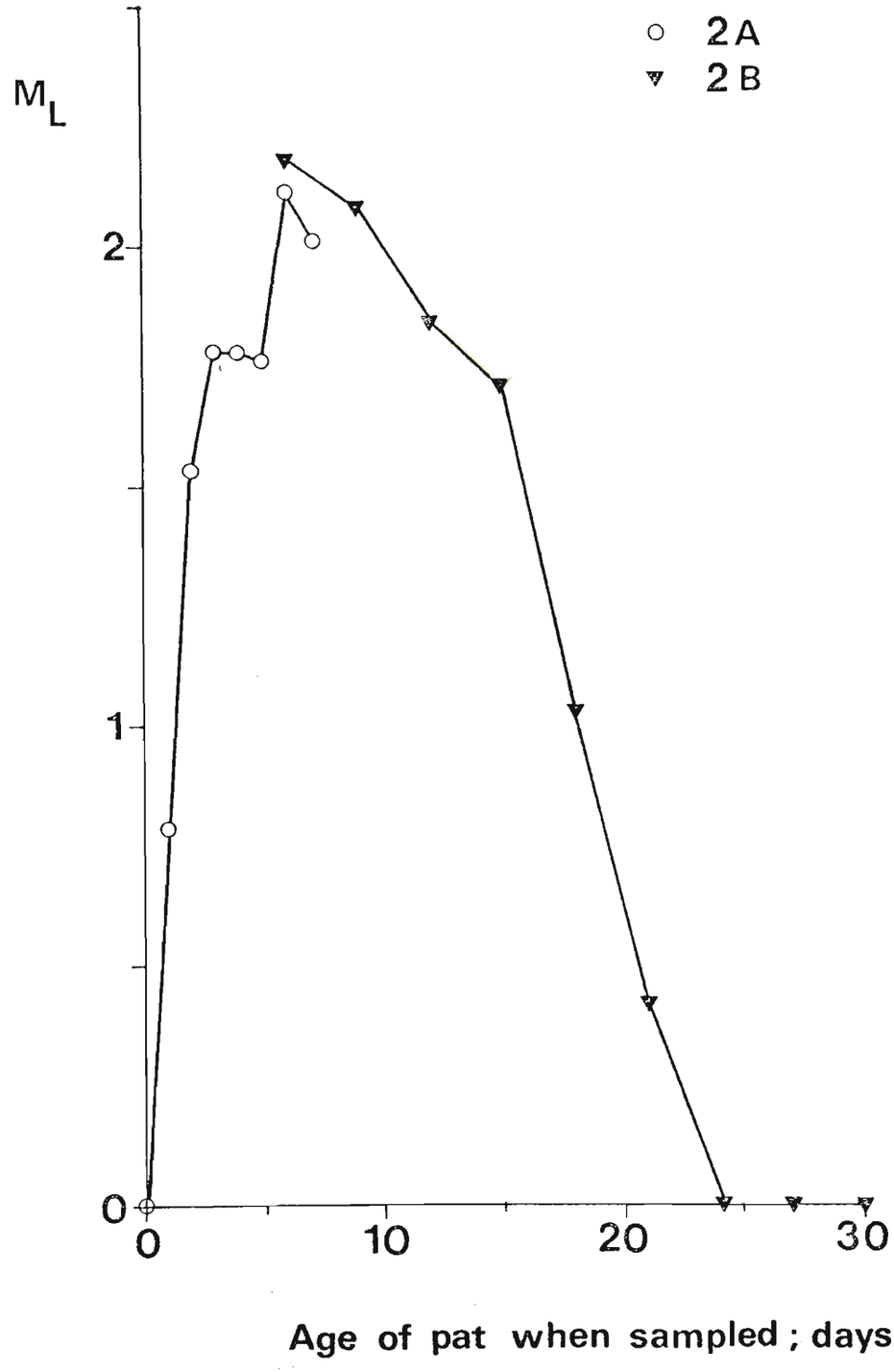
RESULTS

Part 2A

Pats exposed only on the first day produced fewest adults (table 1.5). The number of adults that emerged was doubled when the duration of exposure was increased by 1.7 days (b= 0.180) (table 1.5; fig. 1.II). Significant deviation of the counts away from linearity was recorded and was probably due to the nil increase recorded from days 3 to 5.

All of the replicates produced large numbers of adults (appendix I.2A) except the one set out on 30th March 1973 when the maximum temperature was 29°, the minimum 22°, and the humidity 60% (at 1500h), none of which differed to a marked degree from the conditions on many

Fig. 1.II Number of adults recovered from pats of increasing age at sampling.


2A
2B
M_L
2
1
0
0
10
20
30
Age of pat when sampled ; days

other days. It was the only rainy day with enough falling to leach the surface of the new pats. Leaching occurred only on pats less than one day old and caused the fibrous nature of the surface to become more apparent and the colour to change to grey.

TABLE 1.5 Analysis of variance and means, experiment 2A

Source	DF	MS	VR	P
days	6	1.917	13.9	< 0.001
regression	1	9.085	69.9	< 0.001
deviations	5	0.484	3.5	0.01-0.001
replicates	9	0.926		
residual	54	0.138		

Days	M_W	M_L	
1	5.1	0.79	
2	33.1	1.53	
3	60.8	1.79	LSD (P= 0.05) = 0.33
4	60.2	1.79	
5	59.2	1.78	
6	127.8	2.11	
7	103.6	2.02	

$\hat{y} = 0.952 + 0.180\ x$; $b = 0.180$, $S_b = 0.031$

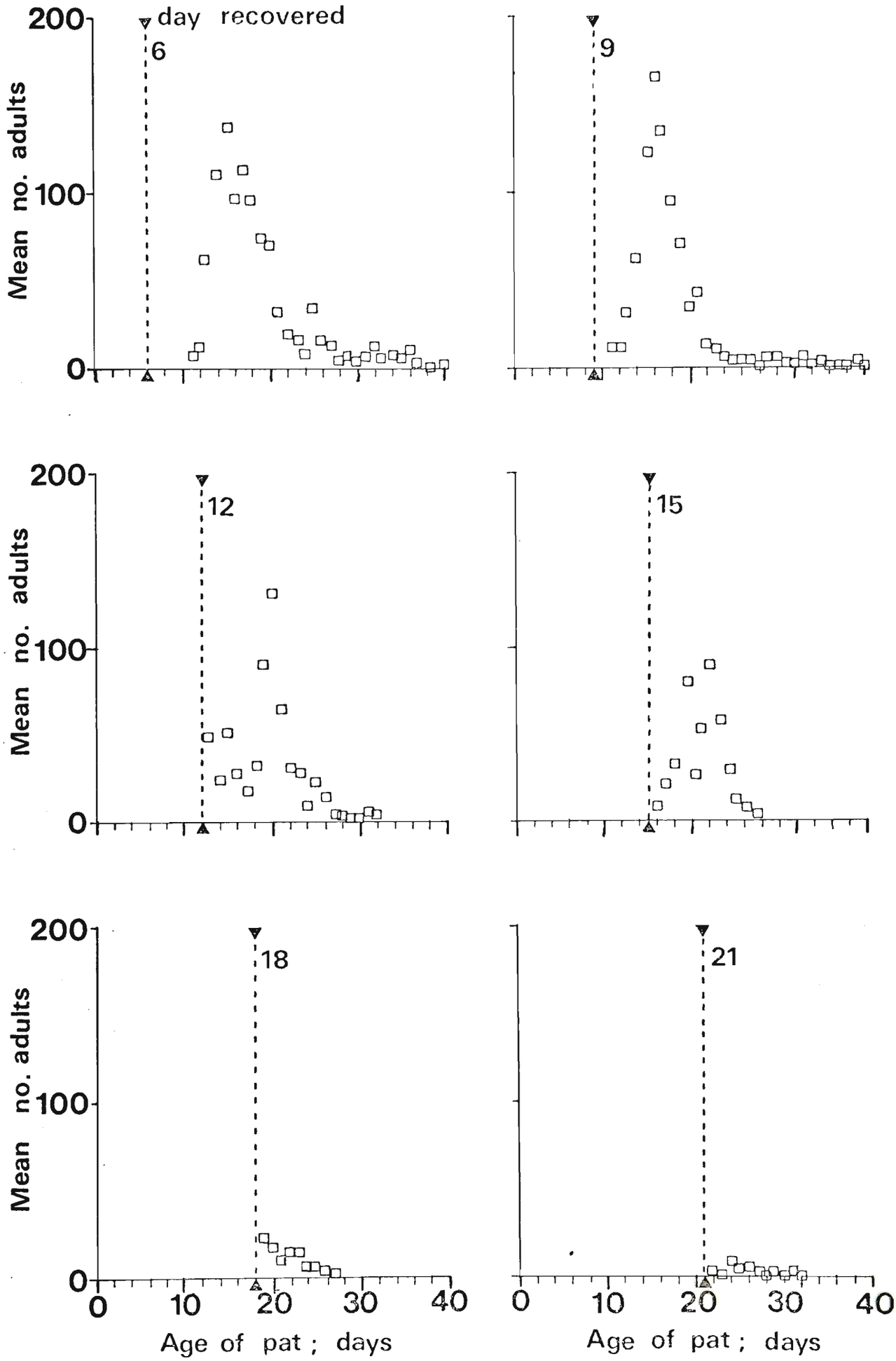
Part 2B

The mean number of adults did not change significantly between days 6 and 15 (table 1.6) but decreased significantly on the 18th and again on the 21st days. No adults emerged from pats sampled on days 24, 27, and 30 hence they were omitted from the analysis. The regression of the means against time was significant; the number of adults being halved every 2.6 days (fig. 1.II).

Temporal pattern in emergence

The mean number of adults that emerged from each treatment on successive days are shown in fig. 1.III. The first adults emerged from treatments 1 and 2 on the fifth and second days respectively after recovery from the field, being the eleventh day after dropping in each case. Therefore eleven days was the minimum time for development at the prevailing temperatures. The first adults emerged from subsequent

Fig. 1.III Daily emergences of adults from cores.


day recovered
6
9
12
15
18
21
Mean no. adults
200
100
0
0
10
20
30
40
Age of pat ; days

treatments within 24 hours of recovery, suggesting that emergence had already started in the field.

TABLE 1.6 Analysis of variance and means; Experiment 2B

Source	DF	MS	VR	P
days	5	2.85	21.46	< 0.001
regression	1	13.01	98.05	< 0.001
deviations	4	0.31	2.31	NS
replication	5	0.40		
residual	25	0.13		

$y = 3.154 - 0.117$; $b = -0.117$, $S_b = 0.046$

Mean days	M_W	M_L	
6	151.7	2.18	
9	118.1	2.08	
12	68.0	1.84	LSD (P=0.05) = 0.43
15	51.7	1.72	
18	9.7	1.03	
21	1.5	0.41	

DISCUSSION

Oviposition into pats continued until the 6th day with the number of eggs doubling every 1.7 days (fig. 1.II). From the 6th to the 9th days there was no difference in yield and as no adults emerged, oviposition could not have occurred.

Because oviposition ceases before emergences begin, the emergence pattern from any treatment should be the same as on the same calendar days for earlier treatments. However the peak of emergence in cores recovered on the 12th and 15th days was three and six days later than in those recovered earlier (fig. 1.III)

The lateness could have resulted from;

(i) oviposition after the 6th day,

or (ii) different rates of development between the field and the laboratory. The first has been discounted. Faster development could cause an earlier peak in the field and could delay the apparent peak in the laboratory for cores recovered after emergences had started.

By the end of the first day a crust has formed over the dung and by the 6th day is quite thick. I could not smell pats that were more than one day old. It seems unlikely that odour would be detectable on the 6th day and that gravid females could find pats only by sensing odour.

If the rain had reduced only the oviposition on that day it would have made a smaller than usual contribution to all the pats exposed, a result that could not have been detected. The rain must therefore have altered the fresh pats in some way, either by reducing their attractiveness to gravid females or their suitability for larval survival.

The places where females hide during oviposition have not been discovered. Pats might be used by females finding them soon after engorgement. Oogenesis takes 40-45h at 25^{o}, therefore two days is a reasonable estimate in the field. Any adult that oviposited in the pat on the 6th day after resting in it during oogenesis had found a four day old pat. It is unlikely that odour would be detectable on the 4th day. The arguments showing that odour is unlikely to be the stimulus used to find pats applies equally well to use of a gradient in temperature or carbondioxide.

In all the following experiments the pat was exposed in the field for seven days unless otherwise specified.

I.3 ARE GRAVID FEMALES ATTRACTED BY ODOUR

INTRODUCTION

Gravid females could find pats by vision, compounds volatolised from the pat (odour, carbondioxide), or temperature although volatiles are unlikely to be involved (p 24). Pats rapidly change from body (cow) to either ambient or ground temperature after dropping and no sensible heat gradient away from the pat would exist one, let alone six, days after dropping.

This section reports an experiment designed to examine the influence of odour on the discovery of a pat.

MATERIALS & METHODS

The method was similar to one used to examine the role of odour in the orientation of Philanthus triangulum (Beusekom, 1948). Two standard volumes of dung were used, on six successive days, to form a normal pat and a sunken pat; the latter was dropped into a hole which it filled level. The exposed surface area of the pats was the same. They were 2.0m apart.

RESULTS

Many more adults emerged from the normal (91.6) than the sunken pats (8.7) (table 1.7) The differences were so large and consistent that statistical analysis was unnecessary. There were fewer adults of Orthellia lauta on the sunken pats but no differences in numbers of Sepsidae and Sphaeroceridae.

I.4 PAT SIZE AND ITS DISCOVERY

TABLE 1.7 Counts from sunken and normal pats

replicate	normal pat	sunken pat
1	154	1
2	140	14
3	88	13
4	51	0
5	70	25
6	86	1
7	28	3
8	42	9
9	113	5
10	144	16
mean	91.6	8.7

DISCUSSION

The few adults recovered from the sunken pats indicates that gravid females did not readily find them, and odour, carbondioxide output, temperature gradients, pat colour, and colour contrast between the pat and its surroundings are not, at least alone, the stimuli by which pats are discovered. If odour is not involved it is likely that very few adults will find pats during the night, a conclusion supported by the absence of *O lauta* from the sunken pats. *O lauta* is present on pats only during daylight hours (Campbell, in preparation).

I.4 PAT SIZE AND ITS DISCOVERY

INTRODUCTION

Pats vary from very small (50mm diameter, 40mm high), those dropped by a moving animal, to very large (up to 500mm diameter), those dropped after camping for some hours. The commonest size approximates that formed from one standard container of dung, i.e. 300mm diameter. Pat size could influence their discovery by gravid females, over and undersized ones not being recognised and used. Alternatively size could influence the number of gravid females finding the pat and hence the number of eggs deposited.

MATERIALS & METHODS

Pats were formed from ½, 1, 2, or 4 volumes of dung. For the larger pats the extra volumes of dung were put alongside each other and then formed into one large pat. Each pat was measured but without excessive accuracy (the shape of the pat is not regular and decreases with age). Their surface area doubled as the volume of dung doubled. Two series of treatments (= replicates) were set out on each of three days.

RESULTS

Pat size did not alter the number of adults that emerged from a core from each pat (table 1.8). The core estimates the density of adults, therefore the number of adults in a pat was directly proportional to its surface area. In this experiment there were 3.3×10^{-2} adults mm^{-2}

TABLE 1.8 EFFECT OF PAT SIZE ON OVIPOSITION; ANALYSIS AND MEANS

Source	DF	MS	VR	P
day	2	0.238	4.58	0.035
size	3	0.109	2.10	NS
day x size	6	0.100	1.92	NS
replicate	1	0.045		
residual	11	0.052		

Means

size	M_W	M_L	
½	61.4	1.80	
1	55.2	1.75	
2	75.2	1.88	LSD (P=0.05) = 0.29
4	112.6	2.06	

DISCUSSION

Oviposition occurs at equal density into pats that vary widely in size hence the number of gravid females finding a pat was directly proportional to its size. That conclusion holds whether or not the female lays all of its at the one time. Equal density of oviposition could also be achieved if each gravid female established a territory of fixed area that she defended in some way and in which only she oviposited. A constant density would result if the whole pat was covered with territories. Defence of a territory would require either the continuous presence of the female for 6 days, but extensive searching has failed to discover them on pats, or definition of the territory by scenting the surface. Scenting must be doubted because of the gross changes to shape and condition of the surface of the pat during the first six days.

Visual discovery could cause the number of gravid females finding a pat to be directly proportional to its size. The female could be level with the side of the pat (low level) and see a profile, or be above it (high level) and see a plan view. The pat could be recognised by its shape, bulk, or outline.

A mean of 91.7 adults emerged from each core (surface area $2800mm^2$) If there had been no mortality, 91.7 eggs were laid on that core, or 2000 eggs on a pat 300mm in diameter. Fecundity averaged 31.3 (p 165), indicating that 63.9 females oviposited on that 300mm pat. With oviposition spread evenly over six days, the number ovipositing per hour can be predicted as

(i) oviposition throughout 24h: 0.49

(ii) oviposition in 12 hours only (daylight); 0.99

(iii) oviposition during 5h around dusk: 2.37.

The time taken for oviposition is unknown, but if it is assumed to be 1 hour and oviposition is restricted to five hours around dusk, the maximum number of adults on a pat at any one time would be five (50% mortality) or 25 (90% mortality). It would be nearly impossible to find them.

Negligible, or at least constant, mortality of larvae between pats has been assumed. Only then can the number of eggs oviposited be equated with or predicted by the adults emerged. To convert counts of adults back to counts of eggs mortality must be known.

I.5 LOCATION OF OVIPOSITION

INTRODUCTION

The apparent absence of *C brevitarsis* from the surface of the pats could be explained by;

(i) Adults were too small, well hidden, and/or too few

or (ii) oviposition does not occur on the upper surface of the pat.

It has already been suggested (p 28) that very few adults are required on the pat at any time to satisfy the needs of oviposition. If ovipositing on the side of the pat, especially close to the ground, they would be even more difficult to see. This section examines the distribution of eggs over the pat.

MATERIALS & METHODS

Eight natural pats were spread out to a uniform depth (30mm) immediately after being dropped (700-800h). As many cores as possible were taken exactly 24h later, one from the centre, a series around the outer edge, and another series in between (inner ring) The number of cores varied between inner and outer rings and between pats.

RESULTS (Table 1.9)

Emergence of adults from the outer ring was 26% (14.8/57.3) and from the inner ring 39% (22.3/57.3) of the number from the centre, indicating a progressive decrease in oviposition towards the edge of the pat.

TABLE 1.9 Number of adults and location on pat; analysis and means.

Source	DF	MS	VR	P
location	2	13.28	1079.9	< 0.001
pat	7	2.96	240.8	< 0.001
residual	110	0.01		

Means

location	M_W	M_L	LSD
centre	57.3	1.77	
			0.88
inner	22.3	1.37	
			0.88
outer	14.8	1.20	

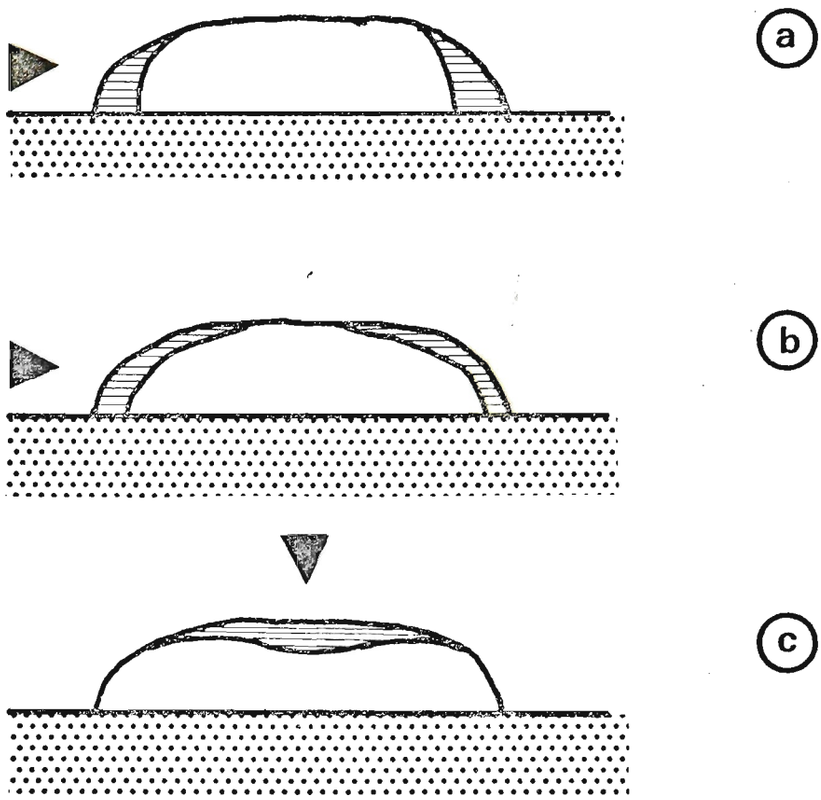
DISCUSSION

Laboratory observation of the eggs of C brevitarsis indicated that they required 36h incubation. Therefore those laid on the pats in this experiment would not have hatched before sampling, hence movement around the pat would not have occurred. The number of adults emerged therefore reflects the number of eggs laid in that sample.

Many adults and therefore much oviposition occurred in the centre of the pat and almost certainly would have been on the surface. The immediately sub-surface dung of 1 day old pats is still very wet and would probably trap any C brevitarsis. The result negates the possibility that oviposition does not occur at the centre. They reinforce the postulate that non-observance of adults is due to their small size and low density.

If the female discovers the pat when at low level, the greatest oviposition should occur on the outer edges of the pat (fig 1.IVa) although the bias towards the edge could be reduced (fig 1.IVb), if adults moved around before or during oviposition. If the pat was discovered by adults at high level, a higher density of eggs could be expected at the centre (fig 1.IVc), the result observed. Pats are probably perceived visually from above.

Fig. 1.IV Alternate patterns of distribution of eggs on pats.



▶ position of discovery

I.6 THE DEPTH OF SURROUNDING GRASS AND OVIPOSITION

INTRODUCTION

It pats are found by females flying at high level, those not easily seen by man should not be found easily by C brevitarsis. Pats in deep grass are not easily found by man. Deep grass should therefore reduce the likelihood that a pat will be found by gravid females, a reduction that could;

(i) reduce the number of adults emerging from the pat

or (ii) cause greater variation between pats in yield of adults thus increasing the variance.

Both could occur. In this section the numbers of adults from pats in a range of conditions from bare ground to deep grass are compared.

MATERIALS & METHODS

Pats were dropped into the following situations:

(1) bare ground

(2) grass less than half the height of the pat (low grass)

(3) grass level with the top of the pat (level grass)

and (4) grass more than twice the height of the pat (deep grass).

One set of treatments was put out on four days.

The estimates of variance for each treatment were compared and the arbitrary numbers 1 (deep grass), 2, 3, and 4 (bare ground) were assigned to allow regression analysis.

RESULTS (table 1.10)

Fewer adults emerged from the pats in the deeper grass but the significance of the differences are in doubt. The means were not different in the analysis of variance but were with the regression analysis. The estimate of variance was greater in deep grass than level grass and bare ground. The variances of other pairs of treatments were not significantly different.

TABLE 1.10 Number of adults from, and depth of grass around pat; analysis and means.

Source	DF	MS	VR	P
depth	3	0.168	1.91	NS
regression	1	0.412	4.68	0.05
deviations	2	0.051	0.58	NS
replicates	4	0.039		
residual	12	0.088		

$\hat{y} = 1.644 + 0.129x$; $b = 0.129$, $S_b = 0.054$

Means

	M_W	, M_L	S^2		VR
deep grass	53.0	1.732	0.184	)	
level grass	99.5	2.002	0.024	)	7.8*
low grass	87.9	1.949	0.067		
bare ground	149.3	2.177	0.028		
		bare ground/ deep grass			6.6*

* significant at P = 0.05

DISCUSSION

The means may or may not have been significantly different but there was a trend to lower means in deeper grass. The insignificance may be due to the high variance of the data which is, at least in part, due to the wide range in counts from deep grass (12-156) The results agree with the postulates of lower means and greater variance when the pat is not easy to find visually.

I.7 THE SHAPE OF THE PAT AND OVIPOSITION

INTRODUCTION

Many objects in nature resemble pats, especially if colour is not important. Gravid females could explore or even oviposit in all such objects with breeding occurring only in suitable media. However that would be very inefficient and should have been eliminated by natural selection. It is much more likely that females oviposit only in suitable sites thus implying that they can recognise suitable sites. How does the gravid female recognise pats? The shape of the pat, especially its outline viewed from above, may be involved; the experiment reported here examines the possibility.

MATERIALS & METHODS

Dung was formed into the following shapes on four days.

(1) round; i.e. normal pat
(2) triangular; from standard volume of dung
(3) elongate; 250mm long, 70mm wide
(4) extra elongate; 500mm long, 40mm wide
(5) hollow centred; same outer diameter as standard pat, only 50mm wide
(6) T-shaped; each arm 180mm long; 70mm wide
(7) six small pats in a circle; each 70mm diameter; circle 300mm diameter.

Two cores were taken from each 'pat' and later amalgamated.

One extra replicate, including only treatments 1, 4, 5, 6, and 7 was set out but sampled on the fourth day to minimise the effects of drying in the smaller pats.

RESULTS (table 1.11)

The dung from treatments 4, 5, and 7 and to a lesser extent 6, and 3, all of which were thinner in cross-section than normal, was drier than treatments 1 and 2. The surface area of pat actually taken in the core was greater in treatments 4, and 7, because of their shape. Therefore the means cannot be examined critically.

Most adults emerged from pats of nearly normal shape (round, triangular, and elongate) while fewest came from pats which were also the driest. Mortality of larvae may have been increased in the drier pats.

Drying was minimal in the pats recovered after four days with light rain during exposure further reducing drying. Treatments 4, 5, and 6 yielded more adults than the normal pat; treatment 7,

the same as the normal pat.

TABLE 1.11 Numbers of adults from pats of different shapes;

shape	mean (first four days)	count (last replicate)
(1) round	110.5	74
(2) triangle	124.0	-
(3) elongate	71.8	-
(4) extra elongate	22.5	134
(5) hollow centre	52.3	138
(6) T - shape	25.5	101
(7) 6 small	54.3	67
esposure (days)	7	4

DISCUSSION

The uncritical nature of the first part of the experiment was highlighted by the different result when drying was minimised. Gravid females successfully found dung in all of the shapes offered, hence shape in plan view is unlikely to be important in recognition. The conclusion is not really surprising because pats in the field vary in shape. Most are round but a moving animal produces a pat that is either elongate or of discrete small units. For the maximum utilisation of breeding material odd-shaped pats should therefore be recognised. The range of shapes tested was greater than is normally found in the field.

Variations to the shape of pats in plan view were easy but other changes in shape were nearly impossible. Fresh dung is viscous and can be formed into different shapes in vertical profile (e.g. very high pats, straight sided pats) but within 24 hours such pats subsided to the more usual, round-shouldered mound (fig. 1.IV), making experimentation with different shapes (in vertical profile) difficult.

1.8 THE SIDES OF THE PAT AND OVIPOSITION

INTRODUCTION

Oviposition occurs into pats of different shape in plan view. A constant feature of almost every natural pat is the shape of its edges in vertical profile, a shape not shared by many other objects in the field. Virtually all pats have rounded shoulders (fig. 1.IV) which were absent from the sunken pats (p 25 et seq) into which oviposition did not occur. This section examines the effect of eliminating the shoulders from the pat on oviposition.

MATERIALS & METHODS

Tins (150mm diameter) without ends were pushed into bare ground leaving 60mm above the ground, then filled with dung and paired with a standard pat (60mm deep at centre). Three replicates were set out.

RESULTS

Many more adults emerged from the normal (107) than the encircled pats (1.3) (table 1.12). Analysis was unnecessary.

TABLE 1.12 Number of adults from standard and encircled pats.

Pair	Normal pat	Encircled pat
1	87	0
2	66	0
3	168	4
mean	107	1.3

DISCUSSION

The sides of the pat may be important to its recognition but how an adult perceives them is unknown. The edges of a pat may be areas of low reflectance (fig. 4.XIII) and hence the pat may appear as a bright object surrounded by concentric areas of decreasing reflectance. If males use areas of low reflectance as swarm markers (p 100), it is reasonable that females should perceive the same sort of difference.

Females use the edges of the pat to recognise it but not as the major area for oviposition. Possibly the pat is recognised as a whole unit that has edges of low reflectance.

INTEGRATING DISCUSSION

Pats are not found by gravid females sensing odour, temperature gradient, colour contrast with the background, colour alone, or shape in plan view although it is possible that the coloureof the pat may play a part in its recognition. The pat is probably seen as a whole with its rounded shoulders involved in recognition. Further experimentation is necessary, the most profitable probably being attempts to trap gravid females on the surface of imitation pats that were designed to test the hypothesis. This would also test the possibility that some females may rest in the pat during oogenesis. Not all females could rest during oogenesis in the pat in which they oviposit because oviposition occurs on the first day pats are exposed.

Two assumptions have been made throughout the experiments:

(i) oviposition occurs immediately after the pat is discovered and (ii) that mortality is relatively constant between pats.

The first assumption is of no real consequence to any of the results or conclusions but did allow the word oviposition to be used as a shortening of 'the discovery of the pat' If oviposition does not immediately follow discovery then it was the time of discovery of the pat that was determined, not the time of oviposition. If larval mortality is in any way density dependent an incorrect conclusion may have been drawn in sections 4, 5, and possibly 6.

The method chosen for the experiments was the best available, the more direct method of counting eggs was precluded by their small size.

In a sense the experiments do not allow the separation of what may be two different facets of the discovery of the breeding site. The first facet involving the discovery of the pat (i.e. its recognition), the second its acceptability as a breeding site. Only dung has been used and therefore attraction to other objects has not been recorded. A wide range of species exhibit some form of test of the medium before ovipositing. Kennedy (1942) showed that mosquitoes fly across the surface of the water occasionally dipping the tip of the abdomen until they find a suitable site. Many of the parasitic hymenoptera (e.g. Aphytis coheni ; Quednau, 1964) select their oviposition site with their antennae. Similar behaviour may well exist in C brevitarsis but its examination awaits the development of a colony in the laboratory.

I.9 TIME OF DAY OF OVIPOSITION

INTRODUCTION

Determination of the daily pattern in oviposition in the field proved to be difficult. Oviposition extends over six or seven days after the pat has been dropped but only at very low rates (p 28), and therefore pats must be exposed for a long time if large numbers of eggs are to be laid. It is difficult to decide whether zero counts are due to no oviposition or a very low rate that was not detected unless large numbers of adults emerge from pats that were continuously exposed.

Exposure of pats on successive days causes problems. If they are removed from the site how can they be stored under field conditions but out of range of gravid females? If they are covered in the field how is it achieved without drastically altering the microclimate around the pat and at the same time ensuring the exclusion of gravid females?

The first attempt was made at Collard using containers filled level with dung and exposed for the same 4 hours on seven days. Between exposure they were stored on the ground approximately 2km from the experimental area (no bovines were closer than the experimental area). No adults emerged, a result easily understood because the pats did not have round shoulders.

Later pats were formed on boards and moved in and out of the field but during transfer flexion of the boards cracked the pats, imposing intolerable differences from normal pats. C. brevitarsis may have found the pats where they were being held between exposure as did Orthellia lauta, a species known to breed in dung.

Pats are discovered visually suggesting that oviposition is more likely during the day, early evening, or dawn. In an attempt to define more accurately the time of oviposition two experiments were carried out and are reported in this section.

I.9.i FIRST EXPERIMENT

MATERIALS & METHODS

Five pats (150 mm diameter) were formed on each of four occasions (=4 replicates) at 800h. Each was exposed during one of the following periods on seven successive days.

(1) 800-1900h
(2) 1900-2200h
(3) 2200- 800h
(4) continuously
(5) never

C brevitarsis was excluded between exposure periods by covering the pat with a tin (150mm diameter x 180mm deep) closed at the upper end by fine red organza. All counts were converted to number emerged per hour exposed per day to eliminate the effect of different durations of exposure.

RESULTS (table 1.13)

The greatest number of adults emerged from pats exposed 800-1900h (3.5), 1900-2200h (2.5), and continuously (1.7). Very few (0.2) emerged from those exposed from 2200-800h and none from pats continuously covered.

DISCUSSION

Oviposition probably occurs between 800 and 2200 h, but the periods of exposure were very long and therefore did not define the period with accuracy. The periods were shortened in the second experiment.

The influence of the tin on the micro-climate around the pat and hence on the pat, especially during the day, is unknown. Excessive heating may cause excessive mortality but, as many adults emerged from the pats exposed from 1900-2200h and which were covered all day, the effect was probably minimal.

TABLE 1.13 Adults / hour exposed, at different times of the day.

Source	DF	MS	VR	P
time exposed	4	0.334	14.5	< 0.001
replicate	3	0.315	13.7	< 0.001
residual	12	0.023		

Means

time exposed	M_W	M_L	Significance
800-1900	3.5	0.657	|
1900-2200	2.5	0.541	|
continuously	1.7	0.438	|
2200-800	0.2	0.080	|
never	0.0	0.000	|

LSD (P=0.05) = 0.240

I.9.ii SECOND EXPERIMENT

INTRODUCTION

Other experimentation (p 36) subsequent to I.9.i. showed that C brevitarsis do not find pats closely surrounded by a tin even if the tin is open at the top. Without gauze on the top disturbances to the micro-climate should be minimal making the results of the experiment more likely to represent reality.

MATERIALS & METHODS

Pats were exposed for one of the following periods on seven successive days.

(1) 700-1400
(2) 1400-1600
(3) 1600-1800
(4) 1800-2000
(5) 2000-2200
(6) 2200-2400
(7) 2400-700
(8) continuously
(9) never

The intervals were not equal, allowing more detailed examination of the time when oviposition was thought to occur.

Nine replicates were set out. When not exposed pats were surrounded by tins (150mm diameter x 180mm high) without tops. Counts were converted to number per hour exposed per day.

RESULTS (Table 1.14)

TABLE 1.14 Counts per hour exposed;short periods of exposure; analysis of variance & means.

Source	DF	MS	VR	P
times	6	0.535	5.3	< 0.001
replicates	8	0.768		
residual	48	0.101		

Means

time	M_W	M_L
700-1400	6.4	0.867
1400-1600	17.3	1.262
1600-1800	18.9	1.299

1800-2000	10.1	1.048	LSD (P=0.05) = 0.303
2000-2200	7.6	0.936	
2200-2400	9.9	1.036	
2400-0700	2.8	0.584	
continuously	5.1	0.782	not in analysis

Oviposition was least from midnight to 7 am (2.8 adults emerged / hour exposed) and increased to a peak from 2 pm to 4 pm (17.3) to 6 pm (18.9) with a slight decrease to midnight (table 1.14 ; fig 1.V). The differences between means were highly significant, although as shown by the significant ranges calculated by Duncans test and marked onto figure 1.V, adjacent means were not significantly different at P = 0.05.

The two controls were excluded from the analysis. Continuously exposed pats yielded 5.1 adults per hour exposed.

DISCUSSION

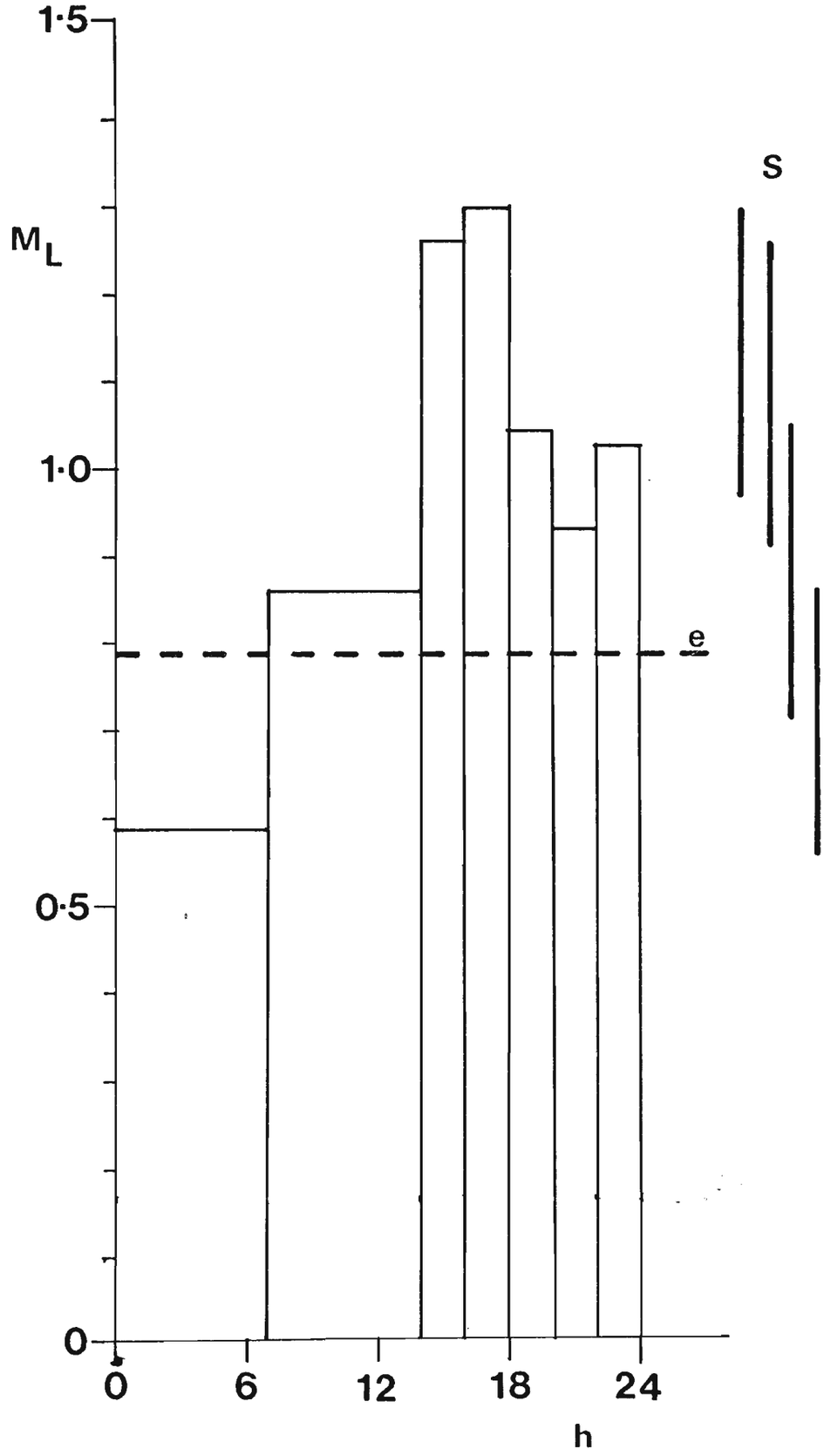
Ovipposition, at least the discovery of the pat, occurred predominantly during the afternoon and evening extending through to midnight, with 48.1% of eggs laid between 2pm and 8pm, 18.3% from 8pm to midnight, 10.3% to 7am, and then 23.3% to 2pm. The estimate of oviposition rate (p 28) can now be improved to the following;

(1) 2pm to 8pm : 1.0 females / hour
(2) 8pm to midnight : 0.5 "
(3) midnight to 7am : 0.2 "
(4) 7am to 2pm : 0.4 "

It is therefore not surprising that gravid females were not seen on pats.

The daylight hours were the time predicted for oviposition from the assumption that vision is used to find the pat. In summer, at least, that period extends from before 7am until 8pm, when 71.4% of oviposition occurred. During the darkest hours (midnight onwards) 10.3% of oviposition occurred, but that does not necessarily negate vision as the means of discovery of the pat. Insect eyes are well adapted to the detection of objects in low intensity light ; oviposition at night may indicate a high degree of dark adaptation in C brevitarsis.

Fig. 1.V Daily pattern in oviposition.



S ; significance at P = 0·05
e ; always exposed

II IMMATURE STAGES OF C brevitarsis

INTRODUCTION

A brief knowledge of the development of larvae and pupae and the eclosion of the adult is necessary before attempts can be made to establish a colony. Eggs are laid on the upper surface of the pat (p 30). In the laboratory eggs laid on wet filter paper hatched in 36 hours. The egg is described on pp 160-5

Cannon and Reye (1966) defined three zones in pats; the outer crust (hard and dry), an inner zone that had the consistency of wet caneite fibre, and a central, viscous, zone. They found larvae only in the wet caneite zone but did not find pupae. The larvae did not swim with the rapid serpentine movement characteristic of other Culicoides, but showed only slow flexion.

II.1 LOCATION OF LARVAE AND PUPAE WITHIN PATS

MATERIALS & METHODS

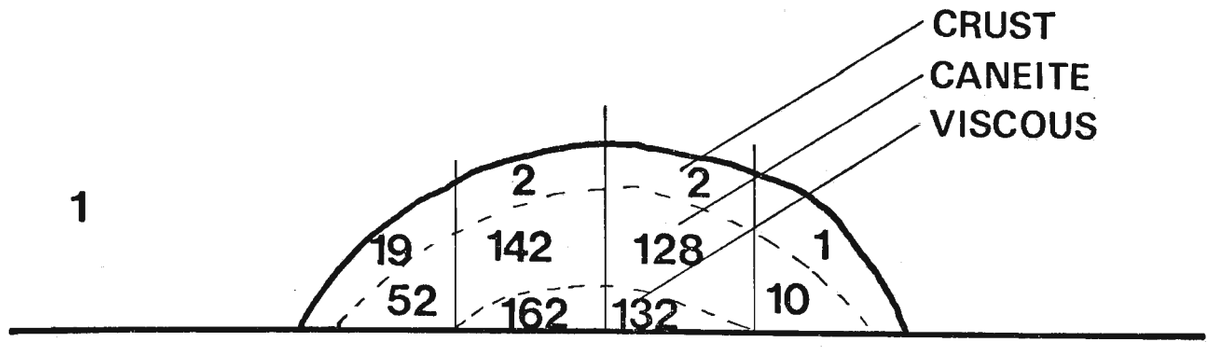
A 50mm thick section was cut to the full depth and width of 8 pats. The section, including the boundaries of the zones, was sketched, then cut vertically into four or five parts. Each zone was collected separately. The position of each subsample was marked on the sketch (fig. 2.I). Larvae and pupae were washed out and counted from each sample. Another section was taken from five of the pats, subsampled in the same way, and used to determine moisture content (as % dry weight) by weighing and drying at 40^{o}

RESULT (figures 2.I ; 2.II ; table 2.1)

Larvae were recovered from five pats, pupae from two. The mean number of larvae per sample was 7.3 in the crust, 21.7 in the wet caneite, and 22.6 in the viscous. The corresponding means for pupae were 4.7, 17.4, and 6.0. The means are not accurate estimates of density because the volume of samples was not standardised. The data suggest low densities of larvae and pupae in the crust, larvae distributed evenly throughout viscous and wet caneite zones, and pupae more dense in the wet caneite than the viscous.

Moisture content of subsamples ranged from 174 - 537% of dry weight. Larvae were found from 190 to 537%, pupae from 184 to 517%, suggesting that both stages can survive in very wet dung.

Fig. 2.I Larvae in pats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CANEITE
VISCOUS
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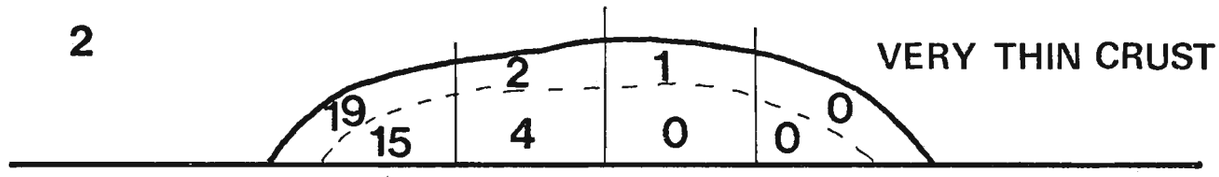
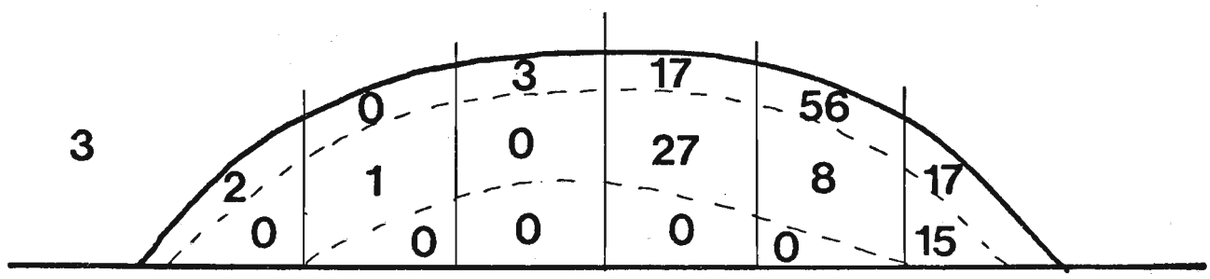
2
2
19
142
128
1
52
162
132
10


2
VERY THIN CRUST
2
1
19
0
15
4
0
0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56
0
27
2
1
8
17
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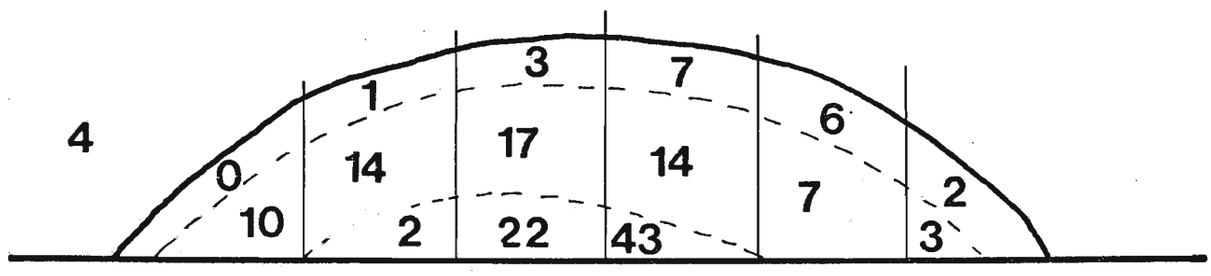
0
0
0
0
15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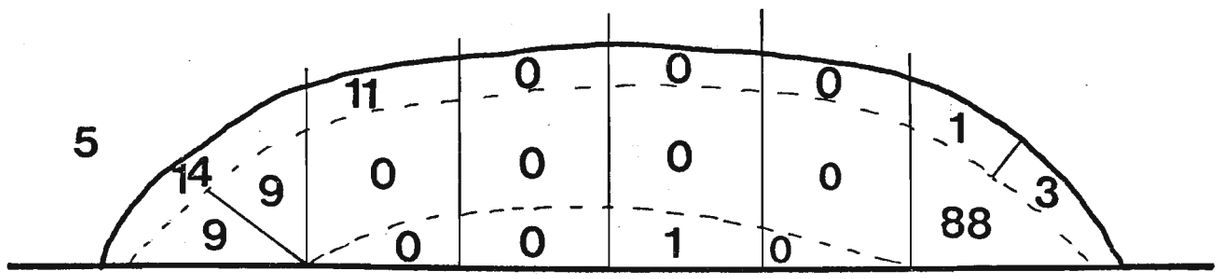
14
7
2
10
2
22
43
3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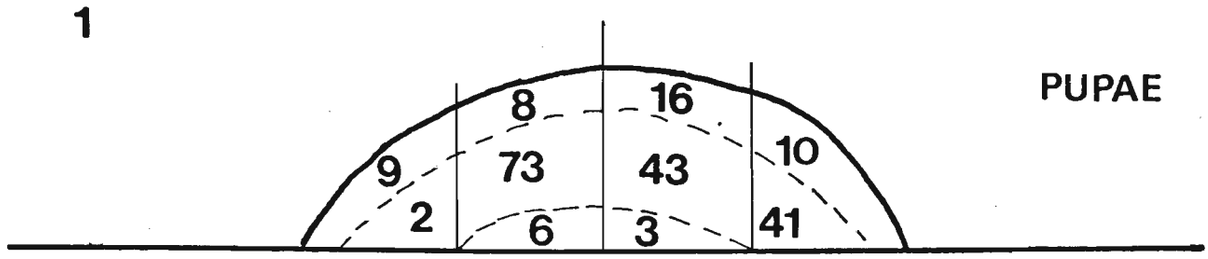
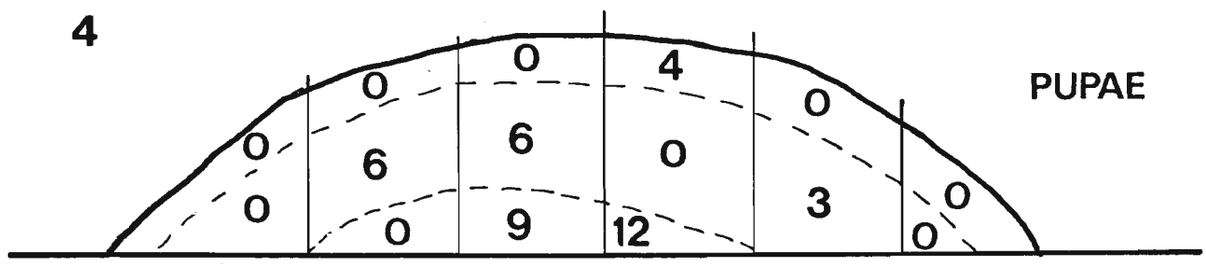
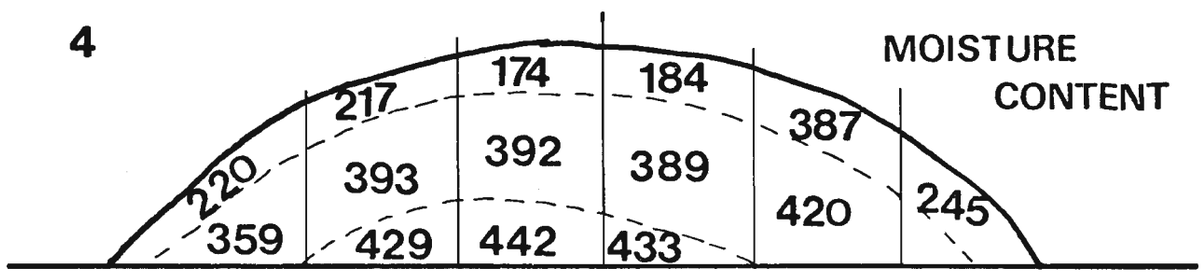
0
0
11
1
14
9
0
0
0
0
3
9
0
0
1
0
88

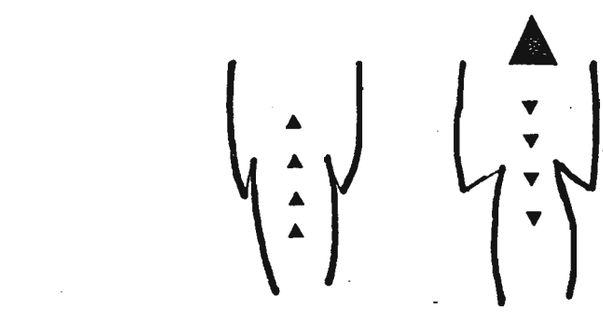
Fig. 2.II Pupae in pats.

Fig. 2.III Movement of abdomen









▲ direction abdomen

▲ direction pupa

TABLE 2.1 Moisture content of samples of dung.

Subsamples containing					
Larvae				Pupae	
238	400	217	391	393	392
429	359	387	174	442	184
346	389	190	243	433	420
537	274	404	467	245	517

DISCUSSION

Both larvae and pupae occur throughout the pat independently of moisture content. The low numbers in the crust imply even lower numbers at the actual surface. Pupae survive in the viscous zone but their ability to move to the surface for eclosion is unknown.

The restriction of larvae to the wet caneite, as reported by Cannon and Reye (1966),was probably a function of the very low numbers they recovered, which in turn was very likely the result of sampling in winter.

II.2 DESCRIPTION OF PUPATION AND ECLOSION

MATERIALS & METHODS

Larvae washed out of dung were transferred to sand slopes in paper cups (3.675×10^4 mm^3), or to agar plates containing nematodes. Larvae of other Culicoides sp. were successfully reared using nematodes on agar. (Kettle et al, in press). Pupation and eclosion were observed, described, and a generalised description prepared.

DESCRIPTIONS

No one position was characteristic of pupation which required approximately 12 hours and tended to occur in the upper parts of the agar plate. When newly formed the pupa was colourless, and shorter than the larva. Shortening occurred after the head and thorax of the pupa had developed.

The young pupa assumed the orientation shown in Figure 2.IV with pupal respiratory horns just protruding through the surface. Every pupa in agar achieved and remained in that position until immediately before eclosion. The pupa moved by flexion and by altering the shape of its abdomen in a fashion similar to the changes which occur in the washer of a pump. The abdomen (fig. 2.III) contracted towards the

thorax, and was then forced backwards but with the posterior margin of the sc lerites of each segment expanded and wider than the anterior part of the next posterior segment, the net result was forward movement of the whole pupa. The viscera of the abdomen, which can be seen through the transparent cuticle, initiated the movements.

Tan colour developed initially and darkest on the head, last and least on the abdomen. Some abdomens never coloured. Approximately 24 hours before emergence the eyes darkened, turned red, then just before emergence, black.

Less than five minutes before eclosion the pupa moved vertically and assumed the eclosion position (fig 2.V). The adult separated from the pelt (the pupa became translucent) and within two minutes the pelt split and the adult emerged as though being squeezed out of a tube with the anterior edge of the dorsal surface of the prothorax the first to emerge (the head was ventral of the prothorax). The adult immediately ran to the top of the nearest object from which it could only be dislodged by considerable violence. Elapsed time, from the initiation of the split to emerged adult, was 30, 35, and 40 seconds in different cases. The wings appeared to harden within 2½ minutes, during which time the adult was stationary and did not respond to light.

Few adults emerged from pupae in positions other than described. Some pupae moved up the side of the containers and were attached by their ventral surface and were effectively in eclosion position. Eclosion succeeded.

Larvae stayed in the moist regions of the sand slope mostly where there was free water at the surface, but moved up the slope before pupation. The pupae moved further up the slope before burying tail first to achieve the resting position.

