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BRITISH SIMULIID GROUP BULLETIN

Number 1

May 1992

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EDITORIAL

The Newsletter of the British Simuliid Group appeared from 1979 until its 13th number in 1987. At subsequent Annual Meetings it was suggested that it should be retitled as 'Bulletin' and that its presentation could be improved through the medium of wordprocessing. Unfortunately, achieving the latter aim has considerably delayed the Bulletin's production - for which my apologies, in particular to the contributors to this first number. I am also glad to acknowledge the suggestions and assistance received from colleagues over the choice of hard and software options, and the continued support of my Department in meeting the costs of printing and distributing the Bulletin.

Accounts of the Meetings held from 1988 to 1991 will be given in the next Bulletin. Subsequent issues will continue to report the Annual Meetings, but perhaps I can remind the readership of the original aims of the Newsletter -"to maintain and develop contacts between those interested in simuliids and to provide for the exchange of news, information, requests and ideas concerning all aspects of simuliid biology" (Gavin Gatehouse, Newsletter **1**). It is to be hoped that the Bulletin will serve a similar purpose and to this end articles and news items will always be welcome.

Trefor Williams : *Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX*

Phone: 051 794 5021 Fax: 051 794 5094

10th ANNUAL MEETING OF THE BRITISH SIMULIID GROUP

[BSG Bull. No. 1, May 1992]

J.A. Bass : *Eastern Rivers Group, Monkswood Experimental Station, Abbots Ripton, Huntingdon PE17 2LS*

About 20 people attended a very successful 10th Simulium Group Meeting on 29th September, 1987, held at the Freshwater Biological Association's River Laboratory in Dorset. Dr. Mike Ladle (F.B.A.) chaired the meeting. Six talks were given and some poster exhibits displayed. Authors' summaries are given below; in addition, Colin Fairhurst and Margaret Curtis (Salford) reviewed the environmental monitoring of the Onchocerciasis Control Programme.

Bob Cheke (Tropical Development and Research Institute) provided a video on Onchocerciasis in West Africa and this was followed by a general discussion on recent developments in the Control Programme.

Thanks go to Lilian Ladle for the excellent buffet lunch and to John Bass for a taxi service for participants (and the food!).

TALKS GIVEN AT THE 10th ANNUAL MEETING

Addresses are those at the time of the 10th Annual Meeting - Editor

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Provisional Study of Simuliids in Speyside, Scotland

James Coupland : *Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB9 2TN*

The Kincraig region of the Spey Valley is subject to serious infestations of 'birchflies' (Simuliidae). While the pest species have been tentatively identified (*S. reptans* and *S. tuberosum*) their ecology is very poorly known. Consequently, since October 1986 research has been conducted into the ecology and biology of the simuliids of this region with the aim of developing safe control measures.

This talk discussed the various methods used to collect larvae and adults and their various shortcomings. One of the major problems faced was the difficulty in obtaining quantitative estimates of simuliid larval density. This is due to the nature of the rivers themselves, which tend to be extremely 'flashy', with very unstable substrate. Adult sampling was done with sticky
traps, suction traps and silhouette traps. The silhouette traps were the most effective at catching large numbers of simuliids especially if baited with carbon dioxide. Emergence patterns of the most common species (*S. reptans*, *S. variegatum*, *Prosimulium hirtipes*) were shown along with
their larval and adult distributions. While there were occasional reports of biting this year the frequency was quite low compared with previous years. This may have been due to the adverse weather conditions.

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Haemolymph Attenuation of Microfilarial Motility (HAMM): An in vitro assay of simuliid immunity to Onchocerca

P.J. Ham : *Department of Medical Entomology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA*

A marked variation in the susceptibility of simuliids to *Onchocerca* infections has been observed, both for British blackflies and *O. lienalis*, and for West African *Simulium damnosum* cytospecies to *O. volvulus* of differing strains. As well as innate variation in susceptibility, an aquired immunity has been demonstrated in which haemolymph from *Onchocerca*-infected *Simulium* confers resistance to susceptible naive recipient flies, following passive transfer. This study describes methods used to look at the in vitro haemolymph properties, in particular by looking at the microfilaricidal properties of haemolymph (a) from different uninfected species of British and West African simuliids (innate immunity), (b) from infected simuliids (acquired immunity). The method used was to bleed simuliids of their haemolymph using fine glass needles, and to deposit the fluid from each fly (1µl approx) in a single well of mini microtitre trays (Terasaki plates). To this haemolymph was added a suspension of fresh living microfilariae. Between 20_30 flies were used for each group. The plates were kept at 21°C in a humid chamber and the motility of the microfilariae monitored at regular intervals.

Innate immunity

A series of experiments revealed significant attenuation of microfilarial motility in certain British species of *Simulium* haemolymph, when compared to others. *S. equinum* and *S. erythrocephalum* both significantly reduced motility in comparison to *S. ornatum*. This correlates with in vivo susceptibility. More interestingly, *S. yahense* haemolymph, a highly susceptible form of the *S. damnosum* complex was relatively non-attenuating to *Onchocerca volvulus*, whereas *S. soubrense* haemolymph was highly microfilaricidal, again correlating with in vivo studies.

Acquired immunity

O. lienalis infected *S. ornatum* haemolymph was found to attenuate *O. lienalis* microfilariae motility to a much greater degree than uninfected groups. Attenuation was not dependent on age of infection. Furthermore nonspecific trauma did not bring on such a response in the haemolymph. The response was however able to attenuate *Brugia pahangi* microfilariae
in vitro. Both types of immunity can be demonstrated, in vitro, to be haemolymph related, but biochemical and physiological causes are probably different.

Thanks go to the Wellcome Trust for a Tropical Lectureship, and to Dr. R. Garms and the staff of the Liberian Tropical Institute for their valuable assistance.

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Attachment and Germination of Trichospores in the Digestive Tracts of Simuliidae Larvae

S.T. Moss : *School of Biological Sciences, Portsmouth Polytechnic, Portsmouth, Hampshire PO1 2DY*

Two aspects of Trichomycetes/Simuliidae relationships were presented:

Germination and attachment of trichospores

The digestive tracts of Simuliidae larvae may be inhabited by up to 14 species of the Harpellales (Trichomycetes). Species of *Harpella* occur attached to the peritrophic membrane lining the midgut whereas species of the Legeriomycetaceae occur attached to the cuticle lining the hindgut. Survival and growth of thalli depend upon the germination and attachment of their trichospores to specific regions of the digestive tract. The mechanisms of trichospore germination and attachment have been studied at the electron microscope level. This has shown the presence of an initial but ephemeral adhesive 'pad' and a subsequently produced persistent holdfast. The initial adhesive 'pad' is produced by secretion of adhesive material through pit fields in the terminal region of the sporangiospore wall. The adhesive accumulates between the sporangio-spore and sporangium walls prior to germination within the gut. The stimulus for germination within a specific region of the host gut has not been determined. However, germinated trichospores adhere immediately to the host cuticle by means of the accumulated adhesive. Basal growth of germinated and attached trichospores produces a basal, persistent holdfast and the initial adhesive 'pad' is lost.

The presence of harpellid-like hyphae in ovary tissue

Transmission electron microscopy of ovary tissue from *Simulium yahense* (collected by R. Garms in Liberia) has indicated the presence of fungal thalli. The septal structure of these hyphae is similar to that characteristic of the Harpellales. If these initial observations are confirmed it will be the first report of a harpellid growing within host tissues.

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Predation of larval blackflies by larvae of *Limnophora riparia*

R.S. Wotton and R.W. Merritt : *Department of Biological Sciences, Goldsmiths' College, University of London, London SE14 6NW*

Larvae of *Limnophora riparia* (Diptera: Muscidae) are often found in mosses, especially those which have a thin film of water passing over, or through them. They are thus associated with lake outlets where they can be the commonest predator of oligochaetes, and larval chironomids, simuliids and psychodids. In experiments we have shown that larval midges (chironomids) and blackflies (simuliids) are preferred prey. They are attacked by means of the mouth hooks of the muscid which subsequently invades the prey's body and removed much of the contents. As larval blackflies were the most abundant prey available in the lake outlets which we studied, we tested the relationship of predator and prey numbers and showed that survivorship of blackfly larvae was always reduced when the ratio of predator to prey was increased. *L. riparia* larvae had a preference for small blackfly larvae when those of a range of sizes were presented, and small prey always sustained higher damage than larger ones.

Oviposition sites of *Simulium posticatum* Meigen

M. Ladle : *Freshwater Biological Association River Laboratory, East Stoke, Wareham, Dorset BH20 6BB*

The eggs of *S. posticatum* have been found in bankside soil above the water surface of the River Stour. The distribution of oviposition sites has been studied in relation to a local medical problem caused by the bites of the female flies. Sites with a vertical bank profile and loamy soil in the shade of trees were favoured for oviposition. The eggs have a diapause which may be broken by four week's exposure to temperatures of less than 5oC. Eggs are not very resistant to desiccation despite the relatively exposed position in which they are deposited.

POSTER PRESENTATIONS AT THE 10th ANNUAL MEETING

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Towards a new method for plotting migration routes in *Simulium damnosum* s.l.

R.J. Post and D.P. Surtees : *Department of Medical Entomology, Liverpool School of Tropical Medicine*

Presently available techniques for plotting migration routes in *S. damnosum* all involve making direct observations on the migrant flies themselves. An alternative method is to determine the genetic similarity between populations, thus making use of the fact that genetic similarity is very strongly influenced by migration.

The example of *S. soubrense* in western Guinea was presented, using a minimum spanning tree linking populations plotted on a map.

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Onchocerca development in blackflies: Surface changes in lectin binding characteristics.

P.J. Ham and A.J. Smail : *Department of Medical Entomology, Liverpool School of Tropical Medicine and Winches Farm Laboratories, London School of Hygiene and Tropical Medicine*

Among the natural functions that lectins have in insects are those of protecting the organism against non-self invaders. These proteins specifically bind to carbohydrate moieties and can be used as biochemical tools. In this study we used FITC labelled lectins to study the surface of developing *Onchocerca lienalis* larvae recovered from infected blackflies, *Simulium ornatum* sl. Live parasites were incubated with 0.005% solutions of 7 lectins (con A, lentil, peanut, helix, wheat germ agglutinin (WGA), Asparagus pea and kidney bean). These lectins have different sugar binding specificities. By examining the larvae after incubation and washing, it was possible to observe patterns of fluorescence on their surfaces, as they develop within the fly. No lectins bound to living microfilariae, but con A, lentil and WGA increased in binding as the worms developed through to the second stage, and thereafter decreased. A second group, helix and peanut agglutinins, increasingly bound only to the third-stage larvae as they progressed from preinfective thoracic to infective head stages. Asparagus, pea and kidney bean lectins failed to bind to any of the developing stages. It appears, therefore that there is changeover in the carbohydrates presented to the fly on the parasite surface as it develops,

particularly as the microfilariae start developing and as the larvae moult from the 2nd to the
3rd stage. This correlates with the presence of mannose and N-acetyl-D-galactosamine specific lectins within the haemolymph of infected flies, whose relative concentrations apparently fluctuate with the presence of such moieties on the parasite surface. Sugar inhibition studies demonstrated that lectin binding in this study was indeed specific. This turnover may be an evasion mechanism by the parasite, thus contributing to its successful development in the fly, and has clear implications in the transmission of *Onchocerca volvulus* by members of the *S. damnosum* complex. (We are grateful to the Wellcome Trust for financial support).

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THE NEWSLETTER AND THE ZOOLOGICAL RECORD

R.W. Crosskey : *Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD*

At the 10th Annual Simuliid Group meeting (September 1987) it emerged that group members were unaware that the Newsletter of the British Simuliid Group is routinely scanned for its scientific content by the indexing staff of Biosis U.K. so that anything of material interest can appear in the Zoological Record. Papers are included in the ZR 'Author Index' (i.e. bibliography) and their content recorded in the appropriate technical index(es), usually the 'Subject Index' or the 'Systematic Index'. The same computerized data base is used to produce the printed version of Zoological Record as to provide ZR on-line, and there is therefore ready public access to Newsletter data both in electronic and hard copy form; the data base is updated bimonthly, and on-line searches provide more up to date information than that obtainable by awaiting the printed record - though ZR has achieved its currency and the printed record is now distributed within four months of closure of annual indexing. The Newsletter started about the time when ZR first became available on-line, and recorded items from all the issues have been loaded; this will continue, except in the event that ZR has to abandon its present policy of covering all forms of biological newsletter as too costly an undertaking. No ZR-recordable material was included in the first issue of the Newsletter, nor has there been since issue 11 (1985).

Matter entered by the indexers into the data base consists of all authored items in each Newsletter issue that have a zoological content and therefore fall within the very wide indexing scope of ZR. The attached list shows the items so far indexed, printed and published in the Record. The Newsletter items are READ by the indexers so that they can reliably pigeonhole the scientific data into the topic classification of the Subject Index - i.e. they do not rely solely on heading titles to gauge content; for instance, Roger Wotton's passing mention of a blackbird feeding on *S. noelleri* larvae was
picked up and indexed in Aves (ZR Section 18) under *Turdus merula* - even though Roger's note was very informal and did not give the bird's Latin name. When appropriate, a contribution is recorded in several places in the Diptera Subject Index, for example Rory Post's note on sexing blackfly larvae (Newsletter No. 8) is entered three times in the Subject Index, viz. under 'Sexual dimorphism', 'Sexing techniques' and 'Histological techniques: Staining'. Minor items such as the transfer of the Lewis Davies collection to the BMNH and a short obituary of Baranov have

been indexed - because such information is virtually impossible to find unless 'gathered' by Zoological Record.

The ZR is not, of course, an abstracting journal, but even so the fact that contributions with any scientific content will be picked up and included in the data base - and become available in bibliographic title and synoptic records - might hopefully induce more group members to provide the Editor with copy!

The Diptera are an independent part of each volume of ZR, viz. Section 13 (Insecta), Part C (Diptera).

A general intoduction to electronic databases in entomology can be found in Gilbert, P. and Hamilton, C. J. (1990) 'Entomology: a Guide to Information Sources', 2nd edition, Mansell Publishing Limited (Chapter 5). The Zoological Record database is stored on the Lockheed DIALOG system at Palo Alto, and information on access procedure can be found on pp. 188_189 of Gilbert & Hamilton, or obtained from the General Manager, BIOSIS U.K., Garforth House, 54 Micklegate, York YO1 ILF.

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ZOOLOGICAL RECORD COVERAGE OF NEWSLETTER ITEMS

Coverage extends up to issue 11 (1985). Later issues included no ZR recordable material.

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ZOOLOGICAL RECORD COVERAGE OF NEWSLETTER ITEMS

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Newsletter Number	Author	Topic	ZR Reference
2 (l979)	Crosskey	Larval identification	Vol.117, p.22 (biblio.), p.353 (syst. index)
3 (l980)	Crosskey	Ardnamurchan records	Vol.117, p.22 (biblio.), p.233 (geog. index)
3 (l980)	Curran	Nematode / Protozoan parasites	Vol.117, p.22 (biblio.), pp.204, 206 (subject index: parasites)

[Graphics File Bull1t1b.gif here]

3 (1980)	Post	*S.ornatum* s.l. chromosomes	Vol.117, p.85 (biblio.), p.182 (subject index: cytogenetics), p.355 (syst. index)
4 (1980)	Mess	Trichomycetes in *Simulium*	Vol.117, p.74 (biblio.), p.204 (subject index: commensalism)
4 (1980)	Williams	Larval microsculpture	Vol.117, p.119 (biblio.), p.138 (subject index: integument), p.174 (subject index: larva)
5 (1981)	Crosskey	Nomenclature of British simuliids	Vol.118, p.24 (biblio.), p.321 (syst. index)
6 (1981)	Biggs	Larval feeding behaviour	Vol.118, p.12 (biblio.), p.159 (subject index: filter feeding)
6 (1981)	Crosskey	Death of Baranov	Vol.118, p.24 (biblio.), p.145 (subject index: obituaries)
6 (1981)	Descals	Simulid fungal parasite	Vol.118, p.30 (biblio.), p.232 (subject index: fungal diseases)
6 (1981)	Raastad	Norwegian weir project	Vol.118, p.99 (biblio.), p.210 (subject index: freshwater habitat rivers)
8 (1982)	Golini	Collecting method for chromosome study	Vol.120, p.45 (biblio.), p.152 (subject index: histol. techniques fixation)

continued

[Graphics File Bull1t1c.gif here]

8 (I982)	Post	Sex of blackfly larvae	Vol.120, p.100 (biblio.), p.152 (subject index: histol. techniques staining), p.154 (subject index: sexing techniques), p.198 (subject index: sexual dimorphism)
8 (I982)	Wotton	Predation of *S.noelleri*	Vol.120, p.141 (biblio.), p.168 (subject index: predators)
10 (I984)	Crosskey	Lewis Davies collection	Vol.121, p.27 (biblio.), p.158 (subject index: museums, collections)
10 (I984)	M.White	Onchocerciasis / *S.damnosum*, Sierra Leone	Vol.121, p.149 (biblio.), p.216 (subject index: breeding places), p.287 (subject index: transmission of parasites), p. 312 (geog. index)
11 (I985)	Bass	'Eusimuliums', keys and pH relationships	Vol.122, p.9 (biblio.), p.186 (subject index: Chemical environment - hydrogen ion conc.), p.387 (syst. index).

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[The above graphics duplicated as text below]

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2 (1979)	Crosskey	Larval Identification	Vol. 117, p. 22 (biblio.), p.353 (syst. Index)
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3 (1980)	Post	S.ornatum a.l. chromosomes	Vol.117, p.85 (biblio.), p.182 (subject index. Cytogenetics), p.355 (syst. Index)
4 (1980)	Moss	Trichomycotes in Simullum	Vol. 117, p.74 (biblio.), p.204 (subject index: commensalism)
4 (1980)	Wililarns	Larval microsculpture	Vol. 117 ,p. 119 (biblio.), p. 138 (subject Index.. integument), p.174 (subject Index: larva)
5 (1981)	Crosskey	Nomenclature of British simullids	Vol. 118, p. 24 (biblio.), p.321 (syst. Index)
6 (1981)	Biggs	Larval feeding behaviour	Vol. 118, p. 12 (biblio.), p.159 (subject index: filter feeding)
6 (1981)	Crosskey	Death of Baranov	Vol. 118, p.24 (biblio.), p.145 (subject index: obituaries)
60981)	Descals	Simulild fungal parasite	Vol. 118, p.30 (biblio.), p.232 (subject index: fungal diseases)

6 (1981)	Raastad	Norwegian weir project	Vol. 118, p.99 (biblio.), p.210 (subject index: freshwater habitat rivers)
8 (1982)	Golini	C o 11 a c t i n 9 method for chromosome study	Vol.120. p.45 (biblio.), p.152 (subject Index: histol. techniques fixation)
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8 (1982)	Wotton	Predation of *S.noellerl*	Vol. 120,p. 141 (biblio.), p. 168(subject Index: predators)
10 (1984)	Crosskey	Lewls Davies collection	Vol.121, p.27 (biblio.), p.158 (subject index: museums, collections)
10 (1984)	M.White	Onchocerclasis/ *S. damnosum,* Sierra Leone	Vol.121, p.149 (biblio.), p.216 (subject index: breeding places), p.287 (subject index: transmission of parasites), p. 312 (geog. Index)
11 (1985)	Bass	'Eusimullums', keys and pH relationships	Vol.122, p.9 (biblio.), p.186 (subject index: Chemical environment - hydrogen ion conc.), p.387 (syst. index).

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RIVER REGULATION AND *SIMULIUM CHUTTERI*, LEWIS 1965

R.W. Palmer and J.H. O'Keeffe : *Institute for Freshwater Studies, Rhodes University, Grahamstown 6140, South Africa*

Since the completion of the Orange/Fish water transfer tunnel in 1975, the pest species *Simulium chutteri* has become the dominant simuliid in the Great Fish River, South Africa. It has largely displaced non-pest species such as *S. adersi* and *S. nigritarse*, except below one of the dams, where *S. chutteri* is scarce, and the simuliid community composition is similar to the pre-transfer community. This condition persists for 36km below the dam, after which *S. chutteri* becomes the most numerous again.

We suspect that the change from a seasonal to a permanent flow caused by the water transfer tunnel has favoured *S. chutteri*. The intermittent flow below the dam, and the fact that adult *S. chutteri* (unlike the other simuliid species present) disperse their eggs into the drift, could mean that the dam is acting as a barrier to downstream distribution of *S. chutteri* eggs. A considerable ammount of work remains to be done, but this could explain why the river immediately below the dam favours pre-transfer simuliid species rather than *S. chutteri*.

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'MOSQUITO ECOLOGY: FIELD SAMPLING METHODS'

M.W. Service : *Vector Biology and Control Group, Liverpool School of Tropical*

Medicine, Pembroke Place, Liverpool L3 5QA

Mike Service writes that he has recently completed writing a revised and updated second edition of the book *Mosquito Ecology: Field Sampling Methods* which first appeared in 1976. The new edition, which contains some 1800 new references, is to be published in January 1993 by Elsevier, London.

As in the first edition, the book contains descriptions of traps and sampling methods for all stages of the life-history of mosquitoes, as well as techniques and procedures for studying the population dynamics of vectors,including survival rates of adults and immature stages, population estimates, dispersal and identification of predators. In addition there are accounts of vectorial capacity, parasite inoculation rates, remote sensing techniques, and methods for identifying blood-meals from engorged mosquitoes. There are also a number of black and white photographs in the new edition.

Every attempt has been made to give worldwide coverage and not to have missed interesting papers in the lesser known journals. Although aimed at mosquito workers, some of the sampling methods, such as for adults, are applicable to other blood-sucking insects including blackflies.

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Preliminary Notice of the 15th Annual Meeting, 1992

The 15th Annual Meeting of the British Simuliid Group will be held at the University of Keele on Wednesday, September 23rd, 1992. Further details will be circulated in due course, but Peter Ham, who is organising the meeeting, would appreciate hearing from anyone wishing to volunteer a talk or poster presentation as soon as may be convenient.

Peter's address is *Professor P.J. Ham, Department of Biological Sciences, University of Keele, Keele, Staffordshire ST5 5BG* and the University's phone number (0782) 621111.

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EDITOR'S NOTE

This second number of the Bulletin brings up to date accounts of the British Simuliid Group's annual meetings held between the demise of the Newsletter and the birth of the Bulletin. Participants' addresses are given as they were at the date of each meeting.

Accounts of the 1992 and 1993 meetings will be included in issue 3. The editor would, as ever, welcome additional copy for the Bulletin!

Trefor Williams : *Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX*

Phone: 051 794 5021 Fax: 051 794 5094

[BSG Bull. No. 2, October 1993]

THE 11TH ANNUAL MEETING (1988) OF THE BRITISH SIMULIID GROUP

Margaret Curtis : *Department of Biological Sciences, University of Salford, Salford M5 4WT*

The 11th meeting of the British Simuliid Group was held in the Department of Biological Sciences at the University of Salford on September 29th, 1988. It was chaired by Professor D.H. Molyneux and attended by some 30 BSG members, staff and students. The meeting was preceded by a festive dinner on the previous evening, which was also well attended.

Six talks were given at the meeting. In addition Professor Molyneux's group demonstrated cuticular hydrocarbon analysis in gas-liquid chromatography and Alice Millest presented a poster on larval morphology in the *S.metallicum* complex.

TALKS GIVEN AT THE 11TH ANNUAL MEETING

[BSG Bull. No. 2, October 1993]

The Onchocerciasis Control Programme

Colin Fairhurst : *Department of Biological Sciences, University of Salford, Salford M5 4WT*

The Onchocerciasis Control Programme, organised by the World Health Organisation in West Africa, has been running for some fifteen years. The use of larvicides to control *Simulium damnosum* has required extensive environmental monitoring to ensure that non-target taxa, both invertebrates and fish, are not affected by the treatment. Data on invertebrate and fish monitoring at selected sites in the treatment zone have been analysed at Salford since the start of the Programme.

Some insect groups besides Simuliidae have been reduced, but it has been shown that other taxa have increased to fill the available niches. As a result, insectivorous fish have not suffered from a lack of food. Other studies have shown little effect of larvicides on fish populations.

There have been great variations in fish numbers and occurrences, which were initially feared to be due to treatment. However, over the lengthy monitoring period these effects, which have also been found in untreated rivers, have been linked to hydrological changes. The prolonged drought in the mid-1980's caused the disappearance of many species, through poor recruitment or migration, and numbers are now recovering, if not yet to the original abundance.

[BSG Bull. No. 2, October 1993]

Ageing Populations of Different Members of the *Simulium damnosum*

Complex by Pteridine Concentrations

R.A. Cheke : *Overseas Development National Resources Institute, College House, Wrights Lane, London W8 5SJ*

The results of using fluorescence spectrometry to measure the pteridine concentrations in adult flies (the head and thorax only, the wings and abdomen having been removed) of different members of the *Simulium damnosum* complex were described. Pteridines were detected in all cytotypes examined (*S.damnosum* s.str., *S.sirbanum*, *S.squamosum*, the Djodji form of *S.sanctipauli* and the Beffa form of *S.soubrense*).

