Chapter 2

THE EFFECTS OF LABORATORY CULTURING ON MUSCALURE QUANTITIES ON FEMALE HOUSEFLIES

Abstract

Using gas chromatography the relative amounts of (Z)-9-tricosene (muscalure) and some other hydrocarbons on the cuticle of 1-20-day-old houseflies (*Musca domestica* L.) from different strains were determined. Flies from a WHO strain, in culture since 1961, and first-generation laboratory-cultured flies from two wild-type strains from a poultry breeding and a cow-house with pigsty, respectively, were compared.

On WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on wild-type females less than 2 % of these compounds was present. Muscalure comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% muscalure was present on the wild-type females.

It is suggested that in mixed populations (both sexes together in a cage) in the course of time muscalure is transferred from females to males and (Z)-9-heptacosene from males to females.

We also compared the amounts of muscalure and some other hydrocarbons on female houseflies, kept in culture in the laboratory for several generations. It appeared that whereas on first-generation wild-type females hardly or no muscalure could be detected, the amounts of this substance had increased considerably after some tens of generations in the laboratory. It is suggested that this was due to selection in subsequent generations of high-density populations. Production of (Z)-9-tricosene and of tricosane was shown to be closely linked. Selection did not affect the production of other cuticular hydrocarbons by the females.

It is concluded that reproduction ability of houseflies does not primarily depend on the amounts of (Z)-9-tricosene on females, although higher amounts of this substance may increase contacts between males and females.

Introduction

The cuticular hydrocarbons of insects provide a barrier to water diffusion (Wigglesworth, 1945; Beament, 1945; Gibbs *et al.*, 1991; Gibbs, 1995) and also play an important part in mate selection by means of chemical communication (Silhacek *et al.*, 1972; Nelson, 1978; Howard & Blomquist, 1982; Blomquist *et al.*, 1987).

Rogoff *et al.* (1964) showed that on the cuticle of female houseflies, *M. domestica*, chemicals are present which influence the behaviour of male houseflies. Since the identification of (Z)-9-tricosene (muscalure) by Carlson *et al.* (1971) and the confirmation that this substance is a part of the female sex pheromone of the house fly by Rogoff *et al.* (1973), many studies have been carried out to reveal the precise effect of the various female cuticular chemicals on the sexual activity of male flies. In 1976 and 1978, Uebel *et al.* showed that cuticular methyl alkanes and the non-hydrocarbon oxidation products of muscalure, (Z)-9,10 epoxytricosene and (Z)-14-tricosen-10-one, also play a role in inducing sexual activity of males towards females. Adams and Holt (1987) found that the cuticular non-hydrocarbon fraction contains sex recognition factors and that the methyl alkane fraction increases contact time between males and females and, thus, has an arresting effect; the latter fraction does not induce mating striking behaviour in males.

Nelson *et al.* (1981) determined the composition of the cuticular hydrocarbon fraction on both females and males and found three groups of long-chain hydrocarbons: (Z)-9-alkenes, *n*-alkanes and methyl alkanes. Methyl alkanes were more abundant in females than in males. Of the total hydrocarbons present on males and females about 97% were components with 23-31 carbon atoms. (Z)-9-tricosene was only present on females, whereas (Z)-9-heptacosene comprised about fifty percent of the total hydrocarbons on adult males and about three percent on females. Nowadays it is widely accepted that the substance inducing sexual behaviour in male *M. domestica* consists of several components, although (Z)-9-tricosene is believed to be the major component of this sex pheromone.

The above studies were all done with flies which had been kept in culture for several years. However, studies of Adler *et al.* (1984) on North American houseflies have shown that 4-11 times less muscalure is present on 10-day-old females than on laboratory-reared females of the same age. It is conceivable that in laboratory-cultured

houseflies and in flies living in more or less isolated environments (e.g. stables) production of cuticular hydrocarbons and sexual behaviour may have changed by genetic drift. In addition, it is known that the production of cuticular hydrocarbons in insects may be affected by environmental factors (Gibbs *et al.*, 1991, Gibbs & Mousseau, 1994, Toolson *et al.*, 1990, Hadley, 1978). Laboratory conditions often differ considerably from environmental conditions in nature and thus may contribute to the differences in production of cuticular hydrocarbons. Thus, for controlling houseflies in, e.g., houses and stables using cuticular hydrocarbons as an attractant, possible differences between laboratory and wild-type strains of houseflies have to be studied.

The present paper reports results of studies on the amounts of hydrocarbons present on houseflies originating from different strains – a WHO laboratory strain and wild-type strains from different sites in The Netherlands - and of different sex and age. In addition, we compared the amounts of hydrocarbons on flies from strains which had been kept in culture for different numbers of generations. One of the main aims of these studies is to find out whether or not selection may play a role in (Z)-9-tricosene production and if and to what extent the production of this substance is related to that of other cuticular hydrocarbons.

Materials and methods

Insects

Experiments were done with *Musca domestica* L. flies from a laboratory strain (WHO Ij2) obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark), and from wild-type strains obtained from a poultry breeding (Van Diermen) and a cow-house with pig-sty (Pesse) in The Netherlands, respectively. In Experiment 1, flies from the WHO strain (in culture since 1961) and first-generation laboratory-cultured flies of the wild-types were used; in Experiment 2 flies from the wild-type strains were used which had been reared in the laboratory for 37, 25, 12 and 12 generations, respectively (‘Diermen 37', ‘Pesse 25', ‘Diermen 12' and ‘Pesse 12' strains). The flies were reared in cages (30 x 30 x 40 cm) in a L12:D12 regime at 25 ^{0}C and r.h. 60%. They were fed (ad libitum) a mixture of sugar, powdered milk and yeast (5 : 5 : 1 by weight). In addition, a fountain filled with tap water was present.

Chemical analyses

In Experiment 1 cuticular hydrocarbons were determined on flies 1, 3, 5, 8, 12 and 20 days after emergence. We mainly concentrated on (Z)-9-tricosene, the major component of the female sex pheromone, and on (Z)-9-heptacosene, which is the most abundant hydrocarbon on males. The flies were taken from cages containing about 30 males and 30 females ('mixed populations'), and from cages containing either about 30 males or 30 females ('isolated populations'). In the latter cases each sex was kept separately from emergence.