Before eclosion a few pupae were lying on their ventral surface on the agar, oriented with head towards the light. If the light was moved they reoriented. Eclosion occurred in that position.

Pupation occurred between 1800 and 700 hours with some occurring as late as 1000 hours. Eclosion occurred in the morning, 2 days later, the pupal stage lasting 48-60h at 25°

DISCUSSION

Larvae of *Culicoides circumscriptus* (Becker, 1961) and *C nubeculosus* (Megahed, 1956) move to drier regions of the sand slope before pupation, but unlike them *C brevitarsis* does not breed in areas where free water is available and therefore movement away is of little

relevance. Pupae of C brevitarsis have the ability, shown also by C nubeculosus (Megahed 1956), to burrow backwards into a substrate to achieve the resting position during pupation. Pupation occurs within the dung pat, therefore the behaviour patterns described in agar, where pupation occurs below the surface, are likely to represent the natural patterns. The resting and eclosion positions are identical whether pupae are in sand or agar, strengthening the belief that they are positions assumed in dung.

The dorsal surface through which the adult emerges is, by virtue of the angle of the pupa, slightly more exposed than the ventral surface. The split extends approximately the exposed length of the pupa. The behaviour described probably occurs in pats, with pupae formed throughout the pat before moving upwards to the surface. Movement of pupae was attributed by Megahed (1956) to wriggling of the abdomen aided by abdominal spines. The latter would be unnecessary in C brevitarsis. Lawson (1951) described pupation in detail; the descriptions above complement his work by describing eclosion.

11.3 THE INFLUENCE OF LIGHT ON ECLOSION

In the initial examination it was noted that many adults emerged within 30 minutes of exposure to light if the pupa was at least 48h old.

MATERIALS & METHODS

Pupae were collected daily at 900h from agar plates containing larvae but those with red eyes were discarded. Six pupae per plate were put onto agar plates stored in black plastic bags in a closed drawer. Four plates were exposed to diffuse light of approximately 2800 lux after each of the following intervals.

(1) 900h; 2nd day
(2) 1100h; "
(3) 1300h; "
(4) 900h; 3rd day
(5) 1100h; "

The adults already emerged and which emerged within 3 hours were counted. Pupae were classified dead if emergence did not occur within 24 hours.

RESULTS (table 2.2)

Pupae which did not undergo eclosion within 3 hours, never did. Of the pupae exposed at 900h on day 2, 21/24 (88%) emerged within 3 hours;

of those exposed at 1100h, 19/20 (95%) emerged in 3 hours as did 14/17 (82%) of pupae exposed at 1300h. For day 2 as a whole, 7/61 had already emerged, the remaining 88% emerged within 3 hours. On day 3, 37/42 had already emerged when exposed (88%) and the rest emerged within 3 hours. Exposure to light accelerated but was not obligatory for eclosion.

One living pupa was exposed, after 2½ hours at 2800 lux on the second day, to light from a microscope lamp (60,000 lux). The adult emerged 5 minutes later.

DISCUSSION

Experience had already indicated that eclosion did not occur during the day after pupation. Emergence soon after exposure to light was verified by the experiment, but in the original observation the adult emerged 5-30 minutes after exposure to the light of a microscope lamp (approximately (60,000 lux) which together with a rapid eclosion from the pupa moved from 2800 to 60000 lux suggests that the greater the light intensity, the sooner eclosion occurs.

TABLE 2.2. Number of adults emerging after exposure of pupae to light.

Time exposed	already emerged	emerged in 3h	dead	total
day 2 ; 900h	3	21	0	24
day 2 ; 1100h	1	19	4	24
day 2 ; 1300h	3	14	7	24
day 3 ; 900h	18	2	4	24
day 3 ; 1100h	19	3	2	24

Eclosion from pupae in pats is probably triggered rapidly by exposure to direct sunlight (60-80000 lux) or more slowly by exposure to diffuse light. The surface of a pat is irregular and penetrated by many cracks, and many pats are in grass that shelters their sides for long periods of the morning and evening. Pupae, especially those in cracks, are more likely to be exposed to bright light during the middle of the day and that should lead to increased emergence at that time.

The pupa orientates to put its dorsal surface uppermost and towards the light, therefore the adult emerges into open space, is less likely to be trapped, and hence its chance of surviving eclosion is increased. At the topeof a nearby object wings and body will dry more rapidly and there will be some protection from predation by organisms on the surface of the pat.

II.4 THE TIME OF DAY OF ECLOSION

INTRODUCTION

Eclosion occurs when pupae are 2-3 days old, probably during daylight and with a peak during the middle of the day, but some could be expected in the evening. The following experiment examines the time of day when adults emerge from dung held in small cages in the field.

MATERIALS & METHODS

Pats, approximately one week old, were placed, one per trap (fig. 2.VI), in 10 emergence traps similar to the ones used by Linley (1968a) The sleeve was coated with olive oil and changed every two hours from 600 to 2400 h daily for four days after emergences began in each trap. Adults were counted but sex was not determined because the olive oil hindered recognition.

RESULTS

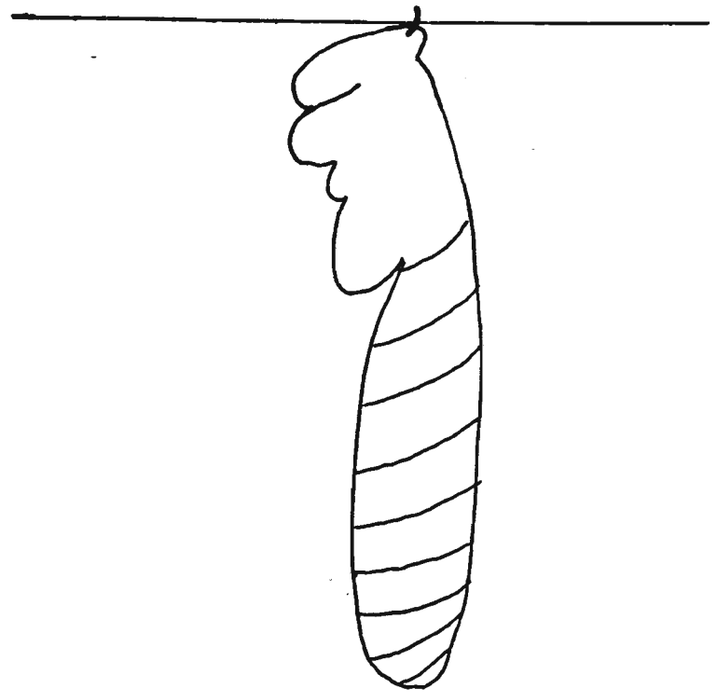
Some adults emerged between 1000 and 1200h, the majority between 1200 and 1600h with a few more to 2000h (table 2.3 : fig. 2.VIII). The periods 16-1800 and 18-200h were conbined because of very small numbers. Emergence peaked on day 2, and subsequently decreased on both days 3,and 4.

There was not one cause of the significant inte raction. Expected values were calculated for each cell of the inte raction table and deviations from expected determined. The greatest deviation was at 12-1400h on day 2; when the yield was much lower than expected. In addition all deviations from 10-1200h were large, with yield higher than expected on days 1 and 2 and lower on days 3 and 4 suggesting that the peak of emergence is delayed on the later days. The time of median emergence, 1314h on day 1, 1424h on day 2, 1438h on day 3, and 1426h on day 4, tends to confirm the belief.

The interraction makes comparisons between the means invalid mathematically, but the pattern of emergence is affected little if at all because the peak of emergence on different days (fig 2.VII) did not move out of the range established for the whole data, 1200h to 1600h.

Fig. 2.IV Pupal position.

Fig. 2.V Eclosion position.



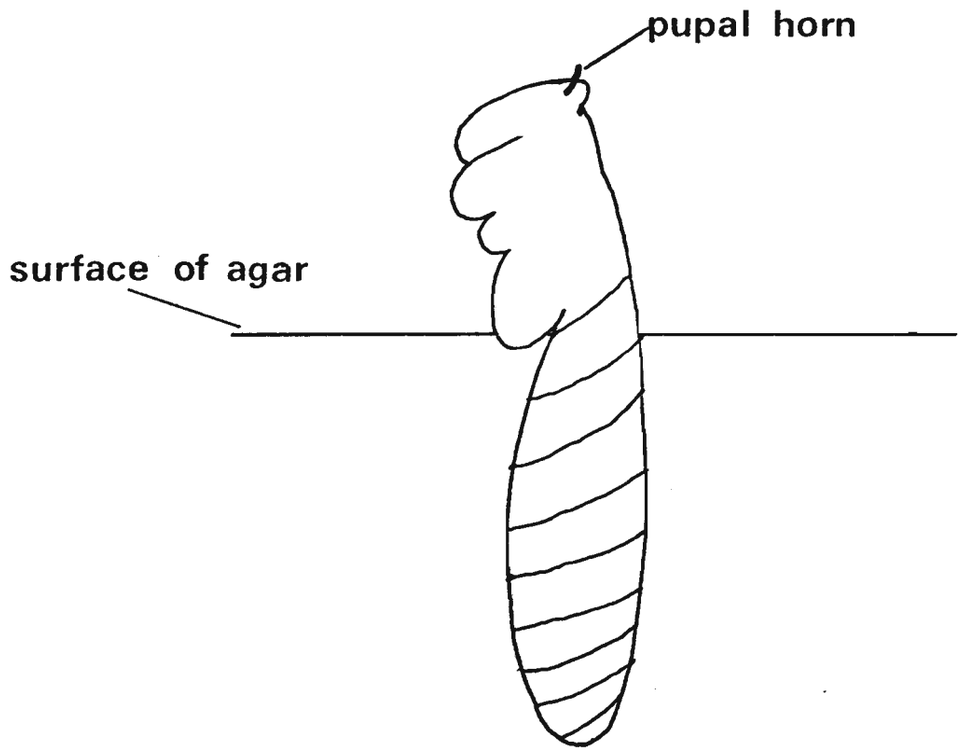

pupal horn
surface of agar

Fig. 2.VI Emergence trap.

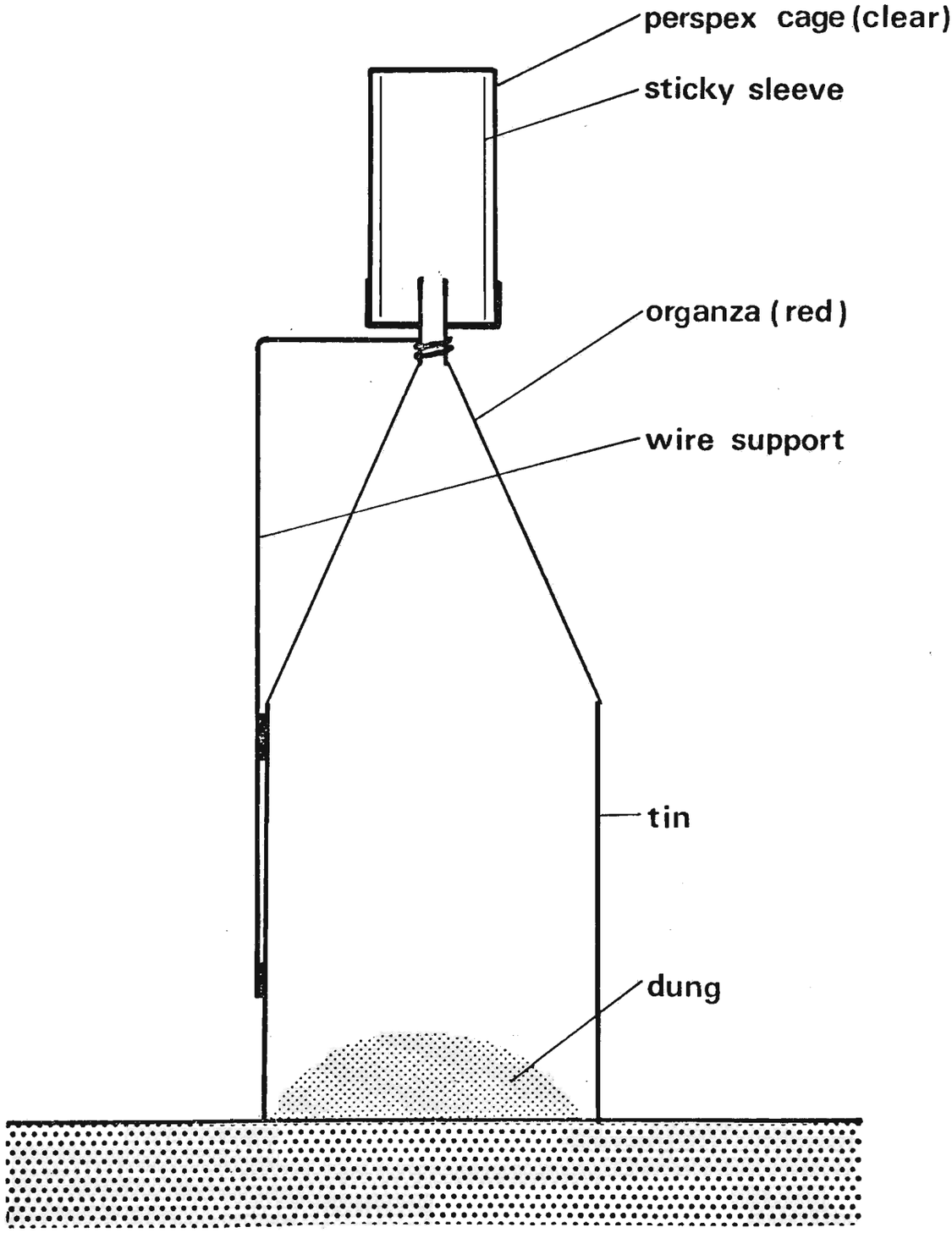

perspex cage (clear)
sticky sleeve
organza (red)
wire support
tin
dung

TABLE 2.3 Number of adults emerged in each period; means and analysis

Source	DF	MS	VR	P
period	3	10.48	63.4	< 0.001
day	3	2.21	13.4	< 0.001
p x d	9	0.53	3.2	0.002
replicates	8	0.85		
residual	120	0.17		

Means

period	M_W	M_L	significance
1000-1200	1.3	o.37	C
1200-1400	21.9	1.36	A
1400-1600	30.7	1.50	A
1600-2000	3.9	0.69	B

LSD (P=0.05) = 0.19

day	M_W	M_L	significance
1	8.9	1.00	B
2	17.4	1.27	A
3	9.0	1.00	B
4	3.6	0.66	C

LSD (P=0.05) = 0.19

period	day				total	deviation
	1	2	3	4		
10-1200	0.61	0.75	0.11	0.00	1.47	1.03
12-1400	1.45	1.35	1.43	1.20	5.43	0.81
14-1600	1.33	2.09	1.44	1.14	6.00	0.59
16-2000	0.59	0.86	1.02	0.30	2.77	0.62
total	3.98	5.05	4.00	2.64	15.67	
deviation	0.64	0.88	0.70	0.83		

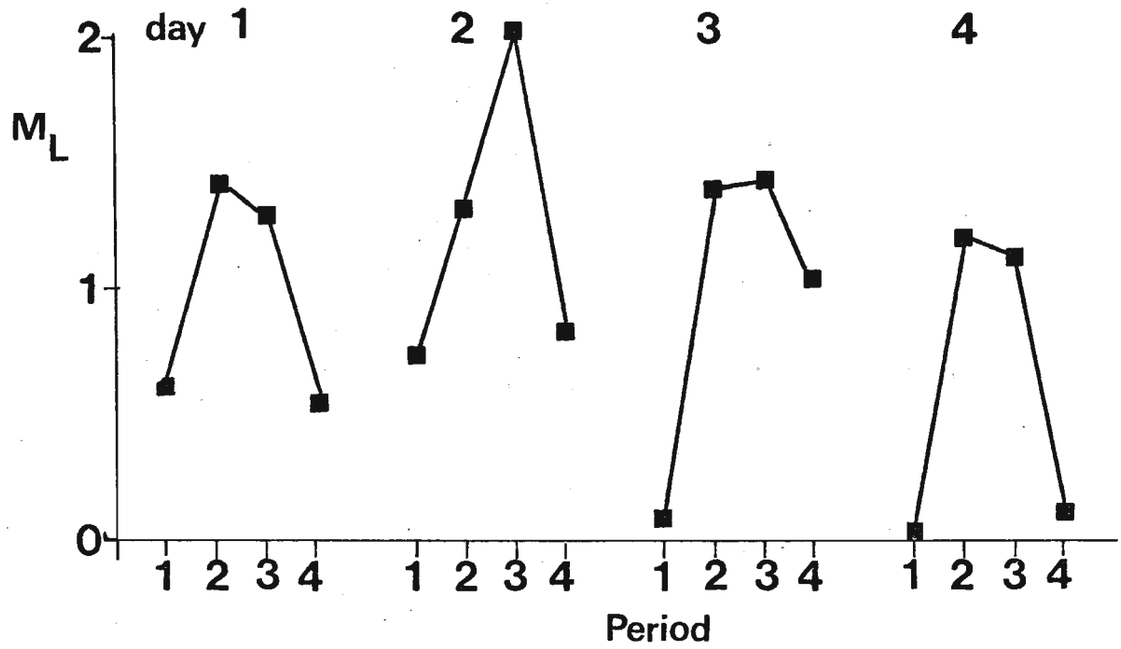
DISCUSSION

The cage itself could influence the time of emergence, as could any delay between emergence and trapping, but the effects are probably minimal as the time determined agrees with that predicted from laboratory observations on eclosion. Changes in sexual composition between days could have been the cause of the interraction. Oviposition occurs on six days, therefore emerging adults will have come from a series of overlapping populations, therefore any differences between sexes in the time to emergence should be masked on all but the first one or two days.

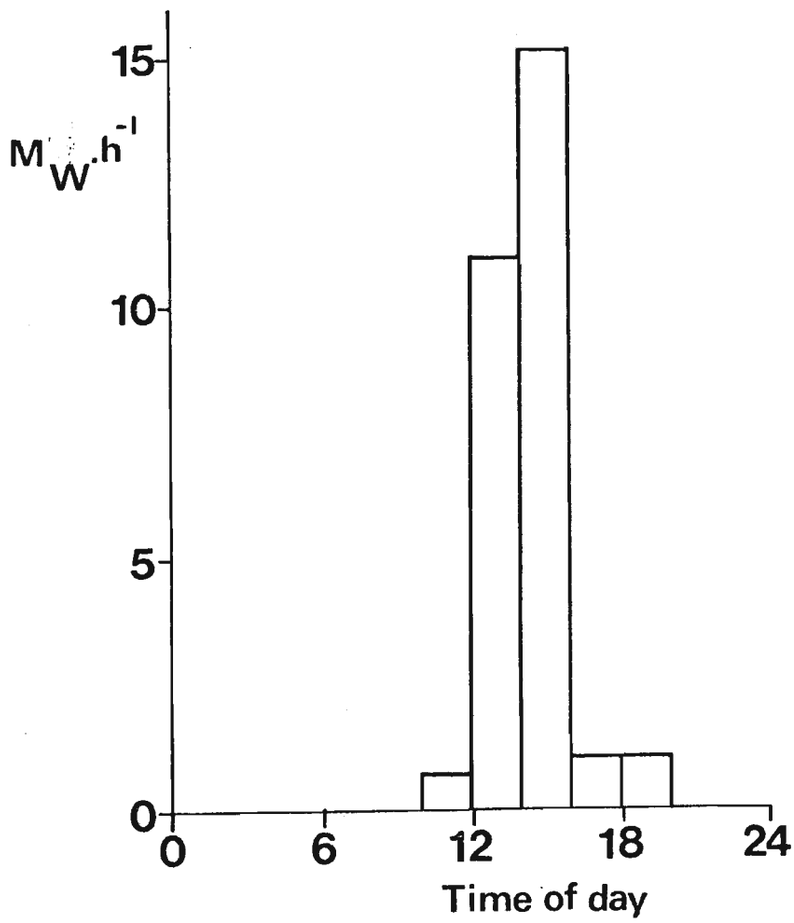
The emergence period for C brevitarsis differs from Leptoconops becquaerti (0300-0700h : Linley, 1968a) and C furens (0700-1100h ; Linley, 1966b). The behaviour of all three coincides in that none of them emerged between 2000h and 0300h.

Fig. 2.VII Mean number emerged for each period of day.

Fig. 2.VIII Emergence period



Period , 1 - 10 - 1200 h
2 - 12 - 1400 h
3 - 14 - 1600 h
4 - 16 - 2000 h



III POST EMERGENCE DEVELOPMENT

INTRODUCTION

The time between emergence and either mating or blood-feeding, whichever occurs first, varies widely throughout the diptera, but has not been studied in detail for Culicoides. It was estimated to be 2 or 3 days in C nubeculosus (Downes, 1950 ; Roberts, 1950) but Megahed (1956 showed that some adults would feed on the day of emergence. In C furens (Linley, 1968b), C guttipennis (Hair & Turner, 1966), and C variipennis (Jones, 1960) it was less than 2 days, and possibly less than 1. C arakawae (Morii & Kitaoka, 1967) fed and mated immediately after emergence. Linley & Adams (1972) showed mating and insemination of C melleus occurred a few minutes after emergence with younger males more efficient than older. C melleus does not mate in swarms. Leptoconops kerteszi (Rees et al, 1971) mated within two or three days of emergence.

Mating occurs shortly after emergence in Culicoides but of the above species only C furens mates exclusively in swarms. Linley and Adams (1972) suggested that mating immediately after emergence may be an adaptation to mating at the place of emergence. If correct, the time lag between emergence and mating may be longer in species that swarm.

If swarming and mating and blood feeding are to be induced in cages the age of the adults used may be important. This part reports an attempt to relate the condition of males caught from swarms to age in the laboratory.

MATERIALS & METHODS

Males reared out of dung collected from the field were incubated at 25^{o} and 75-85% humidity, and offered 10% sucrose. Adults were dissected at the following intervals after emergence; 0-1h, 3h, 20h, 1-6days, and more than 6 days. Preparations for examination of spermatids were stained, by flooding, with aceto-lactic orceine (Itard, 1971: 33ml distilled water + 33ml lactic acid + 33ml acetic acid + 2g orceine). In dissections made to search for spermatids the stain was not used because it stopped movement and they were more easily seen when moving.

RESULTS

Development in the reproductive tract

Spermatids develop within cysts that can be seen in the testes

of most newly emerged males and may have been present but not visible in the rest. Initially the cysts appear full of granules, then they gradually become fibrous. When testes at the latter stage were crushed they contained groups of short, thick, immature spermatids (plate Ia) which were difficult to separate from their clusters. The few that were measured ranged from 30-60 μm long but several seemed to be joined together, therefore the ends of each were difficult to determine. Their most characteristic feature was swollen regions along their length. They were not motile. Later the fibrous nature of the cysts became more obvious and movement of spermatids began. The cyst nearest the lumen of the vas deferens developed first and there were up to four cysts in each testis.

The cyst ruptured at or near the lumen of the vas deferens and spermatids moved head first into and filled the vas deferens (Plate Ib) the vesicula seminalis. The spermatids were fully developed and motile (plate Ic).

Visual comparison suggested that spermatids from more than one cyst can be accumulated in the vesicula seminalis of older males.

About the time of rupture of the first cyst, two zones appear in the accessory gland (within which is located the vesicula seminalis; Pomerantzev 1932) as shown in Plate Id). The posterior section (lower section in Plate Id) appeared granular, the anterior clear. Active spermatids were present in the testes of 30% of males 3 hours after emergence (table 3.1) and in 100% after 20 hours. They were present in the vas deferens and vesicula seminalis of 75% of the males after 20 hours and 100% after 24 hours. The accessory gland developed zones about 24 hours after emergence.

Spermatids were present in the testes, vas deferens, and vesicula seminalis, and the accessory gland was zoned, in every male collected from swarms.

The spermatid

The mean length of spermatids is 90.0 μm (Table 3.1) regardless of source, but they are too thin to be measured or clearly observed with the light microscope. When spermatids in saline were stained by flooding with acetolactic orceine the head and tail absorbed stain, but only the head when the saline was allowed to dry out before staining. The head was curved, 2.4-3.1 μm long. The difference between the head and tail was just enough to distinguish them.

Movement

Three types of movement were observed.

(i) Short wavelength oscillation originating 10 μm from the anterior tip of the spermatid. This type of movement was very common but did not appear to move the spermatid.

(ii) Slow flexion of the flagellum giving rise to oscillation of long wavelength but without an obvious point of origin. It was observed in many spermatids and succeeding in moving them.

(iii) A corkscrew motion imposed on top of that described in (i) was observed, but only rarely.

Striated body

The abdomen of the newly emerged male and female is packed with a structure-less body that, for want of a more accurate name, has been called striated body (plate Ie). There is little doubt it is stored metabolites, probably anologous to pupal fat body. It was found in pupae and in newly emerged adults but was lost within 24 hours of emergence. It stained with neither sudan black B (a fat stain) or acetolactic orceine (stains nuclear material), but dissolved in fat solvents. No structure could be discerned, and the striations were not visible using phase contract illumination. In the pupa and newly emerged adult each piece had sharp edges but 10 to 20 hours after emergence, the pieces were smaller and had rounded edges; as if they were being dissolved away on the outside. Striated body was not found in males collected from swarms or females from hosts.

TABLE 3.1 Development and length of spermatids

age of male	number of dissections			
	total	with active sperm in		
laboratory emerged		testes	vd	ves sem
1h	2	0	0	0
1-3h	10	3	0	0
16-20h	9	8	6	6
24h	48	48	48	47
6 days	31	25	25	25
swarms	34	34	34	34

source of male	length of spermatid (μm)	numbered measured
swarms	90.3	37
mated females	90.9	12
males emerged in lab.	89.0	21
mean	90.0	70

vd = vas deferens ves sem = vesicula seminalis

DISCUSSION

Males held in the laboratory achieved the condition of males in swarms after incubation for 24 hours. If striated body is a metabolite store the rate of its consumption may depend on the level of activity of the adult. Adults within cartons are relatively inactive but if more so in the field they may consume the striated body faster, therefore its presence or absence is not a reliable guide to the minimum age of males in swarms. The development of spermatids should be a more reliable measure.

The processes of sperm transfer have been examined by Pomerantzev(1932) in C nubeculosus and by Linley and Adams (1971) in C melleus, but spermatids have not been described for species of Culicoides. In some of the photographs appended to the paper by Linley and Adams (1971), spermatids are shown as thin and indistinct strands. The definition achieved in Plate Ic is little better because the spermatids are extremely narrow. The head is slightly wider than the tail but in unstained preparation they are difficult to distinguish. At insemination they are full length and fully active. Within the female they appear to be coiled around the outer edge of the spermathecae and at times seem to be moving en masse. More detailed descriptions of spermatids require electron microsope.

Spermatids in some other Diptera, especially Drosophila vary in size within the adult (Beatty and Burgoyne 1971). Variation was not observed in C brevitarsis.

The anatomy of the reproductive system of the male does not differ from C nubeculosus (Pomerantzev 1932) and two Neoforcipomyia species (Chan and Le Roux 1965), but the ejaculatory duct of C nubeculosus and C brevitarsis is much shorter than is figured for N eques and N saundersi. The posterior ends of the vas deferens are tightly appressed to a large structure which has been called the accessory gland. Pomerantzev (1932) examined the structure in detail, including section-

ing, and described it as a combination of the paired accessory glands and seminal vesicles. In C brevitarsis many spermatids are stored within it, therefore it certainly functions as a vesicula seminalis. There is no reason to doubt the conclusion of Pomerantzev (1932)

IV SWARMING

INTRODUCTION

Swarming has been defined as "a formalised flight pattern performed by male mosquitoes within narrow spatial limits" (Nielsen & Haeger, 1960). It is not however restricted to mosquitoes but is widespread in Diptera (and in a few other orders; Downes, 1969) including Ceratopogonidae (e.g. Culicoides nubeculosus ; Downes, 1950), Simuliidae (e.g. Simulium venustum ; Peterson, 1962), Tabanidae (e.g. Tabanus bishoppi ; Blickle, 1959), Cecidomyiidae (e.g. Anarete pritchardi ; Chiang, 1961), Chironomidae (Gibson, 1945), as well as mosquitoes (e.g. Culex pipiens Knab, 1906). Despite observations on many species over the last 30 years by many workers (reviewed by Haddow & Corbet (1961) and Downes (1969)) the function subserved is still debatable. Downes (1958) as a generalisation and Blickle (1959) for T bishoppi believed that it subserved mating, while Cambournac & Hill (1940) and Nielsen & Greve (1950) believed it a relict of a behaviour pattern that once may have subserved mating.

In their summary of previous work Haddow & Corbet concluded that it was "of major importance to the insects concerned, and we find it difficult to agree... that it is a vestigial or ritual activity." They did not appear to be convinced that swarming subserved mating. Corbet (1964) observed swarms but not mating in mosquitoes at the top of a tower in a forest, but in swarms of the same species at ground level, where observations were arranged to maximise visibility, he observed pairing. He proposed that it was improved visibility which made possible the observation of pairing. If swarms of other species were viewed under optimal conditions pairing might be observed more frequently, therefore the failure to observe pairing is not convincing evidence that swarming does not subserve mating.

Gibson (1942, 1945) showed that males of Spaniotoma minima rejoined the swarm after mating but the females did not, rapidly causing the swarm to become predominantly male (93%). If the same process occurs in other species it would satisfactorily explain the predominance of males in swarms, the other objection often raised against swarming subserving mating.

Downes (1958) suggested that swarms were a method of bringing the sexes together for mating. In that context it is interesting that Shannon (1931) found that swarming occurred typically among drab coloured mosquitoes in South America. Predation on swarming males has been recorded with dragonflies (Corbet, 1962; Howard et al, 1913-7;

Thienemann, 1954), Ceratopogoninae (Downes, 1960), and other Diptera (Frohne, 1959). Dragonflies were observed flying back and forth through swarms of C brevitarsis but proof of predation was not sought. Swarming requires flight which in turn demands energy and consequently more frequent feeding than would otherwise be needed. It is almost certainly a dangerous activity to the longevity of the individual, and therefore would be lost by natural selection unless it subserved a critical function. At the present state of knowledge swarming could only subserve mating. I have assumed that it does.

A number of species of Culicoides have been colonised. In most (C nubeculosus, Megahed (1956); C variipennis, Jones (1957); C guttipennis, Hair & Turner 1966); and C arakawae, Morii & Kitoaka (1968)) mating occurred easily, during or close to blood-feeding. C furens, the only species for which swarming is mandatory to have been colonised, was far more difficult. Manipulation of the light regime in cages around sunset (Linley, 1968b) was required and was so arduous that the colony was eventually discarded (Linley, personal communication) L kerteszi, in which swarming may be mandatory, was colonised using a similar technique (Rees et al, 1971).

The same technique was tried with C brevitarsis without success. Pairing was not observed and spermatids were not found in the spermathecae of dissected females. In other attempts to obtain mating, both sexes were crowded into cages of various sizes and subjected to a wide range of conditions, including sunset, without success. Some adults were held for 15 days. Pairs of adults confined in small containers (750 mm^3) and subjected to sunset and to repeated contact did not mate. No response was elicited from headless males suggesting that attempts to copulate females with them would be pointless. The size of C brevitarsis virtually precludes the use of manipulative techniques such as attaching threads to manipulate flight (Weintraub, 1961). Initial attempts to colonise were therefore frustrated partly by the inability to obtain mating in the laboratory.

No information was available about mating in the field. Swarms were observed at Highvale and later at Collard and it was assumed that mating occurred in them, and that the induction of swarming in the laboratory may be necessary to mating, both of which are more likely to be achieved if field conditions are duplicated as closely as possible

MATERIALS & METHODS

Sites

Observations, prior to January 1st 1973 were made at Highvale;

later observations and experiments were at Collard which was more convenient. The population of C brevitarsis at Highvale appeared to be greater than at Collard hence Highvale was the better area during winter and spring.

Highvale

A dairy farm situated on the side of a hill in a valley surrounding Samford, northwest of Brisbane. The dairy ran 30 cows of which 25 or 26 were milked twice daily. At the beginning, 1600h, of the evening milking period 25 cows were herded into the yard (fig 4.I) adjacent to the milking sheds. They were removed a few at a time as required. The yard was used as the experimental area.

Most of the yard (220.9 m^2) was compacted dirt, bare of vegetation except under the rails of the wooden fences. A concrete apron extended from the milking shed into the yard. The owners removed all dung daily, between 900 and 1000 hours, but pats that had been flattened by cows, and a few objects such as sticks and stones were occasionally present. The dairy buildings on the southern and western sides acted as a wind break. A series of high hills to the west effectively advanced the time of sunset by 15 to 25 minutes depending on the time of the year.

Collard

Collard Rural Sheltered Workshops ran five cows, one bull and a few calves on a flat area within Brisbane. The experimental area was a square of side length 7.3 m within which the grass was mown to 20 mm high. In the surrounding areas grass and weeds varied from 100 to 300 mm. Noogoora burr (Xanthium pungens) plants were scattered over the area. The experimental area was near both cattle yard and calf yard (fig.4.II). A long band of trees 6 m tall to the east acted as a windbreak

Fig. 4.I Highvale

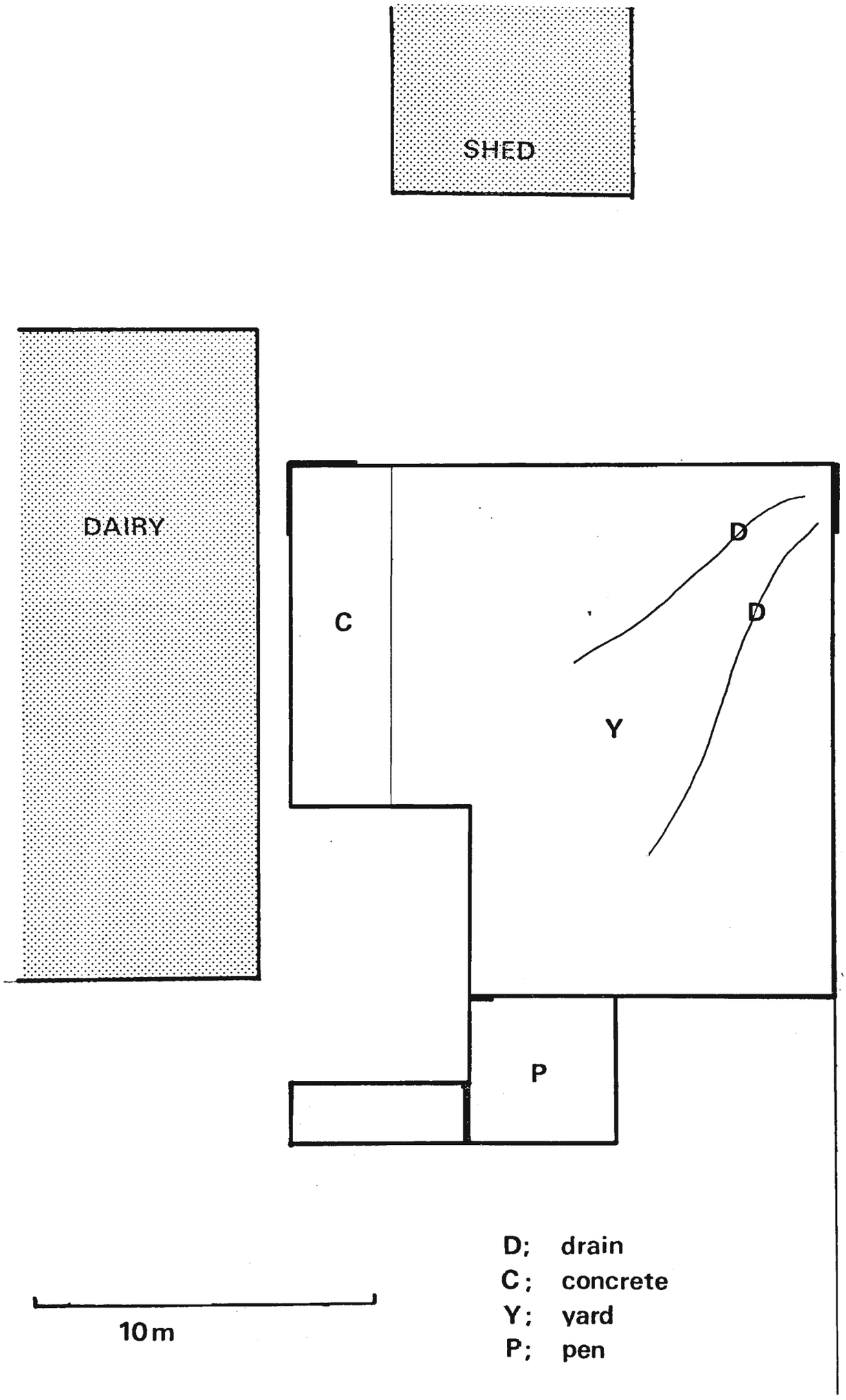
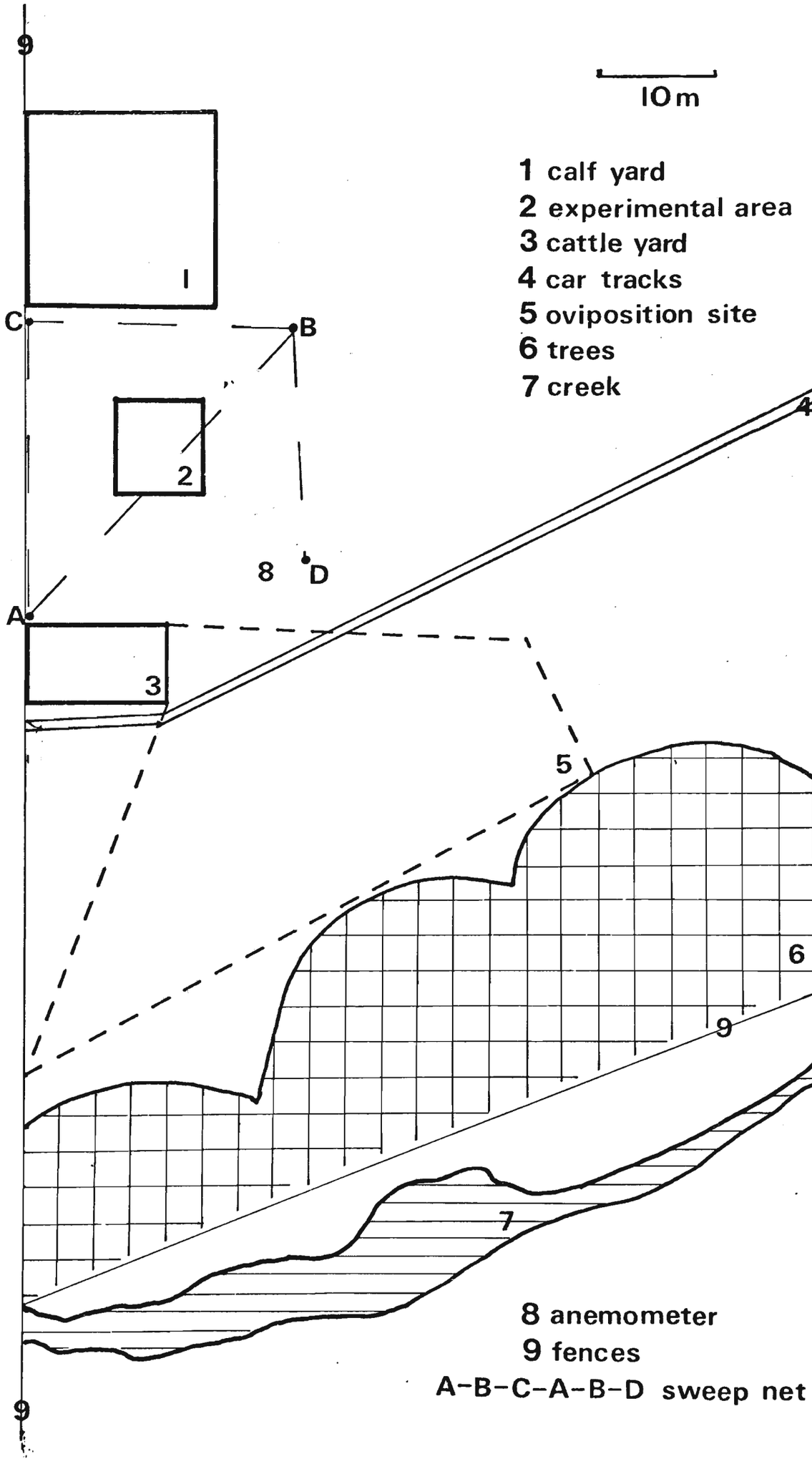

SHED
DAIRY
C
D
D
Y
P
10 m
D; drain
C; concrete
Y; yard
P; pen

Fig. 4.II Collard.


10 m
1 calf yard
2 experimental area
3 cattle yard
4 car tracks
5 oviposition site
6 trees
7 creek
8 anemometer
9 fences
A-B-C-A-B-D sweep net
A
B
C
D
1
2
3
4
5
6
7
8
9

IV.1 GENERAL OBSERVATIONS ON SWARMING

INTRODUCTION

Observations, descriptions, and experiments that do not easily fit into any particular section, but which are used or assumed throughout all, are presented here.

MATERIALS & METHODS

A series of swarms were collected individually at Highvale and Collard by swinging a net upwards through the swarm. The adults collected were held in alcohol (70%) until sexed and counted in the laboratory. Adults that were flying at Collard were collected by sweeping in a horizontal plane 1.5m above the ground with a hand net (opening of $0.12m^2$) while the collector walked A to B, C, A, B, D (fig 4II). The net travelled a standard 300m, sampling $36m^3$ of air space. This sweeping was similar to that used by Parker (1949) and suffered the same disadvantage, viz. the volume sampled varied between swings despite a deliberate attempt to standardise. The volume, therefore, differed between collections but should have done so only to a minor degree. Sweeping was carried out before and after sunset at irregular intervals (when other observations permitted) which were shorter near sunset.

OBSERVATIONS

Swarms were seen by viewing them against either the western sky or a dark background or both. Reflection of light, presumably from their wings, made them visible. Two swarms, over shadows or dark marks on the concrete apron (not over the white posts as the photographs seem to suggest) in front of the milking shed at Highvale are shown in Plate IIa,b. Adults moved rapidly within the swarm but their small size precluded accurate description of flight patterns.

In several swarms observed under the best possible conditions the adults did not appear to be flying in any particular direction, certainly not towards the wind which was nearly too strong. It is possible to see swarms only when facing the sun therefore a side view to help determine if the adults are flying towards the sun is not possible. Swarms were occasionally blown downwind but quickly regained position. Stronger gusts blew them away.

Swarms were high level (base 0.6-1.2m; top 1.5-2.1m above ground) or low level (base 0.1-0.4m; top 0.6-1.2m) (fig.4.III) On several occasions, whole swarms moved en masse from high to low and back again with movement in either direction completed in 5 secs without dispersal.

High and low swarms differed in shape and when they moved between heights their shape changed.

Swarms first formed an hour before and appeared to cease at sunset but a few persisted a little longer. Very few were seen at dawn.

The numbers collected in sweeps at Collard varied widely between evenings and to a lesser extent on the same evening (fig 4.IV). The greatest number of males was collected just before sunset, falling rapidly afterwards, with more males than females in almost every collection.

Swarms were easily netted before sunset but attempts afterwards over the same markers rarely caught either males or females. The few exceptions are discussed later(p 81).

Swarm markers at both sites were modified causing the dispersal of the swarm. On most occasions a swarm of similar size reformed less than 10 sec after the modification was removed or after a previous swarm had been netted.

On two evenings dragonflies flew back and forth through swarms staying within the space occupied by swarms most of the time. It was assumed that they were devouring C brevitarsis.

DISCUSSION

Predation in swarms has been recorded with other species. The rapidity with which swarms reformed was surprising. The collector was satisfied, by visual observation, that most if not all of the adults were collected when the swarm was netted, demanding that the replacement swarm be of different adults. It suggests that many adults were flying close to the swarm marker and that they, or some of them, immediately began to swarm, an explanation posing two questions, neither of which can be answered:

(i) why, if they were flying and able to swarm had they not already joined the swarm and increased its size? i.e. Is there an upper limit to size?

OR

Fig. 4.III Shape and height of swarms.

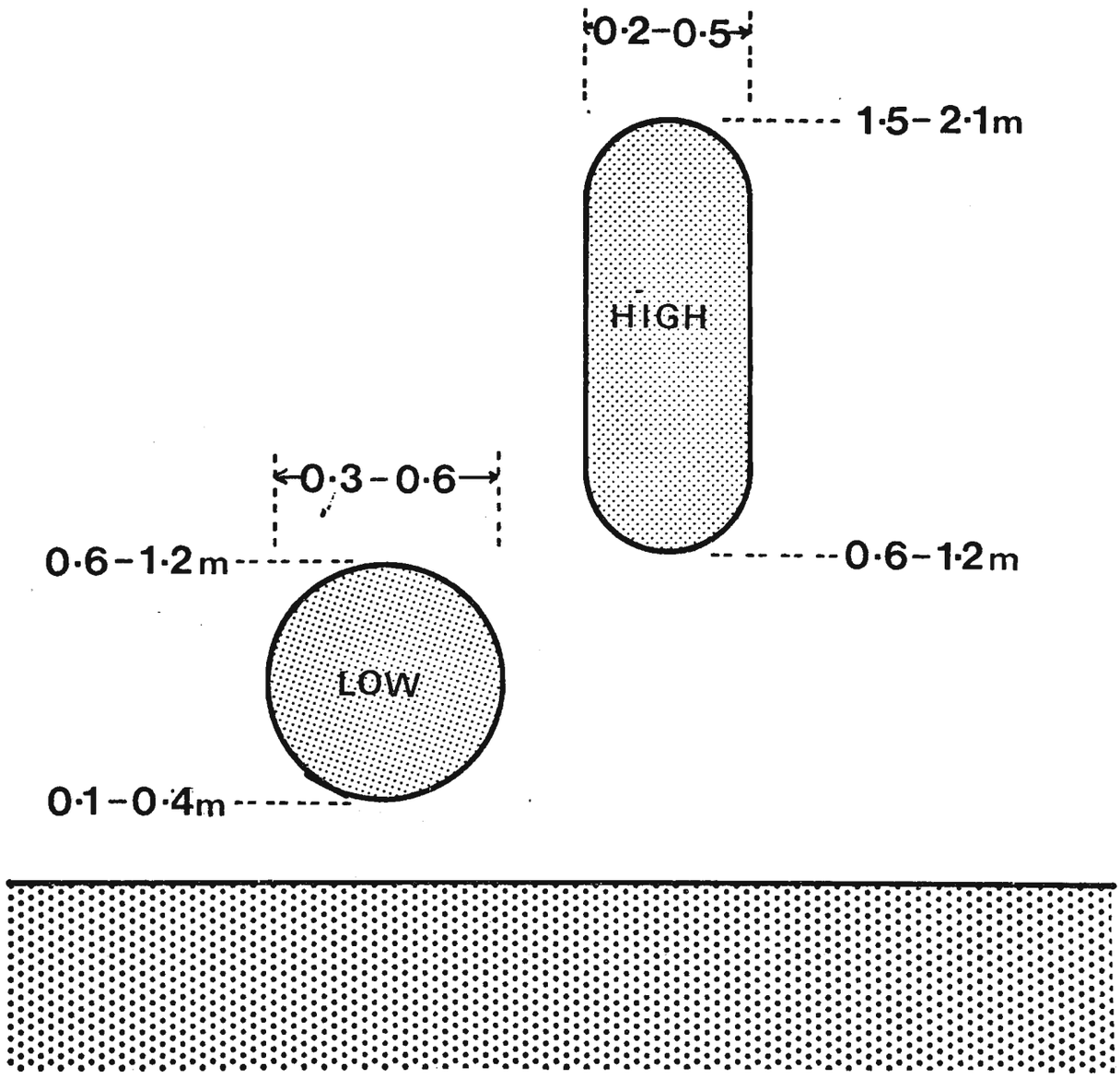
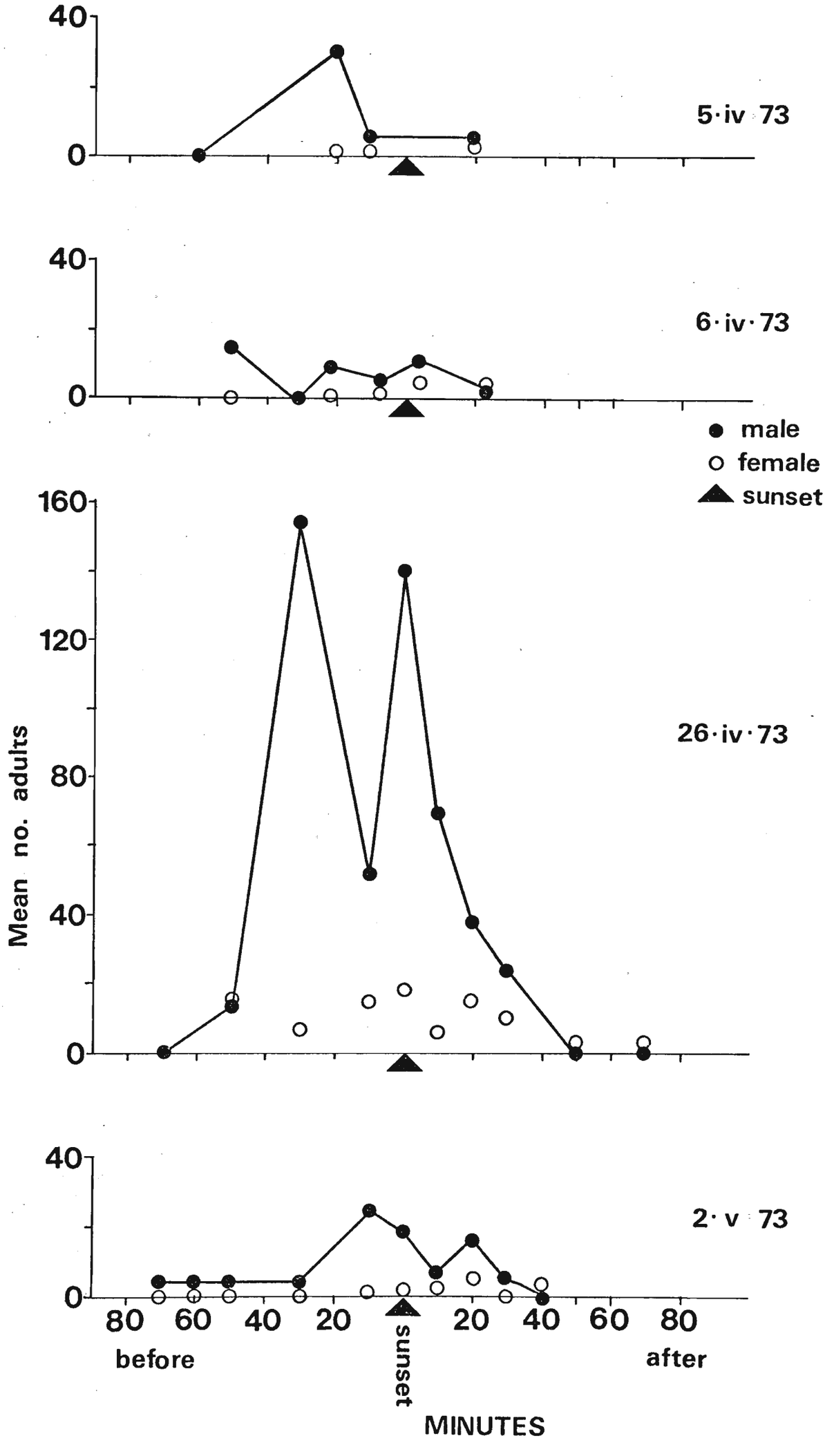

0·2–0·5
1·5–2·1m
HIGH
0·6–1·2m
0·3–0·6
0·6–1·2m
LOW
0·1–0·4m

Fig. 4.IV Sweep net collections; Collard.


40
0
5·iv·73
40
0
6·iv·73
male
female
sunset
160
120
80
40
0
Mean no. adults
26·iv·73
40
0
2·v·73
80 60 40 20
20 40 60 80
before
sunset
after
MINUTES

(ii) are adults continuously moving in and out of swarms at a sufficient rate to replace all or most of the adults every 10 seconds?

Most swarming ceases at sunset but there is no doubt that swarms persist over a few markers well after sunset. Failure to observe swarming after sunset could result if:

(i) swarming ceases over all but a few markers at sunset,

or (ii) swarming continues but is so difficult to see that it appears to cease.

Nil catches over markers where swarms had been present before sunset suggests that the former is more likely. Counts of males caught while sweeping do not help because although a sharp reduction after sunset was recorded in the most reliable data (April 26), males collected may or may not have been swarming. Swarming could not occur more than 50 min after sunset because no males were flying then (fig. 4.IV)

IV.2 SWARM MARKERS

INTRODUCTION

A swarm forms over an object, the swarm marker, on the ground. In many cases it is possible to define a particular point or boundary of the marker that is being used to regulate swarming. I shall call this the optische marke, a term originally coined to describe the whole marker (Wenk 1965). Downes (1958) showed that perception of the marker was visual, and the types of markers described by other workers (Nielsen 1964; Corbet 1964; Chiang 1961 and many others) confirm his conclusion. It has been assumed that adults of C. brevitarsis perceive the marker visually.

MATERIALS & METHODS

Every swarm within the experimental areas at Highvale (four occasions, April 1972; three occasions, November 1972) and Collard (16 occasions, January and February 1973) was noted. Observations began 2 hours before and finished one hour after sunset. On one occasion at both sites all dung pats up to two days old were counted. At each observation the time of day, exact location and height of the swarm were recorded and the marker and the appearance of the swarm described. At Highvale, the position of the swarm relative to the ground was estimated by viewing from two directions but at Collard, a dart was dropped from the centre of the swarm. The distance of the dart from two adjacent corners of the experimental area was measured and the exact location was mapped.

The distances between the centres of contemporaneous swarms were calculated from the coordinates using the formula (Hale 1966)

$$\sin A = \frac{2}{bc}\sqrt{s(s-a)(s-b)(s-c)}$$

where $s = \frac{1}{2}(a+b+c)$

A is the angle opposite side a and adjacent to sides b and c of a triangle.

In the triangles B_1-B_2 was one side of the experimental area and S_1 and S_2 the location of adjacent swarms. The angles A_1 and A_2 were calculated using the formula, then the difference between A_1 and A_2 used to calculate the distance S_1-S_2.

