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RESEARCH ARTICLE

Factors affecting the biosynthesis and emission of a *Drosophila* pheromone

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ABSTRACT

The most studied pheromone in *Drosophila melanogaster*, *cis*-vaccenyl acetate (*c*VA), is synthesized in the male ejaculatory bulb and transferred to the female during copulation. Combined with other chemicals, *c*VA can modulate fly aggregation, courtship, mating and fighting. We explored the mechanisms underlying both *c*VA biosynthesis and emission in males of two wild types and a pheromonal mutant line. The effects of ageing, adult social interaction, and maternally transmitted *c*VA and microbes – both associated with the egg chorion – on *c*VA biosynthesis and emission were measured. While ageing and genotype changed both biosynthesis and emission in similar ways, early developmental exposure to maternally transmitted *c*VA and microbes strongly decreased *c*VA emission but not the biosynthesis of this molecule. This indicates that the release – but not the biosynthesis – of this sex pheromone strongly depends on early developmental context. The mechanism by which the preimaginal effects occur is unknown, but reinforces the significance of development in determining adult physiology and behaviour.

KEY WORDS: *cis*-Vaccenyl acetate, *desat1*, Microbiota, Preimaginal conditioning, Social interaction

INTRODUCTION

The volatile *Drosophila* pheromone (Z)-11-octadecenyl acetate (*cis*-vaccenyl acetate; *c*VA) is one of the most intensively studied pheromones in the animal kingdom. It is synthesized by *Drosophila melanogaster* males (Bontonou and Wicker-Thomas, 2014; Butterworth, 1969; Guiraudie-Capraz et al., 2007; Symonds and Wertheim, 2005) and can be detected both on the male cuticle and in the ejaculatory bulb. It is transferred from males to females during mating and is then introduced into food during egg-laying, playing a series of behavioural roles, altering responses to other compounds (Bartelt et al., 1985a,b; Hedlund et al., 1996; Jaenike et al., 1992; Schaner et al., 1987). The different effects of *c*VA when presented in a blend can be seen in two ways. When combined with food compounds, *c*VA induces aggregation in male and female flies of several *Drosophila* species (Bartelt et al., 1985a; Keesey et al., 2016; Schaner et al., 1987; Wertheim et al., 2002); the neuronal basis of this effect is beginning to be understood (Das et al., 2017). When combined with the *D. melanogaster* male cuticular hydrocarbon 7-T, *c*VA inhibits male courtship (Everaerts et al., 2018; Jallon et al., 1981; Laturney and Billeter, 2016), stimulates female mating and modulates the intensity of *D. melanogaster* male–male aggressive behaviour (Wang et al., 2011).

Although *c*VA detection and processing has been intensively investigated at the cellular level, in particular sexually dimorphic effects (attraction and repulsion) (Datta et al., 2008; Kurtovic et al., 2007; Lebreton et al., 2015; Ruta et al., 2010), the factors involved in *c*VA biosynthesis and emission are poorly understood (Chertemps et al., 2005). *c*VA is produced in the ejaculatory bulb (Butterworth, 1969; Guiraudie-Capraz et al., 2007) and its level increases with age (Bartelt et al., 1985a). There are differing reports about whether physical interactions with another fly of either sex are required before any cVA is released – it cannot be detected in socially isolated males using solid phase micro-extraction (SPME; Everaerts et al., 2010), but traces of cVA on the cuticle and legs have been found using ultraviolet laser desorption/ionization orthogonal time-of-flight mass spectrometry (Yew et al., 2009). In general, only the superficial levels of *c*VA on the outside of the insect – those found on the cuticle or at the male genital opening – have been studied. To our knowledge, the sole exception is Bartelt et al. (1985b), who used long duration (24 h) extractions. There has been no other investigation of internal levels of *c*VA, and studies of *c*VA biosynthesis have thus far been limited to identifying some of the enzymes involved (Chertemps et al., 2005).

