# CUTICULAR HYDROCARBONS OF BUFFALO FLY, *Haematobia exigua*, AND CHEMOTAXONOMIC DIFFERENTIATION FROM HORN FLY, *H. irritans*

RUDOLF URECH*, GEOFFREY W. BROWN, CHRISTOPHER J. MOORE, AND PETER E. GREEN

*Department of Primary Industries and Fisheries (Queensland), Animal Research Institute, 665 Fairfield Rd, Yeerongpilly Qld 4105, Australia*

* To whom correspondence should be addressed. E-mail: rudolf.urech@dpi.qld.gov.au

**Abstract—**We determined the quantity and chemical composition of cuticular hydrocarbons of different strains, sex and age of buffalo flies, *Haematobia exigua*. The quantity of cuticular hydrocarbons increased from less than 1 μg/fly for newly-emerged flies to over 11 μg/fly in 13 d-old flies. The hydrocarbon chain length varied from C21 to C29, with unbranched alkanes and monounsaturated alkenes the major components. Newly emerged flies produced almost exclusively C27 hydrocarbons. Increasing age was accompanied by the appearance of hydrocarbons with shorter carbon chains and an increase in the proportion of alkenes. 11-Tricosene and 7-tricosene were the most abundant hydrocarbons in mature buffalo flies. Cuticular hydrocarbons of buffalo flies are distinctly different from those of horn flies. The most noticeable differences were in the C23 alkenes, with the major isomers 11- and 7-tricosene in buffalo flies and (*Z*)-9- and (*Z*)-5-tricosene in horn flies, respectively. Cuticular hydrocarbon analysis provides a reliable method to differentiate buffalo and horn fly, which are difficult to separate morphologically. The differences in cuticular hydrocarbons also support their recognition as separate species, *H. exigua* and *H. irritans*, rather than as subspecies.

**Key Words—**Cuticular hydrocarbons, *Haematobia*, buffalo fly, horn fly, Diptera, chemotaxonomy, gas chromatography, mass spectrometry, alkenes.

# INTRODUCTION

The buffalo fly, *Haematobia exigua*, occurs in the Oriental and Australian regions (Seddon, 1967; Williams et al., 1985). *H. exigua* are obligate blood feeders spending most of their time on the host (MacQueen and Doube, 1988) and constitute one of the major animal health problems to dairy and beef producers in northern Australia. The horn fly, *H. irritans*, a close relative of the buffalo fly, occurs in the Americas, North Africa and Europe and is also considered a major arthropod pest of pastured cattle in the US and Canada (Drummond et al., 1986; Kunz, 1986).

Morphological differentiation of the adult fly species is difficult and Pont (1973) indicated that the various larval stages were probably indistinguishable. Whilst Zumpt (1973) recognised the two as subspecies of *H. irritans*, Skidmore (1985) subsequently recognised them as two separate species, *H. exigua* and *H. irritans*, although acknowledging their close relationship. Species and subspecies status are currently being applied to the horn and buffalo flies by various authors. One approach to species identification which could clarify this situation is cuticular hydrocarbon profiling (Lockey, 1991), which has been successfully applied to a number of problem species (Brown et al., 1998; Broza et al., 2000; Carlson et al., 1993; Howard et al., 2003). The cuticular hydrocarbons of *H. irritans* have been identified (Mackley et al., 1981), with the major components being odd-numbered, straight-chain C21-C29 saturated hydrocarbons and mono-unsaturated (alkene) C23, C25 and C27 hydrocarbons. Double bond positions were established through oxidative cleavage followed by GC-MS of the resulting aldehydes.

In this study, we report the identification of buffalo fly cuticular hydrocarbons and variations in their composition and quantity depending on the flies' sex and age. We employ a more elegant and direct route to the determination of double bond positions through the formation and GC-MS analysis of dimethyl disulfide (DMDS) adducts (Carlson et al., 1989;

Scribe et al., 1988). Considering the morphological and behavioural similarities between horn and buffalo flies, the resulting information is valuable and useful for taxonomic purposes.

## METHODS AND MATERIALS

*Flies. H. exigua* were obtained from a colony of a field strain at the DPI Oonoonba Veterinary Laboratory, Townsville (DPI strain), and from the closed laboratory colony at CSIRO Entomology, Indooroopilly (CSIRO strain), Queensland, Australia. Both colonies were cultured on bovine hosts (Stegeman et al., 1996) and the CSIRO-strain flies were collected from cattle kept in an insectary. Pupae submitted from Townsville were kept in cardboard cylinders lined with filter paper at 27$^{o}$C, 70-80% RH and emerged flies fed with bovine blood (Anderson, 1995). *H. irritans* were obtained from the colony of Prof. Butler at the University of Florida, Gainesville.

