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 for species-specific communication and mate-finding. A sensory panel finds that synthetic *Z*4-11Al has a characteristic flavour, which we perceive even at the small amounts produced by one 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. Females release *Z*4-11Al at 2.4 ng/h and we readily sense 1 ng synthetic *Z*4-11Al in a glass of wine (0.03 nmol/L), while a tenfold concentration is perceived as a loud off-flavour. This corroborates the observation that a glass of wine is spoilt by a single *D. melanogaster* fly falling into it, which we here show is caused by *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, semiochemicals 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. Evolutionary convergence may result from the widespread occurrence or even physico-chemical properties facilitating information transmission, but is first of all thought to reflect the biological significance of chemicals, including the underlying biochemical pathways and precursors.

Citrus fruit is a preferred oviposition substrate for the vinegar fly *Drosophila melanogaster* (Dweck et al. 2013) provided that yeast is present (Becher et al. 2012). Citrus peel and brewer's yeast both produce linalool (Chisholm et al. 2003; Carrau et al. 2005), which the flies perceive via several odorant receptors (Ors), including DmelOr69a (Münch et al. 2016; Lebreton et al. 2017).

Linalool, commonly found in the headspace of foliage, flowers and fruit, is bioactive in many animals. Plant-produced linalool enhances mate-finding in several phytophagous insects, while other species release linalool as a sex pheromone component (Hefetz et al. 1979; Aldrich et al. 1986; Leal et al. 1993; Yang et al. 2004). Herbivory, on the other hand, upregulates linalool production in plants, which may protect against further infestation (Mithöfer and Boland 2012). The (R) and (S) enantiomers of linalool differentially attract pollinators and herbivores, for feeding and oviposition (Reisenman et al. 2010; Saveer et al. 2012; Raguso 2016), and enantiomeric changes during phenological development modulate our perception of flower aroma (Pragadheesh et al. 2017). In mammals, linalool induces psychopharmalogical effects via glutamate receptors (Elisabetsky et al. 1995; Nakamura et al. 2009), perception via Ors produces a sweet, floral note and makes a prominent contribution to the bouquet of flowers, fruit and wine, where both grape and yeast are a source of linalool (Lewinsohn et al. 2001; Carrau et al. 2005; Swiegers et al. 2005).

The response to food and mate olfactory cues is strongly interconnected in *Drosophila* (Lebreton et al. 2015; Gorter et al. 2016; Das et al. 2017) and DmelOr69a is also tuned to the newly identified female pheromone (*Z*)-4-undecenal (*Z*4-11Al), in addition to food odorants (Lebreton et al. 2017). Curiously, Z4-11Al is also found in citrus essential oil (Chisholm et al. 2003). Unsaturated aldehydes are prominent constitutents of a range of food aromas, including fruit, wine, meat and fish (e.g. Varlet et al. 2006; Cullere et al. 2007, Perez-Cacho and Rouseff 2008; Shi et al. 2013; Yang et al. 2008). Moreover, Z4-11Al is an anal gland volatile in the rabbit (Goodrich et al. 1978).

While collecting volatiles from *D. melanogaster* flies, we discovered that we can reliably distinguish single male from female flies by their scent, which is strongly reminiscent of synthetic *Z*4-11Al. We then employed a sensory panel to verify whether we can indeed discern single flies, and whether the newly discovered pheromone *Z*4-11Al contributes to the scent of the female fly.

Materials and methods

Chemicals

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

Sensory evaluation

Eight members (six men and two women) of the sensory panel for organoleptic tests for the wine-growing area of Baden evaluated the odor of *D*. *melanogaster* and synthetic *Z*4-11Al. Members of this panel have been trained and selected for the official quality assessment of wines produced in Baden, at the Federal Institute for Viticulture, Freiburg, Germany. Each test comprised three glasses, control and two treatments, which were presented in random order. The panel was asked to score odor intensity, ranging from 1 (weak, silent) to 9 (strong, loud) and to comment on odor quality. The local human subjects committee approved sensory evaluation of Z4-11Al by sniffing. The first test compared the odor from single male and female flies. Flies were kept during 5 min in empty wine tasting glasses (215 ml) and were released shortly before tests. The second test compared a glass impregnated with fly odor and *Z*4-11Al (10 ng in 10 µl ethanol), which was applied to an empty glass, the solvent was allowed to evaporate during 2 min. Next, 10 ng *Z*4-11Al 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

