

Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 38 (1981)
Heft: 3

Artikel: Identification of two West African black flies (Diptera: Simuliidae) of the "Simulium damnosum" species complex by analysis of cuticular paraffins
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DOI: <https://doi.org/10.5169/seals-312824>

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Identification of two West African black flies (Diptera: Simuliidae) of the *Simulium damnosum* species complex by analysis of cuticular paraffins³

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Summary

Simulium damnosum is known to be a sibling species complex with at least 24 cytotaxonomically distinct forms in Africa. Of these, six occur in the area of West Africa covered by the World Health Organization Onchocerciasis Control Programme. They are all vectors of *Onchocerca volvulus*, and several are capable of migrating great distances. We report here a comparatively simple chemical method of species identification using individual adult females of pooled samples that have been collected and stored dry in glass vials. Females of two species, *S. sirbanum* and *S. squamosum*, were identified by extraction and analysis of cuticular components using gas chromatography. Statistically significant differences were seen between these two species for each of five sets of consistently-appearing peaks that were quantitated and compared. The technique has considerable potential for use in the field since extracts or specimens may be stored indefinitely at ambient temperature before analysis.

Key words: *Simulium damnosum* spp.; gas chromatography; cuticular; *S. sirbanum*; *S. squamosum*; paraffins.

Introduction

Since 1974 the World Health Organization Onchocerciasis Control Programme has been working to control the *Simulium damnosum* species complex (Diptera: Simuliidae), vectors of onchocerciasis or river blindness in West Afri-

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ca (Walsh et al., 1979). Conventional taxonomic study of the adults of members of the complex has provided valuable characters (Garms, 1978; Quillévére et al., 1977; Dang and Peterson, 1980), but only by studying the polytene chromosomes of the larvae can specific identifications be made (Vajime and Dunbar, 1975; Dunbar, 1976). However, adults of the important savanna vectors *S. damnosum* s. str. and *S. sirbanum* cannot be separated. *S. squamosum* can be positively identified only by enzyme electrophoresis (Townson and Meredith, 1979), a technique requiring that material be stored at very low temperatures until analysis. As these techniques are difficult to use routinely in Africa, a method for the precise identification of biting and migrating adults of the *S. damnosum* complex is required.

A method was recently described to separate single male and female mosquitoes of the *Anopheles gambiae* species complex by using gas chromatography (GLC) to distinguish differences between two species when selected pairs of GLC peaks were compared (Carlson and Service, 1979; Carlson and Service, 1980).

We report here a similar method for the identification of individual females of two members of the complex, *S. sirbanum* and *S. squamosum*, by GLC of their cuticular paraffins. The collecting method requires only that flies are caught, stored dry, and not allowed to come into contact with materials containing plasticizers or hydrocarbons.

Materials and methods

Females from an isolated, pure population of *S. sirbanum* from Upper Volta, hereafter called (A), and a population of *S. squamosum* from Togo, possibly admixed with a few *S. damnosum* s. str. hereafter called (B), were collected from human bait. The flies were captured directly in dry hexane-rinsed glass vials (1.6 × 5.0 cm) and capped with metal foil or paper-lined caps. The vials, labelled externally, were shipped to Florida where analyses were made four months later.

Gas chromatographic analyses were performed on a Varian Model 1440 equipped with a flame ionization detector, a Hewlett-Packard Model 3380A integrator, and a 1.8 m × 2 mm ID glass column packed with 3% OV-1 on 120–140 mesh Gas Chrom Q. Samples were analyzed with the column oven temperature programmed from 200 to 265° C at 6° per minute. Other parameters were: injector port, 310° C; detector, 385° C. The carrier gas was helium, 20 ml/min. Retention indices⁴ and quantitations were determined by using authentic paraffin standards (Analabs Inc., North Haven, Conn. 06473, USA) to ensure that the same GLC peaks were being measured.

Crude lipids were extracted from individual females by soaking each sample in 40 µl of n-hexane. Some extracts were reduced in volume with a stream of dry air to a minimum amount (2–10 µl) for immediate analysis. Others were added to a column prepared from a disposable 5 mm diam. Pasteur pipette, dry-packed with 60–200 mesh silica gel (2 cm) over 20% silver nitrate-impregnated silica gel (1 cm). Saturated paraffins were eluted with n-hexane, olefins with 1% ether in hexane, and other lipids with 15% ether in hexane. The volume of each separated sample of paraffins was adjusted to 2–10 µl for GLC analysis (Fig. 1).

⁴ Kovats indices (KI values) were assigned to compounds eluting from a nonpolar GC column held at one temperature based on their retention times by using a log scale, relative to straight-chain (normal) saturated paraffins (Kovats E., in: *Advances in chromatography*, Vol. 1, ed. by J. C. Giddings and R. A. Keller, p. 229–247. Dekker, New York 1965).

