Supplementary information

Incipient speciation in *Drosophila melanogaster* involves chemical signals

Micheline Grillet[1,2], Claude Everaerts[1], Benjamin Houot[1,3], Michael G. Ritchie[4], Matthew Cobb[2] and Jean-François Ferveur*[1]

1. Centre des Sciences du Goût et de l'Alimentation, UMR6265 CNRS, UMR1324 INRA, Université de Bourgogne, Agrosup Dijon, 6, Bd Gabriel, 21000 Dijon, France.
2. Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK.
3. Present address: Department of Biology, WVU, Life Science Building, 53 Campus Drive, Morgantown, WV 26506, USA.
4. School of Biology, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9TF, UK.

*Correspondence and requests for materials should be addressed to J.F.F.
jean-francois.ferveur@u-bourgogne.fr

Legends

Table S1–Mating frequency and sexual isolation between wild-type strains.

A) Percentage of mating of single pairs of male and female flies from AL, Dij and Z1-Z6 strains. Couples were observed for 60 minutes. Data represent the mating frequency for each cross, between males (horizontal) and females (vertical). n=34-156.

B) Sexual isolation index (I_{psi}, expressed with their SD) and asymmetric isolation index (IA_{psi}) between the eight strains: I_{psi} and IA_{psi} were calculated in JMATING, with 100,000 bootstrapping replicates to access the significance of deviations from the null hypothesis (i.e. random mating and symmetry, respectively). Each I_{psi} was expressed with the corresponding *t*- and *p*- value. Stars indicate significant IA_{psi} at level p=0.05.

Table S2– Production of hydrocarbons in cosmopolitan and Zimbabwe strains.

Data show mean (±SE) percentage of cuticular hydrocarbons and C5/C7 ratio in AL, Dij and Z1-6 males and females. Peaks were identified by mass spectrometry. Key for compounds: A= 7,11-TD, B=23MeBr+9-T, C=7-T, D=5-T, E=n-C23, F=9,13-PD, G=7,11-PD, H=5,9-PD, I=9-P, J=7-P, K=5-P, L=n-C25, M=9,13-HD, N=7,11-HD, O=27MeBr, P=5,9-HD, Q=7-H, R=5-H, S=n-C27, T=9,13-ND, U=7,11-ND, V=29MeBr, W=5,9-ND, X=n-C29. n=15. For other details, refer to Figure 4.

Figure S1- Courtship indices (upper panels) and mating frequency (lower panels) of all four crosses between AL and Z6 flies were measured with control females (left panels) and with 'blind' females whose eyes had been painted with black nail varnish (right panels). Letters indicate significant differences. n = 18-41. The conditions were as in Fig.2.

Figure S2-

A) Effect of chemical signals on sexual isolation. Cumulative mating curves for Z6 females with AL or Z6 males that have received hydrocarbons from AL males (AL*AL*; Z6*AL*, respectively) or from Z6 males (AL*Z6*; Z6*Z6*). n=38-46. Letters indicate significant differences.

B) Discriminant analysis of male flies from AL and Z6 strains, and from AL and Z6 males that had pheromone transfers (AL*AL*; Z6*AL*; AL*Z6*; Z6*Z6*) **projected onto the first two principal components.** Probability ellipses correspond to 95% probability. The various components in the cuticular cocktail were also projected onto these components (arrows) to give a sense of dimensionality. For each sex, we conducted a forward stepwise

discriminant analysis (with an entry threshold value of p=0.05 and a removal threshold value of p=0.10) using the additive/log ratio transformed proportion of the CHs) using the absolute amount of the uncorrelated CHs as quantitative variables and the strain as a qualitative variable. Magenta arrows indicate C7 compounds, cyan arrows indicate C5 compounds, black arrows indicate others compounds.

