Journal of Chemical Ecology
https://doi.org/10.1007/s10886-024-01540-8

RESEARCH

Population Density Affects Drosophila Male Pheromones in Laboratory-Acclimated and Natural Lines

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Received: 19 April 2024 / Revised: 30 July 2024 / Accepted: 12 August 2024

Abstract
In large groups of vertebrates and invertebrates, aggregation can affect biological characters such as gene expression, physiological, immunological and behavioral responses. The insect cuticle is covered with hydrocarbons (cuticular hydrocarbons; CHCs) which reduce dehydration and increase protection against xenobiotics. *Drosophila melanogaster* and *D. simulans* flies also use some of their CHCs as contact pheromones. In these two sibling species, males also produce the volatile pheromone 11-*cis*-Vaccenyl acetate (*c*Va). To investigate the effect of insect density on the production of CHCs and *c*Va we compared the level of these male pheromones in groups of different sizes. These compounds were measured in six lines acclimated for many generations in our laboratory – four wild-type and one CHC mutant *D. melanogaster* lines plus one *D. simulans* line. Increasing the group size substantially changed pheromone amounts only in the four *D. melanogaster* wild-type lines. To evaluate the role of laboratory acclimation in this effect, we measured density-dependent pheromonal production in 21 lines caught in nature after 1, 12 and 25 generations in the laboratory. These lines showed varied effects which rarely persisted across generations. Although increasing group size often affected pheromone production in laboratory-established and freshly-caught *D. melanogaster* lines, this effect was not linear, suggesting complex determinants.

Keywords 7-tricosene · 7-pentacosene · *Drosophila simulans*

Introduction

In nature, animals of the same species tend to aggregate and form groups whose number of individuals depends both on population density and available resources (Parrish and Edelstein-Keshet 1999; Bonabeau et al. 1999; Lof et al. 2008). The size of the group changes the frequency of inter-individual interactions. This induces physiological and behavioral effects which are not always linearly correlated with the number of individuals in the group (Bednekoff and Lima 2004; van der Marel et al. 2019; Beauchamp 2019; Wiedenová et al. 2018; Verschut et al. 2023). Larger vertebrate groups show enhanced vigilance against predation and allow increased food uptake (Wirtz and Wawra 1986; Wilson and Richards 2000; Rieucau et al. 2010; Scott-Samuel et al. 2015). Aggregation also changes body temperature, immune response and gene expression (Andrews et al. 1987; Runcie et al. 2013; Wiedenová et al. 2018; Hamilton et al. 2022). In social insects, the size of the group strongly affects work organization, metabolism, hormone titer and water intake (Hewitt et al. 1971; Mao and Henderson 2010; Fewell and Harrison 2016). Aggregation also impacts development, sexual and aggressive behaviors, pheromonal communication and reproduction (Robson and Traniello 1998; Kikuchi et al. 2008; Yoder et al. 2010; Amsalem and Hefetz 2011; Fujioka et al. 2019; Orlova and Amsalem 2021; Nixon et al. 2022). The study of the sub-social species *Drosophila melanogaster* — a favourable model for genetic manipulation (The FlyBase 2003) — identified genes underlying the aggregation effect involved in sexual and feeding behaviors, learning and memory, gene expression and reproduction (Kent et al. 2008; Lof et al. 2008; Saltz 2011; Billeter et al.

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Published online: 26 August 2024

2012; Lihoreau et al. 2016; Sehdev et al. 2019; Rooke et al. 2020; Churchill et al. 2020; Ebanks et al. 2021; Muria et al. 2021; Bailly et al. 2023).

Virtually all insects carry a mixture of long chain hydrocarbons on their cuticle (cuticular hydrocarbons; CHCs (Qiu et al. 2012; Kefi et al. 2019). CHCs are primarily used in desiccation resistance (Gibbs et al. 1997; Ferveur et al. 2018; Yang et al. 2020) and protection against xenobiotics penetration (Balabanidou et al. 2018; Wang et al. 2017, 2019). CHC-related bidirectional permeability diverges between Drosophila species (Wang et al. 2020a, 2021). CHCs are also involved in intra- and inter-specific communication in many insects including Drosophila species (Howard and Blomquist 2005; Ferveur 2005). In the *Drosophila melanogaster* subgroup of species, some CHCs produced in relatively large amounts are either involved in mate recognition, copulation, or in territory and aggressive male-male behavior (Hoffmann 1990; Greenspan and Ferveur 2000; Wang et al. 2011). *D. melanogaster* males of temperate geographic regions produce a high level of 7-tricosene (7-T; 23 carbons) and much less 7-pentacosene (7-P; 25 carbons) whereas males of tropical and equatorial regions show an opposite pattern (Jallon 1984; Ferveur and Jallon 1996). Differently, *D. melanogaster* females produce low levels of 7-T and 7-P and high amount of 7,11-heptacosadiene (7,11-HD; 27 carbons; (Antony and Jallon 1982). Moreover, in their ejaculatory bulb males produce the volatile pheromone 11-*cis*-Vaccenyl acetate (*c*Va). The terminal steps of CHC and *c*Va biosynthesis depend on distinct enzymatic pathways and tissues (Guiraudie-Capraz et al. 2007; Jallon 1984; Ferveur et al. 1997). In *D. melanogaster* males, some CHCs can quantitatively vary with circadian activity and social interaction (Kent et al. 2008). However, in these experiments, CHCs did not accumulate on the cuticle since their amounts returned to initial levels after a 24-hour long cycle.

Given our limited knowledge on the effect of aggregation in *Drosophila* pheromones, we investigated whether CHCs and *c*Va amounts changed in males competing in groups of different sizes during early adult life. We evaluated this effect on "superficial" and "whole-body" amounts of CHCs and *c*Va, using two extraction procedures. After testing 6 laboratory-acclimated lines (four wild-type and one CHC-mutant *D. melanogaster* lines plus one *D. simulans* line), we tested 21 *D. melanogaster* lines freshly collected in nature after 1, 12 and 25 generations acclimation in the laboratory.

Materials and Methods

Flies

Laboratory Lines

We used five *Drosophila melanogaster* stocks which have been already intensively studied — however not for group-size effect — : the laboratory strains Canton-S (Cs, caught in the 1930's USA), Dijon2000 (Di2, caught in 2000 in France), Oregon-R (Or-R, caught in the 1950's in the USA; provided by Professor Jean-Christophe Billeter) and Zimbabwe30 (Z30, caught in the 1980's in Zimbawbe; provided by Professor Jerry Coyne) and the *desat1*^{1573}-Gal4 homozygous mutant strain (*desat1*; introgressed with the genetic background of Di2 strain). These mutant flies produce a reduced quantity of 7-T, 7-P and 7,11-HD (Marcillac et al. 2005a, b); *desat1* males also show reduced *c*Va emission (Cortot et al. 2022a). We also tested a *D. simulans* stock (#K509; provided by Professor Daisuke Yamamoto). The reason why we compared *D. simulans* males to *D. melanogaster* Cs and Di2 males, is based on the fact that males of both species produce high level of 7-T, low level of 7-P and *c*Va (Ferveur 1991; Schaner et al. 1987). We also compared females of the Cs, Di2 and *desat1* strains, but given that they did not show any group-size effect in our preliminary experiments, we did not pursue further investigation with females. Stocks were maintained on alcohol-free standard cornmeal medium mixed with killed yeast in 30 ml glass vials, at 24 ± 0.5 °C and 65 ± 5% humidity on a 12:12 dark: light cycle. To reduce larval competition, only 8–10 pairs of progenitor flies were kept in each vial and transferred to fresh food vial every 2–3 days to generate enough tester flies. 1- to 2-hour old flies were sexed under light carbon dioxide anaesthesia 2–4 h after lights on and were kept in fresh-food glass vials randomly distributed either in small groups of 5 flies or in larger groups of 25 or 50 flies. In the first experiment, we tested groups of 50 flies only in series2 after finding a clear group-size effect in groups of 25 flies (compared to groups of 5 flies) in series1. Given that no major difference was found between groups of 25 flies and or 50 flies, we only tested groups of 25 flies in the other experiments. All flies tested were 4 day-old, at the age at which they showed a mature CHC profile and a relatively limited lethality when kept in larger groups (Cortot et al. 2022b; this study).

Freshly Caught Lines

In September 2021, flies were caught in traps containing different types of fresh fruits (fig, peach, mirabelle, banana, melon). All groups of founder flies (F0) caught in the same

spot (an suburban orchard in Dijon, France), and trapped in each vial were used to initiate a distinct line in the laboratory (Ferveur et al. 2024a). At the next generation, some F1 males were kept for chemical analysis. In each F1 line, species identity was visually checked under binoculars. Then, we performed reciprocal crosses between F2 flies and control Cs flies to observe the presence of F3 progenies of both sexes. To avoid bottleneck effects, each line was maintained in a minimum of 6 vials (and in 12–18 vials at F11 and F24 generations preceding F12 and F25 extractions). For the sake of clarity, each line was identified with a number.