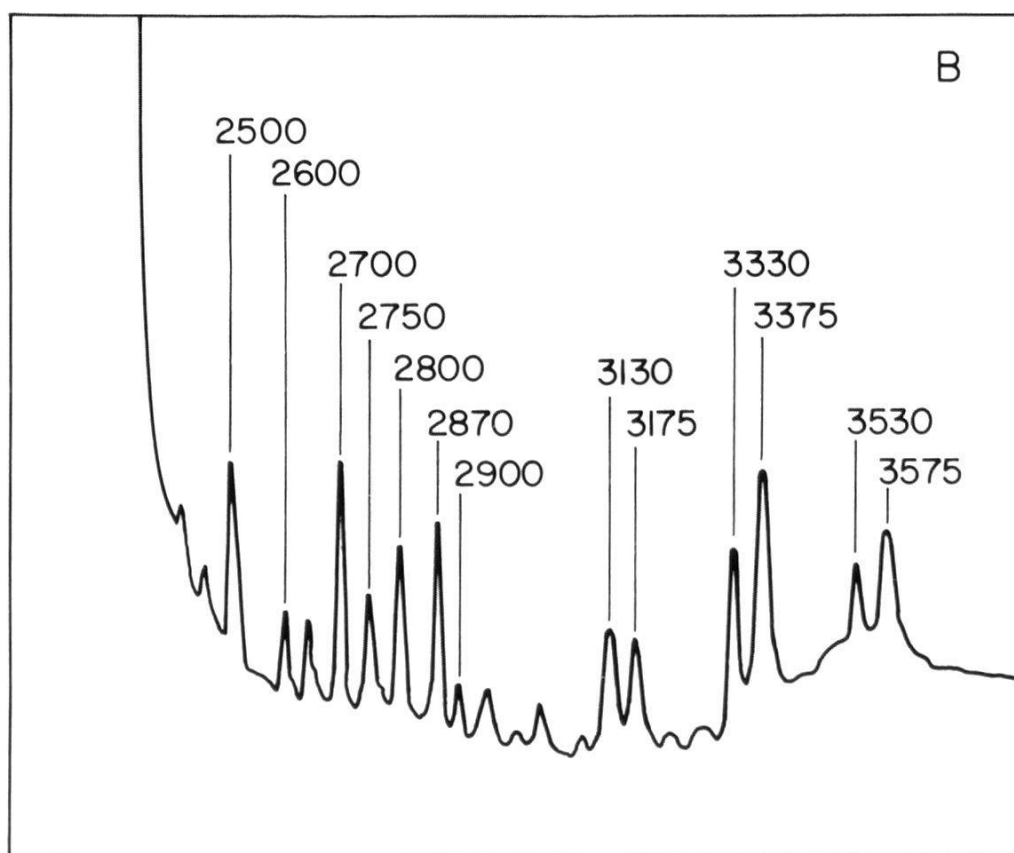
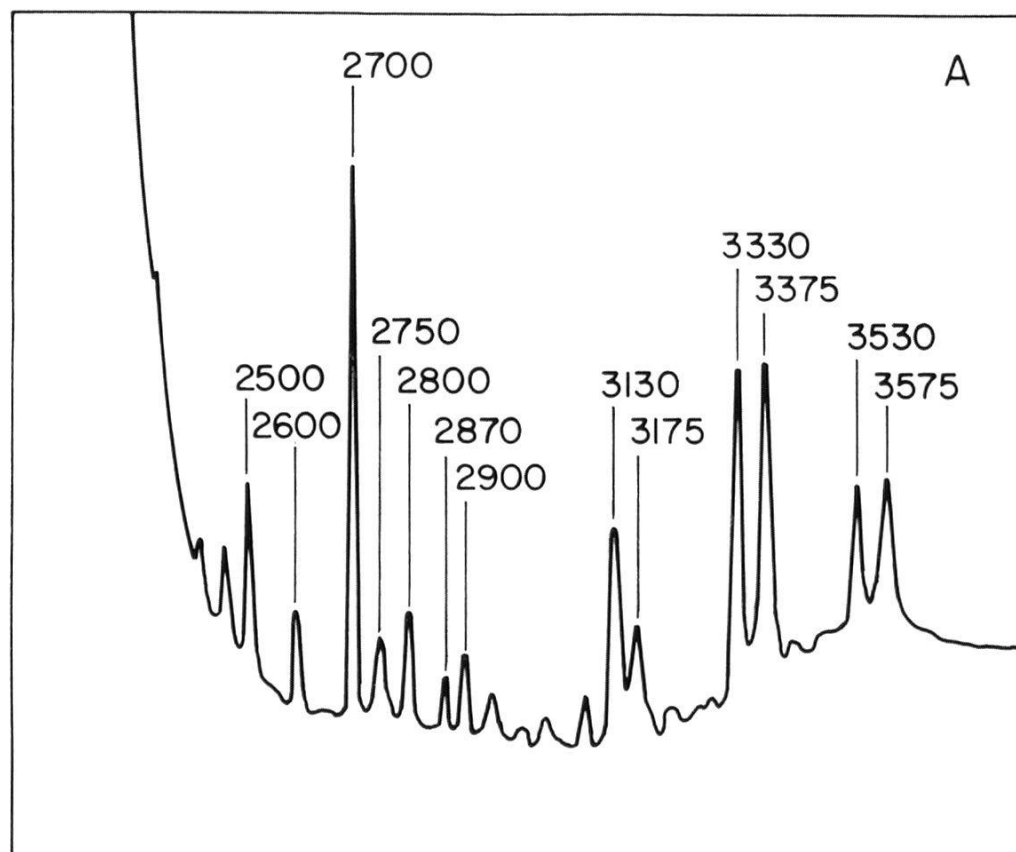