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

Chemical analysis has shown earlier that *Z*4-11Al and its precursor, the cuticular hydrocarbon (*Z,Z*)-7,11-heptacosadiene, are produced by female flies, not by males (Billeter et al. 2009; Lebreton et al. 2017). Our panel tests established that synthetic *Z*4-11Al has a distinctive odor (Fig. 2b). Moreover, a female fly and 10 ng *Z*4-11Al were found to be similar, with respect to odor quality and intensity, when presented in an empty glass, in water or wine (Fig. 2c,d,e). Since 10 ng *Z*4-11Al was assessed as slightly louder than the odor of a fly, we compared *Z*4-11Al at 1 ng and 5 ng, showing that as little as 1 ng *Z*4-11Al in a wine glass (corresponding to ca. 5 ng/L or 0.03 nmol/L at the time of application) 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 similar 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 and solvent extracts of fly cuticula contained 0.3 ng *Z*4-11Al/female (Lebreton et al. 2017).

Discussion

Sensory evaluation confirmed that we sensitively smell *Z*4-11Al, the female-produced pheromone of the fruit fly *D. melanogaster* (Lebreton et al. 2017) and that we reliably distinguish single female from male flies. This supports the observation that one fly spoils a glass of wine, after falling into it - provided it is of the female sex. Other fly volatiles may contribute to our perception of fly odor. However, *Z*4-11Al is the most abundant

compound which is released by females only, other volatiles are found in both sexes (Lebreton et al. 2017).

A straightforward explanation for convergent perception of *Z*4-11Al is, however, not at hand. The occurrence of *Z*4-11Al in nature is probably incompletely known and its possible role in humans remains unclear. *Z*4-11Al may merely be reminiscent of other food aldehydes, or it might avert ingestion of fruit infested with vinegar flies, which may be contaminated with microbes vectored by flies.

A range of mammalian Ors is responsive to aldehydes (e.g. Benbernou et al. 2007; Saito et al. 2009; Nara et al. 2011), including human OR1A1 and OR2W1 (Geithe et al. 2017 a,b). Perception of *Z*4-11Al effects the heart rate of the rabbit, where it has been found in the anal gland (Goodrich et al. 1978). The positional isomer (*E*)-2-undecenal is a bovid body odor (Gikonyo et al. 2002) and olfactory sensory neurons of ticks (Acari, Ixodidae) tuned to aldehydes afford indirect evidence for aldehyes as vertebrate signals (Steullet and Guerin 1994). A characteristic scent wich is reminiscent of tangerine emanates from colonies of crested auklet, a monogamous seabird. Two unsaturated aldehydes, the chain-shortened analog (*Z*)-4-decenal (*Z*4-10Al) and (*Z*)-2-decenal are main odor-active constitutents. In crested auklet, *Z*4-10Al likely plays a role as an ectoparasite repellent and a signal of mate quality (Douglas et al. 2001; Hagelin et al. 2003; Caro and Balthazart 2010).

*Z*4-11Al is also part of clementine aroma (Chisholm et al. 2003) and it may play a dual role as social signal and food cue, not only in flies, but also in other animals. The olfactory sense in animals plays a key role during habitat adaptation. Tuning of Ors to habitat cues will create a bias for mate-finding signals that match or are structurally similar to habitat odorants (Endler 1992). This idea yields a tentative scenario for the convergence of semiochemicals. Insects and other animals feed on fruit, containing associated yeasts that facilitate digestion of plant materials, provide nutrients and protection of food from antagonistic microorganisms. Animal-produced compounds sharing structural motifs may have secondarily been adopted as mating signals, via established sensory channels dedicated to habitat and food odorants.

The olfactory system of *Drosophila* is conveniently accessible to experimental investigation and current research extends beyond the Or ligand repertoire (Münch and Galizia 2016) to neural circuits underlying odor-mediated behavior (Kohl et al. 2013; Auer and Benton 2016; Seki et al. 2017), chemical ecology (Depetris-Chauvin et al. 2015; Mansourian and Stensmyr 2015) and phylogenetic diversification (Shiao et al. 2015; Arguello et al. 2016; Ramasamy et al. 2016). A future challenge is to extend functional, behavioral, ecological and phylogenetic studies to include vertebrates, towards an understanding of the chemical vocabulary that interconnects us with other living things.

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Data accessibility. Data is completely included in the figure.

