

1 **The scent of the fly**

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13 **Abstract**

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

26 **Keywords**

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

28 **1. Introduction**

29 All living things communicate with chemicals. Unlike sounds or sights, chemicals
30 interconnect species across the kingdoms, and enable information exchange between
31 animals, plants and microorganisms [1]. A fascinating, recurrent observation is that the
32 same compound is bioactive in different species and context. The evolutionary
33 convergence of semiochemicals may be due to their physico-chemical properties but is
34 first of all expected to reflect biological significance, including the underlying biochemical
35 pathways and precursors.

36 Linalool, for example, is found in foliage, flowers and fruit of many plants. Herbivory
37 upregulates linalool production, which protects against further infestation [2]. Plant-
38 produced linalool enhances mate-finding in some plant-feeding insects, while other
39 species release linalool as a sex pheromone component [3-6]. The (R) and (S)
40 enantiomers differentially attract pollinators and herbivores, for feeding and oviposition
41 [7-9], and enantiomeric changes during phenological development modulate our
42 perception of flower aroma [10]. In mammals, linalool induces psychopharmacological
43 effects via glutamate receptors [11,12], perception via odorant receptors (Ors) produces
44 a sweet, floral note and makes a prominent contribution to the bouquet of flowers, fruit
45 and wine, where both grape and yeast are a source of linalool [13-15].

46 Citrus fruit is a preferred oviposition substrate for the fruit fly *Drosophila melanogaster*
47 [16], provided that yeast is present [17]. Both citrus peel and brewer's yeast produce
48 linalool [14,18], which the flies perceive via several Ors, including Or69a [19,20].
49 Interestingly, the Or69a olfactory channel encodes in addition the recently identified fly
50 pheromone (Z)-4-undecenal (Z4-11Al) [20], which is also found in citrus peel [18].

51 While collecting volatiles from *D. melanogaster* flies, we discovered that we can reliably
52 distinguish single male from female flies by their scent, which is strongly reminiscent of
53 Z4-11Al. We therefore employed a sensory panel to verify whether we can indeed discern
54 single flies, and whether the newly discovered pheromone Z4-11Al contributes to the
55 scent of the female fly.

56 **2. Materials and methods**

57 **(a) Chemicals**

58 Isomeric and chemical purity of synthetic Z4-11Al were 98.6% and >99.9%, respectively,
59 according to gas chromatography coupled to mass spectrometry (6890 GC and 5975 MS,
60 Agilent Technologies, Santa Clara, CA, USA). Ethanol (redistilled, >99.9% purity; Merck,
61 Darmstadt, Germany) was used as solvent.

62 **(b) Sensory evaluation**

63 Eight members of the sensory panel for organoleptic tests for the wine-growing area of
64 Baden (Germany) evaluated the odour of *D. melanogaster* and synthetic Z4-11Al. Each
65 test comprised three glasses, control and two treatments, which were presented in
66 random order. The panel was asked to score odour intensity, ranging from 1 (weak,
67 silent) to 9 (strong, loud) and to comment on odour quality. The first test compared the
68 odour from single male and female flies. Flies were kept during 5 min in empty wine
69 tasting glasses (215 ml) and were released shortly before tests. The second test
70 compared a glass impregnated with fly odour and Z4-11Al (10 ng in 10 µl ethanol), which
71 was applied to an empty glass, the solvent was allowed to evaporate during 2 min. Next,
72 10 ng Z4-11Al or a female fly were added to a glass filled with either water or white wine
73 (dry Pinot blanc, Freiburg 2013, Staatsweinkellerei Freiburg). The fly was removed after 5
74 min, prior to testing. Finally, 1 or 5 ng Z4-11Al was added to wine.

75 **(c) Statistical analysis**

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

79 **3. Results**

80 *D. melanogaster* females (figure 1) produce a distinctive scent. The sensory panel found
81 the odour of single female flies to be stronger and qualitatively clearly different from male
82 flies (figure 2a).

