Notes

New Trends in Entomological Research in Trypanosomiasis *

by J. M. B. Harley, *Senior Scientific Officer, East African Trypanosomiasis Research Organization, Tororo, Uganda*

One of the major trends in entomological research in trypanosomiasis over recent years has been the gradual change in outlook of the entomologist. This has been the result of a large number of factors, of which one of the most important has been the formation of research institutes where entomologists have been brought into close contact with research staff working on other aspects of trypanosomiasis. This contrasts with the situation pertaining before the Second World War, when entomologists in most parts of Africa only rarely came into contact with medical, veterinary and other professional staff. It has led to a broadening of outlook with the result that the trypanosomiasis problem is increasingly viewed as a whole rather than as a number of separate facets. The creation of separate units to deal with immediate practical control problems has also aided this change of viewpoint, while these units themselves are more and more becoming branches of veterinary, and in some cases medical, departments—this development also resulting in a closer association of entomological staff with others working on the same problems. The greater efficacy of drugs and the increasing use of insecticides, together with better communications, surveillance and other facilities, ensuring that epidemics of sleeping-sickness are more limited in extent and can be brought under control more readily, may also be cited. On the veterinary side a range of curative and prophylactic drugs has made it possible to use many areas that are tsetse-infested for livestock, and it is becoming clearer that the control of trypanosomiasis does not necessarily require the complete eradication of *Glossina*. The necessity for emphasis on the design and application by research staff of control measures directed against *Glossina* as such is therefore reduced and it is now possible to consider more particularly the role of *Glossina* as a vector of trypanosomes, thus broadening the basis of the research effort.

This broadening of viewpoint makes it increasingly difficult to draw a line between entomological and other branches of trypanosomiasis research. Hence a discussion of recent trends in entomology must inevitably encroach to some extent on other fields.

Glossina *as a vector of trypanosomes*

Consideration of *Glossina* as a vector of trypanosomes has led to the concept of man-fly contact in connexion with human sleeping-sickness and of trypanosome risk, which has also been called trypanosome challenge, in connexion with animal trypanosomiasis. While all species of *Glossina* probably bite domestic animals and thus are probably implicated in the transmission of animal trypanosomiasis, attack on man is, in many species, extremely rare. This is especially true of the species of the *fusca* group, none of which has as yet been associated with transmission of sleeping-sickness; in fact only five species of tsetse fly are known to transmit the human disease in the field. Where more than one species of potential vector occur together it is essential to gather objective evidence on the importance of the various species. A case in point is that of a sleeping-sickness area in Uganda where an epidemic of *Trypanosoma rhodesiense* occurred about twenty years ago. *G. pallidipes* was shown to be a vector both during the epidemic and again later when the disease was endemic. However, it was recently noticed in one village that *G. palpalis*, which also inhabits much of the area, was in greater contact with man than *G. pallidipes*. Subsequent isolations of trypanosomes from *G. palpalis* proved that this species was also a vector, the first time it has been proved to transmit *T. rhodesiense* (Southon, 1960). The degree of contact with man of flies old enough to be cyclically infected has been shown to vary both seasonally and between areas in Nigeria (Page & McDonald, 1959) and this is undoubtedly one reason for similar differences in the incidence of the disease.

* Note presented to the WHO Expert Committee on Trypanosomiasis, June 1962.

In animal trypanosomiasis, the trypanosome risk is defined as the degree to which an animal is exposed to being infected in nature. In order to understand the particular level of risk pertaining in any area we have to study both factors influencing the level of the tsetse fly population and factors influencing the transmission of trypanosomes by *Glossina*. While much research on the former has been carried out for many years, work on the latter aspect has, until recently, been less intensive.

Factors limiting the numbers and distribution of Glossina. Climate has long been recognized as perhaps the major factor limiting the numbers and distribution of *Glossina* and has been the subject of much investigation. Recent physiological work has led to a greater understanding of the mode of operation and has obvious application in the evolution of successful methods of tsetse fly control involving modification of the vegetation within the habitat. This and other physiological studies are also beginning to explain many of the field observations on the behaviour and activity of tsetse flies.