*Hydrocarbon extraction.* 1 ml of hexane (UltimAR, Mallinckrodt) containing 5 µg n-tetracosane (internal standard, IS) was added to 20 flies which had been immobilised (-20$^{o}$C or carbon dioxide). After standing for 5 min, the hexane solution was passed through a silica gel plug (1g) confined in a Pasteur pipette which was then washed with 5 x 1 ml hexane. The combined hexane solutions were dried with anhydrous sodium sulfate, filtered, evaporated to dryness and reconstituted with 100 µl hexane. Single fly extractions were carried out analogously using 20-fold lower IS concentration and reconstituted with 5 µl hexane.

(*Z*)-9-Tricosene was obtained from ISP Fine Chemicals, Columbus OH43228.

*Methylthiolation of alkenes.* DMDS adducts were prepared according the method of Carlson et al. (1989) with a reaction time of 16 h at 40$^{o}$C.

*Analysis and identification*: Gas chromatography (GC) was carried out on a HP5890 equipped with a capillary column (DB-5, J&W, 30 m x 0.25 mm, 0.25 µm film) using nitrogen as the carrier gas (column pressure 100 kPa) and with a flame ionisation detector (FID). The oven temperature was increased (5$^{o}$C/min) from 200$^{o}$C to 260C (280$^{o}$C for DMDS

adducts) where it was held for 5 minutes. Data acquisition and processing was done with an HP3392A integrator with peak area used for the quantitation of components (relative to tetracosane). GC-MS was performed on a VG Trio 2000 coupled to a HP 5890 GC fitted with the same column as above. Ionisation was by electron impact (EI) and the total ion current was accumulated from m/z 35 to 400 (500 for DMDS adducts) over 1 sec. Selected ion mass spectrometry of high-intensity mass fragments was used for the detection or confirmation of components at low abundance.

## RESULTS

The GC/FID traces of cuticular hydrocarbon extracts from newly emerged, 2-3 and 13 d-old female buffalo flies are shown in Figure 1. Relevant hydrocarbon peaks are designated with a reference number (for key see Table 1). All alkanes were completely resolved from the alkenes. At least partial, but often complete separation of homologous alkenes with double bonds at 5,7 and 9 positions was achieved, but the 9 and 11 alkenes co-eluted. The hydrocarbon chain length varied from C21 to C29, with a distinct shift to shorter hydrocarbons with increasing age of the flies.

Insert Figure 1 near here.

The quantities of cuticular hydrocarbons recovered from flies of different strains, sex and age are provided in Table 1. From newly emerged flies (DPI strain) only small quantities of hydrocarbons (<1 μg) were extracted, and these were almost exclusively of 27-carbon chain length. The flies, which were kept in cardboard cylinders and maintained on bovine blood, produced larger amounts of hydrocarbons with increasing age, reaching over 11 μg per female fly at 13 days. With increasing age, the DPI strain produced shorter carbon chains (88% C23) and a higher proportion of alkenes (62% at 13 d). The CSIRO strain, which had been kept on cattle post-emergence, contained at the age of 3 d a similar amount of cuticular hydrocarbons

to the DPI strain of the same age, but its hydrocarbon pattern was almost identical to the 13 d-old DPI flies. 80% and 20% of the CSIRO (3 d) and DPI flies (13 d) were gravid respectively.

Unbranched, odd-numbered C21 to C29 alkanes were recovered from most buffalo flies. Heptacosane was the most abundant alkane in young flies, whereas tricosane was found in the highest quantities in mature flies. A series of methyl branched C28 alkanes was detected only in newly emerged flies.