83 Chemical analysis has shown earlier that Z4-11Al and its precursor, the cuticular
84 hydrocarbon (*Z,Z*)-7,11-heptacosadiene, are produced by female flies, not by males
85 [20,21]. Our panel tests established that synthetic Z4-11Al has a distinctive odour (figure
86 2b). Moreover, a female fly and 10 ng Z4-11Al were found to be similar, with respect to

87 odour quality and intensity, when presented in an empty glass, in water or wine (figure
88 2c,d,e). Since 10 ng Z4-11Al was assessed as slightly louder than the odour of a fly, we
89 compared Z4-11Al at 1 ng and 5 ng, showing that as little as 1 ng Z4-11Al was clearly
90 perceptible (Fig. 2f). Even at small amounts, Z4-11Al was perceived as a somewhat
91 unpleasant off-flavour.

92 The detection threshold for Z4-11Al is apparently similar in flies and men, since we
93 clearly sense Z4-11Al released from a single fly (figure 2a). Chemical analysis found that
94 *D. melanogaster* females released Z4-11Al at a rate of 2.4 ng/h and solvent extracts of
95 fly cuticula contained 0.3 ng Z4-11Al/female [20].

96 **4. Discussion**

97 The sensory panel confirmed that we sensitively smell Z4-11Al, the female-produced
98 pheromone of the fruit fly *D. melanogaster* [20] and that we can reliably distinguish
99 single female from male flies. This supports the observation that one fly spoils a glass of
100 wine, after falling into it - provided it is of the female sex. Other fly volatiles may
101 contribute to our perception of fly odour. However, Z4-11Al is the most abundant
102 compound released by females only, whereas other, structurally related compounds are
103 found in both sexes [20].

104 An explanation for convergent perception of Z4-11Al is, however, not at hand. Little is
105 known about the occurrence of Z4-11Al in nature and a possible biological role in humans
106 remains unclear. Z4-11Al has also been found in citrus peel [18] and in the anal gland of
107 the rabbit, where perception effects heart rate [22].

108 A characteristic citrus-like scent emanates from colonies of crested auklet, a seabird
109 (Douglas et al. 2001). Two unsaturated aldehydes, including (Z)-4-decenal (Z4-10Al) are
110 main constituents of this bird odour [23]. In crested auklet, Z4-10Al likely plays a role as
111 an ectoparasite repellent and a signal of mate quality [23,24]. (E)-2-nonenal is another
112 odour-active unsaturated aldehyde, found in mushrooms and wine [25-27].

113 The olfactory sense in animals plays a key role during habitat adaptation. Tuning of Ors
114 to habitat cues is thought to create a bias for mate-finding signals that match or are
115 structurally similar to habitat odorants [28]. This idea yields a tentative scenario for the
116 convergence of semiochemicals. Insects and other animals have long been associated
117 with yeasts that facilitate digestion of plant materials, provide nutrients and protection of
118 food from antagonistic microorganisms.

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

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

126

127 **Data accessibility.** Data is completely included in the figure. **Authors' contributions.**
128 P.G.B. sensed the fly scent, P.G.B., S.L., M.B. and V.J. conceived the idea and
129 contributed to the experiment, F.B. calculated statistics, E.W. and E.H. synthesized the
130 test chemical, P.W. supervised the project and wrote the manuscript, all authors
131 contributed to and approved the final version of the manuscript. **Competing interests.**
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136 **References**