Two samples were prepared each containing two flies of the same strain, sex and age in 0.4 ml hexane. Each sample was shaken during 1 min, after which the flies were kept in the fluid for at least 1 hour.

In Experiment 2 cuticular hydrocarbons were determined on single females. The flies were taken from cages containing about 30 males and 30 females. 10 female flies, 8 days old, from each strain were used for analysis. The weight of each fly was determined after which the flies were individually immersed in 0.2 ml hexane. Each preparation was shaken during 1 min, after which the fly was kept in the fluid for at least 1 hour.

Gas chromatography was performed on a Shimadzu GC 17A gas chromatograph. One µl of a solution was injected into a 10 m, 0.32 mm CP-Sil-5 CB column (Chrompack) with injector at 250 ^{0}C and FID at 300 ^{0}C. The flow rate of the helium carrier gas was approx. 1 ml/min. GC oven temperature was programmed from 50 to 300 ^{0}C at 10 ^{0}C/min. 2-Nonanone was used as an internal standard. In each sample hydrocarbons were identified by comparing the retention time with reference runs of alkanes and (Z)-9-alkenes, or with data from literature, and by mass spectrometry. Test runs with C24-C38 n-alkanes demonstrated that all these long-chain chemicals could readily be recovered using the above technique. The lower detection level of the individual hydrocarbons was in the order of 5 ng. The quantities of the hydrocarbons were expressed as percentages of the total quantity of hydrocarbons or in micrograms.

Results

Experiment 1: Figure 1 shows the relative amounts of cuticular (Z)-9-tricosene and

(Z)-9-heptacosene as a function of age for females and males from the isolated and mixed populations of the WHO laboratory strain, and the average amounts of (Z)-9-heptacosene on the Van Diermen and Pesse strains. The amounts of (Z)-9-tricosene on the latter two strains are not shown, since on males of these strains (Z)-9-tricosene could never be detected and on the females the amounts of this substance were very low or below detection level, and did not increase with age.

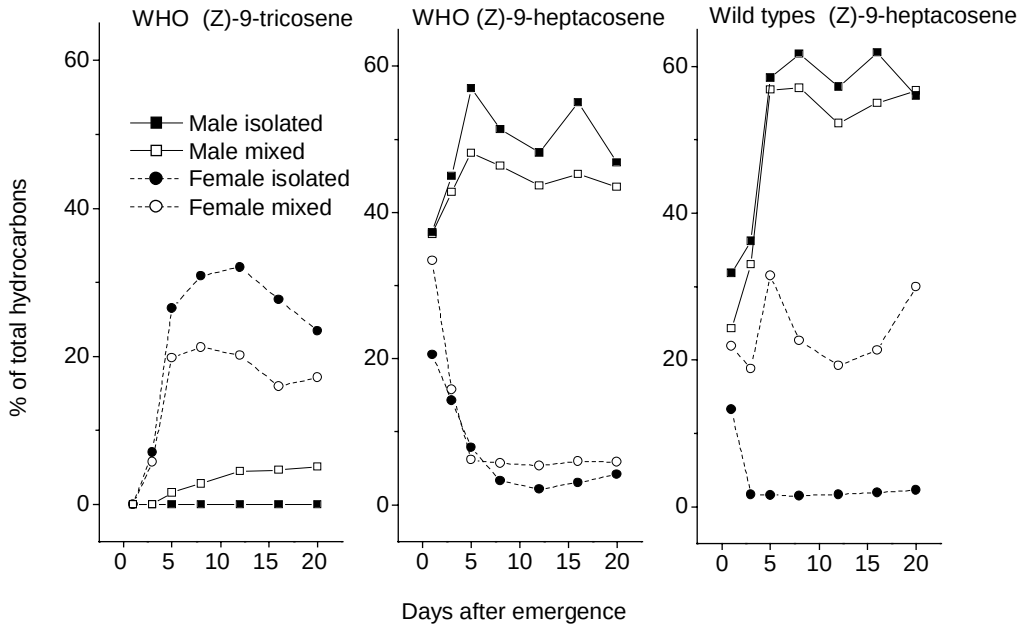

Figure 1. Quantities of (Z)-9-tricosene and (Z)-9-heptacosene as a percentage of the total cuticular hydrocarbons on isolated and mixed populations of the WHO and the wild type strains.

As appears from Fig. 1, on the cuticle of 1-day-old females of both WHO populations no muscalure was present. The relative amounts of (Z)-9-tricosene on females increased with age. On females from both the isolated and mixed populations the relative amount of (Z)-9-tricosene increased up to day 5, remained at about the same level up to day 12 and then decreased slightly. Interestingly, on females of the mixed population the relative amounts were lower than on those of the isolated population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed). Cuticular washes of males of the isolated WHO population never contained (Z)-9-tricosene. However, on males from the mixed population a small amount of (Z)-9-tricosene was found on day 5 after emergence, which gradually increased with age.

On 1-day-old males from both the mixed and isolated WHO populations relatively

large amounts of (Z)-9-heptacosene were present, which increased up to day 5. Beyond that day the relative quantities slightly decreased with age. (Z)-9-heptacosene was found in larger amounts on males of the isolated population than on males of the mixed population ($p < 0.02$; Wilcoxon signed ranks test; 2-tailed). One-day-old WHO females from both populations also contained relatively high amounts of (Z)-9-heptacosene. However, thereafter these amounts gradually decreased on both females of the mixed and the isolated populations up to day 8 and remained at a very low level up to day 20. On females from the mixed population (Z)-9-heptacosene was present in higher quantities than on females from the isolated population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed).

On 1-day-old males from both the mixed and isolated wild-type populations relatively large amounts of (Z)-9-heptacosene were present, which increased up to day 5. Beyond that day the relative quantity remained about the same up to day 20. (Z)-9-heptacosene was found in larger amounts on males of the isolated than on males of the mixed population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed). One-day-old wild-type females from both mixed and isolated populations also contained relatively high amounts of (Z)-9-heptacosene. On females from the mixed population the amounts of this substance remained on this relatively high level up to day 20. However on females of the isolated populations the amounts of (Z)-9-heptacosene were much lower on day 3 and remained at that low level up to day 20.