Distances between swarms near calves were measured from a map of their positions (fig 4.XVII).

Several markers were modified by a partial or complete covering or removal and the effect of the modifications recorded. A range of 'artificial markers' chosen to simulate natural ones were evaluated, including flat objects of various colours and shapes (e.g. thin boards painted different colours and pieces of cloth) as well as objects that cast shadows.

RESULTS

Markers

The markers observed were described and assigned an arbitrary number (Highvale 1-14; Collard 15-26). (table 4.1).

The markers were:-

HIGHVALE (7 evenings) (position of markers shown in fig 4.V)

1. Old (more than 2 days) dung, flattened and dried out to a thin crust lying on bare ground, and covered with a layer of dust from the yard. Their size did not seem relevant. They cast virtually no shadow and were only slightly darker than their background.
   They were used on all seven evenings, six different pats on one evening. They were independent of direct sunlight and the same pat was used on successive evenings.
2. A grey stone 50 mm in diameter, covered with dust, lying on bare ground, was the only stone used as a marker. It did not contrast in colour with its background, The sun was shining and a small shadow was cast by the stone on both evenings when small and unstable swarms were observed.
3. A clump of living grass on the otherwise bare ground of the cow yard was the only large patch of grass (0.35 m diameter, 0.3 m high) within the yard and was present only in April 1972. It was a very effective marker with large and stable swarms forming over it, including the first and last swarms each evening. Its use was independent of direct sunlight.

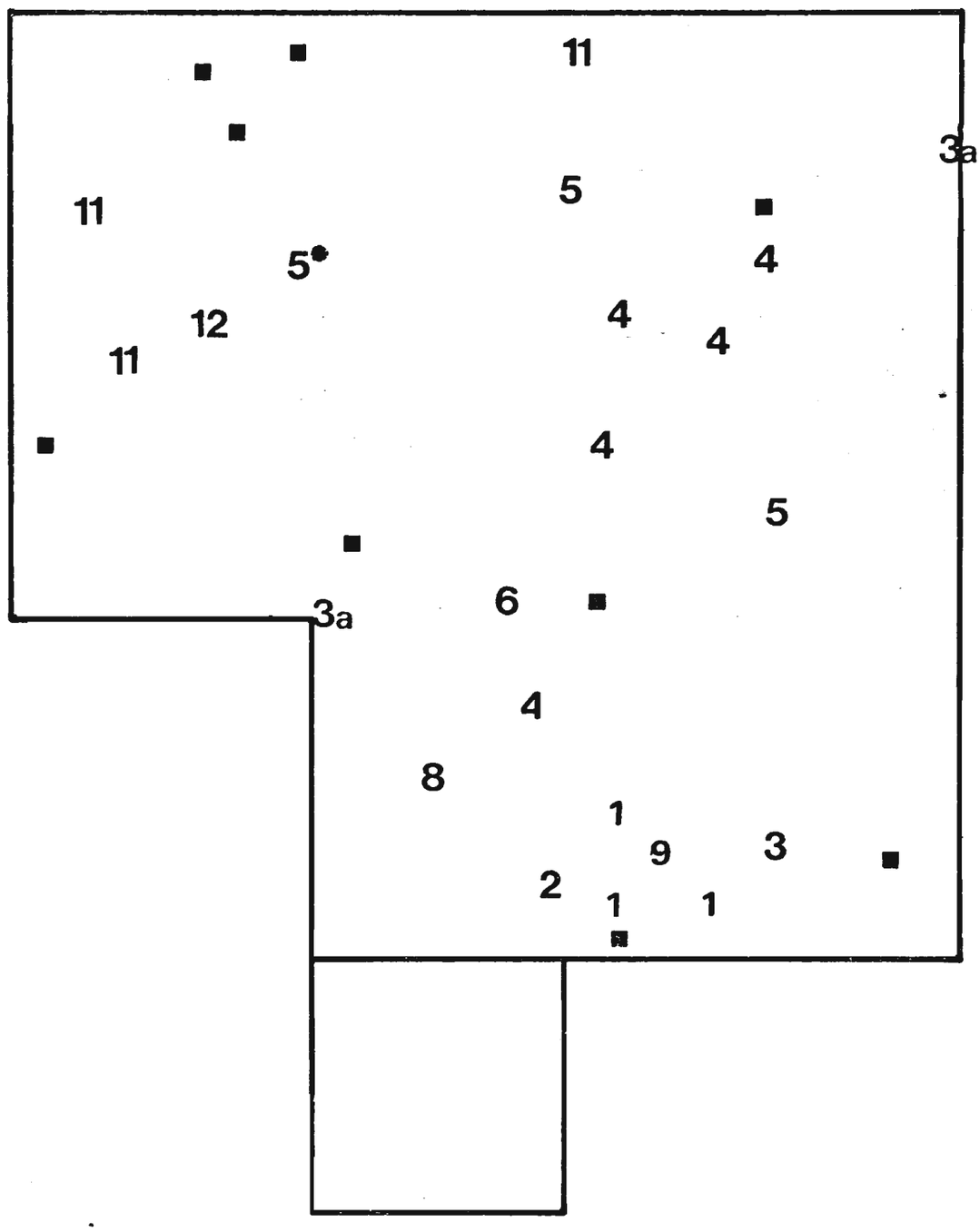
TABLE 4.1 Swarm Markers

	Marker	N	+	-	S	D	NC
1	old flattened dung	16	15	1	0	0	16
2	grey stone/bare ground	2	2	0	2	0	0
3	living grass/bare ground	12	11	1	11	1	0
4	dead grass/bare ground	4	4	0	4	0	0
5	dark ground/lighter	10	8	2	0	10	0
6	nothing	4	4	0	0	0	4
77	new pats/bare ground	6	6	0	6	6	0
8	old wood	1	1	0	1	0	0
9	old grey felt hat	1	1	0	1	0	0
10	cow	3	2	1	2	3	0
11	shadow distant object	9	9	0	9	0	0
12	tall grass/edge concrete	1	1	0	1	0	0
13	Old dung in deep grass	2	0	2	0	2	0
14	grass clump/grass	3	2	1	2	0	1
15	wet ground	1	1	0	0	1	0
16	old dung/short grass	1	0	1	0	1	0
17	new pats/short grass	10	7	3	7	3	0
18	shadow distant object	1	1	0	1	0	0
19	old dung in deep grass	6	5	1	5	1	0
20	grass clump/grass	31	28	3	28	0	3
21	Noogoora burr	16	9	7	9	0	7
22	dark grass/lighter	10	0	10	0	10	0
23	dark dirt/sparse grass	6	3	3	0	6	0
24	green grass/dead grass	5	2	3	0	5	0
25	deag grass/living grass	2	2	0	2	0	0
26	depression in sward	6	6	0	6	0	0

N = total number of swarms; + sun shining; - sun not shining; S swarms over markers casting a shadow; D swarms over markers without shadow but darker than the ground; NC no difference between object and ground in colour

? classification doubtfull

Fig. 4.V Swarm locations: Highvale.



■ dung pats ≤ 2 days old

Other markers of the same type, but growing at the base of the fence and hence not so clearly defined, were used on most evenings (3a in fig 4.V) .

4. Piles of dead grass, the colour of straw, were put in the yard by the owners each evening in April 1972. They varied in size and shape but averaged 0.4 m in diameter and 0.2 m in height, and were used on the three evenings when the sun was shining. They cast a large and obvious shadow. The grass was a light object on the darker ground. The swarms were large and stable.
5. Darker patches of ground, formed when cows urinated, were present on every evening but their position and size varied. Swarms formed over them on five of the seven evenings, but not all of the areas were used. They were independent of direct sunlight. In December 1972 a wet patch was present at 5* (Figure 4.V) on three evenings and each time a swarm formed and persisted for 30 minutes after sunset. The swarms were large and stable.
6. Two swarms were observed over areas where no marker could be seen. The swarms were small but persistent.
7. Pats of fresh dung, usually less than twelve hours old, but with a few up to 2 days old. They had not been flattened by the cows which dropped many pats soon after entering the yard. Nine pats were present on one evening. Swarms formed on 2/7 evenings only and on both the sun was shining. The dark pat contrasted with its light background.
8. A piece of rotted wood, 150 mm x 50 mm x 30 mm covered with the dust of the yard was a marker on one evening when the sun was shining. It cast a small shadow but did not contrast in colour with its background.
9. On one evening a flattened grey felt hat, covered with dust, was used as a marker for a small but persistent swarm. The sun was shining and the hat cast a small shadow.
10. The cows (specifically their heads) were used as swarm markers on 3/7 evenings, independently of direct sunlight. The swarms ranged from small to large but did not persist probably because the cows tended to move around the yard.

Shadows were present on the heads of the cattle which were darker than the ground but compared to other markers, the contrasting surfaces were separated by a vertical distance ranging from 0.9 to 1.2 m.

11. Broad shadows were cast on the ground particularly in the western half of the area, by posts and rails up to 4m away. On three evenings these were used as swarm markers most commonly on the concrete apron. Swarms were moderate in size and persisted but present only when the sun was shining.
12. A tall patch of broad leaved weed 0.45 m high and 0.2 m in diameter was present at the edge of the concrete apron (December 1972 only) above which large and persistent swarms formed until 40 minutes after sunset. The first and last swarms on each evening were over it, independent of direct sunlight.
13. Old dung pats nearly hidden by grass that surrounded them to a height of as much as 0.3 m were used as markers but were only found outside the experimental area.
14. Areas of grass 20 to 100 mm taller than the surrounding sward were used as markers outside the yard. They cast a shadow. Markers 13 and 14 were observed during cursory examination of areas immediately adjacent to the experimental area. They have not been marked onto fig. 4.V.

Collard (16 evenings)

15. A wet patch (urinated ground) in the cattle yard, was noted only once but was in an area not usually examined hence frequent occurrence would not have been recorded. No shadow was or could be cast but the wet ground was much darker than its surroundings.
16. Old dung (2 days old) on grass so short that it was virtually bare ground was used as a marker on one evening in the absence of direct sunlight but occurred only rarely at Collard. The dung was darker than its background.
17. Pats, up to two days old on mown grass, that were clearly visible and not flattened were used on 7/16 evenings, with a maximum of three on any one evening. There were always more pats present than used as markers. On one occasion 10 pats were present but none were used. Their use was

independent of direct sunlight. Shadows formed on the top of some pats, but all were darker than their background. Swarms were of moderate size and persistent.

18. Shadows cast on the ground by distant objects. This type was observed once where the shadow of a fence rail crossed a cement slab. The shadow was long and there was no visible reason why the swarm should have formed at the exact position along the shadow that it did. Shadows of this type were not present in the experimental area, and may have been used as markers more frequently than is implied by this observation.

19. An old dung pat surrounded by tall grass giving the impression of a deeply shaded hole at the edge of the experimental area. Swarms were observed on 3/16 evenings independently of direct sunlight.

20. Areas of grass, 20 to 100 mm higher than the sward, casting a shadow when the sun shone/were used on 10/16 evenings, on seven of which the sun was shining. On sunny evenings swarms formed over 2.5 of them but only 0.6 on clouded evenings. Swarms were stable ranging from small to large, and occurred over the same marker on successive evenings. This was the most common marker at Collard and the type above which the largest swarms were observed. Swarms persisted over the larger markers but tended not to over smaller ones. There were vast numbers of this marker in the paddock, and most of the swarms outside the experimental area formed over them. Many markers apparently suitable were not used. Observation suggested that all were used sometimes but especially with the smaller markers, swarms moved from marker to marker. On most evenings the first swarm observed was over this type.

21. Noogoora burr plants 0.15 to 0.6 m above the general sward were scattered across the area. They or their shadows were markers on 7/16 evenings, four sunny and three clouded. All were on or close to the edge of the experimental area. The plants were lighter green than the background and did not give rise to distinct shadows, However darker zones were formed under the plants by general shading.

22. Areas of darker green grass that did not cast shadows

and were not elevated above the general sward area were used on 4/16 evenings, clouded evenings only. Their diameter ranged from 0.3m to 1.5m. Very large and persistent swarms formed over an area 1.5m in diameter.

23. Dirt darkened by urine or rain showed through areas of sparse grass. Large and persistent swarms formed over areas varying from 0.3m to 1.0m diameter on 3/16 evenings, on one of which the sun was shining.
24. Small areas of green grass surrounded by dead grass, present after mowing on four evenings, were used as markers. The sun was shining on two of the evenings but only shadow cast was small. The marker was a dark area contrasted against a lighter background.
25. Piles of dead grass were present on the sward after mowing and on 2/4 evenings, both sunny, they were used as markers. Close examination showed that shadows cast by the piles on themselves were the actual markers.
26. Depressions in the general grass sward, probably reflecting depressions in ground level, which were sufficient to cast a shadow were used on 3/16 evenings when the sun was shining. They were uncommon.
27. The head of the calf was often used as a marker. It has been included for completeness, even though the calf was excluded from the experiment area.

Direct sunlight and swarming

All the markers were grouped on the basis of whether or not the sun was shining and then on whether or not the predominant characteristic was a shadow or contrast with the background (table 4.2). Shadows were the dominant marker on sunny evenings but the use of dark objects varied little between sunny and cloudy evenings.

TABLE 4.2 Swarms over shadows and darker areas; +/- direct sunlight.

TYPE OF MARKER	SUN SHINING		TOTAL
	yes	no	
shadow	82	-	82
dark area/lighter	29	27	56
total	131	27	158

Size of swarms

Swarms collected distant from calves (p 105) were sorted into those over shadows from grass clumps and dark grass. They were the only types for which enough swarms were collected to allow a detailed comparison. The mean number of males did not differ between markers (table 4.3) but the variance associated with the size of swarm was greater for those over shadows.

Distance between swarms

The distance between a swarm and its nearest neighbour (fig. 4.VI) within the experimental area was determined (table 4.4). The mean distance between simultaneous swarms was 2.2m although there were objects apparently suitable for markers much closer than the nearest swarm in every case. The mean distance between swarms less than 5.5m from calves (p 124) was significantly lower.

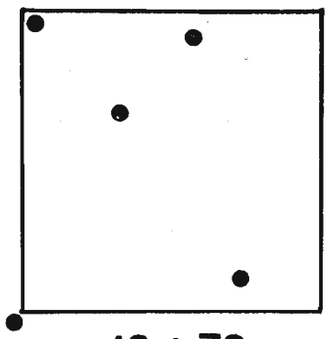
TABLE 4.3 Size of swarms over shadows and dark grass

	shadow	dark grass
number swarms	25	14
total males	648	321
s^2	1968.91	131.30
$s_{\bar{x}}$	8.9	3.1
mean number males	25.9	22.9

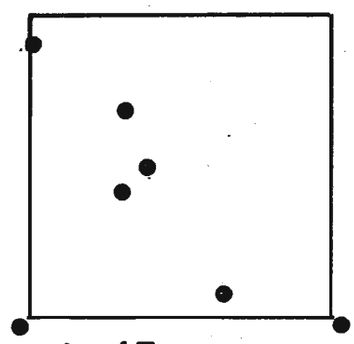
$$s_a^2 \,/\, s_b^2 = 15.0 \; ; \; P < 0.001$$

$$t' = 0.32 \; ; \; NS$$

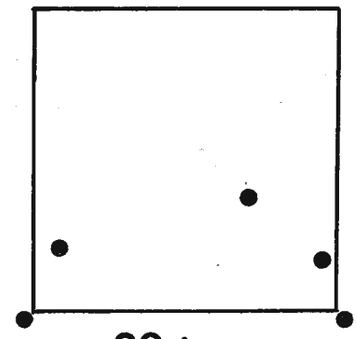
Fig. 4.VI Location of swarms on different evenings; experimental area at Collard.



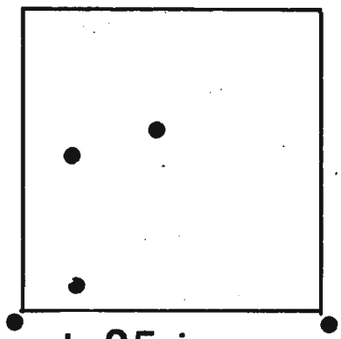
a 10·i·73



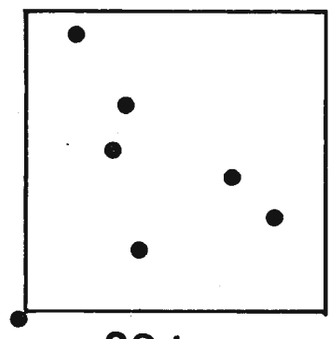
b 15·i·



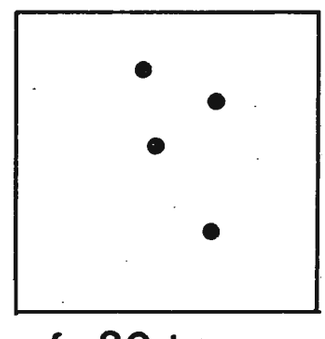
c 23·i·



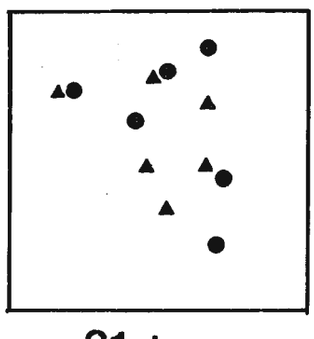
d 25·i·



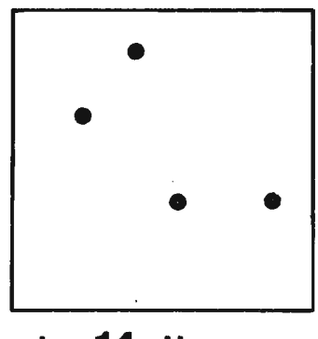
e 26·i·



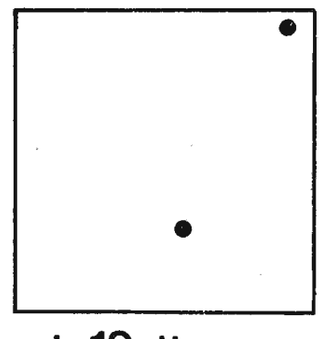
f 30·i··



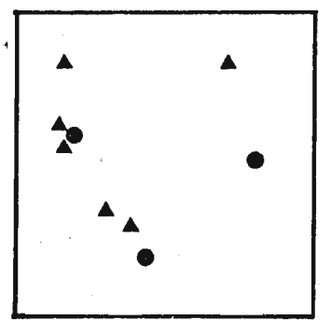
g 31·i·



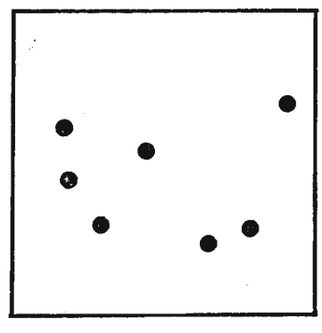
h 14·ii·



i 19·ii·



j 20·ii·



k 23·ii·

● ▲ different times

TABLE 4.4 Distance to nearest swarm

calves distant					calves near	
date (1973)	distance (m)				distance (m)	
10.1	2.9				0.79	0.55
15.1	1.3	0.7	3.4		0.49	0.73
23.1	2.3	2.6			0.91	0.46
25.1	2.2	1.0			0.49	
26.1	2.6	1.0	2.7	1.7		
30.1	2.0	1.9	2.6			
31.1	2.1	1.3	1.6	1.6		
	1.6	1.6	1.6	1.9		
14.2	2.3	3.3	2.0			
19.2	5.7					
20.2	3.6	4.0	2.6	0.9		
	1.9					
23.2	1.6	2.2	2.2	3.6		
total			80.1		4.42	
mean			2.2		0.63	
S^2			0.99		0.03	
$S_{\bar{x}}$			0.17		0.004	

$t'' = 8.8$; $P < 0.001$

$S^2_{distant} / S^2_{near} = 33$; $P < 0.001$.

Markers for swarms containing females

The markers underneath swarms (Collard only) containing females (p 105) were classified as in table 4.1. No swarms were collected above markers of types 15, 21, 23, 24, and 25 which were rare. Females were collected in at least one swarm over each of the other types of markers (table 4.5a). Insufficient swarms were collected above most types to allow comparisons, so the markers were grouped (table 4.5b). There was dependence between the occurence of females and the type of marker, almost all due to the group 'all others combined'. The presence of females was independent of markers 20 and 22 ($\chi^2 = 0.66$; NS) with less than half of the swarms containing females, but the reverse applied to the 'all other markers' group.

TABLE 4.5 Markers under swarms containing females

a.

	Marker	N_t	N_f
16	old dung / short grass	5	2
17	new dung / short grass	1	1
18	shadow of distant object	3	3
19	old pat in deep grass	2	1
20	grass clumps	70	21
22	dark grass / lighter	14	5
26	depression in sward	3	2
27	head of calf	8	5

N_t = total number swarms; N_f = swarms + females

b.

marker	females		
	+	-	total
20	21	49	70
22	5	9	14
all others	14	8	22
total	40	66	106

$\chi^2 = 8.09$; $0.02 > P > 0.01$

Markers after sunset

Swarms occurred after sunset over three markers at Highvale on several occasions and once each over three markers at Collard (table 4.6). Markers 5 and 27 were presumed to function because of the contrast of dark on light ground, contrast which would still be visible after sunset. Markers 3 and 12 were still visible after sunset presumably by their contrast to the background. Continued swarming over 20 and 27 can probably be attributed to some, as yet unknown, effect of cattle. The swarm over marker 27 was level with the head of a cow held in a milking bail, while 20, which before sunset was a shadow, was alongside a calf and persisted for only five minutes.

Centre of Swarm (Optische Marke)

The point directly beneath the centre of swarms at Collard

is shown in figure 4.VII in which sunlight is coming from the right. The markers were allocated to one of the following groups.

(i) Well exposed pats (type 17) (fig 4.VIIa-g).

The pat was a dark object on a lighter background and the swarm was over its sunward edge. The swarm was occasionally over the opposite edge of one pat, but not simultaneously with the sunward edge. Considerable distortion of the pat's shape away from round did not reduce its usefulness as a marker.

(ii) Dung pat surrounded by tall grass (fig. 4.VIIh)

One marker was examined. It was used on successive evenings and each time the swarm was in the same place. The marker, when viewed from above, appeared as a black hole, 0.2m in diameter, in the grass sward.

TABLE 4.6 Markers under swarms after sunset

location	date	type	description
Highvale			
	April 1972	3	living grass, 350mm diam x 300mm high
	Dec 1972	15	weed at edge of concrete
	Dec 1972	5	wet ground
Collard			
	March 1973	27	cow's head
	March 1973	23	dark ground with sparse grass
	April 1973	20	near calf.

(iii) Shadows cast by grass (fig. 4.VII i-q)

In every case the swarm was above the highest point of the marker, which was the centre of the sunward edge of the shadow. Shadows ranged from 60 to 500mm long. Swarms were not observed over shorter or longer shadows but long shadows were rare, and should therefore not be dismissed as unsuitable without further examination.

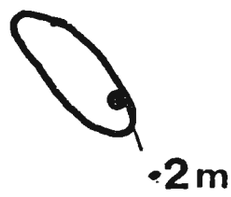
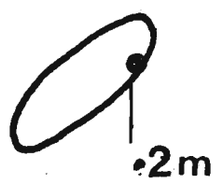
(iv) Areas of dark grass on a light background (fig 4.VII r-u).

The four markers in this group occurred on evenings without full sunlight. They were much larger than other types, ranging from 450 to 900mm diameter. Three of them were used in the absence of directional light, with the swarm above the centre of two, and 1/6 of its diameter from the western edge of the third.

Fig. 4.VII Optische marke

MARKER EXPOSED DUNG PATS

DATE OPTISCHE MARKE

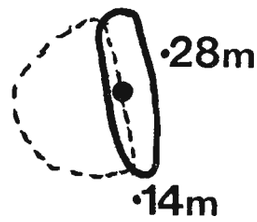
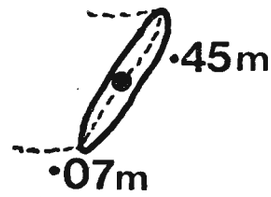
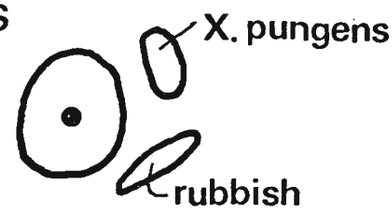
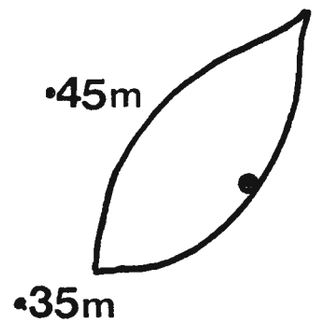
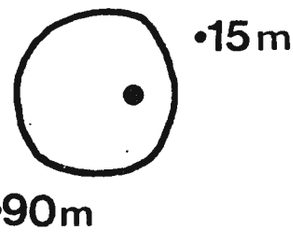
23·i	a		(part)
26·i	b		(full)
26·i	c		(none)
30·i	d		
31·i	e		
31 i	f		
31·i	g		

MARKER DUNG PAT IN TALL GRASS

DATE		OPTISCHE MARKE	SUN
22·ii	h	grass 0·2m above pat	

GRASS CLUMPS CASTING A SHADOW

10·i	i		
26·i	j		
26·i	k	·06m diameter	
26·i	l	sunward edge of shadow	
31·i	m	similar to l	
31·i	n		

MARKER		GRASS CLUMPS CASTING A SHADOW	
DATE		OPTISCHE MARKE	SUN
31•i	o		●
20•ii	p		●
23•ii	q	as for l	●
		DARK GRASS	
15•i	r		○
23•i	s	centre of grass patch	○
30•i	t		◗
19•ii	u		○

Modification of Natural markers

1. A swarm present over marker 3 (fig 4.V) when the grass clump was covered with a net, dispersed immediately giving the impression that each individual broke away and flew off. The swarm did not move away as a whole. A swarm reformed less than ten seconds after the net was removed. The procedure was repeated five times (at five minute intervals) with the same result each time. On two of the occasions a smaller clump of grass 0.6m away was used as a marker, but never when a swarm was present over the larger marker.

2. A clump of grass that had been used on several nights as a swarm marker at Collard was modified. The top of the clump was 150mm above the general sward with four grass spikes and two stems bearing small leaves extended another 150mm above the clump. A similar marker was used as a control with observations of swarming only when swarms were present over the control. On the first day, when there was no direct sunlight, the grass spikes were removed then the stems. Swarming persisted after the first but not the second modification. At sunset and twice more at 10 min intervals a net was swung upwards over the modified marker and the control. A few adults were collected each time over the control but only the last time over the modified marker.

   The next evening the sun was shining. A swarm was present over the modified marker (fig 4.VIIIa) and persisted when quarter of the clump, on the dark side, was removed (fig 4.VIIIb), but when all of the shadowed grass was removed (fig 4.VIIIc) swarming ceased and did not resume during the subsequent two months. The size and shape of the shadow cast was not modified.

3. A large marker (fig 4.VIIId) of dark green grass with some areas of dark dirt (wet), surrounded by dry grass was used when the sun was partially obscured by cloud. A series of reversible and then irreversible modifications were imposed on it. Initially the swarm was above a point half way along the sunny edge of the marker (1, fig 4.VIIId). When a dart dropped from the centre of the swarm was left in place the swarm moved 150mm (2 fig 4.VIIId) and was again marked with a dart left in position. The swarm moved to 3, 150mm on the other side of 1, and when that position was similarly marked the swarm moved to 4, on the opposite side of the marker. It dispersed when

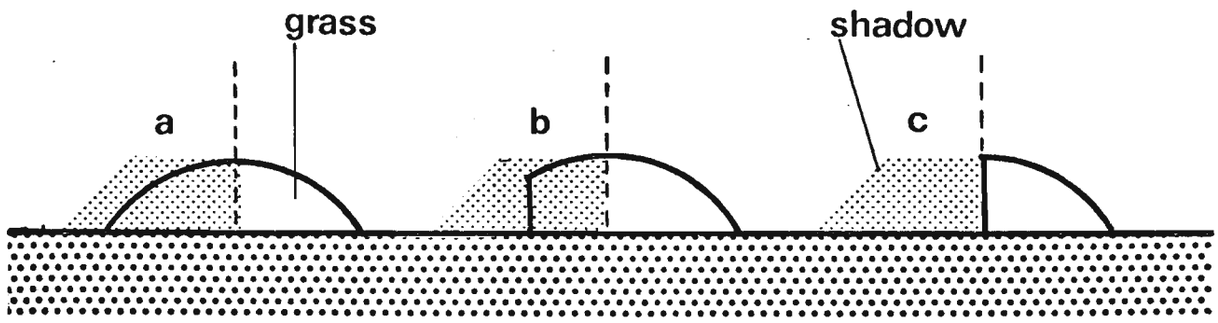
a dart was at position 4. When the darts were removed the swarm reformed at 1. The sequence was repeated twice.
The tallest grass stalks were removed without stopping swarming, but when the marker was covered with a layer of dry grass, making it appear the same as its surroundings, the swarm dispersed, reforming when the grass was removed. Two stalks of dry grass, each 10cm long were placed side by side two cms apart on the marker. When at 4 the swarm continued, but if on the centre or at 1 the swarm was much smaller. The swarm persisted even when all of the green grass within the marker was pruned to below the level of the surrounds, and the marker became predominantly wet dirt; i.e. the marker changed from type 24 to 23 (table 4.1).
The green grass removed from the marker during modification was spread in approximately the shape and size of the original marker over another area of dry grass but a swarm did not form. A shadow was not created by the modification. Swarms formed over the marker on the next night.

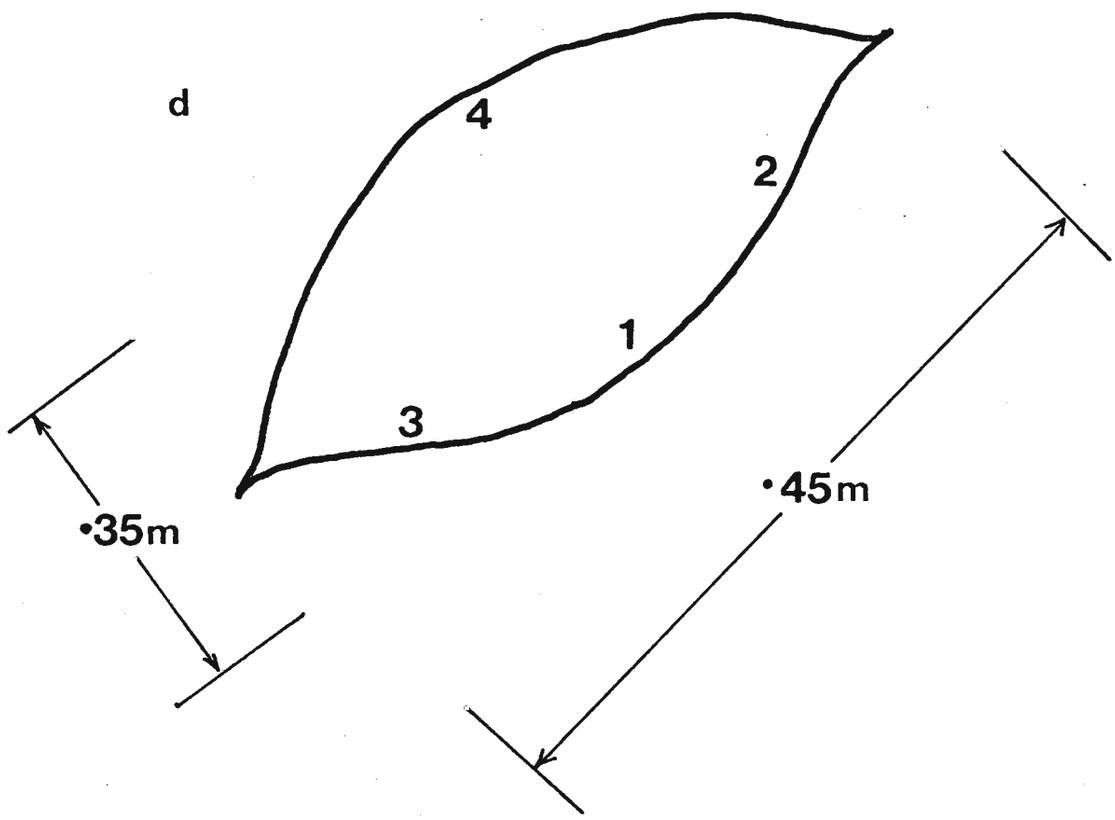
4. The swarm above a marker of type 20 (table 4.1) dispersed when a translucent disc 130mm diameter was placed centrally on the top of the marker and hence over the sunward edge of the shadow which was 300mm long. The same disc, placed in the centre of a pat 370mm diameter, did not interrupt swarming.

5. A swarm dispersed when 4 day old pat, 350mm diameter, under it was covered with dry grass. A smaller swarm formed over the centre of the grass above a shadow.

6. A huge swarm was observed over an area (0.35m wide by 0.60m long) of short grass growing in dark ground which was clearly visible. The swarm dispersed when a net was placed in the centre of the area and reformed when it was removed. The swarm reformed within 10 seconds of removal of the net whether the latter had been present 20 sec or 5 min.

Fig. 4.VIII Modified marker


grass
shadow
a
b
c


d
4
2
1
3
•35m
•45m

Artificial markers.

Artefacts placed in the field are referred to as artificial markers whether or not they were used by C brevitarsis as a locus for swarming. In every case swarms were looked for over these markers only when they were present over natural markers.

Each artificial marker was an attempt to copy one naturally occurring.

(i) Squares of masonite (a mid-brown composition board 4mm thick) of side length 100, 200, and 300mm were painted black or left their natural colour. They were placed flat on the ground in the experimental area at Highvale. Swarms did not form.

(ii) On two successive evenings a series of cloths of different colours (blue and brown check, plain royal blue, and maroon tartan folded into a square (side 150mm) and two triangles (side 150mm or 450mm - equilateral) were dropped onto the mown grass of the experimental area (Collard) and onto a sheet of yellow foam (1.2m x 1.8m). A black umbrella, a dark green cloth 400mm square, a yellow cloth 150mm square, and a light green, deep pile mat 350mm x 480mm were dropped on the experimental area. No swarms formed.

(iii) Pieces (300mm x 60mm) of ply (4mm thick), 2 pieces each colour, painted various colours (red, black, green, blue, and mottled black and green) were put on the ground at Collard with a 20mm square post 300mm high at the centre of the sunny edge of one board of each colour. A swarm formed over the red board without the post on a sunny evening but not on the next (clouded). A shadow formed over the shadow of the post on the black board on the first but not the second evening, and over another black board next to a peg but on the second evening only. The swarms were small, unstable, and present for only very short periods. No other swarms formed. The black and red boards were left out on subsequent evenings but swarms did not form.

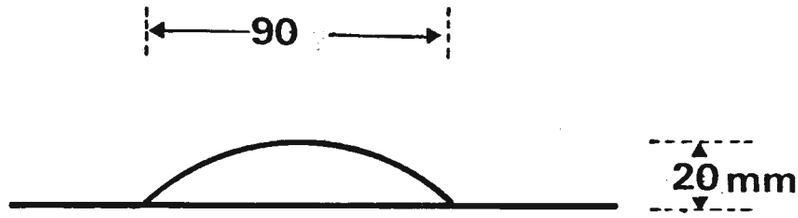
4. Single leaves of noogoora burr were placed on the experimental area at Collard. They were lighter than the grass. Swarms did not form.

5. Boards (4mm thick) were cut to simulate cross sections through grass clumps. They were stood on one edge and arranged at right angles to the direction of the sun, being held upright with a small peg. They case shadows similar to those from grass clumps and other artifacts. The various shapes and sizes were:-

(i) Edge of a circle (600mm diameter), maximum height 200mm (fig. 4.IXa) stood on its straight edge (900mm long). It was white on one side, mid-brown on the other. The length of the shadow increased as the sun declined. Swarms occurred regularly at position 1, rarely at

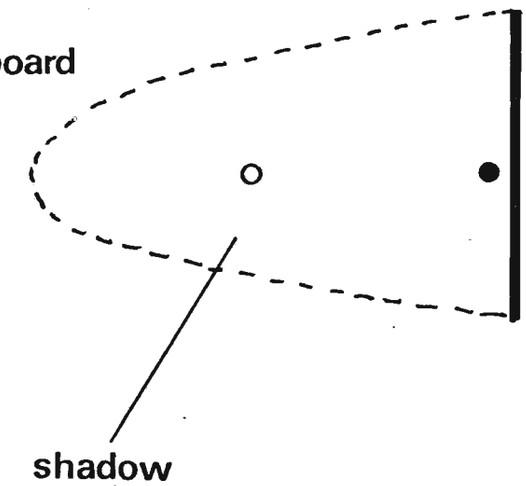
Fig. 4.IX Artificial marker.

(a)

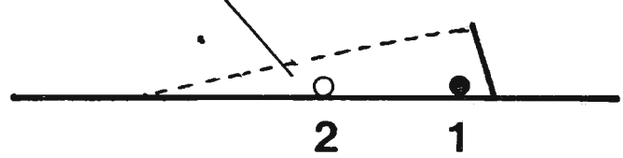


(b)

distance from board
- ● 90 mm
- ○ 330 mm



(c)



1,2,3 centre of swarms

(d)

position 2 (fig. 4.IXb,c), but not simultaneously. The first swarm was observed when the shadow was 1.2m long. Swarms occurred at both heights. When the marker was moved to another area of the grass sward, the swarm dispersed. Another formed at the new location and did so on four more occasions. The board was left in the field and on every night with direct sunlight a swarm formed.

Sometimes, during the five minutes preceding sunset, the swarm moved to position 3 (figure 4.IXd), 30mm sunward of the board, but only when the top of the board tilted slightly away from the sun. Swarms formed irrespective of which side of the board faced the sun.

(ii) A board 90mm high was cut from a circle of the same diameter as (i). Swarms formed in the centre (90mm behind the board) at low level only. The swarm was first observed when the shadow was 0.6m long.

(iii) A board 0.15m high was cut from the edge of a circle 0.30m in diameter. Its shadow was 1.2m long when adults started to swarm over it. The position of the swarm, which formed at low level only, was equivalent to position 1 of figure 4.Xc.

(iv) A rectangular board 200mm high and 450mm long was arranged in the same fashion as the board in 5(i). The first swarm occurred when its shadow was 1.4m long. A huge swarm, at high level formed in a position equivalent to 1 on fig. 4.IXc.

(v) A board was cut from a circle of diameter 300mm but only 60mm high in the centre. Swarms did not form.

6. Several tents (figure 4.Xa) were constructed from ply 4mm thick. They were arranged with their long axis normal to the direction of the sun.

(i) h=100mm l=380mm (fig. 4Xb). Sunward side grey, shaded side black. Adults swarmed in position 1, 90mm behind the top of the marker, at its middle. The swarm was at high level.

(ii) As in 6(i) but with the sides reversed so that the sunny side was flat black. The swarm formed in exactly the same position.

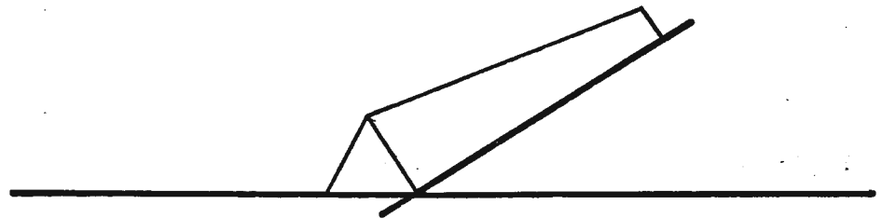
(iii) The same tent as in 6(i) but with its long axis parallel to the direction of the sun. No swarm formed.

(iv) Height 150mm, length 200mm both sides highly reflective grey. Swarms formed at position 1 and moved up and down from low to high level.

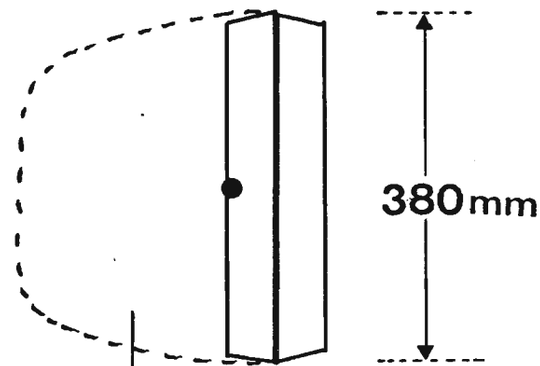
7. A board 230mm wide and 380mm long was painted flat black but 22mm diameter holes at 60mm centres removed 7.5% of the surface. It was arranged as in figure 4.IXa, to case a shadow mottled by patches of light. A swarm formed at position 1.

Fig. 4.X Artificial marker

(a)



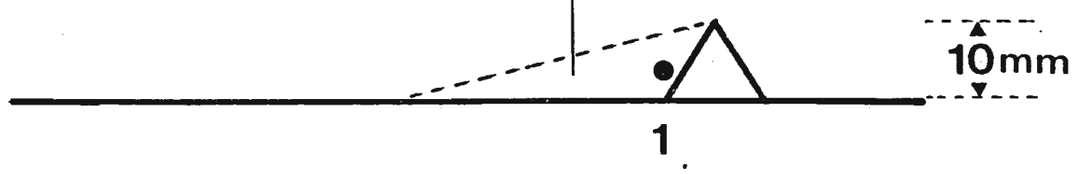
(b)



● swarm

shadow

(c)



1

8. A board 380mm x 130mm was laid flat on the ground, longest side parallel to the sun's rays. Its upper face was painted flat black and bowed upwards in the centre of the long side causing a shadow on the darkward half of the board. Adults swarmed at low level 90mm behind the sunward edge of the shadow.

Swarm location and wind direction.

The direction of the wind varied little on the evenings when observations were made. The exact location of the swarm was recorded for an artificial marker (5(i)) with winds from the south east (10 evenings), the south (2 evenings) and the northeast (2 evenings). In every case (fig. 4XI) the swarm occurred at position 1. Wind from other directions was recorded but in all cases its speed was too great for swarming.

DISCUSSION

The difference between the surface area of the two experimental sites (Highvale 221m^2, Collard 54m^2) is of no significance in this context as no attempt has been made to compare densities of swarms between the sites.

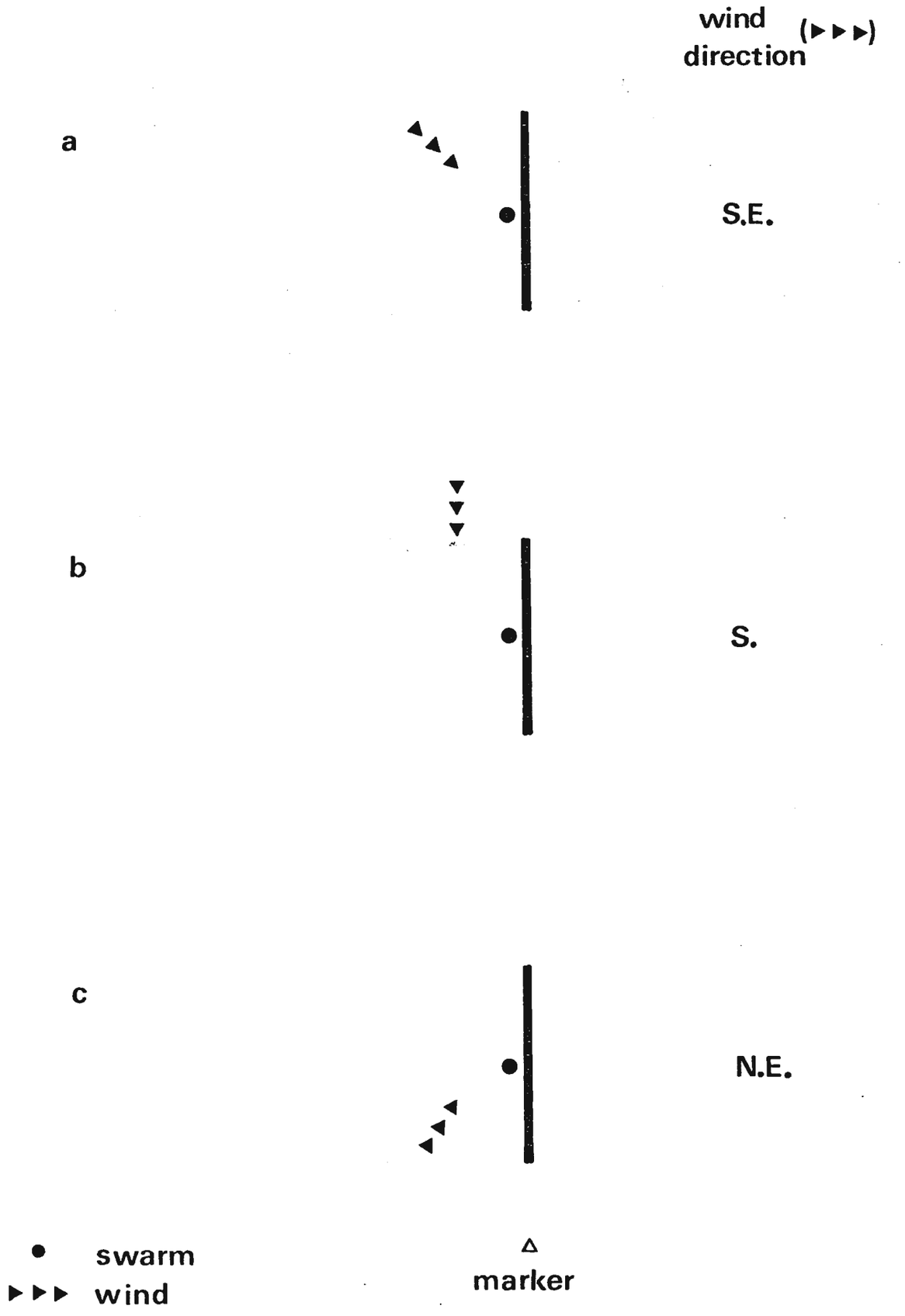
At Highvale the ground was bare and compacted. Very few swarm markers were present but, especially while the cattle were in the yard, many adults were flying. The situation at Collard differed in that many markers were present while cattle were not and the area was always grassed. The two areas are therefore complementary.

Markers were sorted into types according to locality (table 4.1), then regrouped ignoring locality, listed in descending order of occurrence (table 4.7), and assigned a new symbol.

Shadows, and in their absence dark areas on lighter backgrounds, are the dominant markers. In direct sunlight 63% of swarms (82 swarms) were over shadows (table 4.7) and 22% (29 swarms) over dark objects. The remaining 15% (19 swarms) were over markers that fitted neither pattern. When direct sunlight was absent, 71% (27 swarms) were above dark objects, the remainder (19 swarms) were not.

The markers under thirty-one swarms (N_u , 19+12; table 4.7) were neither shadows nor dark objects. Old cow dung (line ii) at Highvale accounted for 16 of them. The pats were flattened to sheets less than 10mm thick lying on the ground and covered with a layer of dust. They were recognised by their shape and very slight contrast with the ground. Any shadow cast was very small. C brevitarsis

Fig. 4.XI Swarm location and wind direction.


wind direction (▶▶▶)
a
S.E.
b
S.
c
N.E.
● swarm
▶▶▶ wind
△ marker

could have seen them either by the slight contrast or the very small shadow. The apparent paucity of markers and the large numbers of adults in the area may have forced swarming over such indistinct objects.

TABLE 4.7 Condensed list of markers

			sun shining				sun not		
line	types	marker	N_+	N_s	N_d	N_u	N_-	N_d	N_u
i	3,14,20	grass clumps	41	41	0	0	5	1	4
ii	1,16	old dung	15	0	0	15	2	1	1
iii	5,15,23	dark ground	12	0	12	0	5	5	0
iv	12,21	tall grass	10	10	0	0	7	0	7
v	7,17	new dung	13	0	13	0	3	3	0
vi	22	dark grass	0	0	0	0	10	10	0
vii	3,19	dung in grass	5	5	0	0	3	3	0
viii	11,18	shadows	10	10	0	0	0	0	0
ix	25,4	dead/ live grass	6	6	0	0	0	0	0
x	26	depression	6	6	0	0	0	0	0
xi	24	live grass	2	0	2	0	3	3	0
xii	6	nothing	4	0	0	4	0	0	0
xiii	10	animal	2	0	2	0	1	1	0
xiv	2	stone	2	2	0	0	0	0	0
xv	8	wood	1	1	0	0	0	0	0
xvi	9	old hat	1	1	0	0	0	0	0
	total		130	82	29	19	39	27	12
	%		100	63	22	15	100	71	29

N_+ total markers when sun shining; N_- total sun not shining
N_s number casting shadows; N_d dark objects; N_u unexplained

Tall grass (line iv) accounted for another seven of the unexplained markers. All seven were Noogoora burr plants at Collard. Their leaves were lighter than the background. Swarms would not form over leaves laid on the sward. Even without direct sunlight the area under the plants is shaded and hence darker than its surroundings. This would present, from above, an area of dark (ground) land and light (leaves) contrasts that may have been the marker.

On clouded evenings (line i) adults formed four swarms over grass clumps that were not casting shadows. Leaves at the top of clumps of

grass are further apart than those at the surface of the sward, allowing more of the shadowed area of the leaves to be visible. The clump appears slightly darker than its surroundings. The difference is small. It seems unlikely that adults at least 300mm above the sward could perceive a variation of as little as 20mm in height at the level of the sward, the only other obvious difference between the swarm marker and its surroundings.

The four swarms, beneath which a marker could not be distinguished (line xii) were at Highvale. The lack of swarm markers at Highvale may have been responsible.

Swarms formed over the top of animals (line xiii) at both sites. The animal being used as a marker cannot easily be defined as either a shadow or a dark object; it is both in almost every case. Shadows are formed on the animal and on the ground by the animal. All of the cattle were darker than the ground and grass. They were markers whether or not the sun was shining and their presence caused major disturbances to the distribution as well as the shape of swarms.

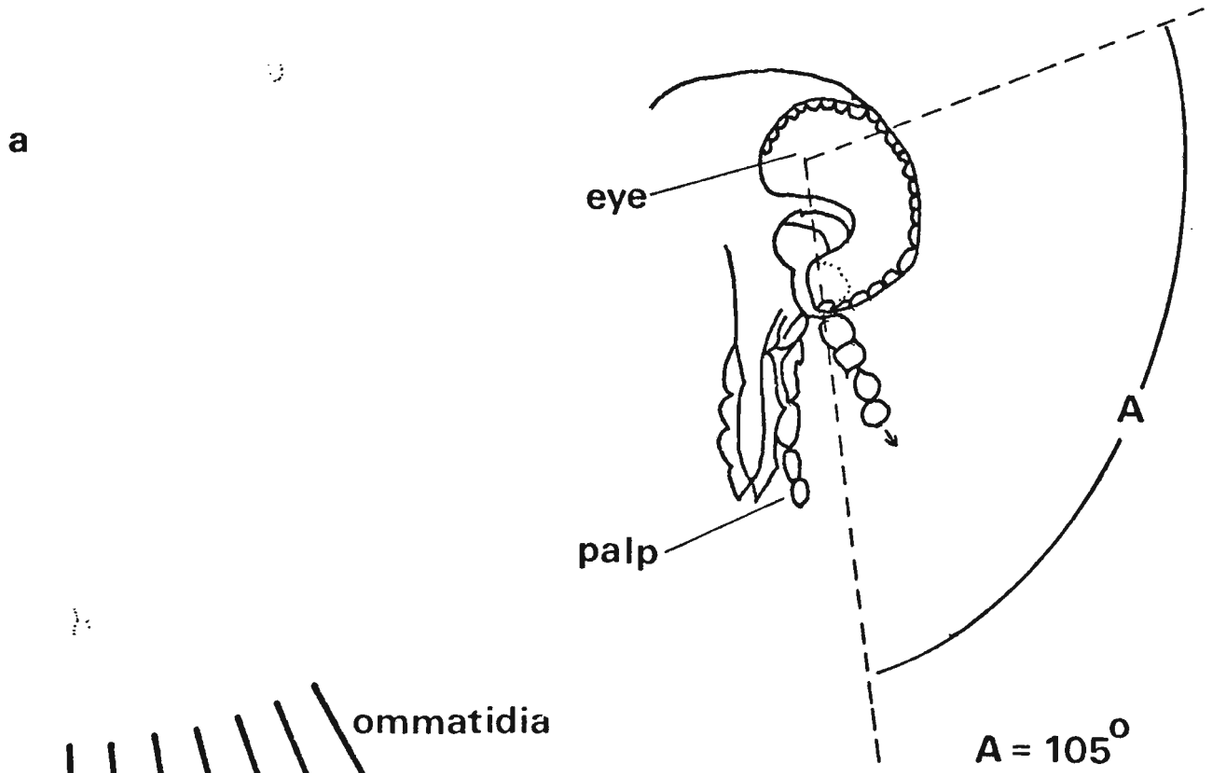
A dart dropped from the centre of a swarm locates the position vertically beneath it, but that position is not necessarily the 'optische marke'. Downes (1958) showed that swarms of Aedes hexodontus leant windward from the marker, hence a dart dropped from the middle of the swarm would land windward of the 'optische marke'. When a dart was dropped from the centre of the swarm and left in place the swarm moved 150mm away to a different part of the marker. When a white disc was placed over the region (vertically beneath a swarm formed over a clump of grass casting a shadow) the swarm dispersed, but when the same disc was placed centrally over a pat but not beneath the centre of the swarm the swarm persisted. Interference with the optische marke should cause dispersal of the swarm while interference near it would not necessarily do so. Swarms did not lean in any direction. It is highly likely that the optische marke is directly below the centre of the swarm.

