Cuticular hydrocarbons corroborate the distinction between lowland and highland Natal fruit fly (Tephritidae, Ceratitis rosa) populations

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Abstract

The cuticular hydrocarbons (CHs) and morphology of two Ceratitis rosa Karsch (Diptera: Tephritidae) populations, putatively belonging to two cryptic taxa, were analysed. The chemical profiles were characterised by two-dimensional gas chromatography with mass spectrometric detection. CHs of C. rosa that originated from the lowlands and highlands of Kenya comprised of n-alkanes, monomethylalkanes, dimethylalkanes and unsaturated hydrocarbons in the range of the carbon backbone from C14 to C37. Hydrocarbons containing C29, C31, C33 and C35 carbon atoms predominated in these two populations. 2-Methyltriacontane was the predominant compound in both populations. Quantitative differences in the distribution of hydrocarbons of different chain lengths, mainly the C22, C32, C33 and C34 compounds of these two populations, were observed despite indistinct qualitative differences in these hydrocarbons. Morphological analyses of male legs confirmed that the flies belong to different morphotypes of C. rosa previously labelled as R1 and R2 for lowland and highland populations, respectively. A statistical analysis of the CH compositions of the putative R1 and R2 species showed distinct interspecific identities, with several CHs specific for each of the lowland and highland populations. This study supports a hypothesis that the taxon C. rosa consists of at least two biological species.
Keywords

*Ceratitis rosa*, cryptic species, chemotaxonomy, GC×GC/MS, integrative taxonomy

Introduction

Sexual selection within populations can play an important role in speciation when divergence in mating signals and corresponding mate preference occur along different evolutionary trajectories in different populations (Jennings et al. 2014). In fruit flies (Diptera, Tephritidae), one potential target of sexual selection may be the blend of hydrophobic compounds on their cuticle, which often show intra- and interspecific variation, sexual dimorphism and may act as short-range pheromones (Carlson and Yocom 1986, Goh et al. 1993, Sutton and Carlson 1993, Vaníčková et al. 2012b, Vaníčková et al. 2014, Vaníčková et al. 2015). These compounds, cuticular hydrocarbons (CHs), play a major role in desiccation resistance, waterproofing, and/or mate choice, and may be under selection if particular components confer a mating advantage or increase the fitness of the resulting offspring (Howard and Blomquist 2005, Blomquist and Bagnères 2010, Gibbs 2011, Jennings et al. 2014). Characteristics of the CHs blend can vary with the diet, sex, age and geographic origin of a species and population (Blomquist and Bagnères 2010, Jennings et al. 2014).

In species of the fruit fly genus *Ceratitis*, courtship generally includes visual, auditory, tactile and olfactory cues (Shelly 2000, Aluja and Norrbom 2001, Yuval and Hendrichs 2001, Shelly et al. 2007). During courtship, male-borne volatiles are recognised in the initial phase and are detected by olfactory sensillae on the fly’s antennae, while less volatile compounds, such as CHs, may be exchanged during later courtship stages, when the male touches the female with legs and proboscis (Aluja and Norrbom 2001). The courted female chooses whether or not to mate with the male based on the quality of the various signals that he emits. Signal-preference co-evolution may provide mechanisms for both mate recognition and sexual selection in the early stages of population divergence, which may eventually lead to speciation (Jennings et al. 2014).

The Natal fruit fly, *Ceratitis rosa* Karsch (Diptera, Tephritidae), is a polyphagous species attacking a wide range of fruits on the African mainland. It has invaded some Indian Ocean islands, where it displaced the similarly introduced *C. capitata* (De Meyer 2000, De Meyer 2001, Duyck et al. 2004). *Ceratitis rosa*, together with *C. fasciventris* and *C. anona*, are a closely related group of morphologically similar taxa known by researchers as the FAR species complex. The FAR complex has recently been studied by chemical, molecular genetic and morphological approaches to allow for discrimination of the putative species of this cryptic species complex (De Meyer 2001, De Meyer and Freidberg 2006, Virgilio et al. 2012, 2013, Vaníčková et al. 2014). Within the FAR complex, five genotypic groups have been identified and labeled as A (for *C. anona*), F1 and F2 (for two *C. fasciventris* populations), and R1 and R2 (for two *C. rosa* populations) (Virgilio et al. 2013).