The samples included flies of both sexes of known age reared from pupae and femal flies of unknown age separated into nulliparous and parous classes. Male flies had significantly higher pteridine concentrations than females of the same age and species. Samples of nulliparous flies had significantly smaller amounts of pteridines than parous samples for each species except *S.soubrense*. For female flies of known age the pteridine concentrations were size dependent.

Significant multiple linear regressions between fluorescence, age and size were found for female *S.damnosum* s.str., *S.sirbanum* and *S.squamosum*. The ages of samples of unknown age were estimated from the regressions. The maximum estimate, 27 days, was for a female *S.damnosum* s.str.

[BSG Bull. No. 2, October 1993]

Onchocerca ochengi in Cattle

P.J. McCall and A.J. Trees : *Department of Veterinary Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA*

Laboratory reared nulliparous female flies of six temperate species of Simuliidae were examined for their susceptibility to infection with *Onchocerca ochengi* by intrathoracic injection of cryopreserved skin microfilariae obtained from cattle in Mali. Three species (*S.equinum*, *S.ornatum* and *S.erythrocephalum*) supported development to the infective stage, one species (*S.variegatum*) allowed partial development and the two remaining species (*S.reptans* and *S.aureum*) were insusceptible to infection. The most suitable surrogate vectors were *S.equinum* and *S.ornatum,* which had survival rates of 44% and 49%, proportions of microfilariae developing to third stage larvae of 6.4% and 3%, and infection rates with infective larvae of 13.5% and 14% respectively. *O.volvulus* infective larvae, produced by intrathoracic microfilarial injection in *S.ornatum*, were 586-760µm (mean 687µm) long and were significantly shorter ($p < 0.02$) than *O.ochengi* infective larvae (645-880µm, mean 756µm). No constant differences in the posterior or anterior morphology, or in the acid phosphatase staining patterns between *O.ochengi* and *O.volvulus*, were seen. These results raise the possibility that the presence of *O.ochengi* in a population of infective larvae from vector flies in endemic onchocerciasis zones might be identified on the basis of their length alone.

Reference: P.J. McCall and A.J. Trees (1989). The development of *Onchocerca ochengi* in surrogate temperate Simuliidae, with a note on the infective larva. *Trop.Med.Parasit.* 40: 295-298.

The Cytotaxonomy of *Onchocerca*

R.J. Post, P.J. McCall, A.J. Trees, C.J. Delves and B. Kouyate
Departments of Medical Entomology and Veterinary Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA

Examination of ovaries and testes from adult *Onchocerca ochengi*, *O.gutturosa*, *O.armillata* and *O.lienalis* revealed five pairs of chromosomes, but in contrast *O.volvulus* and *O.gibsoni* had only four pairs. The number of nuclei increases from approximately 280 in intra-uterine microfilariae to approximately 900 in infective L3 larvae of *O.volvulus*, which suggests that it should be possible to observe mitosis and count the number of chromosomes in the developing stages of the parasite in the vector.

[BSG Bull. No. 2, October 1993]

Recent Developments in the Use of Isoenzyme Electrophoresis

J.B. Davies : *Department of Medical Entomology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA*

The talk described some recent developments in the techniques of isoenzyme electrophoresis in the separation of the sibling species of *Simulium* complexes, and is the result of collaboration with M.C. Thomson and M._G. Basanez.

The classical method used by Meredith and Townson (1981) using the enzymes trehalase and phosophoglucomutase in starch gels although excellent, had serious practical limitations under tropical field conditions. The gels had to be made up a few days before use, and required up to two hours to run, hence refrigeration equipment was required to keep the gels cool to prevent degradation of the enzymes. The system was thus laboratory based, and the flies had to be brought to the laboratory and stored in liquid nitrogen if they could not be processed immediately.

The recent use of cellulose acetate as a substrate by Fryauff and Trpis (1986) prompted us to try using it in Sierra Leone. This substrate reduced the run time to 10 or 20 minutes only, and as a result it was found that cooling was not necessary. This immediately opened up the possibility of assembling a field kit which might be independent of an advanced laboratory and mains electricity.

In Liverpool, Madelaine Thomson experimented with different membranes and buffer systems and at the same time we were most fortunate to find a microchip integrated circuit which would convert 12 VDC to 250 VDC. This enabled us to build a small power pack that would work off a car battery and thus made the system truly portable.

Since then, Madelaine has spent four months in Sierra Leone testing the system with OCP teams trying to identify *S.squamosum* amongst the flies originating from the supposed invasion source area. I have visited Puerto Ayacucho in Venezuela where with Maria-Gloria Basanez we have looked at the enzymes of the suspected vectors of onchocerciasis in the Amazonas Region.

In Venezuela it was immediately obvious that there was a great deal of variation in PGM and TRE within most of the species complexes that we looked at, and this supports the

evidence from behaviour and cytotaxonomy that these are species complexes. Unfortunately there was not time to process enough flies to begin to resolve the situation. It is quite clear that what is now required is a larger scale study combining the techniques of morphology, cytotaxonomy and electrophoresis.

The new portable kits performed well in both field situations, and we are confident that they are now operational, and will be useful to a much wider range of disciplines that of Medical Entomology alone.

References:

Fryauff D.J. and Trpis M. (1986). *Am.J.Trop.Med.Hyg.* 35: 1218-1230.
Meredith S.E.O. and Townson H. (1981). *Tropenmed.Parasit.* 32: 123-129.

[BSG Bull. No. 2, October 1993]

The Present Status of the *Simulium neavei* Group in Kenya -Some Preliminary Notes

J.N. Raybould : *13 Rownham Mead, Hotwells, Bristol, Avon BS8 4YA*

The *Simulium neavei* group was introduced with slides showing a range of habitats and larvae and pupae attached to various riverine crabs.

A recent collecting trip to western Kenya was described. Three *S.neavei* group species are known from the area: *S.neavei* s.s., *S.goinyi* and *S.hightoni*. *S.neavei* s.s., the sole onchocerciasis vector, was virtually eradicated by 1956 except in a very small area on Mount Elgon (McMahon et al, 1958). The other two species returned to their former habitats after control operations ceased. In view of this unique historical background, the area was visited in August-September 1988 to obtain up-to-date distribution data and material for morphological and cytotaxonomic study. Some observations were made on the immature stages and their associated crabs and specimens from different parts of the crab (including the exhalent branchial chambers in which *S.goinyi* occurs) were tubed separately.

The *S.neavei* group has been little studied recently and methods of catching crabs are not widely known. An illustrated account was therefore given of crab trapping techniques.

The collections will be investigated by Mrs Phoebe A.O. Josiah, a Kenyan Ph.D. student working under the supervision of Dr. R.P. Lane at the London School of Hygiene and Tropical Medicine. They will also provide reference material for Drs. A.J. Shelley and W. Procunier at the Natural History Museum in their proposed reclassifiation of the *S.neavei* group as a whole. Mr. T.R. Williams, Liverpool University, will identify the crabs.

Reference:

McMahon J.P., Highton R.B. and Goiny H. (1958). The eradication of *Simulium neavei* from Kenya. *Bull.Wld.Hlth.Org.* 19: 75-107.

[Dr. Josiah was awarded her Ph.D. by the University of London in 1991 for her

thesis titled 'Biological and taxonomic studies of Simuliidae from Kenya, with emphasis on the *Simulium neavei* complex'. Ed.]

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[BSG Bull. No. 2, October 1993]

THE 12TH ANNUAL MEETING (1989) OF THE BRITISH SIMULIID GROUP

The meeting was held at the Liverpool School of Tropical Medicine on November 7th, 1989, and organised by staff from the School's Department of Medical Entomology. Participants were welcomed by R.J. Post before J.B. Davies gave an introductory talk to present the extent of the School's involvement in *Simulium* research.

John Davies has provided the following account of his introduction:

At the present time the School is probably more heavily involved in *Simulium* related research than at any other time.

There are five groups working full time comprising six members of staff and eight PhD students. Two other staff members pursue an active interest. Thus we have here sixteen Simulidologists. The members were introduced in turn by John Davies who said a few words about each project.

The interests of the groups and individual members are summarised as follows:-

R.J. Post - Cytotaxonomy and DNA taxonomy of *S.damnosum*

P. Flook	DNA variation and migration in W. African *S. damnosum*
L. Gomulski	Development and testing morphometric identification techniques
M. Wilson	Identification of *S. damnosum* in the OCP area

P.J. Ham - Vector Immunity to Parasite Infections

A. Baxter	Molecular, biological & biochemical aspects of immune proteins
E. Winbolt	Production of monoclonal antibodies to *Simulium* immune proteins

M.J. Roberts - Identity and Biology of Onchocerciasis vectors in Malawi

J.B. Davies - Dynamics of Onchocerciasis transmission in areas of low endemicity

M.C. Thomson	Use of Isoenzyme Electrophoresis for identifying cytospecies of *S. damnosum* s.l.
M.J. Bockarie	Vectors and transmission levels around a forest village in Sierra Leone
D.C. Chavasse	Effect of ivermectin on the uptake and development of microfilariae in *S.damnosum*

A.J.Trees and P. McCall - *Simulium* oviposition pheromones

Other interested workers are M. Service (trapping methods for *Simulium*) and H. Townson who keeps an active interest in all aspects of *Simulium* and onchocerciasis.

TALKS GIVEN AT THE 12TH ANNUAL MEETING

[BSG Bull. No. 2, October 1993]

A Fossil Simuliid from the Middle Jurassic Period

R.W. Crosskey : *Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD*

I was recently able to borrow from the Palaeontological Institute in Moscow the dipteran fossil pupa described by Kaligina under the name *Simulimima grandis* and assigned tentatively to the extinct family Eoptychopteridae. The fossil comes from the Mongolian border area of the Soviet Union and is of Middle Jurassic age (i.e. about 165-170 million years ago). Close examination and photography of the fossil while under alcohol clearly shows that it possesses the basic diagnostic features of simuliid pupae, i.e. presence of a unique groundplan of pupal abdominal hooks found in no other family and large prothoracic gills. Furthermore, the abdomen has long sinuous terminal hooks exactly like those of *Prosimulium* pupae. The conclusion is inescapable that *Simulimima* is a true simuliid and that the origin of the family can now be dated back at least as far as the Middle Jurassic period (a time much earlier than any other known simuliid fossils).

[Further information on the fossil can be found in Roger Crosskey's book *The Natural History of Blackflies* (pp. 59-60), and a complete account in his 1991 paper in *Systematic Entomology* 16: 401-406. Ed.]

Gene Expression in Larvae of *Onchocerca* in Blackflies

A.E. Bianco : *Department of Pure and Applied Biology, Imperial College, London*

Onchocerca parasites typically require 12-24 months to complete their life-cycles, yet three out of the four moults that occur during development take place within approximately two weeks. Compressed into this early period of larval differentiation and growth are both crucial points of parasite transmission between the invertebrate and vertebrate hosts. In order to identify molecules with specialized functions permitting larvae to make the major transition between hosts, we set out to identify genes expressed in infective larvae immediately prior to transmission. To do this it was necessary to devise a novel method for labelling proteins synthesised by developing stages within the vectors, involving the micro-injection of [^{35}S] methionine into the thorax of infected blackflies. Pulse labellings of *Onchocerca lienalis* larvae within *Simulium ornatum* s.l. have revealed a major acidic protein of 23kD which is developmentally expressed almost exclusively by infective, third-stage larvae. Homologous proteins occur in *O.lienalis* and *O.volvulus*, but these exhibit size polymorphisms both among species and individual organisms. The 23kD molecule continues to be elaborated after terminal differentiation of the parasite in flies, but not by post-infective larvae entering the phase of development in the vertebrate host. A shift in temperature from 26^{o}C to 37^{o}C triggers secretion of the 23kD molecule as a discrete event 24-72 hours after transmission. The labelling technique has been successfully

employed with filarial species that develop in mosquitoes, and in principle should be widely applicable to the study of endoparasite gene expression within arthropods.

[BSG Bull. No. 2, October 1993]

Invasions by Savanna *Simulium damnosum* s.l. into Forest Habitats and by Forest Forms into Savannas in West Africa

R.A. Cheke : *Overseas Development Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB*

The occurrence and possible epidemiological significance of movements of savanna forms of *Simulium damnosum* s.l. (*S.sirbanum* and *S.damnosum* s.str.) into forest zones were described and discussed. Examples include:

(a) populations of savanna forms breeding and biting man at Bong Mine, Liberia, where mining activities have created artificial conditions that have been exploited by the invading flies. The phenomenon is apparently seasonal and it it thought the invaders arrived with the harmattan winds during the dry season. Until recently only *S.yahense* and *S.sanctipauli* were known from the area.

and (b) *S.damnosum* s.str. breeding in forested stretches of rivers in Togo and Ghana, such as the Asuakawkaw, Amou and Dayi, where only forest forms used to occur. This was attributed to habitat changes, mainly de-forestation.

Examples of forest forms moving into the savanna were also discussed. The Djodi form of *S.sanctipauli* and, to a lesser extent *S.yahense*, have been found to migrate out of their forest strongholds, in southwest Togo and southeast Ghana, into savannas during wet seasons. This phenomemon was attributed, partly, to reduced competition from other cytospecies consequent upon the latter's being controlled with insecticides.

Talks were also given at the 12th annual meeting by:

I. Cameron Development of iridescent virus' as potential control agents for *Simulium*

M. Ladle Trials of Bt.I. for blackfly control in the south of England

M. Wood Homologies of labral fans and other structures between simuliids and other Nematocera

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[BSG Bull. No. 2, October 1993]

THE 13TH ANNUAL MEETING (1990) OF THE BRITISH SIMULIID GROUP

C.A. Lowry : *Medical and Veterinary Division, Entomology Department, The Natural History Museum, Cromwell Road, London SW7 5BD*

Some 30 people attended the 13th Simuliid Group meeting held at the Natural History Museum, South Kensington, on September 25th, 1990.
Many of those present had attended an informal meal on the previous evening.

Dr Tony Shelley introduced the meeting at which five talks were presented. In addition R.A. Cheke showed a video made by Evergreen Helicopters describing their operations for the WHO Onchocerciasis Control Programme in West Africa.

The meeting concluded with a small cheese and wine party to commemorate the retirement of Dr Roger Crosskey from the Natural History Museum earlier in the year.

TALKS GIVEN AT THE 13TH ANNUAL MEETING

[BSG Bull. No. 2, October 1993]
Cytology of the *S.exiguum* Complex in Ecuador

M. Charalambous, A.J. Shelley and M. Arzube : *Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD*.

Onchocerciasis was discovered in Ecuador in 1980. An epidemiological

survey showed that it was restricted to a number of foci in Esmeraldas Province. A follow up survey in 1986 showed that the average prevelance of onchocerciasis had risen from 28 to 47.3% in the population, representing an increase of 74% since 1980 and blatently indicating the enormity of the public health problem.

The main vector of onchocerciasis in the foci is *Simulium exiguum* which is in fact a species complex. In Ecuador there are four members of the *Simulium exiguum* species complex. The method by which they were identified, polytene chromosome analysis, and their cytotaxonomy were described.

Only two of the four members of the complex occur in Esmeraldas Province and so are likely vectors. However, a worry of the epidemiology is the fact that widespread migration of people is occuring. If infected migrants settle in areas where there are high biting densities of *S.exiguum*, new foci of
infection may be set up if the cytospecies present in these areas can act as efficient hosts. The results of experimental infection of adults of all four members of the complex with *Onchocerca volvulus* suggest that all of them can host the parasite efficiently.

[BSG Bull. No. 2, October 1993]
Is the Simuliid Pupal Gill a Plastron?

T.R. Williams, C.E. Denley and H.M. Wain : *Department of Environmental and Evolutionary Biology, Liverpool University*

A plastron, or permanent physical gill, is a superficial film of 'air' which, when submerged, allows gases to be exchanged across an extensive air_water interface. These air films, which typically have interface spaces of at least 0.5 µm, are held by hydrofuge structures and can be displaced by wetting agents or by increased water pressure without damage to their supports.

Undoubted plastron surfaces occur on the spiracular pupal gills of many insects (Hinton 1968), but the nature of the simuliid gill is more contentious. Originally described as having an unbroken surface (Pulikovsky, 1927) it was considered to be a plastron by Hinton (1964) who showed that it could be wetted by pressure and by an alcohol of low surface tension, and by Messner and Grafner (1983) from its appearance in the scanning EM. Our work at Liverpool leads us to disagree with these later conclusions.

We have used scanning and transmission electron microscopy to examine the gills of a number of British and African simuliids. In no case has it been possible to resolve a typical plastron surface with the

SEM, although some perforations are almost always to be found. However, the irregular nature of these pores, and their being especially common on the gills of pupae from the field, suggests that they are the result of mechanical damage. Examination of pupae reared and carefully prepared in the laboratory shows the gill surface to be essentially unbroken, at least within the SEM's limit of resolution.

The transmission EM, used with magnifications of up to 150K, provides even stronger evidence of the absence of plastron pores on the simuliid gill. Our results (mainly from *Simulium ornatum* and *S.spinosum*) are similar to those of Hinton (1976) and show that between its supporting filaments the gill surface is a continuous three-layered (at least) membrane, some 70-100 nm in thickness and with its outer margin produced into minute papillae. Hinton (1976) conjectured that this membrane is perforated by small (2_5nm) air-filled pores, though these were not visible in his TEM micrographs. We have found that slightly larger (7nm) channels, similar in size and staining properties to structures in insect cuticle, do occur in the
membrane, but have no evidence (and do not think it necessary to suggest) that they open to the surface or are air-filled.

The experimental evidence for the simuliid gill's being a plastron also appears to be inconclusive. Wetting agents, for example, might penetrate through tears in the surface rather than through plastron pores. By repeating Hinton's (1964) experiments on the effects of pressure, but with laboratory reared pupae, we have found that pressures sufficient to wet the base of the gill, its weakest point, also disrupt the surface and its supporting filaments in the area where water penetrates.

References:

Hinton H.E. (1964). *J.Ins.Physiol.* 10: 73-80.
Hinton H.E. (1968). *Adv. Insect Physiol.* 15: 65-162.
Hinton H.E. (1976). *J.Ins.Physiol.* 22: 1061-1070.
Messner B. & Grafner G. (1983). *Zool.Jb.Anat.* 110: 373-380.
Pulikovsky N. (1927). *Z.Morph.Okol. Tiere* 7: 384-433.

Talks were also given at the 13th annual meeting by:

Richard Baker	Onchocerciasis vectors in Sierra Leone
Desmond Chavasse	Effect of ivermectin treatments on the uptake and development of microfilariae by *Simulium*

damnosum s.l.

Angus McCrae — Host selection by *Simulium posticatum* at Blandford, Dorset

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[BSG Bull. No. 2, October 1993]

THE 14TH ANNUAL MEETING (1991) OF THE BRITISH SIMULIID GROUP

The Simuliid Group met for the 14th time at Portsmouth Polytechnic on September 25th, 1991. Steve Moss organised and hosted the meeting which started with an informal dinner on the previous evening.

The 25 members who attended were joined by 5 local entomologists. Six papers were presented in addition to an entertaining talk entitled 'Onchomania' given by John Davies from the Liverpool School of Tropical Medicine.

The meeting was drawn to a close by Roger Crosskey. Informal discussions then continued in the Yorkshire Grey .....

TALKS GIVEN AT THE 14TH ANNUAL MEETING

Humoral Immunity to *Onchocerca* in British *Simulium* Species

A.J. Baxter and P.J. Ham : *Department of Medical Entomology, Liverpool School of Tropical Medicine, Pembroke Place, L3 5QA*

Parasites and other pathogens transmitted by insects are subject to the vector's own defence mechanisms. We are looking at different levels of the response of British *Simulium* species to *Onchocerca lienalis* infection, including effects on biological function, e.g. pupal emergence and haemolymph protein production.

Haemolymph proteins present in *O.lienalis* infected, but not in untreated *Simulium*, have been demonstrated by SDS-PAGE and further characterised by proteinase gels, Western blots and 2D-NEPHGE (non-equilibrium pH gel electrophoresis) analysis. Radioactive pulse experiments have indicated the time scale of their production.

At the genomic level, DNA sequences homologous to a *Drosophila* cDNA probe coding for the immune protein cecropin, have been detected in *Simulium* species by Southern hybridisation. Three candidate cecropin

clones have been isolated from the genomic library of *S.ornatum* using this heterologous probe and their sequences partially determined.

Using British *Simulium* species and *O.lienalis* as a model system it is hoped to gain information of the mechanisms controlling susceptability to infection in *Simulium*.

[BSG Bull. No. 2, October 1993]
Humoral Immunity in African Blackflies

Hans-E. Hagen : *Department of Medical Entomology, School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA*

Acquired and innate immunity in temperate blackflies is now a well documented fact. In order to carry out comparative trials with African blackflies various cytospecies of the *Simulium damnosum* complex in the rain forest and savanna of Cameroon were intrathoracically injected with microfilariae of *Onchocerca ochengi*, *O.dukei* and *O.volvulus*. Moreover wild-caught flies were collected after taking a bloodmeal on infected cattle. So far the results reveal that:

1. The haemolymph of blackflies in Cameroon reacted in a similar way to infection as European species by showing a distinct band at about 66kD after 24 hours, however without featuring a trauma protein due to the injection itself.

2. This 66kD band is stronger in those flies that were injected with microfilariae of *O.ochengi* than in those that received *O.dukei*. This compares favourably with the different susceptibility of these blackflies towards the two *Onchocerca* species: microfilariae of *O.dukei* do not develop well in *S.damnosum* s.l. even following injection (Wahl *et al*, 1991).

3. Only wild caught, blood fed flies that harboured microfilariae showed this 66kD band.

All trials indicated that this change in the protein pattern of the haemolymph is caused by the infection as such. This reponse might be due to an increase of the phenoloxidase-activity in the haemolymph following the infection with pathogens.

[BSG Bull. No. 2, October 1993]
Differentiation of *Simulium damnosum* s.l. Larvae and Pupae from Nigeria by Cuticular Hydrocarbon Analysis

H.B. Mafuyai : *Department of Biological Sciences, University of Salford, Salford M5 4WT*

Larvae and pupae of three blackfly species in the *S.damnosum* complex from Nigeria were analysed, using a Gas Chromatograph, for their differences in cuticular hydrocarbons and with a view to their classification. Up to 75% correct classification was achieved for larvae, and 73.3% of the pupae of the same species (*S.damnosum* s.s., *S.squamosum* and *S.yahense*) were correctly classified into their three groups. The cuticular hydrocarbon profiles of larvae and pupae showed remarkable similarities in the occurence of common peaks. Evidence for within group cohesion and for differences between the groups showed in scattergrams derived from discriminant function statistics. The possibilities for further exploratory studies into the nature of cuticular hydrocarbons in sibling species complexes of *S.damnosum* s.l. and their value for identification, are highlighted.

Some Thoughts on Larval Feeding

R.S. Wotton : *Department of Biology, University College London, Gower Street, London WC1E 6BT*

Most blackfly larvae feed by holding their cephalic fans into the current of water which passes over their bodies. Materials trapped by the fans by sieving, impaction and adsorption are then removed by the mouthparts and the food passed into the gut.

When viewed under a microscope the gut contents are seen to consist of particles of easily-identified structure (e.g. diatoms and bacteria) and apparently amorphous material. What is this amorphous material, and what is the basis of larval nutrition?

A Phase in the Life-Cycle of the Harpellales (Trichomycetes) Pathogenic to Simuliidae

M. Taylor and S.T. Moss : *School of Biological Sciences, Portsmouth Polytechnic, Portsmouth, Hampshire PO1 2DY*

The Harpellales is an order of fungi obligately associated with the digestive tracts of aquatic insect larvae and nymphs, including Simuliidae. Horizontal transmission of infection is achieved by the production of asexual trichospores. However, few spores are produced by each fungal thallus and spores are released into a lotic environment.

Ingestion of a released trichospore must occur prior to infestation, each spore giving rise to a single determinate thallus. Evidence is presented to support the occurrence of a stage in the life-cycle of the Harpellales that infects and is pathogenic to adult Simuliidae. This stage also enables upstream transport of the fungus, colonisation of ephemeral habitats and an increase in fungal inoculum.

[BSG Bull. No. 2, October 1993]

The Rise and Fall of the Blandford Fly

M. Ladle : *Institute of Freshwater Ecology, The River Laboratory, East Stoke, Wareham, Dorset BH20 6BB*

In the late 1960's a problem arose of people being bitten by insects in the valley of the Dorset River Stour. Extensive biological investigations identified the cause of the biting as *Simulium austeni* (now *Simulium posticatum*) and established most of the basic ecological parameters of the species. Efforts to find a satisfactory method of control for the species were rendered ineffective by environmental, economic and political considerations. Hope of a new avenue, with control potential, arose when the peculiar oviposition behaviour of the species was described, but it was only with the availability of *Bacillus thuringiensis israelensis* that control of *S.posticatum* became a real possibility. Following a small scale trial in 1989 the North Dorset District Council was given permission to treat four main river sites on an experimental basis in 1991. It seems probable that the end is in sight for the notorious 'Blandford Fly'.

[BSG Bull. No. 2, October 1993]

Blackflies and Iridescent Viruses

Trevor Williams, Cathy Doyle and Jenny Cory : *NERC Institute of Virology and Environmental Microbiology, Oxford OX1 3SR*

Viral pathogens of Diptera have been much neglected, and host virus interactions in aquatic systems remain almost completely unstudied. Blackflies make good candidates for viral ecology studies in riverine systems for a number of reasons:

i) geographical ubiquity and abundance
ii) they harbour known viral pathogens, with the potential for intriguing transmission routes involving aerial and aquatic phases
iii) their vector status makes them a target for biocontrol measures.