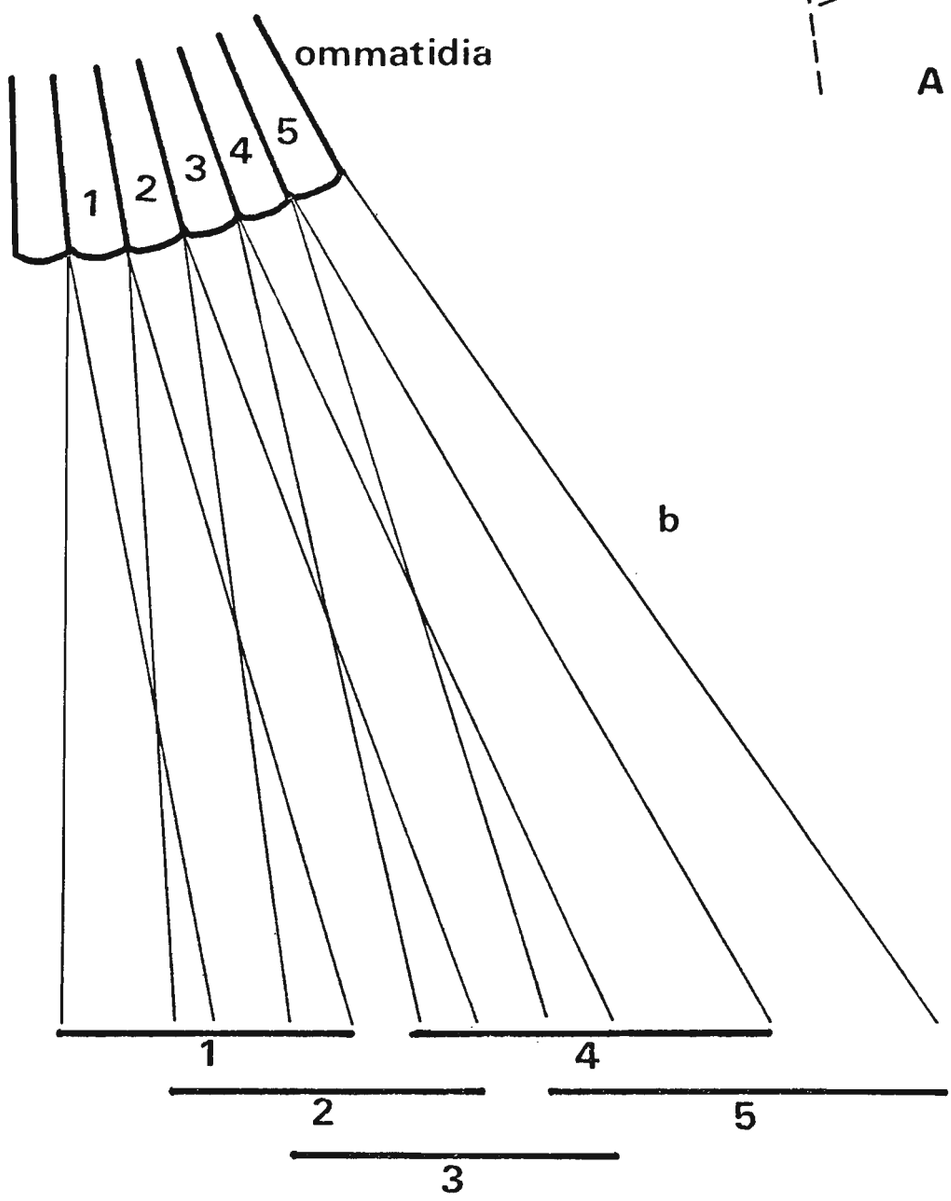
The optische marke was very close to or at the sunward boundary of the dark area (whether dung pat or shadow) when the sun was shining (fig. 4VIII). Where the marker was a round object (e.g. dung pats), the optische marke was at the point nearest the sun. On objects elongated across the direction of the sun, it was always in the centre of the long side. The optische marke was on different sides of the grass clump (fig. 4.VII k and o) but in both cases it was at the sunward edge of the shadow. Therefore the optische marke was not centre of the clump of grass.

Swarms persisted over modified markers provided that neither their shadow nor their colour contrast with surroundings were altered. Tall grass stems overhanging the marker were not essential to swarm formation nor was any of the grass on one marker (fig. 4 VIIId). The modifications imposed on one marker (fig. 4.VIIId) produced interesting results. The swarm initially formed in the centre of the long side nearest the sun even though the marker was angled obliquely to the sun. When darts interfered with the centre of the optische marke the swarm relocated to central between the dart and one end of the marker. The swarm was over the centre of the optische marke of all of the artificial markers. The method of control that keeps the swarm central along the length of the optische marke is not immediately apparent. In frontal view (fig. 6 XIIa) the eye of an adult of C brevitarsis has a field of view from one eye (as measured by the angle a) of at least 105^{o} An adult flying 600mm above the sward needs only 22^{o} to view the whole of the marker in figure 4.VIIId. The head's angle of inclination during swarming flight is unknown but if it is inclined at 45^{o}, proboscis backwards, the field of view could be reduced below 105^{o} but is unlikely to be reduced to less than 22^{o}. The field of view estimated for C brevitarsis is of the same order as that for Culex molestus and Anopheles maculipennis atroparvus, 112.5^{o} of arc in the horizontal plane for each eye (Rao, 1947)

On the basis of the mosaic theory of vision, initially proposed by Muller in 1826, it is possible to show that the central part of the marker could appear darkest. If each ommatidium occupies 5^{o} of arc of the compound eye (Dethier (1968 - p.204) suggested the angle varied from 1^{o} to 4^{o} at various parts of the eye) and the angle of view (fig. 4.XIIb) exceeds the ommatidial angle, causing the field of view of adjacent ommatidia to overlap (Dethier (1968) pp.201, 204), a series of ommatidia view overlapping portions of the optische marke. Ommatidia one and two do not see the end of the optische marke, 3 just sees it, in 4 the end is half way across and in 5 just at the beginning of the field of view. Fig. 4.XIIb shows the right half of the optische marke only and considers an adult flying so that the ommatidia sketched are in the same plane as the long axis of the optische marke. The image of the optische marke composed by the compound eye from single ommatidia viewing overlapping portions could therefore appear darkest in the centre. If the angle of view proposed for each ommatidium is increased relative to the ommatidial angle, the darkest zone (centre) of the optische marke becomes narrower. Burtt and Catton (1954) measured the visual angle (field of view) of each ommatidium in the

Fig. 4.XII Eye of C brevitarsis; frontal view





'optische marke'

locust as 20^{o} and the angle subtended between adjacent ommatidia as 2.4^{o}. Using those two values the effect would become very pronounced.

Undoubtedly the assumptions, especially with regard to the angle subtended by and the angle of view of each ommatidium, and equating a section of the compound eye with a circle, are simplifications of reality. The proposed effect would be non-existent only if the angle of view was equal to or less than the ommatidial angle i.e. the field of view of adjacent ommatidia did not overlap. The observations of Burtt and Catton (1954) and the review of other results included in Dethier (1963 pp.196-207) suggest that in many species, overlapping does occur.

On the basis of the above theory the swarm should be in the middle of the optische marke regardless of fairly wide variation in its length. This is the case in the field.

An alternative hypothesis can be advanced. If adults in the swarm turn away from the end of the optische marke, they would be distant from the ends most of the time. One could expect to observe occasional adults near the ends of the marker and a broader swarm over a wider marker. Neither has been.

Two broad groups of swarm markers have been observed in other insects; protruding upright objects such as trees (e.g. Forcipomyia culipes, Downes 1955), and patterns of lighting on a horizontal surface, usually the ground (Chiang, 1961). The second type can be sorted into dark objects on a light background or vice-versa. A dark object is used by Aedes punctor (Frohne 1953), Uranotaenia alboabdominalis and Mansonioides fuscopennata (Corbet 1964), C nubeculosus and C halophilus (Downes 1958). Light objects were used by C riethi (Downes 1955), Anarete pritchardi (Chiang 1961 as Anarete near felti Pritchard; Anarete pritchardi (Downes 1969))and Anatyolynia algena (Chiang 1961). Contrast between an object and its background was used by Simulium venustum (Peterson 1962) and by Mansonia perturbans (Nielsen 1964) but in neither case did description of the marker allow a decision on whether adults were swarming over dark or light objects.

C brevitarsis swarms over dark objects on a lighter background, a conclusion supported by the examination of individual swarm markers, by the exact location of the optische marke, and by the modification of markers. The postulate is an adequate explanation for evenings without directional light but not those when the sun is shining because of inability to explain the rare use of dark objects such as boards of various colours, the very low utilisation of dung pats which provide strong contrast with the background, and the rare use of a black board but the use of a shadow on the same board. Almost every object above

which swarms formed was elevated, sometimes only slightly, above the surrounding surface. That adults perceive elevation per se must be discounted by the occurrence of swarms over shadows cast by objects up to 4m away. To postulate that the swarm is formed in a position defined jointly by an object on the ground and a distant object elevated above the ground is unnecessary and is disallowed by the occurrence of vast numbers of swarms well away (30m and more) from the nearest vertical object.

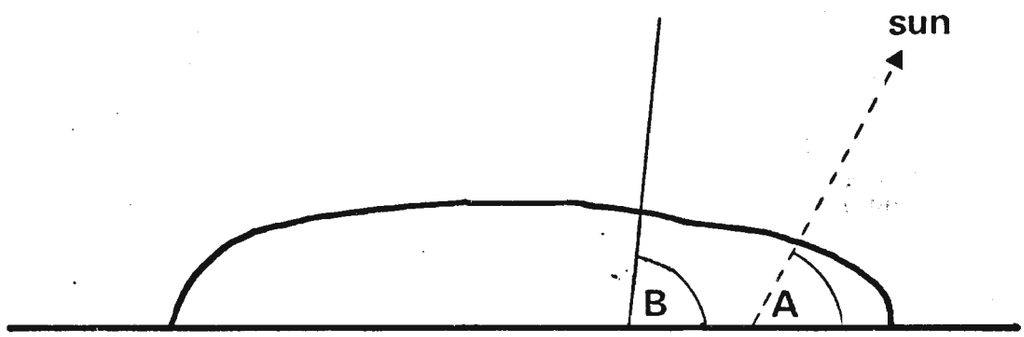
A characteristic of all swarm markers is that they are areas from which very little light is reflected. Dung pats are not areas of low reflectance when the sun is shining nor are boards painted various colours but a shadow cast on a board is. All the markers described can be explained if C brevitarsis swarms over the sunward edge of areas where reflected light is much less intense than the surroundings. Insects in general are able to discriminate objects on the basis of the intensity of reflected light (Imms 1960, p 196) The postulate accommodates the observations that the colour of the background i.e. light coloured dirt, green grass and black boards, is unimportant provided that it reflects more light than the marker. It is still not easy to accommodate occasional swarming over dung pats although it is possible that the sunny edge of a pat has low reflectance because of its orientation (figure 4.XIII). It is highly likely that the optische marke is a line of great difference in reflected light that probably causes flicker response in the males of the swarm.

Blickle (1959) and Downes (1955), with Tabanus bishoppi and Culicoides nubeculosus respectively, observed that the adults always faced into wind. When the wind was very slow (Blickle 1959) the adults faced in any direction. When it was very strong the adults flew lower and eventually settled on or near the marker (Downes 1955). Downes (1956) proposed two patterns of flight in relation to the marker. Below 0.2ms^{-1} adults flew around the marker in either direction, but above that speed they faced into the wind and flew upwind to the optische marke then allowed themselves to drift back (Downes 1956).

Adults of C brevitarsis are so small that they can only be seen with difficulty in swarms but observations suggest that they fly at random.

Downes proposal requires that the location of the swarm can move around the marker with its exact position at any time defined by the direction of the wind. Such movement is not possible when the sunward edge of the shadow is the optische marke because the shadow-line does not alter with changes in direction of the wind.

Fig. 4.XIII Dung pat showing reflection of light from edges.



A › B light reflected to ground

B › A light reflected to sky

To use this optische marke adults must either face the sun or fly in circles. They cannot fly upwind. Swarms do not alter position on the marker with changes in wind direction and do not fly lower and settle on or near the marker as wind spead increases, both of which support the belief that they do not fly upwind. On the basis of the hypothesis that the centre of the optische marke is darkest, swarms should occur over the centre of the whole object when directional light is entirely absent. They did so (fig. 4.VII r and s).

The swarm of C brevitarsis probably maintains its position over the centre of the optische marke in stronger wind by a temporary exaggeration of the flight patterns which normally locate it there, the changes in the wind speed being perceived by each adult by its own displacement relative to the centre of the optische marke. Downes (1969) proposed huge evolutionary changes in swarming behaviour (mostly towards truncation of the process) throughout the Diptera, compared to which the change from orientation to wind to orientation to sun is not massive. Anarete pritchardi (Chiang, 1961) orients towards the sun but at wind speeds near the upper limit for swarming it orients towards the wind and settles on the marker. The behaviour of C brevitarsis and A pritchardi are similar and yet sufficiently different to suggest that they may have evolved separately from the more common pattern suggested by Downes (1969)

Two other conclusions may be made. The data accumulated by using artificial markers which cast shadows suggest that the size of the shadow may not be critical, and there was no apparent difference between the markers below swarms which did or did not contain females.

At Highvale every suitable object within the experimental area was being used as a marker. This may account, as has already been suggested, for adults swarming over objects which do not appear to be good markers e.g. a stone, a piece of wood, a hat, and old flattened cow dung. In this particular situation swarm markers are rare objects (Downes, 1969). The experimental area at Collard was completely different, having very many markers. Adults appeared to move between swarms and whole swarms tended to break up and reform over adjacent markers, the sort of behaviour described for C pallidicornis where markers were more numerous than swarms (Downes 1955)

IV.3 SWARM SIZE

INTRODUCTION

Other workers have collected swarms to look for females, to determine the identity of the species swarming, and to determine the ratio of females to males. Females are usually rare in swarms (p 111) which are usually monospecific. While information is available on the proportion of females in swarms, the proportion of swarms that contain females is unknown. It has been assumed that mating occurs in swarms and the low incidence of females satisfactorily explained (p 60). However with mating in swarms females should occur in a fair proportion of swarms but at very low level in each.

A series of artificial markers were developed (p 89) on the basis of presence of swarming. The swarms above close imitations of natural markers should be of similar size to natural markers.

MATERIALS AND METHODS

Swarms were collected at Collard whenever time was available with at least 15 on any one evening for a total of 122. The height (as low or high), marker, and the time of collection of each swarm were recorded. The swarms were collected by swinging a net,fitted at its apex with a removable container, vertically upwards from ground level to 2.5m through the swarm. Each swarm was stored separately, killed before removal from the container, and stored in 70% alcohol, sexed and counted.

The swarms were sorted into one of four groups depending on their marker.

Group	Markers
i	Two artificial markers left in the field (fig.4.IX & X.)
ii	Shadows cast by grass, dung, wet ground and any other natural marker more than 20m from the nearest animal.
iii	Similar markers to (ii) but within 5m of a calf.
iv	Over calf.

RESULTS

Numbers of Males and Females

C brevitarsis was the only species collected. The mean number of males in the swarms over (i) the artificial markers (26.9) and

(ii) natural markers away from cattle (29.6), were not significantly different (Table 4.8), but increased significantly (85.9) in swarms over markers near the calf (iii). Over the calf (iv) the mean number of males (41.8) did not differ from the number over other markers. The variance of the number of males in swarms was least over the artificial markers. The variance estimates for (ii) and (iii) are significantly different but the coefficient of variation for each are similar (119.3 cf 96.4), suggesting that the degree of variability about the mean is similar.

The mean number of males for all swarms was 49.7. Females comprised 1.3% of all adults but were present in 42 (34.7%) of the swarms.

Single adults performed swarming flight over markers, especially during the period just before the first swarms formed. They did not stay at the swarming site for very long.

TABLE 4.8 Numbers of males and females in swarms.

	marker (i)	(ii)	(iii)	(iv)	sum
no. swarms collected	10	60	43	8	121
no. + females	1	16	20	5	42
= females/total (5)	10.0	26.7	46.5	62.5	34.7
no. adults	270	1796	3694	334	6094
no. females	1	19	52	6	78
mean females	0.1	0.3	1.2	0.8	0.64
no. males	369	1774	3642	328	6016
mean males	26.9	29.6	84.7	41.0	49.7
mean all adults	27.0	29.9	85.9	41.8	50.3
variance total number	184.5	1246.0	6663.4	3672.0	
$S_{\bar{x}}$	4.3	4.6	12.4	21.4	
C.V.	50.5	119.3	96.4	147.8	
regression of size of swarm (y) on time (x)					
b	0.30	-0.65	0.04	1.37	
S_b	0·48	0·38	0·98	2·54	
number of swarms at two heights.[1]					
high level	9	42	4	0	
low level	1	16	39	8	

mean size of swarms

comparison	variances F	P	means t	P
a v b	6.8	< 0.001	0.42	NS
b v c	5.3	< 0.001	4.15	< 0.001
c v d	1.8	NS	1.44	NS
b v d	2.9	0.05-0.01	0.52	NS
a b d	19.8	< 0.001	0.65	NS

comparison swarm heights	χ^2	P
all markers	54.2	< 0.001
ab v cd	50.21	< 0.001

1. 2 swarms near plough deleted from marker (ii)

Time (minutes before sunset) and swarm size.

Within each group regression analysis was carried out between the number of adults (y) and the minutes before sunset (x) when that swarm was caught (fig. 4.XIV). The slope of the regression was not significant in any group (table 4.8).

Swarm height.

Swarms over the artificial markers (i) and those distant from cattle (ii) were predominantly at the high level, while near (iii) and over (iv) the calf they were at low level (χ^2 ab v cd = 50.2; table 4.8). The difference between a and b and also between c and d was not significant.

In the swarms over natural markers away from cattle the mean number of males did not differ between high (30.2) and low (27.8) swarms (table 4.9). The variance, however, was greater in high swarms as was the coefficient of variation. The difference reflects the greater range of numbers in the high (1-223) than the low(5-70) swarms.

Females were present in 25% of the swarms over natural markers away from cattle (24% of high and 31% of low swarms) (table 4.9). The number of swarms containing females did not vary between heights (χ^2 = 0.06).

One or more females were found in 46.5% of the swarms (marker (c)) near calves but only 26.7% away from calves (marker (b)) (table 4.8). The differences, when tested by χ^2 on a contingency table formed by the numbers of swarms with and without females, were not significant. However, if swarms above all markers were compared the difference became significant (χ^2 = 6.0 ; 0.02 P 0.01). The proportion of swarms containing females was greater near and over cattle, but the proportion of females in swarms was independent of the proximity of cattle (χ^2 = 0.89 ; for 2x2 table generated from number of males and females in swarms near and distant from cattle: table 4.8).

Fig. 4.XIV Size of swarms and time to sunset

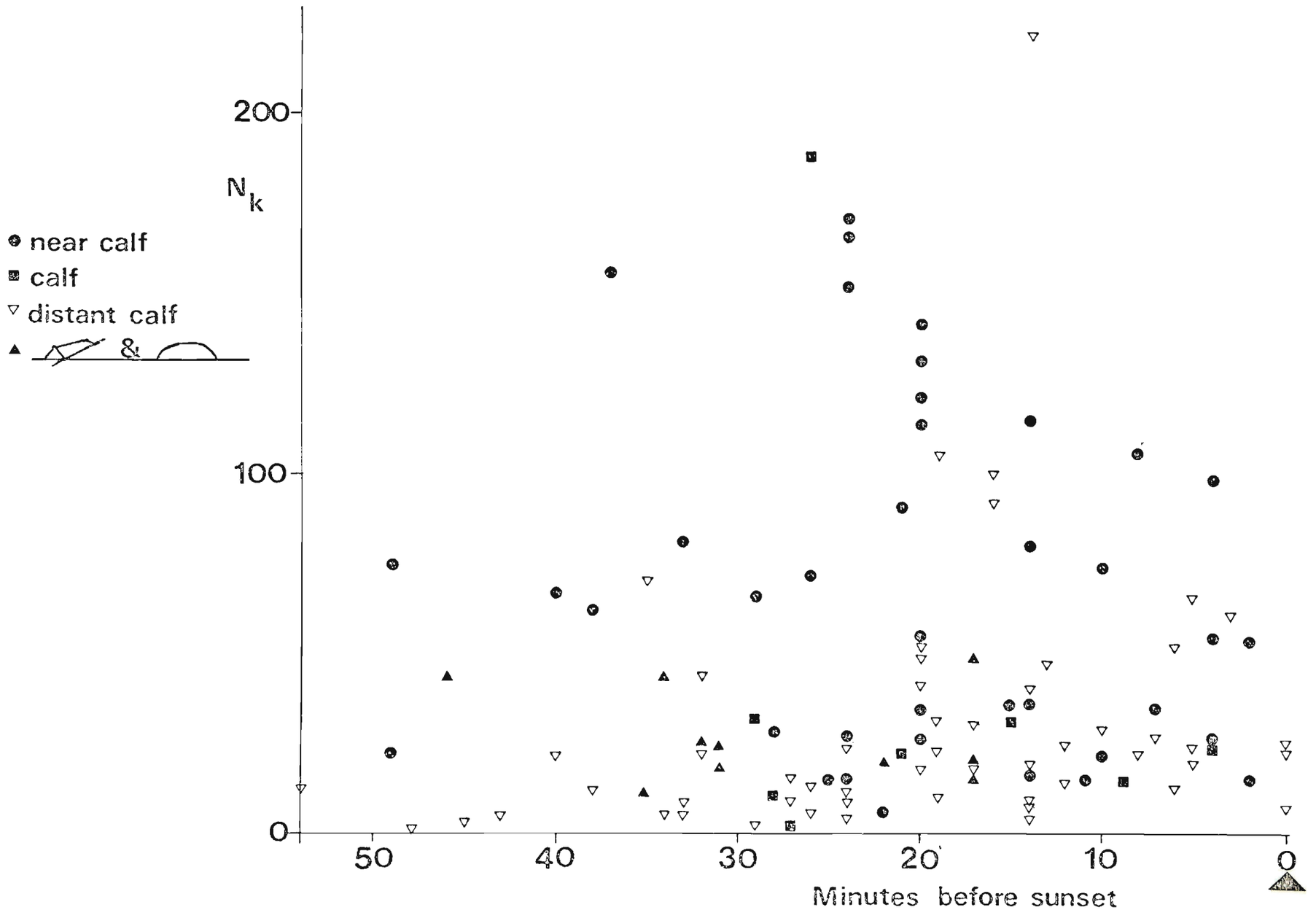

near calf
calf
distant calf
&
200
N_k
100
0
50
40
30
20
10
0
Minutes before sunset

TABLE 4.9 Size and sexual composition of swarms

	height	
	high	low
number swarms	42	16
number males	1270	444
mean number males	30.2	27.8
S^2	1557.1	475.4
CV	130.7	78.4

comparison of variances F = 3.3 P < 0.01

means t' = 0.3 NS

Number of swarms			total
+ females	10	5	15
- females	32	11	43
total	42	16	58

$\chi^2 = 0.06$ NS

Occurrence of females in swarms

Swarms containing one female were infrequent (24% of all swarms) (table 4.10), those containing more than 1 female even fewer (11%). Only 5% contained 3 or more females, but one swarm contained 18. Its occurrence has not been explained.

Swarms were assigned to one of five groups (table 4.10) by their number of males. The number of swarms with females was dependent on the size of the swarm, but the dependence was almost entirely confined to swarms of less than 10 males ($\chi^2 = 7.2$; $0.01 > P > 0.001$ for comparison of 0-9 with rest; table 4.10) in which females were disproportionately rare.

Distribution of females between swarms

Averaged over all swarms there were 0.01295 females for every male. If there is direct proportionality between the number of males and females in a swarm, the number of females in any one swarm would be 0.01295 x the number of males. The number of females thus predicted rarely exceed 1 per swarm, but if predicted and observed numbers of females are to be compared by χ^2 the expected values must exceed 5, which will only occur if the swarm contains more than 387 males. Therefore swarms were pooled in the order in which they were collected until the pool contained more than 387 males. The

expected and observed numbers of females were significantly different (table 4.12), therefore the hypothesis that the number of females in a swarm was directly proportional to its number of males was rejected.

TABLE 4.10 Number of females in swarms grouped on size

number of	number of females						females #	
males	0	1	2	3	4	18	-	+
0-9	18	1	0	0	0	0	18	1
10-19	19	5	2	0	0	0	19	7
20-49	23	11	5	0	1	0	23	17
50-99	10	7	1	0	1	0	10	9
99	9	5	0	1	1	1	9	8
total	79	29	8	1	3	1		
%	65	24	7	1	2	1		

\# χ^2 (all cells) = 11.5 0.05 >P >0.02

(0-9 v rest) = 7.2 0.01 >P >0.02

(within rest) = 2.7 NS

Swarms were regrouped on their number of females (0,1,2, or more than 2). The mean number of males did not differ between the groups (table 4.11).

TABLE 4.11 Number of males in swarms grouped on count of females ; analysis and means

source	DF	MS	VR	P
group	3	7729	2.1	NS
residual	117	3651		

means

number of swarms	number of females	mean number of males
79	0	44.2
29	1	55.7
8	2	40.7
5	> 2	106.2

TABLE 4.12 Grouped swarms.

number males	number females	
	predicted	observed
488	6.3	1
405	5.2	18
470	6.1	2
416	5.4	5
406	5.3	7
431	5.6	4
470	6.1	5
387	5.0	0
417	5.5	1
429	5.6	12
433	5.6	7
390	5.0	9
487	6.3	2
396	5.1	4

$\chi^2 = 31.4$ $P < 0.001$

The frequency distribution of swarms containing various numbers of females (in table 4.10) appeared similar to a Poisson distribution and was compared with the theoretical Poisson generated using the mean number of females (0.6393 per swarm) as μ for the distribution. The expected and observed distribution were significantly different ($\chi^2 = 10.3$; $0.05 > P > 0.02$). When the one swarm of 18 females was excluded because it grossly elevated the estimate of μ ($\mu = 0.4959$ when swarm excluded), the expected and observed distributions were in close agreement ($\chi^2 = 2.21$; NS). The agreement suggests that the observed distribution of females is similar to what would result from random discovery of swarms by females.

DISCUSSION

Females, although rare, were found in approximately 1/3 of all swarms collected but no exclusively female swarms were found. Female swarms have been recorded for *Culex pipiens fatigans* (Goeldi 1945) and for *Aedes punctor*, *Simulium venustum* (Hocking 1953, p244).

Estimates of the numbers of males in swarms are available for only a few species mostly from visual estimates, sometimes (Downes 1955) checked by the collection of a few swarms.

TABLE 4.13 *Size of swarms, other species*

Species	No. males	Source
Anopheles sundaicus	few hundred to 5000	Vendat Rao et al (1942)
Anopheles subpictus	"	"
Anopheles funestus	300 - 500	Harper, J.O. (1944)
Tabanus bishoppi	1 - 75	Blickle, R.L. (1959)
C nubeculosus	1 - 70	Downes, J.A. (1955)
C riethi	1 - 100	"
C halophilus	2 - 20	"
C griscescens	up to 100 or 1000	"
C pallidicornis	few - 100	"

Swarms of *C brevitarsis* ranging from 1 to c.500 males with a mean of 49.7 are similar in size to those of other species of *Culicoides*, but detailed comparisons are not possible. The swarms of the three mosquitoes quoted are much larger.

The general concensus of observations (Downes 1969) is that females are rare in swarms but exact figures are not often quoted. Corbet (1964) found two females amongst 290 males collected from swarms of *Uranofaenia alboadominalis* (0.7%, which is similar to *C brevitarsis* (1.3%)).

Haddow and Corbet (1961) showed a wide range in the percentage of females in swarms of different species (all Diptera). In five species females ranged from 0 to 2.8% of males, a figure of the same order as that found for C brevitarsis, but in three species from 11 - 76%. The low proportion of females in swarms has already been explained (p 60).

There appears to be no linear relationship between the size of swarms and the time before sunset. If, however, such a response was present it could easily be completely masked by the large variation encountered.

The mean number of males in swarms was the same over the artificial and natural markers (in absence of cattle) suggesting that the artificial markers may be reasonably good copies of natural ones. The much lower variance associated with counts over artificial markers is not unexpected. Only two objects were used, both casting approximately the same size shadow. In comparison, swarms over many different natural markers of widely different size were combined for the 'markers away from calves' If the size (however that may be defined) of the marker influences the size of the swarm, a greater range of sizes of swarms, and hence a larger variance, could be expected in the 'markers away from calves' group.

Unquestionably the greatest effect of swarms was imposed by the presence of calf. The size of swarms increased, they were predominantly at low level, and the distance between them decreased. Swarms over animals were relatively rare but when they occurred they were exclusively at low level in respect to the top of the calf. They were intermediate in size between swarms over markers near and distant from cattle. Swarms near calves were more likely to contain females than those further away. Neither the number of males nor the proportion of swarms containing females was influenced by the height of swarms distant from cattle, suggesting that the increased likelihood of females in swarms near cattle is a function of nearness to cattle rather than the height of the swarm.

The data do not justify the proposal that the number of females is directly proportional to the number of males in the swarm, suggesting that the size of the swarm is not important to its discovery by females. If the one swarm containing 18 females is omitted, the observed frequency of occurrence of females in swarms fits a Poisson distribution, which is generated by a rare event

(Li 1964, p.515) occurring at random. It indicates that the end result of discovery of swarms by females is a random distribution of females amongst swarms, but it does not necessarily follow that females discovered swarms at random. The method of discovery of swarms by females is unknown but hearing the wing beat of the male has been excluded, at least for mosquitoes, because females are deaf to the flight tone of the male (Roth, 1948). If discovery was by hearing in C brevitarsis more females should find larger swarms, a result that did not occur. It is probable that females fly at random until they see or are seen by a male.

Every swarm collected contained only C brevitarsis, a strict degree of specificity that is in agreement with other species (Downes 1969).

Swarms of single adults were probably much more common than was observed because of the difficulty of seeing a single adult. It is not possible to compare the time spent by a single adult over one marker when it is alone or when other adults are present, but a shorter than usual stay over the marker was observed for single adults of other species (Corbet 1964). It is pertinent to speculate whether there may be some influence of the swarm itself on adults within it - an influence which may account for their unified movement up and down, the possible increase in time spent over a single marker by an adult when other adults are present, and the apparent mass migration of the swarm when modifications were imposed on a particular marker (Figure 4.VIIId). Swarming flight has been observed in a single midge (Downes 1950) and single mosquitoes (Nielsen & Nielsen, 1958). Downes (1958) suggested that no gregarious factor was needed to explain the formation of swarms but that interractions between adults in the swarm might be expected. Unified movement of swarms has been recorded in C nubeculosus (Downes 1955) and in Aedes cataphylla (Klassen & Hocking, 1964). Unified movement may be some form of aggregation response but it is just as likely to be the response of all the adults as individuals to a specific stimulus that has not yet been discovered. The discovery that females of C brevitarsis are rarer than expected in swarms containing less than 10 males may also point to some form of aggregation response. The equivalence of the number of females in low and high level swarms suggests that density of females may not govern height. It is possible that the swarm pursues a female down and in doing so moves from high to low level. That

explanation is inadequate because it does not explain why most swarms near cattle are at low level continuously.

The different shape of low swarms (fig 4.111) may be due to height alone, ie because it is close to the ground the adults at the bottom fly higher to avoid the ground to a greater extent than the higher adults in the same swarm. Similar change in shape at different height was recorded for Mansonia fuscopennata (Corbet 1964).

IV.4 SWARMS AND CATTLE

INTRODUCTION

During early observations at Highvale it became obvious that at least the number of adults, and possibly the number of swarms, in the experimental area increased when cattle entered the yard. At Collard, swarms were much closer together near the calves. It has already been shown that the presence of cattle reduces the height and increases the size of swarms (p106). Further observation suggested that the spatial arrangement of swarms was different near cattle.

MATERIALS AND METHODS

Five trials were conducted.

(i) Cows and swarms in the experimental area at Highvale were counted at 10 minute intervals (reduced to 5 min near sunset) for 100 minutes before and after sunset in April (3 evenings) and December (5 evenings) 1972. The time when cattle entered the yard differed between evenings but could not be controlled by the observer.

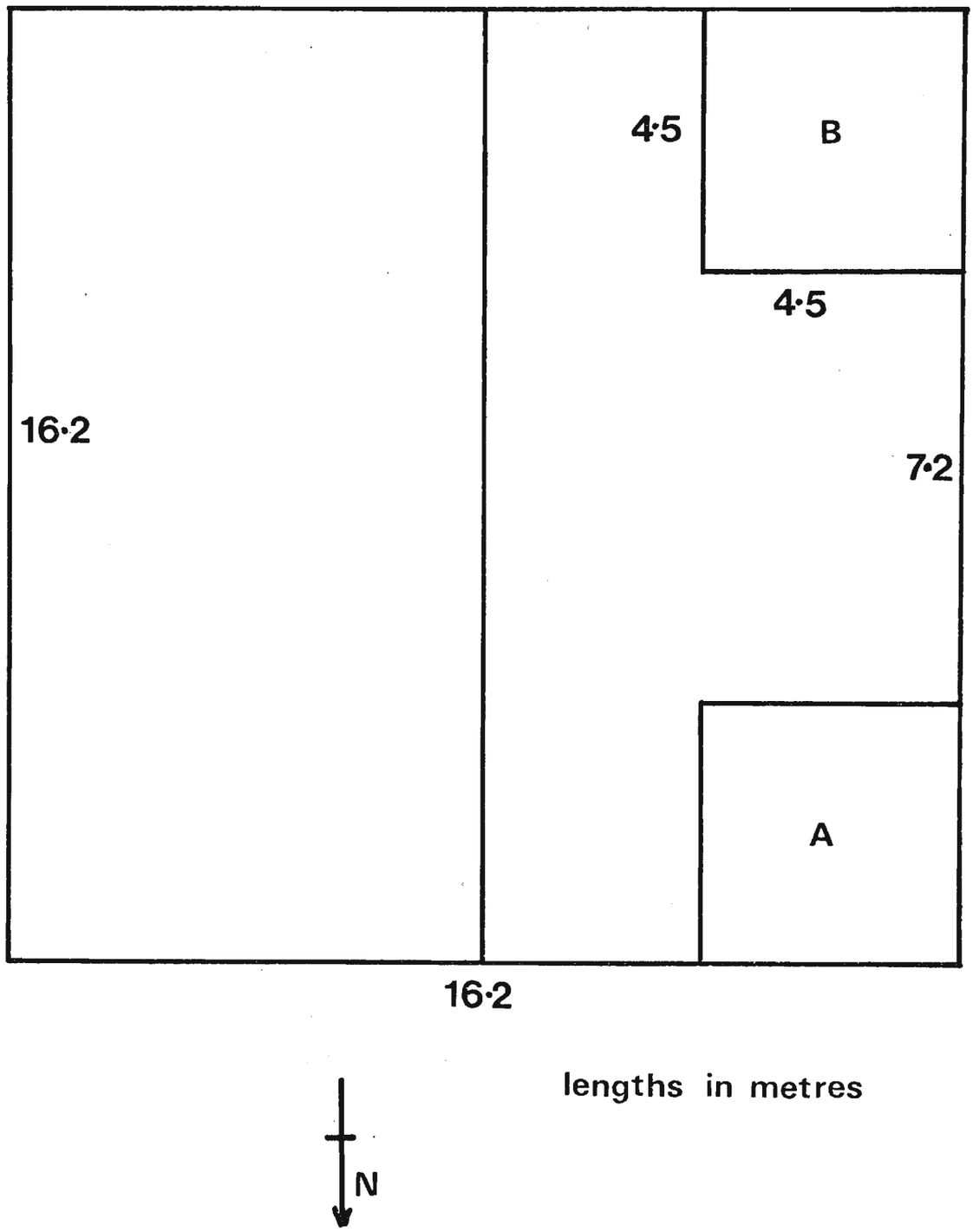
(ii) The distribution of swarms within the calf yard at Collard was observed. Four calves were put in area A (fig. 4.XV) and none in B (situation 1) and every swarm within both areas marked with a dart. The calves were moved to area B (situation 2) and swarms in both areas again marked. Both situations were repeated once on the same evening.

(iii) Within the same area on two successive nights swarms were collected over markers close to the four calves (which always stayed close together). The calves were driven to the other side of the area and the swarm, if any, over the same marker also collected. If no swarm formed within five minutes of the removal of the calves, a zero value was recorded.

(iv) The exact location and height of every swarm within 10m of a calf tied to a peg was recorded.

(v) Two calves were chained to a steel post on a night when the wind speed was just too great for swarming. The location of swarms which formed leeward of the animal where there was a zone of reduced speed was recorded.

Fig. 4.XV Calf yard at Collard.


4·5
B
4·5
16·2
7·2
A
16·2
lengths in metres
N

RESULTS

Trial (i) (figure xvi)

Comparison of the time of entry of cattle and of the occurrence of the first swarm (fig. xvi) shows that the two are not related. Swarms were present before cattle on three evenings (April 19 by 30 min; April 28 by 25min; December 8 by 15min). Swarming started at the same time as cattle entered the yard on two evenings (December 5 and 12) but after cattle entered on three evenings (April 21 by 15 minutes; December 6 by 15 minutes; and December 7 by at least 10 and probably 30 minutes) Similar discrepancy occurred between the end of swarming and cattle leaving the yard. Two groups of cattle entered the yard. The first, itinerant cattle in figure 4.XVI, were present occasionally during the day but were removed before the main herd entered. Swarms did not occur when they were present.

The number of adults within the experimental area increased drematically on the nights of April 19 and December 12 when cattle entered. Swarming began 60 - 80 minutes before sunset (fig. 4.XVI:i), reached a maximum during the 40 minutes before sunset then decreased rapidly. (fig. 4.XVI:i)

Trial (ii)

In both replicates of the two situations (table 4.4a) many more swarms were present near than distant from the calves. The swarms near the calves were on the sunny side of the animals with one exception which was over the shadow cast by the legs of a calf (98% of the swarms were on the sunny side of the calves). The calves themselves were markers for 14 swarms (25% of swarms). Shadows cast by grass clumps were the dominant markers; 20/35 (60%) when calves were present and 7/9 (78%) when calves absent. The area (A or B) did not influence the number of swarms but the presence of calves did (table 4.4c) with many more swarms near the calves.

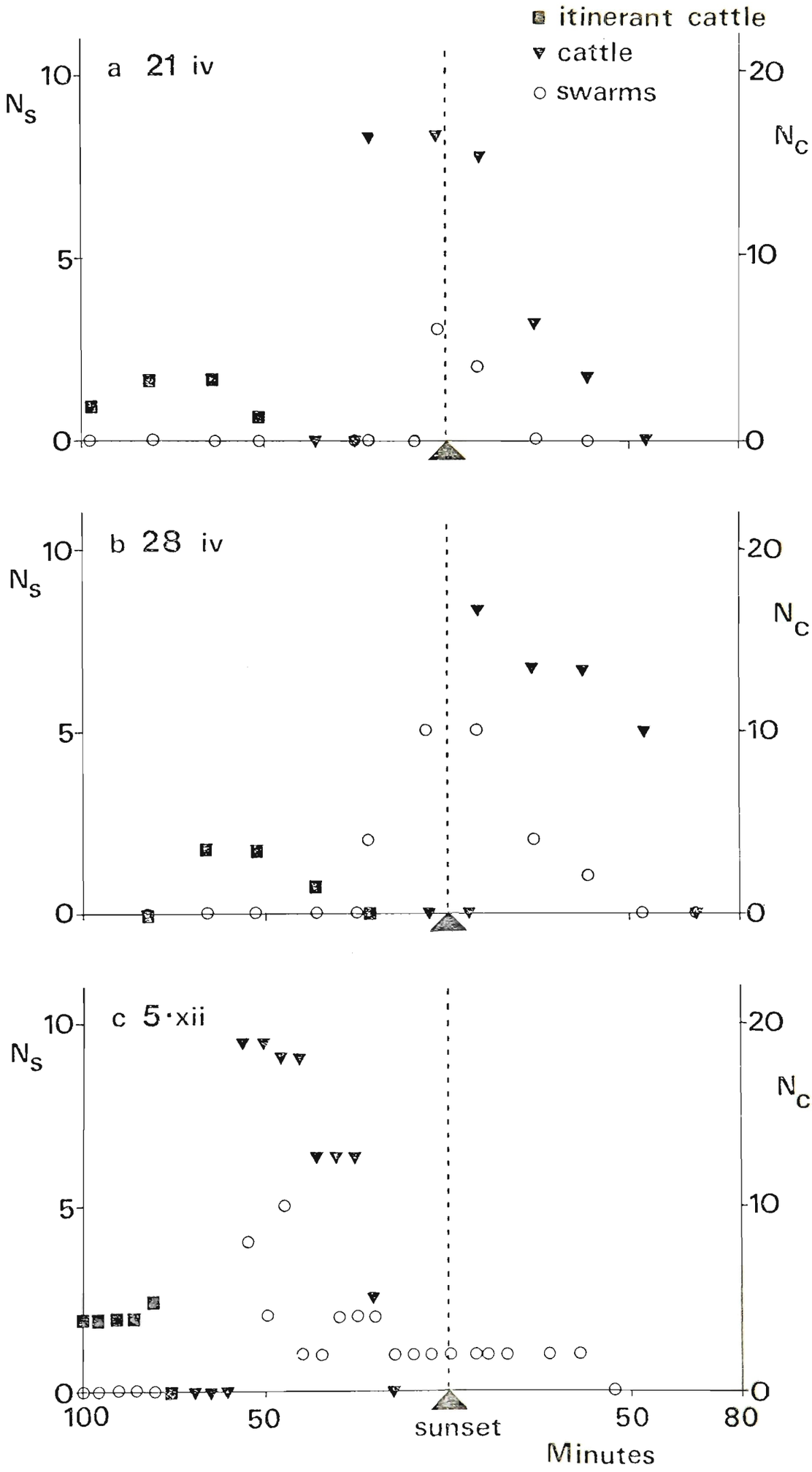
Trial (iii)

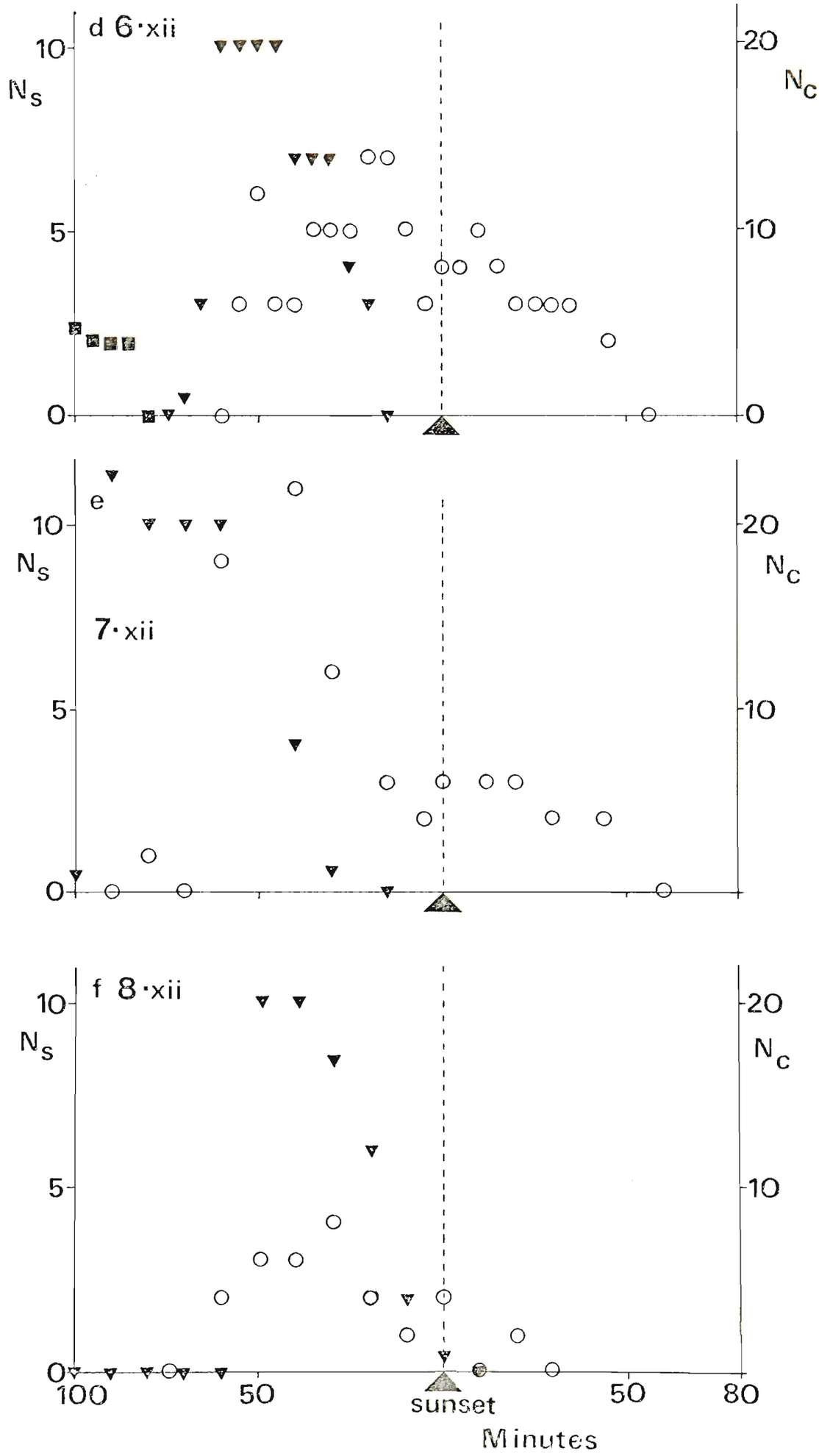
The mean size of swarms near the calves was 141.5 males and 1.0 females and they were collected over 13 different markers. When the calves were moved away swarms were recorded over only 4 of the markers, and size was reduced to 11.3 males and 0.0 females (table 4.15).

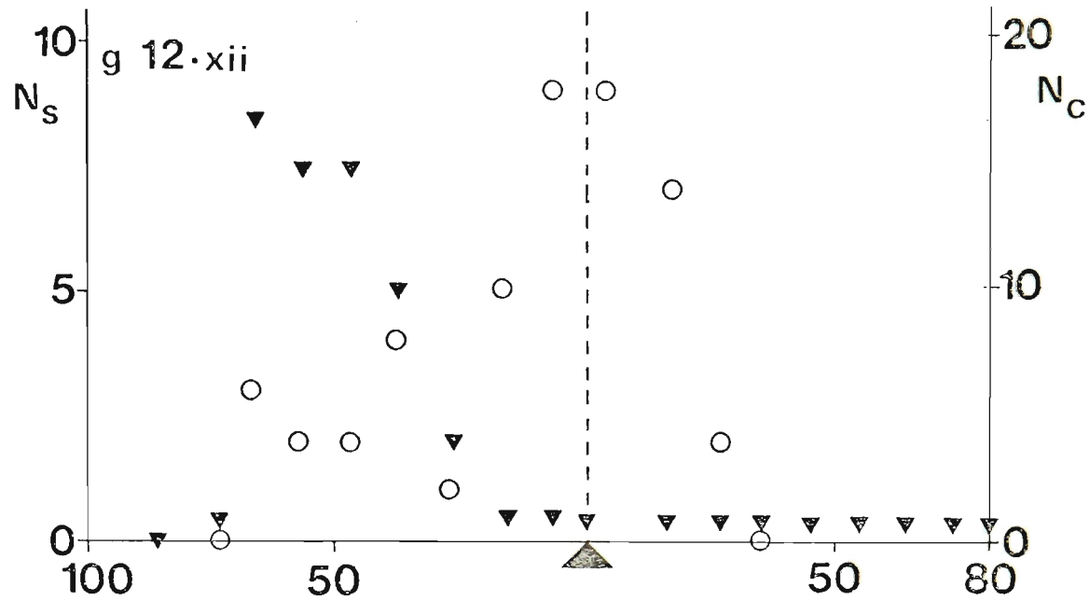
Fig. 4.XVI Swarms and Cattle at Highvale

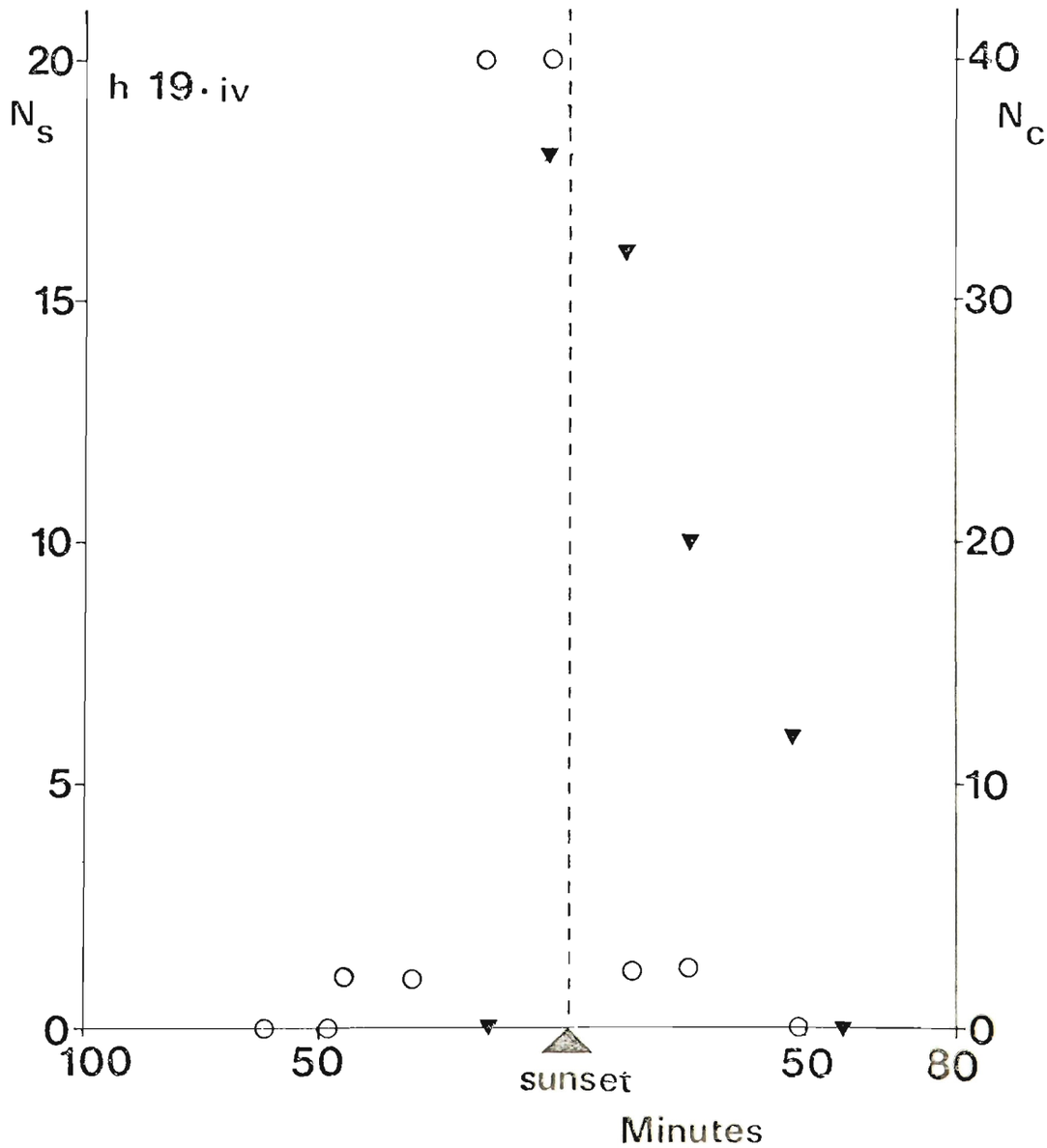
N_s = no. swarms

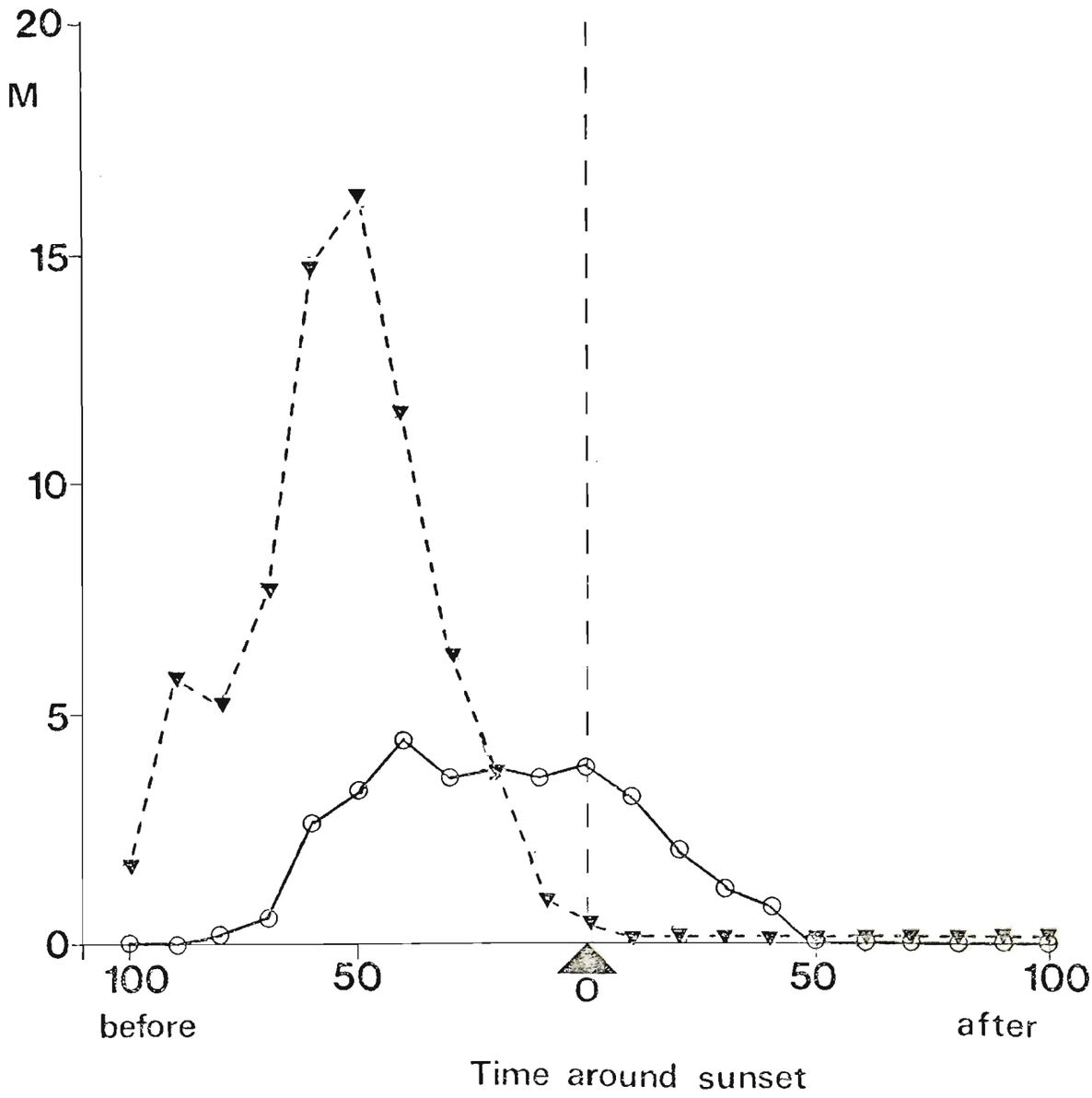
N_c = no. cattle


itinerant cattle
cattle
swarms
a 21 iv
b 28 iv
c 5·xii
N_s
N_c
10
5
0
20
100
50
sunset
50
80
Minutes


d 6·xii
10
5
0
N_s
20
10
0
N_c
e
7·xii
f 8·xii
100
50
sunset
50
80
Minutes


g 12·xii
N_s
N_c
10
5
0
20
10
0
100
50
50
80


h 19·iv
N_s
N_c
20
15
10
5
0
40
30
20
10
0
100
50
sunset
50
80
Minutes



M Mean no.