The chemical analyses of the cuticular hydrocarbon profiles of these putative species found significant differences between the A, F2 and R2 genotypes and characterised
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chemotaxonomic markers to distinguish these groups (Vaníčková et al. 2014). More recently, research has focused on the two C. rosa types, largely because C. rosa is considered the most economically important species within the complex (De Meyer 2001, Quilici et al. 2002, Baliraine et al. 2004). Adult males of the two C. rosa types can be differentiated based on the characters of the male, but not female, mid tibia (De Meyer et al. 2015); while analysis of wing land-marks using geometric morphometrics gives only a partial separation of the five FAR complex genotypes (Van Cann et al. 2015). Additional markers for the R1 and R2 populations are therefore needed.

The literature provides conflicting information regarding developmental physiology and climatic niche for C. rosa. Some studies indicate that C. rosa might be more tolerant of colder and wetter conditions than C. capitata (Duyck et al. 2004), suggesting greater potential for establishment in temperate regions (De Meyer et al. 2008). However, Grout and Stoltz (2007) indicate that C. rosa prefers hot and wet conditions. A re-analysis of the distributional data and historical material in collections shows that this might be because of the failure to differentiate between the two types (R1 and R2) that were indicated by the microsatellite study (Virgilio et al. 2013). R2 appears to occur at lower latitudes on the African continent and at higher altitudes – hereafter referred to as ‘highland’ type. It might be more cold resistant than the R1 type, which is absent from the colder parts (lower latitudes, higher altitudes) within the geographic range of C. rosa – hereafter referred to as ‘lowland’ or ‘coastal’ type (Tanga et al. 2015). The cold resistance may be directly connected to the cuticle composition as previously reported for other Diptera e.g. Drosophila sp. (Gibbs et al. 1997, Rouault et al. 2001, Rouault et al. 2004), and Anopheles sp. (Wagoner et al. 2014). With respect to CHs amount/n-alkane length, it is assumed that a reduction in water loss is the outcome of lower surface-area-to-volume ratio and reduced cuticle permeability, respectively (Rouault et al. 2004, Blomquist and Bagnères 2010, Gibbs 2011). Combining this background knowledge, leads us to hypothesize that CHs are likely to vary between R1 and R2 populations.

The purpose of the present study was, therefore, to identify the chemical constituents of the CHs and to analyse their variation between two populations of C. rosa (one highland and one lowland - based on morphological differentiation) originating from Kenya. These two populations were chosen for this study because they had previously been shown to be sexually incompatible (Ekesi et al. unpublished data), as well as having distinct male-borne volatile profiles (Kalinová et al. unpublished data). Additional to inter-population differences, we also evaluated sexual dimorphism in CHs composition within each population.

**Methods**

**Insects**

Pupae of two laboratory populations of C. rosa were obtained from the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). The source
colonies were established in 2012 and came from one lowland locality [Mwajamba, Msambweni, Coast Province, 04°18.21'S; 39°29.88'E, host fruit *Psidium guajava* (Myrtaceae), altitude 106 m, average temperature 28.1 °C] and one highland locality [Kithoka, Meru, Central Province, 00°05.59'N; 37°40.40'E, host fruit *Mangifera indica* (Anacardiaceae), altitude 1425 m, average temperature 21.5 °C] in Kenya (see Appendix). The pupae (F₁ generation) were kept under identical laboratory conditions at the Institute of Organic Chemistry and Biochemistry (IOCB, Prague, Czech Republic). Flies were separated by sex within 24 hours of eclosion, fed on an artificial diet consisting of cane sugar and enzymatic yeast hydrolysate (in the ratio 3:1) and mineral water and kept at a relative humidity of 60%, at 25 °C, and a 12L:12D photoperiod.

