The scent of the fly

Paul G. Becher[1], Sebastien Lebreton[1], Erika A. Wallin[2], Erik Hedenström[2], Felipe Borrero[3], Marie Bengtsson[1], Volker Jörger[4], Peter Witzgall[1*]

[1]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 102, 23053 Alnarp, Sweden

[2]Department of Chemical Engineering, Mid Sweden University, Holmgatan 10, 85170 Sundsvall, Sweden

[3]Biological Control Laboratory, Colombian Corporation of Agricultural Research, AA 240142 Las Palmas, Bogota, Colombia

[4]Staatliches Weinbauinstitut, Merzhauserstr. 119, 79100 Freiburg, Germany

[*]Correspondence: Peter Witzgall, SLU, Box 102, 23053 Alnarp, Sweden
phone +46 70 2426939, peter.witzgall@slu.se

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 few nanograms per hour, for species-specific communication and mate-finding. Tests with synthetic *Z*4-11Al show that it has a characteristic off-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. A wine-tasting panel finds that we sense as little as 1 ng synthetic *Z*4-11Al in a glass of wine, and 10 ng *Z*4-11Al 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

1. 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 [1]. A fascinating, recurrent observation is that the same compound is bioactive in different species and context. The evolutionary convergence of semiochemicals may be due to their physico-chemical properties but is first of all expected to reflect biological significance, including the underlying biochemical pathways and precursors.

Linalool, for example, is found in foliage, flowers and fruit of many plants. Herbivory upregulates linalool production, which protects against further infestation [2]. Plant-produced linalool enhances mate-finding in some plant-feeding insects, while other species release linalool as a sex pheromone component [3-6]. The (R) and (S) enantiomers differentially attract pollinators and herbivores, for feeding and oviposition [7-9], and enantiomeric changes during phenological development modulate our perception of flower aroma [10]. In mammals, linalool induces psychopharmalogical effects via glutamate receptors [11,12], perception via odorant receptors (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 [13-15].

Citrus fruit is a preferred oviposition substrate for the fruit fly *Drosophila melanogaster* [16], provided that yeast is present [17]. Both citrus peel and brewer's yeast produce linalool [14,18], which the flies perceive via several Ors, including Or69a [19,20]. Interestingly, the Or69a olfactory channel encodes in addition the recently identified fly pheromone (*Z*)-4-undecenal (*Z*4-11Al) [20], which is also found in citrus peel [18].

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 *Z*4-11Al. We therefore 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.

2. Materials and methods

(a) 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.

(b) Sensory evaluation

Eight members of the sensory panel for organoleptic tests for the wine-growing area of Baden (Germany) evaluated the odour of *D. melanogaster* and synthetic *Z*4-11Al. Each test comprised three 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 odour quality. The first test compared the odour 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 odour 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.

(c) 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).

3. Results

D. melanogaster females (figure 1) produce a distinctive scent. The sensory panel found the odour of single female flies to be stronger and qualitatively clearly different from male flies (figure 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 [20,21]. Our panel tests established that synthetic *Z*4-11Al has a distinctive odour (figure 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 (figure 2c,d,e). Since 10 ng *Z*4-11Al was assessed as slightly louder than the odour of a fly, we compared *Z*4-11Al at 1 ng and 5 ng, showing that as little as 1 ng *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 similar in flies and men, since we clearly sense *Z*4-11Al released from a single fly (figure 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 [20].

4. Discussion

The sensory panel confirmed that we sensitively smell *Z*4-11Al, the female-produced pheromone of the fruit fly *D. melanogaster* [20] and that we can 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 odour. However, *Z*4-11Al is the most abundant compound released by females only, whereas other, structurally related compounds are found in both sexes [20].

An explanation for convergent perception of *Z*4-11Al is, however, not at hand. Little is known about the occurrence of *Z*4-11Al in nature and a possible biological role in humans remains unclear. *Z*4-11Al has also been found in citrus peel [18] and in the anal gland of the rabbit, where perception effects heart rate [22].

A characteristic citrus-like scent emanates from colonies of crested auklet, a seabird (Douglas et al. 2001). Two unsaturated aldehydes, including (*Z*)-4-decenal (*Z*4-10Al) are main constitutents of this bird odour [23]. In crested auklet, *Z*4-10Al likely plays a role as an ectoparasite repellent and a signal of mate quality [23,24]. (*E*)-2-nonenal is another odour-active unsaturated aldehyde, found in mushrooms and wine [25-27].

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 similar to habitat odorants [28]. This idea yields a tentative scenario for the convergence of semiochemicals. Insects and other animals have long been associated with yeasts that facilitate digestion of plant materials, provide nutrients and protection of food from antagonistic microorganisms.