In Fig. 2 the GC traces of 8-day-old males and females from the three different strains are shown. The peaks of the substances with more than 29 C atoms are not considered because they comprised only about 2 to 3% of the total hydrocarbons. It is evident that compared to females of the WHO strain, the hydrocarbons with up to about 27 C atoms were underrepresented on females of both wild-type strains. (Z)-9-tricosene was even virtually absent on females of the wild-types. It is also clear that the amounts of pentacosene and (Z)-9-heptacosene on females of the three strains were higher in mixed than in isolated populations. No striking differences are seen between the amounts of the hydrocarbons on the males of the various strains. However, the figure shows that (Z)-9-tricosene was not present on WHO males of the isolated populations, but occurred -in small amounts- on males of the mixed populations.

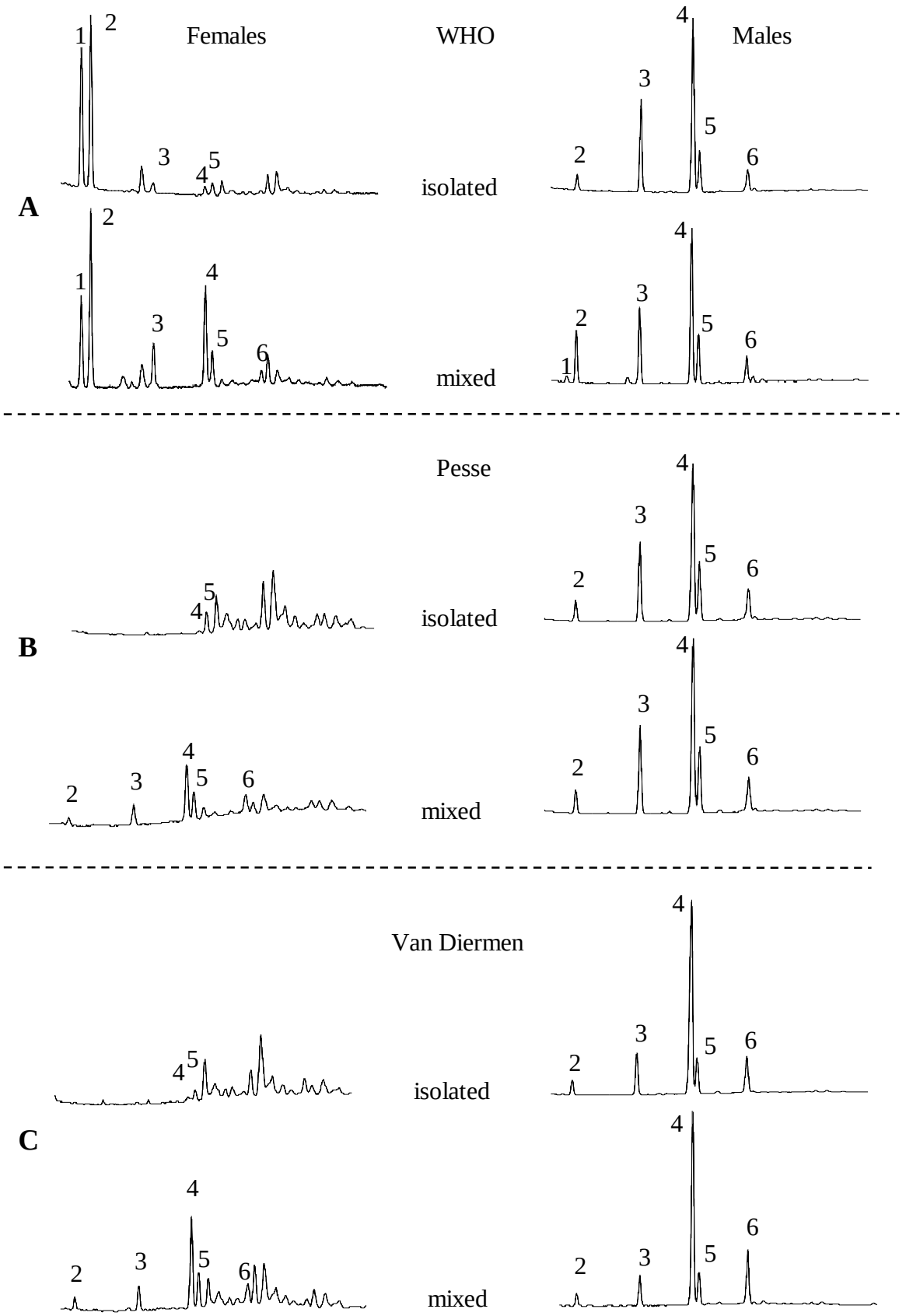

Figure 2. GC traces of the cuticular hydrocarbons of 6-day-old male and female *Musca domestica* from isolated and mixed populations of the 3 strains. A : WHO, B: Pesse, C: Van Diermen.
1: (Z)-9-tricosene, 2: tricosane, 3: pentacosane, 4: (Z)-9-heptacosene, 5: heptacosane, 6: (Z)-9-nonacosene.

Table 1. Relative amounts of a number of hydrocarbons on first-generation laboratory-cultured wild-type and on WHO 8-day-old male and female *M.domestica* from isolated populations.

	WHO		Pesse		Van Diermen	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
(Z)-9-tricosene	0.0	28.2	0.0	0.0	0.0	0.3
tricosane	4.0	34.0	4.9	0.0	3.3	0.0
pentacosane	24.3	2.8	20.6	0.0	10.9	0.8
(Z)-9-heptacosene	52.2	1.6	45.2	1.3	62.9	1.4
heptacosane	11.2	2.9	15.3	6.1	9.5	3.3
(Z)-9-nonacosene	7.5	1.1	9.4	1.3	10.6	0.9
nonacosane	0.9	4.0	0.8	14.3	0.4	8.3
methyl- and dimethylheptacosanes	<0.5	2.8	<0.5	12.6	<0.5	12.4
methyl- and dimethylnonacosanes	<0.5	5.6	<0.5	25.8	<0.5	25.7

In Table 1 the relative amounts of a number of alkanes and alkenes are shown which were found on 8-day-old males and females of the three strains. It appears that on females of the WHO strain the group of hydrocarbons with 23 up to 25 C atoms constituted 65 % of the total hydrocarbons, whereas on females of the wild-type strains less than 2 % of these compounds were present. On females of the wild-type strains nonacosane, and the methyl and dimethyl heptacosanes and nonacosanes were the most abundant chemicals. Males of both laboratory and wild-type strains contain only very small amounts of the methyl and dimethyl heptacosanes and nonacosanes. (Z)-9 heptacosene and pentacosane are the most abundant hydrocarbons on male flies. As in Fig. 2, the table does not show striking differences between the relative amounts of the various hydrocarbons on the bodies of males of the laboratory and wild-type strains.