Behavioural responses to *c*VA can be affected by experience. If males court recently mated females, which are rich in *c*VA, their subsequent courtship of virgin females is reduced in intensity (Ejima et al., 2007; Siegel and Hall, 1979). Developmental factors can shape these courtship responses to *c*VA. When an egg is laid by a recently mated female, it is covered in *c*VA that the male has introduced together with his sperm and other compounds (Billeter and Wolfner, 2018). If this *c*VA is removed from the egg, the males that eventually emerge show a weaker courtship reduction in response to *c*VA (Everaerts et al., 2018). This suggests either that *c*VA directly affects development in the egg, or that early contact with *c*VA as the larva emerges from the egg shapes the adult response. Furthermore, this pre-imaginal effect also seems to be mediated by other maternally transmitted factors present on the outside of the egg, such as microbes (Everaerts et al., 2018).

In this paper, we explored how the link between biosynthesis and emission of *c*VA changes as male flies age. We also studied the effects of early exposure to *c*VA and microbial factors on *c*VA biosynthesis and emission and looked at the role of *desat1*, a gene that simultaneously affects the production, detection and processing of pheromones in *Drosophila* and is expressed in a variety of tissues, including the male genital tract (Nojima et al., 2019). Our results show that early developmental exposure to maternally transmitted *c*VA and microbes strongly decreased *c*VA emission but not the biosynthesis of this molecule. This indicates that the release – but not the biosynthesis – of this sex pheromone strongly

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depends on early developmental context. Similar effects may be found in other chemical communication systems.

MATERIALS AND METHODS

Flies

Drosophila melanogaster Meigen 1830 were raised on yeast/cornmeal/agar medium and kept at 24±0.5°C with 65±5% humidity on a 12 h:12 h light:dark cycle. Unless indicated, flies were transferred every 2 days to avoid larval competition and to regularly provide abundant progeny for testing. Flies were isolated under light CO_2 anaesthesia 0–2 h after eclosion. We tested two wild-type stocks, Canton-S (CS) and Dijon2000 (Di2), and the *desat1*^{1573} homozygous mutant (*desat1*), which was produced by the insertion of a PGal4 transposable element in the regulatory region of the *desaturase1* gene (Marcillac et al., 2005a). Unless specified otherwise, males and females were held separately in fresh glass food vials in groups of five flies until they were studied.

cis-Vaccenyl acetate (cVA)

Flies were frozen for 5 min at −20°C; *c*VA was then extracted from individual flies following two procedures. To characterize superficial *c*VA, individual flies were immersed for 5 min at room temperature using 30 µl hexane. To measure global *c*VA (superficial+internal), individual flies were immersed at room temperature for 24 h in 30 µl dichloromethane. Dichloromethane is a polar solvent widely used in the study of insects (Golub and Weatherston, 1984). In both cases, the solvent solutions contained 3.33 ng µl^{-1} of C26 (*n*-hexacosane) and 3.33 ng µl^{-1} of C30 (*n*-triacontane) used as internal standards. *c*VA amounts were quantified by gas chromatography using a Varian CP3380 gas chromatograph fitted with a flame ionization detector, a CP Sil 5CB column (25 m×0.25 mm, length×internal diameter; 0.1 µm film thickness; Agilent), and a split–splitless injector (60 ml min^{-1} split-flow; valve opening 30 s after injection) with helium as carrier gas (50 cm s^{-1} at 120°C). The temperature program began at 120°C, ramping at 10°C min^{-1} to 140°C, then ramping at 2°C min^{-1} to 290°C, and holding for 10 min. We determined *c*VA amounts on groups of 50 eggs using the hexane-extraction procedure for individual flies.

Egg and fly manipulation

To obtain enough eggs, approximately one hundred 3- to 4-day-old mass-reared females (except for the D1/D5 experiment, see below) of the three strains were allowed to lay eggs for 3 h in an egg-laying device consisting of a 50 mm Petri dish filled with 1 ml 3% agar striped with fresh yeast to stimulate egg-laying. After this period, flies were removed and eggs collected. To determine the influence of the egg chorion on *c*VA levels, eggs were collected and rinsed five times in fresh deionized and sterile water. They were then dechorionated by immersion for a few minutes in a 3% solution of sodium hypochlorite, followed by three washes with sterile deionized water. This procedure substantially reduces the level of microbes on the egg (Farine et al., 2017). As a control, a group of eggs were treated identically except that they were not subjected to washing in either sodium hypochlorite or deionized water. Approximately 50 eggs were transferred in each food vial.