A number of C23 to C29 monounsaturated alkenes was detected. The position of the single double bond was established through derivatization with DMDS, GC-MS analysis of the adducts and correlation of GC retention times of parent compounds and adducts. The presence of abundant fragments (often at or close to 100% abundance) corresponding to cleavage at the original site of unsaturation, now carrying thiomethyl groups at each end, defined the location of the double bond. For instance, four adducts of C23 alkenes showing intense ions at *m/*z 201/215, 173/243, 145/271 and 117/299 indicated the presence of 11-, 9-, 7-, and 5-tricosene respectively. The most abundant hydrocarbon in mature female buffalo flies was 11-tricosene, followed by 7-tricosene, which together constituted about 90% of all alkenes. 7-Tricosene was the most abundant hydrocarbon in mature, male CSIRO flies. Other C23 alkenes included 5-tricosene and (*Z*)-9-tricosene, with the latter being detected only in the CSIRO strain in trace quantities. In the early stages of the DPI flies' development, C27 and C25 alkenes were predominantly being produced, with 7- and 9-heptacosene and 7-pentacosene the major components.

The production of cuticular hydrocarbons was consistent in quantity and composition between individual flies within a sex/age group (Table 2). The standard error of the means of the major components from replicated hydrocarbon extraction from individual flies is small (typically 5%). Furthermore, the mean values from single fly extractions closely match those from the batch extractions.

Single fly extracts of *H. irritans* gave as major components (*Z*)-9-tricosene, 5-tricosene, 9-pentacosene, pentacosane, 9-heptacosene and heptacosane, confirming previous findings (Mackley et al., 1981; Milstrey, 1983).

## DISCUSSION

Analyses of horn fly cuticular hydrocarbons were consistent with those published previously (Mackley et al., 1981; Milstrey, 1983) but were obtained in a more efficient fashion. This was a consequence of the superior resolving power of modern capillary GC columns compared with the packed column, avoiding the necessity for prior column-chromatographic separation of alkenes from alkanes. Structure elucidation of the hydrocarbons was achieved on the basis of GC retention times, methylthiolation and mass spectrometry of the parent compounds and their adducts. Resolution of all unsaturated positional isomers was obtained with the exception of the 9 and 11 isomers, where the parent compounds as well as the DMDS adducts partially co-eluted. However, these isomers could be distinguished by selected ion monitoring of the DMDS adducts which showed clear differences in the major fragments obtained by EI ionisation. The stereochemistry of the double bond could be assigned unambiguously only for (*Z*)-9-tricosene, the only unsaturated reference hydrocarbon we were able to obtain. It is likely that all other alkenes share the *Z* configuration, based on their GC retention times and precedence from the structures of cuticular hydrocarbons from related flies (Mackley et al., 1981).

The cuticular hydrocarbon profile of freshly emerged buffalo flies was similar to the profile of the corresponding colony horn flies (Milstrey, 1983). Normal C25 and C27 and methyl branched C28 hydrocarbons provided the bulk of the alkanes, and 9-heptacosene and 9-pentacosene largely made up the alkenes. A few additional components not detected in horn flies were observed in buffalo flies, including 9-nonacosene, 5- and 7-heptacosene and 7-pentacosene, however they were present only in small or trace quantities. The branched

C28 alkanes were present in newly emerged buffalo and horn flies but were not detected in any other age category in either species. The quantity of alkanes and alkenes extracted was lower in newly emerged buffalo flies than horn flies. Similarly to horn flies, there was no obvious difference in the composition of cuticular hydrocarbons between female and male flies after emergence.

Significant qualitative and quantitative changes in hydrocarbon composition were observed 2-3 d after emergence of buffalo flies. Shorter carbon chains were more prominent and alkenes constituted close to 80% of the total hydrocarbons, compared to less than 35% in freshly emerged flies (Table 1). Similar age-related changes in the production of hydrocarbons had also been observed for horn flies (Milstrey, 1983). However, qualitative differences between the composition of the hydrocarbons in buffalo and horn flies were obvious. 2-3 d-old buffalo flies kept in a cardboard cylinder since emerging (DPI strain, Table 1) produced 5- and 7-tricosene, 7- and 9-pentacosene and 7- and 9-heptacosene, the latter being the major component. Horn flies (3-4 d-old) yielded (*Z*)-5- and (*Z*)-9-tricosene (major components), (*Z*)-9-pentacosene and (*Z*)-9-heptacosene (Mackley et al., 1981; Milstrey, 1983). Thus, none of the Z-9-tricosene, which was the major alkene component in horn flies (except newly-emerged), was found in the DPI buffalo flies. Conversely, 7-tricosene, 7-pentacosene and 7-heptacosene were detected only in buffalo flies but not in horn flies. The extraction of 13 d-old, female DPI flies produced a total of 11 µg cuticular hydrocarbons per fly, with the alkenes comprising about 65% of these. The shift to shorter carbon chain length had continued and two additional components, 11-tricosene and 11-pentacosene were detected. 11-Tricosene was the major component and together with 7-tricosene and tricosane contributed 80% to the total hydrocarbons. An inspection of a sub-sample of this population indicated that 20% of the female flies were gravid.

