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Recent advances and outstanding problems in the biology of phlebotomine sandflies

A review

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Summary

All aspects of the biology of sandflies suffer from a greater or lesser degree of neglect which is explained partly by the special difficulties of studying these small nocturnal flies in the field, and partly by the dearth of functional laboratory colonies. With recent advances in colonization of vector species, rapid progress is likely in the near future. Although the problems of rearing sandflies are not yet solved, closed colonies of three species have recently been established at new centres in Britain, France, the USA and Brazil.

The most important aspect of the biology of a sandfly is whether or not it is a vector of leishmaniasis. The role as vectors of most of the 52 species or subspecies of sandflies thought to transmit leishmaniasis to man requires confirmation.

Genetics of sandflies is a most neglected field, but one which will probably contribute more than any other new study to the solution of the outstanding problems in the biology of sandflies and the transmission of leishmaniasis to man.

Other aspects with outstanding problems include: 1. the physiology of blood digestion; 2. the potential of sandflies to develop resistance to insecticides; 3. the behaviour of sandflies in the field with special reference to (a) dispersion, (b) sources of plant sugars taken by sandflies, (c) factors which stimulate sandflies to seek a bloodmeal, bite and engorge, and (d) the choice of vertebrate hosts as revealed by bloodmeal analyses; 4. population dynamics and ecology with special reference to autogeny, diapause, longevity and age grading.

Key words: Phlebotomus; Lutzomyia; Leishmania; leishmaniasis; sandfly biology.

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Introduction

The selection by WHO of leishmaniasis as one of the diseases of the Special Programme is leading to increasing interest in phlebotomine sandflies, the only known vectors of the various forms of the leishmaniases. As with all large groups of insects of economic importance, the initial work on sandflies was mainly the description of new species and the systematics of the subfamily (Phlebotominae) into which they are grouped. This phase of the work is not yet complete; new species are still being discovered in the Neotropics, and the classification is by no means stable (see Lewis et al., 1977). Attention is, nevertheless, being turned progressively towards the biology of sandflies partly because, with sufficient background knowledge, leishmaniasis can be controlled in some places by attacks on the vector, and partly because the evolution, behaviour and ecology of members of this undoubtedly old group of haematophagous insects are of interest in their own right.

Knowledge of the biology of sandflies is fragmentary and is mainly based on observations on one or other of the few better studied species. It is then tempting to assume that the biology of less well known sandflies is the same, an assumption fraught with error. The diversity of sandflies is, therefore, the backcloth against which generalizations made in the present paper must be viewed.

Two factors other than the problems of taxonomy which have contributed to the comparative neglect of the study of the biology of sandflies are, firstly, the difficulties of establishing truly functional colonies in the laboratory and, secondly, the specialized techniques required to study these small nocturnal insects in the field.

In considering the biology of the known or suspected vector species, special attention should be paid to any facet which suggests specialization, e.g. strong preference for a particular vertebrate as a source of blood; a preferred source of sugar; or marked dependence on specialized larval-breeding or adult-resting sites. New methods of reducing the numbers of a target species by changing the habitat may then present themselves. Specialized species of sandflies dependent on particular habitats or hosts are more vulnerable than less specialized species which are generally widely distributed and flourish in diverse habitats.

Incrimination of vector species

From the medical point of view, the most important aspect of the biology of a sandfly is whether or not it transmits leishmaniasis to man. In the control of sandflies, the first step is to determine the target species, and the second is to discover enough of its biology to mount an economical and effective attack.

In Tables 1 and 2 are listed 52 species or subspecies of sandflies known or suspected to be vectors of leishmaniasis. Two of the most recently incriminated species are *Phlebotomus saheli* in India (Kalra and Lewis, 1975) and *Lutzomyia umbratilis* in Brazil (Lainson et al., 1976; Ward and Fraiha, 1978). In addition,

Table 1. Known or suspected vectors of leishmaniasis of man in the New World (Lainson and Shaw, 1971; Lewis, 1974; Ward and Fraiha, 1977; Carnevali and Scorza, 1977; Lainson, personal communication)