Iridescent viruses (IV's) are icosahedral particles, some 120nm in diameter, containing double stranded DNA, and unlike the better known baculovirus pathogens, are not occluded in a protective protein matrix. The particles form crystalline arrays in host tissues producing a distinct opalescent blue, green or lilac hue, which makes diagnosis of patent infections simple. IV's are typically isolated from the larval stages of invertebrate hosts inhabiting moist or aquatic environments (Kelly 1985, *Current Topics in Microbiology and Immunology* 116: 23-35).

These viruses have been recorded as simuliid pathogens on seven occasions world-wide, but only two isolates have been kept for study. One, named IV22, was found in extremely low abundance (1 in 10^5-10^6 larvae) in mid-Wales (Bateson *et al*. 1976, *J. Invertebrate Pathology* 27: 133-135). Cameron (*Virology* 178: 35-42, 1990) characterised the gene of IV22 coding for the major structural protein (MSP) which itself accounts for some 40% of the protein of a virion. This gene, some 1400 base pairs in length, was used to detect the presence of IV22 DNA in field samples from the rivers Cherwell, Evenlode and Windrush around Oxford.

In the laboratory, field-collected larvae were individually blotted onto a nylon membrane which binds any DNA present in the sample. The MSP gene fragment was then radiolabelled using ^{32}P and allowed to hybridise to simuliid and control DNA on the membrane. Unfortunately, no larvae were identified as positive for IV22 DNA despite a sample size approaching 5000. Likewise, no patently infected larvae were observed in the samples. Probably there is no detectable level of IV22 infection in simuliids from these rivers.

Greater success was achieved in assessing the host range of different iridescent viruses. Several distinct IV's (as shown by restriction enzyme analysis of DNA) were grown in a permissive lepidopteran host, *Galleria mellonella*, and frozen until required. Individual field-collected simuliid egg masses were reared in the laboratory. When egg hatching was near completion, first instar larvae were challenged for 72 hours with high titres (c. 10^6 pfu/ml) of different IV's in the form of macerated *G.mellonella* cadavers. No attempt was made to quantify the relative infectivity of each IV to each simuliid species challenged - a simple yes or no answer was sought as to whether the virus would cause patent infections in simuliid larvae. In this manner, overt infections were observed in each and every combination tested:

Heliothis IV (IV21) against *S.erythrocephalum, S.pseudequinum* and *S.equinum*
Simulium IV (IV22) against *S.ornatum*
Tenebrio IV (IV29) against *S.erythrocephalum*
Isopod IV (IV31) against *S.erythrocephalum*

The frequency of patent infection was consistently low, never rising above 8%, but these results clearly indicate the host range of IV's to be broad (IV21 was tested against a greater number of species simply due to availability). In addition, when larvae exposed to IV21 were squash blotted and probed using IV21 DNA, all showed up as positive - indicating symptomless infection had occurred.

These studies have triggered further work looking at the possibilities of wide scale covert infection in populations of blackfly larvae from elsewhere in the UK.

POSTER PRESENTATION AT THE 14TH ANNUAL MEETING
[BSG Bull. No. 2, October 1993]

Sample Size in Parasitological and Vector Surveys

R.J. Post and A.L. Millest : *Department of Biological Sciences, Salford University*

The interpretation of geographic survey data inevitably involves a great deal of subjectivity. Post and Millest (*Parasitology Today* 7: 141) show one way of assessing the adequacy of sample size. They ask for any given sample size, what would be the maximum likely frequency of a species that had actually not been found in the sample. This is in fact a simple binomial problem and is equivalent to asking, given a sample size x and an estimated frequency of the species as zero (because it is absent from the sample), what is the upper 95% confidence limit on that estimate. It is interesting that for sample sizes of 20, 100 and 500, the maximum likely frequency of undiscovered species is 13.9%, 3.0% and 0.6% respectively. The weakness of this approach is that it assumes an adequate sampling method, and does not take into accoung prior probabilities. Hence, no matter what our sample size, we have virtually zero expectation of finding *Simulium damnosum* breeding in Surrey!

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BRITISH SIMULIID GROUP BULLETIN

Number 3 June 1994

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From the Editor

The Bulletin has finally caught up with the BSG's annual meetings (and the editorial conscience is at last eased). Although it will now appear annually to report the meetings of the Group, it's hoped this is not seen to be its only function. The editor will gladly accept notes, news and articles at any time. Contributions on disk would be particularly welcome!

Trefor Williams : *Department of Environmental and Evolutionary Biology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX*

Phone: 051 794 5021 Fax: 051 794 5094

[BSG Bull. No. 3, June 1994]

THE 15TH ANNUAL MEETING (1992) OF THE BRITISH SIMULIID GROUP

The 15th Annual Meeting of the British Simuliid Group was held at Keele University on Wednesday, September 23rd, 1992. The Centre for Applied Entomology and Parasitology was host to the meeting, which was organised and chaired by Professor Peter Ham. The 60 or so participants were warmly appreciative of Peter's arrangements, which included the customary informal meal on the evening before the meeting.

Speakers included Colin Fairhurst (Salford University) whose talk was titled 'Disturbing News For Blackfly Larvae'.

TALKS GIVEN AT THE 15TH ANNUAL MEETING

Analysis of two cytotypes of ***Simulium exiguum*** **in Ecuador**

M. Charalambous, P.D. Ready, A.J. Shelley, M. Arzube and C.A. Lowry : *Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD*

Sex chromosome evolution is thought to be important in the speciation of simuliids. For example, in the *S.exiguum* species complex the Cayapa cytospecies does not have differentiated sex chromosomes, whereas the Aguarico cytospecies possesses two types of Y-chromosomes which are differentiated by the linkage of inversions.

The Bucay and Quevedo cytotypes of the *S.exiguum* species complex are closely related as they share the same fixed paracentric inversions. However, Quevedo differs from Bucay in having differentiated X_chromosomes. The question this raises is: what degree of reproductive isolation is the X-linked inversion associated with?

A number of populations were sampled in southern and central Ecuador. Larval polytene chromosome analysis confirmed the existence of two chromosomal forms. The cytological results are discussed in relation to isoenzyme analysis of pure populations of each variant collected at the same time.

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Onchocerca **and blackfly reproduction**

Melanie Renshaw : *Centre for Applied Entomology and Parasitology, Keele University, Keele, Staffordshire ST5 5BG*

A reduction in reproductive output has been observed with *Onchocerca* infected *Simulium damnosum* in the wild (Checke *et al.* 1982) and with *O.lienalis* infected *S.lineatum* and *S.ornatum* in the laboratory (Ham & Banya 1984). When blood-fed *S.ornatum* were infected with 20 microfilariae by intrathoracic injection, there was a significant reduction in ovarian protein sequestration at 24, 34 and 50 hours post blood-feeding in comparison with a sham injected group. There was no significant difference in ovarian protein content between sham injected and control groups, indicating that the physical process of injection does not affect sequestration. Ovarian protein profiles showed that *S.ornatum* vitellin consisted of two main subunits, at 200 and 60 kDa, corresponding well to those found in *Aedes aegypti* at 200 and 65 kDa (Raikhel 1992). Microfilariae may affect synthesis of vitellogenin in the fat body, circulation in the haemolymph and/or ovarian sequestration.

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Mite induced protease activity in *Simulium* haemolymph

Jenni Hood and Peter Ham : *Centre for Applied Entomology and Parasitology, Keele University, Keele, Staffordshire ST5 5BG*

Simulium spp. adults infested with ectoparasitic mites of the genus *Sperchon* were investigated. The mites were predominantly attached to the soft intersegmental membranes between the head and thorax. Rates of infestation varied between *Simulium* species. Infestation rates were much higher on *S.ornatum* when compared with *S.equinum* from the same collection site. Differences in infestation are thought to be due to differences in pupal gill structure between *S.ornatum* and *S.equinum*.

SDS substrate gel electrophoresis was carried out on haemolymph from *S.ornatum* infested with mites. Results indicated protease activity consistent with that previously found in *S.ornatum* in response to infection with *Onchocerca lienalis* microfilariae. Protease activity in haemolymph from
uninfested *S.ornatum* (controls) generally showed lower protease activity. Some controls, however, showed similar protease activity to infested flies. This may be explained by previous mite infestation, since mites are known to detach once fully engorged.

[BSG Bull. No. 3, June 1994]

Infections in *Simulium neavei* before and during a mass campaign using Ivermectin in Uganda

Rolf Garms[1], John Yocha[2] and Walter Kipp[3] : [1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg*; [2]*Vector Control Unit, Ministry of Health, Fort Portal, Uganda*; [3]*Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ, Basic Health Services, Fort Portal, Uganda*

Onchocerciasis is a major public health problem in the Kabarole district of Western Uganda where two vectors, *Simulium neavei* and *S.damnosum* s.l., occur. Investigations of the dynamics of the transmission of *Onchocerca volvulus* were begun in 1991 to examine possibilities of vector control and to monitor the effects of a mass treatement campaign with ivermectin launched in 1991 to treat the human population. Distribution of ivermectin was mostly annual, but semi-annual in one area. Onchocerciasis
transmission was found to be very intense prior to and at the start of the campaign in two foci where *S.neavei* is the vector. At three catching sites in a hyperendemic focus northeast of Fort Portal, where regular full day catches were carried out by vector collectors, annual transmission potentials were estimated to be 4500 to 6500 infective larvae per person per year. Of 709 parous flies which were examined 40% were harbouring first or second stage larvae (L1/L2). After the first distribution of ivermectin infection rates of the fly population showed a significant drop, but increased again a few months later. From January to August, 1992, 33% of the

parous females carried L1/L2 larvae, significantly less than in 1991. After the second round of treatments, which began in July 1992, infection rates of the flies dropped further to 21%, but 100 infective larvae of *O.volvulus* were still found in the heads of 1000 parous flies. Investigations will be continued to assess whether transmission in this limited and isolated onchocerciasis focus can be further reduced, or even interrupted, after repeated dosages and an improved coverage of the human population.

Simulium and river blindness in South West Arabia

Frank Walsh : *80 Arundel Road, Lytham St. Annes, Lancashire FY8 1BN*

Between November 13th and December 11th, 1991, I paid a visit to SW Arabia to investigate whether human onchocerciasis was indigenous to Saudi Arabia. The first half of the visit was spent in the known onchocerciasis focus of North Yemen. Onchocerciasis was first reported from Arabia in 1957 by Fawdry who reported cases from South Yemen (the former Aden Protectorate). A member of the *Simulium damnsosum* complex was reported from North Yemen by Merighi *et al.* (1969). Detailed entomological studies were made by Garms and Kerner (1982) and the vector *S.rasyani* was described by Garms *et al.* (1988).

Unfortunately my visit coincided with a very poor rainy season, indeed over much of SW Arabia no rain fell in 1991, and this followed at least seven years of poor rainfall. Many of the sites in North Yemen studied by Garms and Kerner (1982) were not flowing in 1991, and these included places where flow had been perennial until very recently. *S.rasyani* was found only in the Wadi Surdud. However, there it was found in large numbers encrusting the stones which formed the river bed, in the absence of suitable vegetable substrate. Garms and Kerner (1982) had only found it attached to vegetation. The other species collected proved to be *S.ruficorne*, *S.hargreavesi* and *S.yemenense*.

In Saudi Arabia I visited Abha in the Asir Region and was helped by Professor Mohamed Omar who is based there. The drought had been even more severe than in Yemen and I did not see any flowing water which
looked suitable for the *S.damnosum* complex. The only species collected were *S.ruficorne* and *S.yemenense*. Much of the time in Saudi Arabia was spent investigating the provenance of cases of human onchocerciasis reported from various hospitals and clinics. In all cases bar one it seemed likely that the disease had been contracted in Yemen. However, one undoubted case (examined parasitologically by Professor Omar) concerned a nine year old boy from the Asir Region who had never left the Kingdom of Saudi Arabia. Thus it seems certain that in wetter periods in the past there has been some transmission of onchocerciasis within Saudi Arabia. Presumably the vector was *S.rasyani* which, however, has yet to be detected in that country.

Postscript: 1992 proved to be the wettest for about eight years, but a return visit to Asir could not be sanctioned.

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Most iridovirus infected blackflies do not iridesce

Trevor Williams and Jenny Cory : *NERC Institute of Virology and Environmental Microbiology, Oxford OX1 3SR*

Iridescent viruses are icosahedral DNA viruses which assemble in a crystalline arrangement in heavily infected host tissues. Light striking these viral arrays is subject to interference causing an obvious and characteristic iridescent coloration - usually blue. This has traditionally been the criterion for diagnosing iridescent virus infection, and because of the rarity of the phenomenon in most insect populations, iridescent viruses have usually been considered of low infectivity. There exist two genera of the family Iridoviridae which infect invertebrates: the larger *Chloridovirus* (c. 180nm) isolated from mosquitoes, and the smaller *Iridovirus* (c. 130nm) which have
a broader host range (Diptera, Coleoptera, Hemiptera, Lepidoptera, and even terrestrial crustaceans).

In the early 1970's blackfly larvae infected with an iridovirus were reported as rare occurrences in the River Ystwyth, Wales. Virtually nothing is known about the ecology of these viruses or of the nature of their host relations, or even of their route of transmission; mainly because iridescence has been used as the diagnostic criterion. During monthly sampling from the Ystwyth, a total of eight patently infected *Simulium* larvae were discovered. The virus from each of these larvae was extracted, bulked-up in a permissive lepidopteran (*Galleria mellonella*), and the DNA characterized by restriction endonuclease analysis. The restriction profiles showed obvious variation in the frequency and position of Hind III restriction sites: no two isolates were identical (Williams & Cory, *Med. and Vet. Entomol*. in press). When individual extracts from 30 randomly selected apparently healthy larvae were injected into *G. mellonella*, eight caused patent iridovirus infection in the lepidopteran. Restriction profiles produced from these covertly infected *Simulium* larvae showed a very similar pattern of isolate variability to that seen in isolates causing patent infections. Apparently healthy larvae pooled into groups of 10, injected into *G. mellonella* produced patent infections in all the lepidopteran larvae, the restriction profiles of which contained many sub-molar bands - indicative of mixed infection by more than one genotype.

The covert nature of the infection was confirmed by Polymerase Chain Reaction (PCR) targeted at the major structural protein (MSP) gene. This gene codes for the major capsid polypeptide and should be highly conserved despite the obvious genetic variation in

isolates. By carrying out a two-step PCR reaction involving repeated specific amplification of an 816 base fragment from within the MSP gene, followed by a second amplification of a 719 base fragment (from within the initial 816 base product), the presence of iridovirus DNA within simuliid tissues was demonstrated.

The most common species present in larval samples from the Ystwyth was *S. variegatum*. For both the *G. mellonella* bioassay and the PCR work, uninfected control larvae taken from rivers around Oxford were used. The level of covert infection in simuliid populations from the Ystwyth appears to vary both spatially and temporally, but rates approaching 50% infection may be observed. Current work is taking three approaches:

i) using these techniques to elucidate the ecology of this host-virus system
ii) using labelled DNA probes to investigate the exact location of iridovirus in sections of covertly infected larvae and adults
iii) confirmation of the presence of iridovirus particles in host tissues using electron microscopy.

[BSG Bull. No. 3, June 1994]

Ivermectin intervention and transmission of onchocerciasis in Sierra Leone

J. Whitworth : *THEU, London School of Hygiene and Tropical Medicine*

The main aims of wide-scale ivermectin distribution are to decrease clinical morbidity and reduce transmission of *Onchocerca volvulus* infection. Previous studies in Ghana, Guatemala and Liberia have all shown that entomological measures of transmission can be reduced in optimal situations of high coverage of a large human population with a single dose of ivermectin over a short period of time. Our studies in southern Sierra Leone of the effects of 5 six-monthly doses of ivermectin have shown that even with coverage as low as 30% of a small population, there is a highly significant reduction($p < 0.0001$) in the mean number of larvae per infected fly (mainly *Simulium soubrense B*). Other indices: annual biting rate, annual transmission potential, infected and infective parous rates, and mean number of L3 larvae per infective fly, did not alter significantly. We conclude that the mean number of larvae per infected fly is probably the best measure of the effectiveness of ivermectin distribution schemes if the vector has a high parasite carrying capacity. This index is sensitive, changes rapidly after treatment and is relatively simple to carry out in the field.

Estimation of *Simulium damnosum* gonotrophic cycle lengths by time series analysis

R.A. Cheke : *Natural Resources Institute, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TB*

Data on 13 continuous sequences of the numbers of *Simulium damnosum* s.l. caught per day at six different sites in West Africa were analysed by time series

analysis. ThePdata were de_trended by differencing according to the number of significant lags, from 1 to 5, identified from partial autocorrelation functions. Spectral analysis of the differenced data showed evidence of cyclic behaviour significantly different from white noise ($p < 0.05$) in every case. The major peaks in the periodograms corresponded to cycles of between 2.04 and 2.77 days. The possibility that these represent gonotrophic cycle lengths, given their similarity to estimates obtained by other methods, was considered.

POSTER PRESENTATION AT THE 15TH ANNUAL MEETING [BSG Bull. No. 3, June 1994]

Seventeen years of the identification and distribution of cytospecies of the *Simulium damnosum* complex in Nigeria

H.B. Mafuyai[1], R.J. Post[2], D.H. Molyneux[3] and D.H. Davies[1] : [1]*Department of Biological Sciences, University of Salford, Salford M5 4WT*; [2]*University of Wageningen, Netherlands*; [3]*Liverpool School of Tropical Medicine*

Since the description of 8 cytospecies included in the *Simulium damnosum* complex in West Africa (Vajime & Dunbar 1975) it has become known that there are at least 15 cytospecies/cytoforms in the region. It was in this same study that the first cytospecies from Nigeria (*S.damnosum* s.s. and *S.sirbanum*) were reported. More than a decade and a half later, the following cytospecies/forms of the *S.damnosum* complex have been recorded from the country by various authors (Akoh *et al.* 1987; Crosskey 1981; Vajime & Gregory 1990): *S.damnosum* s.s., *S.sirbanum*, *S.sudanense*, *S.squamosum*, *S.soubrense*, *S.soubrense* B, *S.yahense*, and Volta, Beffa and Nile forms.

Cytotaxonomic identifications of the members of this complex routinely utilise the larvae for their salivary-gland polytene chromosomes. By finding differences in the chromosome sequences in relation to the arbitrarily chosen standard (*S.soubrense*) unknown cytospecies may be typed.

New identifications undertaken between 1990-91 from 13 sites across the Nigerian Sudan, Guinea and derived Guinea Savanna, and from 2 other sites from the rainforest, revealed the presence of *S.damnosum* s.s., *S.sirbanum*, *S.sudanense*, *S.squamosum* and *S.yahense*. *Simulium damnosum* s.s., *S.sirbanum* and *S.sudanense* were restricted in distribution to the Savanna zones and *S.yahense* to the forest area, but *S.squamosum* occupied both forest and Savanna areas. This pattern of cytospecies distribution in Nigeria is similar to that reported in the O.C.P. area in West Africa (Vajime & Dunbar 1975).

Acknowledgements: this study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). The authors also wish to thank Professor C.G. Vajime, ABU Zaria Nigeria, for information on some cytospecies' breeding sites.

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THE 16th ANNUAL MEETING (1993) OF THE BRITISH SIMULIID GROUP

The meeting was held at the Natural History Museum, London, on Wednesday, November 17th, 1993. Participants were welcomed by Dr R.P. Lane, Keeper of the Entomology Department.

Dr Magda Charalambous chaired the meeting, which was attended during the day by about 50 members, Museum staff and students. It was a pleasure to see a larger than usual contingent of overseas members of the group. Seven talks were presented at the meeting, four of which were given by speakers from Germany, the Netherlands and Portugal.

Unfortunately, the afternoon session was interrupted by an emergency evacuation of the Museum due to a bomb alert. Happily, it was a false alarm and the meeting was able to be resumed after about an hour without too much disruption.

The meeting was preceded the previous evening by an informal meal at a local restaurant.

TALKS GIVEN AT THE 16TH ANNUAL MEETING
[BSG Bull. No. 3, June 1994]

Dispersal of onchocerciasis in Brazil and its significance to studies on simuliid taxonomy

Tony Shelley : *The Natural History Museum, London*

Since its discovery in Brazil over 20 years ago the distribution, prevalence and dispersal of onchocerciasis are still poorly known. Renewed efforts using these parameters are now being made to assess the public health importance of the disease with the increasing development of rural areas in this country. Studies made in the 1970's showed the disease to be confined to an area in the Brazilian and Venezualan Amazon; about 1400 of the Yanomami Indians in the area were infected. Ten years later a new focus of
the disease at Minaçu, 2500 km to the south of the Amazon focus, was discovered. Surveys are again being carried out in the Amazon focus to
determine the epidemiology of the disease and to formulate control measures.

Transmission of *Onchocerca volvulus* in the Amazon focus is by three or four vector species: *Simulium guianense*, *S.oyapockense*/*roraimense* and *S.yarzabali*. In the highland, mainly hyperendemic areas of the focus, *S.guianense* is the primary and

S.yarzabali the secondary vector, while in the lowland, mainly hypoendemic areas, *S.oyapockense*/*roraimense* is the vector. Experimental infection of these species showed only *S.guianense* to
be a very efficient host to *O.volvulus* while the other species are probably only efficient vectors when skin microfilaria densities and prevalence rates are high. These vector species and *S.exiguum*, the primary vector in Ecuador, also occur in the Minaçu focus.

Distribution of these vectors in Brazil is apparently wide and they also occur in other countries in South America. Development of the Amazon brings with it the danger that individuals infected with onchocerciasis could carry the disease to non-endemic areas and set up new transmission foci if the appropriate simuliid species are present. Identification of each of these vector species is not straightforward. *S.guianense* occurs in both anthropophilic and zoophilic populations, which are indistinguishable morphologically. Chromosome preparations from larvae are currently being studied in an effort to establish whether female biting behaviour is cytospecies linked. *S.yarzabali* is closely related to *S.incrustatum* and may be a form of it. The latter species is highly variable in morphology and behaviour. Again, cytological studies are necessary in order to link female behaviour and vectorial capacity to the possible existence of cytospecies. *S.oyapockense* may be distinguished from *S.roraimense* by morphology in the male, hydrocarbons in the female and chromosomes in the larvae. The lack of a simple method to distinguish females is the major constraint on establishing whether both species are vectors in the Amazon onchocerciasis focus. Work in the Natural History Museum, London, in collaboration with the Oswaldo Cruz and Evandro Chagas Institutes, INPA and the Ministry of Health in Brazil is aimed at clarifying the taxonomy of these species and facilitating their identification so that onchocerciasis transmission may be qunatified and its dispersal assessed.

[BSG Bull. No. 3, June 1994]

Decline of *Simulium neavei* and its associated crabs in the onchocerciasis foci of the Ruwenzori Area, West Uganda, during the past 20 years

Rolf Garms[1], John Yocha[2] and Walter Kipp[3] : [1]*Bernhard Nocht Intitute for Tropical Medicine, Hamburg*; [2]*Vector Control Unit, Ministry of Health, Fort Portal, Uganda*; [3]*Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ, Basic Health Services, Fort Portal, Uganda*

The man-biting members of the *Simulium neavei* group are restricted to forested areas and depend on a dense vegetational cover over their breeding sites. It has been reported, e.g. from Tanzania and Malawi, that deforestation has resulted in a decrease of fly population densities and transmission of onchocerciasis. Selective bush clearing was successfully employed to eradicate *S.neavei* from one of the former foci in Kenya (reviewed by Walsh *et al.* 1993).

Since *S.neavei* is the main vector of onchocerciasis in Kabarole district east of the Ruwenzori mountains in Western Uganda, it was of interest to examine whether recent ecological changes have had an effect on the occurrence of the species. Considerable research work and successful control measures against *S.damnosum* s.l. were carried out in this area during the sixties and seventies (reviewed by Raybould & White 1979) until all activities came to a standstill due to the civil war in

1977. Most of the early data were never published, but records still kept by the Vector Control Unit of the Ministry of Health, Fort Portal, have been used for a comparison of the situations in the periods 1971 to 1975 and 1990 to 1993.

Onchocerciasis is still hyperendemic in two foci in Kabarole district. One is located around the Itwara forest reserve north-east of the district capital Fort Portal, and the other lies to the east of Lake George bordering the Kasioha_Kitomi forest reserve. *S.neavei* is the only vector in both foci and is associated with the river crab *Potamonautes aloysiisabaudiae*, which is the phoretic host of its immature stages. In the northern Itwara focus *S.neavei* is no longer found in some rivers draining westward into the Rift Valley, where crabs have also become rare. Although no control measures were carried out, *S.neavei* has completely disappeared from a third focus (Ruteete area, south of Fort Portal), where onchocerciasis was hyperendemic in 1971 and is now hypoendemic. This change has been accompanied by the establishment of a population of *S.damnosum* s.l. (probably the Nkusi form), which possibly is a poor vector, but may maintain a low level transmission of onchocerciasis. The disappearance of *S.neavei* from this focus could be the result of the destruction of a forest reserve along the Mahoma river. However, the reasons may be more complex, because the crab host has also disappeared. Furthermore, crabs and *S.neavei* are no longer to be found in the nearby Kibale forest reserve, which is still intact. *S.neavei* has also disappeared from at least one river (the Sebwe, Bugoye district) in the foothills of the Ruwenzori, where it was not a vector.