Extraction of CSIRO buffalo flies, kept on cattle for three days post-emergence, yielded a similar amount of hydrocarbons to the caged flies of the same age (Table 1). However, the resultant hydrocarbon profile was much more like that of the 13 d-old DPI flies, although the ratio of alkenes to alkanes was higher in the CSIRO flies. All mature buffalo flies had predominantly C23 alkenes, with 11- and 7-tricosene the most abundant isomers. The 3 d-old female CSIRO flies were 80% gravid, and the close resemblance of their hydrocarbon profiles to those of the DPI flies (13 d, 20% gravid), indicates that the observed change in the hydrocarbon profile is related to developments other than the attainment of sexual maturity. The CSIRO flies also showed quantitative differences between the sexes, with 11-tricosene and 7-tricosene the major components in female and male buffalo flies respectively. However, the results from this study may not fully reflect sexual dimorphism, as the flies were collected from mixed-sex populations, unlike the horn fly study where pupae were individually contained (Milstrey, 1983). Transfer of cuticular hydrocarbons between female and male flies through mating has been documented (Scott et al., 1988). The CSIRO fly extracts also contained small amounts of (*Z*)-9-tricosene, the major alkene component of horn flies. However, (*Z*)-9-tricosene was only detected in buffalo flies with selected ion mass spectrometry, which provided higher sensitivity than total ion current or flame ionisation detectors.

The variability in the quantity of the major cuticular hydrocarbons extracted from individual buffalo flies was low (Table 2). All major components were detected in all samples and the standard errors of the means were all below 10% and typically around 5%. Thus, single fly extractions provide hydrocarbon profiles which are characteristic of the population with a reasonable degree of certainty.

To complete our study, we extracted one female and male horn fly and determined the structure of the cuticular hydrocarbons by methylthiolation followed by GC-MS. We

confirmed the previous findings, obtained by ozonolysis of the parent components, that (*Z*)-9-tricosene, (*Z*)-5-tricosene, (*Z*)-9-pentacosene, pentacosane, (*Z*)-9-heptacosene and heptacosane, are the major cuticular hydrocarbons found in horn flies (Mackley et al., 1981; Milstrey, 1983).

The results presented in this study demonstrate that cuticular hydrocarbon profiles of buffalo and horn flies are almost identical in newly emerged flies, but can readily be distinguished in flies older than 2 days. This dissimilarity is independent of sex, feeding regimen (*in vivo* or *in vitro*) or sexual maturity. Table 3 lists the cuticular hydrocarbons detected in mature buffalo and horn flies respectively. For chemotaxonomic differentiation, a simple analysis of C23 alkenes of flies aged two days or more provides an unambiguous identification, with each species possessing unique positional isomers (shaded section in Table 3). The major alkenes were the 11- and 7-tricosene in buffalo flies (both not detected in horn flies), and the (*Z*)-9- and (*Z*)-5-tricosene in horn flies. Such a chemotaxonomic differentiation is more reliable and robust than the difficult differentiation on morphological characters (Zumpt, 1973).

Cuticular hydrocarbons are utilised by insects as species recognition cues. The clear differences in the cuticular hydrocarbon profiles of buffalo and horn flies thus provide support for their recognition as separate species. Additional cuticular hydrocarbon profiling and molecular, genetic, morphological and cross-breeding studies of global specimens would be desirable to further explore species integrity.

In conclusion, we determined the qualitative and quantitative composition of cuticular hydrocarbons of different strains, sex and age of buffalo flies. We demonstrated that the hydrocarbon profile of mature buffalo flies unambiguously differs from that of horn flies and that by determining the identity of a few key compounds in cuticular hydrocarbon extracts, fly species can be assigned.

*Acknowledgements*—The authors are grateful to WM Doherty (DPI&F), Dr David Kemp (CSIRO) and Prof JF Butler (University of Florida) for provision of flies and Meat & Livestock Australia for partial funding.

REFERENCES

ANDERSON, J. M. E. 1995. Culturing *Haematobia exigua* de Meijere (Diptera: Muscidae) in the laboratory. *J. Aust. Entomol. Soc.* 1:17-21.