Experiment 2: Figure 3 shows the relative quantities of muscalure on females from the different strains. It appears that females of the WHO strain had about 10 times more muscalure on their skin than females of the Pesse 12-, Van Diermen 37- and Van Diermen 12-generation strain (Mann-Whitney U test, $p<0.001$) and about 3.5 times more than the

females of the Pesse 25 strain (Mann-Whitney U test, $p<0.001$). The females of the Pesse 25 strain had produced significantly more muscalure than the Pesse 12 and the Diermen

12 and 37 strains (Mann-Whitney U test, $p < 0.05$). No significant differences in muscalure quantities occurred between the Pesse 12, and the Diermen 37 and 12 strains.

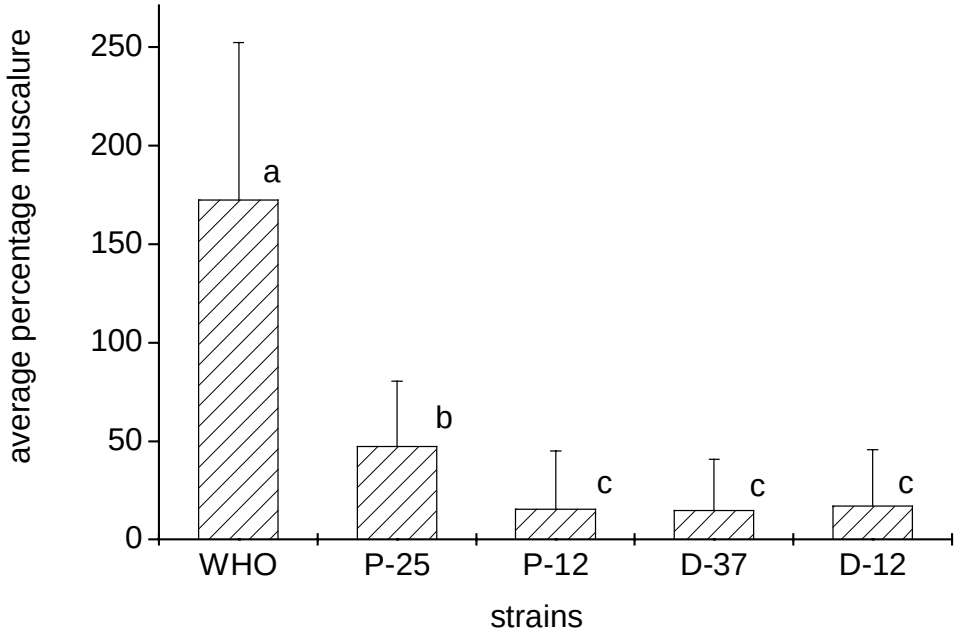

Figure 3. Average percentages (relative to internal standard) of muscalure on females of the WHO strain and the wild-type Pesse 25- and 12-, and Diermen 37- and 12-generation strains. Averages labeled with different letters differ significantly. n=10 for each strain.

In Fig. 4 the correlation coefficients (Pearson correlation test) of the quantities of (Z)-9-tricosene and those of other hydrocarbons on females of the wild-type strains (A) and of the WHO strain (B) are presented. It can be seen that on the wild-type females a high correlation only existed between the amounts of muscalure and tricosane. Quantities of the remaining hydrocarbons showed a low positive or even a negative correlation with muscalure. In the females of the WHO strain, however, the amount of muscalure was significantly correlated to the amounts of 14 out of 22 hydrocarbon GC peaks (Fig. 4B). Here again the correlation between (Z)-9-tricosene and tricosane was by far the highest. Figure 5 shows the relation between the amounts of (Z)-9-tricosene and tricosane on the 40 individual females of the wild-type (A) and the 10 females of the WHO laboratory strains (B).