For the D1 versus D5 experiment, eggs laid by females that had mated either 1 day (D1) or 5 days previously (D5) were transferred to plain food in groups of 50. In order to ensure that eggs were laid by females of the same age, and therefore carrying equivalent internal factors, females laying D1 eggs were 6 days old, while females laying D5 eggs were 2 days old. All eggs in this experiment were therefore laid by 7-day-old females.

In the case of pre-imaginal exposure to *c*VA-rich food, groups of 50 intact eggs were transferred onto regular food with added synthetic *c*VA (15 ng mm^{-3}; Cayman Chemical, Ann Arbor, MI, USA; 50 mg ml^{-1} solution in ethanol, purity >98%) (Everaerts et al., 2018). Adult males eclosing from this food were immediately transferred onto plain food before being tested.

To determine the effect of bacteria on *c*VA levels, we kept the three strains for two generations on food containing an antibiotic mixture (50 µg ml^{-1} tetracycline+200 µg ml^{-1} rifampicin+100 µg ml^{-1} streptomycin; Sharon et al., 2010). Adults produced by this treatment were transferred onto regular food just after eclosion.

For all adult experiments, males produced by the various treatments were collected immediately after adult eclosion and kept in groups of five flies (unless specified otherwise) until they were subjected to *c*VA extraction. To determine the effect of adult social interaction, males eclosing from the control experiment were kept in groups of five flies or alone, and tested when 4 days old.

Statistics

All statistical tests were performed using XLSTAT 2021 (https://www.xlstat.com/en/). We compared the amount of *c*VA in the males of the three genotypes at all ages using the Kruskal–Wallis test followed by Conover–Iman multiple pairwise comparisons (P=0.05, with a Bonferroni correction). The effect of each treatment was estimated by comparison with the corresponding control using the Mann–Whitney test, for each genotype at each age.

RESULTS

The amount of global *c*VA (this corresponds to the sum of *c*VA on the cuticle and in the ejaculatory bulb) can be considered as an indirect measure of *c*VA biosynthesis. In male flies, global *c*VA showed a gradual increase with age, with the main increase being observed in the first day (Fig. 1A; note that for the sake of clarity, on all figures the amount of *c*VA is presented on a log scale; statistical analysis was performed on untransformed data; see Dataset 1). Overall, there were no clear and consistent differences between the three genotypes (the two wild types – CS and Di2 – and the *desat1* mutants) for this measure, with all three strains reaching around 1000 ng of *c*VA per fly at around 4 days old. These levels were maintained not only in 12-day-old flies (Fig. 1A), but also over subsequent weeks of life, up to 40 days in the case of CS males (Fig. S1). Clear differences were observed for the amount of *c*VA detected via a shorter extraction protocol, which measured the superficial *c*VA present on the cuticle and at the genital opening and therefore available for immediate detection by other flies (Fig. 1B). This superficial *c*VA can be considered as the amount of *c*VA emitted by a male, and is evidently dependent on biosynthesis, as measured by the global level of *c*VA. Overall, *desat1* mutant males showed significantly lower levels of superficial *c*VA than the two wild-type strains, indicating that the mutation affects the emission of *c*VA, perhaps by altering hydrocarbons secreted on the inner surface of the genital tract. CS males emitted the highest amounts at all ages.

We next investigated how early experience affected these patterns of *c*VA synthesis and emission. When eggs are laid by a recently mated female, they are covered with two factors that may alter the behaviour of the adult fly: *c*VA and bacteria. *c*VA is transferred into the female during mating and is introduced into the medium along with the eggs, while the female also has her own microbiome, some or all of which is transferred onto the eggs before or during egg-laying. Removing the chorion from eggs, a process that significantly

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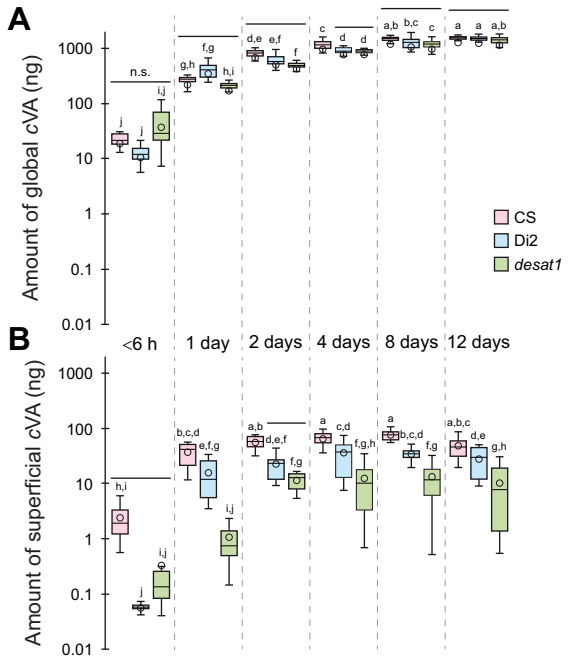