**Chemical analyses**

The extraction of the cuticular hydrocarbons of 20-day-old virgin males (*N* = 10) and females (*N* = 10) of the R₁ and R₂ morphotypes (resulting in *N* = 20 for R₁ and *N* = 20 for R₂) followed the methodology described in Vaničková et al. (2012b) and Vaničková et al. (2014). Flies were frozen at -18 °C and placed for 15 minutes into a desiccator to remove the surface moisture. In order to extract CHs from insect body surface individual fly was placed in small glass vials, which contained 0.5 mL of hexane (Fluka, Germany) and gently agitate for 5 minutes. 1-Bromdecane (Sigma-Aldrich, Czech Republic) was used as an internal standard for quantification (10 ng per 1 µL of the extract). Each extract was concentrated to approximately 100 µL by a constant flow of nitrogen and stored in a freezer (-5 °C) until analysis.

Two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC/MS) was used for the quantification and identification of CH profiles. The analyses were performed on a LECO Pegasus 4D instrument (LECO Corp., St. Joseph, MI, USA) equipped with a non-moving quad-jet cryomodulator. A DB-5 column (J&W Scientific, Folsom, CA, USA; 30 m × 250 µm i.d. × 0.25 µm film) was used for GC in the first dimension. The second-dimension analysis was performed on a polar BPX-50 column (SGE Inc., Austin, TX, USA; 2 m × 100 µm i.d. × 0.1 µm film). Helium was used as a carrier gas at a constant flow of 1 mL min⁻¹. The temperature program for the primary GC oven was as follows: 150 °C for 2 min, then 150–300 °C at 5 °C min⁻¹, and finally a 10 min hold at 320 °C. The program in the secondary oven was 10 °C higher than in the primary one and was operated in an iso-ramping mode. The modulation period, the hot-pulse duration and the cool time between the stages were set to 3.0, 0.4 and 1.1 sec, respectively. The transfer line to the TOFMS was operated at 260 °C. The source temperature was 250 °C with a filament bias voltage of −70 eV. The data-acquisition rate was 100 Hz (scans/sec) for the mass range of 29–400 amu. The detector voltage was 1750V. For each sample, 1µL was injected in splitless mode. The inlet temperature was 200 °C. The purge time was 60 sec at a flow of 60 mL min⁻¹. The data were processed and consecutively visualized on 2D and 3D chromatograms using LECO ChromaTOF™ software. The *n*-alkane standard (C₈–C₃₈; Sigma-Aldrich) was co-injected with authentic samples.
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...to determine the retention indices ($RI$) of the analytes. The hydrocarbons were identified by a comparison of their mass spectra fragmentation patterns and $RI$ (Van Den Dool and Kratz 1963, Carlson and Yocom 1986, Vaníčková 2012, Vaníčková et al. 2014).

**Morphological identification**

Male specimens were shipped to the Royal Museum for Central Africa (RMCA), Tervuren, Belgium, where identifications were confirmed by M. De M. based on the pilosity and coloration of mid tibia (Virgilio et al. 2013, De Meyer et al. 2015).

**Statistics**

The relative peak areas of 46 CH compounds (as identified by the GC×GC/MS in the deconvoluted total-ion chromatogram mode) were calculated in 10 replicate specimens for each sex of the two species ($N = 40$). Following Clarke (1993), we log-transformed the multivariate data in order to reduce the differences in scale between the variables while preserving information on the relative abundance of CHs across specimens.

A heat map was used to visualise the complex data sets organised as matrices. Heat maps make it possible to identify differences in the relative amounts of CHs between populations, with different compounds tending to form small clusters according to their quantities. To achieve this, the heat map performed two actions on a matrix of chromatographic peak areas. First, it reordered the rows and columns so that rows and columns with similar profiles were closer to one another, causing these profiles to be more visible to the eye. Second, each entry in the data matrix was displayed in a different colour, making it possible to view the patterns graphically. The dendrograms were created using correlation-based distances and the Ward method of agglomeration was applied in the present analysis (Key 2012).

To examine the differences between the two populations and sexes further, the percentage contribution of each compound to the average dissimilarity between the aforementioned factors was calculated with similarity percentage analysis (SIMPER) (Clarke 1993). All computations were performed with R 3.1.2 language and environment (R Core Team 2014) and the R packages `gplots` (Warnes et al. 2015) and `vegan` (Oksanen et al. 2015).