It has for many years been considered that water loss of the adult tsetse fly in the field was a major limiting factor. This proposition was suggested by the correlation found between fluctuations in tsetse fly numbers and variations in saturation deficit and also by laboratory experiments on teneral flies. However, recent detailed studies by Bursell (1959) on the comparative water balance of different species of *Glossina*, together with evidence on water available in the residual blood meal, cast doubt on these suggestions. Bursell found that there is no correlation between the resistance of adults of different species to desiccation and the habitat occupied and it would appear that water loss of the adult is neither a hindrance to the occupation of drier areas nor a significant cause of death in the dry season. On the other hand, there is a close correlation between resistance to desiccation of the pupae of different species and the habitat in which they occur (Bursell, 1958). Thus the pupae of the forest-dwelling species of the *fusca* group have a low degree of resistance to desiccation, those of the savannah-living *G. swynnertoni* and *G. morsitans* a high degree of resistance, while the pupae of lacustrine and riverine *G. palpalis* show intermediate values. This suggests that the occupation of more arid areas is limited not by the water balance of the adult fly but by that of the pupa. This conclusion is supported by observations by Jackson (1945) that, while *G. palpalis* adults survived well when released in the more arid habitat of G. *swynnertoni*, no second generation adults were detected, suggesting that pupae from the first generation had not survived. Further evidence suggesting that desiccation of pupae may be an important cause of death of *G. pallidipes* during the dry season in the field has also recently been obtained (Bursell, 1960b).

However, this picture is complicated by the effect of temperature on both adult and pupa. The high temperatures of the dry season in Northern Nigeria for instance have long been associated with restriction of the distribution of tsetse flies and noted as a probable cause of considerable mortality. The effect of low temperatures is less well known but it undoubtedly limits occupation of older areas whether due to latitude or to altitude. Most studies of the effect of temperature have been concerned with the adult and it has only recently been shown that both high and low temperatures acting on the pupa may be important in limiting both distribution and population density. This is due to a combination of the effect of temperature on the metabolic rate and the duration of development, which results in *G. morsitans* emerging from pupae experiencing both higher and lower temperatures than the optimum of 22°-24°C having smaller fat reserves, thus reducing the period in which a blood meal must be obtained before death occurs due to exhaustion of fat (Bursell, 1960a). Smaller emerging individuals also have smaller fat reserves and are thus at a disadvantage compared with larger individuals. This is most likely the explanation of the differential mortality observed in populations of *G. morsitans*, *G. swynnertoni* (Jackson, 1948; Glasgow, 1961) and *G. palpalis* (Bursell & Glasgow, 1960), where the death of up to about 10% of the emerging flies during the dry season, owing to a reduction in the number of the smallest individuals, has been observed. It is therefore probable that temperature influences the distribution of tsetse flies by its effect on pupal development, ensuring that populations are restricted within a defined temperature range.

There are several other causes of death in *Glossina* apart from climate. One of these is predation. Observations made in the past incriminated predators of several groups as occasionally attacking tsetse flies but very little quantitative work was carried out. One recent study has shown that predation can be of much greater importance than has hitherto been suspected. It was estimated that one spider, *Hersilia setifrons*, might account for a daily mortality of 650 *G. swynnertoni* per square mile and it was considered that this might correspond to about 30% of the total daily mortality taking place at the time (Southon, 1959). This is an extremely high death-rate as a result of one factor only and several other predators are known to attack *G. swynnertoni* in this area, although no quantitative data are available.

Factors influencing the transmission of trypanosomes by Glossina. The mammal species present in any area, the degree to which each is utilized as a host and the incidence of infection of each determine their role as reservoirs of trypanosomiasis. The accumulation of data over the last few years on the natural hosts of many species of *Glossina* is therefore extremely pertinent to this study. Very briefly, it has been established that *G. morsitans* and *G. swynnertoni* feed largely on warthog in most areas. *G. pallidipes* and *G. longipalpis* often feed mainly on bushbuck. *G. palpalis* has a wider range of hosts, feeding on man, domestic animals, reptiles and bovids, the proportions varying in different areas. Several species of the *fusca* group in West Africa feed largely on red river hogs and bushbuck while in East Africa hippopotamus is important to *G. brevipalpis* and rhinoceros to *G. longipennis*. These most frequently bitten hosts are, however, not always the most important and several areas are known where they are replaced as the major host by other animals. Nevertheless, it is clear that bushbuck and wild pigs are extremely important as hosts of *Glossina* generally (Glasgow et al., 1958; Jordan et al., 1961; Weitz & Glasgow, 1956).[a] Recent studies on the numbers of potential hosts in an area confirm previous observations that the different species are not attacked in proportion to their relative abundance (Weitz et al., 1958; Lamprey et al., 1962) and it seems likely that it is the coincidence of habits of host and tsetse fly that is the important factor, although some degree of choice may perhaps be exercised by the tsetse fly.[a]