Fig. 1. Age-related variation for global and superficial amounts of *cis*-vaccenyl acetate (*c*VA) in male flies. *c*VA was measured (in ng) in males of the following ages (indicated between the two series of graphs): <6 h, and 1, 2, 4, 8 and 12 days. We measured both the global amount of *c*VA (A; obtained after a 24-h extraction with dichloromethane) and the superficial amount of *c*VA (B; obtained after a 5-min hexane extraction). We compared these values in males of three genotypes: Canton-S (CS), Dijon 2000 (Di2) and *desat1*^{1573} homozygous mutant (*desat1*). 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 plain dot. The ends of the whiskers shown below and above each box represent the limits beyond which values were considered anomalous. For each series of graphs, we compared all values including the three genotypes at all tested ages using a Kruskal–Wallis test followed by Conover–Iman multiple pairwise comparisons (P=0.05, with a Bonferroni correction). N=15 individual flies for each sample. Horizontal bars indicate no statistical difference between genotypes at each age while different letters indicate significant differences for all genotypes and ages considered.

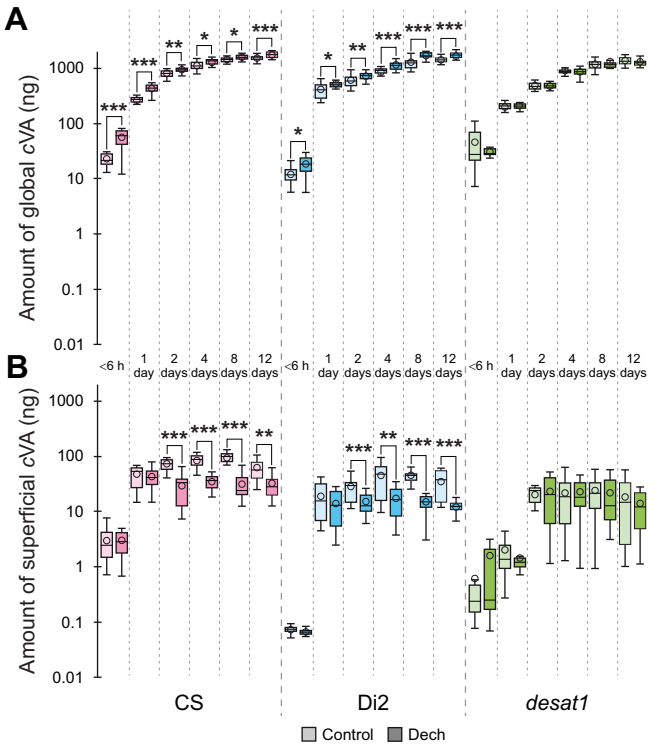

Fig. 2. Age-related effect of dechorionation on global and superficial amounts of *c*VA. The amounts of (A) global and (B) superficial *c*VA were compared between males from dechorionated eggs ('Dech') and from intact eggs (Control). We compared males of the three genotypes and ages as those described in Fig. 1. We used the Mann–Whitney test to compare the dechorionation effect for each genotype at each age: *P<0.05, **P<0.01, ***P<0.001. The absence of asterisks indicates that no significant difference was detected. N=15 flies for CS and *desat1* genotypes; N=13–15 flies for Di2 genotype.

reduces the levels of both cVA and microbes on the egg surface (Bakula, 1969; Farine et al., 2012; Everaerts et al., 2018), led to a slight but significant and consistent increase in the amount of global *c*VA produced by the two wild-type strains throughout adult life (Fig. 2A). However, from 2 days old onwards, the amount of superficial *c*VA immediately available on the cuticle and at the genital opening of males showed slight but consistently and significantly lower levels in wild-type males from dechorionated eggs (Fig. 2B). This suggests that early exposure to chorion-associated factors allows the consistent trafficking of *c*VA to the outside of the fly; in the absence of this exposure, less *c*VA was emitted. No effect of dechorionation was observed for *desat1* mutants (Fig. 2A,B), which showed consistently lower levels of superficial *c*VA compared with the wild-type males, but no significant difference for global *c*VA levels.