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[BSG Bull. No. 3, June 1994]

Prospects for the eradication of *Simulium damnosum* from the island of Bioko (Fernando Po)

R.J. Post : *Wageningen Agricultural University, The Netherlands*

Actual eradication of *Simulium* vectors of onchocerciasis has been achieved on a few occasions. The important factors which have made this possible have been the isolation of the target populations from vector immigration and the accessibility of breeding sites. These factors are both indicated for Bioko, by the endemic nature of the cytotype, prevailing wind pattern, and highly seasonal nature of most of the streams (which possibly restricts dry-season breeding to just five or six perennial rivers).

Recent advances in the identification of adult female *Simulium damnosum* s.l. from West Africa

Mike Wilson : *Wageningen Agricultural University, The Netherlands*

The need to identify reliably the adult female *Simulium damnosum* s.l. has led to the application of a wide range of techniques including morphological studies, cuticular hydrocarbon analysis, DNA probes, etc. We have developed a technique for morphological identification based on multivariate statistical analysis of 15 characters. This morphological scheme can identify *S.damnosum* subcomplex, *S.sanctipauli* subcomplex, *S.squamosum* and *S.yahense* with an overall correct identification of over 98%. However, to achieve the separation of individual members of the *S.damnosum* and *S.sanctipauli* subcomplexes we have been examining DNA sequence variation for species-specific differences, and some have been found between *S.sirbanum* and *S.damnosum* s.str. We are currently screening specimens to confirm the broad applicability of these traits. A non-radioactive detection system was developed specifically to enable large scale use for identifications in both laboratory and field situations.

[BSG Bull. No. 3, June 1994]

The blackflies (Diptera: Simuliidae) of Portugal. Taxonomy, distribution and bioecological data

A.J. dos Santos Grácio : *Instituto de Higiene e Medicina Tropical, Lisbon*

The author presents the results obtained during a survey carried out in Portugal from 1980 to the present time. Based on the material studied (14048 larvae, 10424 pupae and 2646 adults) and bioecological data the
author presents a list of the genera and 30 species obtained and their distributions.

The genera, subgenera and species collected were as follows:

Genera *Prosimulium*[1], *Metacnephia*[2] and *Simulium*

Subgenera *Prosimulium*[2], *Nevermannia*[2], *Eusimulium*, *Wilhelmia*, *Simulium* s.str.[2], *Obuchovia*[1] and *Boophthora*[2]

Species *P.latimucro*[2], *P.tomosvaryi*[1], *M.blanci*[2], *M.nuragica*[2], *S.vernum*, *S.naturale*[1], *S.cryophilum*[1], *S.armoricanum*[1], *S.ruficorne*, *S.pinhaoi* sp.nov.[2], *S.latinum*[3], *S.aureum* s.l., *S.angustipes*[2], *S.equinum*[1], *S.pseudequinum*, *S.sergenti*, *S.lineatum*, *S.ornatum* s.l.[1], *S.nitidifrons*, *S.spinosum*[1], *S.reptans*[1], *S.hispaniola*[1], *S.variegatum*[1], *S.monticola*[1], *S.argyreatum*[1], *S.tuberosum*[1], *Simulium* sp.[2], *S.ibericum*[2], *S.auricoma* s.l.[1], *S.erythrocephalum*[1]

[1]New to Portugal [2]New to the Iberian Peninsula
[3]Confirmed for Portugal (c.f. Beaucournu-Saguez, 1972)

Data on filariae in cattle and horses in southern Portugal are also presented.

The talk ends with a summary of simuliid breeding grounds found in Portugal.

Reference

A.J. dos Santos Grácio (1994 in press). *An.Inst.Hig.e Med.trop.* 11(1/4)

Blood meal identifications from *Simulium posticatum*

Angus McCrae and Nigel Hill : *Warborough, Oxon. and London School of Hygiene and Tropical Medicine*

Previous findings relevant to host selection by *S.posticatum* in Dorset are briefly reviewed. These together with host identifications obtained in 1993 through ELISA tests on blood-meals from 3 flies from Dorset and 28 from Oxfordshire hint that in Dorset *S.posticatum* is primarily ornithophilic, whereas in Oxfordshire *S.posticatum* (identifications need confirmation) seems primarily boviphilic with horse and canine hosts also. Implications and prospects for further work are discussed.

[An extended account of this talk appears on page 23. Ed]

[BSG Bull. No. 3, June 1994]

The predator_prey relationship between adult watermites and *Simulium* larvae

John Mwango[1], Roy Wiles[1] and Trevor Williams[2] : [1]*University of Buckingham*, [2]*NERC Institute of Virology and Environmental Microbiology, Oxford*

Aquatic mites of the family Hydrachnidia are usually present in abundance across the spectrum of freshwater habitats; both lentic and lotic. However, because of their small size and because of problems of identification (taxonomy) they have often been neglected in studies of aquatic ecology. Watermites have a 7_stage lifecycle of which only the larva, deutonymph and adult are active. The ectoparasitic relationship between larval watermites and adult *Simulium* has been well described by Gledhill *et al.* (1982) and Gledhill (1985). Larval watermites enter the pupal cocoon, crawl onto the adult during emergence and feed by piercing the inter_segmental membranes. Specially adapted piercing and cutting appendages, the chelicerae, are carried on the infracapitulum for this purpose. Having engorged, the larval watermites drop off the adult, back into the water to continue the lifecycle. The predator_prey relationship between watermites and *Simulium* larvae, however, has not been previously recognised (Mwango, Wiles & Williams 1993). Adult watermites feed by puncturing the blackfly larval integument and probably inject a proteinaceous toxin to paralyse or subdue their victim. In other mite species, this toxin has been identified as ca. 250 amino acids in length, with neurotoxic activity.

By direct observation of adults in the laboratory, we identified 3 species of watermite from a typical lowland river habitat which showed clear predatory behaviour towards late instar *Simulium* larvae, including the two most abundant species in the river: *Hygrobates fluviatilis* and *Lebertia porosa*. These species were adopted as the model species for the study. Patterns of adult watermite predation were studied by confining mites and *Simulium* larvae together in cells, in flowing water, in the laboratory. No alternative prey items were available to the watermites. Both species showed similar rates of predation of (mostly) *Simulium equinum* larvae at ca. 1.4 larvae/mite/day for 2nd+3rd instar larvae and ca. 0.5 larvae/mite/day for 6th instar larvae. When offered a simultaneous choice between early and late instar *Simulium* larvae, *H.fluviatilis* showed a distinct preference to attack smaller larvae. The reasons for this are uncertain, but may be related to the problems associated

with handling and subduing larger larvae; which may be better able to defend themselves against the mite attacks. By direct observation, and by sampling from the upper part of *Ranunculus* weedbeds, watermites and *Simulium* larvae were observed in the same microhabitat.

Repeated mid_season samples from the *Ranunculus* beds in the River Ouse at Buckingham, demonstrated that *Simulium* larvae were by far the most abundant prey items available for watermites in the river, by an order of magnitude.

The impact of watermite predation on chironomid populations in lakes (in the Netherlands) has been carefully examined by Ten Winkel (1985). Exclusion experiments showed watermites were as important as cyprinid fish (mainly bream) in terms of chironomid predation. Using the adult watermite densitites from 16 weeks of core sampling, the published generation times of *Simulium* spp. in UK rivers, and the laboratory rates of predation in this study, we have tentatively estimated the possible impact which mites may have on populations of *Simulium* larvae. We recognize the criticisms which can be levelled at these data (e.g. availability of alternative prey, elevated temperatures, flow rates, ability of the prey to utilize escape responses such as drift, etc.) but we believe that watermites may represent one of the most influential, yet one of the most neglected of *Simulium* natural enemies.

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POSTER PRESENTATIONS AT THE 16TH ANNUAL MEETING

[BSG Bull. No. 3, June 1994]

P.J. McCall, A.J. Trees and D.H. Molyneux (*Liverpool School of Tropical Medicine*) exhibited a poster with the title 'Chemical mediation of communal oviposition in the river blindness vector *Simulium damnosum* s.l. in Sierra Leone'.

The development of habitat preference curves for blackfly larvae and their use in assessing in-stream flow needs

M.A. Bickerton and M.T. Greenwood : *Freshwater Environments Group,*

Department of Geography, Loughborough University of Technology

1. Introduction

Recent concerns about the ecological impacts on rivers of over-abstraction, drought, regulation and augmentation have led to a 'spate' of new studies on the responses of river fauna to flows. Attempts to quantify habitat preferences and relate these to flows are quite new, and form the subject of this poster.

Habitat suitability can be predicted by integrating information on taxa preferences for flow-related variables (water depth, current velocity, shear stress, substrate characteristics) and how these variables change at a site under different flows.

Selection of appropriate 'target' species for analysing in-stream flow needs is a critical step (Orth 1987), with those having the narrowest range of habitat preferences being most sensitive to flow alterations. The Simuliidae are a classic example of this group.

2. Construction of Preference Curves

Three methods of constructing habitat preference curves which have been compared using Simuliidae by Morin et al (1986) and Skinsley (1993) are:

i) The Incremental Method (Gore and Judy, 1981).

ii) The Polynomial Regression Method (Orth and Maughan, 1983).

iii) The Multiple Regression Method (Gore and Judy, 1981).

In all of these methods a composite habitat suitability can be calculated for a number of variables by multiplying the individually calculated suitabilities.

3. Examples From Two English Streams

Figure 1 shows Skinley's depth and velocity preference curves for Simuliidae using the three methods, using data from field collections from the Wood
Brook, Leicestershire. The Multiple Regression method was found to be the most accurate, with the Polynomial Method next best.

[Graphic File BULL3F1.gif here]

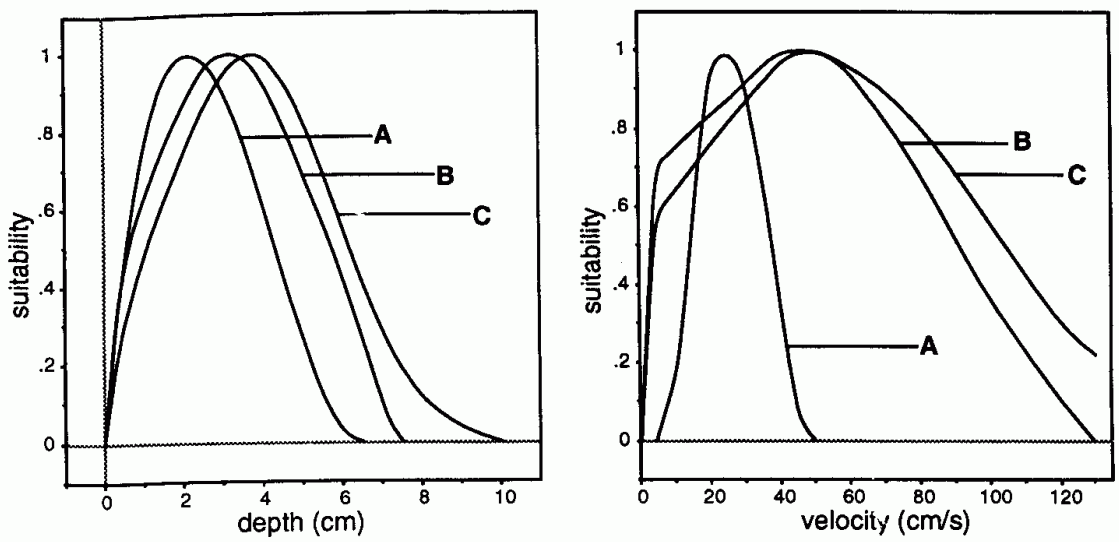

Figure 1. Comparison of methods of preference curve construction for Simuliidae from the Wood Brook, Leicestershire: depth and current velocity preferences: A - Incremental method; B - Polynomial regression method; C - Multiple regression method.

Fig. 1 Comparison of methods of preference curve construction for Simuliidae from the Wood Brook, Leicertershire: depth and current velocity preferences: A – Incremental method: B – Polynomal regression method: C – Multiple regression method.

Figure 2 illustrates preference curves for Simuliidae from the River Wissey, Norfolk (Petts and Bickerton, 1993), together with a plot of the original abundance data against composite suitability calculated from the individual preferences.

Figure 3 shows the uses of these habitat preference curves to predict composite habitat suitability for three sites on the River Wissey under different flows. Chalk Hill Farm is a coarse gravel, asymmetric riffle with areas of relatively high current velocity even under low flows, providing good habitat for Simuliidae. Didlington (sand) is a deep sand- and gravel-bed run of uniform cross-section which requires higher flows to provide reasonable habitat. Didlington (gravel) is intermediate in character.

[Graphics File BULL3F2.gif here]

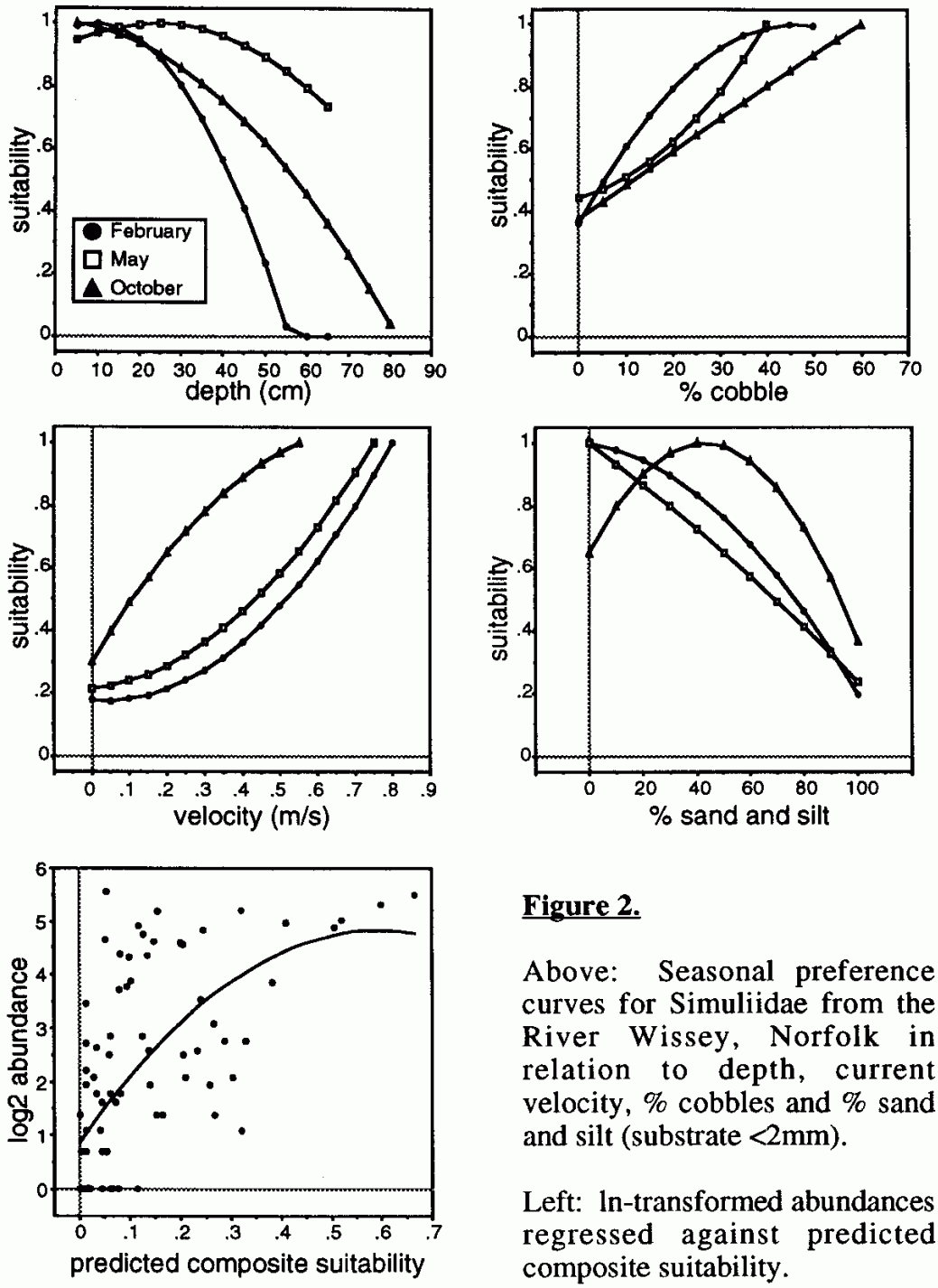

Figure 2.

Above: Seasonal preference curves for Simuliidae from the River Wissey, Norfolk in relation to depth, current velocity, % cobbles and % sand and silt (substrate <2mm).

Left: ln-transformed abundances regressed against predicted composite suitability.

[Graphics File BULL3F3.gif here]

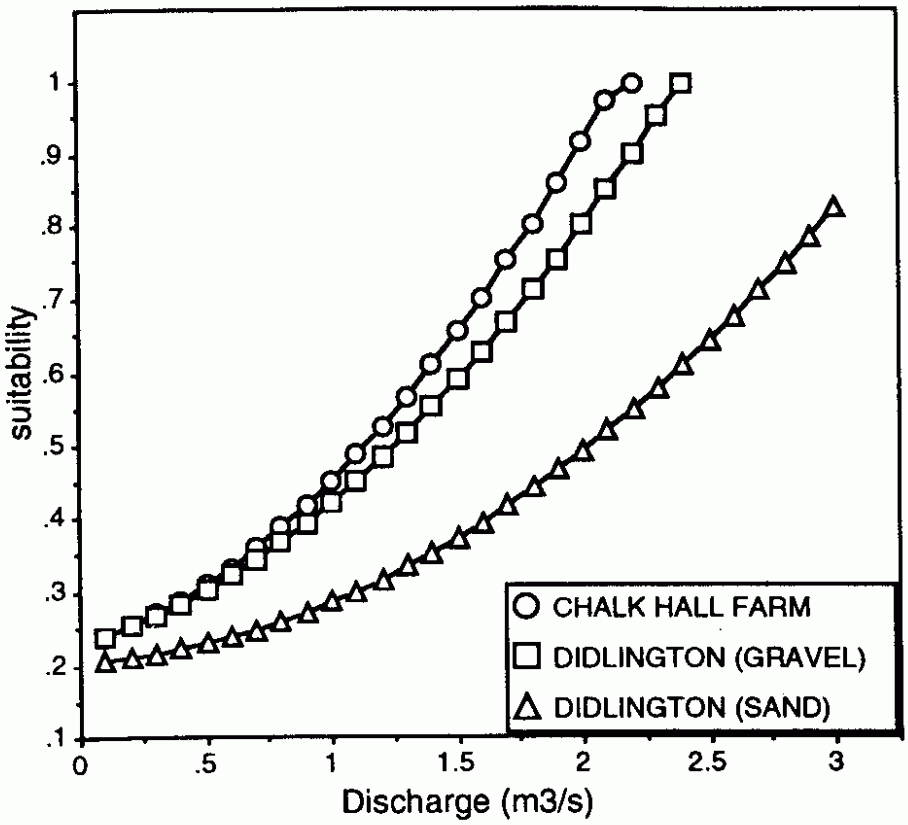

Figure 3. Predicted habitat suitability for Simuliidae with increasing discharge at three sites on the River Wissey, Norfolk, in May.

Fig. 3 Predicted habitat suitability for Simuliidae with increasing discharge at three sites on the River Wissey, Norfolk, in May

4. Conclusions

Table 1 gives the optimum current velocity and depth values for Simuliidae found in a range of studies using comparable methods. Gore (1978) and Orth and Maughan (1983) used a method of weighted habitat means to establish the conditions supporting most larvae at their study sites. Morin et al (1986), Skinsley (1993) and Petts and Bickerton (1993) employed preference curves to predict optimum conditions.

Variability in estimates of optima from different studies probably reflect differences in the methods of calculation, seasonal and, most importantly, species differences. This highlights the need for more species level studies in order to understand the habitat preferences of this important family more fully.

	Velocity (cms-1)	Depth (cm)
Gore (1978) (Montana) *Simulium* sp.	77	27
Orth & Maughan (1983) (Oklahoma) *Simulium* sp.	82	20
Yamagata & Kanayama (1985) (Guatamala) *Simulium ochraceum*	41-66 (max)	0.2-2 (min)
Yamagata (1986) (artificial stream) *S.ochraceum* & *S.horacioi*	40	0.25
Morin et al (1986) (Quebec) *S.aureum*	70 (max)	3 (min)
Skinsley (1993) (Leicestershire) *Simulium* sp.	30	2.8
Petts & Bickerton (1993) (Norfolk) *Simulium* sp.		
February	80 (max)	10
May	75 (max)	25
October	55 (max)	5 (min)

Table 1. Velocity and depth preferences for *Simulium* spp. from published and unpublished studies. Max/min = maximum or minimum observed value.

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Host Selection by the Blandford Fly (*Simulium posticatum* Meigen), with Blood-Meal Identifications

A.W.R. McCrae and N. Hill : *71 Thame Road, Warborough, Oxon. OX10 7EA and London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Despite the severe hypersensitivity that its bites sometime provoke in humans and the adverse public opinions consequently engendered (Hansford & Ladle 1979; Crosskey 1990), field observations (by AM) show clearly that *Simulium posticatum* is actually a 'nervous' or 'dilettante' blood-feeder (see Crosskey 1990 p. 431) on man. Simple calculations based on available data also show that only a minute proportion of the female flies emerging from the River Stour in Dorset actually bite man. Laboratory studies (by AM) have indicated that female *S.posticatum* in Dorset are not autogenous, and this together with the sometimes high parous rates of those coming to man (including those few biting) combine to tell us that other host species must be much more severely attacked. So far, however, field observations have not shown what this (these) host(s) might be: in Dorset such attempted observations have invlolved donkeys (totally negative: Hansford 1977; AM pers. obs.), dogs (occasionally bitten: M. Ladle, pers. comm.; only large, dark and short-haired dogs: AM pers. obs) and cattle (negative: carefully checked by AM because boviphilic simuliids are often also anthropophilic: see Crosskey 1990 for context and examples). Further hosts checked with
negative results in the Stour Valley, though only cursorily, or else merely from a distance when they have shown no indication of disturbance in the presence of abundant females of *S.posticatum*, have been horses, red deer, rabbits and (at Merley Tropical Bird Park near Broadstone) a goat and a wide range of bird species. Certain deer species other than red deer had seemed likely on empirical grounds to be primary hosts, but attempts to secure these for close observation were unsuccessful.

Blood Meals

In a dissected subsample of 30 flies from among abundant *S.posticatum* around man at Broadstone, Dorset, in May 1992, one was found to contain a small but fresh blood-meal. This fly had oocytes of a size typical for a freshly emerged fly and was even unfertilised (though its crop contained an apparent sugar meal), whereas of 6 nullipars captured in the

act of biting man, all had been fertilised and had oocytes appropriate to flies 2.5 to >7 days old. This blood-meal was therefore perceived to hold promise of the first fragment of direct evidence as to the elusive identity of a primary host and so was stored on filter paper until host identification could be attempted.

In May-June 1993, 1242 more flies collected by means of a man-baited table trap (which without bait took virtually no Simuliidae) were dissected,
yielding 2 more blood-meals from 164 flies at Broadstone (where *S.posticatum* is only too well known as the 'Blandford Fly') and 28 from 1078 at Stonesfield in Oxfordshire (where *S.posticatum* breeds widely (Williams 1991) and where females of what is presumed to be this species are known variously as the 'Stonesfield Stinger' - R.H.A. Baker, pers. comm. - or the 'Woodstock Fly'). Blood-meals of smallest size (classed as +/-) and/or blackest colour (BB) (see Table for other classes) were not used; thus, 31 (2.4%) of all dissected flies yielded blood-meals judged by size and colour as usable, as later justified by results (Table). Of these 31 flies, 15 were fully engorged (blood-meals +++ or ++++), 14 with BR blood. The bodies less abdomens of most of these 31 flies were retained for confirmation of their identifications, but were inevitably too damaged by wet dissection for *S.posticatum* then to be distinguishable from other minutely similar species.

Table: Blood-meals and host identifications.

Colour / Size	DB	RB	DR	BR	Total
++++	0	1 (1B)	0	2 (2B)	3
+++	0	0	0	10 (6B) (1H) (3N)	10
++	3 (1H) (2N)	1 (1N)	4 (1Ax) (3N)	4 (3B) (1N)	12
+	2 (1B) (1N)	2 (1D) (1Nx)	1 (1N)	1 (1A*)	6
Total	5	4	5	17	31

Blood-meal colour classes: DB = dark brown, RB= red-brown, DR = dark red, BR = bright red.

Blood-meal size classes: + = small, ++ = medium, +++ = large (engorged), ++++ = very large (hyper-engorged).

Host identifications: A = avian, B = bovine, D = dog, H = horse, N = negative. Note none positive for human or sheep/goat.

Locality/Year: * = Broadstone (Dorset), 1992; x = Broadstone 1993. All others - Stonesfield (Oxfordshire) 1993.