TABLE 4.14 Swarms with and without calves

(a)

marker	number of swarms							
	situation 1				situation 2			
	A		B		A		B	
	1745	1805	1745	1805	1745	1805	1745	1805
grass	10	5	2	1	2	2	11	7
calf	2	0	0	0	0	0	3	9
artificial	0	2	0	0	2	0	2	0
calf's shadow	1	0	0	0	0	0	0	0
dung	1	2	0	0	0	0	0	0
total	14	9	2	1	4	2	16	16

(b)

	area		
	A	B	Total
+calves	23	32	55
-calves	6	3	9
total	29	35	64

$\chi^2 = 1.05$; NS

(c)

	area		
	A	B	total
situation 1	23	3	26
2	6	32	38
total	29	35	64

$\chi^2 = 6.8$; $0.01 > P > 0.001$

Trial (iv)

Nine swarms, all at low level and on the sunny side of the calf were present simultaneously (fig. 4.XVIIa) Three swarms at higher level were present but further from the calf and further apart. All 12 swarms were within 5.4m of the peg to which the calf was tied. No swarms were present more than 5.4m but less than 10.0m from the peg.

Trial (v)

Three swarms were present on the eastern side of the calves (over shadows cast by their legs) but not elsewhere in the paddock. The wind was westerly hence a zone of reduced wind speed would occur on the eastern side of the calves.

TABLE 4.15 Size of swarms near to and distant from calves

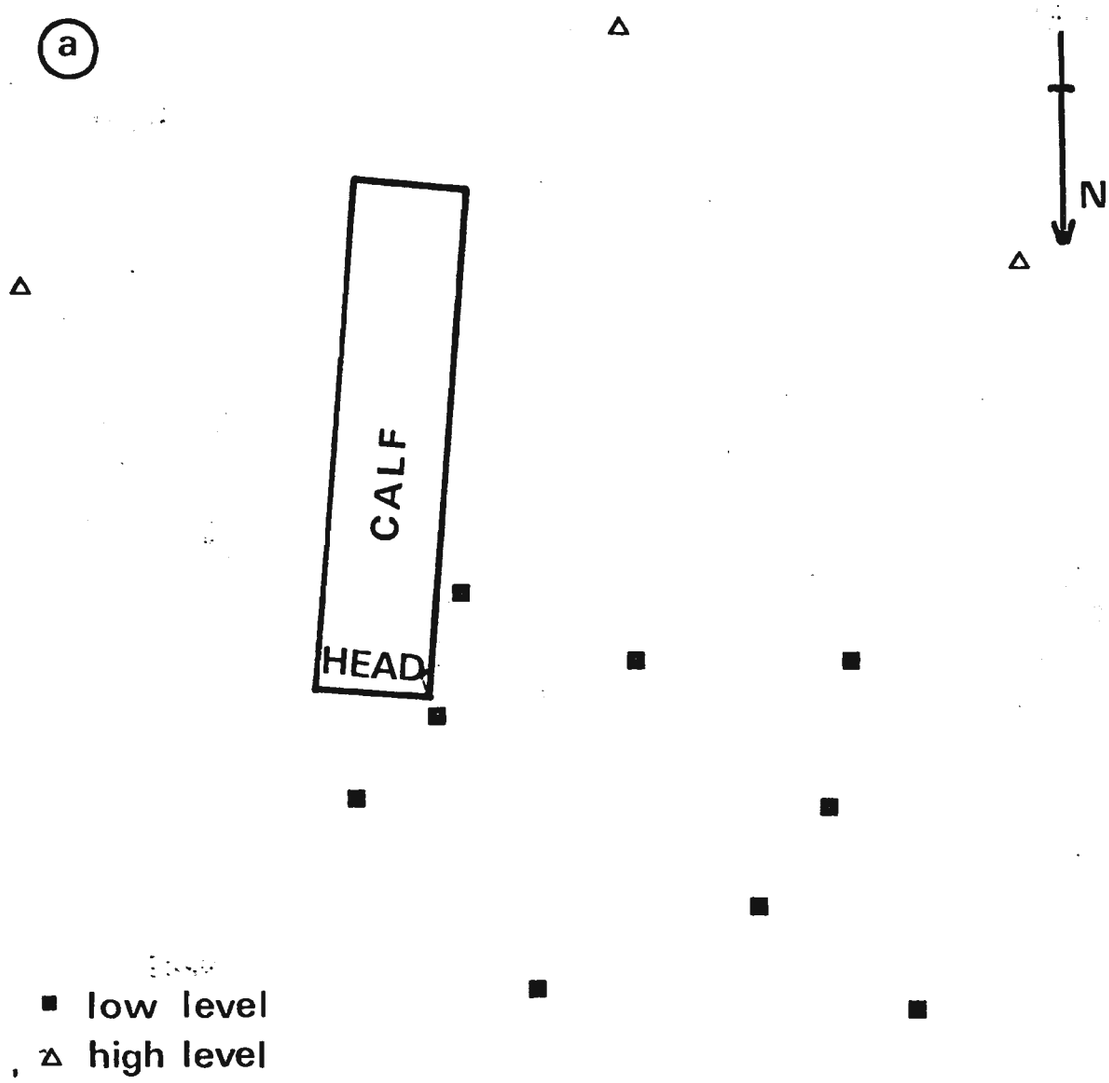
marker	near calf			away calf		
	f	m	t	f	m	t
6.3.73						
dung in deep grass	1	171	172	0	5	5
grass shadow	0	431	431	0	0	0
"	0	80	80	0	9	9
"	0	98	98	0	0	0
"	1	166	167	0	8	8
"	3	152	155	0	23	23
8.3.73						
grass shadow	0	28	28	0	0	0
"	1	36	37	0	0	0
"	4	250	254	0	0	0
artificial	0	261	261	0	0	0
dung in deep grass	0	15	15	0	0	0
"	1	105	106	0	0	0
depression	2	34	36	0	0	0
total	13	1827	1840	0	45	45
mean (n=13)	1.0	140.5	141.5	0	3.5	3.5
(n=4)				0	11.3	11.3

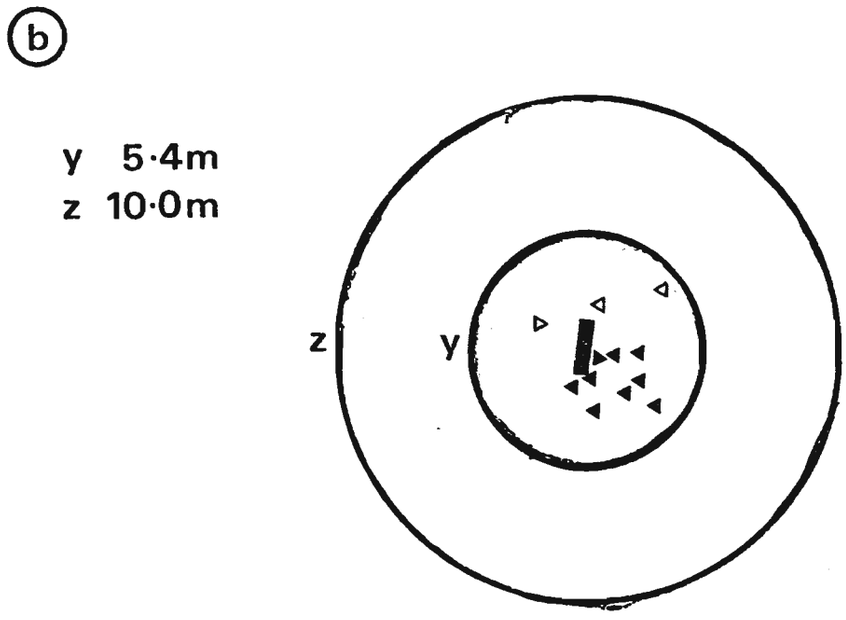
f = female m = male t = total

DISCUSSION

Swarming was observed at places well removed from the nearest bovine. The time at which the first swarm occurred (December 1972 at Highvale) was nearly the same every night, 63 min before sunset (55,55,80,60, and 65 min on different evenings). The time at which the cattle entered the area varied widely. Decreasing light intensity, which does not fluctuate according to the immediate weather, is the factor most likely to be initiating swarming (Corbet 1966). Cattle are not obligatory for the initiation of swarming nor does their absence cause its cessation. Cattle cause gross changes in swarming, by increasing size and the likelihood of females, and by reducing the height of and distance between swarms. Swarms near calves are virtually all on the sunny side of the animal, nearer the head than the tail, but when wind speed was limiting, the preference for the sunny side was lost.

Fig. 4.XVII Swarms near a calf.


a
N
CALF
HEAD
low level
high level


b
y 5·4m
z 10·0m
z
y

Several swarms were seen in the same sort of relationship to a red plough left in the paddock as to a calf but swarms were not always present. The plough may, in a minor way, simulate a calf and may therefore indicate that the calf is perceived visually. It also suggests that a more detailed examination of the cause of the effect of cattle on swarming might allow simulation of cattle and inducement of swarming in the laboratory.

Swarming is very well adapted to keeping males near to a mobile animal. Markers such as dung pats, sticks, stones etc do not migrate with animals, and could be absent from areas to which cattle had moved by the swarming period. Shadows cast by grass clumps, or any other objects, are ubiquitous on all evenings when there is directional light. If cattle move, then the swarms can stay near them, by shifting from one shaddow to the next. Darker areas are not common but are produced when an animal urinates.

Swarming over the sunny edge of shadows demands that the orientation of the insect be towards the sun (p 102) not the wind. The evolutionary change involved would have been part of those needed to keep swarms near to cattle. While the majority of females collected from cattle are already mated (p 153), virgins are collected. Swarms near cattle might be more likely to be found by virgin females thereby giving evolutionary advantage to the males in them. The likelihood of finding females in swarms is greater nearer cattle.

IV. 5 SWARMS AND WIND

INTRODUCTION

Field observations suggested that wind influenced swarming by reducing the likelihood with strong winds. Strong gusts blew away extant swarms but they reformed rapidly (less than 10 seconds) after reductions in wind speed.

MATERIALS AND METHODS

Wind speed was measured using a Casella Sensitive Anemometer (callibration number 2479/c) with the cups 1.2m high, approximately the centre of high swarms, set up in the open, 6m from the cattle pen at Collard. The revolutions of the cups were counted (electronically).

Initially counts were made at intervals of 30 seconds (55 observations) but when it was realised that swarms were responding to changes in wind speed lasting less than 30 seconds, the interval was reduced to 15 seconds (172 observations). Wind speed and the presence of swarms were recorded each 15 seconds for periods ranging from two to ten minutes, during the 45 minutes before sunset.

A calf was tied to a post 3m from the anemometer and the presence of swarms over three specified markers near the animal was recorded. Swarms changed from marker to marker and a zero recorded over the specified markers did not necessarily reflect absence of swarming. Assessment was changed to scanning the area west of the observer for the presence of swarms. The calf, although unecessary, was retained for uniformity. Insects were observed flying at wind speeds exceeding the upper limit for swarming.

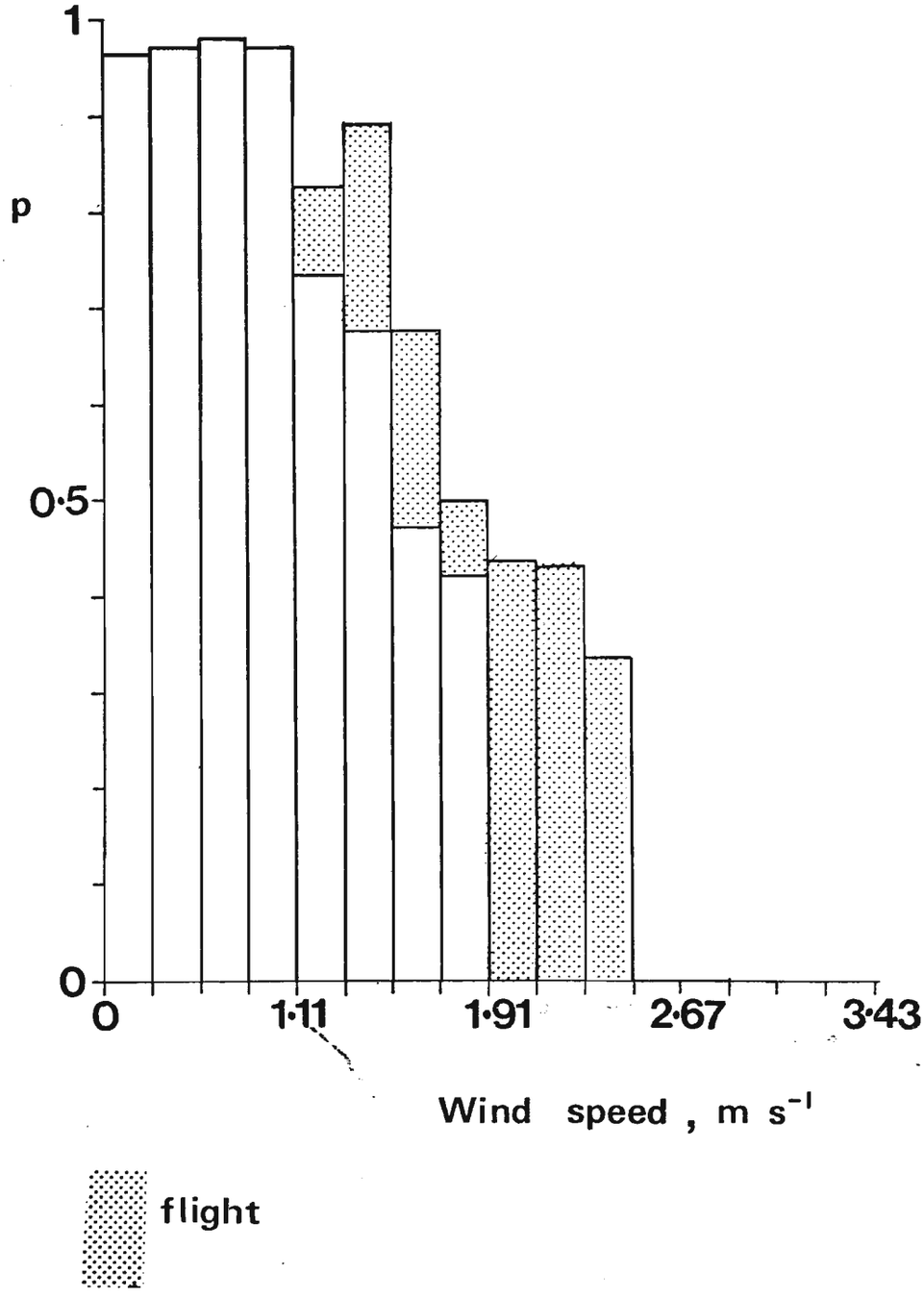
Wind speed was converted to counts per minute and observations were sorted into groups on that basis. The end points of each range were later converted to ms^{-1}.

RESULTS (fig. 4.XVIII)

The probability of swarming was very high (> 0.95) when the wind speed was less than $1.11ms^{-1}$ (2.48 miles h^{-1}) but was reduced to nil when wind speed increased to $1.91ms^{-1}$, (4.28 miles h^{-1}). Adults that were flying but not swarming were not observed below $1.11ms^{-1}$ (although they must have been) but it was the only flight activity between $1.91ms^{-1}$ and $2.47ms^{-1}$ (5.53 miles h^{-1}).

Less than 10 observations were available in each group where

Fig. 4.XVIII Wind speed and swarming.


1
p
0·5
0
0
1·11
1·91
2·67
3·43
Wind speed , m s⁻¹
flight

the wind speed exceeded 1.91ms^{-1} therefore the validity of any generalisations is questionable and conclusions for wind speeds greater than 1.91ms^{-1} can only be tentative.

Stability of swarms.

Swarms were simultaneously present over many markers, but there were many more markers than swarms at Collard. Swarms existed for a short time only over the smaller markers, and moved between markers although there is no evidence that they did so as a whole. One marker (usually large) may have swarms over it on many occasions on any one night, while others (usually small) may be used only for short times on occasional evenings.

DISCUSSION

Flight activity by C brevitarsis was limited to wind speeds below 2.47 metres/second. However, it was not possible to identify flying insects as C brevitarsis to any better degree than to say that they were of approximately the right size. Therefore the speed limit may over estimate the upper speed for flight as the insects observed may have been other species.

Swarms were dispersed instantly by increased wind speed. Hence, when a gust of wind occurred at or immediately before the end of the 15 second period, no swarm would be observed during most of which swarming had occurred. Such a gust would have little influence on the mean wind speed and therefore the probability of swarming at any particular speed could be underestimated. Any underestimation would be minor but could account for the difference between the observed probability (>0.95) and 1.0 with winds of very low speed (e.g. 0-1ms^{-1}).

Downes (1955) commented that at wind speeds of about 2 mph (0.9ms^{-1}) C reithi settled. Other references for Ceratopogonidae give only the vague comment that at high speeds swarming ceases.

Flight without swarming was observed on calm nights during the five to ten minutes before swarming began, but on evenings when strong wind delayed swarming until just before sunset, the initial period of flight was not observed. Flight activity before swarming was observed with both Culex salinarius and Aedes taeniorrhynchus (Nielsen 1964).

Individual swarms over specific markers appear to be quite unstable, the swarm appearing and disappearing continuously. Similar behaviour was described for C pallidipornis (Downes 1955) swarming over fern leaves. Swarms were small and unstable, and markers were more numerous than swarms. In the experimental area at Collard, and in the open paddock on most dairy farms, the situation was similar to that described for the area in which C pallidicornis swarms. Small and unstable swarms may be the result of an oversupply of markers but that conclusion leaves unanswered an important question; why don't smaller swarms form over every marker that is available? Is it that adults swarm over the largest marker within their visual range or is it that there is some aggregation effect (p 113) of adults in swarms which imposes a minimum size on viable swarms? Movement between markers would occur if a larger one came within visual range and drew off the males. Movement to that marker may bring another, even larger, into view and the movement would continue, creating a situation where they are more stable and more frequent over larger markers.

IV. 6 SWARMS AND WEATHER

INTRODUCTION

Swarming was observed on only some evenings and then during a short period, and other observations suggested that weather influenced the occurrence of swarms.

MATERIALS AND METHODS

The number of swarms in the experimental area at Collard was counted at 20 minute intervals, but the interval was reduced close to sunset. Observations started about 2 hours before and finished one hour after sunset. At the same time as the swarms were counted the following climatic factors were measured;

Time as minutes before sunset.

Wind calculated from the number of counts by the anemometer in the previous 20 minutes and 1 minute (on some evenings).

Temperature and humidity using an Assman Hygrometer drawing air 1.5m above the ground.

Cloud cover visually in eighths.

Presence or absence of direct sunlight

Rain recorded as heavy, light, or nil.

Each set of observations was coded on to a punch card for ease of sorting and comparison. The codes used were:

Swarms. 0;1; 2-3, 4-7, 8-15, 16-31, more than 31.

Wind. up to 0.2; -0.4, -0.6, -0.8, -1.0, -1.2, -1.4, -1.6, -1.8, -2.0, -2.4, more than 2.4 ms^{-1}

Temperature. 16^{0}+, 20^{o}+, 28^{o}+, 32^{o}+

Humidity. Less than 50%, 50%+, 60%+, 70%+, 80%+

Cloud cover. eighths

Direct sunlight. + or -

Rain. + or -

A total of 278 observations were made over 41 days.

The punch cards were sorted into those with and those without swarming, then the measures of weather were compared for the groups using the median or the χ^2 test. Where differences were detected that measure was assessed for its effect on the number of swarms using the same χ^2 tests.

The number of occasions when biting and swarming occurred were compared.

RESULTS

Time

The first swarms occurred 60 minutes before sunset but on many nights they were delayed by excessively strong wind.

The last swarms occurred 10 minutes after sunset (table 4.16)

In the following analysis, all data collected after sunset were discarded. Temperature did not differ between the groups of observations with and without swarming, ranging from 20^{o} to 32^{o} Humidity ranged from less than 50% to more than 80%, but did not differ between the groups of observations. Neither cloud cover nor presence of direct sunlight varied between the two groups of observations. Very light rain fell during 12 observations (2 evenings). Swarming was occurring during one observation. No conclusions can be drawn.

The mean wind speed for the previous 20 or 1 minutes varied significantly between the two groups of observations. Most swarming occurred at lower wind speeds (table 4.17), with 90% occurring when speed was less than $1.2ms^{-1}$

The observations were regrouped on the basis of compliance (93 observations) or not (165 observations) with the following requirements:

a. Wind speed over the previous 20 minutes less than $1.2ms^{-1}$

b. Wind speed over the previous 1 minute, if available, less than $1.2ms^{-1}$.

c. Within the 60 minutes before sunset.

Swarming occurred in neither observations, that did not comply nor in 39 observations that met the specifications. The latter were examined.

(i) For 11 observations swarms occurred later on the same evening. It is assumed that the observations were too early.

TABLE 4.16 Number of swarms and time to sunset

time	number of swarms						
	1*	2+	4+	8+	16+	Total	mean
60	3	0	2	0	0	5	3.0
40	2	4	13	0	0	19	4.7
20	1	3	6	4	1	15	7.8
10	1	2	9	6	1	19	8.2
5	0	0	2	0	1	3	12.0
0	4	0	0	0	0	4	1.0
-5	1	2	1	0	0	4	3.0
-10	1	1	1	0	0	3	3.2

* numbers of observations with nominated number of swarms

time = minutes before sunset

TABLE 4.17 Number of swarms and wind speed

wind speed	number of swarms							
ms^{-1}	0	1	2+	4+	8+	16+	total	mean **
0.0-0.4	29*	2	1	1	0	0	33	2.8
-0.6	29	4	5	6	4	0	48	5.4
-0.8	23	0	1	11	3	2	40	9.0
-1.0	33	3	2	7	1	0	46	4.8
-1.2	18	0	2	2	2	1	25	9.6
-1.4	7	1	0	3	0	0	11	4.8
-1.6	19	1	0	0	0	0	20	1.0
-1.8	14	0	0	0	0	0	14	0.0
-2.0	5	1	0	0	0	0	6	1.0
-2.4	7	0	0	0	0	0	7	0.0
2.4-	2	0	0	0	0	0	2	0.0

* numbers of observations

** observations without swarms not included in mean

(ii) Measurements of wind speed over the previous 1 minute were not available on many nights. Wind was gusty and obviously restricting swarming to lulls between gusts in another 11 observations, and it is assumed that gusts were responsible for the absence of swarming.

(iii) In one observation on each of three evenings a heavy bank of cloud near the horizon caused the sun to appear to set five minutes early. Such a dummy horizon may have caused cessation of swarming.

(iv) For three observations on one evening, calves were close to the experimental area. Swarms were absent from the area but present elsewhere.

(v) Swarms were present in other areas during one observation.

The above explains the absence of swarming in 29 observations without modification of the weather requirements. The absence of swarming in ten observations has not been explained. Eight were made on days when very dense cloud covered the sky all day. No swarming was observed on those days, suggesting that some direct sunlight may be necessary.

So far the swarms observed after sunset have not been considered. They were rare and over a restricted range of markers, and not observed more than 10 minutes after sunset (table 4.16)

Swarming and biting

Adults were present on hosts (biting) in more observations than swarming (table 4.18) A significant dependence between biting and swarming was observed.

TABLE 4.18 Biting and swarming

biting	swarming		total
	+	-	
+	59	141	200
-	12	66	78
total	71	207	278

$\chi^2 = 5.2$; $0.05 > P > 0.02$

DISCUSSION

The results allow the prediction of whether or not swarming will occur at any specific time. Swarming occurs in the 60 minutes before sunset, some continuing up to 10 minutes afterward. The influence of wind is indicated but has already been examined more critically. The influence of cloud over during the whole day is not explored elsewhere and must, therefore, remain unproved.

Biting activity would be expected in more observations than swarming because it continues after sunset (101 observations were made after sunset) Those observations cannot, however, account for all of the difference between the number of observations with swarming (78) and the number with biting (200) When the observations were examined for the influence of weather on biting the maximum wind speed was higher for biting (p 152) than for swarming, thus explaining biting without swarming. Flight activity occurs at speeds higher than swarming, therefore biting at speeds in excess of those for swarming is not unexpected.

V. BLOOD FEEDING ON HOSTS

INTRODUCTION

The temporal incidence of adult of C brevitarsis on horses in and near Brisbane was examined by Riek (1954). Adults were not present between 700 and 1600h, and the major period of activity occurred between 1800 and 2200h. Observations (Standfast & Dyce 1968) suggest that Riek's observations apply to cattle.

Kettle (1969a) determined the time of activity of C furens on human hosts. He showed major peaks of activity around sunset and dawn, with a lesser peak at midnight. Little or no activity occurred during the day but there was some between the three peaks during the night. Many studies of the activity of adults of Culicoides species using light traps or truck traps have shown that most species are active at night with very little activity during the day, very often with peaks of activity at either or both sunset and sunrise (Hill 1947, Jobling 1953, Bidlingmayer 1961, Nevill 1967, Kay 1973).

Cursory examination confirmed that many adults were present on cattle around sunset. C brevitarsis on dark coloured host animals were counted at Highvale and Collard throughout the day and night. Standfast (personal communication) suggested that C brevitarsis could be seen most easily on a black animal, an observation that was confirmed, and which may be due to (i) greater numbers or (ii) greater contrast between adults and the host and hence easier viewing. Neither possibility has been investigated, but Davies (1972) reported studies which showed that the landing frequency of females of black flies that were seeking a meal was inversely proportioned to the intensity of reflected light regardless of colour. The same may apply with C brevitarsis.

MATERIALS AND METHODS

At Highvale adults were counted on a black cow which was the only animal that would allow the observer to stand alongside it. At Collard, adults were counted on three animals (i) a large black bull (the biggest animal used), (ii) a black, brown and white heifer (droughtmaster breed) of medium size, and (iii) a dark red - brown cow of moderate size.

C brevitarsis were attracted strongly towards any light used to find them hence continuous use of light would bias the counts.

* 879 flies were collected from backs of cattle; all were C. brevitarsis.

The technique used by Kettle and Linley (1967), collecting all adults that landed, was therefore inappropriate for this species. Because C brevitarsis is the only species* of the genus on the back of cattle in the experimental areas, it was not necessary to collect them for identification. The adults on the animal were counted at the end of a fixed period (15 or 20 minutes).

Most counts were taken within a standard area, side length 100mm. The twelve squares marked on each animal (plate IIc) were (i) one and two on opposite sides, between the croup and the tail, 5 cms down the flank from the backbone (ii) three to ten as a contiguous series straddling the backbone from the croup reaching forwards 3/4 of the way along the back, (iii) eleven straddled the backbone at the withers, and twelve was on the head. Square twelve was abandoned because no adults were found. Variations to the pattern of standard areas are listed where appropriate.

Time has been expressed in solar time measuring from sunset or sunrise, allowing comparison between counts at different times of the year. It appears to be more meaningful biologically. Kettle and Linley (1967) used the same technique.

The following methods were used.

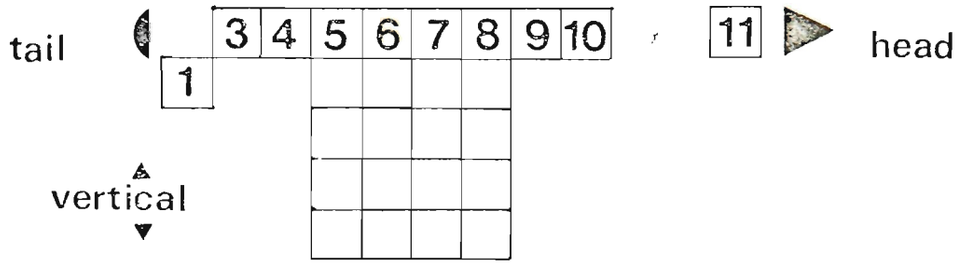
(a) The adults on square seven were counted every 30 minutes throughout most of the day, on all four animals from December 1972 to March 1973. The period of greatest density on the host, 2 hours before to 6 hours after sunset, was examined on every occasion but for the rest of the day counts were made less frequently as one observer and one cow could not be used continuously for 24 hours. Observations on separate occasions included overlapping periods. When a nil count was recorded in the test square during daylight hours, the whole animal was searched. No attempt was made to correct scores for variations due to animal or locality in the way that Kettle and Linley (1967) corrected for collector.

(b) Temperature, humidity, cloud cover and the presence or absence of direct sunlight were measured (p 130) at the same time as counts of adults on a specified area of a black calf, using the same calf each night. The observations were concurrent with those on swarming (p 130). The adults were counted within a triangle defined by three points on the calf, two on the croup and one on the withers (* on plate IIc) The surface area was much greater than the $10^{-2} m^2$ of the standard area.

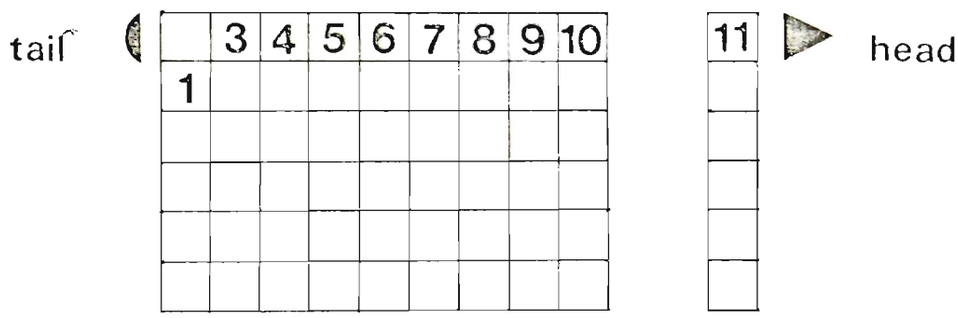
Adults were counted every 20 minutes from two hours before to one or two hours after sunset, occasionally with additional counts 5 and 10 minutes before and after sunset.
Simultaneous observations (i.e. one set of time, weather, and count of adults) (p 130) were coded on to punch cards. The number of adults on the animal was coded as: 0, 1-4, 5-9, 10-19, 20-39, 40-79, 80-159, 160 or more. These approximate a geometric series. The cards were then sorted into those with and those without adults. The parameters of weather were compared, and sorted on whether or not they differed between the two groups. Parameters that differed were examined for their influence on the density of adults. The observations in this part were incidental to other work. They were collected in an attempt to categorise broadly the effects of weather on the density of adults on the host. Observation was not rigorous and neither has been the analysis with the exception of the numbers of adults on the host in relation to time around sunset.
(c) Eleven standard areas were marked on the three animals at Collard, and adults in each area counted each half hour after sunset for 4½ hours, on three nights.
(d) On two occasions a grid of standard squares was drawn on the side of the red cow at Collard. On one 16 squares, 4 along the backbone and 4 down the flank, were drawn (fig. 5Ia). The adults in each square were counted four times when other work permitted, but at least 5 minutes apart (actual separation in time was 13, 6, and 11 minutes). On the other occasion five squares were drawn down the flank from each of the squares on the backbone forming a grid of 60 squares (fig. 5.Ib). The adults in each square were counted twice. The calf was tied to a peg and forced to assume different postures (table 5.9). Adults on a standard square at positions 3 and 12 were counted 10 minutes after the calf assumed each posture. Replication was minimal because it was difficult to force the calf to assume the postures.

Fig. 5.I Location of standard areas on side and back of animal.

(a)


horizontal
tail
3 4 5 6 7 8 9 10
11
head
1
vertical

(b)


tail
3 4 5 6 7 8 9 10
11
head
1

RESULTS

(a) Periodicity of activity on the host animal

A rapid increase of activity started 30 min before and peaked 30 min after sunset (fig. 5IIa), then decreased to nothing 6 hours after sunset. The variability 10 to 13 hours after sunset reflects the low level of activity at dawn, activity that can be seen when the times of dawn were synchronised (fig. 5IIb). Very few adults were present during the day.

(b) Weather

Counts on the calf (fig. 5 III) showed two interesting differences from those on the cow, (i) the peak of activity occurred at sunset, 30 minutes earlier than on the cow and (ii) the rate of decrease after the peak was greater on the calf (reduced to 23% 1 hour after peak) than the cow (37% 1 hour after the peak).

Adults were present in a higher proportion of observations after sunset (χ^2 = 33.3) and in observations when direct sunlight was absent before sunset (χ^2 = 10.9). The mean number of adults on the calf in bright sunlight (4.6) (table 5.1) was less than half the number in its absence (9.4).

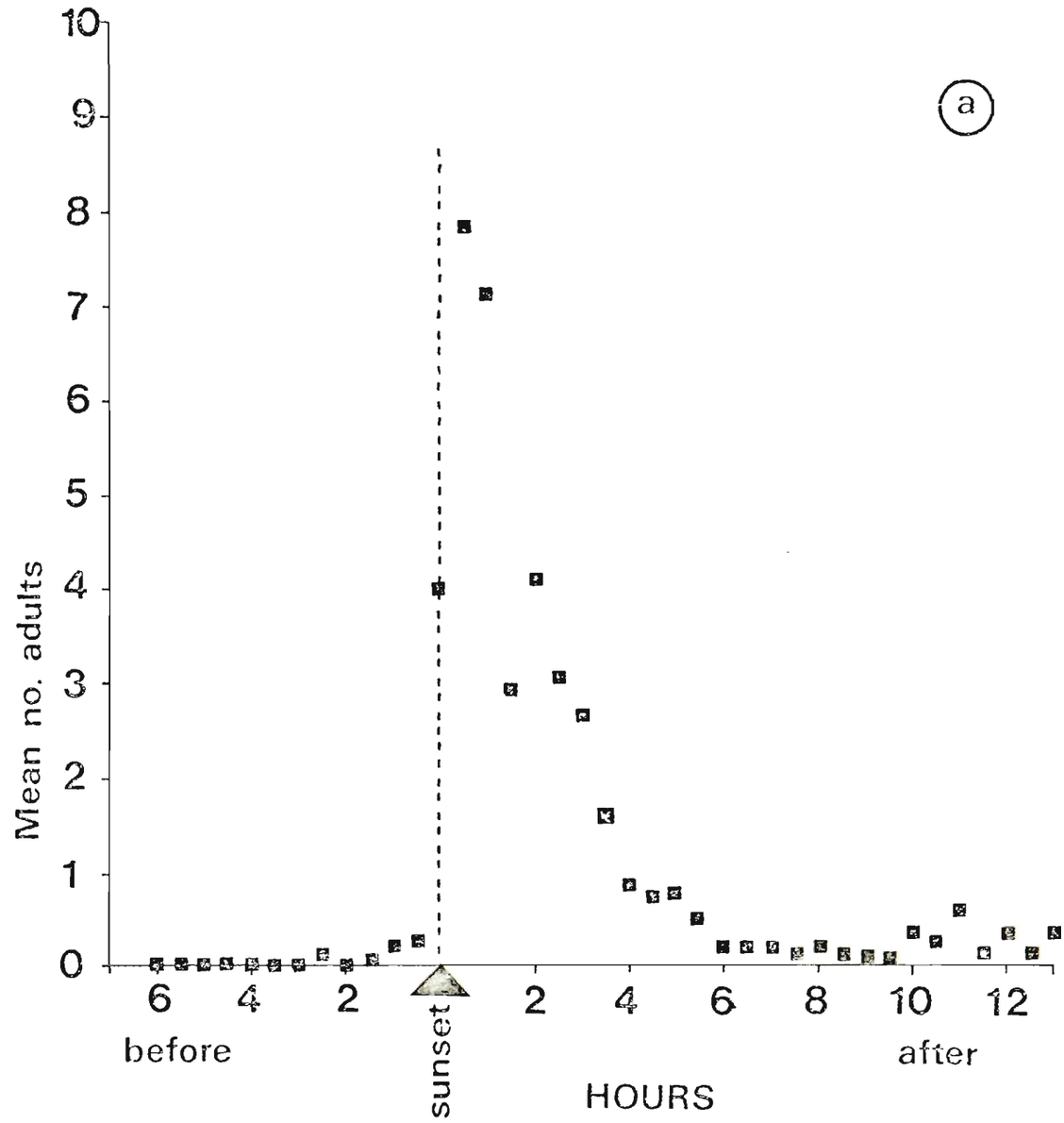
TABLE 5.1 Numbers of adults and direct sunlight

number biting	direct sunlight (number observations)		
	+	-	
0	46	21	
1+	25	21	
5+	9	8	median group 1+
10+	4	13	χ^2 = 9.14 **
20+	3	5	
40+	2	2	
80+	0	1	

none biting v rest χ^2 = 10.9 ***

When all the data were examined, the proportion of observations with biting was greater at lower temperatures (χ^2 = 21.2 ***) but not so when only observations at 40 and 60 minutes before sunset were compared (table 5.2).

Fig. 5.II Mean density of adults on cattle showing changes with time around sunset and dawn.


a
Mean no. adults
10
9
8
7
6
5
4
3
2
1
0
6
4
2
2
4
6
8
10
12
before
sunset
after
HOURS

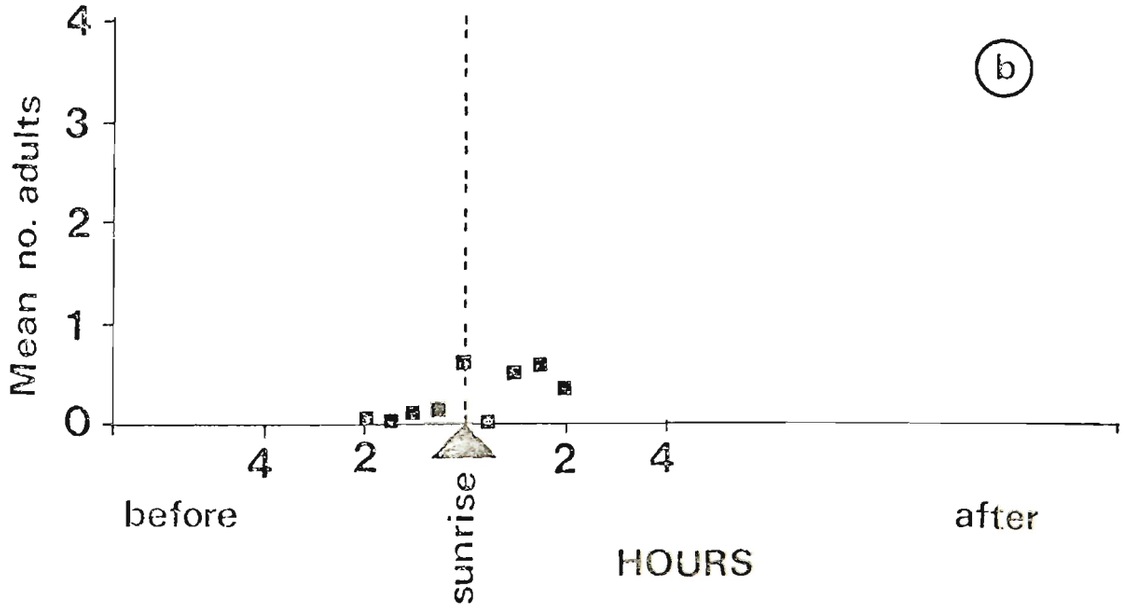

b
Mean no. adults
4
3
2
1
0
4
2
2
4
before
sunrise
after
HOURS

Fig. 5.III Mean density of adults on calf showing changes with time around sunset.

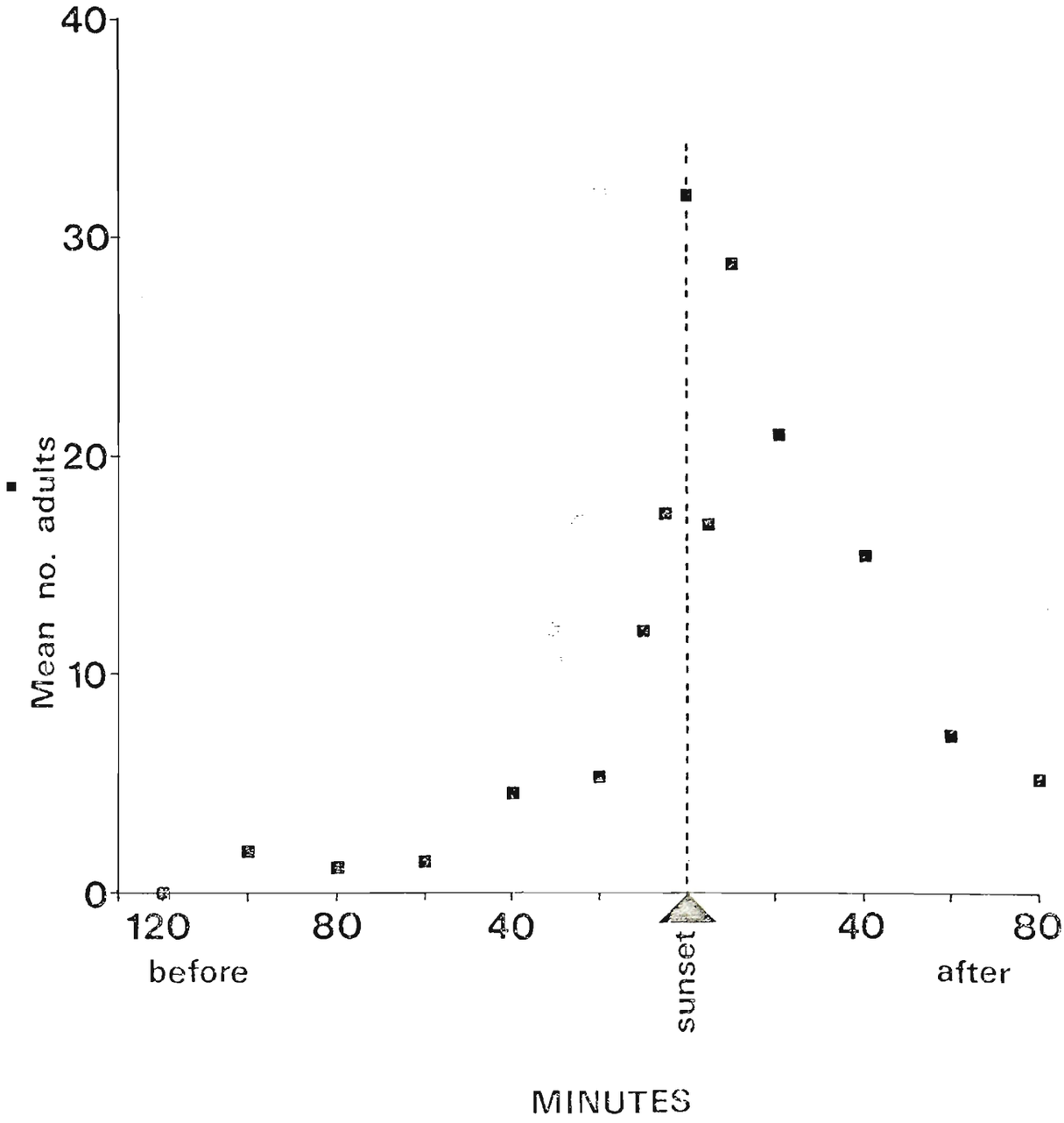

40
30
20
10
0
Mean no. adults
120
80
40
40
80
before
sunset
after
MINUTES

TABLE 5.2 Presence of biting at different temperatures for observations 40 & 60 min before sunset

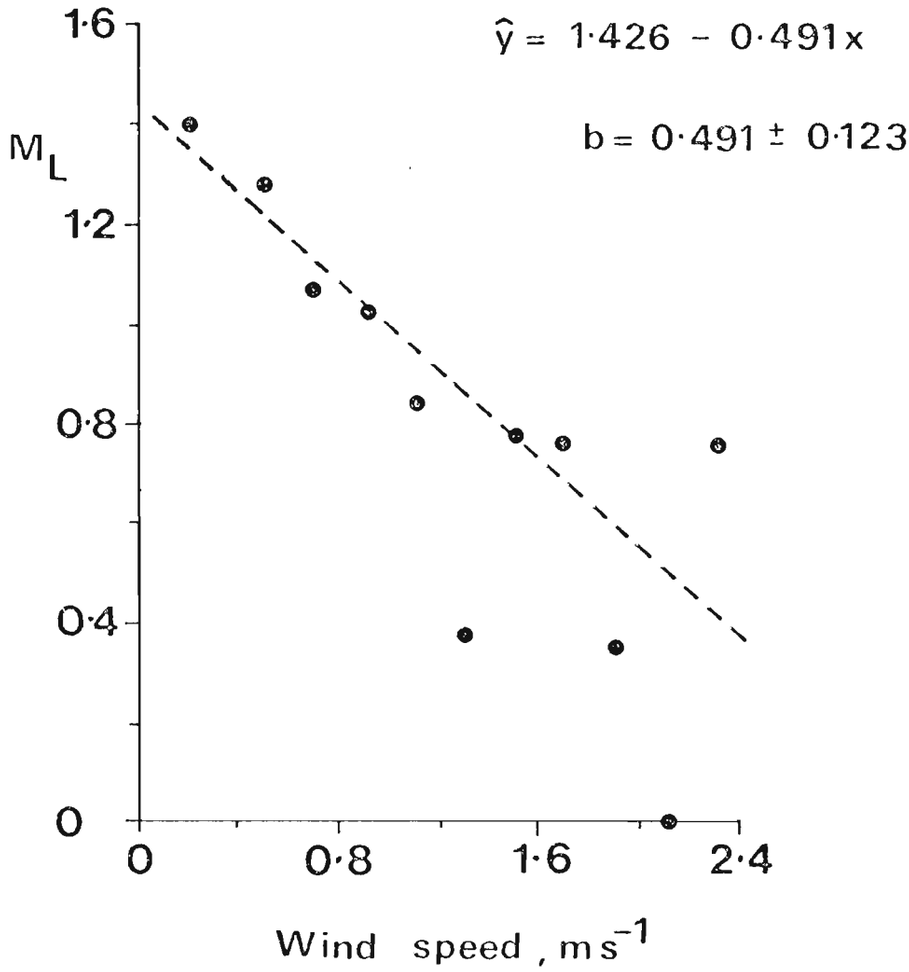
temperature (^{o}C)	biting (no. observations) +	-	
20+	0	2	
21+	0	1	
22+	2	3	
23+	5	3	median group 25+
24+	6	5	
25+	6	5	$\chi^2 = 0.14$ NS
26+	5	5	
27+	5	7	
28+	3	3	
29+	0	4	
30+	0	1	
31+	1	1	

all observations $\chi^2 = 21.2$

Neither humidity nor cloud cover differed between the groups with and without biting.

The number of adults in the test area (fig. 5.III) was halved by an increase of 0.61ms^{-1} in wind speed. Adults were present in some of the observations at the highest wind speed recorded (2.2ms^{-1}).

Fig. 5.IV Mean density of C brevitarsis relative to wind speed.


$\hat{y}$ = 1·426 − 0·491x
b = 0·491 ± 0·123
M_L
1·6
1·2
0·8
0·4
0
0
0·8
1·6
2·4
Wind speed , $m s^{-1}$

(c) Location of biting; variation between animals

The analysis of variance in this experiment was over sensitive undoubtedly due to the very large number of degrees of freedom associated with the error sum of squares (table 5.3). Every source of variation was, as a result, highly significant. Using P = 0.001 as the rejection level only the interraction between times and positions was insignificant, however all of the interractions contributed very little to the total variation compared to the main treatment effects. They have therefore been considered to be biologically insignificant and ignored.

The number of flies was greatest on the heifer and least on the cow with the bull intermediate and not different from either of the others. Days, which were virtually used as replicates, were significantly different from each other, a result that was to be expected. There were significant differences between positions with most flies on positions 1, 3, 4, 5, & 6 followed by a rapid reduction to positions 10 (fig. 5.V).

The number of flies on the hosts in relation to time confirmed earlier results (fig. 5.IIa). On the log scale there was a significant regression between time and mean number of adults the latter being halved each 2.1 hours after sunset.

(d) The distribution of flies on the animal

Occasion 1 (table 5.4).

The differences between the times of the replicates are similar to those already shown (fig. 5.VI) and need no further discussion. All of vertical, horizontal, and their interraction were significantly different. The interraction suggests that the density of flies decreases more rapidly vertically than horizontally (fig. 5.VII) with the greatest concentration along the very top of the back, but with a tendency for the density to be high further down the flank at position 7 (fig. 5.1).

TABLE 5.3 Adults on host, effect of time, position & host; analysis & means.

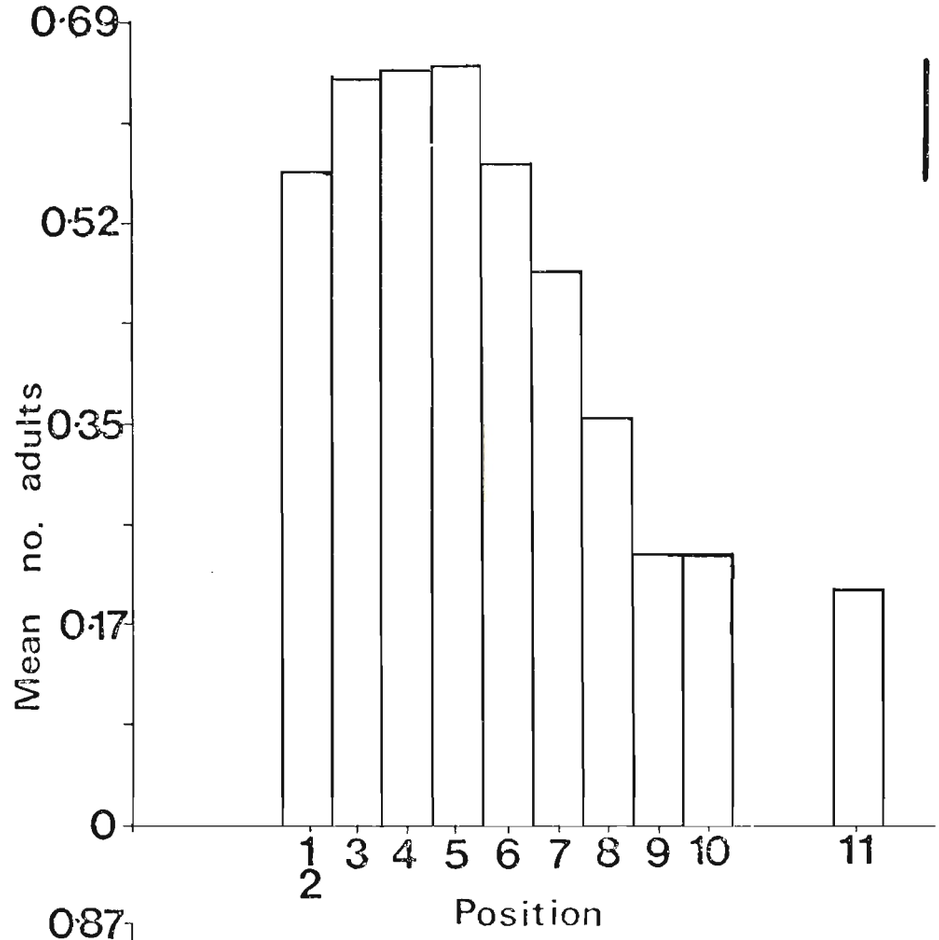
source	DF	MS	F	P
days	2	3.694	81.60	***
treatments	329	0.363	8.03	***
times	9	4.037	89.19	***
positions	10	2.910	64.28	***
animals	2	1.436	31.72	***
cow v others	1	2.430	53.68	***
bull v heifer	1	0.510	11.26	***
txp	90	0.080	1.77	**
p x a	20	0.778	17.18	***
t x a	18	0.400	8.84	***
t x p x a	180	0.092	2.03	***
residual	658	0.045		

means

animals	M_W	M_L	significance	
heifer	2.43	0.53	a	
bull	2.0	0.47	a b	LSD (P=0.001)=0.22
cow	1.5	0.40	b	
position				
5	3.5	0.656		
4	3.5	0.650		
3	3.4	0.643		
1	2.7	0.573		
6	2.7	0.573		
2	2.6	0.556		
7	2.0	0.478		
8	1.2	0.352		
9	0.7	0.235		
10	0.7	0.235		
11	0.6	0.204		

Fig. 5.V Mean number (M_L) of C brevitarsis at each position along the animals back.

Fig. 5.VI Mean number of C brevitarsis related to minutes after sunset.