A **Percentage of mating of single pairs of male and female flies from AL, Dij and Z1-Z6 strains**

FEMALES \ MALES	AL	Dij	Z1	Z2	Z3	Z4	Z5	Z6
AL	59.4	63.3	43.6	25.0	54.3	73.7	28.6	62.4
Dij	88.6	87.7	77.1	55.0	74.3	97.2	65.7	82.9
Z1	80.0	70.5	70.0	30.8	42.9	44.1	20.0	41.2
Z2	41.7	28.6	10.0	22.9	58.5	81.6	48.6	73.5
Z3	25.7	17.1	30.6	57.1	76.5	80.0	35.3	85.7
Z4	2.9	2.7	14.3	44.4	40.0	55.9	20.0	65.7
Z5	8.6	2.9	2.9	31.4	42.9	40.0	20.0	62.9
Z6	6.0	5.6	3.0	52.4	28.6	51.4	51.4	74.4

Couples were observed during 60 minutes. *n*=34-156.

B **Indice of sexual isolation (Ipsi, expressed with their SD) and of asymmetric isolation (IApsi) between the 8 strains**

Mating Pairs a	b	Ipsi (S.D.)	*t* (2755 df)	*p*	IApsi ab/ba
AL	**Dij**	0.02 (0.06)	0.33	*0.7414*	1.01
	Z1	0.04 (0.06)	0.64	*0.5222*	0.98
	Z2	0.06 (0.08)	0.79	*0.4295*	0.97
	Z3	0.28 (0.07)	4.28	*0.0000*	1.23*
	Z4	0.48 (0.06)	7.55	*0.0000*	5.22*
	Z5	0.36 (0.09)	4.16	*0.0000*	1.47*
	Z6	0.49 (0.06)	7.93	*0.0000*	3.27*
Dij	**Z1**	0.03 (0.06)	0.58	*0.5619*	1.00
	Z2	0.06 (0.07)	0.84	*0.4009*	1.03
	Z3	0.38 (0.06)	6.53	*0.0000*	1.75*
	Z4	0.48 (0.06)	8.61	*0.0000*	6.13*
	Z5	0.44 (0.07)	6.47	*0.0000*	3.48*
	Z6	0.49 (0.06)	8.91	*0.0000*	3.87*
Z1	**Z2**	0.39 (0.08)	4.90	*0.0000*	1.50*
	Z3	0.33 (0.06)	5.22	*0.0000*	1.11*
	Z4	0.42 (0.07)	6.29	*0.0000*	1.62*
	Z5	0.63 (0.07)	8.63	*0.0000*	3.11*
	Z6	0.64 (0.06)	11.39	*0.0000*	6.02*
Z2	**Z3**	0.16 (0.07)	2.38	*0.0173*	0.99
	Z4	0.25 (0.07)	3.71	*0.0002*	0.86*
	Z5	0.29 (0.09)	3.37	*0.0007*	0.88*
	Z6	0.20 (0.07)	3.02	*0.0025*	0.94
Z3	**Z4**	0.07 (0.06)	1.13	*0.2585*	1.05
	Z5	0,00 (0.08)	0.03	*0.9760*	1.00
	Z6	0.20 (0.06)	3.29	*0.0010*	1.24*
Z4	**Z5**	0.08 (0.09)	0.98	*0.3271*	0.95
	Z6	0.05 (0.06)	0.82	*0.4122*	1.01
Z5	**Z6**	0.19 (0.07)	2.76	*0.0058*	0.96

I_{psi} and IA_{psi} were calculated in JMATING, with 100,000 bootstrapping replicates to access the significance of deviations from the null hypothesis (i.e. random mating and symmetry, respectively). Each Ipsi was expressed with the corresponding *t*- and *p*- value. Stars indicate significant IA_{psi} at level *p*=0.05.