**Results**

**CHs composition**

The GC×GC/MS analyses identified 46 peaks. The chain-length of the carbon backbones ranged from $C_{14}$ to $C_{37}$. The hydrocarbon profiles of the males and females
included 5 n-alkanes, 19 methylbranched alkanes, 19 unsaturated alkanes, squalene, 1 aldehyde and 1 unidentified compound. The heat map characterised differences in the relative amounts of CHs between the C. rosa flies originating from highland and coastal regions (Figure 1). Marked quantitative differences were observed in the peaks between the two populations and genders. The most prominent peaks in all of the chromatograms were 2-methyltriacontane (2-MeC₃₀, RI 3064, CH₂₃) and tritriacontene (C₃₃:1, RI 3240, CH₃₁) (Figures 1–3).

Sexual dimorphism in CHs

The CH profiles of the virgin males and females differed qualitatively. SIMPER analyses, comparing conspecific males and females, revealed sex-specific compounds. In females the most abundant compounds were docosene (C₂₂:₁, RI 2182, CH₁₁), hentriacontene (C₃₁:₁, RI 3082, CH₂₄), 3-methyldotriacontane (3-MeC₂₃₂, RI 3272, CH₃₂) and tritriacontene (C₃₃:₁, RI 3280, CH₃₃) (Table 1). In males, the compounds shared by coastal and highland flies were identified as tritriacontene (C₃₃:₁, RI 3292,
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Table 1. A comparison of the average abundance of important cuticular hydrocarbons between two morphotypes of *Ceratitis rosa* [coastal R1, highland R2]. The compounds are listed in the order of their contribution ($\delta_i$) to the average dissimilarity $5(\delta_i)$ between the two groups, with a cut-off when the cumulative percent contribution ($\sum \delta_i$%) to $\delta_i$ reaches 70%. The numbering of the compounds corresponds to Figure 1.

| No. | Compound | RI | Abundance | $\delta_i$ | $\delta_i$/SD($\delta_i$) | % contr. diss. | $\sum \delta_i$% |
|-----|----------|----|-----------|-----------|--------------------------|----------------|-----------------|
| 15  | 2-MeC$_{28}$ | 2865 | 1.037 | 1.731 | 0.016 | 3.384 | 0.104 | 15 |
| 11  | C$_{22,1}$ | 2182 | 0.425 | 0.995 | 0.015 | 1.592 | 0.096 | 11 |
| 35  | diMeC$_{31}$ | 3297 | 0.883 | 0.447 | 0.010 | 2.884 | 0.065 | 35 |
| 26  | diMeC$_{28}$ | 3105 | 1.575 | 1.147 | 0.010 | 4.198 | 0.064 | 26 |
| 29  | 3-MeC$_{31}$ | 3178 | 1.093 | 0.797 | 0.007 | 3.241 | 0.044 | 29 |
| 12  | C$_{17,1}$ | 2622 | 0.198 | 0.419 | 0.006 | 1.531 | 0.039 | 12 |
| 16  | diMeC$_{26}$ | 2902 | 0.613 | 0.869 | 0.006 | 2.443 | 0.039 | 16 |
| 30  | diMeC$_{29}$ | 3205 | 0.395 | 0.196 | 0.005 | 2.623 | 0.030 | 30 |
| 37  | MeC$_{33}$ | 3331 | 0.721 | 0.906 | 0.004 | 1.826 | 0.029 | 37 |
| 36  | C$_{34,1}$ | 3308 | 0.815 | 0.650 | 0.004 | 1.389 | 0.028 | 36 |
| 38  | C$_{34,1}$ | 3342 | 0.206 | 0.370 | 0.004 | 1.431 | 0.026 | 38 |
| 23  | 2-MeC$_{30}$ | 3064 | 2.045 | 1.882 | 0.004 | 1.633 | 0.026 | 23 |
| 2   | unknown | 1402 | 0.816 | 0.649 | 0.004 | 2.035 | 0.025 | 2 |
| 27  | 7-/9-MeC$_{31}$ | 3142 | 1.044 | 0.882 | 0.004 | 1.768 | 0.024 | 27 |
| 1   | C$_{14}$ | 1400 | 0.934 | 0.771 | 0.004 | 1.826 | 0.024 | 1 |
| 34  | C$_{31,1}$ | 3291 | 0.378 | 0.261 | 0.004 | 1.134 | 0.024 | 34 |
| 28  | MeC$_{31}$ | 3152 | 0.317 | 0.227 | 0.004 | 1.695 | 0.023 | 28 |