To separate the effect of early exposure to *c*VA from that of exposure to bacteria, we next reared flies on antibiotic-rich food for two generations. The effects were less clear-cut, but there were small age-related variations in the amount of global *c*VA and the amount of superficial *c*VA produced by wild-type males (Fig. 3A,B) that had been reared on antibiotic-rich food, compared with controls. Some significant differences were observed between the two conditions for *desat1* mutant flies, in particular in the first 6 h of life, where control levels of *c*VA were significantly higher than those for antibiotic-treated flies. These findings suggest that the effect of early exposure to maternally transmitted factors on *cVA* levels does not only involve bacteria.

To explore the role of *c*VA in this effect, we indirectly manipulated the amount of *c*VA present on the surface of the egg. We first measured the amount of *c*VA present on eggs laid at 1 and 5 days after mating (D1 and D5, respectively – these females were the same age when they laid their eggs, see Materials and Methods). *c*VA levels on the eggs declined significantly between the two treatments, for all three genotypes (Fig. 4A). By comparing the amounts of *c*VA produced by males that emerged from D1 or D5 eggs, we were therefore able to explore the quantitative impact of early *c*VA exposure on adult male *c*VA production. All three genotypes showed occasional but non-consistent significant differences, unlike the clear effect induced by dechorionation. There were differences between the two wild-type strains: variations of global and superficial *c*VA levels in CS males were more similar to those induced by dechorionation than in Di2 males (compare Fig. 4 with Fig. 2). The effects of low exposure to *c*VA in *desat1* males were not consistent but did show some significant differences (Fig. 4), whereas no exposure to *c*VA resulted in no differences (Fig. 2). These data suggest that exposure to reduced *c*VA level present on the eggs laid 5 days after mating was not sufficient to affect the amount of *c*VA emitted by adult males in a consistent manner. Taken together, these three experiments suggest that the

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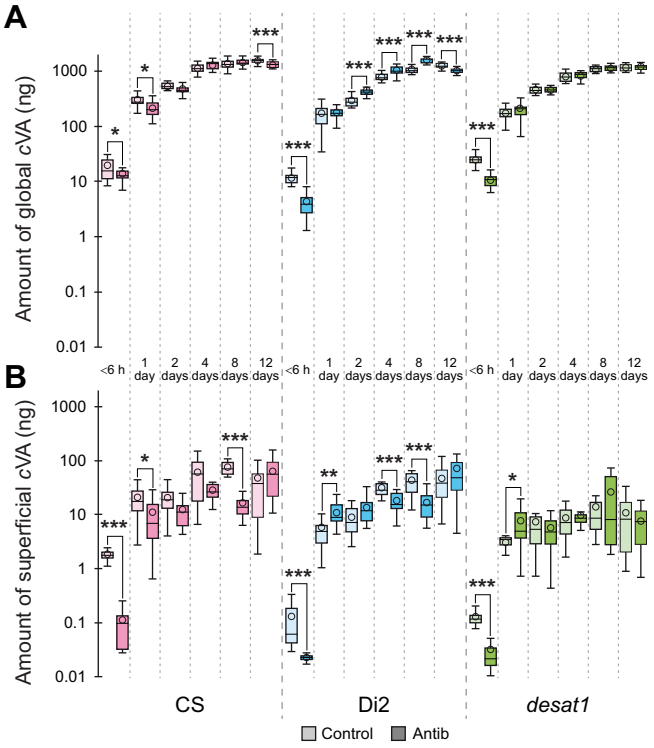

Fig. 3. Age-related effect of antibiotics on global and superficial amounts of *c*VA. The amounts of (A) global and (B) superficial *c*VA were compared between males raised on regular food containing antibiotics (see Materials and Methods) for two generations. These treated males ('Antib') were compared with males raised on plain food (Control) of three genotypes and at different ages (see Fig. 1 caption). For statistics, see Fig. 2 caption. *N*=15 individual flies.