Sandfly[1]	Parasite	Disease in man	Presumed distribution of vector/parasite pair
Lu. olmeca olmeca	Le. mexicana mexicana	cutaneous, Chiclero's ulcer	Belize and other parts of Central America
Lu. permira (?)	do.	do.	do.
Lu. cruciata (?)	do.	do.	do.
Lu. flaviscutellata	Le. m. amazonensis	cutaneous, often diffuse	Amazon Basin, Trinidad
Lu. flaviscutellata (?)	Le. m. pifanoi	cutaneous, diffuse	Venezuela
Lu. olmeca bicolor (?)	Le. mexicana subsp. indet.	(not yet recorded in man)	Panama
Lu. wellcomei	Le. braziliensis braziliensis	cutaneous, espundia	Pará State, Brazil
Lu. paraensis	do.	do.	do.
Lu. amazonensis (?)	do.	do.	do.
Lu. pessoai	do.	do.	São Paulo State, Brazil
Lu. intermedia	do.	do.	do.
Lu. mignonei (?)	do.	do.	S. Brazil
Lu. whitmani (?)	do.	do.	S. Brazil
Lu. umbratilis	Le. b. guyanensis	cutaneous, pian bois	N. Brazil
Lu. anduzei	do.	do.	Guianas, N. Brazil
Lu. anduzei (?)	Le. braziliensis subsp. indet.	cutaneous, espundia	Venezuela
Lu. trapidoi	Le. b. panamensis	cutaneous, simple, metastasis rare or absent	Panama and neighb. countries
Lu. ylephiletor	do.	do.	do.
Lu. gomezi	do.	do.	do.
Lu. sanguinaria (?)	do.	do.	do.
Lu. panamensis	do.	do.	do.
Lu. panamensis (?)	Le. braziliensis subsp. indet.	cutaneous, espundia	Venezuela
Lu. verrucarum	Le. peruviana	cutaneous, uta	Peru
Lu. peruensis (?)	do.	do.	do.
Lu. townsendi	Leishmania sp. indet.	cutaneous, simple	Venezuela (cloud forest)
Lu. longipalpis	Leishmania donovani chagasi	visceral	S. America (not Chile)
Lu. evansi (?)	do.	do.	do.

[1] Including several species, marked "(?)", for which evidence of their roles as vectors is far from conclusive. Lainson and Shaw (in press) give further details of many suspect but unproven vectors in the New World.

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Sandfly[1]	Parasite	Disease in man	Presumed distribution of vector/parasite pair
P. sergenti	Le. tropica	"urban cutaneous"	Parts of Med. Basin. N. Africa. Iran
P. perfiliewi[2]	do.	do.	Parts of Med. Basin. Crimea
P. bergeroti (?)	do.	do.	S. Sahara
P. chabaudi (?)	do.	do.	Algeria
P. papatasi[3]	do.	do.	Parts of Med. Basin
P. papatasi	Le. major	"rural cutaneous" (= "zoonotic cutaneous leishmaniasis")	W. Asia
P. alexandri	do.	do.	Central Asia. Turkey
P. caucasicus[4]	do.	do.	Central Asia, Iran
P. mongolensis[5]	do.	do.	Iran
P. ansarii	do.	do.	do.
P. saheli	do.	do.	Rajistan, N.W. India
P. duboscqi	do.	do.	W. Africa
P. longipes	Le. aethiopica	cutaneous	Ethiopia, Kenya
P. pedifer	do.	do.	do.
P. argentipes	Le. donovani donovani	visceral	N. E. India
P. ariasi	Le. d. infantum	do.	S. France
P. perniciosus perniciosus	Le. d. infantum	do.	W. Mediterranean
P. simici (?)	do.	do.	Israel
P. tobbi	Le. donovani subsp. indet.	do.	E. Mediterranean. Iran. Caucasus
P. longicuspis	do.	do.	Algeria. Tunisia?
P. chinensis chinensis	do.	do.	China
P. c. halepensis	do.	do.	Causasus. Iran? Syria?
P. c. longiductus	do.	do.	Uzbekistan. USSR. Pakistan?
P. kandelakii (?)	do.	do.	Azerbaidjan. USSR
P. langeroni orientalis	do.	do.	Sudan. Chad?
P. major syriacus	do.	do.	Parts of Med. Basin: Turkestan
P. martini	do.	do.	Kenya. Uganda? Somalia?
P. celiae (?)[6]	do.	do.	do.
P. vansomerenae (?)[6]	do.	do.	do.