These studies have to be related to the natural frequency of infection in wild animals, data on which have been summarized recently by Ashcroft (1959). The data are few, were obtained by differing methods of examination and come from widely scattered areas inhabited by several species of tsetse fly, hence the conclusions must be viewed with caution. However, 10% of the warthogs and 31% of the bushbuck examined were found infected and, taking into consideration the frequency of tsetse fly meals derived from these animals, it seems that these must form the major trypanosome reservoir. In this connexion the recent isolation of *T. rhodesiense* from a bushbuck in a sleeping-sickness endemic area where *G. pallidipes* is known to be a vector (Heisch et al., 1958) ties in with the high frequency of feeding of this fly on bushbuck. However, it is interesting that waterbuck were found to have by far the highest frequency of infection with trypanosomes of the *T. brucei* subgroup and, despite the very few tsetse flies that feed on this species, it could perhaps also be an important reservoir of sleeping-sickness in some areas. That cattle are frequently found in close contact with *G. palpalis* confirms the comparative lack of importance of this species as a vector of nagana, and is doubtless linked to the high proportion of feeds that are frequently taken from reptiles and domestic animals to the exclusion of potentially infected wild mammals.

The frequency of infection of the different wild animals, however, requires further study to establish, with some degree of certainty, their role as natural reservoirs of trypanosomiasis. Part of this lack of data is due to the labour involved in collecting reasonable samples of animals—a work which also cannot be undertaken in areas where ecological studies are in progress, because of alteration of the habitat. In this connexion a technique that has recently been devised, and may obviate the necessity to shoot the animals, is of interest. It was found that antibodies against trypanosomes can be detected in part of the blood meals of tsetse flies, the other part of which, at least in the larger species, could be used to determine the identity of the host from which the meal was derived (Cunningham et al., 1962). Thus, once a bank of suitable antigens has been established, the antibody status of the various hosts can be readily determined. A start has in fact already been made on the determination of agglutinins in blood meals of wild *Glossina* using a limited number of antigens (Harley & Cunningham, 1962), and it is hoped to link these with host identifications shortly.

Different degrees of parasitaemia occur in wild animals challenged experimentally with trypanosomes (Ashcroft et al., 1959), presumably due to differing immunological responses. It is not known, however, whether wild animals, which are continuously subject to varying degrees of trypanosome

[a] See also the article by B. Weitz on page 711 of this issue.

risk, show appreciable differences in parasitaemia. There is also little known about the influence of the parasitaemia of the host on the number of tsetse flies that subsequently become infected, and further work on these and related problems is desirable.

The influence of temperature on the pupa is another important factor in determining the infection rate of *Glossina*. Earlier findings that raised temperatures experienced by the pupa resulted in a higher proportion of the emerging *G. morsitans* becoming infected with *T. rhodesiense* are now supported by similar findings from experiments on *T. vivax* and *G. palpalis* (Fairbairn & Watson, 1955). These laboratory results are in turn supported by dissection data from wild flies of the *G. morsitans* group, relating to all parts of Africa, that show a significant correlation between the mean annual temperature of the area of collection and the frequency of infection with *T. vivax* group infections (Ford & Leggate, 1961). It is interesting that there was no significant correlation of mean annual temperature with infections of the *T. congolense* group, and the apparent difference between this group and the *T. vivax* and *T. brucei* groups in this respect would be worth investigating further in the laboratory.

The effect of increased temperatures on the pupa in raising the infection rate of the adult may be at least partly due to the emerging flies having a smaller proportion of food reserves in the form of fat (Bursell, 1960a), and therefore feeding sooner after emergence, recent experiments confirming earlier work that the earlier the first feed is taken, the higher is the resulting infection of *G. palpalis* with *T. gambiense* (Wijers, 1958).