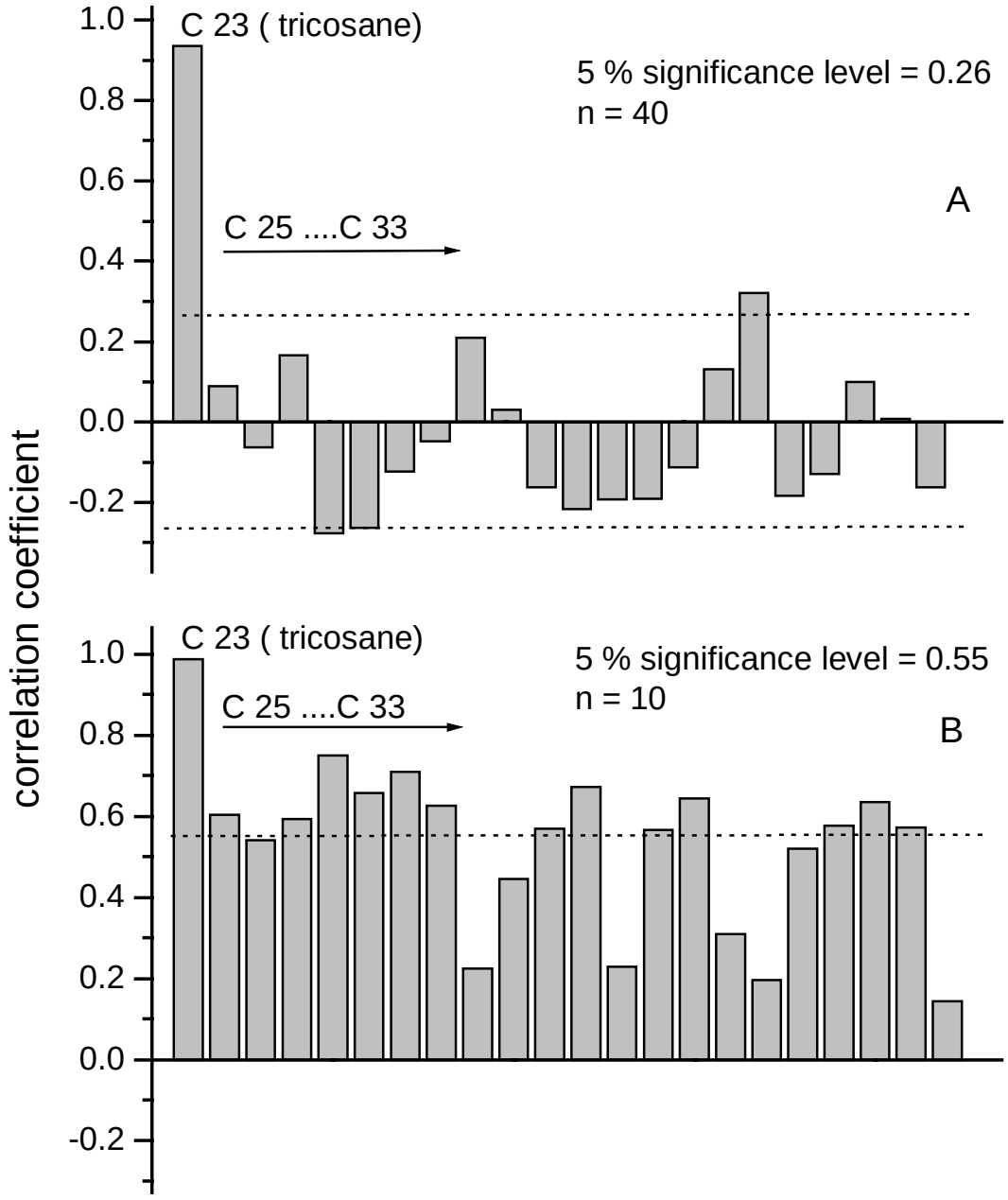

Figure 4. Correlation between (Z)-9-tricosene and other hydrocarbons on the cuticle of 8-day-old females of the wild-type strains (A) and of the WHO strain (B). n=10 for each strain.

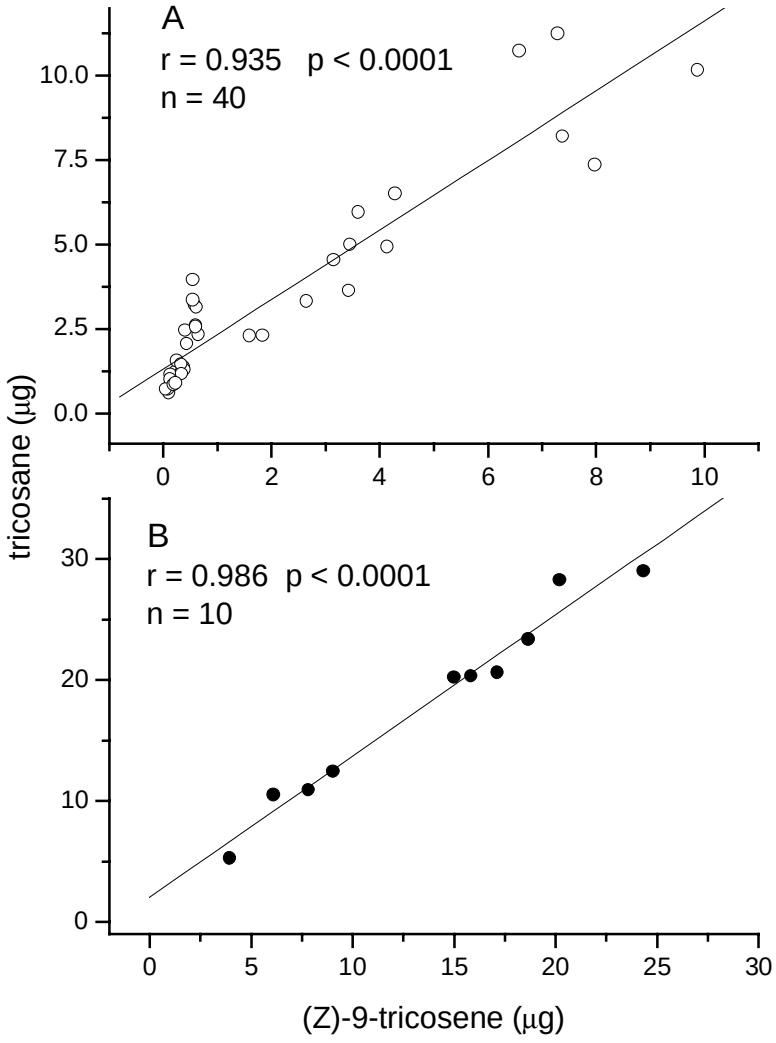

Figure 5. Relationship between the amounts of (Z)-9-tricosene and tricosane on individual females of the wild-type (A) and WHO strains (B).

In Fig. 6 the coefficients of variation (standard deviation as a percentage of the mean) of the hydrocarbons of females of every strain separately are presented. The variation in quantity of (Z)-9-tricosene and to a lesser extent of tricosane was high in Pesse 12 and Van Diermen 12 and 37, the strains in which muscalure production was low (Figure 3). In Pesse 25, in which muscalure production was three times higher, the variations in (Z)-9-tricosene and tricosane quantities were much lower and in the WHO strain these variations were even of the same low order of magnitude as those of the other hydrocarbons identified on the females.