BROWN, W. V., MORTON, R., LACEY, M. J., SPRADBERY, J. P., and MAHON, R. J. 1998. Identification of the geographical source of adults of the Old World screw-worm fly, *Chrysomya bezziana* Villeneuve (Diptera : Calliphoridae), by multivariate analysis of cuticular hydrocarbons. *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 119:391-399.

BROZA, M., NATION, J. L., MILNE, K., and HARRISON, J. 2000. Cuticular hydrocarbons as a tool supporting recognition of *Gryllotalpa tali* and *G. marismortui* (Orthoptera : Gryllotalpidae) as distinct species in Israel. *Ann. Entomol. Soc. Am.* 93:1022-1030.

CARLSON, D. A., MILSTREY, S. K., and NARANG, S. K. 1993. Classification of tsetse flies *Glossina* spp (Diptera, Glossinidae) by gas chromatographic analysis of cuticular components. *Bull. Entomol. Res.* 83:507-515.

CARLSON, D. A., ROAN, C., YOST, R. A., and HECTOR, J. 1989. Dimethyl disulfide derivatives of long chain alkenes, alkadienes and alkatrienes for gas chromatography/mass spectrometry. *Anal. Chem.* 61:1564-1571.

DRUMMOND, R. O., GEORGE, J. E., and KUNZ, S. E. 1986. Control of Arthropod Pests of Livestock: A Review of Technology. CRC Press Inc., Boca Raton Florida.

HOWARD, R. W., JACKSON, L. L., BANSE, H., and BLOWS, M. W. 2003. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: Identification and role in mate choice in *D. serrata*. *J. Chem. Ecol.* 29:961-976.

KUNZ, S. E. 1986. Integrated pest management and Dipteran pests in the new world, pp. 659-664, *in* M. J. Howell (ed.). Parasitology - Quo Vadit. Australian Academy of Science, Canberra.

LOCKEY, K. 1991. Insect hydrocarbon classes: Implications for chemotaxonomy. *Insect Biochem.* 21:91-97.

MACKLEY, J. W., CARLSON, D. A., and BUTLER, J. F. 1981. Identification of the cuticular hydrocarbons of the horn fly and assays for attraction. *J. Chem. Ecol.* 7:669-683.

MACQUEEN, A. and DOUBE, B. M. 1988. Emergence, host finding and longevity of adult *Haematobia irritans exigua* de Meijere (Diptera: Muscidae). *J. Aust. Entomol. Soc.* 27:167-174.

MILSTREY, E. G. 1983. Variation in horn fly, *Haematobia irritans* (L.) hydrocarbons and the effect of altering pheromone levels in the field on wild horn fly populations. MSc dissertation. University of Florida, Gainesville.

PONT, A. C. 1973. Studies on Australian Muscidae (Diptera). IV. A revision of the subfamilies Muscinae and Stomoxyinae. *Aust. J. Zool.* 21:129-296.

SCOTT, D., RICHMOND, R. C., and CARLSON, D. A. 1988. Pheromones exchanged during mating: a mechanism for mate assessment in *Drosophila*. *Anim. Behav.* 36:1164-1173.

SCRIBE, P., GUEZENNEC, J., DAGAUT, J., PEPE, C., and SALIOT, A. 1988. Identification of the position and the stereochemistry of the double bond in monounsaturated fatty acid methyl esters by gas chromatography/mass spectrometry of dimethyl disulfide derivatives. *Anal. Chem.* 60:928-931.

SEDDON, H. R. 1967. Diseases of domestic animals in Australia. Part 2: Arthropod infestations (flies, lice and fleas). Department of Health, Commonwealth of Australia, Canberra.

SKIDMORE, P. 1985. The biology of the Muscidae of the world. Dr W Junk, Dordrecht.

STEGEMAN, D. A., TOZER, R. S., and SUTHERST, R. W. 1996. Procedures for mass rearing the buffalo fly, *Haematobia irritans exigua* de Meijere (Diptera: Muscidae). *Aust. J. Entomol.* 35:77-79.

WILLIAMS, J. D., SUTHERST, R. W., MAYWALD, G. F., and PETHERBRIDGE, C. T. 1985. The southward spread of buffalo fly (*Haematobia irritans exigua*) in eastern Australia and it survival through a severe winter. *Aust. Vet. J.* 62:367-369.