| No. | Compound | RI | Abundance | $\delta_i$ | $\delta_i$/SD($\delta_i$) | % contr. diss. | $\sum \delta_i$% |
|-----|----------|----|-----------|-----------|--------------------------|----------------|-----------------|
| 11  | C$_{22,1}$ | 2182 | 0.435 | 1.415 | 0.022 | 2.485 | 0.133 | 11 |
| 15  | 2-MeC$_{28}$ | 2865 | 1.186 | 1.768 | 0.013 | 2.817 | 0.079 | 15 |
| 29  | 3-MeC$_{31}$ | 3178 | 1.078 | 0.599 | 0.011 | 4.145 | 0.065 | 29 |
| 26  | diMeC$_{28}$ | 3105 | 1.534 | 1.191 | 0.008 | 2.824 | 0.047 | 26 |
| 34  | C$_{34,1}$ | 3291 | 0.352 | 0.112 | 0.007 | 1.789 | 0.044 | 34 |
| 28  | MeC$_{31}$ | 3152 | 0.384 | 0.066 | 0.007 | 3.160 | 0.043 | 28 |
| 33  | C$_{33,1}$ | 3280 | 1.353 | 1.444 | 0.005 | 1.337 | 0.029 | 33 |
| 30  | diMeC$_{29}$ | 3205 | 0.362 | 0.148 | 0.005 | 2.727 | 0.029 | 30 |
| 27  | 7-/9-MeC$_{31}$ | 3142 | 1.043 | 0.835 | 0.005 | 1.875 | 0.029 | 27 |
| 36  | C$_{34,1}$ | 3308 | 0.705 | 0.639 | 0.005 | 1.373 | 0.029 | 36 |
| 1   | C$_{14}$ | 1400 | 0.732 | 0.925 | 0.005 | 1.791 | 0.028 | 1 |
| 42  | C$_{35,2}$ | 3460 | 1.263 | 1.223 | 0.004 | 1.381 | 0.027 | 42 |
| 2   | unknown | 1402 | 0.623 | 0.811 | 0.004 | 1.831 | 0.027 | 2 |
| 35  | diMeC$_{31}$ | 3297 | 0.803 | 0.629 | 0.004 | 1.384 | 0.026 | 35 |
| 16  | diMeC$_{26}$ | 2902 | 0.689 | 0.858 | 0.004 | 1.512 | 0.026 | 16 |
| 38  | C$_{34,1}$ | 3342 | 0.199 | 0.318 | 0.004 | 2.264 | 0.024 | 38 |
| 24  | C$_{31,1}$ | 3082 | 0.414 | 0.354 | 0.004 | 1.426 | 0.024 | 24 |
| No. | Compound | RI  | Abundance | $\delta_i$ | $\delta_i /SD(\delta_i)$ | % contr. diss. | $\Sigma \delta_i$ |
|-----|----------|-----|-----------|----------|-----------------|---------------|----------------|
| 33  | C_{33,1} | 3280| 1.026 | 1.353 | 0.008 | 1.680 | 0.071 | 33 |
| 11  | C_{22,1} | 2182| 0.425 | 0.435 | 0.005 | 1.118 | 0.049 | 11 |
| 24  | C_{31,1} | 3082| 0.196 | 0.414 | 0.005 | 1.633 | 0.045 | 24 |
| 36  | C_{36,1} | 3308| 0.815 | 0.705 | 0.005 | 1.387 | 0.043 | 36 |
| 1   | C_{14}   | 1400| 0.934 | 0.732 | 0.005 | 2.207 | 0.043 | 1  |
| 2   | unknown  | 1402| 0.816 | 0.623 | 0.004 | 2.217 | 0.041 | 2  |
| 15  | 2-MeC_{28} | 2865| 1.037 | 1.186 | 0.004 | 1.220 | 0.040 | 15 |
| 40  | C_{34,2} | 3371| 0.311 | 0.242 | 0.005 | 1.160 | 0.031 | 34 |
| 28  | MeC_{31} | 3152| 0.196 | 0.258 | 0.005 | 1.517 | 0.027 | 28 |
| 32  | 3-MeC_{23} | 3105| 0.378 | 0.352 | 0.003 | 1.160 | 0.031 | 32 |
| 19  | MeC_{29} | 2960| 0.477 | 0.586 | 0.003 | 1.557 | 0.027 | 19 |
| 12  | C_{27,1} | 2622| 0.419 | 0.115 | 0.007 | 1.604 | 0.026 | 12 |
| 34  | C_{36,1} | 3291| 0.995 | 1.415 | 0.013 | 1.324 | 0.093 | 33 |
| 31  | C_{33,1} | 3240| 1.095 | 1.444 | 0.008 | 1.904 | 0.058 | 33 |
| 12  | C_{27,1} | 2622| 1.357 | 1.673 | 0.007 | 2.177 | 0.052 | 31 |
| 38  | C_{34,1} | 3342| 0.198 | 0.101 | 0.003 | 0.915 | 0.026 | 12 |
| 26  | diMeC_{28} | 3105| 1.406 | 1.516 | 0.003 | 1.614 | 0.027 | 31 |
| 16  | diMeC_{26} | 2902| 0.477 | 0.586 | 0.003 | 1.350 | 0.026 | 19 |