[1] See footnote to Table 1.
[2] Possibly also a vector of visceral leishmaniasis in Rumania.
[3] Possibly also a vector of visceral leishmaniasis in Iraq.
[4] Possibly also a vector of visceral leishmaniasis in S.E. Kara Kum.
[5] Possibly also a vector of visceral leishmaniasis in parts of Central Asia.
[6] Females of P. celiae and P. vansomerenae are morphologically indistinguishable from those of P. martini.

overwhelming evidence of the role of *Lu. flaviscutellata* as the vector of *Leishmania mexicana amazonensis* in the Amazon Basin has been presented (Ward et al., 1973, 1978), and experimental transmissions of *Le. donovani chagasi* by the bite of *Lu. longipalpis* have removed any doubts of the part played by this fly in the transmission of visceral leishmaniasis in Brazil (Lainson et al., 1977). In the Old World, the strong epidemiological evidence of the role of *P. ariasi* as a vector of *Le. donovani infantum* in southern France west of the Rhône (Rioux et al., 1969) has been augmented by the demonstration of the complete life cycle of the parasite in the fly (Rioux et al., in press).

Many of the vectors listed in the tables cannot unequivocally be said to transmit leishmaniasis. In some instances, the sole evidence appears to be that a suspect species is the most abundant man-biting sandfly in an endemic area, or unidentified flagellates have been found in the gut of a wild-caught fly, and the complete life cycle and transmission of *Leishmania* by bite has been demonstrated for only a few sandfly-parasite combinations. There is, therefore, a need to prove beyond reasonable doubt which of the suspected vectors are truly responsible for transmission. For this, it should be shown that promastigotes isolated from individual wild-caught flies are the same as those cultured from man, and that isolates of local parasites will complete their development in the suspected vector with migration of the parasites into the head and proboscis of the fly. In the Old World, complete development in a fly is strong evidence of its capacity to transmit the parasite, but in the New World the full life cycle of some species of *Leishmania* is easily demonstrable in sandflies which, because of their distribution or ecology, are known not to be natural vectors (see Killick-Kendrick, in press). In Latin America, therefore, lack of development in a fly is evidence that it is unlikely to transmit a given parasite in nature, but full development cannot be considered to be unquestionable proof of the role of a fly as a vector. Similarly, whereas experimental transmission of an Old World species of *Leishmania* by a local sandfly is strong evidence that the fly can transmit the parasite in nature, in the New World the same weight cannot be given to similar experimental results.

Recent observations on the life-cycles of several *Leishmania* species in sandflies have confirmed and extended earlier findings (Killick-Kendrick et al., 1974, 1977a, c, d; Molyneux et al., 1975, 1977; Lainson et al., 1977a, b; Ward et al., 1978; Rioux et al., in press). These observations are reviewed in detail elsewhere (Killick-Kendrick, in press) but the principal conclusions pertinent to the incrimination of vectors are summarized here. Firstly, there is new evidence to support the view that the life-cycles of all mammalian (and some saurian) leishmaniae culminate in the migration of parasites to the proboscis of the fly and that transmission takes place when these morphologically characteristic forms are deposited in the skin as an infected fly engorges; an old idea that transmission is by parasites regurgitated from the oesophageal valve no longer seems tenable. Secondly, although the life-cycles of most species of *Leishmania*