Whereas flies with partial (interrupted?) blood-meals too small to induce oogenesis could be expected to be host-attracted, it is inexplicable why any fully blood-fed flies could be captured through the method used. Highest blood-meal rates came towards the end of the biting season: a sample of 52 flies from Stonesfield on 5.vi.93 (very few flies behaving typically for *S.posticatum* were evident there by 10.vi.93) included 9 (17%) with usable feeds, plus a further 14 (27%) with unusable feeds (+/- BB); none of these 23 flies was obviously undergoing oogenesis (following Cupp & Collins 1979). (Nor were any of the total of 1272 dissected flies gravid). Although 42 (81%) of these 52 late-season flies were parous, the idea that the attraction of blood-fed flies to the man-baited trap might be thought an aberrant function of fly senescence was contradicted by finding that 2 of the 5 flies with +++/++++ blood-meals were nulliparous.

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ELISA Tests

One of us (NH) tested all 31 usable blood-meals by ELISA in November-December 1993 against human, sheep/goat, bovine, horse, dog and avian antisera, and obtained the following results (and see Table) as confirmed by repeat tests:

(i) No blood-meals were positive for human or for sheep/goat.

(ii) 2 blood-meals from Broadstone (including the one obtained in 1992) were positive for avian and the 3rd was negative.

(iii) 13 blood-meals from Stonesfield were strongly positive for bovine and 2 for horse, 1 was weakly positive for dog and 12 (3 of which had been large and bright red on dissection) were negative.

(iv) No multiple feed from more than one host species was evident, but see (f) below.

Among the implications of these results are:

(a) Paucity of actual anthropophily is supported. This view in no way detracts from the seriousness of the public healsth problem posed by hypersensitised bite reactions in humans. Such hypersensitivity, incidentally, has not been observed in dogs, according to statements from veterinarians in the Blandford area.

(b) Evidence of ornithophily from Dorset is astonishing, all the more so since *S.posticatum* females possess the untoothed tarsal claws characteristic of mammalophilic Simuliidae (Crosskey 1990).

(c) If the bovine-positive feeds from Stonesfield were from cattle (none of which were kept near the catching site), then a marked difference in host selection between the Oxfordshire and Dorset populations is indicated.

(d) Although every bovine-positive blood sample tested strongly, we cannot yet exclude the possibility of some cross-reaction with deer, using this particular technique.

(e) The single dog-positive Stonesfield feed reacted quite weakly, probably owing to its being small (+) and partly digested (RB). It is possible, however, that some

cross-reaction with fox blood may occur using this method.

(f) In that 3 of the 12 negative Stonesfield blood-meals had been +++BR (Table), at least one species of non-cross-reactive host is indicated. Thus, multiple feeds (see (iv) and (d), above) remain possible.

(g) In combination with other results, the 2 horse-positive results suggest that the Stonesfield flies represent a population of generalist feeders on large mammals including canines ((e) above) and humans (occasionally: direct observations, R.H.A. Baker, pers. comm.), a view subject to future identifications ((3) below) confirming the flies' conspecificity.

Future Needs and Prospects

1. Considerably more blood-meals need to be tested against more hosts, particularly from Dorset. Control operations in Dorset (Ladle 1993) might make this unfeasible there in future, but not so to date.

2. Sufficient has been retained from most if not every blood-meal for several further tests to be run, dependent on ELISA plates for appropriate hosts being obtainable. Especially needed are tests for muntjac and roe deer.

3. In view of the surprising nature of these preliminary findings (notably (b), (c) and (g) above), the flies' identifications should be double-checked in future studies. Dissection procedures particularly need modification to allow this.

Summary: Only a minute proportion of female *S.posticatum* actually bite humans, despite their notoriety for provoking severe hypersensitive bite reactions in man. In Dorset, dogs are also slightly attacked, but cattle are unattractive. Blood-meals from 31 (2.4%) of 1272 female flies attracted to man (mostly sampled by man-baited table trap) were tested by ELISA against human, sheep/goat, bovine, horse, dog and avian antisera. Of the 3 blood-meals from Dorset, 2 were positive for avian and the other negative, whereas of the 28 from Oxfordshire, 14 were positive for bovine, 2 for
horse and 1 for dog. Some of the negatives indicated further hosts untested for. Implications and prospects for future studies are discussed.

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[BSG Bull. No. 3, June 1994]

Dense Aggregations of Blackfly Larvae in Lake Outlets

Roger Wotton : *Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT*

Streams are frequently impounded by weirs and dams to create pools, or larger bodies of water. When water flows as an outlet stream from the surface of an impoundment, conditions are ideal for the development of high densities of suspension-feeding animals. In summer, when lakes become thermally stratified, the outlet stream water is constantly warm and contains large numbers of nutritious particles - zooplankton, phytoplankton, bacterioplankton and particles of detritus, all of which are swept from the lake.

Some outlets, especially larger ones with higher discharge, support high densities of net-spinning caddisfly larvae which selectively ingest the most nutritious particles captured on their secreted nets. Where the stream bed consists of sediment, many bivalves will be buried and these capture particles brought in by feeding currents. Where, in contrast, the substratum is better consolidated, and where discharge is low, the fauna will be dominated by midges of the genus *Rheotanytarsus* and/or by blackfly larvae. Both use passive feeding techniques although the midges must move to ingest the silk strands that are attached to their tubes, and which are used to capture particles. Sometimes, the densities of blackfly larvae are so high that their aggregations appear like a carpet, with larval bodies apparently crowded in upon each other.

Aggregations of blackfly larvae in impounded lake outlets are often dominated by *Simulium noelleri* Friederichs, and may be exclusively of this species. They can form tight masses on the surface of dams and spillways, and rows where the substratum is near horizontal and where flow does not undergo any marked acceleration. On the horizontal surfaces of dams made of planks it is usual to find larvae clumped into aggregations and this may be a result of the hydrodynamic conditions present just before water plunges vertically. Interestingly, while larvae of *S.noelleri* can dominate impoundment outlets in the Palaearctic this habitat is characteristic of *Simulium decorum* Walker in the United States and in Canada. As the two species are impossible to tell apart morphologically as larvae (at least by me), and are closely related cytotaxonomically, it suggests that this habit is one which developed a long time ago, and that living in thin films of water pre-adapted these species for life in impoundment outlets.

I would be most interested to hear from anyone who has records of dense aggregations of *S.noelleri* at impoundment outlets, especially in the south of England. Roger Crosskey has kindly provided me with a list of sites where he collected this species, but I would welcome information from other blackfly workers.

Preliminary Notice of the 17th Annual Meeting, 1994

The 17th Annual Meeting of the British Simuliid Group will be held at the Liverpool School of Tropical Medicine and the Department of Environmental and Evolutionary Biology, Liverpool University, on Wednesday, September 21st, 1994. Further details will be circulated in due course, but John Davies, who is organising the meeeting, would appreciate hearing from anyone wishing to volunteer a talk or poster presentation.

John's address is *Dr. J.B. Davies, Division of Parasite and Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA*. The School's phone and Fax numbers are (051) 708 9393 (phone) and (051) 708 8733 (Fax).

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BRITISH SIMULIID GROUP BULLETIN

Number 4 December 1994

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[BSG Bull. No. 4, December 1994]

From the Editor

Trefor Williams has been both the secretary of the British Simuliid Group and the editor of the Newsletter and the Bulletin since the Group was formed in 1979. At our last meeting Trefor expressed the wish to hand over the editorship and so I find myself the new editor.

The transition from a cyclostyled newsletter to Bulletin, a more polished production, has not been an easy one. I am fortunate that Trefor overcame the production problems before handing over, so I have the relatively easy task of continuing where he has left off.

We all owe Trefor a sincere vote of thanks for his editorship over the past 15 years. So, Trefor, from all of us - thanks for your unstinted efforts in keeping the Newsletters and Bulletin going - we really appreciate it!

I suppose it is inevitable that a new editor will be fired with enthusiasm and want to make changes, but we should remember the objectives of the Group as expressed by Gavin Gatehouse in the first issue of the Newsletter

"to maintain and develop contacts between those interested in simuliids and to provide for the exchange of news, information, requests and ideas concerning all

aspects of simuliid biology".

Now that the production problems are over I think we have in the Bulletin an opportunity to widen the range of interests that it covers. You will notice that I have opened a "News and Correspondence" section to which I invite all members to submit short contributions - anything that might be of interest that other members may have missed or should know about, and that might stimulate some correspondence. This could also include any personal experiences of an anecdotal nature, or simuliid related extracts from newspapers or books.

Past Bulletins have been predominantly concerned with reporting the proceedings of our meetings. I would like to get some reaction from the membership as whether we should try to bring out a second number each year which would be made up of submitted or invited papers and correspondence. I believe that two issues a year, say in December and June, will keep interest going better, and would provide a vehicle for rapid publication. At present we can accept papers up to about 6 weeks before the puplication date. But this depends on **YOU**. If you send in the material I will do my best to get it published. I already have offers for about 12 pages for an additional number, so we only need another 4 or 5 pages to make it worth while.

As organiser of the 17th Meeting, I placed a notice of the meeting on an entomlogical electronic bulletin board (entomo-l) run by the University of Guelph through the Internet (see *Antenna* 18 (3): 102, July 1994 for details). This resulted in a number of requests for membership from Canada, U.S.A., Australia, and I am very pleased to say, from South Africa. Because we have to keep costs down I hope that those overseas members that joined via e-mail will agree to accept their copy of the Bulletin via e-mail. Unfortunately the e-mail version will lack illustrations and figures which we cannot at the moment transmit this way. But should you wish to receive the figures by conventional mail, please let me know. How about a page or two from our new overseas members telling us what is going on in your country?

We must move with the times. Electronic data transference via the Internet is becoming widely accepted and used. Trefor has opened a news list called **simuliidae** which anyone with access to the Internet can join (see his note). Both he and I are concerned that those members to whom this service is not available may feel left out, so we will publish in the Bulletin items that we think are of general interest and may generate some correspondence.

John Davies : *Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.*

Phone: 051 708 9393 Fax: 051 708 e-mail: daviesjb@uxb.liv.ac.uk

THE 17th ANNUAL MEETING (1994) OF THE BRITISH SIMULIID GROUP

The 17th Annual Meeting of the British Simuliid Group was held at Liverpool University on 21 September 1994. It was hosted jointly by the Department of Environmental and Evolutionary Biology and the Liverpool School of Tropical Medicine. About 45 persons participated in the meeting. The customary dinner, held the evening before, was attended by 35 members, spouses and friends.

In the discussions that followed the meeting a number of members expressed concern that the content of the last few meetings had been strongly parasite orientated and feared that this was discouraging those members who were interested in other disciplines such as freshwater biology. This bias is understandable since the majority of funding available to simuliidologists these days is onchocerciasis or filariasis motivated.

It was felt that one way to attract a wider membership might be to invite someone to present a review paper on a specific subject at each meeting, and to widen the scope of the Bulletin, which might then appear twice a year, with the second number containing submitted papers. We should also consider holding our meetings jointly with other groups.

TALKS GIVEN AT THE 17TH ANNUAL MEETING

The meeting was opened by Professor Brian Moss, Head of the Department of Environmental and Evolutionary Biology, who gave a short and entertaining address of welcome which ended with a reading of a poem which had come to light during a recent renovation of his department. This is reproduced under "Notes and Correspondence".

[BSG Bull. No. 4, December 1994]

"Going through the mill" A further look at the effect of the cibarial armature on ingested microfilariae

J.B.Davies: *Liverpool School of Tropical Medicine*
R. Luján: *Centre for Health Studies, Universidad del Valle de Guatemala.*

The cibarium is a structure found at the posterior end of the pharynx, which in some species of *Simulium* bears a cluster of sharp pointed teeth which project into the lumen of the food canal. The effect of these teeth in damaging microfilariae (mf.) of *Onchocerca volvulus* during blood feeding is well known and has been described by Omar and Garms (1975, 1977) and Collins et al. (1977) amongst others. It has been stated that less than 2% of ingested mf. may develop to the infective stage in the fly because of this damage Shelly (1994).

During our attempts to monitor the effect of a nationwide ivermectin distribution campaign by the Guatemalan National Onchocerciasis Elimination Campaign on the continued transmission of the disease, we have been counting the numbers of mf. ingested by the principle vector *Simulium ochraceum* when feeding on treated and untreated volunteers. After feeding to repletion, flies were immediately preserved in 100% ethanol. Later, blood meals were dissected out, then stained and cleared in lactoproprionic orcein using the technique described by Arzube and Shelly (1989).

The preliminary results indicate that when flies were allowed to feed as they would in nature, that is anywhere above the waist, the numbers of mf. ingested from the same person varied widely, and the proportion that appeared to be undamaged varied fron 0% to 30%. Two examples are given in detail. On a moderately infected volunteer with a mean skin mf. density of 18.5 mf./snip, 6 out of 10 flies ingested 37 mf. between them, only one of which was undamaged (Table 1). At the other extreme, from a very heavily infected volunteer with 463.5 mf./snip, all 9 out of 10 flies ingested between 98 and 980 mf. and numbers of undamaged mf. varied between 0 and 277. The 10th fly ingested a surprising 980 mf. of which 277 (28.3%) appeared to be undamaged.

[Graphics File BULL4T1.gif here]

Table 1 - Numbers of undamaged, damaged and portions of microfilariae counted in the blood meal of *S. ochraceum* that had fed on two volunteers with different levels of skin microfilariae.

Fly No.	Entire Undam.	Entire Dam.	No. of Portions	Est. Total
Volunteer No. 1 - 18.5 microfilariae/snip				
1	0	0	0	0
2	0	0	1	1
3	0	0	0	0
4	1	5	17	12
5	0	0	0	0
6	0	1	3	2
7	0	1	5	3
8	0	0	42	14
9	0	0	0	0
10	0	3	4	5
Volunteer No. 2 - 463.5 microfilariae/snip				
1	5	39	417	183
2	20	214	829	511
3	4	32	184	98
4	5	69	400	208
5	42	144	849	469
6	1	41	350	159
7	42	173	395	347
8	24	249	1213	678
9	0	30	242	111
10	277	352	1053	980

It is considered that the wide variation in uptake is a function of the variety of sites on which the flies fed, in comparison to the restricted sites employed in experiments by other workers. There is also the possibility that in some flies the cibarial mechanism may be less efficient at destroying mf. than in others. Statistical analyses to reveal any trends or density dependency will be carried out when a greater volume of data has been assembled.

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[BSG Bull. No. 4, December 1994]

Density-dependent processes in the transmission of human onchocerciasis. Entomological aspects.

María-Gloria Basáñez: *University of Oxford, Department of Zoology, South Parks Rd, Oxford OX1 3PS.*

The transmission success of *Onchocerca volvulus* is thought to be influenced by several regulatory or density-dependent mechanisms that act at various points in the two-host life cycle. We examine here the evidence for density-dependence in the *Simulium* host, namely, in the processes of microfilarial uptake by the flies, the development of the ingested parasites to the infective stage, and the fate of infected vectors. Analyses are based on published and unpublished data from three endemic areas: Guatemala, where the main vector is *S. ochraceum s.l*. -with a well developed cibarial armature-, West and Central Africa (*S. damnosum s.l*.), and South Venezuela (*S. guianense*) -the latter two vector species with unarmed cibaria-. The ultimate goal is to construct an analytical mathematical model to explore the relative contribution of the various biological processess regulating the population dynamics of human onchocerciasis. In particular, we want to test the assumption that the most important density-dependent mechanism in the transmission of this infection takes place within the simuliid host[1], and to explore its consequences for onchocerciasis control programmes.

The results indicate that in the three *Onchocerca-Simulium* combinations studied and range of parasite loads examined, the intake of dermal mff by the blackflies is essentially proportional to the intensity of the skin burden[2], and that mff ingestion by the vector may not be as strongly density-dependent as previously thought[3]. However, the relationship between the numbers of ingested mff and the numbers of parasites that successfully reach the haemocoele of the flies and develop to L3 larvae in the thoracic muscles is markedly non-linear, suggesting that density-dependence does operate at some point of this stage in the life-cycle. In the three parasite-vector combinations explored, the average number of successful parasites per fly increases initially with mff intake to level off at about 1-3 L3/fly at higher intakes. This phenomenon, known as 'limitation'[4] is confirmed for *S. damnosum s.l*. and found to apply equally to *S. guianense* (the two vectors without prominent cibarial teeth), whilst a pattern of initial 'facilitation'[5] is required to better describe the relationship between mff intake and L3 development in the range of low to moderate parasite loads in *S. ochraceum*. It is possible to explain this initial pattern in terms of density-

dependent damage to the ingested mff by the cibarial armature exhibited by this species[6]. A marked difference between armed and unarmed blackflies is again found when the evidence for parasite-induced vector mortality is investigated. Mean survival times and life expectancies, measured at the beginning of survival experiments, decrease significantly with mff load in the three vectors, the decrease being more pronounced in those species without the protection afforded by the cibarial teeth. Mortality rates are well described by non-linear functions of time post-engorgement, in which increasingly higher death rates are experienced during the first 24 hr PE by those groups of flies fed on more heavily infected subjects. However, the amount of density-dependent parasite regulation in the simuliid host which really takes place in the field, will depend on how frequent is the acquisition of high parasite intakes by vectors in endemic areas. Frequency distributions of skin mff per person are usually highly overdispersed, with most people harbouring low to moderate mff loads[7].

The functional relationships thus found and the parameter values estimated from above have been included in a deterministic mathematical model describing pre-control equilibrium mean worm burdens per human and per fly host. Preliminary results suggest that density-dependence in the vector only may not be sufficient to explain the trends of Community Microfilarial Load (CMFL) vs Annual Transmission Potential (ATP) observed in the field[8,9]. Any speculation on the existence of a transmission threshold, or breakdown point, for epidemiological settings where the main vector has a cibarial armature, must be based on formal stability analysis of dynamic models. These models ought to incorporate the features described for the relationhip between *Onchocerca* and *Simulium*, as well as reasonable assumptions about the processes affecting the parasite in the human host. In the case of highly overdispersed distributions, the parasite intensities at which unstable equilibria occur, may be too low to have meaningful epidemiological relevance[7].

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[BSG Bull. No. 4, December 1994]

Aggregated oviposition in the blackfly S*imulium damnosum* s.l. is mediated by a pheromone.

Philip J. McCall: *Veterinary Parasitology, Division of Parasite & Vector Biology, The Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.*

Although many blackfly species are known to aggregate during oviposition, the nature of the phenomenon has not been studied, since the experiments of Walsh

with *S. damnosum s.l.* in 1984 (Aspects of the biology and control of *S. damnosum s.l.* in West Africa, PhD thesis, Univ. Salford). Here, data are presented from a series of studies carried out using wild-caught *Simulium damnosum* s.l. in Sierra Leone, an important vector species which exhibits strong communal oviposition behaviour. Wild-caught flies were blood-fed on pigs and maintained for 3 days until gravid, when they were tested in a specifically designed behavioural bioassay. A series of two-choice bioassays showed that gravid flies preferred and responded more quickly to substrates already containing eggs over the relevant control, and that this response was related to the number of eggs present. The volatiles collected from freshly-laid eggs elicited similar behavioural responses but volatiles from eggs aged 12 hours post oviposition failed to elicit any response, in the same bioassay.

Gas chromatographic profiles of the egg volatiles showed 2 compounds which consistently emanated from fresh eggs, but which were significantly lowered after 12 hours. The same compounds were also found only in the ovaries of flies at 2 and 3 days post blood-feeding, and never in any other body region, at any other age, or in males. The possibility of either or both of these compounds being responsible for the behavioural responses is therefore very likely. A unique closed purified air system was designed to collect volatiles from actively laying blackflies in running water, and showed that only the same 2 compounds were occurring, indicating the probable absence of any compounds emanating from adult flies being involved in this aggregation behaviour.

Results showing the bioassay data, the gas chromatographic profiles of the extracts and volatile collections will be presented. The proposed advantages of an aggregated oviposition strategy for blackflies are considered. The possible use of such a pheromone is also discussed.

[BSG Bull. No. 4, December 1994]

A novel method for age_grading blackflies by egg_sac relic enumeration

Andrew I.Gryaznov: *Prophylactic toxicology and Disinfection Research Institute, Moscow and Liverpool School of Tropical Medicine*

The described method for physiological age determination in black flies is a modification of the age_grading method suggested by M.I.Sokolova for use in mosquitoes (Sokolova, 1983; Sokolova, 1994). The method is based on specific staining with neutral red of the morphological structures in ovarioles which indicate preceding gonotrophic cycles. It is used to distinguish the gonotrophic dilatations (follicular relics) and zones of granulation which result from egg_sac resorption. The presence or absence in ovarioles of these structures or their combinations allows identification of blackflies which have completed 1, 2 or 3 (or more) gonotrophic cycles. The first publication devoted to applying this method in blackflies was in Russian (Gryaznov, 1993).

Methods

Ovariolar structure was investigated in newly emerged females as well as in females captured from hosts. The formation of gonotrophic dilatations was studied

in facultatively autogenous females *Simulium (Wilhelmia) equinum* L. maintained in the laboratory. Morphology of egg_sac and zones of granulation were studied in parous females kept in the laboratory after oviposition and in the obligatory autogenous blood_sucking species *Simulium (Psilozia) vittatum* Zett. maintained in the laboratory after oviposition.

The dissecting technique is presented in scheme in Fig.1. Females were dissected in a drop of isotonic solution to extract the ovaries. The wall of the ovary was cut and the ovary was stretched with needles. In a drop of isotonic solution, neutral red was added at a concentration 1:7000. Then, the ovary was transferred to neutral red solution for staining for 30_40 s. After turning over the ovary calyx side up and additional stretching with the needles, a small drop of mineral oil was put on the ovary and the preparation was additionally stretched with needles. Then a cover slip with paraphinum legs was put on the preparation. Microscopic examination of the preparations was done under a magnification of x70 to x400.

Results

Ovarioles of blackflies (the same way as in mosquitoes) are located between the ovarian wall and calyx surface (Fig.2). In nulliparous females, a terminal follicle is connected the to calyx surface by a pedicel. The internal tip of the pedicel is located approximately in the centre of a tiny ring that is the place where the ovariole sheath attaches to the calyx surface. The resorbing egg_sac forms a zone of granulation that is presented in the form of a cluster of brightly stained granules of various sizes, bound to the ovariolar sheath at the place where it is attached to calyx. The follicular relic looks like a small ball surrounded by a transparent cover. On the stained preparation, red and yellow granules are inside a follicular relic. The follicular relic is always located inside the ovariolar sheath and connected with connecting stalks (or pedicel) both to the terminal follicle and to the calyx surface.

[Graphics File BULL4F1.gif here]

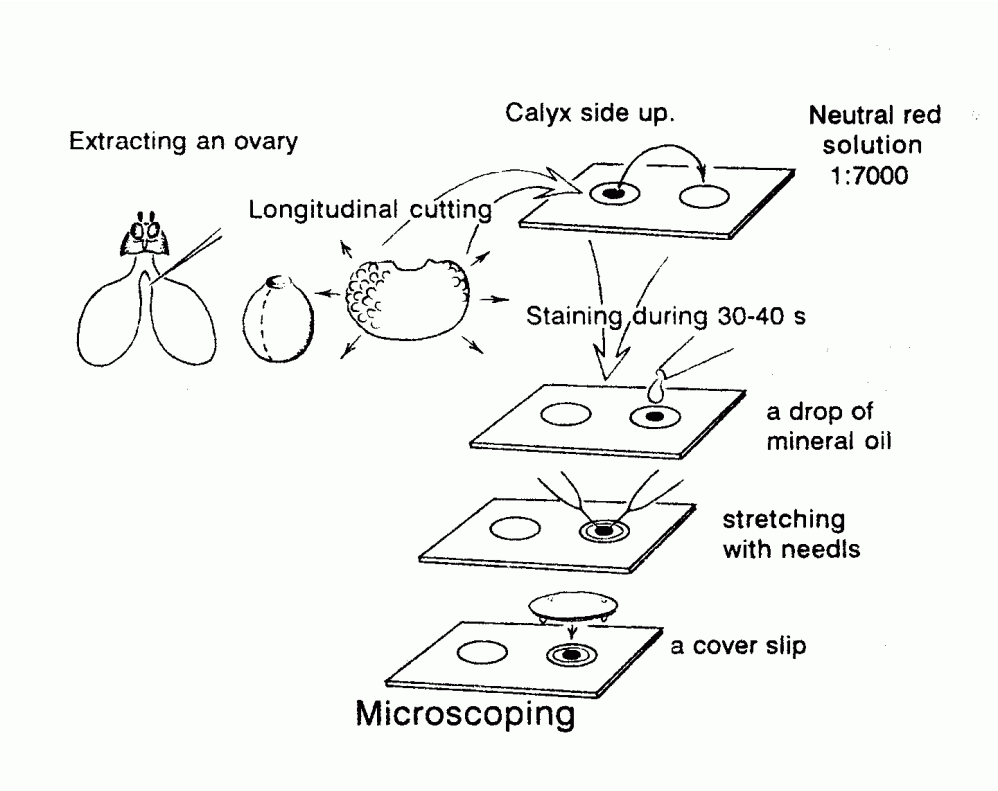


Fig. 1 Dissecting technique

Two different processes can occur in the ovariole during the gonotrophic cycle: either egg development with ovulation or degeneration of a terminal follicle. Egg_sacs are resorbed and form zones of granulation which accumulate in the ovariole with each gonotrophic cycle. After degeneration of the terminal follicle, a follicular relic (=gonotrophic dilatation) is formed. Number of zones of granulation and their combinations with follicular relics indicate the number of completed gonotrophic cycles. However, number of follicular relics in ovarioles does not correspond to parity of female.