$\text{RI}$ – retention index on the DB-5 column.
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Differences in the CH profiles between the highland and coastal C. rosa

Different patterns of CHs were detected between the two populations when constructing the heat map (Figure 1). The coastal (R1) population had higher amounts of dimethyloctacosane (diMeC28, RI 3105, CH26), 7-/9-methylhentriacontane (7-/9-MeC31, RI 3142, CH27), 3-methylhentriacontane (3-MeC31, RI 3178, CH29), 3-methyldotriacontane (3-MeC32, RI 3272, CH32) and pentatriacontadiene (C35:2, RI 2416, CH42), whereas the highland (R2) flies had higher amounts of docosene (C22:1, RI 2182, CH11), 2-methyloctacosane (2-MeC28, RI 2865, CH15) and dimethylhexacosane (diMeC26, RI 2902, CH16) on their cuticle. When the data were compared by SIMPER analyses, a pairwise comparison of the males or females between the two populations revealed the presence of two specific compounds that mostly contributed to the overall dissimilarity, suggesting these CHs to be potential chemotaxonomic markers. These compounds were identified as docosene (C22:1, RI 2182, CH11) and 2-methyloctacosane (2-MeC28, RI 2865, CH15) (Table 1, Figures 1–3).

Discussion

Significant quantitative differences in the chemical CH profiles of the two populations of C. rosa have been demonstrated and complementary morphological analyses have confirmed that these two populations belong to two different morphotypes/genotypes, previously labelled by Virgilio et al. (2013) as R1 and R2.

The characteristic compounds of the lowland R1 type, diMeC28 and 3-MeC31, were present in higher relative amounts, whereas the highland R2 flies were characterised by high amounts of C22:1 and 2-MeC28. The compounds found in the present study correspond to the estimated chain lengths of the CH clusters identified in our earlier work for C. rosa, C. anona, C. fasciventris and C. capitata, where the C. rosa R2 type could be determined based on the presence of even methylbranched hydrocarbons and the absence of odd methylbranched CHs when compared with the other three Ceratitis species (Vaníčková et al. 2014).