Seasonal changes in frequency of infection of *G. palpalis* with *T. vivax* in West Africa, with a peak during the rains, are probably influenced by temperature fluctuations (Fairbairn & Watson, 1955). However, the mean life of the tsetse fly population increases during the cooler weather of the rainy season and this is also of importance in determining the infection rate of the population (Squire, 1951). Age is particularly relevant to infections due to the *T. brucei* group during severe hot seasons, when probably only a limited number of flies live long enough to become cyclically infected. In this connexion a new technique for determining accurately the physiological age of female tsetse flies up to about 40 days old (Saunders, 1960) is of interest as there has previously been no accurate method for aging of individuals.

Sampling methods

The fly-round has long been a valuable tool in survey and research work to determine tsetse fly distribution and density and to provide samples for studies on hunger and infection rate. Fly-rounds are also frequently used to determine the effect of season and of control measures on the population. The usual routine is for a party of trained assistants to follow a defined path, stopping at intervals to catch following flies. This routine, however, represents little of the natural activity of the local human population, and it has long been known that many of the tsetse flies caught on fly-rounds are males that are not ready to feed. It has also been shown that the addition of a bait animal to the party frequently results in changes in the number and sex ratio of the samples obtained, while recent, more precise studies on the comparison of various methods of catching have revealed differences between samples in respect of food reserves (Bursell, 1961), activity (Smith & Rennison, 1961) and age (Saunders, in preparation) as well as sex ratio. Thus each sampling method results in a different section of the population being caught, and it is clear that the sampling method used in any situation must be determined by the information required. When studying the transmission of trypanosomes what is needed is investigation of the infective act—that is, the biting attack on man or domestic animal in situations representative of natural contact with *Glossina*—and examination of flies so collected for determination of infection rates. In particular it would seem that the measure of trypanosome risk to domestic stock would be more precise if catches from bait animals were made rather than data from fly-rounds used. While there has been an increase in the use made of such baited catches recently to estimate biting attack, it appears that the data for infection rates to relate to these catches have frequently been derived from fly-rounds. In view of differences in the age structure of these two types of samples, the infection rates may well be different.

Although it had been long realized that tsetse flies, particularly females, must spend a considerable proportion of their lives at rest or at least in not actively following potential hosts, including man, and thus that only a small proportion of the population is ever seen at one time, it is only in the last decade that a real effort has been made to locate flies in the inactive phase. When it was shown that resting flies of several species could be found fairly readily (Nash, 1952; Isherwood, 1957) impetus was added to the search by the realization that this method of sampling has three attributes of particular value: (*a*) it facilitates the location of certain species, particularly of the *fusca* group, that do not readily appear to man; (*b*) such samples include a high proportion of recently gorged flies of both sexes and are thus of value for the collection of blood meals for host identification; (*c*) the resting-sites are excellent places for the application of residual insecticides, allowing a considerable reduction in the volume necessary per unit area and a corresponding reduction in cost.

The natural resting-sites used during daylight are better known but knowledge is also accumulating on the location of resting flies during darkness. Thus, while the majority of flies apparently rest on the boles and under the branches of trees during the day, during darkness these sites are largely deserted, the flies moving to the upper surface of leaves (Jewell, 1958; Rennison et al., 1958; McDonald, 1960), the changeover apparently taking place at approximately sunset and sunrise. In hotter areas such as the Zambesi Valley there are changes in the type of resting-site utilized at different times of day and at different seasons (Pilson & Leggate, 1960). Thus during the hot season the proportion of *G. pallidipes* resting on the boles of trees increases during the afternoon. This change may be in response to increasing temperature and, if so, may be linked with observations made by Nash in Nigeria that at times of high temperature *G. morsitans* and *G. tachinoides* could be found resting on trees extremely close to the ground where the air is appreciably cooler.