Authors' contributions. P.G.B. sensed the fly scent, P.G.B., S.L., M.B. and V.J. conceived the idea and contributed to the experiment, F.B. calculated statistics, E.W. and E.H. synthesized the test chemical, P.W. supervised the project and wrote the manuscript, all authors contributed to and approved the final version of the manuscript.

Competing interests. Authors declare no competing interests.

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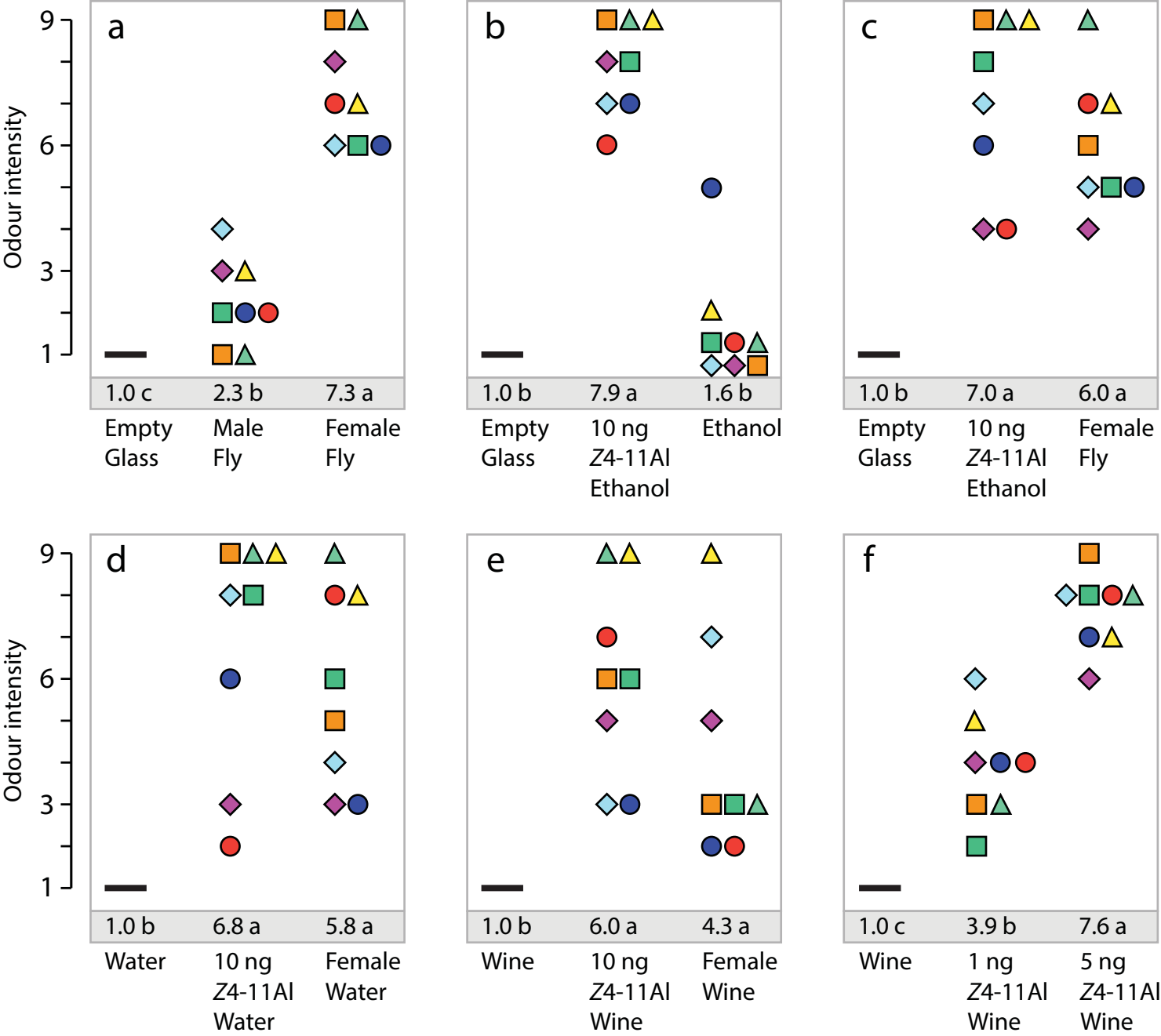
Legends

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

Fig. 2 Sensory evaluation of fly odor and synthetic (*Z*)-4-undecenal (*Z*4-11Al). 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 odor 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 odor 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).


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