are complete within a single ovarian cycle of the fly, and parasites may therefore be transmitted at the first bloodmeal after an infective feed, the development of some (*Le d. donovani* and some strains of *L. d. infantum*) is not complete until after the second bloodmeal; the full life cycles of these and possibly other parasites are not demonstrable in the laboratory if captive flies do not survive oviposition. Thirdly, parasites of the *braziliensis* group (*sensu* Lainson and Shaw, 1972) uniquely colonize the pylorus and ileum of the fly before spreading forwards to the oesophageal valve, pharynx, cibarium and mouthparts; in wild-caught flies, therefore flagellated forms of *Leishmania* posterior to the midgut of sandflies which feed on mammals may, on present evidence, be assumed to belong to the *braziliensis* group. Fourthly, in the oesophagus, pharynx and cibarium of the sandfly all species of mammalian *Leishmania* appear to be small rounded, sessile flagellates (paramastigotes) which are not easily seen in fresh preparations; infections in this site are strong evidence that a wild-caught fly is a vector, and stained smears of pharynges thought possibly to be infected should be prepared to confirm the presence of parasites.

Colonization

In the past eight years, considerable progress has been made in the colonization of sandflies. A distinction must be made between rearing sandflies for only a few generations, and the establishment of a colony regularly producing enough flies for experimental work. Most of the newly established colonies are in the second category. They have proved invaluable in studies on the life-cycles and transmission of leishmaniae but, with few exceptions (e.g. Johnson and Hertig, 1961; Foster et al., 1970; ,Ward, 1974b; Chaniotis, 1974, 1975; Gemetchu, 1976; Ready, 1977), studies on the biology of colonized flies have hardly begun.

The principal recent advances may be summarized as follows. Foster et al. (1970) reported the successful colonization of *P. longipes* from Ethiopia and, in the following year, Gemetchu (1971) showed the value of desiccated liver powder as a food for the larvae of this sandfly; he later (1976) published full details of his methods of maintaining a colony of *P. longipes*. Liver powder has been found to be an ideal food for the larvae of some other colonized species, but is not suitable for all (Killick-Kendrick, 1973; Ward and Killick-Kendrick, 1974). Christensen (1972) in Panama, founded colonies of *Lu. vespertilionis* and *Lu. trinidadensis* and described a new container for holding adult flies. In 1972, Killick-Kendrick et al. (1972, 1977b) established a closed colony of *Lu. longipalpis* from Brazil in England which, eight years later, is in the 47th generation. No signs of reduced vitality have been detected, and more than 1,000 flies are readily produced each generation. Innovations which raised productivity include methods of maintaining gravid females, which improved numbers of eggs laid, and the use of snap-top containers for the control of water in larval pots. A line of this colony is now established in the Department of Tropical Medicine,

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Letterman Army Institute of Research, San Francisco, USA (Rutledge et al., 1976a).

A highly productive colony of another strain of *Lu. longipalpis* from Brazil was founded by Ward and Lainson at the Instituto Evandro Chagas, Belém, Brazil (Ward, 1974a, b). More recently, Ward (1977) has, for the first time, succeeded in colonizing *Lu. flaviscutellata* in the same Institute. In a study of *Lu. trapidoi* in Panama, Chaniotis (1975) described modifications in methods of culture which show promise in the colonization of other difficult sylvatic species.

New colonies of *P. perniciosus* have been bred at the Faculty of Medicine, Montpellier, France; the addition of dried *Daphnia* to larval food composed of soil, faeces and leaf litter was found to improve larval survival (Ready and Croset, 1977). In Brazzaville, Trouillet (1976) is currently studying the colonization of several African sandflies which can be reared by the methods used for *Lu. longipalpis*.

Species of sandflies which have been bred in good numbers as closed colonies for more than ten consecutive generations include: *P. argentipes*, *P. papatasi*, *Lu. longipalpis*, *Lu. sanguinaria*, *Lu. gomezi* and *Lu. flaviscutellata*. Others which could almost certainly be reared with similar success are: *P. longipes*, *P. perniciosus*, *Lu. vespertilionis* and *Lu. trinidadensis*. Unsuccessful attempts have been made to found colonies of *P. perfiliewi*, *P. ariasi*, *Sergentomyia bedfordi*, *Lu. lainsoni* and *Lu. wellcomei* (references in Killick-Kendrick et al., 1977b).

Laboratory colonies of sandflies are labour intensive and, therefore, expensive. There is a need for mass-rearing methods which do not require daily attention to larvae. The methods used by Ready and Croset (1977) to rear *P. perniciosus* show that such developments are possible.