Pheromones

We used two extraction procedures on different insects to measure both the superficial and whole-body amounts of pheromones. The superficial amounts of pheromones are the quantities immediately available by other flies during the sexual or social interactions. Whole-body amounts correspond to the total of internal and superficial pheromones at a given time and provide a good indication of pheromone biosynthesis. Frozen flies were individually immersed either for 5 min into vials containing 30 µl hexane, or for 24 h in 30 µl dichloromethane at room temperature. This allowed us to respectively extract the superficial or the whole-body amount of *c*Va and of CHCs present on (in) each fly (Cortot et al. 2022a, b; Ferveur et al. 2024b). Each solvent contained 3.33 ng/µl of C26 (*n*-hexacosane) and 3.33 ng/µl of C30 (*n*-triacontane) used as internal standards (ISs). Amounts of *c*Va and CHCs were quantified by gas chromatography using a Varian CP3380 gas chromatograph fitted with a flame ionization detector, a CP Sil 5CB column (25 m x 0.25 mm internal diameter; 0.1 µm film thickness; Agilent), and a split–splitless injector (60 ml/min split-flow; valve opening 30 s after injection) with helium as the carrier gas (50 cm/sec at 120 °C). The temperature program began at 120 °C, ramping at 10 °C/min to 140 °C, then ramping at 2 °C/min to 290 °C, and holding for 10 min. The chemical identity of each peak was determined according to (Everaerts et al. 2010). The amount (ng/insect) of each compound was calculated based on the readings obtained from the ISs.

Beside the absolute amounts of 7-T and 7-P, we measured the level of n-tricosane (23Lin), a linear saturated CHC structurally close to 7-T) in some laboratory lines. We also determined the 7-T/7-P ratio that provides a relative measure of these two principal CHCs independently of their absolute quantities. In females, we also measured the amounts of 7,11-heptacosadiene (7,11-HD) and 7,11-nonacosadiene (7,11-ND). In males of freshly caught lines, we calculated the rate of variation between the "x5" (small) and "x25" (large) group sizes with the [("x25" – "x5")/ "x5"] formula.

Statistics

The amounts (and ratios) of the various chemicals were compared using *Mann-Whitney* test or *Kruskal-Wallis* test (with Monte-Carlo simulations, followed by Conover-Iman multiple pairwise comparisons —$p = 0.05$— with Bonferroni's correction), where applicable. The amounts of *c*Va, 7-T and 7-P in whole body or superficial extracts were used as variable to compute principal component analysis (PCA; Pearson's correlation matrix type; standardized values) with the type of fly (line and group size) used as individuals. All statistical analyses were performed using XLSTAT Premium 2021.5.1.1220 (Addinsoft 2021).

Results

Group-size Effect in Laboratory Lines

Repeated Analysis in Three well-studied Lines

We measured whole-body and superficial amounts of *c*Va, 7-T, 7-P and the 7-T/7-P ratio in 4 day-old males of three well-studied laboratory lines: Canton-S (Cs), Dijon2000 (Di2) and the *desat1* mutant. Two series of analysis, separated by 7 months, were performed: the series1 was performed with groups composed of 5 or 25 males (for Cs and Di2) and also of 50 individuals for *desat1*, while the series2 was carried out on groups of 5, 25 or 50 individuals (indicated as "x5", "x25", "x50", respectively).

Whole-body Compounds

We first measured whole-body amounts of CHCs and *c*Va (Fig. 1).

In Cs males, cVa slightly decreased in series2 (between "x5" and "x50" groups; $p = 0.041$). 7-T increased in series1 (between "x5" and "x25" males; $p = 0.001$) and in series2 (between "x5" and "x50" males; $p = 0.018$). 7-P gradually increased with the group size in the two series ($p < 0.0001$). The 7-T/7-P ratio decreased in males of larger group-size in the two series ($p < 0.0001$).

In Di2 males, *c*Va slightly decreased in larger group-size in series1 and 2 ($p = 0.015$; $p = 0.024$, respectively). 7-T decreased in larger groups of series1 ($p < 0.0001$) and showed no change in series2 ($p =$ ns). In males of larger groups, 7-P slightly decreased in series1 ($p = 0.016$) and slightly increased in series2 ($p = 0.012$). The 7-T/7-P ratio did not change in series1 whereas it gradually decreased in the larger groups of series2 ($p < 0.0001$).

No significant variation was found in *desat1* males. In series1, the 7-T/7-P ratio slightly increased just below the

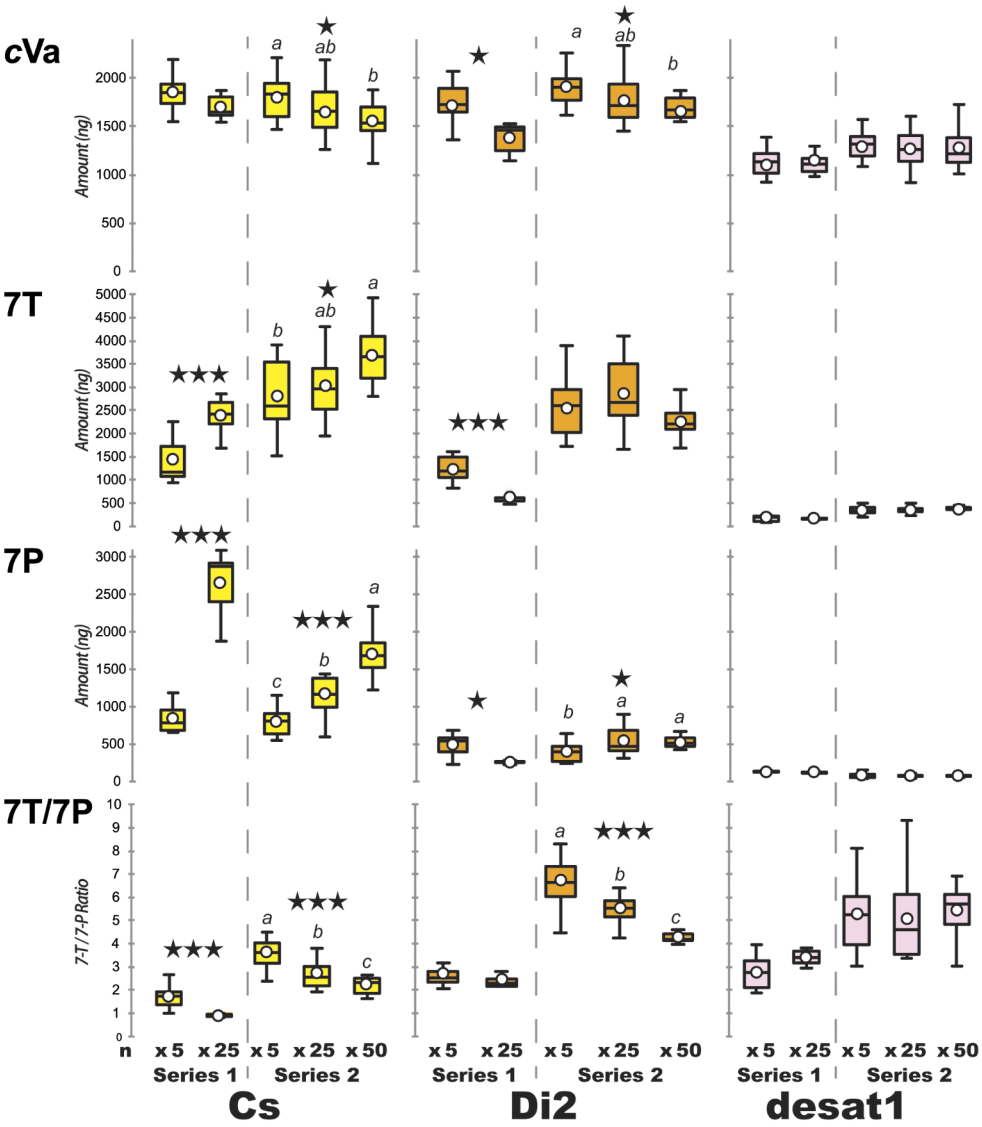

Fig. 1 Group size effect on whole body pheromones in males of three laboratory lines. The data shown (from top to bottom) correspond to the whole-body amounts (in ng) of *cis*-Vaccenyl acetate (*c*Va), 7-tricosene (7-T) and 7-pentacosene (7-P) extracted in single 4 day-old males (with dichloromethane during 24 h). At the bottom, we show the 7-T/7-P ratio. Using three laboratory-established lines: Canton-S (Cs), Dijon2000 (Di2) and the *desaturase1* mutant (*desat1*), we compared males kept in groups of 5, 25 or 50 individuals ("x5"; "x25"; "x50"). We performed two experimental series (series1; series2) separated by 7 months. Data are shown as box and whisker diagrams. The lower and upper box edges represent the first and third quartiles, while the median value is indicated by the inner small horizontal bar and the mean by the empty dot. The ends of the whiskers above and below each box represent the limits beyond which values were considered anomalous. The levels of the various chemicals or ratio were compared using *Mann-Whitney* or *Kruskal-Wallis* test, where applicable. The significance levels are $p=0.05$ (★), $p=0.01$ (★★), $p=0.001$ (★★★). Different letters indicate significant differences between males of the different lines at each generation. For series 1, $N=10$ except for *desat1* × 25 and x50 ($N=5$); for series 2, $N=15$

limit of significance ($p=0.051$) between "x5" and "x25" males.

