The scent of the fly

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Abstract

(*Z*)-4-undecenal (*Z*4-11Al) is the volatile pheromone produced by females of the vinegar fly *Drosophila melanogaster*. Female flies emit *Z*4-11Al at a few nanograms per hour, for long-range and species-specific communication and mate-finding. Tests with synthetic *Z*4-11Al show that it has a characteristic scent, which we perceive even at the small amounts produced by a female fly. Since only females produce *Z*4-11Al, and not males, we can reliably distinguish between single *D. melanogaster* males and females, according to their scent. A wine-tasting panel finds that we sense as little as 1 ng of synthetic *Z*4-11Al in a glass of wine, and 10 ng *Z*4-11Al is perceived as a loud odour. This corroborates the observation that a glass of wine is spoilt by a single *D. melanogaster* fly falling into it, and we here show that is caused by the off-flavour *Z*4-11Al. The biological role of *Z*4-11Al or structurally related aldehydes in humans and the basis for this semiochemical convergence remains yet unclear.

Keywords

pheromone, semiochemical, odorant, off-flavour, olfaction, wine

Introduction

All living things communicate with chemicals. Unlike sounds or sights, chemicals interconnect species across the kingdoms, and enable information exchange between animals, plants and microorganisms (Schultz and Appel 2004). A fascinating, recurrent observation is that the same compound is bioactive in different species and context. This evolutionary convergence of semiochemicals may be due to their physico-chemical properties but is first of all expected to reflect their biological significance, including the underlying biochemical pathways and precursors.

Linalool, for example, is found in foliage, flowers and fruit of many plants. Herbivore infestation leads to augmented production of linalool, which helps plants to repel aphids and other herbivores (Mithöfer and Boland 2012). Release of the enantiomers, (R)- and (S)-linalool, differs among plant parts and changes during phenological development; they play different roles in attraction of pollinators and herbivores, for feeding and oviposition (Okamoto et al. 2007; Reisenman et al. 2010; Saveer et al. 2012; Raguso 2016; Pragadheesh et al. 2017).

A background of plant-produced airborne linalool enhances mate-finding and pheromone response in some plant-feeding species, while other insects produce linalool as a sex pheromone component (Hefetz et al. 1979; Aldrich et al. 1986; Leal et al. 1993; Yang et al. 2004; Ju et al. 2017). In mammals, linalool induces psychopharmalogical effects via glutamate receptors (Elisabetsky et al. 1995; Nakamura et al. 2009); perception via odorant receptors (Ors) produces a sweet, floral note that makes a prominent contribution to the bouquet of flowers, fruit (Pichersky et al. 1994; Lewinsohn et al. 2001) and wine, where both grape and yeast are a source of linalool (Carrau et al. 2005; Swiegers et al. 2005).

Citrus fruit is a preferred oviposition substrate for the fruit fly *Drosophila melanogaster* (Dweck et al. 2013), provided that yeast is present (Becher et al. 2012; Gorter et al. 2016). Both citrus peel and brewer's yeast produce linalool (Chisholm et al. 2003; Carrau et al. 2005). The flies perceive linalool via several Ors, including Or69a, and they are attracted to it (Hallem et al. 2004; Dweck et al. 2013; Lebreton et al. 2017). Interestingly, the Or69a olfactory channel encodes not only linalool, but also the recently identified fly pheromone (*Z*)-4-undecenal (*Z*4-11Al) (Lebreton et al. 2017), which is also found in citrus peel (Chisholm et al. 2003).

During volatile collections from pheromone-releasing *D. melanogaster* flies, we discovered that we can reliably distinguish single male from female flies by their smell and that this smell is strongly reminiscent of synthetic *Z*4-11Al. We therefore employed a sensory panel to verify whether we can indeed sense the pheromone emanating from single *D. melanogaster* females, and whether the newly discovered pheromone *Z*4-11Al contributes to the scent of the female fruit fly.

Methods

Chemicals

Isomeric purity of synthetic (*Z*)-4-undecenal (*Z*4-11Al) was 98.6% and chemical purity >99.9%, according to gas chromatography coupled to mass spectrometry (6890 GC and 5975 MS, Agilent technologies Inc., Santa Clara, CA, USA). Ethanol (redistilled, >99.9% purity; Merck, Darmstadt, Germany) was used as solvent.