ZUMPT, F. 1973. The Stomoxyine Biting Flies of the World. Diptera: Muscidae. Taxonomy, biology, economic importance and control measures. Gustav Fischer Verlag, Stuttgart.

TABLE 1. QUANTITIES OF CUTICULAR HYDROCARBONS EXTRACTED FROM BUFFALO FLIES OF DIFFERENT STRAINS, SEX AND AGE (μg/FLY)

| Ref. No. | Hydro-carbons | Strain/sex/age | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | DPI F ne | DPI M ne | DPI F 2-3 d | DPI M 2-3 d | DPI F 13 d | CSIRO F 3 d | CSIRO M 3 d |
| 1 | C21 | D | D | | D | 0.15 | 0.09 | 0.05 |
| 2 | 11-C23:1 | | | T | | 4.73 | 2.32 | 0.77 |
| 3 | Z9-C23:1 | | | | | | T | T |
| 4 | 7-C23:1 | | | 0.12 | 0.34 | 1.53 | 0.74 | 2.03 |
| 5 | 5-C23:1 | | | D | 0.05 | 0.21 | 0.12 | 0.33 |
| 6 | C23 | D | D | D | 0.09 | 2.73 | 0.71 | 1.03 |
| 7 | 11-C25:1 | | | | | 0.26 | 0.06 | D |
| 8 | 9-C25:1 | D | D | 0.08 | 0.07 | | | |
| 9 | 7-C25:1 | T | | 0.25 | 0.13 | 0.09 | D | D |
| 10 | C25 | 0.08 | 0.08 | 0.22 | 0.22 | 0.57 | 0.12 | 0.20 |
| 11 | 9-C27:1 | 0.21 | 0.14 | 2.73 | 1.90 | 0.17 | D | |
| 12 | 7-C27:1 | D | D | 0.64 | 0.42 | D | D | D |
| 12 | 5-C27:1 | D | | | | | | |
| 14 | C27 | 0.19 | 0.14 | 0.62 | 0.48 | 0.76 | 0.17 | 0.14 |
| 15 | C27-Me | 0.21 | 0.03 | | | | | |
| 16 | 9-C29:1 | D | | | | | | |
| 17 | C29 | D | D | 0.18 | 0.11 | D | | D |
| | Total alkanes | 0.48 | 0.25 | 1.02 | 0.81 | 4.21 | 1.09 | 1.42 |
| | Total alkenes | 0.21 | 0.14 | 3.82 | 2.91 | 6.99 | 3.24 | 3.13 |

F female; M male; ne newly emerged; D detected but not quantified (small quantities); T trace quantities (selected ion monitoring)

TABLE 2. MEAN AND STANDARD ERROR OF QUANTITIES OF CUTICULAR HYDROCARBONS EXTRACTED FROM FEMALE AND MALE BUFFALO FLIES[a]

| Cuticular hydrocarbon | Quantity (µg/fly) | |
|---|---|---|
| | Female | Male |
| 11-C23:1 | 2.3 (0.15) | 0.82 (0.05) |
| 7-C23:1 | 0.79 (0.06) | 2.02 (0.10) |
| C23 | 0.70 (0.06) | 1.11 (0.06) |
| C25 | 0.13 (NA) | 0.25 (0.03) |
| C27 | 0.17 (0.01) | 0.16 (0.01) |

[a] CSIRO flies, 3 d-old, N=5

TABLE 3. IDENTITIES OF CUTICULAR HYDROCARBONS IN EXTRACTS FROM MATURE BUFFALO AND HORN FLIES

| Cuticular hydrocarbon | Buffalo fly | Horn fly[a] |
|---|---|---|
| C21 | ✓ | ✓ |
| 11-C23:1 | ✓✓ | nd |
| Z9-C23:1 | * | ✓✓ |
| 7-C23:1 | ✓✓ | nd |
| 5-C23:1 | ✓ | ✓✓ |
| C23 | ✓ | ✓ |
| 11-C25:1 | ✓ | nd |
| 9-C25:1 | ✓ | ✓ |
| 7-C25:1 | ✓ | nd |
| C25 | ✓ | ✓ |
| 9-C27:1 | ✓ | ✓ |
| 7-C27:1 | ✓ | nd |
| C27 | ✓ | ✓ |