Superficial Compounds

Simultaneously to whole-body compound analysis, we measured the amounts of compounds superficially present on the cuticle (Fig. 2). Basically, despite of the much lower quantities detected, compared to whole-body amounts, grouping induced very similar effects on 7-T and 7-P. Differently, *c*Va showed no group-size related variation whereas the 7-T/7-P ratio showed a slight divergence between the two extraction modes.

In Cs males, 7-T and 7-P increased with the size of the group in series1 ($p<0.0001$) and in series2 between "x25" and "x50" males ($p<0.0001$). The 7-T/7-P ratio was not affected. In Di2 males, 7-T decreased in series1 (between "x5" and "x25" males; $p=0.008$) and did not change in series2 ($p=$ ns). 7-P was not affected in series1 while it increased in series2 (between "x5" and "x25"/"x50" males; $p<0.0001$). The 7-T/7-P ratio decreased in larger groups in series1 ($p=0.005$), and between "x5"/"x25" and "x50" males in series2 ($p<0.0001$). *desat1* mutant males only showed increased 7-T/7-P ratio in series1 (between "x5" and "x25"/"x50" males; $p<0.0001$).

The data shown (from top to bottom) correspond to the superfical amounts (in ng) of *c*Va, 7-T and 7-P extracted in single 4 day-old males (with hexane during 5 min). For genotypes, statistics, and experimental conditions, see the legend of Fig. 1. For series 1, $N=10$ except for Di2 × 25 ($N=7$); for series 2, $N=15$.

To check the specificity of the compound(s) affected by the size of the group, we measured the level of n-tricosane (23Lin; a saturated linear CHC with 23 C structurally close

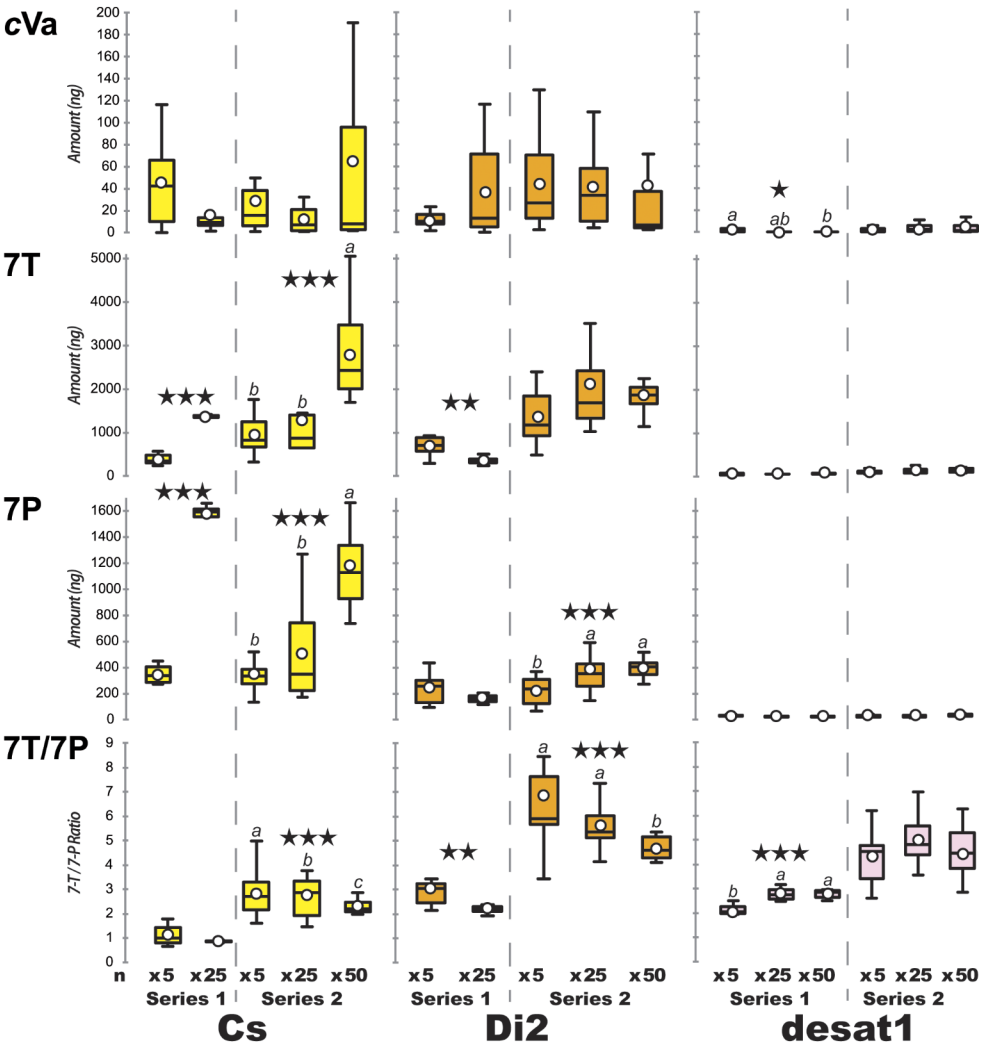

Fig. 2 Group size effect on superficial pheromones in males of three laboratory lines. The data shown (from top to bottom) correspond to the superfical amounts (in ng) of cVa, 7-T and 7-P extracted in single 4 day-old males (with hexane during 5 min). For genotypes, statistics, and experimental conditions, see the legend of Fig. 1. For series 1, N = 10 except for Di2 × 25 (N = 7); for series 2, N = 15

to 7-T). In series1, 23Lin significantly increased between "x5" and "x25" Cs males (whole-body = 557 and 1058 ng, $p < 10^{-4}$; superficial = 210 and 584 ng, $p < 10^{-4}$) while it decreased in Di2 males (whole-body = 398 and 161ng, $p < 10^{-4}$; superficial = 207 and 101 ng, $p = 0.001$). We performed a similar analysis on *desat1* males which showed no 23Lin difference either in series1 (whole body compounds between "x5" and "x50" males: 1379 and 1406 ng, $p = 0.978$; superficial "x5", "x25" and "x50": 943, 999 and 853 ng, respectively, $p = 0.843$) and in whole-body 23Lin of series2 between "x5" "x25" and "x50" males (1737, 1709 and 1757 ng, respectively, $p = 0.717$).

The levels of 7-T, 7-P, 7,11-HD and 7,11-ND, measured in 4 day-old Cs, Di2 and *desat1* females showed no difference between "x5" and "x25" groups (Suppl. Figure 1).

In summary, Cs and Di2 males kept in groups of larger size showed frequent variations of 7-T and 7-P. These changes were more constant in Cs males while they sometimes diverged between the two series, in Di2 males.

Single Analysis of Three Other Laboratory Lines

Beside the Cs, Di2 and *desat1* lines, we compared (in a single series) "x5" and "x25" males in two other *D. melanogaster* wild-type lines: Oregon-R (Or-R) and Zimbabwe30 (Z30) and in one *D. simulans* line (Suppl. Figure 2).

In larger groups of Or-R males, whole-body *c*Va ($p = 0.002$), 7-T ($p < 0.001$) and 7-P ($p < 0.0001$) decreased while the 7-T/7-P ratio increased ($p = 0.004$). Superficial *c*Va was not affected whereas superficial 7-T and 7-P decreased in larger groups ($p < 0.0001$). 7-T/7-P ratio increased in larger size groups ($p < 0.0001$).

In Z30 males kept in larger groups, whole-body 7-T and 7-P decreased ($p = 0.001$ and $p < 0.0001$, respectively) while *c*Va and 7-T/7-P ratio did not vary. Only superficial 7-P slightly decreased in larger groups ($p = 0.032$).

D. simulans males showed no effect either for whole-body or superficial compounds (p = ns).

In summary, group-size generally induced parallel effects on whole body and superficial compounds in the six lines

tested. Each line showed a specific pheromone variation pattern. Also, the comparison of whole-body and superficial levels shows a relatively similar dispersion in flies of these six laboratory-acclimated lines between the two extraction modes (Fig. 3).

Group-size Effect in Freshly Collected Lines

Since males in the four wild-type *D. melanogaster* laboratory-acclimated lines — but not the *desat1* mutant and the *D. simulans* males — showed a significant group-size effect, we tested males in 21 freshly-caught lines, after 1, 12 and