- 137 1. Schultz JC, Appel HM. 2004 Cross-kingdom cross-talk: hormones shared by plants
138 and their insect herbivores. *Ecology* **85**, 70-77. (doi:10.1890/02-0704)
- 139 2. Mithöfer A, Boland W. 2012 Plant defense against herbivores: chemical aspects.
140 *Annu. Rev. Plant Biol.* **63**, 431-450. (doi:10.1146/annurev-arplant-042110-103854)
- 141 3. Hefetz A, Batra SWT, Blum MS. 1979 Linalool, neral and geranial in the mandibular
142 glands of *Colletes* bees - an aggregation pheromone. *Cell. Molec. Life Sc.* **35**, 319-
143 320. (doi:10.1007/BF01964324)
- 144 4. Aldrich JR, Lusby WR, Kochansky JP. 1986 Identification of a new predaceous stink
145 bug pheromone and its attractiveness to the eastern yellowjacket. *Cell Molec Life Sc.*
146 **42**, 583-585. (doi:10.1007/BF01946714)
- 147 5. Leal WS, Sawada M, Matsuyama S, Kuwahara Y, Hasegawa M. 1993 Unusual
148 periodicity of sex pheromone production in the large black chafer *Holotrichia*
149 *parallela*. *J. Chem. Ecol.* **19**, 1381-1391. (doi:10.1007/BF00984883)
- 150 6. Yang Z, Bengtsson M, Witzgall P. 2004 Host plant volatiles synergize response to sex
151 pheromone in codling moth, *Cydia pomonella*. *J. Chem. Ecol.* **30**, 619-629.
152 (doi:10.1023/B:JOEC.0000018633.94002.af)
- 153 7. Reisenman CE, Riffell JA, Bernays EA, Hildebrand JG. 2010 Antagonistic effects of
154 floral scent in an insect-plant interaction. *Proc. R. Soc. B* **277**, 2371-2379.
155 (doi:10.1098/rspb.2010.0163)
- 156 8. Saveer AM, Kromann S, Birgersson G, Bengtsson M, Lindblom T, Balkenius A, Hansson
157 BS, Witzgall P, Becher PG, Ignell R. 2012 Floral to green: mating switches moth
158 olfactory coding and preference. *Proc. R. Soc. B* **279**, 2314-2322.
159 (doi:10.1098/rspb.2011.2710)
- 160 9. Raguso RA. 2016 More lessons from linalool: insights gained from a ubiquitous floral
161 volatile. *Curr. Op. Plant Biol.* **32**, 31-36. (doi:10.1016/j.pbi.2016.05.007)
- 162 10. Pragadheesh VS, Chanotiya CS, Rastogi S, Shasany AK (2017) Scent from *Jasminum*
163 *grandiflorum* flowers: investigation of the change in linalool enantiomers at various
164 developmental stages using chemical and molecular methods. *Phytochemistry* **140**,
165 83-94. (doi:10.1016/j.phytochem.2017.04.018)
- 166 11. Elisabetsky E, Marschner J, Souza DO. 1995 Effects of linalool on glutamatergic
167 system in the rat cerebral cortex. *Neurochem. Res.* **20**, 461-465.
168 (doi:10.1007/BF00973103)
- 169 12. Nakamura A, Fujiwara S, Matsumoto I, Abe K. 2009 Stress repression in restrained
170 rats by (R)-(-)-linalool inhalation and gene expression profiling of their whole blood
171 cells. *J. Agric. Food Chem.* **57**, 5480-5485. (doi:10.1021/jf900420g)
- 172 13. Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam KH, Amar O,
173 Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E. 2001 Enhanced
174 levels of the aroma and flavor compound S-linalool by metabolic engineering of the
175 terpenoid pathway in tomato fruits. *Plant Physiol.* **127**, 1256-1265.
176 (doi:10.1104/pp.010293)