Some outstanding problems inhibiting progress may be solved by information gathered in field studies. The principal difficulties are:

Death at oviposition. Death of colonized females at oviposition hampers experiments in which transmission by bite is attempted, and reduces the productivity of colonies.

Losses of larvae. More work on larval habitats and food in the field would help in improving methods of rearing larvae in the laboratory. Death of larvae in colonies is a major source of loss.

Diapause of larvae or eggs. Holarctic sandflies with the exception of *P. papatasi* have seldom been established as functional closed colonies largely because of winter diapause as 4th instar larvae or, uncommonly in the Old World, as eggs.

Engorgement of females. Females of some species cannot easily be persuaded to take bloodmeals in the laboratory. If the stimuli which trigger host-finding, biting and engorgement were understood, it might become possible to persuade reluctant females to engorge.

Digestion of blood

Apart from observations on the formation and structure of the peritrophic membrane (Feng, 1951; Gemetchu, 1974; Killick-Kendrick et al., 1974) and the inactivation of complement of the bloodmeal in the midgut (Adler and Theodor, 1926), no work has been done on the physiology of the digestion of blood by the sandfly. Since the alimentary tract is the microhabitat of *Leishmania* in the invertebrate host, the relationships between the parasite and the fly would be better understood if more were known of the conditions in the gut of the flies during the whole process of digestion.

There are several outstanding problems which may be illuminated by such work. The stimuli which lead the parasites to migrate forwards towards the proboscis of the fly are unknown, as are the reasons for the differing susceptibilities to leishmaniae of sandflies; some appear to be totally insusceptible, while others are susceptible to some species of parasites but not to others. The unique but unexplained development of the *braziliensis* group in the pylorus (hindgut triangle) of the fly (see Killick-Kendrick et al., 1977d; Lainson et al., 1977b) is presumably related to the physiology of the gut and the metabolic requirements of the parasites. Barnet and Suyemoto (1961) suggested that one factor which might enhance the virulence of a strain of *Leishmania* may be the particular species of sandfly transmitting it; if this is shown to be true, the explanation may lie in differences in the physiology of different species of phlebotomines.

Susceptibility to insecticides

Ward (1977) warned that, although sandflies are known normally to be highly susceptible to insecticides, prolonged contact might lead to the appearance of resistance. He suggested that regular tests should be made on flies from areas in which leishmaniasis is endemic.

In Brazil, Falcão (1963) found that *Lu. longipalpis* from Minas Gerais had an LD_{50} of 0.55% to DDT and 0.24% to Dieldrin. In 1961, Fedder and Alekseev (quoted by Perfil'ev, 1968) noted that different species of sandflies vary in susceptibility to DDT and BHC and that there was resistance of sandflies to chlorinated insecticides in some parts of the USSR; this was, however, uncommon and of little importance in control.

In the light of these observations, there is a need to assess the potential of sandflies to develop resistance which could cause problems in control campaigns. It is especially important that tests should be made before and after flies have been under known insecticide pressure.

Behaviour in the field

Many aspects of the behaviour of sandflies in the field are inadequately studied. There is, in particular, a need to know more about the sylvatic Neotropical species, the control of which cannot feasibly be undertaken by means of

insecticides. Five neglected aspects with outstanding problems are discussed below.

Dispersion. From limited studies on a few species and the manner in which sandflies are seen to move on short flights, it is known that sandflies seldom travel great distances (Perfil'ev, 1968). Precise information is, however, lacking for almost all known or suspected vectors and, most importantly, no work appears to have been done on dispersal by wind currents. An old report of a single male specimen of *P. perniciosus* (the identity of which is not in doubt) on the island of Jersey (Marett, 1923) suggests that sandflies can be dispersed by wind (in this instance from the mainland of France).

Information on dispersal is of importance in control campaigns in which the probability of re-infestation must be assessed. Similarly, in genetical studies, it may be necessary to judge the degree of geographical isolation of two apparently similar populations. Since sandflies are easily marked with fluorescent powders (references in Killick-Kendrick et al., 1978) which probably persist for life, there are no technical limitations on studies on movement. Attention must, however, be paid to the type of terrain and to the meteorological conditions during the period of observation.