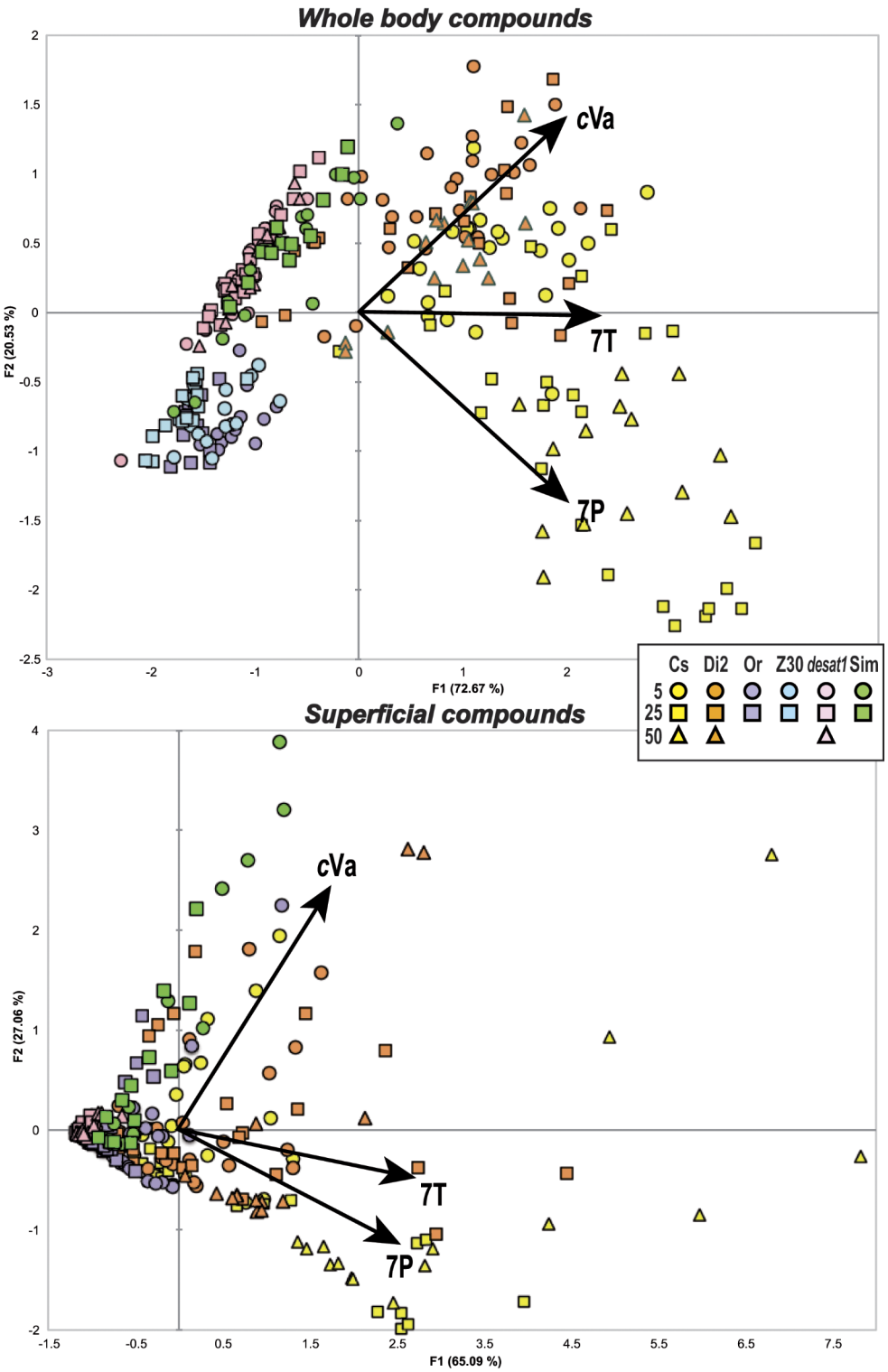

Fig. 3 Principal Component Analysis (PCA) of pheromones in males of 6 laboratory lines. Data show the dispersion of single males according to their genotype (see the color code) and social experience (group sizes are represented by different symbols). The PCA shown on top represents the whole-body compounds analysis and the PCA at the bottom shows the superfical compound analysis

25 generations acclimation in the laboratory (F1, F12, F25, respectively; Fig. 4; Suppl. Figure 3). This allowed us to test the significance of our finding from the field. Since each line resulted of flies trapped with different fruits, we also analysed lines grouped according to their fruit preference (Fig. 5; Suppl. Figure 4). Our study focused on whole-body compounds which showed more robust data than superficial compounds in laboratory lines (see above). For the sake of clarity, we show the rate of variation between "x5" and "x25" groups (see Material and Methods; The complete data set is shown in Suppl. Figure 3).

Line by line Analysis

F1 Generation

After one generation in the lab (F1) on standard food, "x 25" males showed significantly increased 7-P in 7 lines (among 19 lines tested; lines #19 and 20 were not tested; Fig. 4). 7-T increased in "x25" males of four lines and decreased in one line (#5) whereas *c*Va slightly decreased in "x25" males of one line (#11). The 7-T/7-P ratio significantly decreased in "x25" males of three lines.

F12 Generation

F12 males showed a group-size effect in fewer lines, compared to F1 and the variation was not always found in similar lines. In particular, "x25" males showed lower *c*Va in five lines and lower 7-T in four lines. In "x25" males, 7-P increased in three lines and decreased in one line (#10). The 7-T/7-P ratio decreased in "x25" males of six lines. In summary, the three compounds decreased in "x25" males of the line #10 while *c*Va and 7-T decreased in two lines (#5, 6). 7-T and 7-P showed a reciprocal variation in the line #1. Please note that the absolute amounts of all compounds were particularly low at F12 generation.

F25 Generation

At F25, 7-P increased in "x25" males of eight lines and decreased in one line (#21). In x25" males, 7-T decreased in

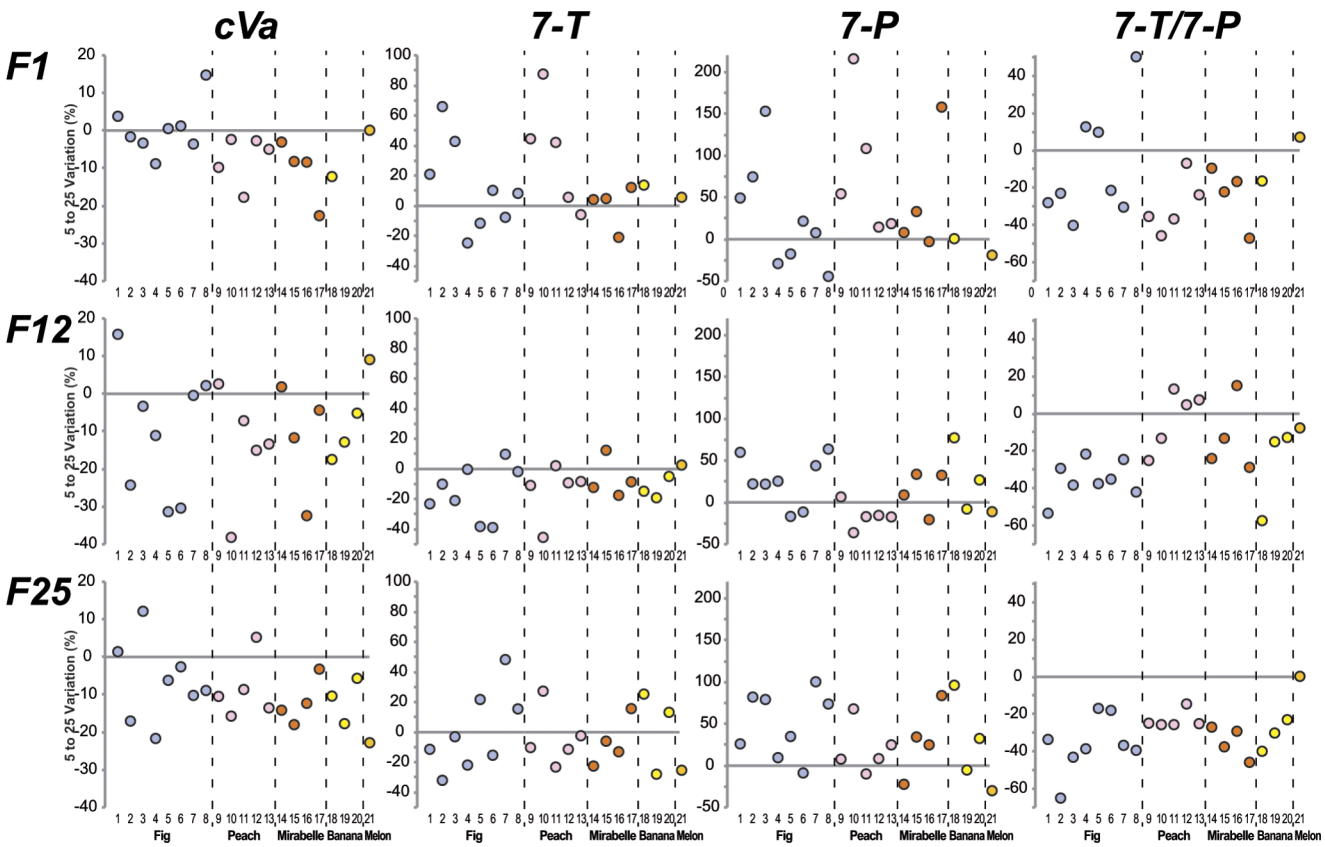

Fig. 4 Group size effect on whole body pheromones in males of 21 natural lines after laboratory acclimation. Each dot corresponds to the pheromone difference measured between 4 day-old males kept in small (x5) and in larger groups (x25) based on the [("x25" – "x5")/ "x5"] formula, in each line (corresponding line # are shown below the graph). Whole body amounts (in ng) of *c*Va,7-T, 7-P, and the 7-T/7-P ratio were analysed after 1, 12 and 25 generations acclimation in the laboratory (F1, F12, F25; shown on the top). The dotted lines show the separation between lines caught on different types of fruits. $N = 8$ (Fig), 5 (Peach), 4 (Mirabelle), 1 or 3 (Banana) and 1 (Melon). Detailed data are shown on Supplemental Fig. 3