Fig. 1. Gas-liquid chromatograms of cuticular paraffins from individual female *S. sirbanum* (A) and *S. squamosum* (B) on 3% OV-1 liquid phase.

Silica gel and argentation-liquid chromatography was conducted to improve resolution of GLC peaks by removal of nonparaffins. Major constituents of the paraffin fraction were C₂₅–C₂₉ n-paraffins and C₂₇–C₃₈ branched paraffins. Patterns of peaks appeared to be similar in all samples. There were no branched paraffins that appeared to be species-specific. Olefins were present, but in quantities too small to be useful.

Results and discussion

Quantitative data on the distribution of GLC peaks suggests that there are at least five relationships that can be used for identification of females of these (two) species. One useful marker is the relative abundance (R) of materials that elute with n-pentacosane at KI 2500⁴, compared to a peak that elutes at KI 2700 with n-heptacosane. The mean relative abundance values for R₁ were 0.48 ± 0.12 in paraffins of single female *S. sirbanum* (A) and 0.78 ± 0.18 in female *S. squamosum* (B). The ratios are highly significantly different by the one way analysis of variance at the 0.005 level (Steele and Torrie, 1960) (Table 1).

A second useful marker is the relative abundance of a peak at KI 2750 compared to the KI 2700 peak. The mean values for R₂ in paraffins of single females were 0.18 ± 0.06 for (A) and 0.43 ± 0.16 for (B), significantly different at the 0.01 level.

A third marker is the relative abundance of a peak at KI 2870 compared to a peak at KI 2900. The values for R₃ were 1.82 ± 0.71 for (A) and 2.62 ± 0.67 for (B), significantly different at the 0.01 level.

A fourth marker is R₄, the relative abundance of materials that elute with 2-methylhentriacontane (KI 3130) compared to a peak at KI 3175. The values for R₄ were 2.24 ± 0.59 and 1.59 ± 0.23 , significantly different at the 0.01 level.

A fifth marker is R₅, the relative abundance of materials that elute with 2-methyltritriacontane (KI 3330) compared to a peak at KI 3375. The values for R₅ were 0.90 ± 0.04 and 0.70 ± 0.11 , significantly different at the 0.005 level.

The technique is thus satisfactory for the confident separation of individual adult females of the *S. sirbanum*, sample (A), and *S. squamosum*, sample (B). There was no evidence for the presence of two species in sample (B). A correct

Table 1. Relative abundance (R) of paraffins in individual female black flies

	R ₁ 2500/2700		R ₂ 2750/2700		R ₃ 2870/2900		R ₄ 3130/3175		R ₅ 3330/3375	
	A	B	A	B	A	B	A	B	A	B
\bar{x}	0.48	0.78	0.18	0.43	1.82	2.62	2.24	1.59	0.90	0.70
s^1	0.12	0.18	0.06	0.16	0.71	0.67	0.59	0.23	0.04	0.11
s^2	0.01	0.03	0.00	0.02	0.50	0.45	0.35	0.05	0.00	0.01
p	0.005		0.01		0.01		0.01		0.005	

A = *S. sirbanum*
B = *S. squamosum*

\bar{x} = mean value (n – 14)
 s^1 = standard deviation

s^2 = variance
p = probability level

identification could be made by measurement or visual inspection of the paraffin peaks. Use of an automated data system is not obligatory, since simple measurement of GLC peak heights from the strip-chart recorder gives the same information. The technique has considerable potential since extracts, pinned or dried specimens stored in glass vials can all be readily mailed to the analytical laboratory. It is hoped that further work will lead to the separation of the adult females of *S. sirbanum* from those of *S. damnosum* s. str. This will be possible only if a source of unmixed populations of the latter can be found in the field.

Acknowledgments. The collections were made while J. F. Walsh was a member of the World Health Organization Onchocerciasis Control Programme team. We are especially grateful to Dr. C. Vajime for making the essential cytotaxonomic determinations of larval samples. Thanks are also owing to Prof. D. H. Molyneux for advice, and S. King for technical assistance.

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