It was shown that zones of granulation in the ovarioles of parous females are usually in the form of clusters of brightly stained granules of various sizes bound to the ovariolar sheath. They form a fragmented ring, while the calyx wall is stretched. It is possible to distinguish two zones of granulation in the each of the ovarioles in females after 2 (or more) ovipositions.

Females collected in nature were arranged in three categories: (a) nulliparous; (b) 1_parous; (c) multi_parous. In investigated multi_parous females, most of the ovarioles have no more than two zones of granulation, or a combination of one zone of granulation plus a follicular relic. The females of this category were suggested to be 2_parous. 568 host_seeking females were investigated at the Center of Eastern Europe. Among *Simulium (Odagmia) ornatum* Mg., *S. (Boophthora) erythrocephalum* De Geer and *S. (Schoenbaueria) nigrum* Mg. females 3.8%, 4.3% and 2.3% respectively were 2_parous (in the 3rd gonotrophic cycle). 6.5% of females in *S. verecundum_venustum* complex were found on the 3rd (or possibly older) gonotrophic cycle.

In parous females, some ovarioles drop germarium and terminal follicle and cannot be used for physiological age determination. The different variants of ovarioles were found in wild females of *Simulium verecundum_venustum* complex are shown in Fig.3.

[Graphics File BULL4F2.gif here]

Fig.2. Morphology of blackfly ovariole.

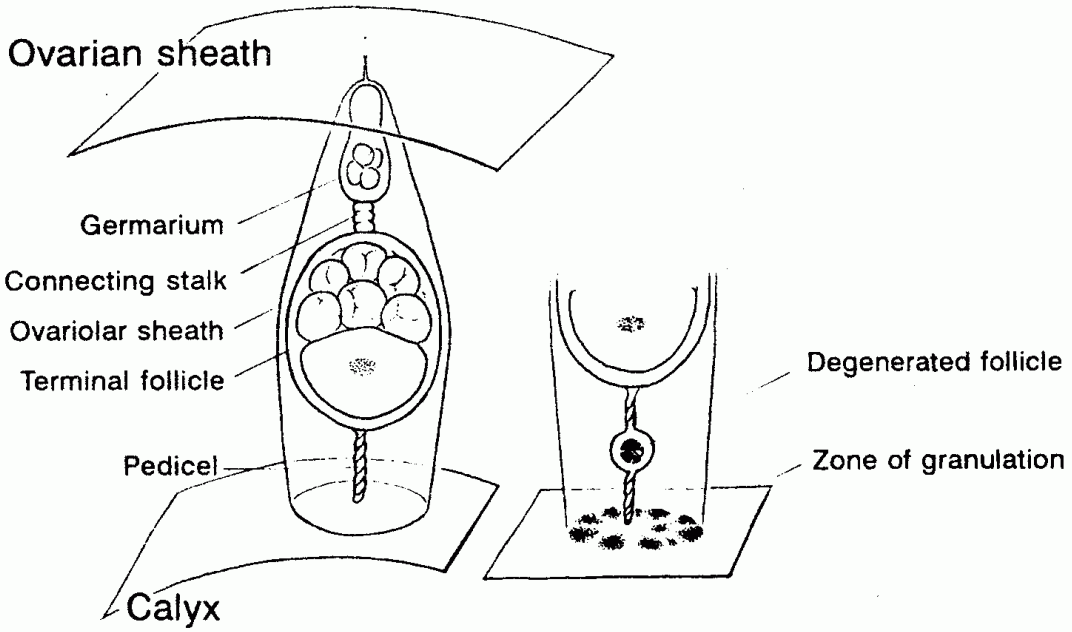
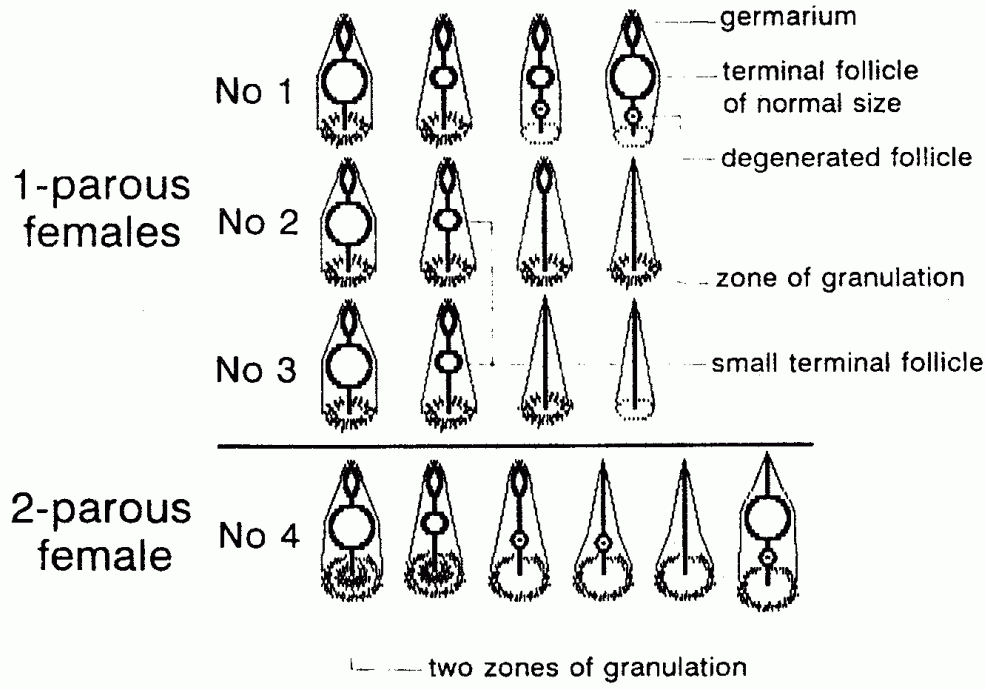


Fig.3. Variability of ovarioles in four females of *Simulium verecundum-venustum* complex attracted to the host.



The main principles of the age_grading method are as follows:

1. In black fly females, normal ovarioles respond to each gonotrophic cycle.

Therefore, either a granular zone or a degenerated follicle form in the ovariole with each gonotrophic cycle.

2. In an ovariole, the egg_sac is continuously reabsorbed and forms a granular zone.

3.A granular zone (as well as degenerated follicles) may be stained with neutral red and counted for parity determination in vital preparation.

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[BSG Bull. No. 4, December 1994]

Characterisation and purification of immune peptides and proteins produced by simuliids in response to infection.

Bianca Smithies and Peter Ham: *Centre for Applied Entomology and Parasitology, Keele University.*

Insects have been found to mount a strong humoral response to infection, this reaction includes the rapid release of antibacterial peptides and proteins into the haemocoel, where they act directly on potential pathogens (see review by Cociancich *et al.*, 1994). We are trying to identify the substances produced by blackflies and to elucidate their role in immunity (Ham, 1992).

We have isolated a lysozyme which is present in the haemolymph of *Simulium equinum*. This has the same molecular weight as hen egg white lysozyme and cross reacts with antiserum to silk moth lysozyme (Hultmark *et al*., 1980) Native polyacrylamide gels overlaid with bacteria have shown that, like other lysozymes, this molecule lyses the gram-positive indicator bacteria *Micrococcus luteus* (both when viable and when lyophilized) but does not lyse the gram-negative *Escherischia coli*. In addition to this role of protection against infection with gram-positive bacteria, this molecule is thought to be responsible for enhancing haemolymph antibacterial activity against *E. coli*.

In addition to *S. equinum* lysozyme, we have also identified two small, inducible peptides with anti-*M. luteus* activity. Native-PAGE indicates that one of these molecules has the same mobility as synthetic cecropin B (3.8kDa), the other has a lower gel mobility. HPLC purification followed by antibacterial assays have revealed three inducible peaks with anti-*M. luteus* activity. Two of these peaks contain molecules ranging from 8 to 14kDa (as determined by Mass spectrometry and SDS-PAGE). The peak with the highest activity contains a major peptide of 4.5kDa (determined by SDS-PAGE), this coincides with a 4.5kDa inducible peptide as determined by SDS-PAGE of whole haemolymph.

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[BSG Bull. No. 4, December 1994]

Effect of zooprophylaxis on *Onchocerca* transmission by *Simulium* spp. In Cameroon.

A. Renz, *University of Hohenheim, Stuttgart, Germany*

The microepidemiology of human onchocerciasis in the various bioclimatic zones of Cameroon depends on the biology of the local *Simulium damnosum* s.l. vector populations and on the species and strains of *Onchocerca* larvae they transmit.

In the grass highlands, the Sudan savanna, and in the rain forest areas of Cameroon, the prevailing species, namely *S. squamosum, S. damnosum s.s., S. sirbanum* and *S. mengense* exhibit different degrees of zoophagy (i.e. proportion of bloodmeals taken from non_human hosts): In cattle raizing areas like the Adamawa highland, the local *S. squamosum* population feeds predominantly on cattle and transmits mainly a bovine filarial species, *O. ochengi* (WAHL et al. 1994)

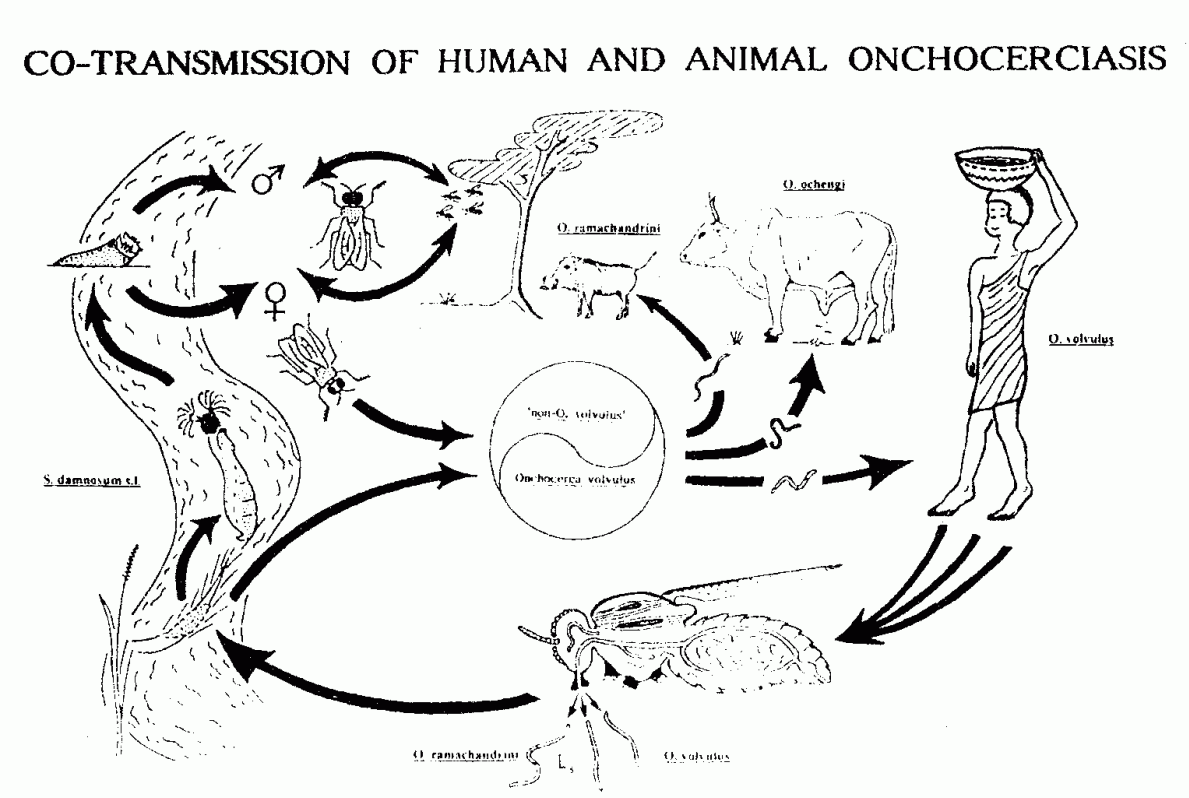
If compared to the standard human fly collector, cattle attract twice as many flies. Taking into account that cattle are five times more frequent than the human population in the Adamaoua highlands, this divertion of vector flies strongly reduces the vectorial capacity of the local fly population (zooprophylaxis, RENZ et al. 1987). Despite an extremely large *Simulium squamosum* vector population (ABR 120.ooo flies/man year at the river) the prevalence of human onchocerciasis is low (WAHL et al. 1994). In addition to the beneficial effects of this reduction of the vectorial capacity of the flies, the infective larvae of *O. ochengi,* which constitute over 90 % of all larvae in the estimate Annual Transmission Potential (25.ooo L3/man,year) may stimulate, when transmitted on man, an immune response which then reduces the chance of development of the forthcoming infective larvae of *O. volvulus* (crossreactive 'concomitant' immunity, RENZ et al. 1994, HOCH et al. 1993).

In the Sudan savanna, on average half of all infective larvae found in man_biting flies belong to *O. volvulus* (DUKE, 1967, RENZ, 1987), one third to *O. ochengi* and the rest to *O. ramachandrini*, a newly described species from the warthog (BAIN et al. 1993, WAHL et al. 1994). *S. bovis* occasionally comes to land on man in large numbers, but only rarely takes a bloodmeal. The frequent infections found in this vector stem from cattle (*O. dukei*, WAHL & RENZ, 1991) or from the warthog (*O. ramachandrini* ?). A third filarial

transmission cycle in *S. griseicolle* is yet to be described.

In the rain_forest, only very few autochthonous cattle are kept nowadays. Thus, local *Simulium squamosum* and *S. mengense* populations presumably take the large majority of bloodmeals on the human population. They are nevertheless capable of transmitting *O. ochengi*. The on_going deforestation and the recent immigration of savanna *S. damnosum* s.s. into this forest area will certainly influence the future of forest onchocerciasis. Ornithofilariae are occasionaly observed (mainly in *S. kenyae*) but seem not to be of epidemiological importance.

[Graphics File BULL4F3.gif here]



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POSTER PRESENTATION AT THE 17th MEETING

[BSG Bull. No. 4, December 1994]

Black fly control through vegetation management in the Thyolo Highlands of Southern Malawi

M. J. Roberts: *Liverpool School of Tropical Medicine*

Onchocerciasis affects some 150,000 of 750,000 people living in the 2,500 square km of the economically important tea_growing area of the Thyolo Highlands (Roberts, 1990).

The Vector Biology and Control section of the Malawi Onchocerciasis Control Project instituted a control programme in July 1992 aimed at reducing biting caused by *Simulium damnosum* cytospecies in the Highlands and this is run in conjunction with mass distribution of ivermectin.

The main component of the control programme is a scheme for management of stream vegetation associated with black fly larvae and pupae, to be supplemented by limited use of insecticides.

The vegetation management programme presently consists of a repeated schedule of manual removal of the aquatic plant *Hydrostachys polymorpha* from 126 black fly breeding sites along a 14 km stretch of the Nswadzi River. Removal of the plant is carried out by five teams of five men, each team being assigned a 2 to 3 km stretch of the river. A cycle of weed removal is carried out at 4 week intervals and each cycle normally lasts 4 to 6 days.

In the first two years, July 1992 to June 1994, vegetation management has been used

exclusively, and has been accompanied by a significant reduction both in the size of immature black fly populations and in the intensity of black fly biting . Over the twelve month period, July 1993 to June 1994, black fly biting has stabilised at about 8% of pre_control levels and immature black fly populations at 5 to 6% of pre_control levels. Daily Biting Rates are down from a mean of 51 over the Highlands for the six_year period July 1986 to June 1992, to a mean of 4 for the twelve_month period July 1993 to June 1994.

In the 1994_95 season, the Project plans to introduce supplementary larviciding with BtH14 or temephos to control black fly populations at sites that are difficult of access including waterfalls and stretches of deeper water.

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NOTES AND CORRESPONDENCE

[BSG Bull. No. 4, December 1994]

An Electronic News List For Simuliidologists

Trefor Williams: Department of Environmental and Evolutionary Biology, Liverpool University, PO Box 147, Liverpool L69 8BX, UK

Although it seems that most members don't have access to e-mail, my recent suggestion of an electronic news list for the BSG met with enough support to make this feasible. Let me say straightaway (and before you skip to the next article) that electronic news won't replace the Bulletin and, if it gives the BSG a higher profile and increased membership, should be to everyone's benefit. Items of general or long-term interest sent as e-mail will be published later in the Bulletin and, who knows, this might even lead to the Bulletin's appearing more often.

The list, with the listname **simuliidae**, is established on Mailbase, 'the UK's major electronic mailing list service' at Newcastle University. In fact, Mailbase does more than this suggests and provides archiving, file retrieval and a number of other services. It is funded by the Joint Information Systems Committee (JISC) of the Higher Education Funding Councils for England, Scotland and Wales, and is effectively free to the academic community. Although it stipulates that its lists are UK-based, Mailbase welcomes users from throughout the world.

You can join the list without further ado by sending this command as the text of an e-mail message to **mailbase@mailbase.ac.uk**

join simuliidae *your-firstname(s) lastname*
stop

Your usual e-mail address should be given in the 'From:' field of your message. Mailbase won't recognise you if you subsequently send mail from another address. 'Stop' must be entered as a separate line, but can be omitted if your e-mail has no

automatic signature.

Information on how to use Mailbase is sent on joining the list. However, if you'd prefer to know more about Mailbase before taking the plunge I suggest you obtain the fully detailed User Guide by e_mailing to **mailbase@mailbase.ac.uk**

send mailbase user-guide
stop

Mailbase's documentation can also be viewed by gopher (server **mailbase.ac.uk**), the World Wide Web (URL **http://mailbase.ac.uk/**) and anonymous FTP (details in the User Guide).

To contribute to the list you must send your message to the listname, i.e.

To: **simuliidae@mailbase.ac.uk**

Please include a descriptive 'Subject:' field when mailing to the list - this will assist anyone browsing through the list's archives. Messages are distributed to all list users, though it's possible to suspend mail for a time. Replies are directed to the sender of a message by default, but this can be overridden if so wished.

I hope this will turn out to be a worthwhile venture - please join the list as and when you can and send in your news, requests, or indeed anything that might be of interest to other members of the BSG!

[BSG Bull. No. 4, December 1994]

Spot the Deliberate Mistake

Roger Wotton: *Dept. of Biology, University College London.*

In a court case in the U.S.A a husband was put on trial for the murder of his wife, her body having been found in a car dumped in a river. As the water was cold it was not possible to date the time of death accurately and the husband claimed that his wife had disappeared in June. It was noted that there were blackfly cocoons on the front bumper of the car and Richard Merritt of Michigan State University was called in to identify them. His findings were used in the prosecution case as the pupal cocoons and exuviae of overwintering blackflies showed that the car must have entered the river in April or May. This evidence was partly responsible for a murder conviction being brought by the jury.

The BBC used this story as part of their programme "The Witness Was A Fly" broadcast earlier this year. The filming of the blackfly sequences was by Sinclair Stammers and he used a periscope for tracking shots of larvae and pupae along a car bumper placed in a laboratory aquarium tank. But how to get film of pupation? A prepupa was located spinning initial strands of silk for its cocoon and the camera focused on it until the cocoon had been completed and ecdysis had occurred. It sounds easy but there were anxious moments of waiting to see if we had the results required by the producer. After several failures we managed to get enough film of pupation for the short sequence in the programme.

Which brings me to the subject of the deliberate mistake. Sharp-eyed readers of the

Bulletin who watched the programme will have noticed that the blackflies used in the filming were not from North America but were of *Simulium noelleri* (collected in Kent).

[BSG Bull. No. 4, December 1994]

Poem

Found during renovations and read to the meeting by Professor Brian Moss.

Advice from the Guardian Angel of University Heads of Department based on observations of the Lower Creatures

The blackfly is a nasty beast,
which bites you where you 'spect it least.
In shorts, by bubbling streams and rills,
Upon your tender bum it mills.

A pity that it's reprehnsible,
when its young is quite so sensible,
providing morals plain to see
for any watching H o D.

The larva's head fans catch each mote,
like hints of gossip you should note.
And ultimately should enquire
if they're just smoke or really fire.
It hangs quite tightly on with wads
of silk in which its hooks it prods.
Thus surviving currents fleeting
not unlike each term's staff meeting.

And when it has to, loops its girth
to more congenial bits of Earth,
where its head fans won't be hit
by flying lumps of words unfit.

However, there's a bad prognosis
if you achieve metamorphosis,
and then at times of grating stress
you get yourself into a mess.

No matter what your senior state,
the canvas straitjacket's your fate,
if, feeling in the darkest dumps,
you start to bite your colleagues' rumps!

Anon.

Specimens wanted

I am a black fly systematist currently engaged in doctoral research. My project is a

genus_level phylogenetic reconstruction of the Simuliidae using rDNA sequence data. I also have morphologically_based revisions underway involving *Ectemnia* (with Peter Adler) and *Simulium (Psilopelmia)* sensu Coscaron. Soon to be submitted is a paper encompassing my Master's research, a revision of the *S. jenningsi* group, which was done in Peter Adler's lab.

My current DNA research is aimed towards identifying the major clades within the Simuliini sensu Currie 1988. If my phylogenetic analysis gives a well_supported tree, I will perhaps propose a classification scheme. Resolution of the groupings at the tips of the tree is critical for the latter. I am also interested in superimposing morphological characters onto the phylogeny in order to answer questions about character evolution in the family.

I am lacking suitable material of a few critical taxa, namely Paracnephia, Procnephia, and the Australian "Cnephia". Inclusion of these taxa is especially important since they have been cited as some of the most primitive taxa within the Simuliini sensu Currie. Any assistance in the
acquisition of 95_100% ethanol_preserved material of these taxa for DNA analysis would be very appreciated.

Kevin Moulton
Department of Entomology
Building #36
University of Arizona
Tucson, AZ 85721 e_mail MOULTONK@CCIT.ARIZONA.EDU

Request for comments on apparent residual activity of *Bacillus thuringensis*

I have spent 3 years examining the rate of blackfly larval development following single_site field applications of larvicides. The data were used to determine the timing of successive larvicide applications for large_scale control purposes. Although there was considerable variability in development rate, water temperature was the strongest determinant. I then went ahead and booked a helicopter well in advance to ensure successful control. Imagine my surprise to find larval development much slower after large_scale field applications. It seems that there is some sort of residual toxicity following Bti applications. In addition, it seems that the downstream carry of larvicides is enhanced by multiple_site applications. The preliminary single_site trials seem to underestimate things a bit, and I was wondering if anyone else has experienced similar responses, or has ideas of how to correct for multiple_site applications.

Rob Palmer tel: +27_54_25605
Onderstepoort Vet. Inst. fax: +27_54_25609
P. Bag X5912 email: palmerr@aqua.ccwr.ac.za
Upington, 8800
South Africa

[These last two items have been filtered out of the Internet - Ed]

[BSG Bull. No. 4, December 1994]

A Turn-up for the Book: Blackflies in the Galapagos

Roger W. Crosskey and Anthony J. Shelley: The Natural History Museum, Cromwell Rd., London, SW7 5BD, U.K.

Received truth is that there is no running water in the Galapagos Islands. The encyclopedias, the geographies, the expedition reports have said so at least since Darwin's time: thirst-maddened sailors quaffed the urine of the giant tortoises in these 'frying pan hot' islands. Given this, and the fact that simuliids had never been found in such an exhaustively researched archipelago, one of us ventured the comment in his book (Crosskey, 1990)

that "these islands are streamless and have no blackflies". Against the odds, however, one species *Simulium bipunctatum*, has recently been found breeding on San Cristobal, the easternmost island - and not only that, biting humans too (Abedraabo et al., 1993).

Darwin and Fitzroy gave San Cristobal (=Chatham Island) a pretty poor press. The interior was like the 'iron furnaces near Wolverhampton', a wasteland of volcanic chimneys and bare and naked lava flows, rough and uncolonized. There were no swarming insects so the birds were seed-eaters. Still, it turns out that blackflies, in their resourceful way, have a toehold even in this inhospitable place where the lava (as on the other islands) is mainly too porous for flowing water. No streams reach the sea, but precipitation at higher altitudes on the southeasterly part of the island produces running water sufficient (at least in the last few years) to permit the development of *S. bipunctatum* and the fly is now well established in the uplands above 300 m. Here it troubles the inhabitants on the banana farms, who associate the appearance of the flies with banana cultivation. Angus McCrae (1968) noticed in Uganda that *S. damnosum* s.l. females swarmed in places where banana beer was brewed - so we like to think that something similar could be going on with *bipunctatum* in San Cristobal. Attraction to humans is 'out of character' given that this species is markedly zoophilic in the coastal parts of mainland South America.

References

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[BSG Bull. No. 4, December 1994]

Tepuis, Sundews and Simuliids

J.B. Davies: *Liverpool School of Tropical Medicine.*

Tepuis are rocky plateaux which rise a sheer 1000 feet or more out of the Amazonian rain forest of southern Venezuela. They are thought to have provided the inspiration for Conan Doyle's "The Lost World".