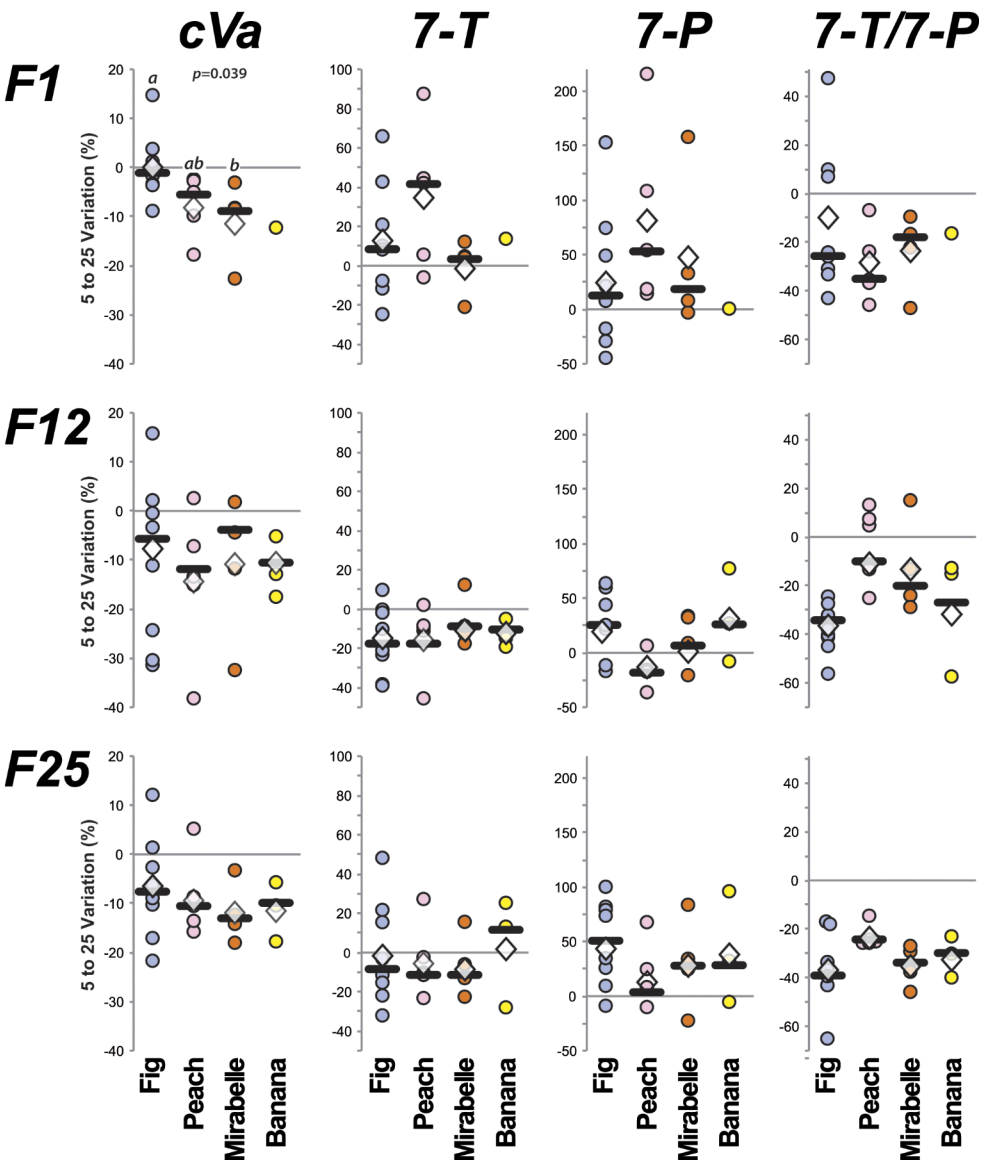

Fig. 5 Group size effect on whole body pheromones in males of four "Fruit lines" groups after laboratory acclimation. We compared the pheromonal productions (amounts of *c*Va,7-T, 7-P and the 7-T/7-P ratio) in lines grouped according to the fruit preference of their founder flies (Fig; Peach; Mirabelle; Banana; Melon included only one line and was excluded). Data correspond to pheromone difference measured between 4 day-old males kept in small (x5) and in larger groups (x25; see the legend of Fig. 3). Each dot corresponds to a line and the group is shown with its median (small horizontal bar) and mean (diamond) values. The levels of variation were compared using *Kruskal-Wallis* test. Different letters indicate significant differences for *c*Va between males of the different lines at F1 generation. $N = 8$ (Fig), 5 (Peach), 4 (Mirabelle) and 1 or 3 (Banana). Detailed data are shown on Supplemental Fig. 4

four lines and increased in one line (#7) while *c*Va decreased in three lines (#4, 10, 21). The 7-T/7-P ratio decreased in "x25" males of ten lines. The ratio decrease was frequently found in lines showing increased 7-P. In summary, the three compounds decreased in "x25" males of the line #21 while both 7-T and 7-P increased in the line #7. A reciprocal variation was observed in "x25" males of the line #2 (decreased 7-T and increased 7-P) and of the line #10 (decreased *c*Va and increased 7-P).

Effect Across Generations

Several lines showed a similar group-size effect across generations (Suppl. Figure 3). In "x25" males, 7-P increased in the lines # 2, 3, 10, 17 (at F1 + F25) and in the lines #7 and 8 (F12 + F25). Note that 7-P decreased at F12 in the line #10. In "x25" males, 7-T decreased in the line #5 (F12 + F25) and showed a reciprocal variation in the lines #2 and 11 (F1 + F25) and in the line #10 (F1 + F12). All significant changes of the 7-T/7-P ratio led to decreased values in "x25" males. The frequency of this effect increased between F1, F12 and F25 (in 3, 6 and 10 lines, respectively). The 7-T/7-P ratio was affected in the lines #3, 8 and 18 (at F12 + F25), and in the lines #10, 11 and 17 (at F1 + F25). In "x25" males of the line #10, *c*Va decreased at F12 + F25. The lines #12, 14 and 15 showed no group-size effect at any generation (Line #20 was not considered).

"Fruit lines" group analysis

We also analysed the lines grouped according to their initial fruit preference (Fig. 5; Suppl. Figure 4). *c*Va showed

no effect at F1, but a general decrease occurred in "x25" males at F12 and F25 (except in "Fig" males at F25). 7-T increased in "x25" males of "Fig" and "Peach" lines at F1 whereas it decreased at F12 in these two groups together with the "Mirabelle" group. No 7-T variation was noted at F25. Across generations males of "Banana" lines showed no effect. All 7-P variations in "x25" males corresponded to increased amounts: at F1 in the "Fig", "Peach" and "Mirabelle" groups; at F12 in "Fig" males; at F25 in "Fig", "Mirabelle" and "Banana" males. "x25" males of the "Fig" group showed a constant increase from F1 to F25.

All variations of the 7-T/7-P ratio corresponded to decreased values in "x25" males: at F1 in "Peach" and "Mirabelle" males; At F12 in "Mirabelle" males; At F25 in all groups. Across generations, "x25" males of the "Mirabelle" group showed a persistent decrease.

Discussion

Our study reveals that *D. melanogaster* males kept in large groups during early adult development frequently changed the production of their two principal 7-monoenes (7-T, 7-P) — less often that of *c*Va — when compared to males kept in smaller groups. Increasing the density of males in laboratory-acclimated lines and in freshly caught lines induced effects varying (*i*) between lines taken at a given generation and (*ii*) between generations in given lines.

Increasing the size of the group in laboratory-acclimated males induced parallel effects on whole-body and superficial compounds with clearer effects on whole-body compounds which showed a relatively lower intra-treatment variability than superficial compounds (Figs. 1, 2 and 3). The group-size effect on whole-body 7-T and 7-P diverged between lines. Cs males kept in larger groups showed the most robust effect with strongly increased 7-T and 7-P (up to +220% and +120%, respectively) compared to smaller groups. The parallel variation of 7-T and 23Lin indicates that increased Cs male density similarly affected desaturated and saturated CHCs with 23 C (23 carbon chain length). However, the decreased 7-T/7-P ratio (in larger groups of Cs males) indicates that 7-P increased relatively more than 7-T. In Di2 males, the group-size effect was not constant: 7-T and 7-P differently varied within and between generations. This likely explaining why their 7-T/7-P ratio differently varied between the two series. The different "reproductibility" of the variations noted between Cs and Di2 males could be due to the possible presence of transposable elements in Di2 males — but not in Cs males — this possibly affecting gene regulation (Anxolabéhère et al. 1988; Quesneville and Anxolabéhère 1998). Or-R males kept in larger groups showed a mirrored variation compared to Cs males: 7-T and 7-P decreased while the 7-T/7-P ratio increased. This suggests that 7-T decreased relatively less than 7-P in Or-R males kept in larger groups. Z30 males kept in larger groups showed a parallel decrease of the two 7-monoenes without changing their ratio. The whole-body *c*Va amount decreased in three of the four wild-type males (Cs, Di2, Or-R) kept in larger groups.