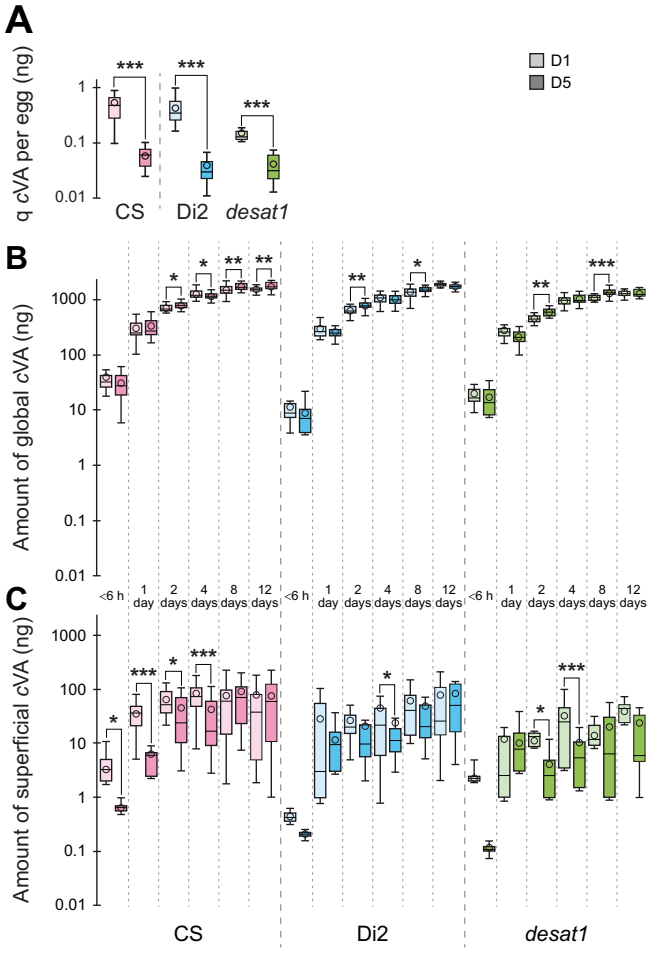

Fig. 4. Effect of egg exposure to maternally transmitted *c*VA. (A) The amount (q) of *c*Va was measured in groups of 50 eggs either laid 1 day (D1) or 5 days (D5) after copulation. The amounts of (B) global and (C) superficial *c*VA were also determined on adult males originating from D1 and D5 eggs. We compared eggs and adult males of the CS, Di2 and *desat1* genotypes (see Fig. 1 caption). For statistics, see Fig. 2 caption. *N*=13–15 groups of eggs (A); *N*=15 individual flies (B,C).

emission of *c*VA in adult male flies is regulated by pre-imaginal exposure to maternally transmitted factors – *c*VA and microbes.

Finally, the social control of the biosynthesis and emission of *c*VA was explored by comparing the amount of global and superficial *c*VA on males that were held in groups of five flies, as in the rest of our study, or singly. There were no differences in the biosynthesis of *c*VA, as measured globally (Fig. 5A), but in all three strains there were significantly lower levels of superficial *c*VA in males that were reared alone (Fig. 5B), highlighting the role of male–male interactions in the emission of *c*VA.

DISCUSSION

This study is the first exploration of the relationship between the production and emission of a *Drosophila* pheromone. We show that although there is an evident necessary relationship between these two factors (emission requires biosynthesis), it is not reciprocal – material can be synthesized but not released. This is partly a consequence of our experimental design: males were housed together, allowing for the emission of small quantities of *c*VA during male–male interactions, but had no contact with females, leading to an accumulation of *c*VA inside the males, as none was introduced into females during mating.

We were able to compare production and emission by using extremely different extraction durations: 5 min for superficial extraction, which will have measured material on the cuticle and in the genital tract, and 24 h for measuring global *c*VA levels, which will have included both superficial levels and *c*VA that had been synthesized in the ejaculatory bulb. These two aspects correspond to different functions of *c*VA: global *c*VA measures material that could be transferred to a female during mating, altering the responses of male flies to her and enabling her to attract other flies to the food site where she has laid her eggs, whereas superficial *c*VA corresponds to material that could immediately be detected by another fly of either sex, playing a role in aggression and courtship. The global amount of *c*VA increased with age, as previously reported (Bartelt et al., 1985a). The increase in the amount of *c*VA seen between 6 h and 8 days could be due to the accumulation of *c*VA in the ejaculatory bulb (Guiraudie-Capraz et al., 2007). Conversely, the amount of superficial *c*VA remained more or less constant after 2 days. Superficial levels of *c*VA on the cuticle can be detected by SPME fibres only after mating (Everaerts et al., 2010; Farine et al., 2012).