On a recent collecting trip to Guatemala I was trapped in my hotel room by a thunder storm and whiled away the time by skipping through the 54 channels on the cable television. One of them "The Discovery Channel" happened to feature an interesting account of a helicopter expedition to a tepui named Kukanam which is close to mount Roraima which itself forms the point at which the borders of Venezuela, Guyana and Brazil meet.

Apparently there is very little soil on the tepuis as any humus that forms is washed off by the frequent heavy rain. this has led to an abundance and great variety of insectivorous plants. One shot showed species of *Drocera*, a sundew-like plant capturing a small flying

insect. There was no doubt that this was a species of *Simulium*, and a female at that. Furthermore, from what I could see from the few seconds the shot was on the screen, it looked uncommonly like *S. guianense*, but I could be wrong since in this area the females of many species are similar in appearance.

S. guianense is one of the suspected vectors of onchocerciasis in S. Venezuela, and it is usually found breeding on the lip and face of waterfalls and the rapids below them. Could it be breeding in the dramatic waterfalls which fall off the edge of tepuis, and does it actually breed in the rocky rivers which flow across these plateaux?

Source: *"Terra-X - Tepuis - Kukanam"* produced by John Borst for the Discovery Channel.

BRITISH SIMULIID GROUP BULLETIN

Number 5 June 1995

CONTENTS

From the Editor

At the last meeting of the British Simuliid Group, it was suggested that it might be a good idea to try to issue the Bulletin twice a year, in order to maintain interest. As a result, this current number is the first which is not dependent on abstracts of presentations for its content.

I would like to thank those who have provided material for this number, and repeat my earlier request for any snippets of news that readers may come across during their activities in the field or in the library.

This publication is issued in numerous simultaneously obtainable copies for permanent scientific record.
It is with great sadness that this Bulletin has to record the death of Colin Fairhurst. He was always a very supportive member of the British Simuliid Group from its inception in 1979 and took an active part in organising the meetings of the Group that took place in Salford.

I would like to remind readers that details of an e-mail list service for simuliidologists can be obtained from Trefor Williams sp36@liv.ac.uk

John Davies: *Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.*

Phone: 0151 708 9393 FAX: 0151 708 8733
e-mail: daviesjb@liv.ac.uk

[BSG Bull. No. 5, June 1995]

AN APPRECIATION OF COLIN FAIRHURST

Stan Frost, *Environmental Resources Unit, University of Salford.*

Those who knew Colin have their own memories and reminiscences. He had a capacity to relate at different levels and adapted quickly to different circumstances. He was always ready with a laugh and a joke.

We first met when he was teaching in Stockport. He was a guest lecturer sharing ecological insights with our MSc in Environmental Resources. It was no surprise when he moved over to Salford to take an active part in coordinating the programme. The academic challanges of the mid- to late '70s, coupled with financial disruptions of the early '80s, gave Colin opportunities to consolidate his position in the new Biological Sciences Department and to develop his research interests in Dutch Elm Disease, soil ecology and aquatic monitoring.

There always seemed to be a group of research assistants in his office and Colin proved to be loyal and supportive both to his students and to staff colleagues. His teaching reflected his personality. His interests ranged from old fast cars to millipedes. He was able to couple the data gathered by the pedestrian biologist with his statistical vision. His predictive analysis had significant potential for the impact assessment of the Onchocerciasis Control Programme.

Changes during the last ten years of Colin's life affected his family, his work and his colleagues. We were watching an indefinable shift which none seemed able to prevent. His death on Boxing Day 1994 marked the passing of a much loved colleague and friend who had so much further to travel.

MEETINGS

The 18th Annual British Simuliid Group Meeting: 1995

The meeting this year is being held at the University of Birmingham on Wednesday, 13th September.

Kindly treat this preliminary notice as a 'call for papers' and poster contributions. If the more 'established' workers in University departments and in Institutes have students just entering Simuliid work, then please encourage them to attend and to offer a poster or contribution.

Overnight accommodation is available at Lucus House (Conference Centre): Bed and Breakfast @ £35 to £40 per night. For those arriving on the Tuesday evening it has been suggested that our usual informal dinner be held in a local, highly recommended Balti House. The Birmingham 'Balti' is something of a culinary delight!

Please get in touch with us as soon as possible, letting us know your requirements, together with a submission (title/abstract) of any presentation.

Malcolm Greenwood **Melanie Bickerton**

Department of Geography University of Birmingham
Loughborough University of Technology
Loughborough Tel: 021 4145538
Leicestershire LE11 3TU Fax: 021 4145528

Tel: 01509 223015
FAX: 01509 262192

E_Mail: M.T.Greenwood@lut.ac.uk

[BSG Bull. No. 5, June 1995]

The 1995 Annual Meeting of NE118.

Douglas A. Craig, *Department of Biological Sciences, University of Alberta, Alberta, CANADA T6G 2E9*

The annual meeting of the North Eastern Regional Project (NE118) was held between 23_25th, February, at the Rancho de la Osa, Sasabe, Southern Arizona. These meetings which have been convened since 1977, have essentially become the annual meeting for North American workers on Black Flies.

Curious about why an oddly named "project" should attract an international group of some 30 entomologists, or why it should be meeting at a place half a mile north of the Mexican border and distinctly isolated to say the least? Well, the United States Department of Agriculture, Agricultural Reseach Service, via the Deans of the land grant universities, establishes "Technical Advisory Boards", or working groups, to help deal with agricultural problems.

Nine Northeastern States joined together in 1977 to form what was termed

"NE118", with the five year mandate to work on "Black Fly Damage Threshold, Biology and Control". The mandate has changed over the years and is not likely to be renewed at the end of the present term, which is next year. Black Flies are not viewed with as much concern as they were in 1977 _ in large part because of the work of this group. A satisfactory reason to be disbanded _ maybe?

Right from the beginning, at the Dixville Notch, New Hampshire meeting, there has always been an interesting mix of people who travel to the NE118 meetings. Importantly there is a good mix of graduate students, university professors, government employees, company representatives, and even occasionally self_employed people. Canadians have always been present, often in considerable numbers, and have held "office" in NE118. Over the years, it has become expected that most attendees will present something of their work, or comment on that of others. One can never tell when a useful piece of information will turn up from an unexpected scource. This had made these meetings extremely useful for keeping up with the current state of Black Fly work in North America, and occasionally elsewhere _ this year Dr. Roger Crosskey, London, England attended. Talks ranged all the way from a US$3,000.00 control programme in Pennsylvania, to a video presentation of pharate pupae cocoon_spinning behaviour, to the most arcane molecular biology. Everyone stayed awake and listened, even after a fine lunch! Yes, food was one of the reasons NE118 was held at the Rancho de la Osa.

Of great value at NE118 is that there is always time for talking in detail after the more formal presentations. So, over the years there have been a number of firm friendships formed and very productive research collaborations.

This time after the formal sessions and at the "Business Meeting", held in the 200 year old cantina (= bar) on the ranch, it was clear that NE118 was coming to the end of its final mandate. This leaves one more year to go and there was considerable discussion about what should be done in 1997. One suggestion was to see if a more international black fly meeting could be held somewhere. Otherwise, it was felt that the group could probably keep going on an informal basis. Time will tell.

Part of wanting to keep the group going is that in the last few years the meetings have been held in warmer venues _ perhaps one of the reason why Canadians attend. Last year in central Florida at the Archbold Biological Station, there wasn't a black fly larva to be found within hundreds of miles. So even inveterate collectors had to concentrate on the formal sessions. Not so at the Rancho de la Osa, for larvae were collected some 22 miles away. Quite interesting, since Sasabe is in the middle of a desert and most of the waterways in the vicinity are highly eroded and subject to flash floods.

So, in 1996, give some thought to attending what will probably be the last meeting of NE118. Where will it be held? Well, that is not settled, but Texas is top of the list, then the Florida Everglades, then Archbold Station again. For further information about this meeting and to get yourself on the mailing list, contact the new Secretary, Dr. Jim Sutcliffe, Department of Biology, Trent University, Peterborough, Ontario, Canada. K9J 7B8. PHONE (403) 748_1424. FAX (403) 748_1205. E_mail_ "jsutcliffe@trentu.ca".

NOTES, NEWS, VIEWS AND CORRESPONDENCE

[BSG Bull. No. 5, June 1995]

Birchflies and Bothys

James Coupland *CSIRO Biological Control Unit, Campus international de Baillarguet, Montferrier sur Lez, 34982, France*.

Canadian colleagues who study blackflies occasionally ask me (a Canadian) why I worked on these beasts in Scotland when obviously Canada has an overabundance of them. Which is exactly the reason with which I usually answer them. While the blackflies in Scotland may not be quite as ferocious as their colonial brethren, Scotland offers in its own way unique experiences for the intrepid simuliidologist. Of which I was following in the footsteps of C.B. Williams, A.E.R. Downe (my Professor at Queens in Canada),and L. Davies (though perhaps he has a different view!).

My abode during my studies in the Highlands was a small bothy on the property of Brigadier Curtis just below the "big house". The Brigadier was a slightly eccentric fellow owning several miles of the Spey and Feshie rivers which he was forever patrolling in his war against canoeists and poachers. He greatly despised canoeists and since poachers were only hypothetical, directed most of his energies against the former. He could often be seen in animated discussion with paddlers as they made their way down the river. Since I worked on the river daily, I was immediately deputised as "bailiff" and basic foot soldier. Sundays meant I was woken at 4:30 a.m. to patrol against possible Glaswegian poachers and early morning canoeists I was given a two_way walkie-talkie and code named "blackfly" (very clever). The only saving grace in all this was that the Brigadier also dragged in other estate owners and gave them ludicrous code names (cock_robin, pinecone etc..).

My sampling techniques for biting blackflies often raised a few eyebrows both among the locals and tourists. I would often borrow children for a morning to catch attacking blackflies. My presence in various woods brandishing a large white sweepnet, while circling the children gave rise to the rumour that ancient pictish sacrificial rites were being carried out in the region. The kids didn't mind as they always got their bag (sack) of candy. One tourist couple from Italy I'm sure returned home with an odd story. On this day I was taking samples off an adult in as many habitats as possible. At our first stop, this Italian couple caught me on my knees trying to poot several landing flies off the lower back of my subject. An embarrassed silence followed, and the couple beat a hasty retreat. At our second site (4 kms away!) the same couple again came across us in an even more embarrassing situation (off the lower leg) and again beat a hasty retreat. To top it off 5 hours later at a site high in the Cairngorms this same couple strides over the hill and there we are again! An expletive in Italian was heard and that was the last we saw of them.

While the Scottish weather was not very accommodating, I was afraid that the locals were going to be similarly inclined especially as my first visit to the "local" pub was similar to the famous scene in "An American Werewolf in London". However, after assuring them that I had nothing to do with either the RSPB or NCC I was grudgingly accepted and indeed made some lasting friends. In my first field season (the wettest summer on record) I wondered often why I ever chose to come to Scotland (as I sat shivering in the drizzle in mid_August). I collected few flies that season and was pretty depressed. The most interesting affair during that year was

my attendance at the BSG meeting in Wareham, Dorset where the reverse on my ancient Volvo failed. I had to be pushed out of the parking lot and pointed towards Scotland (I made it too!). As luck would have it the next summer was warm and sunny and I was able to collect some flies and even had visitors including Mike Service who gave me the benefit of their wisdom.

I look back fondly on my time in Scotland (from the warmth of southern France!). There are still a lot of interesting questions to be answered there and perhaps there will be yet another brave simuliidologist to follow in my tracks. If so, beware of the highland cattle!

[My Collins English Dictionary gives: "Bothy. Scot. a small roughly built shelter or outhouse esp. a hut on a mountain slope" - Ed.]

SCIENTIFIC COMMUNICATIONS

[BSG Bull. No. 5, June 1995]

On the European blackfly *Simulium lundstromi* and inclusion of *S. latigonium* as a new synonym within this species

Jon A.B. Bass: *Institute of Freshwater Ecology, Monks Wood, Abbots Ripton, Huntingdon PE17 2LS, UK*
Roger W. Crosskey: *Department of Entomology, Natural History Museum, Cromwell Road, London SW7 5BD, UK*
Doreen Werner: *Museum für Naturkunde, Humboldt Universität, Invalidenstrasse 43, 10115 Berlin, Germany*

A problem that has had to be decided before finalizing new keys to the larvae and pupae of British blackflies (JABB, in preparation) has been whether or not *Simulium latigonium* (Rubtsov) and *S. lundstromi* (Enderlein) are one and the same species. Lewis Davies thought not, and in his coverage for Britain (Davies, 1966, 1968) treated them as separate valid species (the latter under the misapplied name *angustitarse* Lundström): he recorded '*angustitarse*' (= *lundstromi*) as widespread in Britain but *latigonium* from only one site (Sunbiggin Tarn outlet in Cumbria). Our investigations have led us to the opposite conclusion, viz. that only one species is involved and that *latigonium* should be treated as a (new) synonym of *lundstromi*. The story is complicated, so we think it proper for establishing the synonymy to detail the facts underlying the decision and to give a short general account of *Simulium lundstromi* now that it subsumes *S. latigonium*. (We had hoped to dispose of the matter much sooner but were stalled until this year by being unable to obtain the loan of the holotype and topotypic material of *latigonium* from Russia. This has now been resolved through the courtesy of Dr A.V. Yankovsky.)

Apart from the scientific aspect of how to assess variability, the *lundstromi/latigonium* issue has the elements of classical taxonomic muddle - irregular but available proposal of a specific name, misidentification, problems with types and a large but relevant faunotaxonomic literature of varying quality (163 literature items bearing on *lundstromi* have been found and considered). We need not enter into detail, but as background to the faulty use of names by Davies the following explanation is pertinent. Edwards (1920), in the first account of the immatures of British blackflies, described a species with 4-filament pupal gill and 'horned' cocoon under the name *angustitarse*; Enderlein (1921), however, correctly considered that this was a misidentification and (there being no other applicable name) proposed *lundstromi* as a name for *angustitarse* sensu Edwards. Enderlein's citation was the soul of brevity - "N. Lundströmi Enderl. 1921 [angustitarsi Edw. 1920, nec Lundstr.] England" - but his name (now correctly rendered *lundstromi* under the rules of nomenclature) is available because of the back-reference to Edwards' description as *angustitarse*. Unfortunately, Puri (1925), Smart

(1944) and Davies (1966, 1968), in their influential key works, omitted any mention of *lundstromi* and continued with the old Edwards misidentification of *angustitarse*.

Simulium lundstromi/latigonium, though widely distributed in lowland England, is not common at any site and this makes it impossible to obtain material 'to order'. However, over the last twenty or so years two of us (JABB and RWC) have collected specimens from various sites, and this material, combined with new material from Germany (collected by DW), the loan to us of Russian material, re-examination of Davies' collection (now part of the NHM collection in London), and copies of old but very relevant correspondence between our colleagues Heide Zwick and Lewis Davies, has enabled us to assess the likely significance of morphological variation within *lundstromi/latigonium*. The upshot is that we attribute no interspecific significance to the ostensible specific differences indicated by Davies (see later) and have found no other characters to suggest the existence of two distinct species; such variation as exists we consider intraspecific. This view is shared by Heide Zwick, who (in litt. to RWC, 29.9.93) has written "I fully agree and support your establishment of synonymy between *lundstromi* and *latigonium*".

[BSG Bull. No. 5, June 1995]

Type localities and type specimens

The type locality of *lundstromi* is the Lark river, Mildenhall, Suffolk, England, in accordance with the designation by Zwick (1974) of an original specimen of *angustitarse* sensu Edwards from the specimens that under the rules of nomenclature constitute the *lundstromi* type material, i.e. all English specimens recorded as *angustitarse* by Edwards (1920). The lectotype is an adult male in alcohol (genitalia missing, ? reason) with its pupal skin. It was collected by Edwards on 25.iv.1916. Several paralectotypes, some from the same sample as the lectotype, are in NHM, London (one also in Berlin Museum). These have been listed by Zwick (1974). Three of them, non-reared females from Bovisand (Devon), Corfe Castle (Dorset) and Stokenchurch (Oxford), are technically paralectotypes of *lundstromi* but actually (as shown by the subcostal hairing character mentioned later) specimens of the true *angustitarse*.

Whether *lundstromi* still exists at its type locality is questionable. Attempts to find it in the Lark river (by RWC on 17.iv.93 and 10.v.94) were unsuccessful, though *S. equinum*, *S. erythrocephalum* and *S. ornatum* were present at and near Mildenhall. The river, though, has been much changed in regard to habitat by flood control measures since 1916.

The type locality of *latigonium* is the Sitenka river in Luga District, St Petersburg (ex Leningrad) Region. The holotype was collected here by Rubtsov on 22.viii.l955. It consists of larval parts (head and mouthparts, anal sclerite and circlet, pharate pupal gills) on one slide; this bears the red 'Holotypus' label of the St Petersburg Academy of Sciences and the name 'Eusimulium latigonium', the number '8812' and the collecting data in Rubtsov's hand. We have also seen a similar larval paratype slide with the same data (except number '8760') and seven pinned adult flies (3 _, 4 _). The latter were collected by Rubtsov at the Sitenka river type locality on 8.vii.1960 and each has its dry pupal skin. These topotypic adults have no type status but they have enabled us to form a correct understanding of *latigonium*. Examination of these specimens has confirmed - as we long suspected - that Rubtsov's (1956, 1962) figures of the pupal gill and the ventral plate of the male genitalia are somewhat misleading. Unfortunately, Rubtsov's original slide of *latigonium* male genitalia is lost or apparently so (Yankovsky in litt. to RWC, 23.2.1993).

***Simulium (Nevermannia) lundstromi* (Enderlein)**

lundstromi Enderlein, 1921: 200 (*Nevermannia*) [no description, name available by back-reference to description by Edwards (1920) as *angustitarsis* Lundström]. LECTOTYPE _, with pupal skin (designation Zwick, 1974: 94), ENGLAND: in Natural History Museum, London [examined].
kerteszi Enderlein, 1922: 68 (*Nevermannia*). LECTOTYPE _ (designation Zwick, 1974: 96), HUNGARY: in Museum für Naturkunde, Berlin [examined and synonymized with *lundstromi* by

Zwick (1974)].
latigonium Rubtsov, 1956: 830 (*Eusimulium*). HOLOTYPE larva (parts only), RUSSIA: in Academy of Sciences, St Petersburg [examined]. **Syn. n.**
[*angustitarse*: authors, not Lundström (misidentification): Edwards (1920), Puri (1925), Smart (1944), Grenier (1953), Carlsson (1962), Davies (1966, 1968) and in many minor pre-1970s works.]

Recognition

Member of *Simulium (Nevermannia) ruficorne* species-group for which principal characters are: __ radius fully haired, fore tarsi slender, postnotum bare: _ genitalia with strongly keeled lamellate ventral plate, one main parameral spine, long truncate styles and rod-like median sclerite: _, claws toothed, hind tibia with sub-basal dark band, abdomen fully haired: *pupa*, 4-filament pupal gill and non-collared cocoon: *larva*, head spots positive, postgenal cleft extending forwards not more than half way to hypostomial base, abdomen with ventral papillae. *Simulium lundstromi* is distinguished from other western Palaearctic species of the group by (1) cocoon with well developed anteromedian projection ('horn') and closely woven, (2) pupal gill with uppermost filament strongly arched basally (angle 75°-95° with base of filament 2: Fig. 3), (3) larval postgenal cleft well developed, extending about one-fifth of distance to base of hypostomium, usually more or less square (Figs 1 and 2), (4) head venter nearly always with saddle-like or slightly H-like pigmented mark anterior to and around postgenal cleft (Figs. 1 and 2).

Distribution

Palaearctic species widely but locally distributed from western Europe (including British Isles) to eastern Siberia and northern China. Absent in Mediterranean and Atlantic islands. Reported* from: Algeria[c], Austria [c], Belgium[d], Britain[a,c,d], Bulgaria[d], China[c], Czech Republic[c], Denmark[a,c,d], France[a,c], Germany[a,b,c,d], Hungary[b], Ireland[c,], Italy[a,c,d?], Morocco[c], Norway[a], Poland[a], Portugal[a], Romania[a,c], Russia[a,b,c,d], Serbia[a,d], Slovakia[c], Spain[c,d], Sweden[a], Switzerland[c], Turkey[c], Ukraine[a,b,c]. [North African reports dubious, perhaps involving allied species.]

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* Superscripts show *lundstromi* has been reported under the name(s): a, *angustitarse* (in misidentified sense); b, *kerteszi*; c, *latigonium*; d, *lundstromi*, [Contact RWC if country references required.]

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In Europe *S. lundstromi* is not uncommon in the plains of Denmark and northern Germany. From Germany we have seen numerous adults with pupal skins (in Berlin Museum) obtained in Havelland by Enderlein and identified by him as *lundstromi*, and in the last three years one of us (DW) has collected material from many sites throughout Brandenburg; Seitz (1992) has found the species widespread in Bavaria. In Britain *S. lundstromi* occurs in lowland areas from Dorset and Wiltshire northwards at least to Yorkshire. It has been collected in all counties of South East England (47 known sites in RWC survey records) and East Anglia; Post (1981) records *lundstromi* from several sites in Norfolk. One of us (JABB) recently confirmed that it is still present at the Sunbiggin Tarn exit stream in Cumbria whence Davies recorded *latigonium*. This is a curious site since occurrence in a generally acid moorland environment is unexpected; the site lies, however, on Carboniferous limestone and both the water-plants and streamside vegetation of the habitat are more typical of alkaline areas (JABB, personal observation). (Distribution in southwest England, Wales and Scotland is unclear: some record spots on Davies' (1968: 109) map for '*angustitarse*' are apparently based on old records of wild-caught flies probably misidentified.)

Descriptive notes and variation

The most useful illustrated taxonomic descriptions of the larva, pupa and adults are those of Davies (1966, as *ang*. + *lat*., in English), Grenier (1953, as *ang*., in French) and Rivosecchi (1978a, as *lat*., in Italian). Knoz (1965, as *lat*.), and Davies (1968, as *ang*. + *lat*.) give illustrated coverage in English-language keys. The following works are also useful for their illustrations of significant features (some reproduced in this article). (1) postgenal cleft - Beaucournu-Saguez (1975, *lat*.), Clergue-Gazeau (1991, *lat*.), Jensen (1984, *lat*. + *lund*.), Jensen & Jensen (1973, *lat*.), Knoz (1980, *lat*.), Rivosecchi (1966, ? *lund*., 1967, 1978b, *lat*.), Zwolski (1959, *ang*.); (2) pupal gill - Adler & Wang (1994, *lat*.), Beaucournu-Saguez (1975, *lat*.), Clergue-Gazeau (1991, *lat*.), Jensen (1984, *lat*. + *lund*.), Jensen & Jensen (1973, *lat*.), Rivosecchi (1966, ? *lund*., 1978b, *lat*.), Rubtsov (1962, *lund*.), Zwick (1974, *lund*.); (3) cocoon (showing 'horn') - Adler & Wang (1994, *lat*.), Clergue-Gazeau (1991, *lat*.), Jensen (1984, *lat*. + *lund*.), Rivosecchi (1966, ? *lund*., 1967, 1978b, *lat*.), Zwick (1974, *lund*.); (4) _ genitalia - Beaucournu-Saguez (1975, *lat*.), Clergue-Gazeau (1991, *lat*.), Knoz (1980, *lat*.), Rivosecchi (1966, ? *lund*.).