The intraspecific variation in the CH profiles between the two types reported here might be a result of several different factors, such as the effects of temperature, the social context and diet (Ferveur 2005, Kather and Martin 2012, Bontonou and Wicker-Thomats 2014, Vaníčková et al. 2015). Considering that the R2 type of C. rosa appears to be more cold resistant than the R1 type (Tanga et al. 2015), we assume that temperature...
Figure 2. Section of the GC×GC/MS analysis of the female (A) and male (B) cuticular hydrocarbon profiles of the highland population (R2) of *Ceratitis rosa* from Kenya. The intensity of the signals is colour-coded from green (zero) to red (maximum). The compounds are assigned according to Table 1.
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Figure 3. Section of the GC×GC/MS analysis of the female (A) and male (B) cuticular hydrocarbon profiles of coastal population (R1) of Ceratitis rosa from Kenya. The intensity of the signals is colour-coded from green (zero) to red (maximum). The compounds are assigned according to Table 1.
may be one of the main sources of variation in R1 and R2 CH profiles. The coastal population of *C. rosa*, living at an average temperature of ~28 °C was characterised by greater amounts of long-chain CHs with carbon backbones from C_{30} to C_{35} when compared with the highland population living at an average temperature of ~21 °C. Long-chain CHs have higher melting points, which give them a superior capacity to limit water loss as compared to short-chain CHs (Bontonou and Wicker-Thomas 2014) and insect species or populations living in warmer, drier environments lose water less rapidly and have longer-chain CHs than mesic ones (Ferveur 2005). A recent study of six South American fruit fly populations has shown that the CH profile varies significantly with relative temperature, relative humidity and altitude (Vaníčková et al. 2015).

In *C. rosa*, we found that the differences in cuticular hydrocarbon profiles between the two populations were greater than those between the sexes, although there was still a significant quantitative sexual dimorphism. Our findings are in agreement with studies conducted on *Drosophila* sp., where differences between *D. montana* populations were found to be considerably greater than those between the sexes (Veltos et al. 2012, Jennings et al. 2014). Mating compatibility studies of the *C. rosa* flies from the same lowland and highland populations examined here have revealed a high degree of mating incompatibility between the two populations, where the index of sexual isolation (ISI) values ranged from 0.84 to 0.93, inferring reproductive isolation (Ekesi et al. unpublished data). The sex-specific differences in the quantitative composition of the *C. rosa* CH profiles identified in the present study indicate that these compounds might serve as short-range pheromones and thus could be directly involved in the mating compatibility/incompatibility within and between populations. Since the CHs involved in mating and courtship are not selectively neutral, reinforcing selection may cause closely related species to have distinct CH profiles (Blomquist and Bagnères 2010). A divergence in CH profiles between populations and sexes can lead to assortative mating and reproductive isolation, as shown in two populations of *D. mojavensis* (Stennett and Etges 1997, Etges 1998). Studies on *D. mojavensis* have demonstrated how even short-time isolation events can result in significant changes in CH composition (Stennett and Etges 1997, Etges 1998, Etges and Jackson 2001, Havens and Etges 2013).

It is important to note that the two populations of *C. rosa* studied here originate from different host plants, nevertheless they were reared during two generations on identical laboratory diet. The identified differences in the abundance of the CH between the populations and between the sexes may be, in addition to temperature and reproductive isolation factors, a result of the effects of host plants from which they originated (Stennett and Etges 1997, Vaníčková 2012, Vaníčková et al. 2012a). In *Drosophila* sp., the variation of CH profiles between closely related species of *D. mojavensis* on varied cactus plants or between populations of these species reflects the adaptation to different host plants (Etges and Jackson 2001). The ratio of the principal CHs changed rapidly with laboratory acclimation and influenced courtship mating in *D. mojavensis* (Stennett and Etges 1997). These CH changes depend on enzymes whose level could represent a metabolic adaptation to host-plant chemicals.
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(Higa and Fuyama 1993, Jones 2001, Houot et al 2010). In tephritids, it is not known how are the CHs modified by diet composition and/or laboratory acclimation and whether any observed changes may impact the attractiveness of CH profiles. Therefore, future work needs to be conducted in order to elucidate the complex mechanisms involved in these events.

**Conclusion**

Our data on cuticular hydrocarbon profiles, along with the previously published studies on morphology, genetics and sexual compatibility suggest that there exist two different entities, almost certainly unique biological species, within the taxa *C. rosa* from Kenya. In order to determine whether the different entities observed are consistent, the study needs to be extended to other populations of the two entities throughout their geographic and host ranges.

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Appendix

Map of the *Ceratitis rosa* collection area. Flies were picked up in Kenya, in Mwajamba, Msambweni (Coast Province) and in Kithoka, Meru (Central Province).