0·69
0·52
0·35
0·17
0
Mean no. adults
1
2
3
4
5
6
7
8
9
10
11
Position

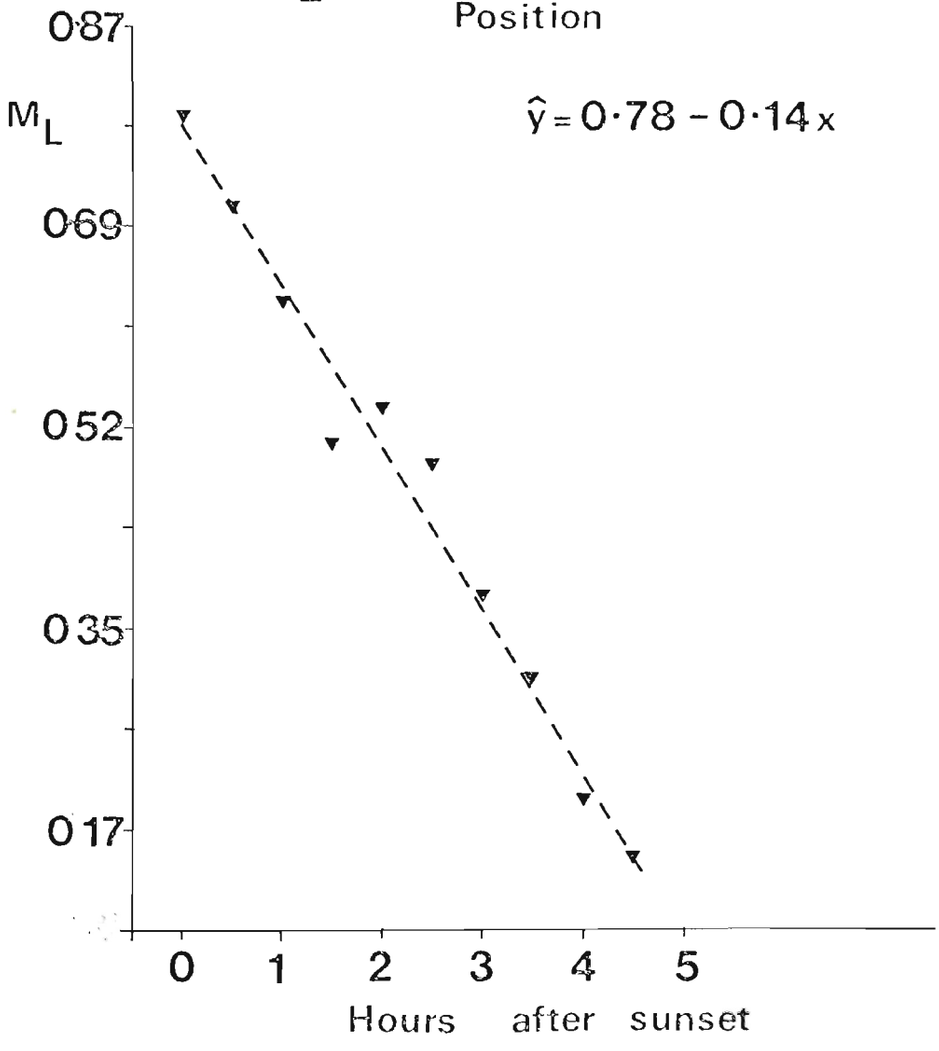

0·87
0·69
0·52
0·35
0·17
M_L
$\hat{y} = 0{\cdot}78 - 0{\cdot}14x$
0
1
2
3
4
5
Hours after sunset

TABLE 5.4 Distribution of flies, occasion 1; analysis and means

source	DF	MS	VR	P
time	3	0.420	13.1	< 0.001
vertical	3	0.832	25.9	< 0.001
horizontal	3	0.264	8.2	< 0.001
v x h	9	0.129	4.0	< 0.001
residual	45	0.032		

Occasion 2 (table 5.5)

As on occasion 1 the effect of the time of observation was significant, as were vertical and horizontal and the interraction between the latter two. The interraction was similar in general to that on occasion 1 although it varied slightly in exact detail. The region of highest density (fig. 5.VII) extended further down the flank in position 3, 4, & 5, but did not extend to position 7. The rate of decrease was greater vertically than horizontally.

TABLE 5.5. Distribution of flies, occasion 2; analysis

source	DF	MS	VR	P
time	1	0.29	13.7	< 0.001
vertical	5	1.84	85.5	< 0.001
horizontal	9	0.46	21.4	< 0.001
v x h	45	0.07		
residual	59	0.02		

Posture of back and head of calf (table 5.6)

When standing, calves' heads are level with their backs and the greatest concentration of flies was on the back changing little, if at all, when the animal's head was bent partly downwards. When the head was bent down to the ground the difference between the number of flies on the head and the back became even greater, however, adults were almost exclusively on the head when that was much higher than the back.

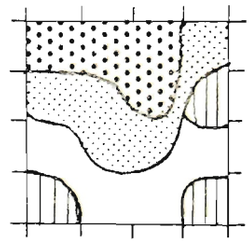
Fig. 5.VII Distribution of flies: occasion 1

Fig. 5.VIII Distribution of flies: occasion 2

◂ horizontal ▸

Occasion 1

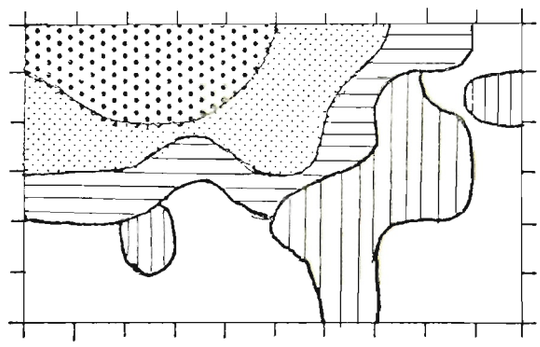
▲
vertical
▼



tail 

 head

Occasion 2



	1	2
	›1·0	›0·7
	›0·6	›0·4
	›0·3	›0·7
	›0·0	

TABLE 5.6 Posture of animal & distribution of flies

posture	head height	replicate	number of flies	
			head	back
standing	level	1	4	16
	level	2	7	12
	½ down	1	3	12
	down	1	0	10
	down	2	1	8
lying	high	1	77	22

Sex and condition of flies on hosts

All 879 adults collected from hosts were female. Of 453 nulliparous females dissected, 97% were mated. From 3-80% of females were parous.

Behaviour

When a fly landed on the host it walked on top of the hairs for a short time before burrowing towards the skin by walking down between hairs, thus becoming hidden. Some reappeared almost immediately, walked around and burrowed again, others not until they were full of blood. When blood fed, flies struggled to the outer edge of the hairs and immediately flew away.

No flies on the host appeared to be inconvenienced by or to react in any way to movements by the host.

Man biting

On one occasion another observer and on several occasions the author were bitten by C brevitarsis while collecting or counting adults on the host animal. One female was allowed to remain on the author for five minutes after apparently biting (as determined by pain) but did not feed on blood.

Host reaction

None of the hosts observed gave any visible reaction to the presence of C brevitarsis even though there were times when at least one thousand adults were present. The typical reaction by cows to other insects (e.g. mosquitoes), swinging their tail, was never observed with C brevitarsis. Twitching of sub-cutaneous muscle did not appear to be any more frequent in areas where the density of

adults was greatest.

Success at blood feeding on different animals

Observations, and the results given earlier in this section, show that more adults were attracted to the heifer than to the other two hosts but engorged females were readily caught on the bull and the cow, rarely on the heifer. The wooden surfaces of the race surrounding the heifer and the bull were searched carefully after the animals were released. At least 10x as many blood fed flies were collected from the race used to hold the bull.

DISCUSSION

Activity peaked about half an hour after sunset then was slowly reduced to almost nothing 6 hours later. Adults were recorded on the host at dawn in much smaller numbers. The pattern of activity is similar to that found for C furens (Kettle 1969a) except that the peak of C furens in the middle of the night was not observed and the significant peak near dawn was much less pronounced in C brevitarsis.

The observations based on counts on the calf did not entirely match those for the older animals. The peak of activity half an hour earlier on the calf, and the rate of decrease in following hour was greater. If the host was perceived visually during daylight hours, calves should be found as readily as cows, a result that would still occur if orientation were both visual and chemosensory during daylight hours. Gillies & Wilkes (1969) showed that mosquitoes were attracted to calves after dusk by host odours up to 40 yards, reinforced by carbondioxide at shorter range, and the warm, moist air at very close range.

A larger animal should exude more odour and carbon dioxide than a smaller animal and ought to be more attractive, and therefore discovered by more flies. A change from predominantly visual to olfactory discovery of hosts near sunset could explain the difference between the cow and the calf if the results for mosquitoes apply to C brevitarsis. A similar proposal was made by Kettle & Linley (1969a) with respect to C barbosai.

The greatest concentration of C brevitarsis occurs on the upper surfaces at or near the croup of the animal (about ¼ of the way along the backbone from the tail) then numbers decrease very rapidly down the flank and less so towards the head. The area of activity can change to the side of the animal if that is highest but does not change to the head unless the latter is forced much higher than

the rest of the animal.

Adults might land on the highest point of the largest part of the animal if they perceived it from above and aimed for the whole animal. The ability to move the area of highest concentration of insects around the host precludes any specific olfactory attraction to the croup/or adjacent areas.

The area of greatest density on the host varies between insect species, e.g. Culicoides marksii, alsoin Australia feeds from the legs and belly region (H.A. Standfast, personal communication). Nielsen (1971) showed that reasonably well defined, but not exclusive, areas were used by different species of Culicoides on cattle. Most species attacked on the legs and belly but, surprisingly, none were restricted to the same region as C brevitarsis. Obviously other species are not attacking the highest point of the animal suggesting that that hypothesis may be an oversimplification

The position of highest density of adults varies between the animals and is possibly a result of difference in shape and size of the croup and tail region. The peak density on the bull occurred on squares 1, 2, and 3, the broadest level surface on the animal. The shape of the tail region of the heifer and the cow differed from the bull but on both the peak density was on the broadest flat area. The animals differed in colour from each other but colour could not be correlated with changes in the position of peak density of adults. The possibility that the different colours of the animals influenced the position of the peak density of adults cannot be ignored.

The small numbers of blood fed females collected around the heifer suggest that, despite high counts, few adults were imbibing blood. There was one obvious difference between the heifer and the other two animals; the hair on its back was much more tightly pressed to the skin. A reduction in the number of females imbibing blood could result either from incorrect stimuli to feed, or physical prevention by the surface condition of the host, due to difficulties in penetrating closely matted hairs. Clements (1963) proposed three stages in host finding by mosquitoes; activation, orientation, and alighting. The observations suggest that a fourth stage may be involved after the host is reached but before feeding, a stage analagous to the host selection stage proposed for parasites on insect hosts (Salt, 1938). Inability to feed at the first burrowing may indicate the rejection of feeding on the host at that particular point suggesting that "Host selection" may differentiate between

areas on the same host. Dirt packed around the base of the hairs and peeling skin could easily prevent C brevitarsis reaching the skin and feeding.

It is always possible when making arbitrary divisions of a continuous process such as host discovery to subdivide the whole process into smaller and smaller sections. Subdivision adds nothing to an understanding of the dynamics of the whole process, unless it is made part of a research programme in which the process is divided, analysed, and then integrated into a complete explanation of the process involved. The additional stage suggested above may be of minimal importance where haematophagous insects are finding a host especially when that host has enough blood to feed many of them. It is additional to those suggested by Clements (1963) and increases our understanding of the whole process as well as indicating a degree of similarity between discovery of a host by a haematophagous insect "seeking" a blood meal and by a parasite "seeking" a site for oviposition.

C brevitarsis was present on the host for a long period each day. The density increased rapidly in the hour around sunset when the temperature fell on all nights (e.g. from 25.4^{o} to 22.2^{o} on five nights selected at random; all with cloud cover less than 4). The increase in density of flies on the host at lower temperatures may not be related to the change in temperature but rather to a change in solar time accompanying the temperature change. At 40 and 60 minutes before sunset the temperature did not differ between the observations with and without adults while temperatures ranged from 20^{o} to 30^{o}. Changes in light intensity were minimal, suggesting that temperature does not control the initiation or cessation of biting.

The increased activity on the host animal in the absence of direct sunlight (during daylight hours) complements the observation, (Standfast personal communication) of C brevitarsis on hosts when heavy cloud reduced sunlight.

Wind speed appears to be the most important factor of the environment in modifying the density of adults on the host. The slope of the regression line (b) can be used as a measure of the rate of reduction in numbers on the ordinate relative to shifts along the abscissa and can be used to compare the effects between different situations or species. The number of C brevitarsis was halved by an increase of $0.6ms^{-1}$ in wind speed compared to $0.67ms^{-1}$ for C furens and $0.85ms^{-1}$ for C barbosai (Kettle, 1969b).

Males were not collected from the host but with only 800 determinations of sex it cannot be stated categorically that males never land on the host but it is reasonable to reject the possibility that mating occurs on the host as it does in C nubeculosus (Pomerantzev, 1932).

The very high proportion of nulliparous females that were already mated (97%) when collected from the host indicates that, in the field, mating precedes blood feeding.

Riek (1954) did not while Lee and Reye (1953) did record instances when men were bitten by C brevitarsis. The instances were rare; Reye (1964) did not list man as a host.

Biting of humans is increased when they are within 2 metres of a suitable host. As previously noted Clements (1963) suggested division of host discovery in mosquitoes into three phases. The latter two being orientation to the host and alighting. Biting of man close to but not distant from cattle suggests that the initial phases of orientation are to the specific host (as found for mosquitoes to calves; Gillies & Wilkes (1969)) with the aberrant few then alighting on man. Orientation to humans from a distance does not appear to occur.

The absence of a reaction in the hosts being bitten suggests that C brevitarsis is very well adapted to feeding on cattle. Perhaps more so than horses where it leads to a dermatitis reaction (Riek 1954) Horses seem to react to the attack of C brevitarsis by twitching muscles, flicking their tail, and rubbing against posts, none of which were observed with cattle.

Standfast and Dyce (1968) reported that the animals (cattle) appeared to be distressed by the joint attack of mosquitoes and midges (mostly C brevitarsis), reacting with vigorous stamping of feet, switching of tail, and movement. Mosquitoes were rare on animals during the examination of the incidence of C brevitarsis in these studies; the restlessness found by Standfast & Dyce may have been a reaction to the mosquitoes alone.

BLOOD FEEDING & MATING

In many species of Culicoides the sequence of mating and blood-feeding does not appear to be important. C nubeculosus may mate in swarms or with females in the process of feeding on the host (Pomerantzev, 1932; Downes, 1950). Mating of C variipennis (Jones, 1960), C guttipennis (Hair, & Turner, 1966), C arakawae (Morii & Kitoaka, 1967), as well as L kerteszi (Rees et al 1971) occurs before,

during, and after feeding on blood. Newly engorged females of C arakawae were particularly attractive to males. The two functions may be associated but do not appear to depend on each other in the cases cited above. It must be remembered, however, that all the above observations were made on individuals in artificial situations such as colonies in the laboratory. and therefore may not be directly applicable to nature. None of the species listed above require that mating occurs within swarms. Mating may precede feeding in species that require mating to occur in swarms.

Of the nulliparous females on the host 97% are already mated hence mating usually precedes feeding. It does not necessarily follow that that order is essential but does suggest that it is the order in which they should be attempted in the laboratory.

VI OOGENESIS

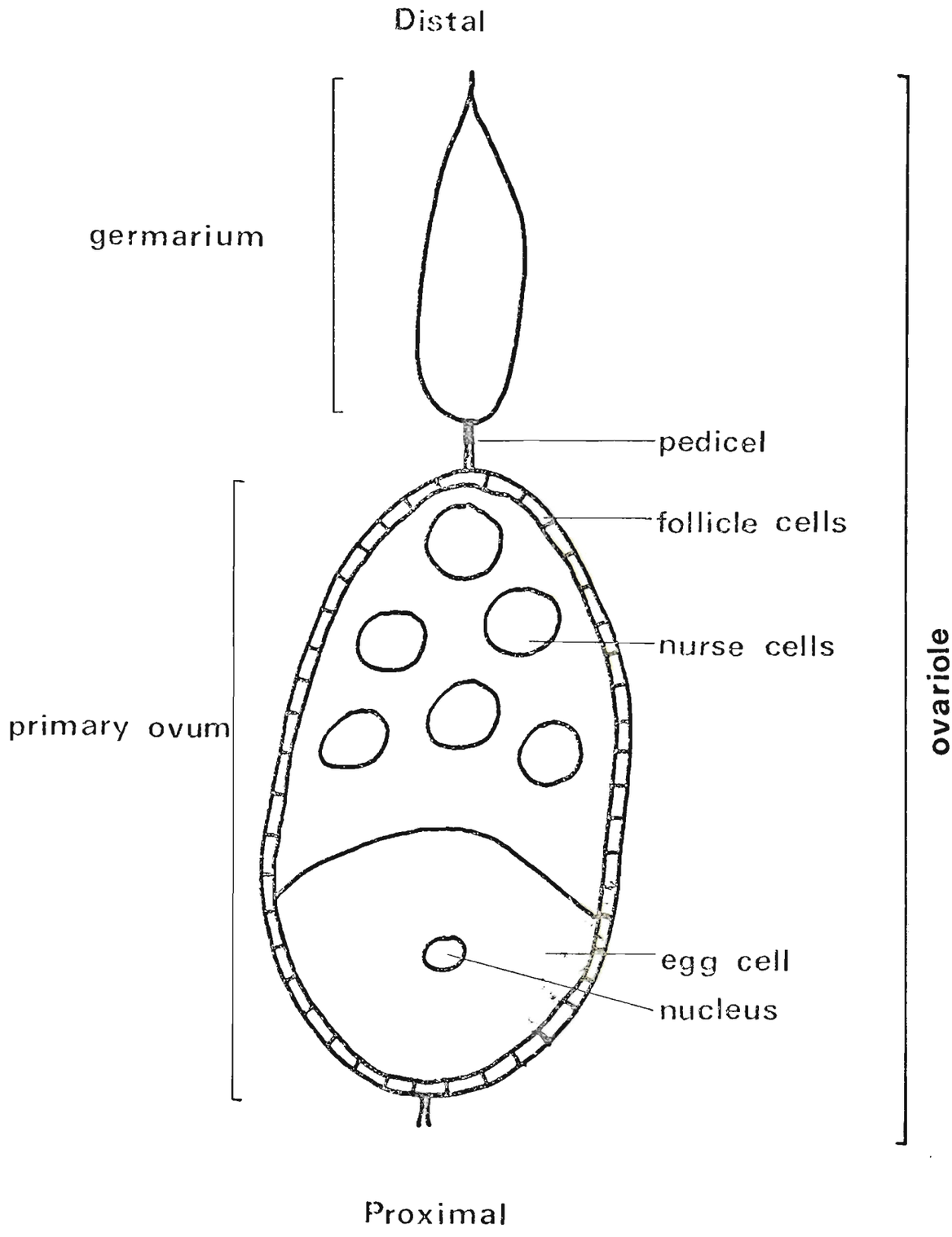
INTRODUCTION

Oogenesis has been studied in a number of species of Culicoides and related genera (Glukhova 1958, Amosova 1959, Linley 1965 and 1966; and Kay 1973). The relationship between the digestion of the blood meal and the development of the ovule, gonotrophic harmony, was examined by those workers. Linley (1965, 1966a) made a detailed study of gonotrophic harmony at different temperatures. Other authors (Hill 1947, Jobling 1953, Jamnback 1961, Becker 1961, Nevill 1967) have estimated the duration of the oogenesis cycle as the time between the taking of a blood meal and oviposition, and have described the egg. Only one blood meal is required to mature a batch of eggs (one egg from each ovariole) in C brevitarsis as in many other Culicoides species (Amosova 1959, Glukhova 1958). The ovarioles of C brevitarsis are meroistic. Nurse cells are grouped with the egg cell within a sheath, to form the ovum.

Different authors have used different names to describe the various structures within the ovary. In order to prevent confusion the names used in this paper are defined in Fig. 6.I (Linley (1965) and Insects of Australia (CSIRO 1970 p.69)). The ovum developing to maturity, as the result of a blood meal, is called the primary ovum. It is well demarked from the germarium and from the next ovum to develop, the secondary ovum, which will develop to maturity after the primary ovum has been oviposited and a blood meal consumed.

The appearance of the surface of the ovum changes in the late stages of oogenesis as chorion develops and ansulae are formed (Becker 1961). Ansulae were described by Hill (1947), Parker (1950). and Jobling (1953) Becker (1961) coined the term ansulae because it had been suggested that they were analogous to small suckers, and their function was to attach the egg to the substrate, but doubted that they were responsible for adhesion.

Fig. 6.I Diagrammatic representation of follicle.


Distal
germarium
pedicel
follicle cells
nurse cells
primary ovum
ovariole
egg cell
nucleus
Proximal

MATERIALS AND METHODS

Adults of C brevitarsis, already blood fed, were collected from cows in the field, incubated for specified times, killed, and dissected. The stage of development and the size of the ova within each female were described and measured.

Adults were collected using a 'suction sorting gun' (Dyce et al 1972), and in a light trap modified from that described by Dyce et al (1972) and Sudia and Chamberlain (1962) by substituting an ultra violet light (8 watt fluorescent) for the incandescent globe. Adults were not killed in the trap which was operated in a small cattle yard containing one or more animals from dusk until 2200h. Very few of the females collected were engorged with blood. While feeding,adults were hidden in the hairs on the animal's hide, then they moved to the surface and immediately flew away making their collection difficult. Only 20-40 engorged females were collected on any one night, even though many thousands had been on the animal. Engorged adults landed on surfaces near the host, and stayed there for at least half an hour. The collector was a moderately good collection site, but the wooden rails of the surrounding fences were much better. Engorged adults were collected from those rails between dusk and 2200h.

The females were stored in microsemple tubes in a vacuum flask over ice until 900h the next day when they were sorted, retaining only females which had fed to repletion. The engorged females were stored at 24-26^{o} and 75-85% humidity, in dim light and offered 10% sucrose solutions.

Incubation ranged from 0-70h in 5h intervals, twenty adults incubated for each period. Females were dissected within 20 minutes of death. The ovaries and the spermathecae were separated and each ovary was dissected.

The degree of digestion of the blood meal and the degree of development of the ova were classified (Linley 1965). Like Linley (1965), but unlike Glukhova (1958), stage I-II of ovum development could not be recognised. Unlike Linley (1965), the stages of digestion of the blood meal were difficult to evaluate externally because the blood slanted across the abdomen (as recorded by Amosova (1959) and Glukhova (1958)), and development of pigment within the abdomen masked the changes within the mid-gut in the later stages of digestion. Stages 1 and 2 of digestion (Linley 1965) were easily distinguished before dissection, stages 6 and 7 easily after dissection, but 3, 4 and 5 must be treated with reserve. The

stages of development of the ova did not differ from those described (Linley 1965) for Leptoconops becquaerti. They are:-

N_0; no differentiation of cells within the ovum.

N; cells of the follicular epithelium differentiated.

I; the cells within the ovum are differentiated.

IIa; yolk begins to appear within the ovum.

IIb; yolk occupies up to half the length of the ovum.

III; yolk occupies up to three-quarters of the length of the ovum.

IV; yolk occupies more than three-quarters of the length of the ovum.

V; chorion starts to develop around the ovum.

The greatest length and width were measured on each ovum. Width was measured between the inside edges of the ansulae layer (which corresponded to the outside edges of the chorion layer) of ova at stage V. Ansulae were photographed, at various stages of development, using a light microscope and those on mature eggs oviposited on wet filter paper,using a scanning electron microscope.

Adults were collected from the host before they had had sufficient time to feed to repletion, but only very few were obtained because of difficulties in both discovery and dislodgement of feeding adults.

Those collected were sorted into one of three classes (Pillit and Jones 1972 defined the stages).

1. Fully blood fed; the abdomen was extensively distended and appeared to be full of blood. This class approximated stages 4- and upwards.
2. Moderately blood fed; slight distention of the abdomen, approximating stages 2+ to 3+
3. Very small blood meal; blood was present in the mid-gut but did not distend the abdomen. Approximated stages 0-2.

The adults of each class were held for 60h before dissection.

Quantitative measures were not available for (i) the degree of the development of pigment in the abdomen, and (ii) the degree of development of curvature and ansulae on the ovum at stage V. In each case development was arbitrarily divided into three classes, defined as:

Class I; no development

Class II; partial development

Class III; complete development.

Forty females which had emerged in the laboratory, were held under similar conditions. They were dissected 0, 1, 5, 12, 14, or 18 days after emergence.

RESULTS

The development of the primary ovum

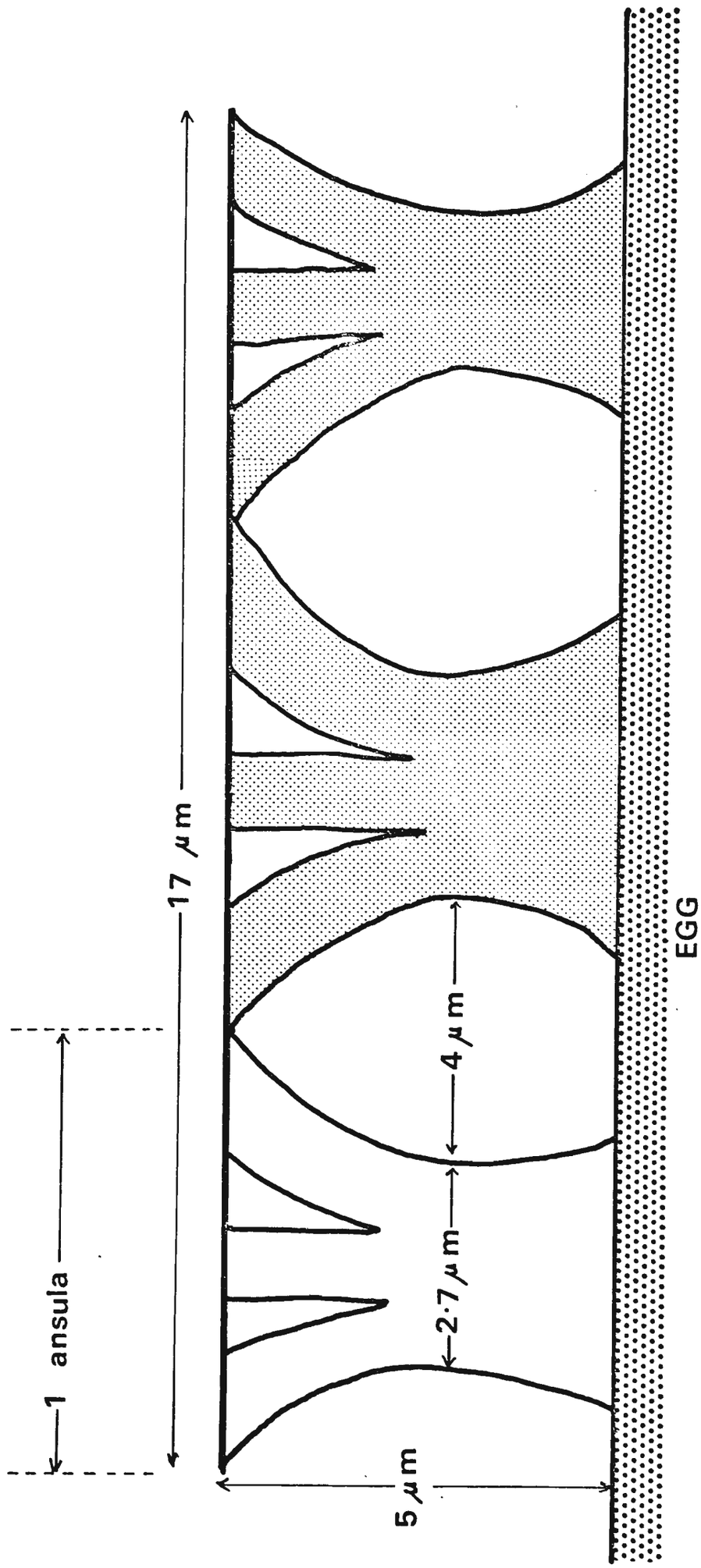
No differentiation can be seen in ova at stage N_0. Differentiation develops (stage N) then the egg cell becomes visually different (stage I), yolk appears (it can be seen very easily using dark field illumination) and gradually extends throughout the ovum. When it occupies less than half the ovum, the ovum is at stage IIb and by stage IV yolk fills the ovum. A central clear spot appears in the yolk (plate IId) of most ova up to the end of stage IV and can sometimes be seen in early stage V.

The shape of the ovum is not modified by pressure from the coverslip up to stage III but the shape of stage IV ova is malleable and they are easily ruptured. Stage V ova do not burst and their shape is not malleable, allowing them to be easily separated from stage IV. The chorion develops beneath the sheath surrounding the ovum (plate IIe). Small protuberances then appear (plate IIf) on the surface and gradually extend outwards towards the sheath, They appear to branch at the ends (fig. 6.II). The sheath ruptures at one end (plate IIf) and progressively degenerates until it disappears. A hexagonal pattern on the surface of the mature ovum appears to be derived from the arrangement of ansulae, but really only from some of them (plate IIIb). Before ovaries in two females were dissected the primary ova were grouped at the proximal end of the ovary, the ovarioles at the distal end.

Ansulae are longer on the concave (8-9μm) than the convex surface (5-6μm) and least at the ends (2-2.5μm). There is a clear line of demarcation between the two regions of ansulae (plate IIIa) with the hexagonal pattern only on the convex side. The ansulae on the convex surface have rounded or bent tops (plate IIIe) often with small tubercules on them, or threads of cuticle joining adjacent ones (plate IIId). The ansulae on the concave surface are quite different and as a whole resemble a miniature forest (plate IIIe). Their outer edges are expanded and appear as two concentric circles joined by cross walls (plate IIIf). The outer edges are nearly contiguous, nearly forming an outer layer to the surface of the egg.

During the change from IV to V, ova develop variable degrees of curvature, but it was difficult to define any temporal relationship between the development of curvature and ansulae (table 6.1). Some stage V ova do not appear to be curved but still show

Fig. 6.II Schematic representation of ansulae.


17 µm
1 ansula
4 µm
2·7 µm
5 µm
EGG

differences between the surfaces, the concave flat and the convex curved. Observations of curvature depends on the orientation of the ovum under the cover slip. Poor orientation was probably responsible for some of the apparent lack of curvature.

TABLE 6.1 Changes to stage V ova;

ansulae*	curvature*		
	0	1	2
0	37**	1	0
1	6	5	7
2	3	3	38

*degree of development
** number of females.

The development of the secondary ovum

The secondary ovum started as a slight swelling of the proximal end of the germarium (stage B) which increased in diameter (stage C) and gradually became distinct from the germarium (stage D) At stage A there was no change in the germarium. At the resting stage (the ultimate development of the ovum before oviposition of the primary ovum and the consumption of another blood meal), the secondary ovum was distinct from the germarium and at stage N or I.

Little or no development of the secondary ovum was observed before the primary ovum reached stage III. The secondary ovum reached stages D, N, or I about the same time as the primary reached stage V. It underwent very little further development except that those at stage D developed further before resting. There was good harmony between development of the primary and secondary ova (Table 6.2). Secondary ova were very small and arranged at various angles under the cover slip causing difficulties in measurement. Size approximately doubled from the undeveloped germarium to the ovum and germarium at the resting stage (table 6.2), when the secondary ovum is sub-spherical (40μm long, 34μm wide).

TABLE 6.2 Development of germarium and primary ovum

primary ovum	secondary ovum					
	N_o					
	A	B	C	D	N	I
I	7					
IIa	25	2				
IIb	4					
III	6	7	12	2	10	
IV			1	13	21	
V				6	40	125
size (μm)						
overall length min.	34	40	45	45	55	
max.	40	50	55	55	65	
secondary ovum only						
min.		-	20	20	24	40
max.		20	26	26	30	45
width		20	17	20	20	34

Changes in the primary ovum

The minimum period for oogenesis was 30h (table 6.3); for (17/20) 85% of females completed it in 40h, while all did after 45h.

Fig. 6.III Size of ova at each stage of development

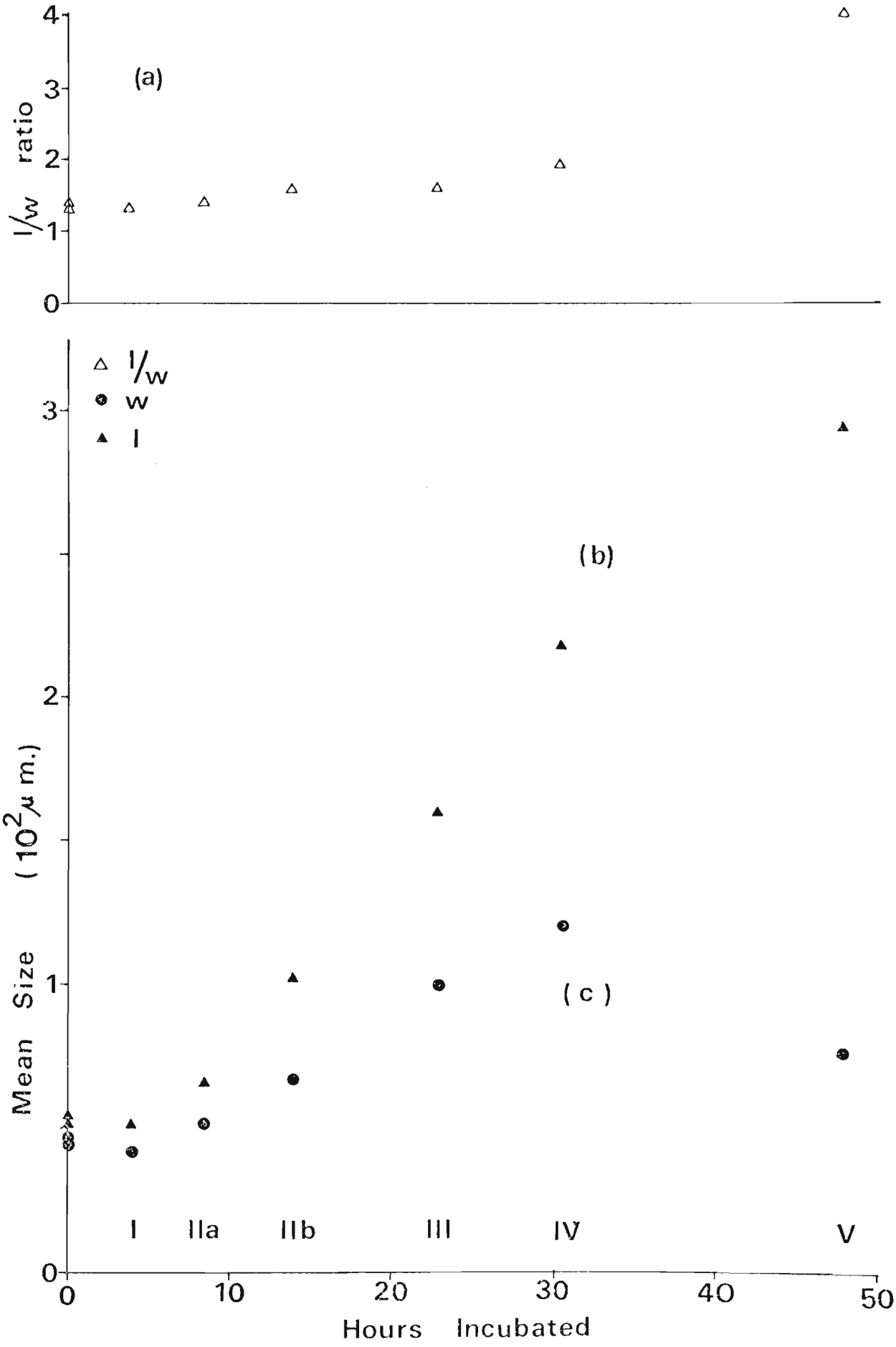

(a)
l/w ratio
4
3
2
1
0
△ l/w
● w
▲ l
(b)
(c)
Mean Size (10² μ m.)
3
2
1
0
I
IIa
IIb
III
IV
V
0
10
20
30
40
50
Hours Incubated

TABLE 6.3 Development after blood feeding

time	development							
	N_o	N	I	IIa	IIb	III	IV	V
0	2	8	5					
5			1	22				
10			1	9	9			
15			1	3	4	11		
20					2	8	1	
21½			2	2		3		
25						14	4	
30					1	5	14	2
35						1	3	2
37½						1	2	5
40							3	17
43½								5
45							1	11
50								19
55								12
60								8
65								6
70								10

There was a linear relationship between the mean length (L) of each stage and the period of incubation (fig 6.IIIb). The mean width (W) increased linearly to stage IV but was reduced in stage V (fig. 6.IIIc) The L/W was unchanged until stage IV but it increased in stage V (fig. 6.IIIa). The average ovum was 293.7 μm (SE = 2.4) long and 77.0 μm (SE = 1.5) wide at stage V.

Fecundity of female

Gravid females contained 31.3 ova (SE = 0.99), the maximum being 54. The most common size for egg batches was 32 to 36, but the frequency did not decrease fast on either side of that range (fig 6.IV). The distribution was a reasonably good fit to the normal distribution: (S = 8.9) $\bar{x}$ + s contained 41% of scores (expected 33.3%), $\bar{x}$ - s contained 28% (expected 33.3%) $\bar{x}$ + s to $\bar{x}$+2s contained 14% (expected 17%), and $\bar{x}$-s to $\bar{x}$ contained 17% (expected 17%)

Gonotrophic harmony

Harmony between the development of the ova and the digestion of the blood meal was apparent (table 6.4). In nine females ova developed to stage V before digestion was completed, while in three the reverse occurred.

TABLE 6.4 Gonotrophic harmony

digestion	development							
(stage)	N_o	N	I	IIa	IIb	III	IV	V
1		2	13					
2	2	8	10	23				
3			1	11	16	15		
4					1	20	4	
5						7	7	1
6						2	12	8
7							3	91

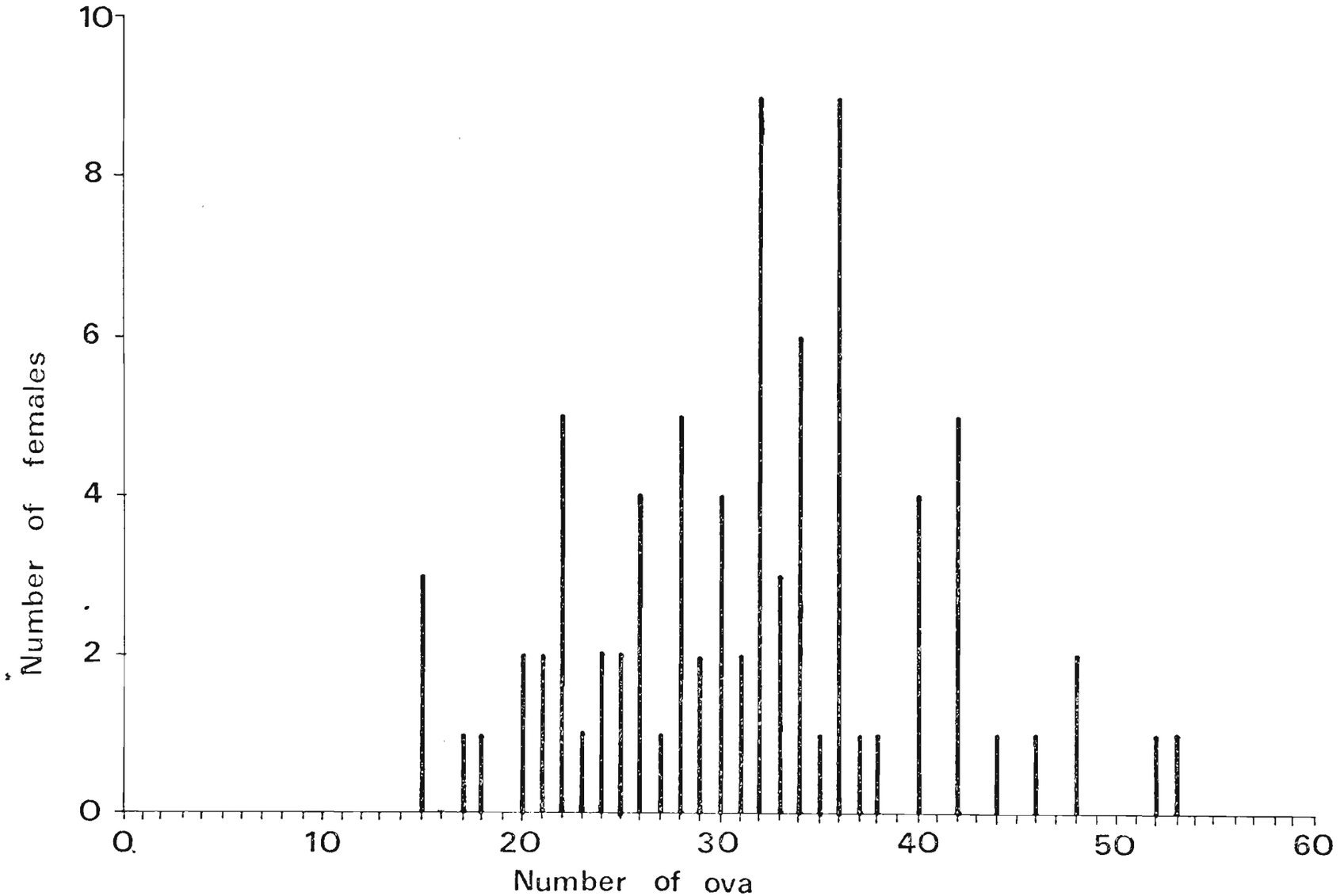
Pigmentation

Pigmentation was first seen in abdomens of females in late stage IIb(table 6.5) and was completed by the end of stage III. It appeared first at the anterior end, excluding the first 2 segments, and then progressed posteriorly to the tip of the abdomen.

TABLE 6.5 Development of pigment

ovum	pigmentation		
	0	1	2
N_o	2		
N	7		
I	9		
IIa	10		
IIb	3	3	1
III	4	8	12
IV			13
V			38

Fig. 6.IV Number of females containine specified number of ova at Stage V.


10
8
6
4
2
0
Number of females
0
10
20
30
40
50
60
Number of ova

Location of blood meal

In every female the blood meal was in the mid-gut, and the sugar meal in the ventral diverticulum. The difference between the locations was easily distinguished in the whole adult e.g. the female in Plate I f contained a small blood meal (black area) in the mid-gut and a small sugar meal (dark area) in the ventral diverticulum.

Oogenesis in virgin females

The females collected from the hosts already blood fed were almost all mated (271/283 = 95.8%). Of the virgins collected, ova in the five incubated for less than 21h (table 6.6) were at the same stage as in mated females. In another seven, incubated for 40h or more, no development had occurred in one, another's ova were at stage IV, while in the other five, degeneration had occurred but pigment was present indicating that ova had developed to stage IV.

TABLE 6.6 Oogenesis in virgin females

incubation	N	development	pigment
0	3	N N_o N_o	0
5	1	IIa	0
20	1	III	1
40	2	IV	2
		I	0
45	2	*	2
55	1	*	2
70	1	*	2
110	1	*	2

N = number of dissections

* = degeneration.

Oogenesis after a partial blood meal (table 6.7)

The adults were incubated for 60h. In ten females which had fed to repletion the ova were at stage V, but ova had developed to stage V in only five of eight which had consumed a moderate meal, and one of three which had consumed a small meal. The mean number of ova was reduced from 34.1 (full meal) to 2.0 (very small meal). All of the females were mated.

In all three females which had consumed a moderate blood meal, and in which ova had not developed to stage V, degeneration had begun. In one the secondary ova had developed to stage I (they were not found in the other two dissections). Ova had not developed in two of the three females which had consumed a small meal. In the other female, two ova in the same ovary, had developed to stage V the rest were at stage I, and no degeneration had occurred. The secondary ova had developed to resting stage behind the primaries at stage V, but not those at stage I.

TABLE 6.7 Oogenesis after a partial blood meal

size of meal	N	ova * at V	fecundity
full	10	10	34.1
moderate	8	5	12.4
small	3	1	2.0

* number of females with at least 1 ovum at stage V
N number females dissected.

Oogenesis without a blood meal (table 6.8)

All of the adults dissected were virgins. Development did not proceed past the resting stage when blood was not consumed and degeneration was observed in females incubated longer than 12 days. None developed pigment, an observation reaffirmed by the thousands of adults, which did not development pigment when maintained in the laboratory on sugar solutions.

TABLE 6.8 Oogenesis in virgin females

age of female (days)	n	number of females with ova at stage				degeneration	
		N_o	N	I	N&I	little	lot*
0-1	6	6					
1	17	1	11	2	3		
5	6		4		2		
12	17		1	5	11		
14	14			10	1	1	3
18	10			8		8	2

* not classified to stage; included in number of females with ova in each stage.
n number of females.

DISCUSSION

The proximal end of the germarium swells, separates into a distinct entity or ovum, and then differentiation occurs and the secondary ovum reaches resting stage. These developmental changes have not previously been described in C species although they were drawn on sketches of the primary ovum by Linley (1965) for L becquaerti. The development of the secondary ovum did not occur without development in the primary. Where degeneration of the primary ova had started, or was well advanced, the secondary ova had developed to the usual resting stage. In a female that had consumed a very small meal, the primary and secondary ova had developed in two ovarioles but neither ova developed in the remaining ovarioles. This suggests that the initiation of development of the secondary ovum is controlled by development within its primary. Visible development within the germarium had occurred by the time the primary ovum had reached stage III, but initiation of development and even development at the cellular level within the germarium may, and probably does, precede the morphological changes. An evolutionary advantage accrues to the individual in which the development of the secondary ovum is initiated by changes in the primary ovum, because such control ensures that there will be a primary ovum at resting stage in each ovariole.

The observations, while by no means convincing because of their paucity, indicate that development of the secondary ovum will proceed to the resting stage even though the primary ovum may degenerate before its own development is completed. This, too, has an evolutionary advantage. In the case, particularly, of virgin females which have taken a blood meal (4.2% of all blood fed females) development proceeded in the primary ovum but did not necessarily reach stage V before degeneration occurred. Degeneration of primary ova also occurred in females which consumed only a partial blood meal and has been observed in other species (Amosova 1959, Glukhova 1958, Linley 1965 and 1966a). It almost always occurs in C barbosai (Linley 1966a). If control of development after initiation of the secondary ovum resided in the primary, then the secondary may not develop to the resting stage if the primary degenerated, a result that would be disadvantageous to the genotype. How the control of the initiation of development within the secondary ovum is exercised remains unknown. The suggested control of development in the secondary ovum is probably common to other Nematocera which show gonotrophic concordancy.

Ova do not develop past the resting stage without consumption of a blood meal. At the resting stage, ova are at stages N or I (the few at sub-stage D probably develop to stage N before resting). Ova may be in different stages in the same ovary at resting. The resting stage is the same in C brevitarsis, C marmoratus (Kay, 1973), and C barbosai (Linley 1966a), but in L becquaerti (Linley 1965) and C furens (Linley 1966) it ranged from N_0 to IIa. In C griscescens and C obsoletus (Glukhova 1958) it is exclusively stage IIa.

None of many thousands of adults fed sucrose solutions in the laboratory developed eggs, therefore autogenous development of ova in C brevitarsis is extremely unlikely.

While the stages of development of the primary ovum differed little from other species of Culicoides (Glukhova 1958, Amosova 1959, Linley 1966a) or even from L becquaerti (Linley 1965), fecundity and the time required for oogenesis differed. The fecundity of C brevitarsis is lower than that of most other species of Culicoides, except C barbosai (Linley 1966a) in which many ova degenerate the result of very small blood meals. C obsoletus (Jamnback 1961, Glukhova 1958) with 17-36 ova in each female is similar to C brevitarsis. Fecundity has been determined for many species (Hill 1947, Jobling 1953, Glukhova 1958, Anosova 1959, Becker 1961, Jamnback 1961, Linley 1965 and 1966a, Nevill 1967, Kay 1973). It exceeds 100 in many species, the maximum being 252 in C circumscriptus (Becker 1961), an autogenous species.

In length and width the eggs of C brevitarsis are smaller than in many other Culicoides, being similar in length to those of C obsoletus (Glukhova 1958) and C obsoletiformis (Amosova 1959). The largest eggs in the genus are 490 μm long and 80 μm wide (C nubecolosus, Hill 1947).

The fecundity of C pallidipennis (Nevill 1967) thought to be conspecific with C brevitarsis by some workers (A.L.Dyce; E.M. Nevill, and H.A.Standfast personal communication), is 69 (range 41-86). Its eggs are 400 microns long (but the data is ambiguous and it is possible that this figure is not a mean for C pallidipennis but rather for eggs laid by a pool of Culicoides including several different species (Nevill 1967). The difference between the measurements and counts for the two species seems too great to be explained on the basis that the specimens of each were reared in a different continent. (Glukhova(1958) in Russia determined a mean of 39 eggs while Jamnback (1961) in New York State determined 17 to 36 eggs for C obsoletus, two measurements which are in much closer

agreement than those for C pallidipennis and C brevitarsis). The difference in fecundity and egg size need to be reconciled before C brevitarsis and C pallidipennis can be declared conspecific.

The time required to complete oogenesis has been determined in one of two ways in other species. Either the adults have been incubated and dissected to observe the development of the ova, or the time between the consumption of a blood meal and oviposition has been recorded. The latter method probably overestimates the time especially if mature eggs are retained in the female as illustrated by the lapse of 11 days between the end of oogenesis and the start of oviposition (Glukhova, 1958). Changes in temperature can cause large changes in the time required for oogenesis (Linley 1965), for which reason I have listed the temperature together with the time required for oogenesis in the species below.

C marmoratus	3d	20-29°*	Kay 1973
C impunctatus	15d	16-19°*	Hill 1947
C pallidipennis	2-3d	?	Nevill 1967
C barbosai	3d	33°	Linley 1966a
C furens	1½d	33°	Linley 1966a
C obsoletus	4-5d	21°	Jamnback 1961
C obsoletus	3d	?	Glukhova 1958
C griscescens	2-3d	25°	Glukhova 1958

* Adults incubated at ambient temperature

? Temperature not quoted.

The estimates for C impunctatus, C pallidipennis and C obsoletus, were made by counting the number of days between blood feeding and oviposition. Oogenesis probably occurs faster in C brevitarsis than in most other species (at comparable temperatures). Comparison with C furens is difficult because of the much higher temperature at which it was incubated

The greatest elongation of the ovum occurs during stage IV. The width increases similarly to length until the change from stage IV to stage V when there is a marked reduction in width which results from the formation of chorion well below the outer edge of the follicle cells or sheath. In the change from stage IV to stage V, the width of the ovum was reduced from 118 µm to 77 µm but some of the difference was due to greater flattening of the stage IV ovum by coverslip pressure.

The differences in the curvature between ova is partly the result of different orientation underneath the cover slip but some reflects real differences in curvature. The ova of late stage IV and early stage V are packed tightly into the ovary where they presumably are under pressure to conform to a shape imposed by the position they occupy within the ovary. Sclerotisation occurs at this time and may fix a shape already imposed. In that case ova in the outer parts of the ovary would assume greater curvature than those in the inner parts.

Ansulae appear to develop as a continuation of sclerotisation into the sheath material. If sclerotisation proceeded out along the pore canals it could form ansulae from the materials surrounding the canals. On the convex surface ansulae form a hexagonal pattern which probably reflects the outline of the follicle cells. There is a group of ansulae within the hexagon. The same pattern may be present on the concave surface but disguised by the more uniform size and shape of ansulae. Wigglesworth (1953) suggested that pore canals appeared, in some insects, to be 1μm in diameter but at that size they may really have been a group of pore canals. Sclerotisation of the walls of a canal 1 m in diameter would lead to a structure somewhat thicker - a size not inconsistent with the 2.7μm of the ansulae, which appear to be hollow at the top. Ansulae on the convex surface have domed outer edges. Both of these properties could be produced by outward sclerotisation. If the hexagonal pattern reflects the follicular cells, then there are certainly fewer ansulae than the least number of pore canals per cell (Wigglesworth 1953, p23—50-75, Sarcophaga larva).

The separation of the outer margins of sheath and chorion was greatest on the concave side. If ansulae are formed by sclerotisation between the chorion and the sheath, longer ones would develop on the concave surface. This does not explain the differences in appearance.

The ansulae on the concave surface are similar to the structures described as plastron on the eggs of Musca domestica (Hinton 1967). The outer perforated sheath supported on the outer meshwork layer of Musca domestica was not found on the eggs of C brevitarsis. Magnifications up to 10,000 times was used in an attempt to find such a layer seen at 3,000 times magnification by Hinton. It is highly likely that the layer of ansulae on the concave surface of the egg of C brevitarsis functions as a plastron,

subserving air exchange. Because of their greater separation it is less likely that ansulae on the convex surface, act similarly.

The development of ansulae in the space between the chorion and the sheath indicates that the whole of the difference in size between ova at stages IV and V is not due to cover slip pressure. Ansulae have been described in other Culicoides species (Becker 1961, Hill 1947, Parker 1950, Jobling 1953). The suggestion that they may be responsible for attaching the egg to the substrate is unlikely. It is more probable that adhesion would be achieved by secretions (Becker 1961), probably from the colleterial glands (Wigglesworth 1953).

It was not possible to examine the location of the concave surface in relation to the orientation of cleavage. It was therefore not possible to determine whether or not the concave surface was fixed in position relative to the embryo.

The rupturing of the sheath around the mature ovum may have been a result of pressures during dissection. However, the re-grouping of germaria and secondary ova at the distal end of the ovary before dissection in two instances, make this explanation unlikely. Observation suggests that, during the rupture of the sheath, the vast majority of it was destroyed. The remnants of the sheath form the follicular relict used to separate parous from nulliparous females in Culicoides (Dyce 1969). The rare occurrence of follicular relicts during the dissection of parous females of C brevitarsis is explained by the destruction of the sheath.

The 'hole' which appeared central in the yolk granules of the developing ovum may have been its nucleus. This was unexpected because in several other species, C barbosai, C furens (Linley 1966a) and C obsoletus, C griscescens (Glukhova 1958), the nucleus of the egg cell could not be seen once yolk granules appeared.

The greater range in stages of development of the ovum at any one stage of digestion of the blood meal in C brevitarsis than in L becquaerti (Linley 1965) may be due to the difficulty of clearly defining the stage of digestion in C brevitarsis.

The development of pigment started at stage IIb in C brevitarsis and C marmoratus (Kay 1973), and was completed in the former by the end of stage III but not until early stage IV in the latter. Pigment developed in females which had matured only a small batch of eggs and in virgin females which had consumed a blood meal. Kay (1973) found pigmentation in C marmoratus females which had

developed a batch of eggs autogenously, therefore its development appears to be a function of ovarian maturation rather than digestion of blood.