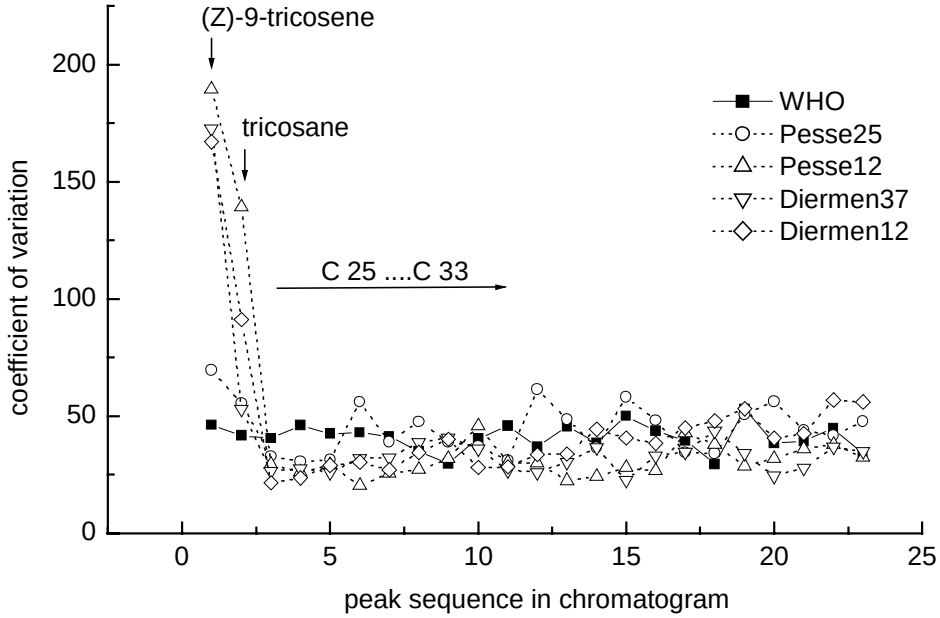

Figure 6. Coefficients of variation of the relative quantities of the hydrocarbons on female flies of the wild-type (dotted lines) and of the WHO strain (solid line). n=10 for each strain.

Cuticular hydrocarbons provide the primary barrier to water loss in terrestrial insects (Beament, 1945; Gibbs *et al.*, 1991; Gibbs, 1995). Therefore, a positive relationship may be expected between the surface of the cuticle of the flies and the quantities of hydrocarbons present on their cuticles. Toolson and Hadley (1979) calculated the surface of scorpions using the equation $S = 15\ (M)^{0.68}$ in which S is the surface area in cm^2 of a scorpion of mass M g. Hadley and Schultz (1987) used the equation $S = 12\ (M)^{0.67}$ as proposed by Edney (1977) to estimate the surface of beetles (M is the mass of a beetle in g and S its surface area in cm^2). We applied both equations on our data but within the measured range of weights (10 to 35 mg) the estimated surface areas showed a strong linear relationship with the body weights of the flies ($r = 0.998$, n=40). Therefore, we chose the body weight of the flies instead of the surfaces as a measure of size.

Figure 7 shows the relationship between the total quantity of cuticular hydrocarbons and bodyweight of 8-day-old females of the wild-type strains. It appears that there is a significant correlation between the weight of the flies and the total amount of hydrocarbons present on their bodies (Fig. 7A). This correlation is a bit stronger when (Z)-9-tricosene and tricosane are excluded (Figure 7B).

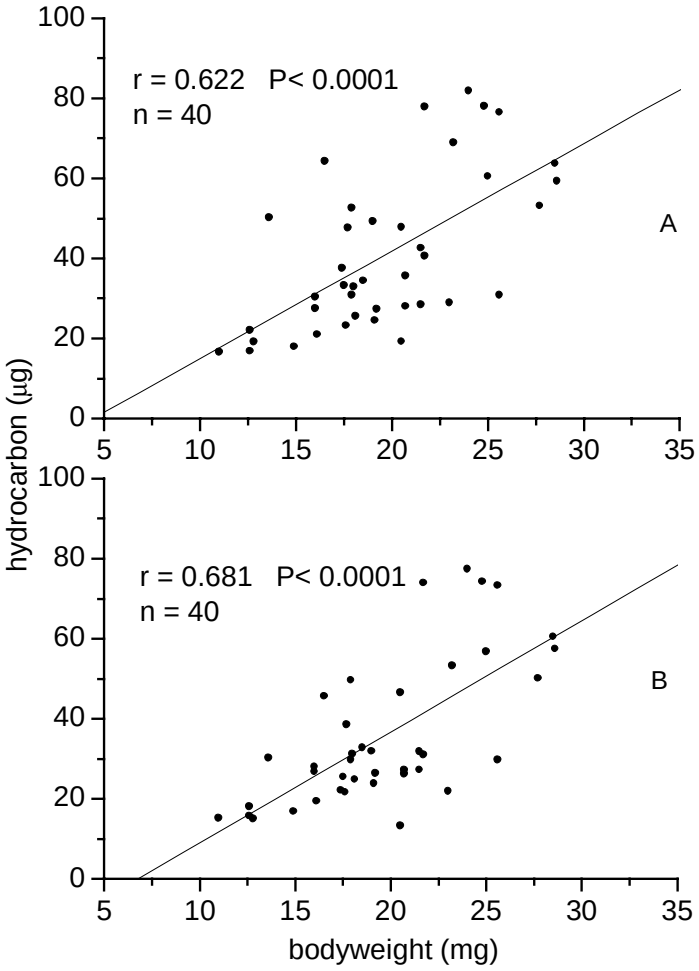

Figure 7. The total quantities of cuticular hydrocarbons (A) and the quantities of cuticular hydrocarbons with (Z)-9-tricosene and tricosane not taken into account (B) as a function of bodyweight of 8-day-old female wild-type houseflies.

To get more insight into the relation between body weight and individual cuticular hydrocarbons we calculated the correlation coefficients of the quantities of 23 cuticular hydrocarbons peaks and body weight of 8-day-old wild-type females (Figure 8). The weight of the flies and the amount of each of cuticular hydrocarbon (or hydrocarbon group) are positively correlated except for (Z)-9-tricosene and tricosane. The latter pair showed a negative although not significant correlation with bodyweight (muscalure: r = -0.148, tricosane: r = -0.009, n = 40). Correlation between bodyweight and cuticular hydrocarbons for females of the WHO strain is not presented, since the range in bodyweight of these flies was to small to draw any conclusions.