In contrast to wild-type *D. melanogaster* males, increasing the group size in *desat1* mutant and *D. simulans* males induced no or a very limited effect. This suggests that the aggregation effect (*i*) depends on gene(s) involved in the biosynthetic pathway leading to final CHC products and (*ii*) can vary between closely related species. Could the difference observed between the two sibling species be related to their different biosynthetic pathway leading to sexually dimorphic CHCs in *D. melanogaster* but not in *D. simulans* flies (Jallon 1984; Chertemps et al. 2007)? This is unlikely since females kept in small and large groups showed no significant CHC differences. Instead, we believe that species difference is due to the higher plasticity of CHC production in *D. melanogaster* as compared to its sibling species. This difference may be reflected by the fact that the variation of the 7-T/7-P ratio depends on several genes *in D. melanogaster* males (Scott and Richmond 1988; Ferveur and Jallon 1996) and on a single gene in *D. simulans* flies (Luyten 1982; Ferveur 1991). However, we cannot exclude that the absence of effect observed in *D. simulans* is due to the limited number of *D. simulans* line tested here.

Males of freshly caught lines kept in larger groups showed varied effects on CHCs and *c*Va, depending on the line considered (Figs. 4 and 5). One or two (rarely three) compounds were affected at one, sometimes at two of the examined generations (F1, F12 or F25). Indeed, these effects were rarely repeated across generations (our interpretation remains reserved for F12 flies which produced much less compounds than F1 and F25 flies). Overall, the three pheromones showed distinct variation patterns. 7-P showed the most coherent variation during laboratory acclimation. In "x25" males, 7-P increased in 35% F1 and F25 lines whereas it only decreased in one line (#21; "melon") at F25. 7-T showed a more contrasted variation: it increased in four F1 lines and one F25 line while it decreased in one F1 line and four F25 lines. Therefore, the frequently decreased 7-T/7-P ratio in "x25" males during laboratory acclimation (in three F1, six F12, and ten F25 lines) was mostly due to increased 7-P (in 8 lines). This effect was eventually enhanced by decreased 7-T (in 4 lines). Whole-body *c*Va varied less frequently in freshly-caught lines than in laboratory-acclimated lines (3/4): "x25" males produced less *c*Va in only one F1, five F12 and three F25 lines. The fact that, during acclimation, increased group-size induced distinct effects on *c*Va and CHCs suggests that the social

interaction, and male-male competition, differently affected their biosynthesis. Such dissociation maybe due to the distinct enzymatic pathways, biosynthesis sites and/or externalization canals involved in CHCs and *c*Va production and emission (Jallon 1984; Ferveur et al. 1997; Howard and Blomquist 2005; Guiraudie-Capraz et al. 2007; Wang et al. 2020b; Cortot et al. 2022a).

Group-size related variation of pheromones may be — at least partly — under hormonal control. The juvenile hormones (JHs) are pivotal gonadotropic hormones involved in several aspects of insect reproduction (Uzsák and Schal 2013). Their synthesis depends on brain associated glands: the *corpora allata* (CA; (Feyereisen 1999). The density and caste of social insects can modulate the hormone level affecting both the behavior and CHCs. For example, while the increased density of Formosan subterranean termites showed elevated JH titer in individual workers, the subsequent introduction of soldiers lessened this level (Mao and Henderson 2010). A specific JH hormone (JH III) changed task-specific CHC profile in the ant *Myrmicaria eumenoides* (Lengyel et al. 2007). Both increased JH titer and CA size were correlated with higher reproductive dominance in a eusocial polistine wasp (Sledge et al. 2004).

The influence of hormones was often reported on reproduction and CHCs in non-social insects such as Diptera. For example, *D. melanogaster* females present in large groups laid egg faster; this effect is dependent on light and JH activity (Bailly et al. 2023). In the female house fly, *Musca domestica*, and likely in other Diptera species, ovarian-produced ecdysteroids regulate CHC synthesis by affecting specific elongases (Tillman et al. 1999). The surgical removal of ovaries or of CA in *M. domestica* induced a drastic change in the internal level of its principal CHC pheromone (9-tricosene; 9-T); a close-to-normal level of 9-T was restored after the injection of ecdysone (Adams et al. 1995). Similarly, the decapitation of immature *D. melanogaster* adults strongly affected the CHC profile of mature adults; this profile was partly restored by the application of a JH synthetic analog (Wicker and Jallon 1995). The manipulation of the bursicon neurohormone receptor (Rickets) affected the externalization of CHCs (Flaven-Pouchon et al. 2016). Also, the sex-ratio of *D. serrata* populations affected the CHC profile of their males (Gershman and Rundle 2017) while *D. melanogaster* flies tested in a social context showed inter-individual variation of their chemical communication depending on the genotype X environment interaction (Kent et al. 2008).

Similarly to *desat1* and *D. simulans* males, males of three freshly caught lines (#12, 14 & 15) showed no pheromone variation at any generation. How can we interpret the stability (or absence of effect) observed in these lines? Since the amount of 23Lin was not affected in larger groups of *desat1* males (differently to Cs males), the absence of effect may not be related to the bias caused by their very low levels of 7-T and 7-P. We rather hypothesize that beside its "canonical" effect on the carbon chain desaturation (Marcillac et al. 2005a), the defective expression of the *desaturase1* gene also altered mechanisms underlying CHC plasticity. This novel effect of *desat1* can be added to another unexpected effect induced by this mutation on the altered externalization of *c*Va (Cortot et al. 2022a).

The increased frequency of lower 7-T/7-P ratio during laboratory acclimation (from F1 to F25) observed in freshly-caught line males kept in larger groups seems to mostly result of increased 7-P levels. We recently found a frequently decreased 7-T/7-P ratio in "x5" males of freshly-caught lines during laboratory acclimation (until F40; (Ferveur et al. 2024a). In these lines, the "across-generations" acclimation effect on 7-T/7-P decrease was found in males of the "Fig", "Mirabelle" and "Banana" lines but not of the "Peach" lines. Taken together these data indicate that the 7-T/7-P ratio can be affected by the (*i*) increased group-size, (*ii*) duration of laboratory acclimation, (*iii*) initial fruit preference, and (*iv*) genetic background.

This study leaves several questions open. Beside genes and hormones, which factors activate or repress CHCs and *c*Va production? Since the finely tuned CHC biosynthesis depends on many biotic and abiotic factors, we believe that any subtle change of their interaction could explain the frequent and somewhat fluctuating variation of pheromone levels within and between generations. The regulation of some of the biosynthesis genes could be affected by the presence of transposable P-elements as found for metabolic and reproductive functions (Clark et al. 1995; Serrato-Capuchina et al. 2021). Could the natural fruit preference impact the subsequent CHC evolution in the laboratory where the food and environment are more constant? Since the different microbiota associated with different fruits drive the fly preference (Becher et al. 2012), the varied microbes ingested in nature could differently affect CHCs plasticity depending on their transmission and conservation in laboratory lines. This hypothesis is supported by the relationship found between the cuticle chemistry and the composition of bacterial microbiota in *D. melanogaster* flies (Mokeev et al. 2021). Overall, the genetic background variation in the "fruit lines" tested in this study may be responsible for the observed differences (Ferveur et al. 2024a). Indeed, most flies caught in the field might have a more variable genetic background than "old established" laboratory lines. Thus, allele variation in genes directly or indirectly responsible for pheromone production and externalization may account for the different responses to male density.

In summary, changing the number of *D. melanogaster* males in a limited space affected the amount of their sex

pheromones in laboratory-acclimated wild-type lines and in freshly caught lines, but not in a CHC mutant and in the *D. simulans* species. During laboratory acclimation, the production of the CHC with the longer carbon chain showed the highest plasticity while the internally-produced pheromone less frequently varied. In conclusion, our study stress the importance of (1) keeping flies in small groups for pheromone analysis and (2) not extrapolating natural CHC phenotype with insects acclimated for many generations in the laboratory.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-024-01540-8.

Author Contributions J-F.F. and J.C. performed the experiments. J-F.F and C.E. analyzed the data. J-F.F, B.M. and C.E. wrote the ms text. C.E. prepared the figures. All authors reviewed the manuscript.

Data Availability An xlsx file containing all raw data will be available as supplemental material.

Declarations

Competing Interests The authors declare no competing interests.