The difficulty of obtaining females which had consumed only a partial blood meal precluded a detailed examination of oogenesis under those conditions. The size of the egg batch was probably determined by the size of the blood meal, a response also shown by C obsoletiformis (Amosova 1959), C griscescens, and C obsoletus (Glukhova 1958) and C barbosai (Linley 1966a). As with C griscescens and C obsoletus (Glukhova 1958) very small meals did not initiate development in some females. There was development of only two ova (and then in only one ovary) in one female of C brevitarsis suggesting that any number of ova can develop beyond the resting stage following a blood meal, the actual number determined by the size of the meal.

The low incidence (4.2%) of virgins amongst the females caught from a host animal after blood feeding suggests that mating normally precedes blood feeding. Ova developed to maturity in virgin females but some degenerated, however insufficient observations were available to compare the rate of degeneration in mated and virgin females.

In every case the blood was in the mid-gut, and carbohydrate in the ventral diverticulum in agreement with observations in other Nematocera (Gooding 1972).

VII LONGEVITY IN THE LABORATORY

INTRODUCTION

The requirements of adults for food and liquid varies widely between different insects and may be assessed on the basis of either longevity or production of spermatids and ova. Females of many Culicoides, in common with all blood sucking Nematocera, require a blood meal before they can mature ova (Kettle 1962) but some are autogenous (e.g. C bermudensis; Williams 1961) Males do not require a blood meal for the maturation of spermatids and indeed do not feed on blood.

Males develop fully active spermatids (p. 56) when fed 10% sucrose, and some will do so when offered no food at all. The food offered for adult maintenance, as distinct from blood for development of ova, is most commonly 10% sucrose, 5% sucrose, or 10% honey solution (Jones 1960, Morii & Kitoaka 1967, Linley 1968b). Only split raisins were offered to C nubeculosus (Megahed 1956), in contrast to a complex diet of raisins, sugar and adult house fly diet (LaBrecque and Gouck 1963) offered to C guttipennis (Hair & Turner 1966).

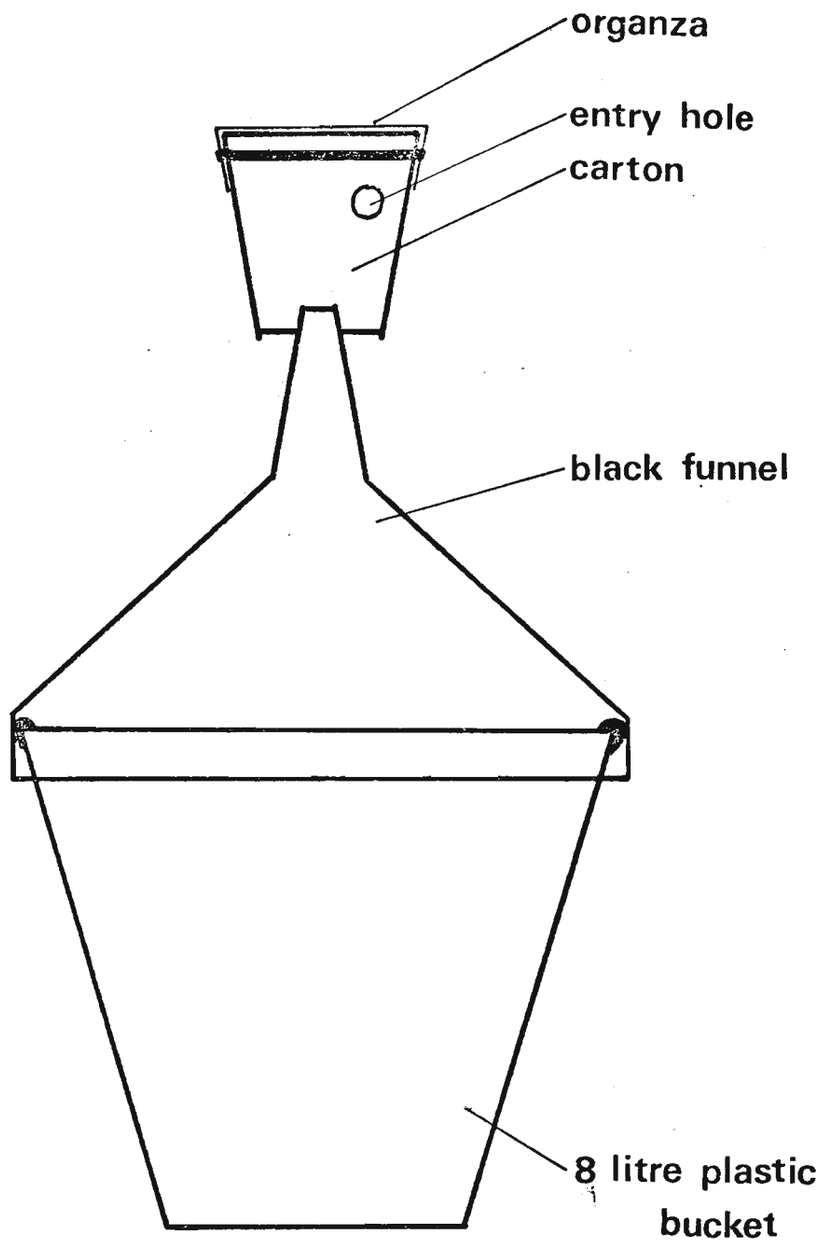
The failure of various techniques to induce swarming in cages (p. 61 et seq.) and the possibility that the age of the male may be important demanded the development of a technique which would allow rapid discrimination between adults of various ages. Techniques such as dusting with powders (Southwood 1966) involve considerable difficulties when used with adults as small as C brevitarsis. The most easily applied method, mass dusting of adults with powder, is unacceptable because, especially with frenzied activity near dusk, powder could be transferred between adults either directly or via the cage (e.g. Senior White et al, 1945).

The most obvious feature of adults of C brevitarsis that had fed on sucrose solutions was an extremely enlarged and translucent crop in which the ingested solution was stored. Colouration of the feeding solution could provide an easy method for marking adults.

MATERIALS AND METHODS

Pats were collected when approximately one week old and held in the laboratory at 25° and 75-85% humidity in emergence containers (fig. 7.I) designed by H.A. Standfast. Most adults emerged between 1000 and 1600 hours and were removed at 1600h daily with a suction sorting gun sorted by sex on a chill table and transferred to cages.

Fig. 7.I Emergence bucket


organza
entry hole
carton
black funnel
8 litre plastic
bucket

The solutions fed to the adults were absorbed into a 30mm long by 2mm wide strip of "wettex" placed on top of the gauze. Free liquid was not allowed.

(a) Age at Death.

Ten males were put in each of thirty cages, ten females in another thirty. Ten cages of each sex were allocated at random to the following treatments,

(i) no food

(ii) water on wettex

(iii) 10% sucrose on wettex pad

The feeding solution was replaced daily when the dead adults were removed and counted, and continued until no adults remained alive in each cage. Age at death, in days, was recorded for each adult.

(b) Selection of marking agents

Fifty unsexed adults were placed in a carton and offered 10% sucrose to which sufficient of one of the dyes (Table 7.2) had been added to colour the solution strongly. The dyed solution was offered only during the first 24 hours after which sugar solution only was offered. The living adults were sorted into coloured or not on the second and eighth days after the start of the experiment.

(c) Attraction to Sucrose

Approximately 100 newly emerged unfed adults were put into a carton covered by gauze on which four concentric circles were drawn. They were 40 (1), 20 (2), 10 (3), and 5 (4) mm diameter. To test for attraction to sucrose observations were made with nothing and then with a drop of 10% sucrose solution at the centre.

To measure the insect's response the first ten adults moving into circle 4 were watched and the sequence of circles they crossed recorded. Recording stopped when each adult moved away from the centre. The same was repeated with ten adults crossing circles 2 and 3. Then sucrose was placed in the centre and the whole proceedure repeated. The experiment was replicated 3 times, each with a new carton of new flies.

RESULTS

(a) Age at Death (Table 7.1)

The mean age at death of males and females was the same. Adults fed on 10% sucrose lived longer (M_W = 8.9 days) than those fed water (2.3 days) which in turn lived longer than those not fed (1.2 days). There was a significant interraction between sex and the type of food caused by the greater survival of females than males

when only fed on water. Seven flies lived more than thirty days. On 10% sucrose the greatest mortality occurred in the first 24 hours (23%).

TABLE 7.1 Age at death of adults; analysis and means

source	DF	MS	VR	P
treatment	5	9.4	94	< 0.001
sex	1	0.3	3	NS
food	2	22.4	224	< 0.001
s x f	2	0.9	9	< 0.001
residual	594	0.1		

treatments

sex	food	M_W	M_L	significance
m	sucrose	9.2	1.01	|
f	sucrose	8.6	0.98	|
f	water	3.1	0.62	|
m	water	1.6	0.42	|
m	none	1.4	0.37	|
f	none	1.1	0.33	|

food

sucrose	8.9	0.99	|
water	2.3	0.52	|
none	1.2	0.35	|

(b) Selection of marking agents (table 7.2)

Colour was obvious, and persistent, in the crop of females fed with some dyes. Few dyes were suitable, as judged by percentage of adults coloured, persistence of colour, and survival of adults, i.e. lack of toxicity in the dye.

The most suitable were:

(i) Orange G:- colour distinct on the sixth but not on the eighth days. Colour did not change.

(ii) Food colours red, yellow, green, and blue:- still distinct on the eighth day. Colours did not change.

(iii) Light green:- no change in colour by eighth day.

(iv) Eosin (light red):- no change in colour by eighth day.

(v) Fuchsin (red);- no change in colour by eighth day.

Eosin, Fuchsin and food colour red could not be separated visually nor could food colour green and light green.

TABLE 7.2 Screening of dyes

dye	N	2nd day			8th day		
		n_a	n_c	%	n_a	n_c	%
rhodamine B	200	113	102	90.3	45	7	15.6
methylene blue	50	36	1	2.8	8	0	0.0
chlorazol black	50	38	32	84.2	0	0	0.0
janus green	50	23	15	65.2	1	0	0.0
orange G	100	71	71	100.0	36	25	69.4
food yellow	50	23	21	91.3	5	5	100.0
food red	50	22	22	100.0	16	14	87.5
food green	50	32	30	93.8	2	1	50.0
food blue	50	32	32	100.0	8	4	50.0
none	50	23	0	0.0	6	0	0.0
light green	50	15	15	100.0	6	6	100.0
carmine	50	21	19	90.5	0	0	0.0
gentian violet	50	18	0	0.0	14	0	0.0
safarin 0	50	26	0	0.0	14	0	0.0
congo red	50	28	13	46.4	13	0	0.0
crystal violet	50	0	-	-	0	-	-
indigo carmine	100	60	23	38.3	0	-	-
eosin	100	58	56	96.6	21	19	90.5
lissamine green	100	39	32	82.1	17	17	100.0
acid fuchsin	50	28	28	100.0	12	11	91.7
toluidine blue	50	23	0	0.0	19	0	0.0
aniline blue	50	25	19	76.0 (very small meals)			
black ink	50	10	2	20.0 (48 did not feed)			
blue ink	50	18	9	50.0 (32 did not feed)			

N_a = number alive N_c = number coloured N number initially fed.

% = N_c as % of N_a

(c) Attraction to Sucrose (table 7.3)

The proportion of adults that crossed between circles was influenced by the presence of sugar only for the innermost two circles (i.e. only from circle 3 to the sugar solution. When adults crossed circle 3 they were 3.5mm from the sugar solution.

TABLE 7.3 Number of adults crossing rings +/- sucrose at centre

rings	crossed		didn't		expected	
	+s	-s	+s	-s	cross	didn't
(a) crossing ring 1						
1-2	17	15	13	15	16	14
2-3	6	8	11	7	7	9
3-4	2	5	4	3	3.3	3.3
	$\chi^2 = 2.5$ NS					
(b) crossing ring 2						
2-3	16	16	14	14	16	14
3-4	8	12	8	4	10	6
	$\chi^2 = 2.1$ NS					
(c) crossing ring 3 (20 flies only +s)						
3-4	13	19	17	1	16	9
	$\chi^2 = 15.3$ $P < 0.001$					

+s sucrose at centre -s no sucrose

In early experiments up to 50% of adults were stuck (and killed) to sugar syrup from the cottonwool pad on which it was offered. Three methods were found by which adults could be fed without being stuck:-

(i) sucrose offered on damp wettex

(ii) water offered on wettex strip and sugar as a sugar cube (if R.H. exceeded 90% the sugar deliquesced and the adults became stuck), or

(iii) from a streak, applied with a syringe, of the following mixture; 40mls of 1% agar + 40 grams sucrose + 25 ml honey + 0.1 g Nipogen M.

The last mixture remains and is fed upon for three days, reducing the frequency at which solutions must be replaced.

DISCUSSION

Adults required a source of carbohydrate if they were to survive more than one or two days in the laboratory. Water alone may prolong life, but does so only marginally. The mean age at death for adults not offered food and held in the laboratory was 1.1 days while the time, under the same conditions, required for the disappearance of all of the striated body was also 1 day (p 57). Striated body has been regarded as stored metabolites; if that is correct then it may be used for both survival and sexual development during the first day of life and may be the major form in which metabolites are stored.

A number of dyes were selected, which clearly mark the crop of C brevitarsis for eight days but no longer. A similar technique was used to mark mosquitoes (0.01% rhodamine B in sugar solution) by Reeves et al (1948) and the gut of Drosophila (Wave et al 1963) with rhodamine B. Southwood (1966) comments that in some cases where materials have been ingested, only a small proportion of the insects would feed. In this study dyes such as Black and Blue Ink reduced the proportion of adults that fed but the dyes selected for use did not. The wide range of dyes tested allowed the selection of a number of different colours that were readily ingested by a large proportion of adults.

The behaviour of adults was not influenced by a drop of sugar solution 3.5 mm or more away from them therefore attraction could only operate over less than 3.5 mm. The apparent attraction of the sugar drop results because adults spend a long time at it, hence large numbers accumulate.

INTEGRATING DISCUSSION

Wide ranging features of the biology and behaviour of C brevitarsis have now been studied allowing the critical path plan to be filled out (fig. 8.1).

Eggs are laid on the surface of dung pats up to 6 days old, throughout the day but with a distinct concentration during the afternoon. Oviposition may have occurred into older pats, but if so eggs laid after the sixth day did not increase the number of adults that emerged. Eggs hatch approximately 36 hours later (at 25°). Larvae and pupae are distributed throughout the pat regardless of its moisture content with the exception that densities are very low in the crust. Pupation occurs anywhere in the pat, but may be concentrated in its upper regions, a minimum of nine days after

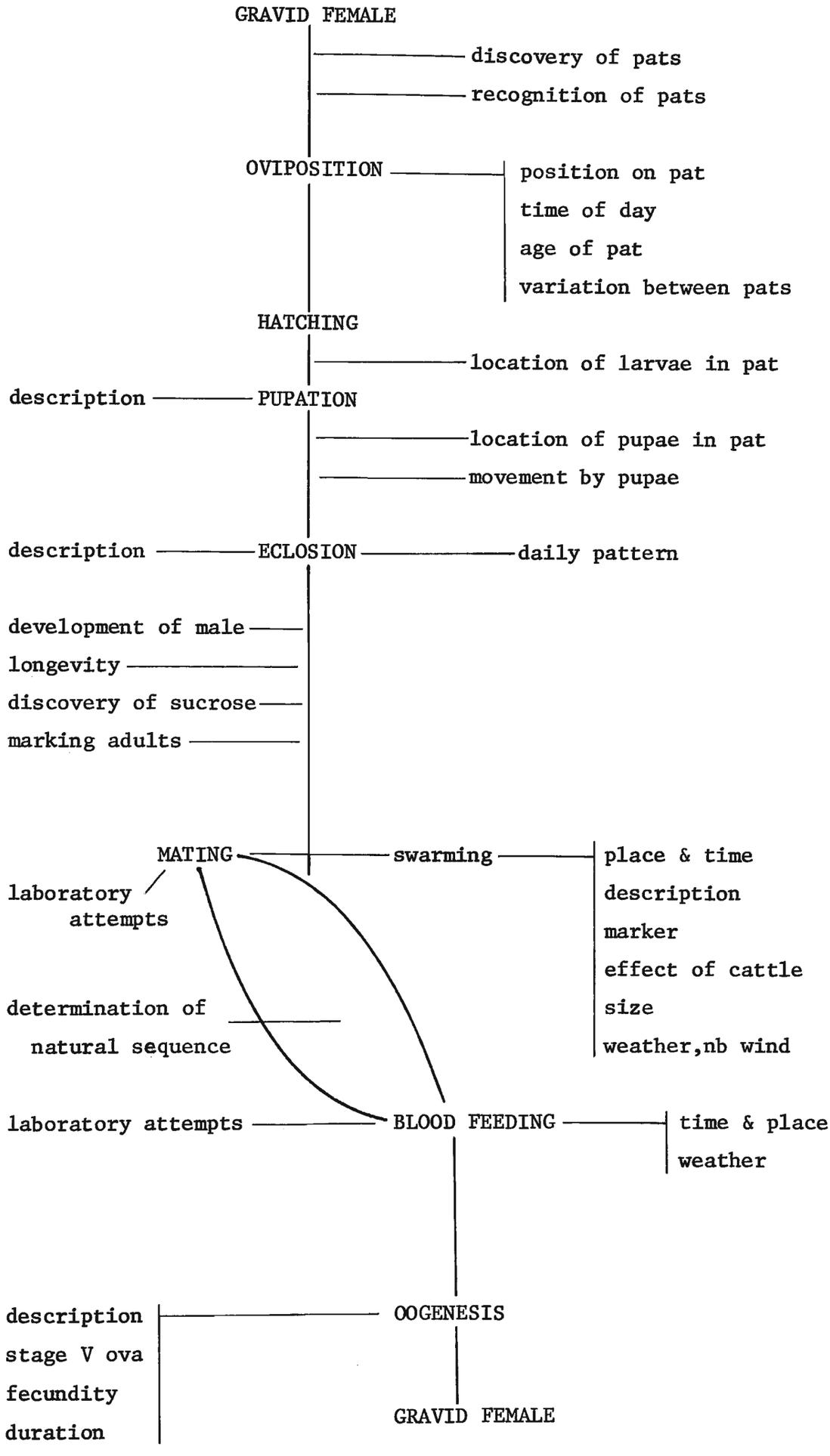


FIGURE 8.1 Critical path plan.

oviposition. The pupal stage lasts from 48-72 hours with exposure to light accelerating eclosion but not being obligatory. At higher intensities of light, eclosion is earlier.

Adults emerge mostly in the early afternoon although a few not until early evening. Spermatids are not fully developed until 20-24 h after emergence, at which time the metabolite stored (striated body) in the abdomen of both sexes has been completely utilised. Males in swarms always contain fully active spermatids.

Swarming occurs during the hour preceding sunset provided that wind speed does not exceed 1.91 ms^{-1}, but there is a high probability for swarming only when wind speed does not exceed 1.11 ms^{-1}. Swarms were predominantly male and were formed at high level when distant from calves or at low level when near calves, using as markers areas where light reflected from the ground was of very low intensity. The presence of cattle grossly altered the characteristics of swarms. Mating precedes the first blood meal in 97% of females but oogenesis can proceed in virgins. Blood feeding starts just before sunset and continues for up to six hours followed by oogenesis in the subsequent 40-45 hours (25^{o}). Mature eggs are covered with ansulae which, on the concave surface, probably act as a plastron, subserving air exchange. The gravid female finds pats visually and recognises them probably by their rounded shoulders which may appear as rings of decreasing intensity of reflected light.

C brevitarsis is multivoltine and anautogenous. The studies confirmed earlier observations on the time of blood feeding but refuted suggestions that oviposition occurred only in the evening into pats less than 24 hours old. Circumstantial evidence suggests that pupae are able, contrary to earlier suggestions, to move through the pat.

The life-cycle of C brevitarsis is one of the shortest in the genus (many Culicoides are univoltine). Life cycles of colonised species are (at 25^{o} where a choice of temperature was possible):-

(i) C furens (Linley, 1960): egg 3 days, larva 21 days, pupa 2 days, oogenesis 8-10 days: total 34-36 days (27^{o}).

(ii) C guttipennis (Hair & Turner, 1966): egg 2-3 days, larva 12+ days, pupa 3 days, oogenesis 4-5 days: total 25±4 days (27^{o}).

(iii) C nubeculesus (Megahed, 1956): egg 3-4 days, larva 10-26 days, pupa 4 days, oogenesis 3-4 days: total 3-8½ weeks ($20-25^{o}$).

(iv) C variipennis (Jones, 1957): eggs 2 days, larva 21 days, pupa 3 days, oviposition 4 days: total 30 days (25°).

(v) C arakawae (Morii & Kitaoka, 1967): egg 2-3 days, larva 18-26 days, pupa 2-3 days, oogenesis 3-5 days: total 5-6 weeks.

In comparison, in mid-summer the life cycle of C brevitarsis requires a minimum of:-

egg 2 days + larval and pupal periods 9 days + oogenesis 2 days = total 13 days.

The times of occurrence of eclosion, mating, and blood feeding show an interesting sequence that suggests that all could occur on the day of eclosion. Eclosion occurs in the early to mid afternoon, mating (=swarming) in the hour before sunset, and blood feeding in the six hours afterwards. Available evidence however is equivocal. In the males both striated body and spermatogenesis do not reach the condition in the swarming male for 24h after emergence, the same applying to striated body in the female. Activity in cages is definitely lower in the absence of sunlight, therefore activity may be greater in the field in which case striated body may be consumed faster. It is possible that females could emerge, mate, and blood feed on the same day, and that males join the swarms 24h after emergence. While the phases of eclosion, mating, and blood feeding occur over short periods, oviposition which cannot occur immediately after blood feeding occurs over a much broader period enhancing the belief that the first three occur in sequence.

Biology and behaviour have been explored just enough to show many avenues for further research. The hypotheses about the discovery and recognition of the pat need to be explored by attempting to develop objects that mimic the essential features of the pat and measuring their attractiveness to C brevitarsis. Flight patterns, particularly with regard to height, need to be investigated. The effect of cattle on swarming has been described and quantified but not explained. Further experimentation, initially by attempting to mimic cattle, should be carried out to try to understand the cause of the observed effect, and to attempt to develop artificial calves that could improve the chances of inducing swarming in captivity. The control of the height of swarms is unknown but would best be examined once colonisation had been achieved. Movement between markers needs to be quantified.

Colonisation is frustrated by the twin problems of mating and feeding both of which have been extensively attampted in small

cages but without success. The information now available needs to be applied within large cages in which dusk can be simulated.

If an insect is to transmit a pathogen it must fulfil certain criteria:-

(i) it must occupy the same segments of space-time as the pathogen and the host, particularly infected hosts,

(ii) it must consume two blood meals, at least, and live long enough for multiplication of the pathogen, and

(iii) the pathogen must multiply in the insect.

C brevitarsis complies with (i) and (ii) but its performance in (iii) is unknown and cannot be accurately assessed until laboratory colonies are available.

Resting places of adults, mortality estimates during development, proof that mating occurs in swarms, attraction to hosts are but some of the features of biology and behaviour yet to be explored.

PLATE I

a immature spermatids

b testis showing developing cysts

c spermatids from ruptured vas deferens
sp = spermatids

d accessory gland showing paired vas deferens (vs), clear zone (cz), and granular zone (gz)

e striated body

f C brevitarsis female with sucrose meal in crop and small blood meal in mid-gut

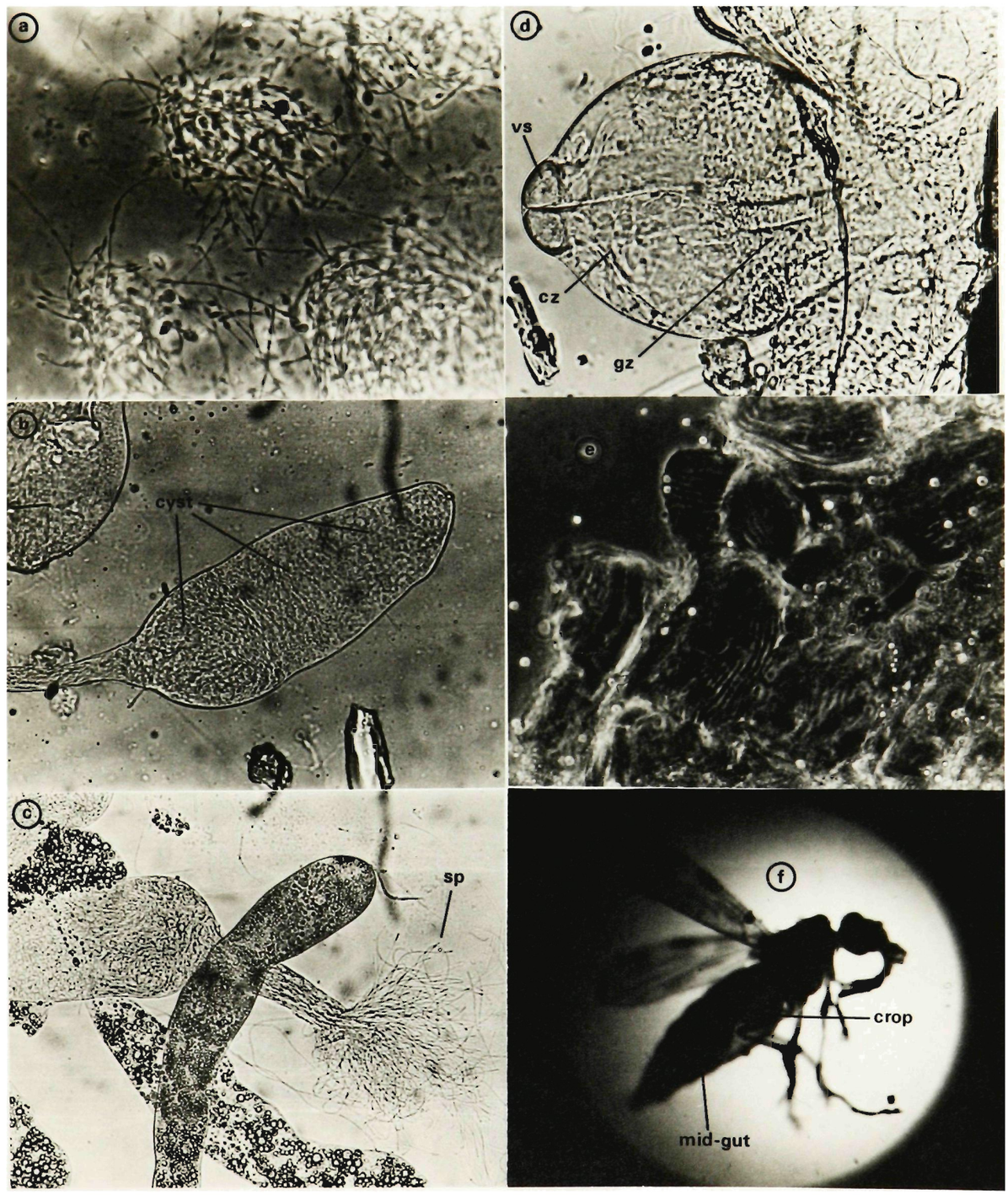

a
d
vs
cz
gz
b
cyst
e
c
sp
f
crop
mid-gut

PLATE II

a swarm over shadow on concrete, Highvale

~~b swarm over different shadow on concrete Highvale~~

c cow used as host at Highvale showing position of standard squares

d developing ovum dissected from C brevitarsis showing central hole (ch)

e early stage V ovum showing sheath (sh), very small ansulae (an), and the concave surface.

f stage V ovum ; ansulae fully developed, sheath partly ruptured

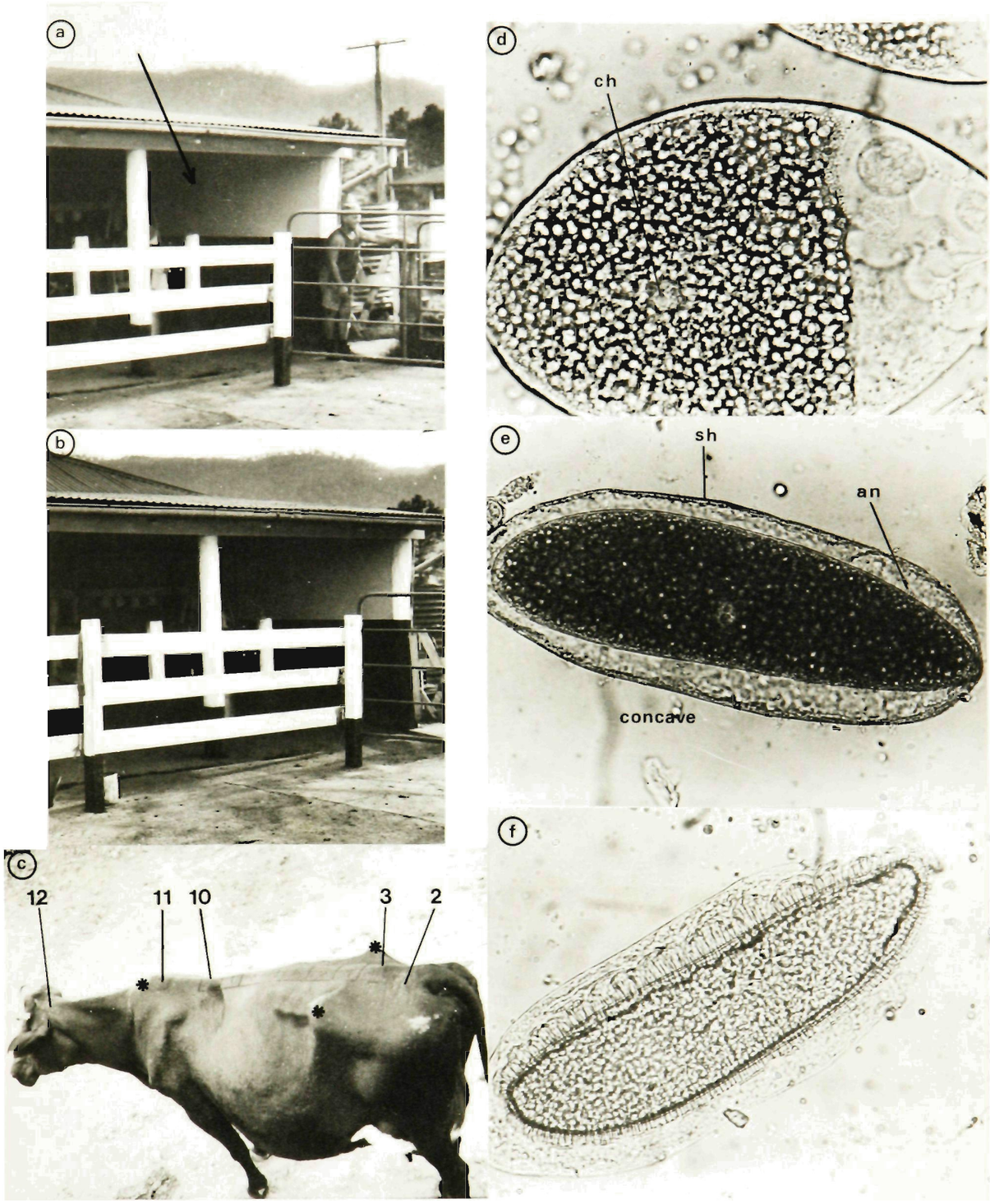

a
b
c
12
11
10
3
2
d
ch
e
sh
an
concave
f

PLATE III

egg photographed with scanning electron microscope

a whole egg showing two types of ansulae
magnification 370x

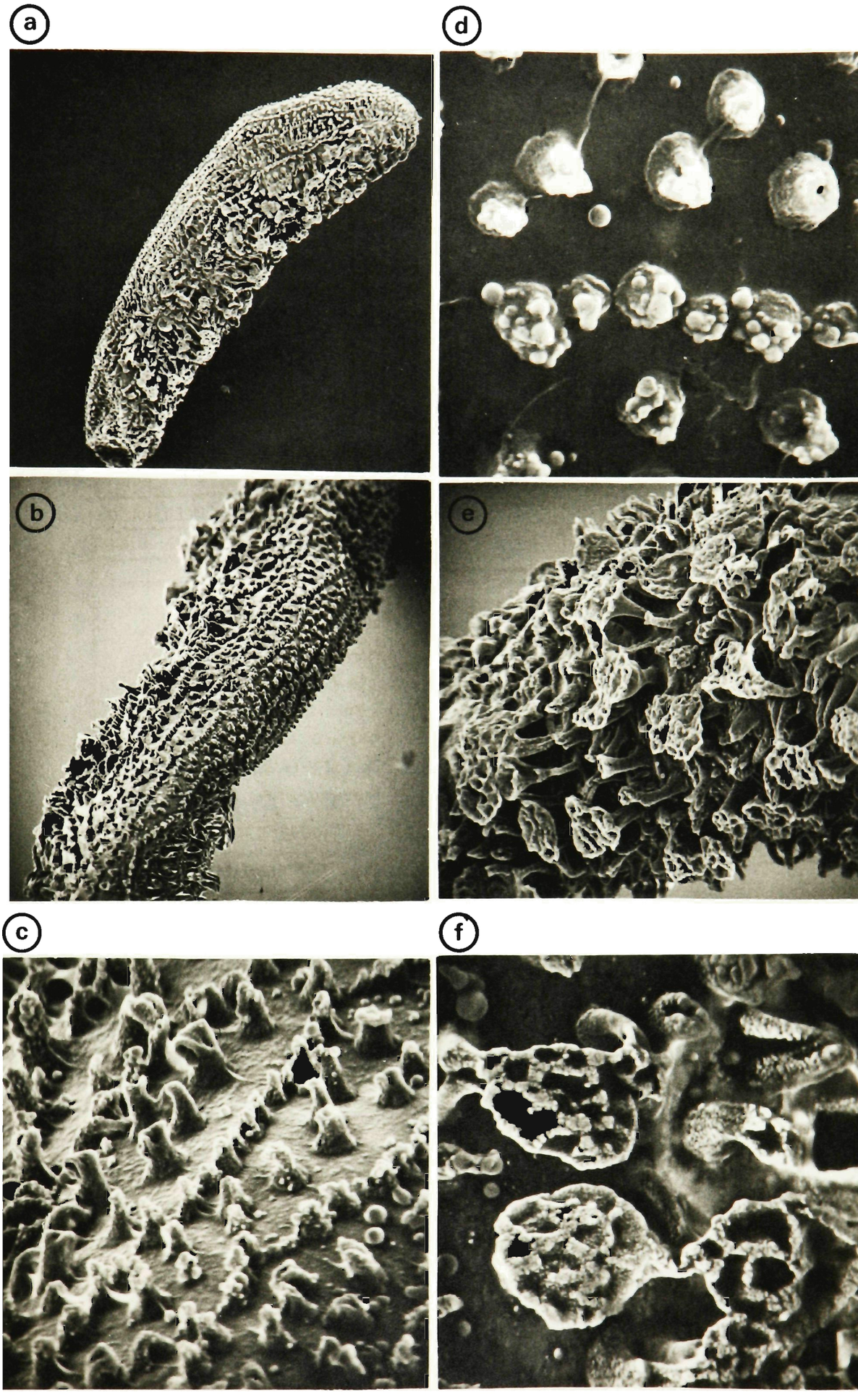
b part of egg showing hexagonal pattern of ansulae on convex surface and demarcation between types of ansulae
magnification 675x

c individual ansulae on convex surface
magnification 3450x

d smallest ansulae on convex surface
magnification 6400x

e ansulae on concave surface
magnification 1350x

f outer surface of individual ansulae on concave surface of egg
magnification 3600x


a
d
b
e
c
f

APPENDIX I.1 EXAMINATION OF TECHNIQUES

A Comparison of techniques to determine the number in the core

washing	emerged	washing	emerged	washing	emerged
28	158	9	58	62	188
5	10	0	7	1	0
47	138	8	144	15	89
32	69	0	33	0	2
59	189	49	197	22	115
28	93	2	16	1	14
36	276	14	52	25	188
55	77	13	10	15	118
31	114	0	6	1	27
0	1	39	91	99	275
13	41	3	134	2	28
0	2	0	0	0	0

n = 36
mean washed = 19.8 larvae (= by flotation)
mean emerged = 82.2 adults

APPENDIX I.1 EXAMINATION OF TECHNIQUES

B Variability between cores from the same pat

series 1 pats

pat	core number			
	1	2	3	4
1	40	30	11	26
2	64	54	77	49
3	157	93	134	58
4	78	56	86	37
5	30	21	124	117
6	190	378	330	370
7	142	135	289	185
8	1	22	4	3
9	80	28	39	52
10	266	111	100	143
11	107	130	127	44
12	100	145	160	171
13	307	191	313	166
14	299	293	208	168

series 2 pats

pat	core number					
	1	2	3	4	5	6
1	60	107	113	67	99	152
2	388	155	117	53	66	198
3	118	96	100	30	96	33
4	73	128	33	50	86	50
5	61	84	124	104	8	45
6	99	133	111	117	106	89

APPENDIX I.2 Number of adults that emerged from a core of dung from a pat exposed in the field for 1 to 7 days after dropping.

SECTION 1 PART 2A

R!	Age of dung when core removed (days)							
	1	2	3	4	5	6	7	$\bar{x}$
0	40	64	157	78	30	190	142	100.1
1	1	80	266	107	110	307	299	167.1
2	24	125	58	336	132	137	269	155.9
3	46	26	20	65	171	181	90	85.6
4	2	43	65	98	42	100	146	70.9
5	0	0	41	20	5	8	6	11.4
6	2	8	35	63	68	98	58	47.4
7	0	144	44	32	93	129	124	80.9
8	3	113	78	98	103	345	158	128.3
9	21	17	43	8	45	209	122	66.4
$\bar{x}$	13.9	62.0	80.7	90.5	79.9	170.4	141.4	

SECTION 1 PART 2B

R!	Age of dung when core removed (days)								
	6	9	12	15	18	21	24	27	30
1	158	58	188	10	7	0	0	0	0
2	138	144	89	69	33	2	0	0	0
3	188	197	115	93	16	14	0	0	0
4	276	52	184	77	10	0	0	0	0
5	118	114	6	27	0	1	0	0	0
6	91	275	41	134	28	2	0	0	0
$\bar{x}$	161.5	140.0	104.5	68.3	15.7	3.2	0.0	0.0	0.0

! replicate number

APPENDIX I.2B DAILY PATTERN OF EMERGENCE

day	cored day 6						cored day 9					
	replicate						replicate					
	1	2	3	4	5	6	1	2	3	4	5	6
7	0	0	0	0	0	0						
8	0	0	0	0	0	0						
9	0	0	0	0	0	0						
10	0	0	0	0	0	0	0	0	0	0	0	0
11	3	0	2	0	0	0	0	1	0	3	5	2
12	5	5	6	0	0	0	0	0	1	3	1	6
13	15	9	13	9	13	2	0	3	0	13	18	0
14	26	11	37	13	20	4	0	17	1	6	21	17
15	24	17	30	13	43	11	1	38	5	9	14	55
16	15	24	22	12	14	10	3	43	40	9	16	54
17	10	9	22	50	13	10	8	11	39	9	16	54
18	6	9	12	50	13	7	4	10	39	0	15	26
19	6	13	16	18	2	19	4	7	35	0	3	21
20	6	18	1	20	0	26	4	3	16	0	2	11
21	11	3	9	9	0	2	17	1	17	0	0	7
22	4	4	3	9	0	20	6	3	0	0	3	4
23	4	1	3	9	0	17	7	2	0	0	3	12
24	3	0	3	2	0	8	1	1	0	0	3	5
25	3	1	2	31	0	37	0	1	0	0	3	4
26	3	2	5	5	0	15	1	0	1	0	0	2
27	5	1	2	4	0	12	0	0	0	0	2	2
28	0	1	0	2	0	3	0	0	0	0	1	1
29	2	0	0	2	0	0	0	1	0	0	2	3
30	1	0	1	0	0	0	1	0	0	0	2	3
31	1	0	0	3	0	0	0	0	0	0	1	1
32	1	0	0	6	0	0	0	0	1	0	0	0
33	1	0	0	3	0	0	1	0	2	0	0	0
34	2	2	0	2	0	0	0	0	0	0	0	0
35	0	3	0	1	0	0	0	1	0	0	0	0
36	1	4	0	1	0	0	0	0	0	0	0	0
37	0	0	0	1	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	1	0	0	0	0
40	0	1	0	0	0	0	0	0	0	0	0	0

day	cored day 12						cored day 15					
	replicate						replicate					
	1	2	3	4	5	6	1	2	3	4	5	6
7												
8												
9												
10												
11												
12												
13	0	20	13	1	5	9						
14	0	7	9	2	0	5						
15	0	30	11	5	0	4						
16	0	9	10	4	0	4	0	6	0	0	0	0
17	0	4	9	1	0	4	0	0	8	0	0	12
18	0	4	21	4	0	3	0	1	0	3	18	7
19	10	11	22	36	1	11	5	0	2	0	2	70
20	53	0	15	63	0	1	0	3	0	7	4	11
21	51	0	3	9	0	0	4	3	13	19	2	11
22	22	4	0	9	0	0	1	37	22	19	0	11
23	25	0	0	8	0	0	0	6	22	19	1	10
24	7	0	0	3	0	0	0	6	21	2	0	0
25	6	0	1	14	0	0	0	5	0	5	0	2
26	6	0	1	10	0	0	0	0	5	2	0	0
27	6	0	0	0	0	0	0	1	0	1	0	0
28	1	0	0	2	0	0	0	1	0	0	0	0
29	1	0	0	2	0	0	0	0	0	0	0	0
30	0	0	0	2	0	0	0	0	0	0	0	0
31	0	0	0	5	0	0	0	0	0	0	0	0
32	0	0	0	4	0	0	0	0	0	0	0	0

APPENDIX I.2B cont.

day	cored day 18						cored day 21					
	replicate						replicate					
	1	2	3	4	5	6	1	2	3	4	5	6
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19	0	1	0	6	0	15						
20	0	6	2	2	0	8						
21	1	5	2	0	0	2						
22	1	9	3	0	0	1	0	1	0	0	0	0
23	4	4	3	2	0	1	0	0	0	0	0	0
24	0	4	2	0	0	0	0	0	8	0	0	0
25	0	3	3	0	0	0	0	0	1	0	1	0
26	1	0	0	0	0	1	0	0	4	0	0	0
27	0	0	1	0	0	0	0	1	0	0	0	1
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	1	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	1	0	0

cored day 24 no adults
cored day 27 no adults
cored day 21 no adults

APPENDIX I.3 Numbers of adults that emerged from a core of dung taken from pats that were either normal or sunk level with the ground.

SECTION 1.3 PART 3

REPLICATE	NORMAL PAT	SUNKEN PAT
1	154	1
2	140	14
3	88	13
4	51	0
5	70	25
6	86	1
7	28	3
8	42	9
9	113	5
10	144	16
$\bar{x}$	91.6	8.7

APPENDIX 1.4 Number of adults that emerged from a core of dung taken from pats of different sizes.

SECTION 1 PART 4

REPLICATE	SIZE OF PAT !			
	½	1	2	4
1	38	86	47	100
2	96	103	77	388
3	12	31	91	188
4	54	28	43	73
5	196	65	139	61
6	110	56	91	99
$\bar{x}$	84.3	61.5	88.5	139.8

! measured as volumes of dung used to make the pat.

APPENDIX I.5 Number of adults that emerged from cores taken from different areas of a pat.

SECTION 1 PART 5

PAT NUMBER	CENTRE	AREA OF PAT INNER		OUTER		
1	54	4	14	2	13	1
		9	4	6	48	41
				4	6	
2	68	8	15	4	0	14
		12	0	1	0	18
		5	4	0	12	6
		26		0		
3	24	29	39	17	2	2
		38		2	30	11
				15	8	7
4	45	68	55	14	60	20
				14	51	0
				26	61	19
5	53	37	3	13	4	8
		15		18	3	6
				20		
6	39	30	4	20	11	105
		18	2	22	0	
7	91	60	0	63	10	57
		127	121	78	49	8
		113	25	53	32	94
		29	16	44	104	
		60				
8	152	61	89	21	72	37
		132	67	61	68	23
		105	263	94	84	91
		165	42	61	126	100
		59				

APPENDIX I. 6 DEPTH OF SURROUNDING GRASS & OVIPOSITION

treatment	replicate				
	1	2	3	4	5
deep grass	156	90	78	12	30
level grass	159	99	81	121	63
low grass	49	43	161	114	134
bare ground	89	212	226	137	128

APPENDIX I.7 SHAPE OF PAT & OVIPOSITION

shape	replicate			
	1	2	3	4
round	101	120	134	87
elongate	73	105	100	9
extra elongate	53	17	15	5
triangular	127	200	97	72
hollow centre	103	32	68	6
T - shape	39	0	56	7
ring of pats	125	46	32	14

APPENDIX I.8 SIDES OF PAT & OVIPOSITION

pair of pats	normal	encirclrd
1	87	0
2	66	0
3	168	4

APPENDIX I.9.i TIME OF DAY OF OVIPOSITION

period exposed	replicate # 1	2	3	4
never	0	0	0	0
800-1900	1.9	1.8	6.6	5.9
1900-2200	0.3	15.7	0.0	5.7
2200-800	0.3	0.6	0.0	0.0
always	1.5	1.6	2.0	1.8

APPENDIX I.9.ii TIME OF DAY OF OVIPOSITION

period exposed	replicate # 1	2	3	4	5	6	7	8	9
700-1400	2.1	6.4	0.1	6.1	28.6	17.6	14.6	5.7	5.7
1400-1600	3.0	8.0	16.5	68.0	38.5	18.5	66.5	41.0	24.5
1600-1800	1.0	18.0	19.5	26.0	43.5	11.0	81.0	17.5	28.5
1800-2000	1.5	8.5	5.0	31.5	22.5	9.0	1.5	12.0	77.5
2000-2200	1.5	0.5	3.5	40.0	13.5	9.0	26.0	6.5	12.0
2200-2400	9.0	8.0	5.0	18.0	9.0	3.5	37.0	14.0	7.5
0000-700	0.0	2.3	0.4	10.6	4.9	6.0	5.0	1.0	6.5
always	1.7	1.3	2.0	9.3	6.7	7.7	1.8	4.1	5.4

values in body of table are counts / hour exposed on any 1 day.

APPENDIX II IMMATURE STAGES OF *C brevitarsis*

II.1 data in text

II.2 descriptions in text

II.3 data in text

APPENDIX II.4 DAILY PATTERN OF EMERGENCE

period	replicate								
	1	2	3	4	5	6	7	8	9
day 1									
0000-1000	0	0	0	0	0	0	0	0	0
1000-1200	0	39	5	1	7	2	0	0	27
1200-1400	4	65	22	43	86	15	26	4	180
1400-1600	40	10	19	30	24	6	86	0	208
1600-2000	10	0	4	0	0	2	6	0	185
2000-2400	0	0	0	0	0	0	0	0	0
day 2									
0000-1000	0	0	0	0	0	0	0	0	0
1000-1200	2	22	19	8	1	3	1	1	13
1200-1400	5	73	16	37	21	22	8	15	69
1400-1600	58	265	70	127	112	157	161	47	355
1600-2000	20	6	5	5	5	9	26	0	6
2000-2400	0	0	0	0	0	0	0	0	0
day 3									
0000-1000	0	0	0	0	0	0	0	0	0
1000-1200	0	0	0	0	0	0	0	0	0
1200-1400	14	29	66	60	31	11	25	18	21
1400-1600	7	20	24	43	70	22	56	23	23
1600-2000	24	12	13	16	8	8	56	1	1
2000-2400	0	0	0	0	0	0	0	0	0
day 4									
0000-1000	0	0	0	0	0	0	0	0	0
1000-1200	0	0	0	0	0	0	0	0	0
1200-1400	2	23	24	51	6	16	47	2	42
1400-1600	11	8	16	39	11	31	39	0	15
1600-2000	1	1	13	0	1	1	1	0	0
2000-2400	0	0	0	0	0	0	0	0	0

APPENDIX IV.1

data in text

APPENDIX IV.2

data in text

APPENDIX IV.3 SWARM SIZE

swarm number	level[#]	type of[##] marker	time[###]	males	females	total
artificial markers						
1	L	-	35	11	0	11
3	H	-	31	18	0	18
4	H	-	31	24	0	24
41	H	-	17	16	0	16
54	H	-	17	49	0	49
55	H	-	17	19	1	20
66	H	-	22	19	0	19
69	H	-	46	44	0	44
74	H	-	34	44	0	44
75	H	-	32	25	0	25
markers near calf						
2	L	26	33	81	1	82
5	L	20	10	74	1	75
6	L	20	8	106	1	107
7	L	20	24	166	1	167
8	L	20	24	152	3	155
11	L	19	24	171	1	172
13	L	20	21	431	0	431
14	L	20	14	80	0	80
16	L	20	4	98	0	98
17	L	26	28	28	0	28
18	L	20	28	261	0	261
19	L	16	25	15	0	15
21	L	20	15	36	1	37
22	L	22	20	121	18	139
24	L	20	5	250	4	254

APPENDIX IV.3 cont.

swarm number	level	type of marker	time	males	females	total
25	L	26	7	34	2	36
27	L	20	22	27	0	27
30	L	20	10	22	0	22
39	L	20	22	7	0	7
56	H	18	14	17	1	18
57	H	18	14	112	1	113
58	H	18	14	37	2	39
60	L	20	4	53	0	53
62	L	20	4	26	4	30
65	H	20	26	72	1	73
71	L	20	40	67	4	71
72	L	20	37	157	0	157
76	L	20	29	66	0	66
77	L	20	24	15	0	15
84	L	20	38	61	0	61
86	L	20	21	90	0	90
99	L	20	11	15	2	17
145	L	20	60	27	0	27
146	L	20	49	75	0	75
147	L	20	49	23	0	23
148	L	20	20	54	0	54
149	L	20	20	35	2	37
150	L	20	20	131	0	131
151	L	20	20	114	0	114
152	L	20	20	141	0	141
153	L	20	20	26	1	27
154	L	20	2	53	1	54
155	L	20	2	15	0	15
markers distant from calves						
9	L	20	24	8	0	8
10	L	20	24	23	0	23
15	L	20	14	9	0	9
12	L	19	24	5	0	5
23	H	16	19	105	1	106
26	H	20	26	6	0	6

APPENDIX IV.3 cont.

swarm number	level	type of marker	time	male	female	total
28	L	20	14	8	0	8
29	L	20	12	25	1	26
31	H	20	8	23	0	23
32	H	20	5	66	0	66
35	H	20	cloud	3	0	3
36	L	17	35	70	1	71
37	H	20	33	6	0	6
38	H	20	27	9	0	9
40	L	20	20	52	1	53
43	H	22	20	18	1	19
44	H	22	19	23	0	23
46	H	22	17	20	0	20
47	H	22	14	18	0	18
48	H	22	13	47	1	48
49	H	22	7	26	0	26
50	L	22	6	13	0	13
51	H	20	32	22	0	22
52	H	20	26	13	1	14
53	H	20	17	30	1	31
63	H	20	33	8	1	9
67	H	20	19	10	0	10
68	H	20	48	1	0	1
73	L	20	5	24	1	25
78	H	20	20	41	0	41
79	H	20	14	223	0	223
81	H	20	14	40	0	40
83	H	20	40	21	4	25
87	H	20	16	91	1	92
92	H	22	45	3	0	3
93	H	22	32	43	1	44
95	H	wood	27	14	0	14
97	H	20	20	49	0	49
98	H	20	16	100	0	100
100	H	20	10	28	0	28
102	H	20	5	19	0	19
103	H	20	0	7	0	7

APPENDIX IV.3 cont.

swarm number	level	type of marker	time	males	females	total
106	H	16	29	3	0	3
107	H	nothing	24	11	0	11
108	L	nothing	12	5	0	5
110	L	16	6	51	2	53
111	L	20	3	60	0	60
112	L	16	0	23	0	23
113	L	20	-3	16	0	16
114	L	20	-7	52	0	52
125	H	20	54	13	0	13
126	H	20	43	5	0	5
127	H	20	32	5	0	5
128	H	22	19	31	0	31
129	H	22	17	19	0	19
130	H	22	12	14	2	16
131	H	22	0	24	0	24
134	H	20	39	12	0	12
swarms above calves						
20	L	-	15	31	1	32
33	L	-	28	11	0	11
34	L	-	27	3	0	3
42	L	-	21	23	1	24
61	L	-	4	25	2	27
64	L	-	29	31	1	32
85	L	-	26	189	0	189
109	L	-	9	15	1	16
above a plough						
59	H	-	7	41	2	43
70	H	-	43	19	0	19

High or Low ## as in table 4.1

minutes before sunset

APPENDIX IV.4

Data in text

APPENDIX IV.5 SWARMS & WIND

wind count	swarms	flight#	wind count	swarms	flight#
30 second intervals					
15	+		32	+	
19	-		19	+	
40	-		34	-	
29	-		24	+	
27	+		19	+	
8	+		7	+	
15	+		14	+	
9	+		2	+	
7	+		13	+	
11	+		11	+	
5	+		13	+	
13	+		7	+	
6	+		6	+	
6	+		4	+	
12	+		10	+	
12	+		5	+	
17	-		31	+	
17	+		13	+	
15	+		11	+	
26	+		18	+	
46	-		25	+	
19	+		19	+	
39	-		24	-	
27	+		39	-	
44	-		30	-	
28	+		28	-	
33	-		31	-	
36	-				
15 second intervals					
5	+		4	+	

APPENDIX IV.5 cont.

wind count	swarms	flight	wind count	swarms	flight
5	+		3	+	
5	+		5	+	
9	+		4	+	
3	+		5	+	
7	+		8	+	
8	+		7	+	
2	-		4	-	
8	+		3	+	
1	+		0	+	
1	+		1	+	
1	+		3	+	
2	+		1	+	
0	+		1	+	
1	+		1	+	
0	+		0	+	
2	+		1	+	
2	+		6	+	
5	+		6	+	
5	+		6	+	
3	+		2	+	
4	+		7	+	
6	+		9	+	
11	+		8	+	
11	+		9	+	
7	+		8	+	
5	+		15	+	
11	+		7	+	
4	+		3	+	
2	+		3	+	
4	+		14	-	
16	-		16	+	
10	+		8	+	
11	+		12	+	
5	+		5	+	
5	+		9	+	
9	+		11	-	+
12	+		7	+	

APPENDIX IV.5 cont.