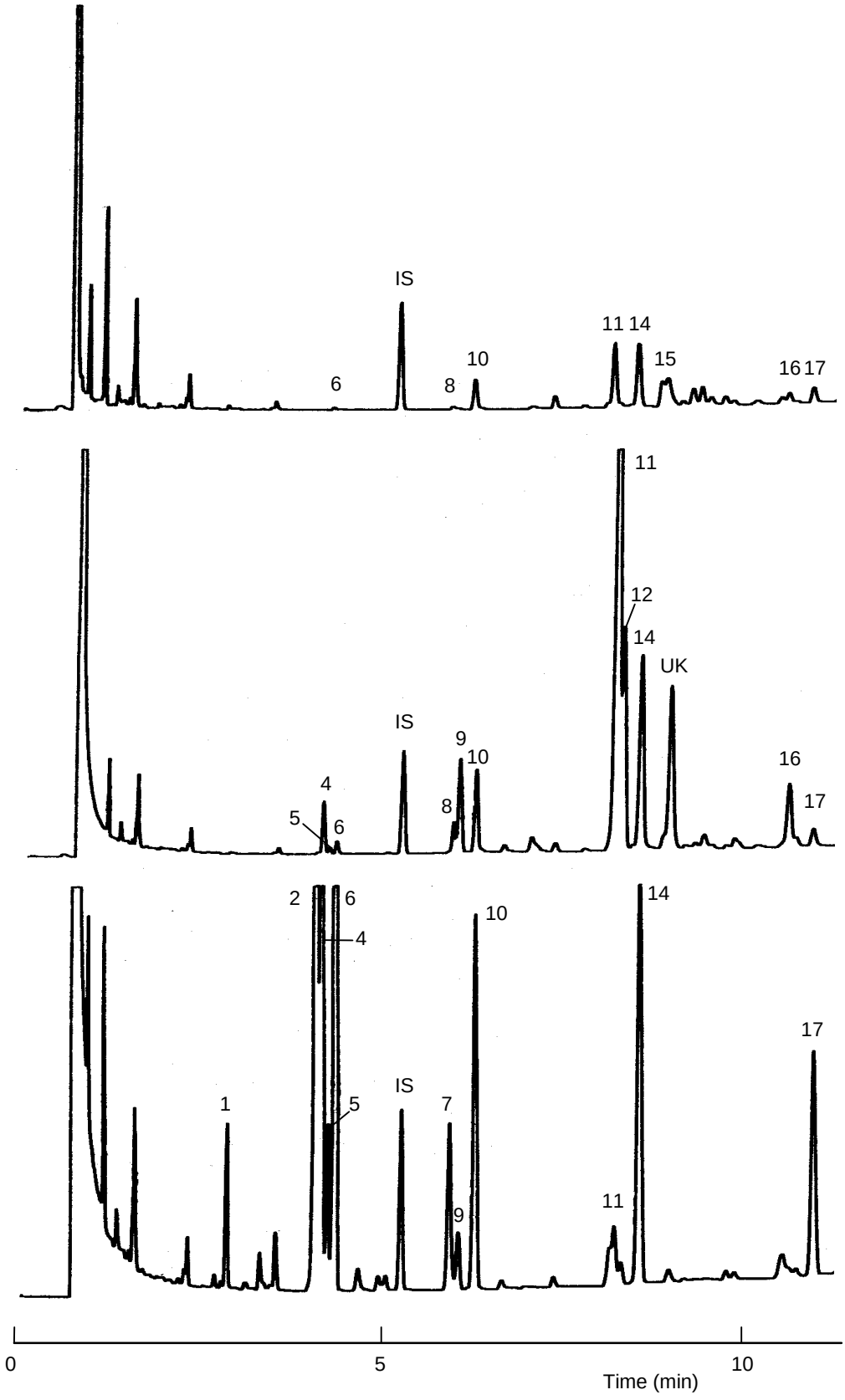
[a] (Mackley et al., 1981; Milstrey, 1983)

✓ present; ✓✓ major component (>25% of total alkenes)

nd not detected; * trace amounts in some fly extracts

Shaded rows indicate key differences between species

FIG. 1. Gas chromatography/flame ionisation detector traces of cuticular hydrocarbon extracts from female buffalo flies; Top: newly-emerged; Centre: 2-3 d-old; Bottom: 13 d-old (IS = internal standard; for assignments of reference numbers see Table 1; UK = unknown).


IS
11 14
15
16 17
10
6
8
11
12
14
UK
IS
9
10
16
17
4
5
6
8
2
6
4
10
14
17
IS
1
5
7
9
11
0
5
10
Time (min)