The detection of tsetse flies at very low population densities is a problem of considerable practical importance and much effort may be expended before one specimen is discovered even in areas where trypanosome transmission is known to take place. Conversely, it is difficult to be certain that tsetse flies are completely absent from areas where the vegetation, climate and animal density are apparently suitable. Also, in control schemes involving the use of insecticide, the rapid discovery of any residual population is essential so that adequate measures can be undertaken before the whole of the treated area becomes reinfested. Cattle are frequently used as indicators of the transmission of trypanosomiasis but may not be practicable or suitable on all occasions and it is clearly desirable to catch the flies themselves. Attractants are proving valuable in this field, and in Kenya traps coated with benzene extracts of pig skins have recently been reported as more effective, as well as considerably cheaper, than fly-boy patrols in detecting the few remaining *G. pallidipes* and *G. palpalis* after the application of insecticide (MacOwan, 1961).

Other studies

The maintenance and breeding of tsetse fly colonies in the laboratory has been extended by several studies, particularly those on *G. palpalis* in Nigeria, where the successful production of large numbers of clean flies for transmission experiments is now a routine procedure (Nash et al., 1958). It has not, however, proved possible to maintain a completely closed colony for a long period in Africa. Such a colony is desirable in that a more standardized insect, such as has been developed in mosquitos, would become available for reproducible laboratory experiments. Further studies on the causes of abnormalities in digestion and build-up of food reserves appear necessary before the gradual reduction in insect size found in closed laboratory populations can be overcome. The recent development of a membrane feeding technique (Cockings, 1961) offers several advantages compared with feeding a large laboratory colony on mammals and is also valuable for the collection of metacyclic trypanosomes.

Two recent attempts have been made to raise the infection rate in laboratory tsetse flies artificially. The first, by introducing infected blood into the hind gut *via* the anus, succeeded in raising the infection rate of surviving flies but mortality was such as to nullify the effect when compared with results from infecting flies by feeding normally (Wijers & McDonald, 1961). Preliminary investigation of the simple technique of intra-abdominal injection of flies with infected blood appears promising at this stage (Webber, 1962).

In the control field, studies on the susceptibility of tsetse flies to various insecticides have led to the discovery that old females may be up to nine times more tolerant of dieldrin than young flies (Burnett, 1961). In practice this means that the kill of the older breeding females is smaller than expected and that larvae continue to be deposited in larger numbers than expected. It also means that, if intervals of the order of one pupal period are used between successive aerial applications of insecticide, a significant proportion of the females present in the residual population will be relatively tolerant and the population reduction less than that expected, a result corresponding with field observations.

One other study of interest is that of the effect of gamma-irradiation on the development of the reproductive system of the tsetse fly, a study suggested by the successful eradication of the screw-worm, *Callitroga*, by the introduction of irradiated pupae in Curaçao. While a high degree of sterilization with little increase in mortality was achieved (Potts, 1958), it seems unlikely that this can become a practical method of control, largely because of the very large numbers of irradiated pupae that it would be necessary to introduce even in an area with a small natural population and because of the difficulty of obtaining them.

REFERENCES

Ashcroft, M. T. (1959) *E. Afr. med. J.*, **36**, 289

Ashcroft, M. T., Burtt, E. & Fairbairn, H. (1959) *Ann. trop. Med. Parasit.*, **53**, 147

Burnett, G. F. (1961) *Nature (Lond.)*, **191**, 1317

Bursell, E. (1958) *Phil. Trans. B*, **241**, 179

Bursell, E. (1959) *Trans. roy. ent. Soc. (Lond.)*, **111**, 205

Bursell, E. (1960a) *Bull. ent. Res.*, **51**, 583

Bursell, E. (1960b) *Bull. ent. Res.*, **51**, 39

Bursell, E. (1961) *Proc. roy. ent. Soc. (Lond.) A*, **36**, 9

Bursell, E. & Glasgow, J. P. (1960) *Bull. ent. Res.*, **51**, 47

Cockings, K. L. (1961) *Laboratory maintenance of* Glossina pallidipes. In: East African Trypanosomiasis Research Organization, *Annual report, 1960*, Nairobi, p. 32

Cunningham, M. P., Harley, J. M. B., Southon, H. A. W. & Lumsden, W. H. R. (1962) *Science*, **138**, 32

Fairbairn, H. & Watson, H. J. C. (1955) *Ann. trop. Med. Parasit.*, **49**, 250

Ford, J. & Leggate, B. M. (1961) *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 383