Sugar feeding. As with other haematophagous dipterans, sandflies feed on plant sugars (Lewis and Domoney, 1966), but neither the frequency with which sugars are taken nor their sources are known. A possible source which has been largely overlooked is honeydew of aphids or coccids (Killick-Kendrick, in press). In preference trials in the laboratory, Chaniotis (1974) showed that *Lu. trapidoi* takes fructose or sucrose in preference to other sugars and that survival and engorgement of this species is greatly enhanced by the provision of sugar (Chaniotis, 1975). Using a simple test devised for mosquitoes (Van Handel, 1972), it was recently demonstrated that almost all female *P. ariasi* collected from dogs as the flies sought a bloodmeal contained demonstrable fructose (Young et al., in press). Presumably the flies take sugar as a source of energy before dispersing to seek blood. Ashford (1974) reported that some Ethiopian species of sandflies appear to take plant juices by piercing the leaves of plants. Observations by Ready (1977), however, suggest that sugars are probably simply sucked up, and not taken by piercing with the mouthparts.

The relevance of sugar taking by sandflies in the epidemiology of leishmaniasis is twofold. Firstly, possible preferences by a sandfly for particular plants may restrict its distribution, and thus the distribution of the parasite it transmits. Secondly, the types of sugars and the frequency with which they are taken by a particular species may be a factor in the insect's ability to transmit leishmaniasis. Promastigotes require carbohydrate for their development, and after the bloodmeal is digested and passed, plant sugars may be essential for survival of the parasites. The well-known finding by workers in India that letting sandflies feed on raisins was the key to the successful transmission of *Leishmania d. donovani* by the bite of experimentally infected *P. argentipes* (see review by

Killick-Kendrick, in press), suggests that plant sugars may influence the anterior migration of parasites to the head and proboscis of the vector.

Host-finding and feeding stimuli. The stimuli which attract sandflies to a vertebrate host, trigger biting and lead to engorgement have not been well studied, although a few incidental observations point the way to new work. In persuading *Lu. longipalpis* to engorge on tiny lesions on hamsters infected with *Le. b. braziliensis*, it was found that flies could be "conditioned" to take a blood-meal by narcotizing them with CO_2, removing them at once from the gas and permitting them to recover in the dark (Killick-Kendrick et al., 1977c). Flies left for more than several minutes in CO_2 became over-stimulated and seldom took blood. Chaniotis (1975) reported that female *Lu. trapidoi* would rarely engorge in the laboratory unless they had been previously provided with a sugar solution.

Ready (1977) showed that, unexpectedly, the addition of ATP to fluids taken by *Lu. longipalpis* through a chick membrane appeared not to enhance engorgement.

Bloodmeal analyses. In spite of the comparatively small size of the bloodmeal taken by sandflies, it is possible to identify bloodmeals and thus determine the range of possible reservoir hosts on which a population feeds, and to show degrees of anthropophily (e.g. Tesh et al., 1951, 1952; Živković et al., 1971). An outstanding example of such work was recently published in which it was shown that *P. argentipes* in India which, until recently, appeared to have been largely controlled by spraying against mosquitoes, had re-acquired man-biting habits (Das et al., 1976). It was correctly predicted that this could lead to a new epidemic of kala azar.

The preferred hosts of only few known or suspected vectors have been studied by bloodmeal analyses, and more work is needed. A recently published latex agglutination method for the identification of the small bloodmeals of *Culicoides* (Boorman et al., 1977) may be of use with sandflies.

Oviposition and larval breeding sites. Information on the behaviour of sandflies at oviposition would be invaluable in attempts to keep females alive after laying eggs in the laboratory and thus forge models of transmission by bite, a valuable technique to challenge laboratory animals or human volunteers given experimental vaccines or prophylactic drugs.