References

Adams TS, Nelson DR, Blomquist GJ (1995) Effect of endocrine organs and hormones on (Z)-9-tricosene levels in the internal and external lipids of female house flies. Musca domestica J Insect Physiol 41:609–615. https://doi.org/10.1016/0022-1910(95)00003-D

Addinsoft (2021) XLSTAT 2021: data analysis and statistical solution for Microsoft Excel. Addinsoft, Paris, France

Amsalem E, Hefetz A (2011) The effect of group size on the interplay between dominance and reproduction in Bombus terrestris. PLoS ONE 28. 0.1371/journal.pone.0018238

Andrews RV, Phillips D, Makihara D (1987) Metabolic and thermoregulatory consequences of social behaviors between Microtus townsendii comparative biochemistry and physiology part A. Physiology 87:345–348. https://doi.org/10.1016/0300-9629(87)90133-2

Antony C, Jallon JM (1982) The chemical basis for sex recognition in Drosophila melanogaster. J Insect Physiol 28:873–880. https://doi.org/10.1016/0022-1910(82)90101-9

Anxolabéhère D, Charles-Palabost L, Fleuriet A, Periquet G (1988) Temporal surveys of French populations of Drosophila melanogaster: P–M system, enzymatic polymorphism and infection by the. Sigma Virus Heredity 61:121–131. https://doi.org/10.1038/hdy.1988.97

Bailly TPM, Kohlmeier P, Etienne RS, Wertheim B, Billeter JC (2023) Social modulation of oogenesis and egg laying in Drosophila melanogaster. Curr Biol 33:2865–2877. https://doi.org/10.1016/j.cub.2023.05.074

Balabanidou V, Grigoraki L, Vontas J (2018) Insect cuticle: a critical determinant of insecticide resistance. Curr Opin Insect Sci 27:68–74. https://doi.org/10.1016/j.cois.2018.03.001

Beauchamp G (2019) On how risk and group size interact to influence vigilance. Biol Rev 94:1918–1934. https://doi.org/10.1111/brv.12540

Becher PG et al (2012) Yeast, not fruit volatiles mediate Drosophila melanogaster attraction. Oviposition Dev Funct Ecol 26:822–828. https://doi.org/10.1111/j.1365-2435.2012.02006.x

Bednekoff PA, Lima SL (2004) Risk allocation and competition in foraging groups: reversed effects of competition if group size varies under risk of predation. Proceedings: Biol Sci 271(1491–1496). https://doi.org/10.1098/rspb.2004.2739

Billeter J-C, Jagadeesh S, Stepek N, Azanchi R, Levine JD (2012) Drosophila melanogaster females change mating behaviour and offspring production based on social context Proceedings of the Royal Society B: Biological Sciences 279:2417–2425 https://doi.org/10.1098/rspb.2011.2676

Bonabeau E, Dagorn L, Fréon (1999) Scaling in animal group-size distributions. Proc Natl Acad Sci USA 96:4472–4477. https://doi.org/10.1073/pnas.96.8.4472

Chertemps T, Duportets L, Labeur C, Ueda R, Takahashi K, Saigo K, Wicker-Thomas C (2007) A female- biased expressed elongase involved in long-chain hydrocarbon biosynthesis and courtship behavior in Drosophila melanogaster. Proc Natl Acad Sci 104:4273–4278. https://doi.org/10.1073/pnas.0608142104

Churchill ER, Bridle JR, Thom MDF (2020) Spatially clustered resources increase male aggregation and mating duration in Drosophila. Melanogaster Anim Behav 169:45–50. https://doi.org/10.1016/j.anbehav.2020.09.002

Clark JB, Altheide TK, Schlosser MJ, Schlosser MJ, Kidwell MG (1995) Molecular evolution of P transposable elements in the genus Drosophila. I. The saltans and willistoni species groups. Mol Biol Evol 12:902–913. https://doi.org/10.1093/oxfordjournals.molbev.a040267

Cortot J, Farine J-P, Cobb M, Everaerts C, Ferveur J-F (2022a) Factors affecting the biosynthesis and emission of a Drosophila pheromone. J Exp Biol 225. https://doi.org/10.1242/jeb.244422

Cortot J, Farine J-P, Ferveur J-F, Everaerts C (2022b) Aging-related variation of cuticular hydrocarbons in wild type and variant Drosophila melanogaster. J Chem Ecol 48:152–164. https://doi.org/10.1007/s10886-021-01344-0

Ebanks B et al (2021) Exercising D. Melanogaster modulates the mitochondrial proteome and physiology. The effect on lifespan depends upon age and sex. Int J Mol Sci 22. https://doi.org/10.3390/ijms222111606

Everaerts C, Farine J-P, Cobb M, Ferveur J-F (2010) Drosophila cuticular hydrocarbons revisited: mating status alters cuticular profiles. PLoS ONE 5:e9607. https://doi.org/10.1371/journal.pone.0009607

Ferveur J-F (1991) Genetic-control of pheromones in Drosophila simulans.1. Ngbo, a locus on the 2. nd Chromosome Genet 128:293–301. https://doi.org/10.1093/genetics/128.2.293

Ferveur JF (2005) Cuticular hydrocarbons: their evolution and roles in Drosophila pheromonal. Communication Behav Genet 35:279–295

Ferveur JF, Jallon JM (1996) Genetic control of male cuticular hydrocarbons in Drosophila melanogaster. Genet Res 67:211–218. https://doi.org/10.1017/s0016672300033693

Ferveur JF, Savarit F, O'Kane CJ, Sureau G, Greenspan RJ, Jallon JM (1997) Genetic feminization of pheromones and its behavioral consequences in. Drosophila Males Sci 276:1555–1558. https://doi.org/10.1126/science.276.5318.1555

Ferveur J-F, Cortot J, Rihani K, Cobb M, Everaerts C (2018) Desiccation resistance: effect of cuticular hydrocarbons and water content in Drosophila melanogaster adults PeerJ. 6:e4318–e4318. https://doi.org/10.7717/peerj.4318

Ferveur J-F, Cortot J, Cobb M, Everaerts C (2024a) Natural diversity of cuticular pheromones in a local population of Drosophila after laboratory acclimation. Insects 15:273. https://doi.org/10.3390/insects15040273

Ferveur J-F, Cortot J, Moussian B, Cobb M, Everaerts C (2024b) Replenishment of Drosophila male pheromone after mating. J Chem Ecol. https://doi.org/10.1007/s10886-023-01468-5

Fewell JH, Harrison JF (2016) Scaling of work and energy use in social insect colonies. Behav Ecol Sociobiol 70:1047–1061. https://doi.org/10.1007/s00265-016-2097-z

Feyereisen R (1999) Insect P450 enzymes. Annu Rev Entomol 44:507–533. https://doi.org/10.1146/annurev.ento.44.1.507

Flaven-Pouchon J, Farine JP, Ewer J, Ferveur JF (2016) Regulation of cuticular hydrocarbon profile maturation by Drosophila tanning hormone, bursicon, and its interaction with desaturase activity. Insect Biochem Mol Biol 78:87–96. https://doi.org/10.1016/j.ibmb.2016.10.007

Fujioka H, Abe MS, Okada Y (2019) Ant activity-rest rhythms vary with age and interaction frequencies of workers. Behav Ecol Sociobiol 73:30. https://doi.org/10.1007/s00265-019-2641-8

Gershman SN, Rundle HD (2017) Crowd control: sex ratio affects sexually selected cuticular hydrocarbons in male Drosophila serrata. J Evol Biol 30:583–590. https://doi.org/10.1111/jeb.13028

Gibbs AG, Chippindale AK, Rose MR (1997) Physiological mechanisms of evolved desiccation resistance in Drosophila melanogaster. J Exp Biol 200:1821–1832. https://doi.org/10.1242/jeb.200.12.1821

Greenspan RJ, Ferveur JF (2000) Courtship in Drosophila. Annu Rev Genet 34:205–232. https://doi.org/10.1146/annurev.genet.34.1.205

Guiraudie-Capraz G, Pho DB, Jallon J-M (2007) Role of the ejaculatory bulb in biosynthesis of the male pheromone cis-vaccenyl acetate in Drosophila. Melanogaster Integr Zool 2:89–99. https://doi.org/10.1111/j.1749-4877.2007.00047.x

Hamilton A, Rizzo R, Brod S, Ono M, Perretti M, Cooper D, D'Acquisto F (2022) The immunomodulatory effects of social isolation in mice are linked to temperature control. Brain Behav Immun 102. https://doi.org/10.1016/j.bbi.2022.02.022

Hewitt PH, Nel JJC, Schoeman I (1971) Influence of group size on water imbibition by Hodotermes mossambicus alate termites. J Insect Physiol 17:587–600. https://doi.org/10.1016/0022-1910(71)90035-7

Hoffmann AA (1990) The influence of age and experience with conspecifics on territorial behavior in Drosophila melanogaster. J Insect Behav 3:1–12. https://doi.org/10.1007/BF01049191

Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol 50:371–393. https://doi.org/10.1146/annurev.ento.50.071803.130359

Jallon JM (1984) A few chemical words exchanged by Drosophila during courtship and mating. Behav Genet 14:441–478. https://doi.org/10.1007/BF01065444

Kefi M, Balabanidou V, Douris V, Lycett G, Feyereisen R, Vontas J (2019) Two functionally distinct CYP4G genes of Anopheles gambiae contribute to cuticular hydrocarbon biosynthesis. Insect Biochem Mol Biol 110. https://doi.org/10.1016/j.ibmb.2019.04.018

Kent C, Azanchi R, Smith B, Formosa A, Levine JD (2008) Social context influences chemical communication in D. Melanogaster Males Curr Biol 18:1384–1389. https://doi.org/10.1016/j.cub.2008.07.088

Kikuchi T, Nakagawa T, Tsuji K (2008) Changes in relative importance of multiple social regulatory forces with colony size in the ant Diacamma Sp. Japan Anim Behav 76:2069–2077. https://doi.org/10.1016/j.anbehav.2008.08.029

Lengyel F, Westerlund SA, Kaib M (2007) Juvenile hormone III influences task-specific cuticular hydrocarbon profile changes in the ant Myrmicaria eumenoides. J Chem Ecol 33:167–181. https://doi.org/10.1007/s10886-006-9185-x

Lihoreau M, Poissonnier L-A, Isabel G, Dussutour A (2016) Drosophila females trade off good nutrition with high-quality oviposition sites when choosing foods. J Exp Biol 219:2514–2524. https://doi.org/10.1242/jeb.142257