Sensory evaluation

Eight members (2 women, 6 men) of the sensory panel for organoleptic tests for the wine-growing area of Baden (Germany) evaluated the odour of *D. melanogaster* flies and synthetic *Z*4-11Al. Each test comprised three visually identical glasses, control and two treatments, which were presented in random order. The panel was asked to score odour intensity, ranging from 1 (weak, silent) to 9 (strong, loud) and to comment on differences in odour quality between the three glasses. The first test compared single male and female flies. Flies were kept singly during 5 min in empty wine tasting glasses (215 ml), they were released before tests. The second test compared synthetic *Z*4-11Al and a glass impregnated with the odour of a single female fly, *Z*4-11Al (10 ng in 10 µl ethanol) was applied to an empty glass and the solvent was allowed to evaporate during 2 min. Next, 10 ng *Z*4-11Al diluted in ethanol or a female fly were added to a glass filled with either water or white wine (dry Pinot blanc, Freiburg 2013, Staatsweinkellerei Freiburg). The fly was removed after 5 min, prior to testing. Finally, 1 or 5 ng *Z*4-11Al was added to wine.

Statistical analysis

Odor panel data was analyzed using one-tailed analysis of variance (ANOVA) followed by a Tukey test. Normality was tested using Shapiro-Wilk and homoscedasticity was tested using Levene's test. All analysis were carried out using SPSS v. 20 (IBM Corp, 2011).

Results and Discussion

D. melanogaster female flies (Figure 1) produce a distinctive scent. The sensory panel found the odour of single female flies to be stronger and qualitatively clearly different from single male flies (Fig. 2a).

Chemical analysis has shown earlier that *Z*4-11Al (Lebreton et al. 2017) and its biosynthetic precursor, the cuticular hydrocarbon (*Z,Z*)-7,11-heptacosadiene, are produced by female flies, not by males (Billeter et al. 2009). Our panel tests establish that synthetic *Z*4-11Al has a distinctive odour (Fig. 2b). Moreover, a female fly and 10 ng *Z*4-11Al were found to be similar, with respect to odour quality and intensity, when presented in an empty glass, in water or wine (Fig. 2c, d, e). Since the wine panel perceived 10 ng *Z*4-11Al as slightly louder than the odour of a single fly, we compared *Z*4-11Al at 1 ng and 5 ng, showing that as little as 1 ng of *Z*4-11Al was clearly perceptible (Fig. 2f). Even at small amounts, *Z*4-11Al was perceived as a somewhat unpleasant off-flavour.

The detection threshold for *Z*4-11Al is apparently in the same order in flies and men, since we clearly sense *Z*4-11Al released from a single fly (Fig. 2a). Chemical analysis found that *D. melanogaster* females released *Z*4-11Al at a rate of 2.4 ng/h in a glass aeration apparatus and solvent extracts of the fly cuticula contained 0.3 ng *Z*4-11Al/female (Lebreton et al. 2017). We cannot exclude that other fly volatiles, including saturated and unsaturated aldehydes contribute to our perception of fly odour. However, *Z*4-11Al is the most abundant compound found only in effluvia of female flies, whereas other, structurally related compounds are released by both sexes (Lebreton et al. 2017).

Taken together, the sensory panel corroborated our initial observation that we sensitively smell *Z*4-11Al, the female-produced pheromone of the fruit fly *D. melanogaster* (Lebreton et al. 2017) and that we can reliably distinguish single female from male flies. This validates the observation that one fly spoils a glass of wine, after falling into it - provided it is of the female sex.

An explanation for the convergent perception of *Z*4-11Al in flies and men is, however, not at hand. The occurrence of *Z*4-11Al in nature is certainly incompletely known and a possible biological role in humans remains unclear. In addition to *D. melanogaster* flies, *Z*4-11Al has also been found in citrus peel (Chisholm et al. 2003) and in the anal gland of the rabbit, perception of *Z*4-11Al leads to changes in rabbit heart rate (Goodrich et al. 1978).

Colonies of crested auklet, a seabird, produce a characteristic citrus-like scent, which we sense from far (Douglas et al. 2001). Two unsaturated aldehydes, including (*Z*)-4-decenal (*Z*4-10Al) are main constitutents of this bird-produced odour (Douglas et al. 2001). In crested auklet, *Z*4-10Al likely plays a role as an ectoparasite repellent and a signal of mate quality (Douglas et al. 2001; Caro & Balthazart 2010). (*E*)-2-nonenal is another odour-active unsaturated aldehyde, found in mushrooms and wine (Wood et al. 1994; Chatonnet & Dubourdieu 1998; Ferreira et al. 2004).