In the laboratory, most gravid females lay eggs and die either as they lay or shortly afterwards. In nature, where more than one gonotrophic cycle is considered normal (Dolmatova, 1942), it is possible that eggs are laid one by one over a comparatively wide area; this is suggested by an incidental observation by Foster et al. (1970) who found that survival at oviposition and the numbers of eggs laid by *P. longipes* were improved when gravid females were held in a large holding cage ("1 × 2 m" in size).

Natural larval breeding sites are in damp soil, humus or leaf litter. Precise information on the exact places is, however, inadequate and any possibility of

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controlling sandflies by destroying their larvae can rarely be considered. In recent work on the forest flies of Panama, in which soil emergence traps were used, larval breeding sites were demonstrated of 13 species, 10 of which were previously unknown. In this habitat, it was concluded that control measures aimed at larvae were not feasible (Rutledge and Ellenwood, 1975a, b, c). In other instances, however, alterations in the habitat which change larval breeding sites (e.g. drainage or selected felling of trees) might be found to reduce the population of sandflies.

Population dynamics and ecology

Long-term studies on the population dynamics of a vector species are of inestimable value in planning a control strategy. The number of annual generations and population peaks (when transmission of leishmaniasis is most probable) determine the most effective time to mount an attack.

Following classical work in the USSR, and stimulated by the concept of landscape epidemiology (Pavlovsky, 1966), Rioux et al. (1969) undertook a penetrating study of the ecology of sandflies in France west of the Rhône. A wide variety of the techniques used in observations on the dynamics of sandfly populations are described in detail in their monograph. Other notable contributions during the past ten years were by Disney (1968) and Williams (1970) in Belize; by Shaw and Lainson (1972), Shaw et al. (1972), Ward et al. (1973) and Lainson et al. (1973) in Brazil; and Rutledge et al. (1976b) in Panama.

Four of the more neglected facets of studies on population dynamics and ecology are discussed below.

Autogeny. Although perhaps less common than in mosquitoes, autogeny in sandflies has been reported for some species (e.g. Johnson, 1961; Schmidt, 1965) and suspected for others (Lewis et al. 1970); an ability to produce eggs without a bloodmeal is of obvious importance in population dynamics (Lewis, 1971). The frequency of autogeny in species established in the laboratory should always be observed and attempts be made to breed autogenous lines similar to a colony of *Lu. gomezi* selected by Johnson (1961). This would provide material for further study.

Diapause. In temperate countries, sandflies overwinter as eggs or larvae. Similarly, diapause of one or other of these stages is probably the means by which many tropical sandflies survive periods of drought or heavy rain (Ward and Killick-Kendrick, 1974). Johnson and Hertig (1961), working with laboratory colonies of Panamanian sandflies, believed that lack of water was a possible reason for quiescence of larvae, but their observations are best interpreted as arrested development rather than as true diapause which would not be immediately reversible by the provision of water. Ready (personal communication) has recently concluded a short study of diapause of *P. ariasi* and *P. perniciosus* undertaken at the Faculty of Medicine, Montpellier, in which, in addition to temperature, the photoperiod experienced by adult females was found to be an

important factor in initiating diapause of 4th instar larvae developing from their eggs. No similar studies have been undertaken with other species.

Longevity of adult sandflies. The longevity of sandflies probably varies from species to species. Although death at oviposition is invariable in the laboratory, it is obviously not usual for vector species (Dolmatova, 1942) which must feed at least a second time to transmit an infection. There are no data on the age structure of populations of sandflies in nature, and the life expectancy of sandflies cannot, therefore, be calculated.

There is reason to suppose that infections of some species of *Leishmania* may be pathogenic to sandflies and thus shorten their life (Molyneux et al., 1977), although only one comparison of the longevity of infected and uninfected flies appears to have been made in the laboratory (Alekseev et al., 1975). In mark-release-recapture studies of *P. ariasi* in the south of France (Rioux et al., in press), it was recently found that sandflies infected with *Le. d. infantum* can survive in nature for at least 29 days; this period represents 3 or 4 gonotrophic cycles depending upon the ambient temperature. The longevity of uninfected *P. ariasi* in nature is not known.