- 177 14. Carrau FM, Medina K, Boido E, Farina L, Gaggero C, Dellacassa E, Versini G,
178 Henschke PA. 2005 De novo synthesis of monoterpenes by *Saccharomyces cerevisiae*
179 wine yeasts. *FEMS Microbiol. Lett.* **243**, 107–15. (doi:10.1016/j.femsle.2004.11.050)
- 180 15. Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005 Yeast and bacterial
181 modulation of wine aroma and flavour. *Austral. J. Grape Wine Res.* **11**, 139-173.
182 (doi:10.1111/j.1755-0238.2005.tb00285.x)
- 183 16. Dweck HK, Ebrahim SA, Kromann S, Bown D, Hillbur Y, Sachse S, Hansson BH,
184 Stensmyr MC. 2013 Olfactory preference for egg laying on citrus substrates in
185 *Drosophila*. *Curr. Biol.* **23**, 2472–80. (doi: 10.1016/j.cub.2013.10.047)
- 186 17. Becher PG, Flick G, Rozpedowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC,
187 Hansson BS, Piskur J, Witzgall P, Bengtsson M. 2012 Yeast, not fruit volatiles mediate
188 attraction and development of the fruit fly *Drosophila melanogaster*. *Funct. Ecol.* **26**,
189 822-828 (doi:10.1111/j.1365-2435.2012.02006.x)
- 190 18. Chisholm MG, Jell JA, Cass DM. 2003 Characterization of the major odorants found in
191 the peel oil of *Citrus reticulata* Blanco cv. Clementine using gas chromatography-
192 olfactometry. *Flavour Fragrance J.* **18**, 275–281. (doi:10.1002/ffj.1172)
- 193 19. Münch D, Galizia CG. 2016 DoOR 2.0 - comprehensive mapping of *Drosophila*
194 *melanogaster* odorant responses. *Sci Rep.* **6**, 21841. (doi:10.1038/srep21841)
- 195 20. Lebreton S, Borrero-Echeverry F, Gonzalez F, Solum M, Wallin E, Hedenström E,
196 Hansson BS, Gustavsson A-L, Bengtsson M, Birgersson G, Walker WB, Dweck H,
197 Becher PG, Witzgall P. 2017 A *Drosophila* female pheromone elicits species-specific
198 long-range attraction via an olfactory channel with dual specificity for sex and food.
199 *BMC Biology* **15**, 88. (doi:10.1186/s12915-017-0427-x)
- 200 21. Billeter JC, Atallah J, Krupp JJ, Millar JG, Levine JD. 2009 Specialized cells tag sexual
201 and species identity in *Drosophila melanogaster*. *Nature* **461**, 987-U250.
202 (doi:10.1038/nature08495)
- 203 22. Goodrich BS, Hesterman ER, Murray KE, Mykytowycz R, Stanley G, Sugowdz G. 1978
204 Identification of behaviorally significant volatile compounds in the anal gland of the
205 rabbit, *Oryctolagus cuniculus*. *J. Chem. Ecol.* **4**, 581-594. (doi:10.1007/BF00988922)
- 206 23. Douglas H, Jones T, Conner W. 2001 Heteropteran chemical repellents identified in
207 the citrus odor of a seabird (crested auklet: *Aethia cristatella*): evolutionary
208 convergence in chemical ecology. *Naturwissenschaften.* **88**, 330-332.
209 (doi:10.1007/s001140100236)
- 210 24. Caro SP, Balthazart J. 2010 Pheromones in birds: myth or reality? *J. Comp. Physiol.*
211 *A* **196**, 751-766. (doi:10.1007/s00359-010-0534-4)
- 212 25. Wood WF, Brandes ML, Watson RL, Jones RL, Largent DL. 1994 trans-2-Nonenal, the
213 cucumber odor of mushrooms. *Mycologia* **4**, 561-563. (doi:10.2307/3760750)
- 214 26. Chatonnet P, Dubourdieu D. 1998 Identification of substances responsible for the
215 'sawdust' aroma in oak wood. *J. Sc. Food Agric.* **76**, 179-188.
216 (doi:10.1002/(SICI)1097-0010(199802)76:2<179::AID-JSFA924>3.3.CO;2-Y)
- 217 27. Ferreira V, Culleré L, López R, Cacho J. 2004 Determination of important odor-active
218 aldehydes of wine through gas chromatography–mass spectrometry of their O-(2, 3,
219 4, 5, 6-pentafluorobenzyl) oximes formed directly in the solid phase extraction

220 cartridge used for selective isolation. *J. Chromatogr. A* **1028**, 339-345.
221 (doi:10.1016/j.chroma.2003.11.104)
222 28. Endler JA. 1992 Signals, signal conditions, and the direction of evolution. *Am.*
223 *Naturalist* **139**, S125-S153. (doi:10.1086/285308)

224 **Legends**

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

227 (colour & high resolution version available).

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