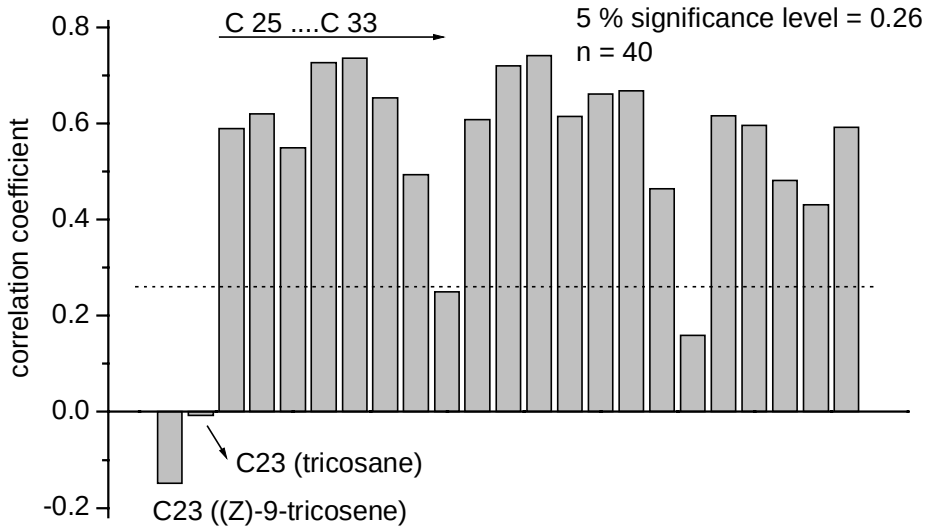

Figure 8. Correlation between the amounts of cuticular hydrocarbons and bodyweight in 8-day-old wild-type female flies (10 flies from each strain).

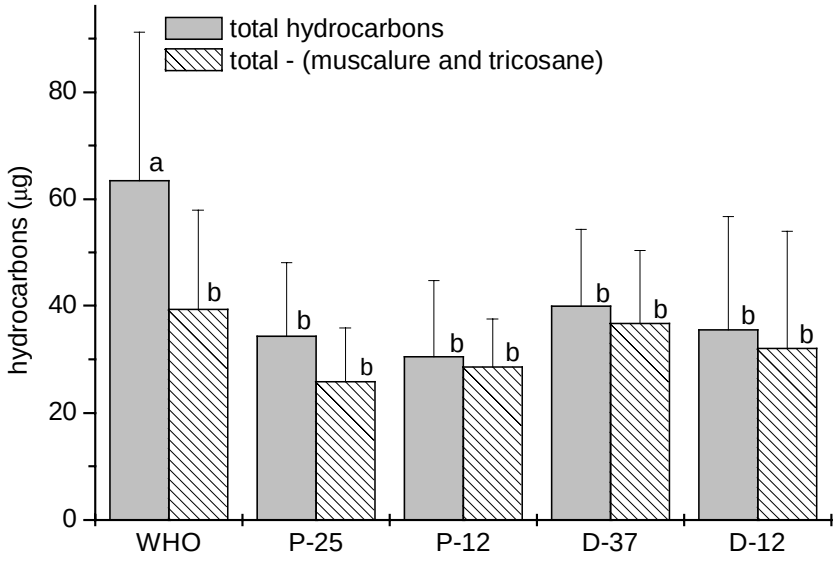

Figure 9. Average relative hydrocarbon quantities on laboratory WHO and wild-type female (*Musca domestica*) corrected for bodyweight. Averages labeled with different letters differ significantly (10 flies from each strain).

Figure 9 presents data for the total amounts of cuticular hydrocarbons and for the total amounts minus (Z)-9-tricosene and tricosane on the body of females from each

strain. For a reliable comparison the amounts are corrected for bodyweight. Significant differences only occurred between the total hydrocarbon amounts of the WHO strain and the four wild-type strains. However, Fig. 9 also shows that these differences were caused by the high amounts of muscalure and tricosane present on the skin of females from the WHO strain compared to the wild-type strains.

Discussion

We found remarkable differences in the composition of cuticular hydrocarbons of females from the WHO laboratory strain on the one hand and from two wild-type strains on the other hand. Whereas in the cuticular washes of 5-20-day-old females of the WHO strain (Z)-9-tricosene comprised up to 20-30 % of the total cuticular hydrocarbons, no (Z)-9-tricosene was found on females of the Pesse strain and less than 0.5 % on those of the Van Diermen strain. These differences were even larger than those observed by Adler *et al.* (1984) who found 4-11 times more muscalure on laboratory strains of houseflies than on those of wild-type strains. The amounts may differ between laboratory strains as well. Nelson *et al.* (1981), e.g., found 4.3 % (Z)-9-tricosene on 4-day-old females of a laboratory strain, whereas around 10% of this substance was present on our WHO females of that age.

Adams and Nelson (1990) studied the effect of diet on pheromone production in female houseflies of the so-called 'Orlando Regular' strain. These authors found that the amounts of (Z)-9-tricosene were not affected by the food offered to the flies, which consisted of either sugar or protein. This was in contrast to the amounts of (Z)-9-heptacosene, which appeared to be significantly higher on females fed with sugar than on those fed with protein. Since all our strains were fed the same mixture of sugar, lipids and proteins, it is very unlikely that the differences in (Z)-9-tricosene quantities between WHO and wild-type females were due to differences in essential food constituents. Assuming that females with higher quantities of (Z)-9-tricosene on their bodies are more attractive to males leads to the suggestion that selection over the years in laboratory cultures may have led to the relatively high production of (Z)-9-tricosene in the laboratory strain.

The amounts of (Z)-9-tricosene on WHO females from the mixed populations were

lower than those on WHO females from the isolated populations, whereas some (Z)-9-tricosene was present on males of the mixed populations but absent on males of the isolated populations. The amounts of (Z)-9-tricosene on males gradually increased and those on females decreased in the course of time, which suggests that transfer of (Z)-9-tricosene from females to males took place due to physical contact between the sexes. This agrees with the results of Ahmad *et al.* (1989) who showed that during copulation an average of 4.1 % topically applied radiolabelled (Z)-9-tricosene on the abdomen of a female was transferred to the body of a male. A similar effect can be observed for the most abundant hydrocarbon in males, (Z)-9-heptacosene. In this case transfer may have taken place from males to females. This is strongly suggested by the fact that higher amounts of this substance were present on females from mixed populations than on those from isolated populations. The large differences in relative amounts of (Z)-9-heptacosene on females from the mixed and isolated wild-type strains compared to those on the mixed and isolated WHO females (Fig. 1) is due to the very low absolute amounts of (Z)-9-tricosene on wild-type females. The relatively high amounts of (Z)-9-heptacosene we found on 1-day-old males and females are in accordance with the findings of Tillmann-Wall *et al.* (1992). These authors found that microsomes prepared from 1-day-old males and females produced (Z)-9-heptacosene as the major alkene. So far, the behavioural function of (Z)-9-heptacosene is unknown. The large differences in the amounts of this substance between the sexes suggest some role in sexual behaviour. Although Rao *et al.* (1990) suggested that (Z)-9-heptacoseneis a component of the male sex pheromone the data presented by these authors, in our opinion, does not support that conclusion. It is not clear why the quantities of hydrocarbons with backbones consisting of 23 to 25 C atoms found on 8-day-old wild-type females are much lower than those found on WHO females. This difference also holds true for flies of other ages (chromatograms not shown).