Alekseev (in Perfil'ev, 1966) suggested that infections of *Leishmania* may actually benefit sandflies, but more recent work suggests the contrary. From field observations, Ashford et al. (1973) believed that *Le. aethiopica* was pathogenic to *P. longipes*. Molyneux et al. (1977) postulated that leptomonads may penetrate the midgut epithelium, and thus damage the sandfly. In ultrastructural studies on *Le. b. braziliensis* in a Brazilian vector, *Lutzomyia wellcomei*, Killick-Kendrick et al. (1977a) found promastigotes in the gut cells of the fly and noted that the cells appeared damaged. Double infections of two species of *Leishmania*, one mammalian and one saurian, were found by Alekseev et al. (1975) to kill artificially fed *P. papatasi* and *Sergentomyia murgabiensis*; in particular, each of these flies seemed most adversely affected by the parasite not normally transmitted by it in nature.

There are several accounts of the behaviour of infected sandflies at a potentially infecting feed which show that, when heavily infected, flies appear to find it difficult to engorge, and tend to probe many times before either taking a small meal or not feeding at all. This behaviour perhaps explains occasional clusters of *Le. tropica* lesions of the same size which are presumed to be the result of one infected fly probing several times. Killick-Kendrick et al. (1977c) postulated that parasites obscuring the cibarial sensilla interfere with full engorgement. Multiple probing by such an inhibited fly could increase the number of times parasites were transmitted.

In any attempt to create a mathematical model of the epidemiology of leishmaniasis, it is essential to know the life expectancy of sandflies, particularly those which are carrying leishmaniae, and to determine the number of gonotrophic cycles. Differences between various combinations of fly and parasite can confidently be predicted.

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Age grading. There is no reliable rapid method of estimating the age and parous state of sandflies. Dissection of ovaries is difficult and slow, and is of little practical value when dissecting large numbers of wild-caught flies in searches for natural infections of *Leishmania*. In practice, therefore, it is not possible to determine the proportion of infected parous flies.

Recognition of parous flies by the presence or absence of granules in the accessory glands is said to be possible with some species (references in Chaniotis and Anderson, 1967; see also Lewis et al., 1970), but doubt is cast on the reliability of this method by laboratory studies on *P. longipes* (Foster et al., 1970), *Lu. longipalpis* (Ward, 1974a) and sandflies of northern California (Chaniotis and Anderson, 1967).

Ashford (1974) noted that in 0.7% saline parous flies tended to sink, and nulliparous flies to float. This method may be useful and should be further investigated.

Genetics

The genetics of sandflies is a neglected subject. Studies were presumably delayed by a dearth of laboratory colonies. White and Killick-Kendrick (1975) demonstrated giant polytene chromosomes in the salivary glands of 4th instar larvae of *Lu. longipalpis* and, although the chromosomes were fragile, it was concluded that their patterning was differentiated well enough to be of use cytotaxonomically. Ward and Killick-Kendrick (in White and Killick-Kendrick, 1975) crossbred two lines of Brazilian *Lu. longipalpis*, one from a peridomestic habitat in N.E. Brazil and the other from a cave in Minas Gerais, Brazil, and found they were reproductively compatible and therefore probably conspecific.

Of all aspects of the biology of sandflies a study of their genetics is the most likely to produce results of widespread significance. There may be sibling species varying in their roles as vectors, or there may be differing susceptibilities to *Leishmania* between different populations of the same species. Differences in behaviour of apparently the same species in different parts of its distribution sometimes suggest why leishmaniasis is absent from some places where a known vector is prevalent. *P. argentipes*, the vector of kala azar in India, is abundant in Sri Lanka where the disease is absent. In the latter country the fly is not anthropophilic. Interestingly, minor morphological differences of the two populations have been noted (Lewis and Killick-Kendrick, 1973), and a genetical analysis would clearly be revealing. Other widespread species which are known to inhabit differing habitats in various parts of a wide geographical range (e.g. *Lu. longipalpis*, *P. papatasi*) should be similarly examined.

A study of the genetics of sandflies is likely to throw light on all aspects of the biology of sandflies, including differences in host preferences and breeding sites, vector potential, choice of resting places, ease of colonization, and susceptibility to insecticides – all subjects with outstanding problems.

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