Studies of other insects may shed light on this balance between synthesis and emission. In many moths, female pheromones are synthesized in an abdominal gland and released with a circadian rhythmicity (for example in *Trichoplusia ni*, *Pectinophora gossypiella* and *Argyrotaenia velutinana*; Groot et al., 2014; Jurenka, 2017). Pheromone synthesis depends on the pheromone biosynthesis activating neuropeptide (PBAN; Jurenka, 2017), whereas pheromone emission – usually associated with 'calling' behaviour (Allison and Cardé, 2016) – is controlled either directly

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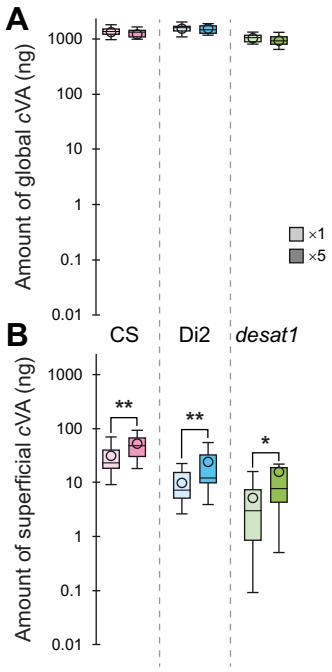

Fig. 5. Effect of adult grouping on global and superficial amounts of *c*VA. The amounts of (A) global and (B) superficial *c*VA were compared between 4-day-old males kept either isolated (×1) or grouped (×5) during adult life. For genotypes, ages and statistics, see Figs 1 and 2 captions. *N*=15 flies.

from the terminal nerve input (Christensen et al., 1994) or indirectly through the muscular contraction of the gland (Raina et al., 2000; Solari et al., 2007). In female cockroaches, secretory cells may synthesize pheromones during periods of sexual receptivity but regress during sexual inactivity (Schaal et al., 2003). Neuroendocrine factors such as PBAN, ecdysteroids and juvenile hormone can regulate pheromone synthesis (Blomquist and Vogt, 2003; Rafaeli, 2002; Tillman et al., 1999), while neuronal signals either descending from the central nervous system or ascending through the ventral nerve cord can also modulate sex pheromone synthesis and/or emission (Teal et al., 1989). The situation may be different in *Drosophila* males, which appear to synthesize *c*VA continuously while they emit *c*VA only during encounters with conspecifics (Everaerts et al., 2010; Farine et al., 2012; Laturney and Billeter, 2016; Wang et al., 2011). In contrast, *Drosophila* cuticular hydrocarbon biosynthesis depends on the circadian activity of the *desat1* gene (Krupp et al., 2008).

Further investigation of the neuroendocrine control of *c*VA physiology will be necessary, but our results already show that significant developmental factors are involved. This can be seen through our observation that the levels of *c*VA are affected by very early experience. Removing the chorion from eggs, thereby significantly reducing both the paternal *c*VA – which was present in the female's genital tract and had covered the eggs as they were laid – and the maternal microbiome, led to a slight but significant and consistent increase in the amount of global *c*VA produced by males. This was shown by both wild-type strains throughout the life of the fly. We were able to explore the relative roles of paternal *c*VA and the maternal microbiome in producing this effect by rearing flies on antibiotic-rich food, and by observing the effects of reducing the amount of *c*VA present on the eggs. Both manipulations altered the global levels of *c*VA, but to a much lesser extent than when both *c*VA and the maternal microbiome were simultaneously removed from the eggs. The levels of *c*VA on eggs laid 5 days after mating were still apparently above the threshold required to affect *c*VA emission in adult males; future experiments will be needed to identify this threshold, by reducing *c*VA levels on eggs still further, for example by extending the gap between mating and laying to 10 days.