Glasgow, J. P. (1961) *J. anim. Ecol.*, **30**, 87

Glasgow, J. P., Isherwood, F., Lee-Jones, F. & Weitz, B. (1958) *J. anim. Ecol.*, **27**, 59

Harley, J. M. B. & Cunningham, M. P. (1962) *Detection of antibodies against trypanosomes in blood meals of wild* Glossina. In: East African Trypanosomiasis Research Organization, *Annual report, 1961*, Nairobi

Heisch, R. B., McMahon, J. P. & Manson-Bahr, P. E. C. (1958) *Brit. med. J.*, **2**, 1203

Isherwood, F. (1957) *Bull. ent. Res.*, **48**, 601

Jackson, C. H. N. (1945) *J. anim. Ecol.*, **14**, 46

Jackson, C. H. N. (1948) *Bull. ent. Res.*, **39**, 441

Jewell, G. R. (1958) *Nature (Lond.)*, **181**, 1354

Jordan, A. M., Lee-Jones, F. & Weitz, B. (1961) *Ann. trop. Med. Parasit.*, **55**, 167

Lamprey, H. F., Glasgow, J. P., Lee-Jones, F. & Weitz, B. (1962) *J. anim. Ecol.*, **31**, 151

McDonald, W. A. (1960) *Nature (Lond.)*, **185**, 867

MacOwan, K. D. S. (1961) Veterinary Department, *Annual report, 1960*, Nairobi, p. 47

Nash, T. A. M. (1952) *Bull. ent. Res.*, **43**, 33

Nash, T. A. M., Page, W. A., Jordan, A. M. & Petana, W. (1958) *The rearing of* Glossina palpalis *in the laboratory for experimental work*. In: International Scientific Committee for Trypanosomiasis Research, *Seventh meeting, Brussels*, Commission for Technical Co-operation in Africa South of the Sahara, p. 343

Page, W. A. & McDonald, W. A. (1959) *Ann. trop. Med. Parasit.*, **53**, 162

Pilson, R. D. & Leggate, B. M. (1960) *The diurnal activity of* Glossina pallidipes *(Aust.)*. In: International Scientific Committee for Trypanosomiasis Research, *Eighth meeting, Jos*, Commission for Technical Co-operation in Africa South of the Sahara, p. 231

Potts, W. H. (1958) *Ann. trop. Med. Hyg.*, **52**, 484

Rennison, B. D., Lumsden, W. H. R. & Webb, C. J. (1958) *Nature (Lond.)*, **181**, 1354

Saunders, D. S. (1960) *Trans. roy. ent. Soc. (Lond.)*, **112**, 221

Smith, I. M. & Rennison, B. D. (1961) *Bull. ent. Res.*, **52**, 165, 183

Southon, H. A. W. (1959) *Studies in predation on* Glossina. In: East African Trypanosomiasis Research Organization, *Annual report, 1958*, Nairobi, p. 56

Southon, H. A. W. (1960) *Ecology of* T. rhodesiense *sleeping sickness in Busoga, Uganda*. In: East African Trypanosomiasis Research Organization, *Annual report, 1959*, Nairobi, p. 29

Squire, F. A. (1951) *Bull. ent. Res.*, **42**, 371

Webber, W. A. F. (1962) *Intrahaemocoelic inoculation of* G. pallidipes *and* G. morsitans *with trypanosomes of the* T. brucei *subgroup*. In: East African Trypanosomiasis Research Organization, *Annual report, 1961*, Nairobi

Weitz, B. & Glasgow, J. P. (1956) *Trans. roy. Soc. trop. Med. Hyg.*, **50**, 593

Weitz, B., Langridge, W. P., Bax, P. N. & Lee-Jones, F. (1958) *The natural hosts of* Glossina longipennis *Corti and of some other tsetse flies in Kenya*. In: International Scientific Committee for Trypanosomiasis Research, *Seventh meeting, Brussels*, Commission for Technical Co-operation in Africa South of the Sahara, p. 303

Wijers, D. J. B. (1958) *Ann. trop. Med. Hyg.*, **52**, 385

Wijers, D. J. B. & McDonald, W. A. (1961) *Ann. trop. Med. Hyg.*, **55**, 46