wind count	swarm	flight	wind count	swarm	flight
8	+		11	+	
7	+		5	+	
5	+		4	+	
9	+		16	+	
11	+		6	+	
12	+		7	+	
9	+		10	+	
9	+		9	+	
5	+		8	+	
4	+		14	+	
7	+		8	+	
8	+		7	+	
12	+		6	+	
9	+		2	+	
6	+		2	+	
5	+		6	-	
3	+		9	+	
6	+		14	+	
5	+		6	+	
8	+		3	+	
4	+		5	+	
6	+		17	-	
4	+		8	+	
8	+		5	+	
11	+		4	+	
6	+		3	+	
3	+		5	+	
3	+		6	+	
5	+		6	+	
15	-		3	+	
12	-		7	+	
10	-	+	9	-	
13	-		16	-	
13	-		10	-	
9	-	+	9	-	
9	-	+	12	-	+
12	-	+	14	-	+

APPENDIX IV.5 cont.

wind count	swarm	flight	wind count	swarm	flight
10	+		11	-	+
21	-	+	18	-	+
16	-	+	23	-	
33	-		33	-	
27	-		20	-	+
24	-		22	-	+
24	-		19	-	
17	-	+	19	-	+
18	-		17	-	+
14	-	+	19	-	+
14	-	+	17	-	+

only when observed and only when swarming absent

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind		temp		RH	C	R	S	swarm	host
	20min	1min	wet	dry	%					
day 1										
86	-	-	22.2	22.9	93	8	+	-	0	0
75	69.4	-	22.3	22.9	94	8	+	-	0	0
55	46.3	-	22.4	22.9	95	8	+	-	0	0
35	67.4	-	21.9	22.4	96	8	+	-	0	0
11	47.7	-	22.0	22.5	96	8	+	-	0	0
- 5	59.1	-	21.8	22.2	97	8	+	-	0	0
-25	52.9	-	21.7	22.2	96	8	+	-	0	0
day 2										
95	-	-	24.5	27.0	81	7	-	-	0	1
75	34.5	-	24.3	26.8	81	7	-	-	0	1
55	40.7	-	24.2	26.4	82	7	-	+	0	3
35	30.0	-	24.2	26.0	85	5	-	-	3	2
15	15.1	-	24.0	25.2	90	3	-	-	4	5
- 5	8.8	-	23.6	24.9	89	3	-	S	0	13
-25	6.3	-	23.8	24.7	93	3	-	S	0	6
day 3										
90	-	-	21.0	24.7	71	7	-	-	0	0
70	16.2	-	20.5	24.3	70	7	-	-	0	0
50	13.8	-	21.2	23.8	78	7	-	-	2	1
30	10.7	-	20.7	23.8	75	7	-	-	3	3
10	6.2	-	19.9	22.7	77	7	-	-	1	14
-10	3.2	-	20.0	22.2	81	5	-	S	0	17
-30	1.4	-	19.7	21.8	81	6	-	S	0	22
day 4										
80	-	-	20.5	27.5	51	1	-	+	0	0
60	23.6	-	20.8	26.1	61	1	-	+	0	0
40	19.0	-	20.4	25.0	64	1	-	+	4	0
20	16.3	-	20.8	24.9	68	1	-	+	12	2
0	13.4	-	19.5	23.0	71	1	-	+	1	7
-20	4.9	-	19.2	22.2	74	1	-	S	0	29
-40	5.2	-	19.0	20.7	85	1	-	S	0	15

time = min before sunset wind = counts / min

C = cloud cover R = rain

S = sun swarm = number of swarms host = no. on host

APPENDIX IV.6 SWARMS?, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 5										
77	[illegible]	-	22.0	24.1	83	8	-	-	0	1
51	12.2	-	21.4	23.5	83	8	-	-	0	0
37	13.1	-	22.0	23.7	86	8	-	-	0	3
17	14.2	-	21.5	23.0	87	8	-	-	0	15
- 3	14.4	-	21.5	22.9	88	8	-	S	0	5
-23	13.6	-	21.2	22.4	89	8	-	S	0	16
-43	2.5	-	21.4	22.5	91	8	-	S	0	5
day 6										
73	-	-	23.3	28.7	63	1	-	+	0	0
56	32.7	-	23.3	28.0	66	1	-	+	0	0
36	52.0	-	22.5	27.2	66	1	-	+	0	0
16	45.5	-	22.7	26.4	65	1	-	+	1	0
- 4	37.5	-	23.0	26.2	76	1	-	S	0	1
-24	33.2	-	22.1	25.3	75	1	-	S	0	15
-44	24.8	-	22.1	25.2	76	1	-	S	0	2
day 7										
76	-	-	22.6	24.8	82	7	-	-	0	1
56	22.0	-	22.6	24.0	88	7	-	-	1	1
36	17.0	-	21.4	22.8	88	8	-	-	5	8
16	9.7	-	22.3	23.3	91	7	-	-	6	12
- 4	6.1	-	21.5	22.7	90	7	-	S	2	35
-24	8.0	-	22.2	23.1	91	7	-	S	0	39
-44	12.8	-	22.0	23.2	91	7	-	S	0	8
day 8										
75	-	-	23.3	28.7	62	1	-	+	0	0
55	23.4	-	23.2	27.7	67	1	-	+	0	1
31	27.5	-	22.0	27.0	64	1	-	+	0	0
15	34.6	-	-	-	-	1	-	+	20	7
- 5	21.8	-	22.4	25.4	76	1	-	S	2	4
-25	40.3	-	22.0	25.0	77	3	-	S	0	2

APPENDIX IV.6 SWARMS, BITING & WEATHER

time			temp		RH	C	R	S	swarm	host
	20min	1min	wet	dry	%					
day 9										
72	-	-	-	-	-	2	-	+	0	0
52	35.0	-	23.3	27.8	67	2	-	+	0	0
28	42.2	-	23.0	26.2	76	2	-	-	0	1
12	10.4	-	23.2	25.8	80	3	-	-	0	5
- 8	3.8	-	23.1	25.1	84	3	-	S	0	12
-28	10.6	-	23.5	24.9	88	5	-	S	0	7
day 10										
65	-	-	23.6	27.5	71	2	-	+	0	0
50	21.0	-	24.4	28.0	74	3	-	+	0	0
38	41.5	-	24.7	27.9	76	5	-	-	0	12
10	33.8	-	24.6	26.6	84	5	-	-	0	18
-11	30.7	-	24.4	26.2	86	5	-	S	0	78
-22	9.4	-	24.4	26.2	86	6	-	S	0	16
day 11										
54	-	-	24.8	30.3	63	0	-	+	0	0
48	21.7	-	25.0	29.4	69	0	-	+	0	0
29	21.3	-	24.7	28.4	73	0	-	+	7	3
9	23.0	-	24.5	27.4	78	0	-	+	7	2
-11	11.3	-	25.9	27.9	85	1	-	S	4	35
-31	13.2	-	24.2	26.3	84	1	-	S	0	27
-51	14.1	-	24.3	26.4	84	2	-	S	0	1
day 12										
59	-	-	21.1	27.0	56	6	+	-	0	0
46	48.8	-	20.3	26.2	57	6	-	-	0	0
26	33.1	-	20.0	25.7	58	6	-	-	10	1
6	17.7	-	20.3	24.8	64	5	-	+	16	8
-14	15.9	-	20.2	24.6	66	5	-	S	0	46
-36	20.5	-	19.8	23.7	69	2	-	S	0	14
-54	7.6	-	19.4	23.1	70	2	-	S	0	7

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 13										
45	-	-	-	-	-	1	-	+	5	7
23	17.1	-	19.6	25.6	56	0	-	+	15	4
9	13.2	-	20.0	24.4	65	0	-	+	15	15
-11	7.8	-	19.9	24.1	67	0	-	S	3	125
-31	4.7	-	19.7	22.8	75	0	-	S	0	132
day 14										
68	-	-	20.8	27.0	55	6	-	-	0	1
47	25.4	-	20.7	26.0	60	6	-	-	4	18
25	18.6	-	21.1	25.6	65	7	-	-	5	19
8	23.9	-	21.0	25.0	69	7	-	-	3	38
-13	23.8	-	20.9	24.7	70	8	-	S	0	82
-32	32.9	-	20.6	24.2	71	8	-	S	0	15
day 15										
103	-	-	23.0	28.9	60	3	-	+	0	0
65	7.5	-	23.0	28.6	61	4	-	+	0	4
45	31.3	-	22.5	27.0	67	5	-	-	6	2
21	15.5	-	22.5	26.4	71	4	-	-	0	25
2	22.1	-	22.3	26.0	72	3	-	+	7	21
-17	16.3	-	21.5	25.2	72	3	-	S	0	43
-35	20.9	-	21.4	25.0	72	4	-	S	0	5
day 16										
74	-	-	24.2	29.5	64	0	-	+	0	0
59	35.2	-	24.0	29.3	64	0	-	+	0	0
39	45.7	-	23.6	28.9	63	0	-	+	1	0
19	44.3	-	23.4	27.7	69	0	-	+	0	0
- 1	30.0	-	23.0	27.0	70	0	-	S	0	0
-34	26.1	-	23.4	26.3	77	0	-	S	0	3
day 17										
51	-	-	20.2	31.6	33	1	-	+	0	0
35	52.8	-	19.7	31.6	30	1	-	+	0	2
15	67.7	-	18.8	30.9	29	1	-	+	0	2
- 5	85.8	-	23.4	29.2	61	1	-	S	0	5
-10	66.4	-	23.2	28.2	64	1	-	S	0	9

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 18										
52	-	-	20.3	26.8	54	1	-	+	0	0
32	26.1	-	19.7	26.1	53	1	-	+	0	3
12	34.4	-	19.5	25.0	58	1	-	+	6	0
- 3	18.5	-	19.5	24.0	64	1	-	S	6	9
- 8	11.2	-	19.5	24.0	64	1	-	S	1	15
-28	16.7	-	19.5	23.5	68	1	-	S	0	6
day 19										
35	-	-	21.2	27.0	58	6	-	-	0	0
31	10.8	-	21.2	27.0	58	6	-	-	4	8
11	29.3	-	20.9	26.4	59	6	-	-	4	34
- 9	24.6	-	21.3	25.5	68	6	-	S	0	60
-29	21.4	-	21.3	25.2	70	5	-	S	0	5
-51	20.8	-	21.3	24.7	74	6	-	S	0	0
day 20										
112	-	-	23.9	30.8	55	3	-	+	0	0
92	31.7	-	23.4	30.1	56	3	-	-	0	7
52	21.8	-	22.2	28.9	54	1	-	+	1	1
32	15.6	-	21.8	27.6	59	1	-	+	7	9
12	8.1	-	21.5	26.6	63	1	-	+	9	15
-10	7.1	-	21.5	25.6	69	1	-	S	0	56
-31	5.2	-	21.1	25.0	69	0	-	S	0	43
day 21										
53	-	-	23.8	29.3	62	0	-	+	0	0
48	28.0	-	23.8	29.3	62	0	-	+	0	0
28	23.7	-	22.6	27.7	64	0	-	+	0	0
7	25.8	-	21.8	26.2	67	0	-	+	7	21
-12	18.6	-	21.9	25.8	71	0	-	S	0	27
-32	17.7	-	22.0	25.8	71	1	-	S	0	10
-52	13.3	-	21.8	25.0	75	1	-	S	0	12
-72	5.7	-	21.7	22.7	91	1	-	S	0	8

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 22										
63	-	-	-	-	-	-	-			
46	53.9	63	21.4	24.8	74	3	-	-	0	2
25	58.5	51	21.5	25.0	73	3	-	-	0	0
6	55.2	62	21.3	24.4	75	5	-	-	0	1
-14	67.3	71	21.0	24.2	74	8	-	S	0	0
-34	58.8	74	20.9	23.9	76	8	-	S	0	0
day 23										
37	-	48	19.0	24.0	61	5	-	+	0	0
17	49.0	26	19.2	22.3	75	5	-	-	0	18
- 3	33.9	23	19.3	22.7	72	3	-	S	0	38
-23	20.6	-	19.3	23.2	68	6	-	S	0	28
-46	28.3	-	19.6	22.7	74	6	-	S	0	15
day 24										
46	-	18	21.4	26.0	66	4	-	+	5	0
26	15.9	35	20.8	25.1	67	3	-	-	7	16
6	12.5	12	20.5	24.2	70	3	-	+	8	68
-17	4.8	-	20.2	21.7	87	3	-	S	0	125
-34	12.2	-	20.1	21.8	85	3	-	S	0	11
day 25										
40	-	16	21.2	25.7	67	7	-	-	0	1
16	15.4	32	20.8	25.1	67	7	-	-	0	12
- 1	14.9	42	20.4	24.7	67	7	-	S	0	62
-20	2.1	1.1	20.3	24.0	71	7	-	S	0	24
-40	1.4	0	20.6	23.6	76	6	-	S	0	50
-60	1.6	13	20.4	23.3	76	6	-	S	0	12
day 26										
38	-	13	20.9	25.2	65	8	-	-	0	2
19	12.7	4	20.0	24.0	68	8	-	-	0	7
1	10.1	1	20.4	24.0	71	8	-	-	0	82
-20	5.4	3	20.0	23.5	72	8	-	S	0	55
-44	1.9	0	20.1	22.9	76	8	-	S	0	2
-64	1.2	4	20.1	22.6	79	8	-	S	0	11

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 27										
55	-	22	19.1	24.2	60	1	-	+	5	0
35	15.8	15	18.5	23.6	60	2	-	p	6	15
15	26.9	61	18.2	22.6	64	2	-	-	0	16
- 5	16.0	10	17.6	21.3	69	2	-	S	0	17
-25	4.4	3	17.5	20.8	72	2	-	S	0	20
-45	2.2	4	17.0	19.2	80	2	-	S	0	0
day 28										
79	-	49	19.4	26.0	52	1	-	+	0	0
57	48.8	34	18.5	25.4	50	1	-	+	0	0
40	49.6	59	-	-	-	1	-	+	0	1
20	22.9	19	19.7	23.6	69	1	-	+	15	0
10	12.3	21	18.2	23.0	62	1	-	-	6	18
0	13.0	43	18.2	23.0	62	1	-	S	0	39
-10	15.4	11	17.7	21.5	68	1	-	S	0	70
-22	11.0	6	17.8	21.2	71	1	-	S	0	49
day 29										
69	-	25	-	-	-	2	-	+	0	0
54	58.1	54	19.8	25.0	61	2	-	+	0	2
31	36.8	43	19.7	24.5	63	2	-	+	0	2
14	26.9	21	19.0	22.8	68	2	-	+	5	15
4	48.7	72	19.3	23.4	61	2	-	-	0	23
- 6	48.1	31	18.6	22.9	64	2	-	S	0	48
-16	28.0	52	18.6	22.5	68	2	-	S	0	33
-27	32.9	23	18.2	21.7	71	2	-	S	0	18
day 30										
70	-	88	-	-	-	6	-	-	0	0
55	23.8	12	20.0	25.7	58	6	-	-	0	1
35	21.8	20	19.5	25.0	58	6	-	+	6	0
15	19.5	1				6	-	+	11	8
4	21.4	16	18.8	23.8	61	6	-	+	0	45
- 5	14.1	4	18.7	23.2	64	6	-	S	0	125
-15	11.1	2	18.3	22.7	65	6	-	S	0	85
-25	6.1	10	18.5	22.7	66	6	-	S	0	41

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 31										
28	-	4	20.0	24.0	68	3	-	-	1	0
21	9.3	8	19.6	23.5	68	3	-	-	2	4
1	11.9	11	19.5	23.1	71	3	-	-	2	9
-19	7.6	14	19.5	23.1	71	2	-	+	9	33
-29	11.0	8	18.9	22.6	70	2	-	S	0	125
-49	5.8	4	18.5	21.3	64	2	-	S	0	135
-59	3.4	1	18.0	20.6	78	2	-	S	0	15
day 32										
30	-	11	20.0	26.0	56	1	-	+	5	2
10	15.8	17	19.5	24.0	59	1	-	+	10	2
0	10.0	11	19.4	23.8	65	1	-	S	1	22
-10	6.6	16	19.0	23.0	67	1	-	S	0	40
-25	10.5	1	19.0	22.1	74	1	-	S	0	55
day 33										
41	-	36	18.9	21.1	82	8	-	-	0	0
28	54.1	43	18.6	20.8	82	8	-	-	0	0
10	73.7	70	18.5	20.3	84	8	-	-	0	0
0	55.7	31	18.5	20.0	87	8	+	-	0	0
-10	51.4	31	18.4	19.8	88	8	-	S	0	4
-20	38.1	45	18.5	19.8	89	8	-	S	0	11
-30	28.5	30	18.5	19.8	89	8	+	S	0	7
day 34										
70	-	89	18.3	23.4	60	6	-	-	0	0
46	68.1	99	18.3	23.0	62	4	-	-	0	0
30	70.0	68	18.5	23.0	64	3	-	+	0	0
10	50.1	43	17.7	22.0	65	3	-	-	0	3
- 2	21.8	8	17.8	21.0	73	3	-	S	0	28
-10	9.1	8	17.6	20.7	71	3	-	S	0	26
-22	12.9	22	17.6	20.6	75	3	-	S	0	8
-30	17.9	18	17.6	20.6	75	2	-	S	0	4

APPENDIX IV.6 SWARMS, BITING & WEATHER

time	wind 20min	wind 1min	temp wet	temp dry	RH %	C	R	S	swarm	host
day 35										
90	-	25	18.2	23.6	58	3	-	+	0	1
70	34.4	23	18.8	24.2	58	4	-	-	0	2
50	47.8	11	18.9	24.5	57	3	-	+	0	5
30	39.4	15	18.1	23.6	58	2	-	+	5	4
10	22.6	28	17.4	22.5	60	2	-	-	0	28
- 2	55.0	36	17.2	22.0	62	3	-	S	0	68
-10	23.0	6	17.3	21.0	69	3	-	S	0	57
-20	5.3	19	17.3	21.0	69	3	-	S	0	44
-30	6.7	26	16.7	19.8	75	3	-	S	0	52
day 36										
70	-	74	17.5	25.3	44	6	-	-	0	0
50	41.7	23	17.9	25.1	48	6	-	+	5	1
30	18.3	23	17.3	24.0	50	6	-	+	6	3
20	16.8	6	17.0	22.7	55	6	-	+	6	17
0	9.9	6	16.5	22.3	54	6	-	S	0	15
-10	6.5	4	16.6	22.4	54	6	-	S	0	14
-20	4.7	3	16.4	21.4	60	6	-	S	0	17
-30	1.6	7	16.3	21.1	61	6	-	S	0	9
day 37										
132	-	45	-	-	-	2	-	+	0	0
112	51.9	43	19.6	28.3	42	3	-	+	0	0
92	48.1	55	20.0	28.2	45	3	-	+	0	0
52	43.3	50	20.0	26.5	53	1	-	+	0	0
32	37.7	12	19.5	25.2	57	1	-	+	5	1
12	30.4	28	18.8	24.0	60	1	-	+	9	0
3	21.7	14	19.0	23.0	64	1	-	+	0	5
- 8	21.6	24	18.5	23.3	62	1	-	S	0	3
-18	24.5	14	18.4	23.0	63	1	-	S	0	0
-28	11.0	5	-	-	-	1	-	S	0	27

time	wind		temp		RH	C	R	S	swarm	host
	20min	1min	wet	dry	%					
day 38										
77	-	22	26.0	20.4	58	3	-	+	0	0
69	31.0	23	26.0	20.3	57	3	-	+	0	0
49	17.7	10	25.3	20.0	60	3	-	+	3	0
29	19.9	24	24.0	20.3	71	3	-	+	5	0
9	11.3	3	19.2	23.0	69	4	-	+	5	9
- 2	1.3	4	21.8	19.5	80	5	+	S	1	0
-12	10.9	36	19.6	22.2	78	5	-	S	0	5
-22	22.2	19	19.2	21.2	83	5	-	S	0	0
-32	12.6	16	18.8	21.0	81	5	-	S	0	0
day 39										
69	-	21	19.6	25.0	59	2	-	+	0	0
49	29.4	50	19.6	24.6	61	2	-	+	0	0
29	30.1	13	19.7	24.2	64	2	-	+	3	4
9	18.1	9	19.6	23.2	70	2	-	-	0	8
- 1	21.3	9	19.4	23.0	71	2	-	-	0	10
-21	19.3	9	19.2	22.1	76	2	-	-	0	6
day 40										
30	-	0	21.1	22.5	87	6	-	-	5	68
10	3.0	0	20.2	21.3	90	6	-	-	5	42
0	0.8	3	-	-	-	6	-	-	0	46
-10	0.8	0	20.2	21.2	91	6	-	S	0	26
-30	0.4	1	20.1	21.0	92	6	-	S	0	8
day 41										
70	-	74	20.5	28.5	43	5	-	-	0	0
50	52.0	47	20.6	28.9	43	5	-	+	0	1
30	75.5	43	-	-	-	5	-	+	0	0
17	62.5	49	18.9	27.0	44	5	-	+	0	2
10	55.6	64	18.6	27.0	42	4	-	+	0	9
0	56.9	96	18.6	26.8	43	3	-	S	0	34
-12	93.3	61	18.6	26.6	45	3	-	S	0	5
-20	30.8	51	18.3	25.6	48	3	-	S	0	5
-30	46.4	46	18.2	25.1	50	2	-	S	0	12
-40	33.3	32	18.2	24.8	51	2	-	S	0	6

APPENDIX V LOCATION OF BITING, VARIATION BETWEEN ANIMALS

time#	position										
	1	2	3	4	5	6	7	8	9	10	11
bull, day 1											
0.0	0	5	4	10	5	1	3	3	1	5	5
0.5	1	10	4	5	3	4	0	3	0	1	0
1.0	3	8	10	3	4	1	1	0	0	1	1
1.5	1	12	2	3	3	1	0	1	0	1	0
2.0	9	6	5	6	4	6	2	1	1	6	3
2.5	7	11	5	6	5	7	4	0	0	0	2
3.0	5	3	6	4	5	4	0	3	0	0	1
3.5	3	7	2	1	2	4	2	2	1	0	0
4.0	3	5	1	0	1	1	2	0	0	1	0
4.5	1	3	2	1	2	0	0	0	0	0	1
bull, day 2											
0.0	5	10	4	5	4	2	4	4	9	8	10
0.5	7	10	7	11	6	8	12	10	9	7	4
1.0	8	7	8	6	4	3	4	5	6	5	1
1.5	6	12	7	6	2	3	3	4	2	3	3
2.0	5	6	3	1	3	1	0	3	4	0	1
2.5	7	5	9	4	4	5	0	3	1	4	2
3.0	3	4	7	4	4	2	0	1	0	4	3
3.5	9	6	4	2	3	0	1	2	1	0	2
4.0	1	0	0	0	1	0	0	0	0	0	0
4.5	0	0	2	2	0	0	0	0	1	1	0
bull, day 3											
0.0	5	12	7	10	16	10	4	5	4	0	0
0.5	9	13	14	8	18	12	7	7	3	0	0
1.0	18	10	22	16	18	10	8	1	3	0	1
1.5	14	16	8	10	7	10	6	0	0	0	1
2.0	7	6	7	5	1	2	1	1	0	1	1
2.5	4	4	5	2	1	3	2	1	0	0	0
3.0	0	1	2	0	0	0	0	0	0	0	0
3.5	0	1	0	0	0	0	0	0	0	0	0
4.0	0	0	0	1	1	0	0	0	0	0	0
4.5	0	0	0	2	0	0	0	0	0	0	0

hours after sunset

APPENDIX V cont.

time	position										
	1	2	3	4	5	6	7	8	9	10	11
heiffer, day 1											
0.0	5	5	4	6	8	8	9	4	1	3	0
0.5	5	2	7	15	10	4	5	0	0	0	1
1.0	1	1	4	3	14	8	6	2	1	0	2
1.5	1	3	3	12	3	9	2	1	1	0	0
2.0	10	12	22	42	26	16	20	14	4	10	8
2.5	3	1	1	14	9	8	3	0	0	5	3
3.0	2	4	6	10	6	7	4	5	0	1	2
3.5	5	2	10	16	19	5	12	2	0	1	2
4.0	1	4	7	4	7	3	1	5	0	0	1
4.5	5	2	4	10	12	3	5	1	0	1	1
heiffer, day 2											
0.0	16	10	13	10	8	9	25	11	7	4	1
0.5	12	3	7	16	4	8	6	12	9	1	4
1.0	10	2	9	14	16	10	16	7	6	2	5
1.5	5	2	4	13	5	8	6	6	3	1	3
2.0	1	0	10	3	7	8	6	9	0	3	1
2.5	4	0	5	7	6	8	2	2	1	2	0
3.0	6	1	8	10	12	14	7	5	0	0	1
3.5	3	3	2	5	4	1	3	0	0	0	0
4.0	1	0	0	1	3	1	1	1	0	1	0
4.5	0	0	0	0	0	0	0	0	0	0	0
heiffer, day 3											
0.0	9	8	11	8	7	5	7	6	3	3	1
0.5	18	14	6	2	10	6	2	5	1	1	2
1.0	3	7	3	5	12	5	4	1	0	0	3
1.5	8	8	2	10	4	4	4	1	3	1	0
2.0	1	2	1	1	0	2	0	0	0	1	0
2.5	2	2	3	3	5	1	3	1	1	0	0
3.0	0	0	0	1	1	1	0	0	0	0	0
3.5	0	0	0	1	0	1	0	0	0	0	0
4.0	1	0	0	0	0	0	0	0	0	0	0
4.5	0	0	0	0	0	0	0	0	0	0	0

APPENDIX V cont.

time	position										
	1	2	3	4	5	6	7	8	9	10	11
cow, day 1											
0.0	10	11	10	9	18	13	10	7	9	5	2
0.5	12	8	10	8	8	12	9	7	1	2	0
1.0	1	2	4	7	7	6	5	2	2	1	0
1.5	4	2	3	6	5	3	3	2	0	0	0
2.0	6	10	7	6	7	4	2	3	2	1	1
2.5	3	2	4	6	6	1	0	3	0	1	0
3.0	3	3	5	6	9	6	7	0	2	1	1
3.5	2	1	1	7	5	9	2	2	3	1	0
4.0	2	2	2	2	3	7	1	1	0	3	0
4.5	4	3	1	1	2	4	2	0	1	0	0
cow, day 2											
0.0	8	5	16	10	15	6	4	1	2	1	1
0.5	6	7	9	8	5	3	1	0	1	1	0
1.0	8	5	4	3	3	4	0	0	0	0	0
1.5	6	3	3	3	1	0	0	0	0	0	0
2.0	2	1	3	5	2	0	0	0	0	1	0
2.5	4	1	4	3	2	2	1	1	2	0	0
3.0	2	3	3	2	1	3	0	0	0	0	0
3.5	1	3	3	0	0	0	0	0	0	0	0
4.0	2	0	1	0	1	0	1	0	0	0	0
4.5	2	0	0	0	0	0	0	0	0	0	0
cow, day 3											
0.0	2	3	14	6	21	12	13	3	4	1	1
0.5	2	3	15	16	12	8	8	2	0	0	0
1.0	4	1	8	5	8	14	12	2	0	1	0
1.5	0	1	1	3	5	1	3	0	1	0	0
2.0	2	0	3	2	1	3	5	0	0	0	0
2.5	0	0	1	3	3	3	4	0	1	0	1
3.0	0	0	0	0	1	0	1	0	0	0	0
3.5	0	0	0	0	0	0	0	0	0	0	0
4.0	0	0	0	0	1	0	1	0	0	0	0
4.5	0	0	0	0	0	0	0	0	0	0	0

APPENDIX V DISTRIBUTION OF FLIES , OCCASION 1

vertical	horizontal							
	4	5	6	7	4	5	6	7
	17.22				17.35			
1	3	3	2	0	8	13	9	7
2	1	1	1	0	8	7	5	2
3	0	1	2	0	2	8	2	0
4	1	1	0	0	1	0	0	3
	17.41				17.52			
1	6	4	8	2	8	7	6	1
2	5	4	6	0	4	3	7	2
3	0	2	1	1	0	5	3	1
4	1	0	0	1	1	2	1	1

APPENDIX V DISTRIBUTION OF FLIES, OCCASION 2

vertical	position									
	1	3	4	5	6	7	8	9	10	11
	17.36									
1	12	11	15	9	11	6	7	4	3	0
2	6	8	12	10	5	4	2	2	0	1
3	5	2	4	4	2	5	1	1	0	0
4	3	3	3	0	3	1	1	1	1	0
5	0	0	2	0	0	0	1	0	0	0
6	0	0	0	0	0	0	1	0	0	0
	18.10									
1	10	6	7	10	8	7	2	1	0	0
2	6	7	7	5	4	4	2	0	0	0
3	3	7	8	2	5	5	2	0	1	0
4	2	2	2	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0

APPENDIX VI Original data for each dissection ; Oogenesis examination

a	b	c	d	e	f	g	h	i	j	k	l	m
1	24	6	IV	M	31	-	-	-	2	6	209.8	131.7
2	24	4	III	M	#	-	-	-	#	6	143.7	97.7
3	45	7	V	M	10	2	2	+	2	5	296.4	78.6
4	0	2	N_o	M	#	-	-	+	0	3	59.0	46.7
5	0	2	N	V	#	-	-	-	0	3	79.3	51.3
6	0	2	N_o	M	#	-	-	-	0	3	74.0	49.0
7	0	2	N	M	#	-	-	-	0	3	62.3	45.3
8	0	2	?	V	#	-	-	-	0	0	#	#
9	0	2	?	V	#	-	-	-	0	0	#	#
10	0	2	N	M	#	-	-	-	2	3	91.0	61.3
11	0	2	N	M	#	-	-	-	2	3	57.0	35.3
12	0	2	N	M	#	-	-	-	0	3	61.7	46.7
13	0	2	N	M	#	-	-	-	0	3	47.7	36.7
14	0	2	N	M	#	-	-	-	0	0	#	#
15	0	2	N	?	#	-	-	-	0	3	49.0	41.0
16	21	3	I	M	#	-	-	-	0	3	72.0	44.3
17	21	3	IIa	M	#	-	-	-	0	5	88.6	62.2
18	31	6	III	M	#	-	-	-	#	9	221.0	136.7
19	44	7	V	M	40	1	2	-	2	5	302.2	93.2
20	60	7	V	M	36	1	2	+	2	6	333.2	118.2

\# not measured
? could not be determined
\- applicable only to ova at stage V
a dissection number
b duration of incubation (hours)
c stage of digestion of blood
d stage of development of ova
e M = mated ; V = virgin
f total number of ova
g stage of development of ansulae
h degree of curvature of ova
i chorion ; + = present, - = absent
j degree of pigmentation
k number of ova measured
l mean length of ova (um)
m mean width of ova (um)

APPENDIX VI (cont.)

a	b	b	d	e	f	g	h	i	j	k	l	m
21	1	2	I	M	#	-	-	-	0	0	#	#
22	20	3	III	M	#	-	-	-	0	0	#	#
23	3	2	I	M	#	-	-	-	0	4	66.5	47.5
24	21	3	III	M	#	-	-	-	0	3	177.0	111.0
25	0	1	I	M	#	-	-	-	0	3	66.7	46.0
26	4	2	I	M	#	-	-	-	0	2	64.5	47.0
27	38	4	IIb	M	#	-	-	-	2	1	138.0	108.0
28	38	7	V	M	#	#	#	+	2	5	324.0	87.8
29	20	4	III	V	#	-	-	-	1	3	207.0	129.3
30	20	4	III	M	#	-	-	-	1	2	214.0	133.5
31	0	1	I	?	#	-	-	-	0	3	53.0	43.0
32	40	7	V	M	8	2	2	+	2	4	291.0	74.8
33	40	6	III	M	#	-	-	-	1	1	223.0	138.0
34	0	1	I	V	#	-	-	-	0	2	42.0	29.5
35	0	1	N	M	#	-	-	-	0	3	53.7	38.3
36	0	1	I	M	#	-	-	-	2	4	47.5	36.5
37	40	7	IV	M	#	-	-	-	2	3	273.0	73.3
38	0	1	I	M	#	-	-	-	2	1	68.0	31.0
39	0	1	I	M	#	-	-	-	2	3	45.7	35.7
40	0	1	I	M	#	-	-	-	2	1	35.0	28.0
41	0	1	I	M	#	-	-	-	2	3	48.0	36.7
42	0	1	I	M	#	-	-	-	2	4	56.5	36.8
43	0	1	I	M	#	-	-	-	2	0	#	#
44	44	7	V	M	37	#	#	+	2	6	317.5	95.8
45	44	7	V	M	20	2	2	+	2	4	321.0	86.0
46	0	1	I	M	#	-	-	-	2	1	67.0	48.0
47	44	7	V	M	40	2	2	+	2	4	333.3	101.3
48	0	1	I	M	#	-	-	-	2	1	47.0	38.0
49	44	7	V	M	34	1	0	+	2	0	#	#
50	44	7	V	M	20	0	0	+	2	3	302.3	76.0
51	110	7	V	V	#	?	?	?	2	0	#	# !
52	110	7	V	M	#	?	?	?	2	0	#	# !
53	110	7	V	M	29	2	2	+	2	0	#	#
54	38	6	V	M	23	1	0	+	2	6	287.1	78.3
55	38	1	I	M	38	-	-	-	0	5	45.3	36.0
56	38	6	V	M	36	0	0	+	2	6	288.5	92.1

! decomposition evident within ova

APPENDIX VI (cont.)

a	b	c	d	e	f	g	h	i	j	k	l	m
57	38	6	IV	M	#	-	-	-	2	0	#	#
58	38	6	V	M	34	1	0	+	2	2	290.1	92.1
59	38	7	V	M	31	1	0	+	2	4	306.2	110.5
60	38	5	III	M	26	-	-	-	0	5	189.7	110.5
61	38	5	IV	M	#	-	-	-	2	6	164.2	92.1
62	38	6	V	M	12	0	0	+	2	2	262.5	92.1
63	10	3	IIb	M	33	-	-	-	0	5	88.4	57.7
64	10	3	IIb	M	62	-	--	-	0	5	98.1	68.8
65	10	3	IIa	M	#	-	-	-	0	5	64.4	40.8
66	10	3	IIa	M	24	-	-	-	0	5	86.6	54.2
67	10	3	IIb	M	57	-	-	-	0	5	83.5	59.9
68	10	3	IIb	M	41	-	-	-	0	5	86.1	61.3
69	10	3	IIb	M	#	-	-	-	0	0	#	#
70	10	3	IIb	M	31	-	-	-	0	5	95.0	61.3
71	10	3	IIa	M	33	-	-	-	0	5	83.5	59.5
72	25	?	III	M	42	-	-	-	0	5	147.4	83.9
73	25	5	III	M	49	-	-	-	0	5	173.2	111.0
74	25	4	III	M	18	-	-	-	0	5	163.3	103.0
75	25	5	IV	M	24	-	-	-	0	3	204.2	129.3
76	25	5	III	M	36	-	-	-	0	5	173.6	126.5
77	50	7	V	M	42	2	0	+	2	5	300.8	64.7
78	50	7	V	M	36	#	#	+	2	5	294.8	73.7
79	50	7	V	M	32	#	#	+	2	5	291.0	63.9
80	50	7	V	M	34	#	#	+	2	5	276.0	63.9
81	50	7	V	M	12	#	#	+	2	5	290.3	66.2
82	40	7	V	M	42	#	#	+	2	5	286.5	66.2
83	40	7	V	M	32	#	#	+	2	5	284.3	63.9
84	40	7	V	M	26	1	0	+	2	5	290.0	59.4
85	40	7	V	M	26	1	0	+	2	5	277.0	54.9
86	40	7	V	M	40	0	0	+	2	5	272.2	69.2
87	15	3	III	M	50	-	-	-	0	5	119.0	78.1
88	15	3	III	M	60	-	-	-	0	5	146.5	96.3
89	15	3	III	M	38	-	-	-	0	5	140.3	82.4
90	15	4	III	M	#	-	-	-	0	5	110.9	65.3
91	15	4	III	M	43	-	-	-	0	5	137.6	90.6
92	55	7	V	M	38	1	2	+	2	5	305.3	70.7

APPENDIX VI (cont)

a	b	c	d	e	f	g	h	i	j	k	l	m
93	55	7	V	M	30	2	0	+	2	5	275.2	66.2
94	55	7	V	M	28	2	0	+	2	5	309.1	75.2
95	55	7	V	M	26	2	0	+	2	5	284.3	70.7
96	55	7	V	M	22	0	0	+	2	5	304.3	80.5
97	30	6	IV	M	34	0	-	-	2	5	251.2	72.9
98	30	6	V	M	48	1	0	+	2	5	281.2	70.7
99	30	6	IV	M	#	-	-	-	2	5	224.8	115.8
100	30	6	V	M	#	#	#	+	2	5	276.0	75.0
101	30	6	IV	M	36	-	-	-	2	5	247.4	82.5
102	15	3	IIb	M	48	-	-	-	0	5	112.0	70.1
103	15	3	IIb	M	#	-	-	-	0	5	117.3	79.7
104	15	3	IIa	M	#	-	-	-	0	0	#	#
105	15	3	IIb	M	32	-	-	-	0	0	#	#
106	15	2	I	M	#	-	-	-	0	5	54.1	45.2
107	15	3	IIa	M	#	-	-	-	0	5	87.3	63.9
108	10	3	IIb	M	#	-	-	-	0	5	106.8	51.8
109	10	3	IIa	M	#	-	-	-	0	5	80.5	52.3
110	10	3	IIa	M	#	-	-	-	0	5	85.0	58.7
111	10	3	IIa	M	#	-	-	-	0	5	82.7	59.8
112	10	2	IIa	M	#	-	-	-	0	5	58.7	46.6
113	35	6	III	M	#	-	-	-	0	0	#	#
114	35	7	IV	M	#	-	-	-	2	5	186.5	98.7
115	35	6	IV	M	#	-	-	-	2	5	267.0	114.2
116	35	7	IV	M	#	-	-	-	2	5	212.8	140.5
117	35	7	V	M	32	2	1	+	2	5	302.3	88.1
118	60	7	V	M	42	2	2	+	2	5	279.0	75.2
119	60	7	V	M	46	2	2	+	2	5	276.0	61.6
120	60	7	V	M	52	2	2	+	2	0	#	#
121	60	7	V	M	33	2	2	+	2	5	265.5	69.2
122	60	7	V	M	32	2	2	+	2	5	278.0	56.4
123	60	7	V	M	36	2	2	+	2	5	294.0	72.2
124	60	7	V	M	22	1	2	+	2	5	318.8	88.0
125	20	4	III	M	#	0	-	-	2	2	131.6	80.9
126	20	4	III	M	#	@	-	-	2	5	152.7	123.0
127	20	5	IV	M	#	-	-	-	2	5	180.5	142.0
128	20	4	III	M	#	-	-	-	2	5	159.4	94.9
129	20	4	III	M	#	-	-	-	2	5	150.4	100.8

APPENDIX VI (cont.)

a	b	c	d	e	f	g	h	i	j	k	l	m
130	15	3	III	M	#	-	-	-	0	5	130.8	84.3
131	15	3	IIb	M	#	-	-	-	0	5	110.5	73.0
132	15	3	III	M	#	-	-	-	1	5	172.2	120.8
133	15	3	IIb	M	#	-	-	-	1	5	127.8	82.2
134	15	3	III	M	#	-	-	-	0	5	120.3	82.8
135	15	3	III	M	#	-	-	-	1	5	122.6	87.4
136	15	3	IIa	M	#	-	-	-	0	5	77.5	54.2
137	15	3	III	M	#	-	-	-	1	5	98.5	60.2
138	15	3	IIb	M	#	-	-	-	1	5	139.1	86.6
139	45	7	V	M	34	2	2	+	2	5	285.1	65.5
140	45	7	V	M	29	2	2	+	2	5	273.0	67.8
141	45	#	#	#	#	#	#	#	#	#	#	#
142	45	7	V	M	44	2	0	+	2	5	320.4	100.2
143	45	7	V	M	22	2	1	+	2	5	294.8	81.2
145	45	7	V	M	36	2	2	+	2	5	314.3	92.6
146	45	7	V	M	30	2	2	+	2	5	327.9	84.3
145	45	7	V	M	24	2	1	+	2	5	303.1	82.7
146	45	7	V	V	#	1	0	+	2	5	301.6	112.4
147	70	7	V	M	32	2	2	+	2	0	#	#
148	70	7	V	M	34	2	2	+	2	0	#	#
149	30	6	IV	?	#	-	-	-	2	0	#	#
150	70	7	V	M	30	2	2	+	2	0	#	#
151	70	7	V	M	36	2	2	+	2	0	#	#
152	70	7	V	M	25	2	2	+	2	0	#	#
153	70	7	V	M	35	2	2	+	2	5	281.2	62.1
154	70	7	V	M	33	2	2	+	2	0	#	#
155	70	7	V	M	32	2	2	+	2	0	#	#
156	70	7	V	M	40	2	2	+	2	0	#	#
157	70	7	V	M	9	2	2	+	2	0	#	#
158	70	7	?	V	#	#	#	#	2	0	#	# !
159	40	5	IV	V	12	-	-	-	2	5	168.4	86.2
160	40	5	V	M	#	0	1	+	2	5	282.8	71.7
161	40	7	V	M	#	1	2	+	2	5	277.5	64.2
162	40	7	V	M	#	1	1	+	2	5	306.1	85.4
163	40	7	V	M	#	1	0	+	2	0	#	#
164	40	7	V	M	#	1	2	+	2	5	293.3	63.5
165	40	7	V	M	#	1	2	+	2	5	284.3	54.0

APPENDIX VI (cont.)

a	b	c	d	e	f	g	h	i	j	k	l	m
166	40	7	V	M	#	2	2	+	2	5	269.2	64.9
167	40	6	V	M	#	1	2	+	2	0	#	#
168	40	5	IV	M	#	-	-	-	2	0	#	#
169	5	2	I	M	#	-	-	-	0	1	71.1	57.8
170	5	2	IIa	M	#	-	-	-	0	5	63.9	44.4
171	5	2	IIa	M	#	-	-	-	0	5	74.6	50.7
172	5	2	IIa	M	#	-	-	-	0	5	74.6	53.3
173	5	2	IIa	V	#	-	-	-	0	0	#	#
174	5	2	IIa	M	#	-	-	-	0	5	67.5	46.6
175	5	2	IIa	M	#	-	-	-	0	0	#	#
176	5	2	IIa	M	#	-	-	-	0	5	75.5	55.1
177	5	2	IIa	M	#	-	-	-	0	5	62.6	47.2
178	65	7	V	M	53	2	2	+	2	5	281.2	65.6
179	65	7	V	M	42	2	2	+	2	5	277.5	66.2
180	65	7	V	M	33	2	2	+	2	5	291.8	70.7
181	65	7	V	M	32	2	2	+	2	5	263.2	66.8
182	65	7	V	M	28	2	2	+	2	6	281.2	61.6
183	65	7	V	M	32	2	2	+	2	5	285.0	66.8
184	30	6	IV	M	#	-	-	-	2	5	261.7	105.7
185	30	5	IV	M	#	-	-	-	2	5	231.6	135.9
186	30	5	III	M	#	-	-	-	2	5	147.4	91.2
187	30	5	III	?	#	-	-	-	0	5	161.7	86.8
188	30	6	IV	M	#	-	-	-	2	5	224.2	135.2
189	5	2	IIa	M	#	-	-	-	0	5	62.2	43.3
190	5	2	IIa	M	#	-	-	-	0	5	75.5	52.2
191	5	2	IIa	M	#	-	-	-	0	0	#	#
192	5	2	IIa	M	#	-	-	-	0	0	#	#
193	30	5	III	M	#	-	-	-	0	5	189.6	120.5
194	30	5	III	M	#	-	-	-	0	5	210.1	121.7
195	30	3	IIb	M	#	-	-	-	0	5	98.9	78.1
196	30	6	IV	M	#	-	-	-	2	0	#	#
197	30	6	V	M	#	0	0	+	2	5	313.8	84.5
198	30	5	IV	M	#	-	-	-	2	0	#	#
199	5	2	IIa	M	#	-	-	-	0	5	63.4	47.1
200	5	2	IIa	M	#	-	-	-	0	5	70.9	53.2
201	5	2	IIa	M	#	-	-	-	0	0	#	#
202	5	2	IIa	M	#	-	-	-	0	5	67.5	47.4

APPENDIX VI (cont.)

a	b	c	d	e	f	g	h	i	j	k	l	m
203	5	2	IIa	M	#	-	-	-	0	5	62.7	42.9
204	5	2	IIa	M	#	-	-	-	0	5	72.3	54.8
205	5	2	IIa	M	#	-	-	-	0	5	62.1	41.6
206	5	2	IIa	M	#	-	-	-	0	5	77.7	55.9
207	5	2	IIa	M	#	-	-	-	0	5	74.3	52.5
208	5	2	IIa	M	#	-	-	-	0	5	60.7	40.9
209	30	4	III	M	#	-	-	-	#	5	150.0	102.2
210	30	4	IV	M	#	-	-	-	#	3	213.8	137.5
211	30	4	IV	M	#	-	-	-	#	5	180.7	122.9
212	30	4	IV	M	#	-	-	-	#	0	#	#
213	30	6	IV	M	#	-	-	-	#	5	200.5	132.3
214	30	6	IV	M	#	-	-	-	#	5	218.0	140.8
215	55	7	V	M	21	2	2	+	2	5	309.1	70.7
216	55	7	V	M	22	2	2	+	2	5	318.4	81.5
217	55	7	V	M	36	2	2	+	2	5	309.1	77.6
218	55	7	?	V	#	#	#	#	2	0	#	#
219	55	7	V	M	15	2	2	+	2	5	318.4	93.7
220	55	7	V	M	24	2	2	+	2	5	335.3	77.6
221	55	7	V	M	15	2	2	+	2	5	359.9	109.3
222	55	7	V	M	17	2	2	+	2	0	#	#
223	0	2	I	M	#	-	-	-	0	5	56.6	38.9
224	0	2	I	M	#	-	-	-	0	5	66.8	47.2
225	0	2	I	M	#	-	-	-	0	5	46.4	33.8
226	0	2	I	M	#	-	-	-	0	5	64.8	47.7
227	0	2	I	M	#	-	-	-	0	0	#	#
228	10	3	IIa	M	#	-	-	-	0	5	76.4	51.2
229	10	3	IIb	M	#	-	-	-	0	5	77.7	54.6
230	10	3	I	M	#	-	-	-	0	5	66.8	51.2
231	10	3	IIa	M	#	-	-	-	0	5	63.4	47.1
232	10	3	IIb	M	#	-	-	-	0	5	79.1	49.8
233	20	3	IIb	M	#	-	-	-	1	0	#	#
234	20	3	IIb	M	#	-	-	-	0	5	121.4	85.3
235	20	3	III	M	#	-	-	-	0	5	133.0	90.6
236	20	3	III	M	#	-	-	-	#	5	139.1	79.7
237	20	3	III	M	#	-	-	-	#	5	120.0	79.1
238	20	3	III	M	#	-	-	-	#	1	154.6	109.4
239	20	3	III	M	#	-	-	-	#	5	128.9	80.5

APPENDIX VI (cOnt.)

a	b	c	d	e	f	g	h	i	j	k	l	m
240	25	6	III	M	#	-	-	-	2	5	216.2	127.2
241	25	4	III	M	#	-	-	-	1	5	175.3	115.8
242	25	4	III	M	#	-	-	-	1	5	179.4	116.8
243	25	6	IV	M	#	-	-	-	2	5	261.9	123.3
244	25	4	III	M	#	-	-	-	2	0	#	#
245	25	4	III	M	#	-	-	-	2	0	#	#
246	25	4	III	M	#	-	-	-	2	0	#	#
247	25	4	III	M	#	-	-	-	2	5	174.6	113.0
248	25	6	IV	M	#	-	-	-	2	3	234.3	122.2
249	25	4	III	M	#	-	-	-	2	0	#	#
250	25	4	?	PARASITISED								
251	25	4	?	PARASITISED								
252	25	4	III	M	#	-	-	-	2	5	195.1	122.2
253	25	4	III	M	#	-	-	-	2	4	159.6	105.8
254	25	4	III	M	#	-	-	-	2	5	141.9	84.6
255	25	4	IV	M	#	-	-	-	2	5	233.2	156.2
256	35	6	V	M	#	1	0	+	2	5	286.1	76.9
257	40	4	I	V	#	-	-	-	0	0	#	#
258	40	6	V	M	#	1	0	+	2	5	309.1	104.5
259	40	6	IV	M	#	-	-	-	#	0	#	#
260	40	6	V	M	27	1	0	+	2	5	249.2	67.7
261	40	7	V	M	26	1	0	+	2	0	#	#
262	40	7	V	M	21	1	1	+	2	0	#	#
263	45	7	V	M	42	2	0	+	2	5	289.1	67.7
264	45	7	V	M	3	2	2	+	2	3	284.5	74.5
265	45	6	V	M	22	0	0	+	2	0	#	#
266	45	6	IV	M	#	-	-	-	2	0	#	#
267	45	6	?	V	#	#	#	#	#	#	#	# !
268	50	7	V	M	26	2	2	+	2	5	295.3	56.9
269	50	7	V	M	8	2	2	+	2	0	#	#
270	50	7	V	M	28	2	2	+	2	5	284.5	78.4
271	50	6	?	M	#	-	-	-	2	0	#	# !
272	50	7	V	M	15	2	2	+	2	5	299.9	78.4
273	50	7	V	M	18	2	2	+	2	0	#	#
274	50	7	V	M	31	2	2	+	2	5	279.9	69.2
275	50	7	V	M	28	2	2	+	2	5	302.5	73.8

APPENDIX VI (cont.)

a	b	c	d	e	f	g	h	i	j	k	l	m
276	50	7	V	M	36	2	2	+	2	5	289.1	75.2
277	50	7	V	M	30	2	2	+	2	5	283.0	67.7
278	50	7	V	M	28	2	2	+	2	5	303.0	64.5
279	50	7	V	M	12	2	2	+	2	5	207.6	92.2
280	50	7	V	M	36	2	2	+	2	5	304.5	66.1
281	50	7	V	M	25	2	2	+	2	5	330.7	81.5
282	50	7	V	M	12	2	2	+	2	0	#	#

APPENDIX VII AGE AT DEATH OF ADULTS OFFERED VARIOUS DIETS. EXPERIMENT 1.

FOOD	SEX	AGE AT DEATH (days)									
REPLICATE 1											
none	f	1	1	1	1	2	2	2	2	2	3
water	f	1	1	2	3	7	9	10	12	18	20
sucrose	f	1	1	1	3	19	25	26	27	33	36
sucrose	m	1	2	3	11	15	17	18	22	22	24
none	m	1	1	1	1	1	1	1	1	1	1
water	m	1	2	2	2	3	4	9	9	10	11
REPLICATE 2											
water	f	1	1	1	3	3	9	9	10	10	12
none	f	1	1	1	1	1	1	1	1	1	1
sucrose	f	1	1	2	2	9	20	23	30	31	34
none	m	1	1	1	1	1	1	1	1	1	1
sucrose	m	1	1	1	11	17	23	24	25	26	26
water	m	1	1	1	1	1	1	1	1	1	1
REPLICATE 3											
sucrose	f	1	1	2	3	12	17	23	23	24	25
water	f	1	2	3	4	4	4	4	10	14	15
none	f	1	1	1	1	1	1	1	1	1	1
sucrose	m	1	1	1	11	19	20	22	22	22	22
water	m	1	1	1	1	1	1	1	1	1	1
none	m	1	2	2	2	2	4	8	9	9	10
REPLICATE 4											
sucrose	f	1	2	8	9	17	18	22	25	27	28
water	f	1	1	9	22	22	22	22	25	26	27
none	f	1	1	1	1	1	1	1	2	2	3
water	m	1	1	1	1	1	2	8	9	9	9
none	m	1	1	1	1	1	1	2	8	9	9
sucrose	m	12	12	14	14	15	17	19	19	20	21

APPENDIX VII (cont.)

FOOD	SEX	AGE AT DEATH									
REPLICATE 5											
sucrose	f	1	1	1	1	11	11	14	17	18	20
water	f	1	1	1	1	10	11	12	16	16	16
none	f	1	1	1	1	1	1	1	1	1	1
none	m	1	1	1	1	1	1	1	1	1	2
water	m	1	1	1	1	1	2	4	9	9	9
sucrose	m	1	1	1	6	9	9	9	9	10	23
REPLICATE 6											
water	f	1	1	1	1	1	1	2	2	2	2
sucrose	f	1	1	1	1	2	9	19	20	21	21
none	f	1	1	1	1	1	1	1	1	1	1
none	m	1	1	1	1	1	1	1	1	1	2
sucrose	m	8	8	13	14	14	21	21	22	22	23
water	m	1	1	1	1	1	1	1	2	2	2
REPLICATE 7											
none	f	1	1	1	1	1	1	1	1	2	2
water	f	1	1	1	1	1	1	1	1	1	2
sucrose	f	3	6	9	10	13	13	21	24	24	24
sucrose	m	1	1	1	1	1	9	11	21	25	26
none	m	1	1	1	1	1	1	1	1	1	1
water	m	1	1	1	1	2	2	2	2	2	2
REPLICATE 8											
water	f	1	1	1	1	2	2	2	2	2	2
none	f	1	1	1	1	1	1	1	1	1	1
sucrose	f	1	1	1	1	1	15	21	22	23	23
none	m	1	1	1	1	1	2	2	2	2	2
sucrose	m	1	1	1	3	8	10	12	18	19	21
water	m	1	1	1	1	1	1	1	1	1	1

APPENDIX VII (cont.)

FOOD	SEX	AGE AT DEATH									
REPLICATE 9											
sucrose	f	1	1	1	9	14	14	15	15	16	21
water	f	1	1	1	2	2	2	2	2	2	3
none	f	1	1	1	1	1	1	1	1	1	2
sucrose	m	1	1	1	10	10	22	24	24	30	32
water	m	1	1	1	1	1	1	1	1	2	2
none	m	1	1	1	1	1	1	1	1	1	1
REPLICATE 10											
sucrose	f	1	3	16	16	21	21	21	22	23	24
none	f	1	1	1	1	1	1	1	1	1	2
water	f	1	1	1	1	1	1	2	2	2	2
water	m	1	1	1	1	1	1	1	1	1	2
sucrose	m	1	1	8	14	19	21	23	24	24	24
none	m	1	1	1	1	1	1	1	1	1	1

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