Adding synthetic *c*VA to the laying medium produced no effect on either global or superficial *c*VA of resultant wild-type males (Fig. S2). This suggests that the effect of *c*VA depends on it being maternally transmitted on the chorion and/or non-homogeneously present in the food; the higher levels of *c*VA present on the chorion compared with our experimental treatment may account for the lack of effect we observed. We suggest that the emission of *c*VA in adult male flies is regulated by pre-imaginal exposure to a range of maternally transmitted factors, including *c*VA and microbes (there may also be other factors that we have yet to identify, which are also removed by dechorionation). Additionally, the effects of microbe manipulation on *c*VA production and emission seen in Figs 2 and 3 could be partly due to a loss of nutrition represented by the absence of the microbes or the lack of some key microbial byproduct (Shin et al., 2011; Douglas, 2019).

One way of exploring this possibility, and of revealing the precise mechanism by which microbes exert their effect, would be to remove microbes non-destructively (for example using a water wash) and then returning either the whole wash, or an identified part of the microbiome (such as a particular class of microbe) in order to rescue the effect observed with the removal of microbes. This would be technically challenging, but would open new mechanistic approaches, including potential effects on gene regulation and/or as modulators of protein function. *c*VA in particular seems to be significant in its pre-imaginal effects on adult behaviour: pre-adult exposure to maternally transmitted *c*VA alters how adult male courtship is affected by *c*VA (Everaerts et al., 2018).

The three genotypes we studied did not show the same responses. The differences between the two wild-type strains – CS and Di2 – were relatively minor and presumably reflect differences in genetic background. For example, the different responses of the two wild types to antibiotic treatment suggests that the two wild-type males are differently sensitive to early exposure to *c*VA and microbes. The differential response of *desat1* flies, which show defects in both production and detection of cuticular hydrocarbons (Marcillac et al., 2005b), was of far greater biological significance. However, this effect does not appear to be related to the known effect of *desat1* on cuticular hydrocarbon biosynthesis: we found no effect on *c*VA production, only on its emission. This suggests that *desat1* does not affect *c*VA biosynthesis. It is possible that, given that social interactions appear to be necessary for *c*VA to be emitted, the pleiotropic effects of the mutation on cuticular hydrocarbon perception may be involved. This will require further investigation.

desat1 flies have the same genetic background as CS, allowing us to impute differences in their responses to the mutation in *desat1*. While all males of all three strains showed very similar global *c*VA levels, wild-type males had higher superficial levels of *c*VA, suggesting that the *desat1* mutant interferes with the trafficking of *c*VA from the ejaculatory bulb to the exterior, thereby reducing the emission of the pheromone. This effect could be due to the mutation leading to the absence of desaturated hydrocarbons in the male genital tract, where they may play a role akin to lubrication. The lack of any effect of dechorionation on *c*VA emission in *desat1* males is at least partly because the levels of superficial *c*VA were already extremely low.

For practical reasons, most of the males tested here were kept in small groups of five flies, which inevitably allowed for male–male

interactions. The comparison of their *c*VA levels with those of males kept alone (Fig. 5) showed a slight decrease of superficial – but not global – *c*VA, suggesting that this effect is dissociated from that induced by egg dechorionation. Additional experiments will be required to test the effect induced by other social interactions such as male–female sexual behaviour, the role of individual preimaginal development, and so on.

We have shown that the biosynthesis and the emission of *c*VA show different changes with aging and with social context. Furthermore, simultaneous exposure to microbes and *c*VA during egg development, and perhaps during larval life, is involved in the regulation of *c*VA emission in adult males. Our next challenge will be to investigate the biological mechanisms underlying this effect and to explore the effect of early exposure to *c*VA on female behaviour. Researchers working on other systems of chemical communication may be inspired to investigate whether early experience also affects production and emission of the pheromones they study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.-P.F., J.-F.F.; Methodology: J.C., J.-P.F., C.E., J.-F.F.; Validation: M.C., C.E.; Formal analysis: J.-P.F., C.E.; Investigation: J.C., J.-P.F., C.E., J.-F.F.; Data curation: C.E.; Writing - original draft: M.C., C.E., J.-F.F.; Writing - review & editing: M.C., C.E., J.-F.F.; Visualization: C.E.; Supervision: J.-F.F.

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