Lof ME, Etienne RS, Powell J, de Gee M, Hemerik L (2008) The effect of chemical information on the spatial distribution of fruit flies: I model results. Bull Math Biol 70:1827–1849. https://doi.org/10.1007/s11538-008-9327-0

Luyten I (1982) Variations intraspécifiques et interspécifiques des hydrocarbures cuticulaires chez Drosophila simulans. CR Acad Sci Paris 295:723–736

Mao L, Henderson GC (2010) Group size effect on worker juvenile hormone titers and soldier differentiation in Formosan subterranean termite. J Insect Physiol 56:725–730. https://doi.org/10.1016/j.jinsphys.2009.12.014

Marcillac F, Bousquet F, Alabouvette J, Savarit F, Ferveur JF (2005a) A mutation with major effects on Drosophila melanogaster. sex Pheromones Genet 171:1617–1628

Marcillac F, Grosjean Y, Ferveur JF (2005b) A single mutation alters production and discrimination of Drosophila sex pheromones Proc. R Soc Biol Sci Ser B 272:303–309. https://doi.org/10.1098/rspb.2004.2971

Mokeev V, Flaven-Pouchon J, Wang Y, Gehring N, Moussian B (2021) Ratio between Lactobacillus plantarum and Acetobacter pomorum on the surface of Drosophila melanogaster adult flies depends on cuticle melanisation. BMC Res Notes 14:351. https://doi.org/10.1186/s13104-021-05766-7

Muria A et al (2021) Social facilitation of long-lasting memory is mediated by CO2 in Drosophila. Curr Biol 31:2065–2074. 0.1016/j.cub.2021.02.044

n der Marel AM, López-Darias M, Waterman JM (2019) Group-enhanced predator detection and quality of vigilance in a social ground squirrel. Anim Behav 151:43–52. https://doi.org/10.1016/j.anbehav.2019.02.017

Nixon L et al (2022) Behavioural responses of diapausing Halyomorpha halys (Hemiptera: Pentatomidae) to conspecific volatile organic compounds. J Appl Entomol 146:319–327. https://doi.org/10.1111/jen.12955

Orlova M, Amsalem E (2021) Bumble bee queen pheromones are context-dependent scientific reports 11. 16931. https://doi.org/10.1038/s41598-021-96411-7

Parrish JK, Edelstein-Keshet L (1999) Complexity, pattern, and evolutionary trade-offs. Anim Aggreg Sci 284:99–101. https://doi.org/10.1126/science.284.5411.99

Qiu Y et al (2012) An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. Proc Natl Acad Sci USA 109:14858–14863

Quesneville H, Anxolabéhère D (1998) Dynamics of transposable elements in metapopulations: a model of P element invasion in Drosophila. Theor Popul Biol 54:175–193. https://doi.org/10.1006/tpbi.1997.1353

Rieucau G, Morand-Ferron J, Giraldeau L-A (2010) Group size effect in nutmeg mannikin: between-individuals behavioral differences but same plasticity. Behav Ecol 21:684–689. https://doi.org/10.1093/beheco/arq039

Robson SK, Traniello JFA (1998) Resource assessment, recruitment behavior, and organization of cooperative prey retrieval in the ant Formica schaufussi (Hymenoptera: Formicidae. J Insect Behav 11:1–22. https://doi.org/10.1023/A:1020859531179

Rooke R, Rasool A, Schneider J, Levine JD (2020) Drosophila melanogaster behaviour changes in different social environments based on group size and density. Commun Biology 3:304. https://doi.org/10.1038/s42003-020-1024-z

Runcie DE, Wiedmann RT, Archie EA, Altmann J, Wray GA, Alberts SC, Tung J (2013) Social environment influences the relationship between genotype and gene expression in wild baboons. Philosophical Trans Royal Soc B: Biol Sci 368:20120345. https://doi.org/10.1098/rstb.2012.0345

Saltz JB (2011) Natural genetic variation in social environment choice: context-dependent gene-environment correlation. Drosophila melanogaster Evol 65:2325–2334. https://doi.org/10.1111/j.1558-5646.2011.01295.x

Schaner AM, Bartell RJ, Jackson LL (1987) (z)-ll-octadecenyl acetate, an aggregation pheromone in Drosophila simulans. J Chem Ecol 13:1777–1786

Scott D, Richmond RC (1988) A genetic-analysis of male-predominant pheromones. Drosophila melanogaster Genet 119:639–646. https://doi.org/10.1093/genetics/119.3.639

Scott-Samuel NE, Holmes G, Baddeley R, Cuthill IC (2015) Moving in groups: how density and unpredictable motion affect predation risk. Behav Ecol Sociobiol 69:867–872. https://doi.org/10.1007/s00265-015-1885-1

Sehdev A, Mohammed YG, Tafrali C, Szyszka P (2019) Social foraging extends associative odor–food memory expression in an automated learning assay for Drosophila melanogaster. J Exp Biol 222:jeb207241. https://doi.org/10.1242/jeb.207241

Serrato-Capuchina A, D'Agostino ERR, Peede D, Roy B, Isbell K, Wang J, Matute DR (2021) P-elements strengthen reproductive isolation within the Drosophila simulans. Species Complex Evol 75:2425–2440. https://doi.org/10.1111/evo.14319

Sledge MF, Trinca I, Massolo A, Boscaro F, Turillazzi S (2004) Variation in cuticular hydrocarbon signatures, hormonal correlates and establishment of reproductive dominance in a polistine wasp. J Insect Physiol 50:73–83. https://doi.org/10.1016/j.jinsphys.2003.10.001

The FlyBase C (2003) The FlyBase database of the Drosophila genome projects and community literature. Nucleic Acids Res 31:172–175. https://doi.org/10.1093/nar/gkg094

Tillman JA, Seybold SJ, Jurenka RA, Blomquist BJ (1999) Insect pheromones — an overview of biosynthesis and endocrine regulation. Insect Biochem Mol Biol 29:481–514. https://doi.org/10.1016/s0965-1748(99)00016-8

Uzsák A, Schal C (2013) Social interaction facilitates reproduction in male German cockroaches. Blattella germanica Anim Behav 85:1501–1509. https://doi.org/10.1016/j.anbehav.2013.04.004

Verschut TA et al (2023) Aggregation pheromones have a non-linear effect on oviposition behavior in Drosophila melanogaster. Nat Commun 14:1544. https://doi.org/10.1038/s41467-023-37046-2

Wang L et al (2011) Hierarchical chemosensory regulation of male-male social interactions in Drosophila. Nat Neurosci 14:757–762. https://doi.org/10.1038/nn.2800

Wang Y, Carballo RG, Moussian B (2017) Double cuticle barrier in two global pests, the Whitefly Trialeurodes vaporariorum and the bedbug Cimex lectularius. J Exp Biol 220:1396–1399. https://doi.org/10.1242/jeb.156679

Wang S, Li B, Zhang DX (2019) NlCYP4G76 and NlCYP4G115 modulate susceptibility to desiccation and insecticide penetration through affecting cuticular hydrocarbon biosynthesis in Nilaparvata lugens (Hemiptera: Delphacidae) frontiers in physiology 10. https://doi.org/10.3389/fphys.2019.00913

Wang Y et al (2020a) Transcriptional control of quality differences in the lipid-based cuticle barrier in Drosophila suzukii and Drosophila melanogaster frontiers in Genetics. 11. https://doi.org/10.3389/fgene.2020.00887

Wang Y et al (2020b) Dysfunction of Oskyddad causes Harlequin-type ichthyosis-like defects in Drosophila melanogaster. PLoS Genet 16:e1008363. https://doi.org/10.1371/journal.pgen.1008363

Wang Y, Ferveur J-F, Moussian B (2021) Eco-genetics of desiccation resistance in Drosophila. Biol Rev 96:1421–1440. https://doi.org/10.1111/brv.12709

Wicker C, Jallon J-M (1995) Hormonal control of sex pheromone biosynthesis. Drosophila melanogaster J Insect Physiol 41:65–70. https://doi.org/10.1016/0022-1910(94)00074-Q

Wiedenová P, Šumbera R, Jan O (2018) Social Thermoregulation and socio-physiological effect in the subterranean Mashona mole-rat (Fukomys darlingi). J Therm Biol 78:367–373. https://doi.org/10.1016/j.jtherbio.2018.10.020

Wilson WG, Richards SA (2000) Consuming and grouping: recource-mediated animal aggregation. Ecol Lett 3:175–180. https://doi.org/10.1046/j.1461-0248.2000.00135.x

Wirtz P, Wawra M (1986) Vigilance and group size. Homo sapiens Ethol 71:283–286. https://doi.org/10.1111/j.1439-0310.1986.tb00592.x

Yang Y, Zhao X, Niu N, Zhao Y, Liu W, Moussian B, Zhang J (2020) Two fatty acid synthase genes from the integument contribute to cuticular hydrocarbon biosynthesis and cuticle permeability in Locusta Migratoria. Insect Mol Biol 29:555–568. https://doi.org/10.1111/imb.12665

Yoder JA et al (2010) Use of an alarm pheromone against ants for gaining access to aphid/scale prey by the red velvet mite balaustium sp. (Erythraeidae) in a honeydew-rich environment. J Exp Biol 213:386–392. https://doi.org/10.1242/jeb.035642