The olfactory sense in animals plays a key role during habitat adaptation. Tuning of Ors to habitat cues is thought to create a bias for mate-finding signals that match or are structurally related to habitat- and resource-associated odorants (Endler 1992; Boughman & Svanbäck 2017). This idea yields a tentative scenario for the convergence of semiochemicals. All animals, including the vinegar fly and ourselves, have long been associated with yeasts that facilitate digestion of plant materials, provide nutrients and protection of food from antagonistic microorganisms. Unsaturated aldehydes may have initially signalled presence of fruit and yeast, leading to conspecific aggregations at feeding sites. Structurally similar aldehydes, derived from fatty acid biosynthesis in animals, may then have been secondarily adopted as mating signals via established sensory channels.

The phylogenetic divergence of Ors, including the Or69a channel, which is dedicated to *Z*4-11Al in the *Drosophila* clade, is accessible to investigation of the underlying molecular and physiological mechanisms (Lebreton et al. 2017). A current research challenge is to further develop studies of odorant perception and phylogenetic divergence in animals and to improve our understanding of the chemical vocabulary that interconnects us with other living things.

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Compliance with ethical standards

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Conflict of interest

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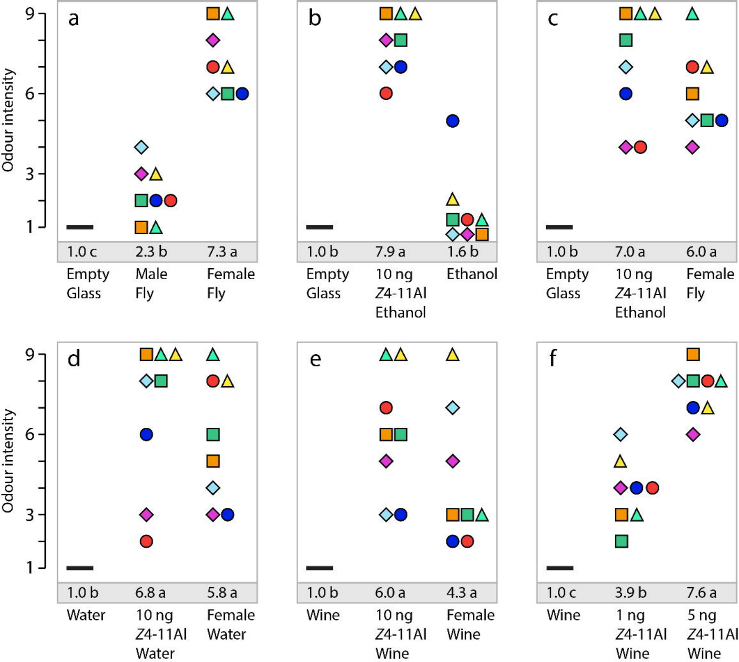
Legends

Fig. 1 Fruit fly *D. melanogaster* female with exposed ovipositor on blueberry (Picture by Cyrus Mahmoudi).

(colour & high resolution version available).

Fig. 2 Sensory evaluation of fly odor and synthetic (*Z*)-4-undecenal (*Z*4-11Al) according to a sensory panel of eight. Odor intensity scale ranges from 1 (weak) to 9 (strong). Symbols show evaluation by individual test panel members, mean intensity ratings followed by different letters are significantly different ($p<0.001$. Olfactory intensity of (a) the odour of a single *D. melanogaster* male and female fly adsorbed during 5 min in an empty wine glass ($F=96.711$), (b) 10 ng synthetic Z4-11Al and solvent (ethanol) ($F=106.732$), (c) 10 ng Z4-11Al and the odour of a single *D. melanogaster* female fly in an empty glass ($F=34.720$), (d) in a glass with water ($F=16.689$), (e) in a glass with wine ($F=12.952$), (f) 1 ng and 5 ng Z4-11Al in a glass with wine ($F=110.694$).


Odour intensity
9
6
3
1
a
1.0 c
2.3 b
7.3 a
Empty Glass
Male Fly
Female Fly
b
1.0 b
7.9 a
1.6 b
Empty Glass
10 ng Z4-11Al Ethanol
Ethanol
c
1.0 b
7.0 a
6.0 a
Empty Glass
10 ng Z4-11Al Ethanol
Female Fly
d
1.0 b
6.8 a
5.8 a
Water
10 ng Z4-11Al Water
Female Water
e
1.0 b
6.0 a
4.3 a
Wine
10 ng Z4-11Al Wine
Female Wine
f
1.0 c
3.9 b
7.6 a
Wine
1 ng Z4-11Al Wine
5 ng Z4-11Al Wine