Yeast and fruit volatiles could have mediated aggregations at feeding sites, while fly-produced compounds sharing structural motifs, may have been secondarily been adopted

as mating signals via established sensory channels dedicated to habitat odorants. The phylogenetic divergence of *Drosophila* Ors is accessible to experimental investigation. A current challenge is to extend studies of Or phylogenetic divergence from insects to mammals, towards an understanding of the chemical vocabulary that interconnects us with other living things.

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. **Funding**. Supported by the Linnaeus initiative "Insect Chemical Ecology, Ethology and Evolution" (Formas, SLU). **Acknowledgements**. We thank the members of the sensory panel (Freiburg, Germany) for evaluating the fly scent and Cyrus Mahmoudi (comgraphix.de, Germany) for sharing a fruit fly photograph.

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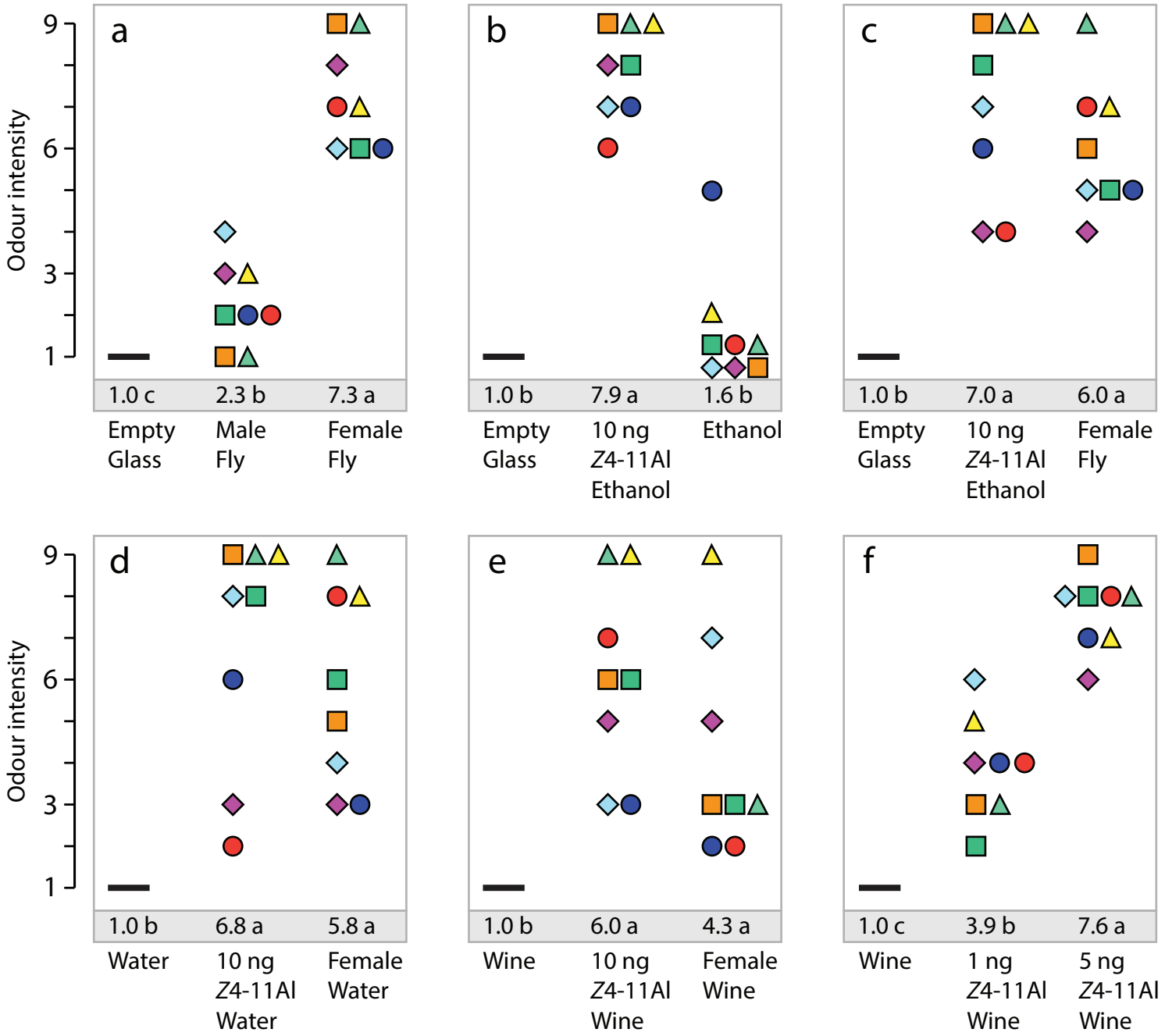
Legends

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). 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 ($F=110.694$).


a
b
c
d
e
f
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