Transfer of hydrocarbons between the sexes in the course of time, as appears to occur with (Z)-9-tricosene and (Z)-9-heptacosene may result in modification of the original male and female cuticular hydrocarbon composition. Possibly this enables one sex to recognise whether the other sex has already copulated or not.

Both the Van Diermen and Pesse strains easily survived in their ‘natural’ environments and, in addition, can readily be maintained in the laboratory. This questions the role (Z)-9-tricosene may actually play in mating behaviour. (Z)-9-tricosene enhances

sexual activity in male houseflies (Mansingh *et al.*,1972; Uebel *et al.*, 1976; Adams and Holt, 1987; La-France *et al.* 1989; Lemke *et al.*,1990; Islam and Port, 1994) which may explain its relatively high production by the females in the laboratory where environmental conditions are constant and not extreme and were selection pressure is high. However, our results suggest that it is not decisive for mating. To investigate this, studies on the sexual behaviour of the various strains have been initiated.

From the results of Experiment 2 it can be concluded that after some tens of generations in the laboratory the amounts of (Z)-9-tricosene had increased considerably. Now, females of the Pesse 25-generation contained only about 3.5 times less (Z)-9-tricosene and those of the Pesse 12- and Van Diermen 12- and 37-generation about 10 times less (Z)-9-tricosene than the WHO females (Fig. 3). This appears to confirm our suggestion that selection in subsequent generations of high-density populations may lead to increased production of (Z)-9-tricosene by the females. Apparently, higher amounts of this substance increase the attractiveness of females to males.

As already said above, Adams and Nelson (1990) showed that sucrose-fed and protein-fed *M. domestica* females did not differ in amount of cuticular (Z)-9-tricosene. However, the sugar-fed females contained significantly higher quantities of (Z)-9-heptacosene. In our laboratory cultures, both the wild-type and WHO flies were fed the same diet, containing proteins as well as sucrose, and they were reared at the same temperature and r.h. Thus, food and environmental factors are not likely to account for the differences in the production of (Z)-9-tricosene which we observed between the Pesse 25- and the Van Diermen 37-generation. Obviously, selection pressure differed between these two strains.

Toolson *et al.* (1990) found that on both male and female *Drosophila majovensis* collected from the field, lower quantities of total hydrocarbons were present than on flies from laboratory strains. Gibbs *et al.* (1991) showed that variation in melting temperature of cuticular lipids of the grasshopper *Melanoplus sanguinipes* was mostly determined by geographic distribution, followed by family effects and rearing regimes. Their results provided evidence for genetic differences in the biophysical properties of surface lipids in natural populations. Toolson and Hadley (1979) observed marked seasonal changes in the relative abundance of cuticular hydrocarbons of the scorpion *Centruroides sculpturatus*, the percentage of *n*-alkanes in the epicuticular hydrocarbon decreasing considerably in warmer months, whereas the proportion of branched alkanes increased.

These seasonal changes in epicuticular hydrocarbon composition probably account for much of the concomitant changes in cuticular permeability to water. Hadley and Schultz (1987) also observed a correlation between water loss rates and quantities and composition of cuticular hydrocarbons. In the tiger beetle *Cicindela obsoleta*, a summer-active species that inhabits dry grasslands, the cuticular hydrocarbon fractions only contains saturated hydrocarbons, whereas on the body of *C. oregona*, a spring- and fall-active species which lives along water courses, about 50% of the hydrocarbons are unsaturated. The presence of unsaturated hydrocarbons is thought to increase the permeability of the hydrocarbon layer to water. Indeed, Hadley and Schultz (1987) found a significant negative correlation between water loss rate and the quantity of saturated hydrocarbons. Gibbs *et al.* (1995) suggested that a potential consequence of pheromone production by female *Musca domestica* may increase cuticular transpiration because the pheromone components are unsaturated or methyl-branched. This tends to lower melting temperatures. It may be that in nature, where high temperatures and low humidities may occur, the production of (Z)-9-tricosene by female houseflies is low or absent, as the presence of this substance may negatively interfere with the water barrier function of the cuticular lipid layer.

In both the wild-type and WHO strains the quantities of (Z)-9-tricosene and tricosane on the females were strongly correlated (Table 1, Figs. 4 and 5). Although in the WHO females the amounts of (Z)-9-tricosene also showed a significant positive correlation with several other hydrocarbons, the correlation between (Z)-9-tricosene and tricosane was by far the highest. In addition, we found no positive correlation between the weight of the females and the (Z)-9-tricosene and tricosane quantities on their bodies, whereas a positive correlation did exist between female weight and the amounts of other cuticular hydrocarbons (Figures 5 and 6). Comparison of the total cuticular hydrocarbons quantities on females of the various strains, after correction for body weight, revealed that selection led tot an increase in both (Z)-9-tricosene and tricosane quantities but did not affect the total amount of the other cuticular hydrocarbons (Figure 7). Moreover, the variation in (Z)-9-tricosene and tricosane quantities between females decreased and ultimately reached the same, relatively low level of that of the other hydrocarbons on the females (Figure 7). These results again strongly suggest that the production processes of (Z)-9-tricosene and tricosane are closely linked and confirm that (Z)-9-tricosene is only synthesized in large amounts under certain environmental conditions and/or selective

pressure. This, however, does not seem to affect the production of other cuticular hydrocarbons by the females. Since the quantities of these substances are positively related with size (body weight) of the females, we assume that these chemicals may (mainly) act as a water barrier preventing desiccation of the flies.

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