Most larval characters, including the positive head-spot pattern with its elongate posteromedian mark, are very constant, but the shape of the postgenal cleft and the intensity of the pigmented area on the head capsule venter show some variation (Fig. 1). Some authors have attributed interspecific significance to this, but we consider this variation as all intraspecific. The cleft usually has parallel sides and is approximately square (Fig.1c,d,i) or slightly wider than deep (Fig.1b,f,g,k); sometimes, however, the sides diverge slightly towards the rear, so giving the cleft a more trapezoidal (Fig. 1l) or even subtriangular form. Davies (1968) and Jensen (1984) distinguished *latigonium* from *lundstromi* (*angustitarse* sensu Davies) on this basis, the former having the deeper and more square cleft (cf. figs 1i,j and 1k,l);

[Graphics File BULL5F1.gif here]

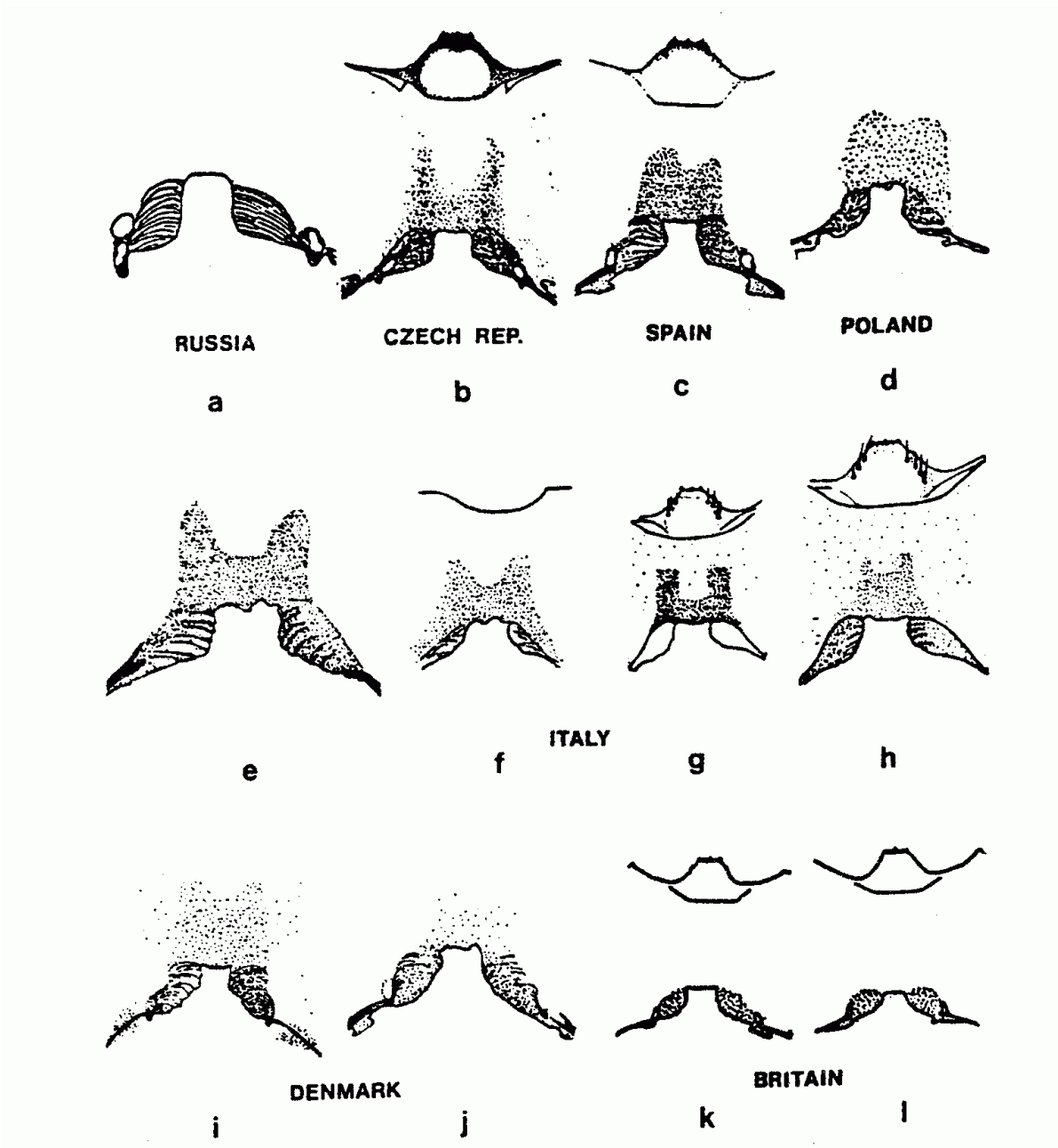

Fig. 1. Larval postgenal cleft and part of head venter, from various authors, countries as shown: a, Rubtsov (1956, *lat.*); b, Knoz (1980, *lat.*); c, Beaucournu-Saguez, 1975, *lat.*); d, Zwolski (1959, *ang.*); e, Rivosecchi (1966, '? *lund*'.); f, Rivosecchi (1967, *lat.*); g, Rivosecchi (1978b, *lat.*); h, Rivosecchi (1978a, *lat.*); i, Jensen (1984, *lat.*); j, Jensen (1984, *lund.*); k, Davies (1968, *lat.*); l, Davies (1968, *ang.*).

[Graphics File BULL5F2.gif here]

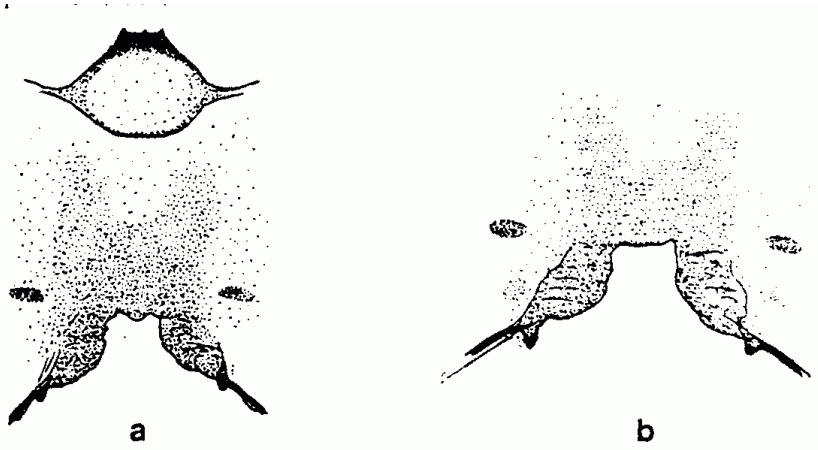

Fig. 2. Larval postgenal cleft and pigment mark of head venter: a, from *latigonium* holotype slide preparation; b, from Sunbiggin Tarn specimen (*latigonium* of Davies). Drawings original (RWC).

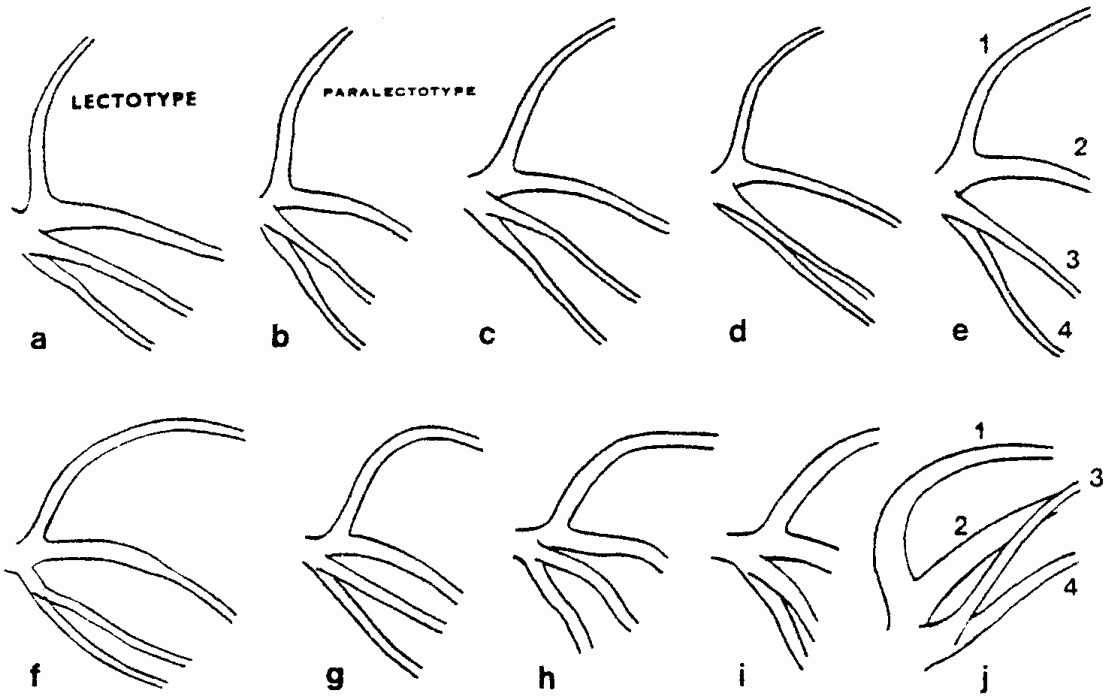

Fig. 3. Pupal gill base: a and b, from lectotype and a paralectotype of *lundstromi* (Britain); c, pupa from China (near Beijing); d, pupa from Britain (Herts, R. Rib); e, pupa from Germany (Brandenburg, Burg, Sudumflüter); f, Zwick (1974, *lund.*, Germany); g, Rubtsov (1962, *lund.*); h, Jensen (1984, *lat.*, Denmark); i, from Jensen (*lund.*, Denmark); j, pharate pupal gill of a larva from Sunbiggin Tarn, Cumbria, England. Drawings a-e and j original (RWC).

connectant states exist, though, even among larvae in the same sample and we see no significance in this feature. Rubtsov's (1956) figure of the cleft in the original description of *latigonium* is poor (Fig. 1a) so we give a new drawing here (by RWC) showing the cleft in the *latigonium* holotype larva (Fig. 2a). (Fig. 2b shows the similar cleft in a larva from Davies' Sunbiggin Tarn material identified as *latigonium*.) The pigmented mark on the undersurface of the head is quite variable in its size, shape and intensity (Fig. 1b-j) and we do not think that its development is a taxonomically significant feature. The same sample can show larvae with varying degrees of pigmentation, some in which the mark is essentially absent. (We do not exclude the possibility of some correlation with annual generation or larval age.)

The pupal stage varies in the length of the cocoon 'horn' and in the relative diameters of the upper pair of gill filaments compared to the lower pair (Fig. 3a-i). Filament 2 in all pupae is fatter at the extreme base than filaments 3 and 4 but in some pupae the basal thickening extends for some distance along filament 2 (Fig. 3,a,b,f) and thickening is apparent also on the upwardly arching filament 1 (Fig. 3a,h). When filament 2 is thickened in this way it either tapers more or less evenly along its length (as in *lundstromi* lectotype) or narrows rather abruptly after some distance (Fig. 3f); the latter condition is rather unusual in British pupae but not uncommon in Germany and Denmark (as illustrated by Zwick (1974) and Jensen (1984)). Davies (1968) and Jensen (1984) have used the difference between 'thick' and 'thin' upper filaments as a key character separating *lundstromi* (thick) and *latigonium* (thin) but our observations do not suggest that this is a specific difference. All states exist from conspicuously thin to rather thick, sometimes in pupae of the same sample, and we see no essential difference in pupae collected as far apart as China (Fig. 3c) and the English *lundstromi* type locality. Gill filament variation is such, even among the *lundstromi* paralectotype pupal skins, that it is impossible to categorize the individuals one way or the other on the Davies/Jensen criteria. A pharate pupal gill we prepared from a larva in Davies' material from Sunbiggin Tarn (his 'thin-filament' *latigonium*) has in fact much thickened upper filaments (Fig. 3j) and some of the pupal skins we have seen from the Russian type locality of *latigonium* have indistinguishable gills from those of some of the *lundstromi* paralectotype pupal skins. We conclude that this minor variation in the thickness of the upper gill filaments is intraspecific.

In the adult stage there is some variation in the male genitalia that deserves comment. Rivosecchi (1966) noticed that the profile shape of the ventral plate in what he called '*? lundstromi*' is somewhat variable, particularly in the outline of the haired blade which varies in its curvature; he observed that winter-emerging males seemed to have a less rotund blade profile (Fig. 4h) than spring-emerging males (Fig.4j). We have not detected such a correlation but have noticed some variation in plate shape. We have compared the male genitalia of *latigonium*with those of *lundstromi*, using specimens from the respective type localities (cf. Figs 4a and 4c) and observing the dissected parts of the genitalia both while freely movable in mountant (to ensure comparable orientation) and after permanent slide preparation, and have also examined the genitalia of males from Britain (including *latigonium* from Sunbiggin Tarn) and

[Graphics File BULL5F3.gif here]

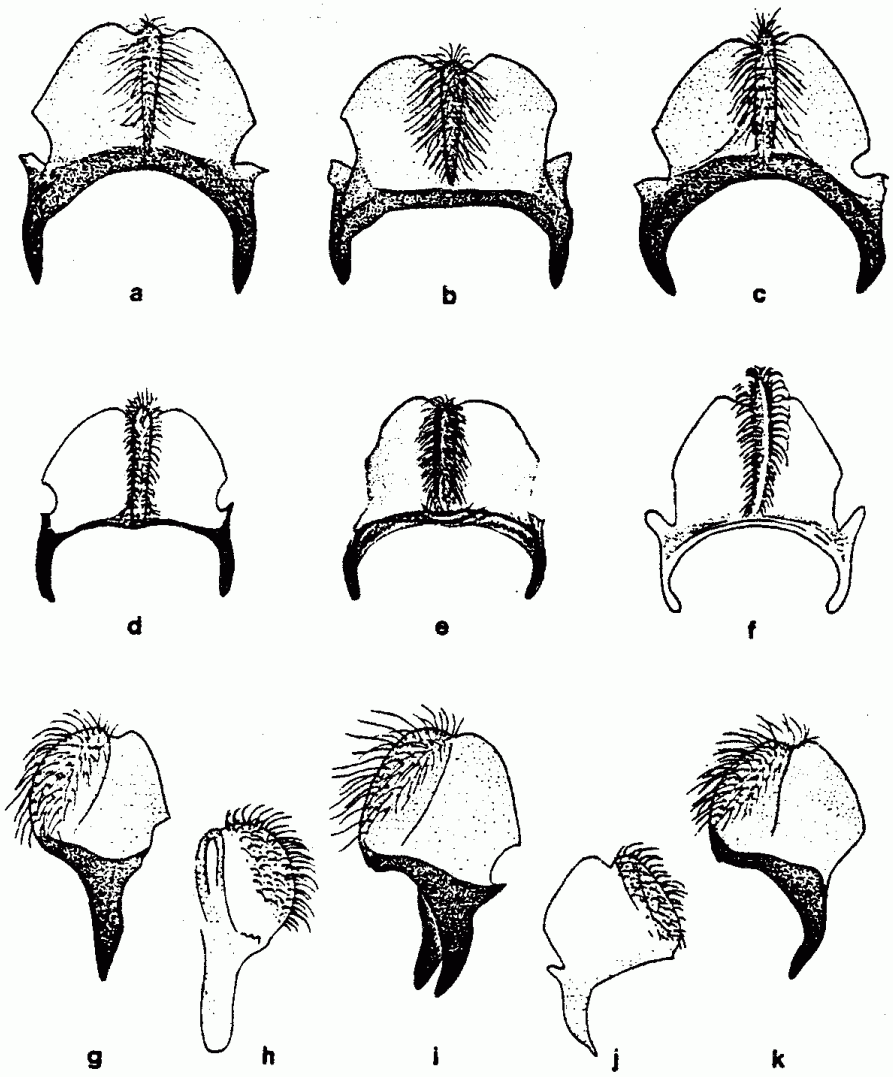

Fig. 4. Ventral plate of ♂ genitalia of *S. lundstromi*, a-f in ventral view and g-k in profile: a, in *lundstromi* paralectotype from Mildenhall, Suffolk; b and c, in specimens from *latigonium* type locality (R. Sitenka, Russia) (see legend note below); d, from Knoz (1965, Czechoslovakia, as *latigonium*); e and f, from Rivosecchi (1966, Italy, as *? lundstromi*); g, in specimen from R. Stor, Pulborough, Sussex; h and j, from Rivosecchi (1966, Italy, as *? lundstromi*, respectively 'winter' and 'spring'); i, in specimen from R. Meon, Hants; k, in specimen from Sunbiggin Tarn outflow stream, Cumbria (*latigonium* of Davies). In b and c the orientation is different, the plate apex tilting upwards in b (giving a relatively broader appearance) and slightly downwards in c. In the Rivosecchi sketches (h and j) the haired keel is reversed compared to the other profiles. Drawings a-c, g, i and k, original (RWC).

g h i j k

Fig. 4. Ventral plate of ♂ genitalia of *S. lundstromi*, a-f in ventral view and g-k in profile: a, in *lundstromi* paralectotype from Mildenhall, Suffolk; b and c, in specimens from *latigonium* type locality (R. Sitenka, Russia) (see legend note below); d, from Knoz (1965, Czechoslovakia, as *latigonium*); e and f, from Rivosecchi (1966, Italy, as *? lundstromi*); g, in specimen from R. Stor, Pulborough, Sussex; h and j, from Rivosecchi (1966, Italy, as *? lundstromi*, respectively 'winter' and 'spring'); i, in specimen from R. Meon, Hants; k, in specimen from Sunbiggin Tarn outflow stream, Cumbria (*latigonium* of Davies). In b and c the orientation is different, the plate apex tilting upwards in b (giving a relatively broader appearance) and slightly downwards in c. In the Rivosecchi sketches (h and j) the haired keel is reversed compared to the other profiles. Drawings a-c, g, i and k, original (RWC).

Germany that have been reared from pupae with 'thick' and 'thin' filament types. We have found the same sort of variation in ventral plate
profile as Rivosecchi mentioned but cannot correlate it with pupal gill calibre or any other features and we do not attribute interspecific importance to it. Comparison in particular of male genitalia from the type locality samples shows nothing contra-indicating the proposed synonymy of *latigonium** with *lundstromi*. Fig. 4 shows some examples of ventral plates. Interestingly, in ventral plates from Davies' Sunbiggin Tarn samples (seven preparations) the basal arms are more slender and strongly curved than usual (Fig. 4k); in this respect the ventral plate profile seems to differ slightly from that in true *latigonium* from the type locality and from males of other English populations (Figs. 4g and 4i show plate profile in specimens from two southern English sites). Davies (1966, 1968) thought he detected a specific difference between *latigonium* and *lundstromi* in the shape of the gonostyles and dorsal plate and used this as a key character. However, it does not hold and Lewis Davies himself later concluded (see below under comparison with *angustitarse*) that the putative specific differences are unsound.

In adult females the only notable variation is that of the colour of the scutal vestiture varying from pale silvery or greyish yellow to golden and the fact that while the subcosta is totally bare ventrally in most specimens the occasional female possesses a very few (up to three or four) tiny subcostal hairs near the base (at most on the basal third).

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Biology

The aquatic stages of *Simulium lundstromi* occur principally in weedy larger streams and rivers that flow smoothly and steadily over muddy or gravelly beds in agricultural areas, marshlands and fens, usually within the velocity range 30-50 cm/sec and the size width range 1-15 metres. However, the species also occurs quite commonly in very small streams: for example, one of us found larvae in Ireland in a stream only 15-30 cm wide that was draining from a bog (Bass, 1990, as *latigonium*). In Britain, breeding often occurs in streams and rivers of the chalk, including the winterbournes of southern England. The outflows of fish-ponds are a habitat reported from Slovakia (Illésová, 1992). Larvae and pupae attach to vegetational substrates, particularly bed-rooted linear water-plants such as the bur-reed *Sparganium erectum* and to a lesser extent *Sparganium emersum*; interestingly, the type material from Suffolk was collected from *Sparganium* (Edwards, 1920, as *angustitarse*); other

[*Rubtsov did not state the meaning of his name *latigonium* but we presume it to refer to a wide male ventral plate. This would accord with the wide appearance of the plate as illustrated by Rubtsov (1956) in the original description and repeated in Rubtsov (1962). We believe this appearance to be artefactual, due to the plate not being orientated horizontally (we cannot prove this because, as mentioned above, Rubtsov's original slide of male genitalia is missing from the St Petersburg slide collection.) The shape shown by Rubtsov does not accord with that of ventral plates from other males from the type locality; however, a somewhat similarly broad appearance results when the plate is drawn with its apex tilted upwards (as in a preparation by RWC, see Fig. 4b).]

substrates include reed-grass (*Phalaris arundinacea*) and *Glyceria fluitans*. Immatures are rarely abundant, usually occurring at rather low density, often in association with more abundant riverine species such as *S. lineatum*, *S. erythrocephalum* and *S. ornatum*; in the small-stream habitats the early stages are sometimes associated with *S. angustipes* and *S. velutinum* (members of the *S. aureum* group).

Pupae and emerging adults can be found through much of the year and *S. lundstromi* appears to be multivoltine almost everywhere that it occurs in western Europe. We have seen wild-caught and reared British specimens that have been collected in all months from April to November. One of us (DW) has found in Brandenburg area of Germany that there are pupation peaks in April and August/September, strongly suggestive of at least two annual generations; Seitz (1992), in Bavaria, reports pupation in all months from March (water temperature 8oC) to October with a triple-peak pupation regime (phanology diagram given) and over-wintering in the larval stage. Up to three annual generations occur in Italy, where there is winter emergence (Rivosecchi, 1978a, under *latigonia*). Davies (1966) thought that '*latigonium*' at the Sunbiggin Tarn locality probably had "a univoltine cycle", but we find this puzzling because his extensive material from this site (in NHM, London) includes many reared adult flies that emerged on 11 May, 12 and 17 July and 19 August [1963]; to our minds this date spread is indicative more of a multivoltine than a univoltine regime. It should be added that spring-emerging adults were obtained by one of us (JABB) in late April/May 1984 during a special re-sampling of Davies' Sunbiggin Tarn site (O.S. grid reference NY675073). Illésová (1992) records from one to three generations at various sites in Slovakia and that at one site in one particular year there was apparently only an 'autumnal generation'.

The bloodsucking toothed-claw females feed on birds. Baker (1970), under the name *angustitarse* (identified for Baker as such by Davies) has shown that *lundstromi* is a vector among rooks (*Corvus frugilegus*) of the protozoan blood parasite *Leucocytozoon sakharoffi*. (Fourteen female flies from Baker's research are in the NHM collection: we have confirmed their identity as *lundstromi*on the basis of the subcostal character mentioned in the next section.) Unlike the females of *angustitarse*, those of *lundstromi* are strongly attracted to carbon dioxide and can be readily caught in traps baited with carbon dioxide, especially when there is no wind and the sky is overcast (Rivosecchi, 1978a, under *latigonia*).

Comparison between *Simulium lundstromi* and *Simulium angustitarse*

As a consequence of our conclusion that *lundstromi* and *latigonium* are conspecific there are only two species of the *S. ruficorne* group in Britain and northwestern Europe, *S. lundstromi* (Enderlein) and *S. angustitarse* (Lundström) (some others occur in southern Europe, including *ruficorne* in Spain and Portugal). The habitats differ, *S. angustitarse* almost always occupying much smaller (usually first-order) streams than the more 'riverine' *S. lundstromi*. This is well shown by Crosskey's survey records for South East England in which (as confirmed by their very different pupae) the two species have only been found together at two sites among 47 sites recorded for *lundstromi* and 41 sites for *angustitarse*.

The two species are best distinguished as follows: pupal cocoon with loose net-like weave and without anteromedian 'horn' in *angustitarse* (closely woven and with 'horn' in *lundstromi*); larval postgenal cleft virtually absent, at most only a tiny notch in the head hind margin, in *angustitarse* (well developed in *lundstromi*, Figs 1 and 2); adult female subcosta with fine hairs ventrally along at least half its length in *angustitarse* (female subcosta completely bare ventrally or with at most only a very few hairs on the basal part in *lundstromi*).

The subcostal character of the female just mentioned was discovered by Zwick (1974). Though seemingly an improbable difference between such closely allied species we have found the same difference among females reared from pupae of known identity. The fine hairs are not as easily rubbed off as would be imagined and we think the subcostal character is reliable enough that it can be used for distinguishing between wild-caught females of the two species. Wild-caught males, on the other hand, cannot be reliably differentiated. The subcostal character does not apply (male *angustitarse* having similarly bare or virtually bare subcosta as in *lundstromi*). Davies (1968) suggested in his key (where *angustitarse* = *lundstromi* and *cambriense* = *angustitarse*) that differentiation is possible on the shape of the genital styles and dorsal plate but other workers have been unable to confirm this. Davies later (1970, in litt. to Zwick) agreed that "the 3 species [the third '*latigonium*'] show very little difference in male genitalia". Zwick (1974) illustrated the variability of the dorsal plate. We agree with Davies' later view, with Zwick, and with Rivosecchi's (1978a) comment (given under *latigonium*) that "Quaesta specie che allo stadio adulto è molto difficile distinguere de *angustitarsis*". (It is noteworthy that Rivosecchi nevertheless illustrates a difference between females in the shape and attachment of the ovipositor lobes: we have not studied this character.)

Cytologically *S. angustitarse* and *S. lundstromi* are extremely similar. Chubareva (1977) studied *angustitarse* from Lithuania and *lundstromi* (as *latigonium*) from the St Petersburg area and reported that karyotypes of both species show 2n = 6 chromosomes (as in almost all Simuliidae), weak pairing of homologues, very strongly differentiated centromeres and a connection zone on chromosome I to the nucleolus: specific differences lie in two homologous inversions, and in the stronger homologue pairing and weaker chromosome puffs occurring in *angustitarse*. [Note: larvae of both species collected in England were communicated at various times by Davies, Crosskey and Bass to the late K.H. Rothfels for chromosome study but findings (if any) were never published and the material has disappeared since dissolution of the Toronto cytotaxonomic 'school'.]

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Appendix

Correctly identified *lundstromi* type specimens in NHM, London:

Lectotype _, SUFFOLK: Mildenhall, R. Lark, 25.iv.1916 (*Edwards*) (in alcohol, with pupal skin). [Genitalia missing, specimen accompanied by Zwick's red determination label.]

4 _, 2 _ (pinned), 13 pupal skins (alcohol), data as lectotype. [Two _ with genitalia in Edwards' balsam preparations on celluloid mounts, one _ with genitalia in micro-vial, one _ with genitalia on slide (_ number 5): two _ and one _ with Zwick's red determination labels.]

1 _, SUFFOLK: Mildenhall, 13.v.1909 (*Yerbury*) [genitalia on slide].

1 _, SUFFOLK: Timworth, 17.v.1913 (*Nurse*).

2 _, BEDS: Shefford, x.1917 (*Edwards*).

1 _, BEDS: Shefford, 17.xi.1917 (*Edwards*).

1 _ (with dried pupal skin), CAMBS: Stapleford, 28.iv.1916 (*Edwards*).
2 _ (with dried pupal skins), HERTS: Hatfield, R. Lea, v.1916 (*Edwards*)
2 _, HERTS: Radwell, 15.vi.1917 (*Edwards*).