MALES

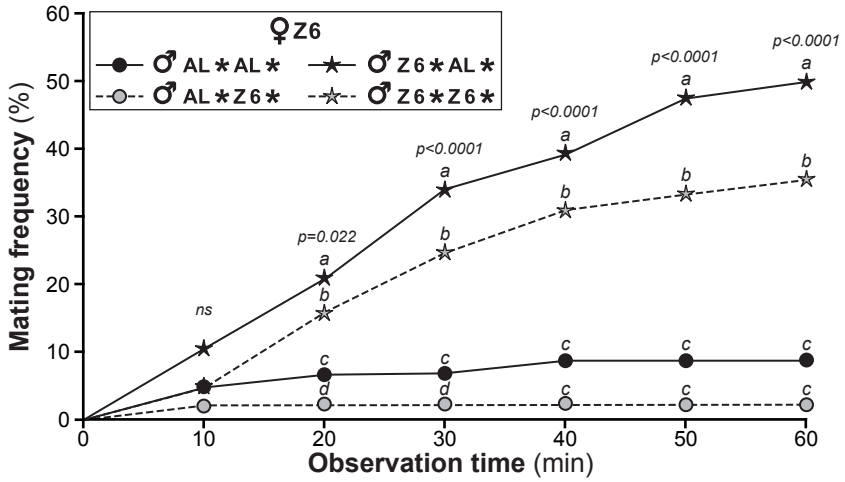
	Compounds	AL	Dij	Z 1	Z 2	Z 3	Z 4	Z 5	Z 6
B	23MeBr + 9-T	3.1 ± 0.1	2.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.6 ± 0.2
C	7-T	49.7 ± 0.8	45.7 ± 1.3	38.3 ± 0.5	36.1 ± 0.6	40.1 ± 1.1	42.1 ± 0.4	22.8 ± 0.9	24.2 ± 1.5
D	5-T	3.1 ± 0.1	2.9 ± 0.2	3.5 ± 0.1	4.7 ± 0.2	5.0 ± 0.1	5.8 ± 0.2	10.5 ± 1.1	20.3 ± 0.8
E	*n*-C23	11.6 ± 0.3	14.8 ± 0.5	10.1 ± 0.7	10.0 ± 0.3	11.3 ± 0.4	14.5 ± 0.3	8.1 ± 0.6	9.3 ± 0.5
I	9-P	6.7 ± 0.2	7.9 ± 0.4	8.8 ± 0.1	8.5 ± 0.2	9.2 ± 0.3	6.6 ± 0.1	7.5 ± 0.4	7.9 ± 0.2
J	7-P	5.5 ± 0.2	7.3 ± 0.4	15.5 ± 0.6	21.4 ± 0.4	15.8 ± 0.6	11.0 ± 0.4	23.5 ± 0.9	9.5 ± 0.4
K	5-P	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.3	1.1 ± 0.1
L	*n*-C25	2.2 ± 0.1	3.1 ± 0.2	2.1 ± 0.1	3.7 ± 0.2	3.5 ± 0.2	3.3 ± 0.1	3.2 ± 0.2	3.0 ± 0.2
O	27MeBr	10.8 ± 0.3	8.4 ± 0.3	9.9 ± 0.2	7.9 ± 0.2	7.2 ± 0.2	8.1 ± 0.2	8.8 ± 0.3	12.7 ± 0.9
Q	7-H	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	1.1 ± 0.2	0.0 ± 0.0
S	*n*-C27	1.0 ± 0.0	1.3 ± 0.2	1.0 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	0.1 ± 0.1	1.9 ± 0.2	1.5 ± 0.1
V	29MeBr	6.5 ± 0.3	5.6 ± 0.3	6.9 ± 0.2	3.8 ± 0.1	4.1 ± 0.3	4.9 ± 0.2	8.1 ± 0.8	8.7 ± 0.8
X	*n*-C29	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.5 ± 0.2	0.1 ± 0.1
	C5/C7 ratio	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.11 ± 0.00	0.27 ± 0.03	0.65 ± 0.03

FEMALES

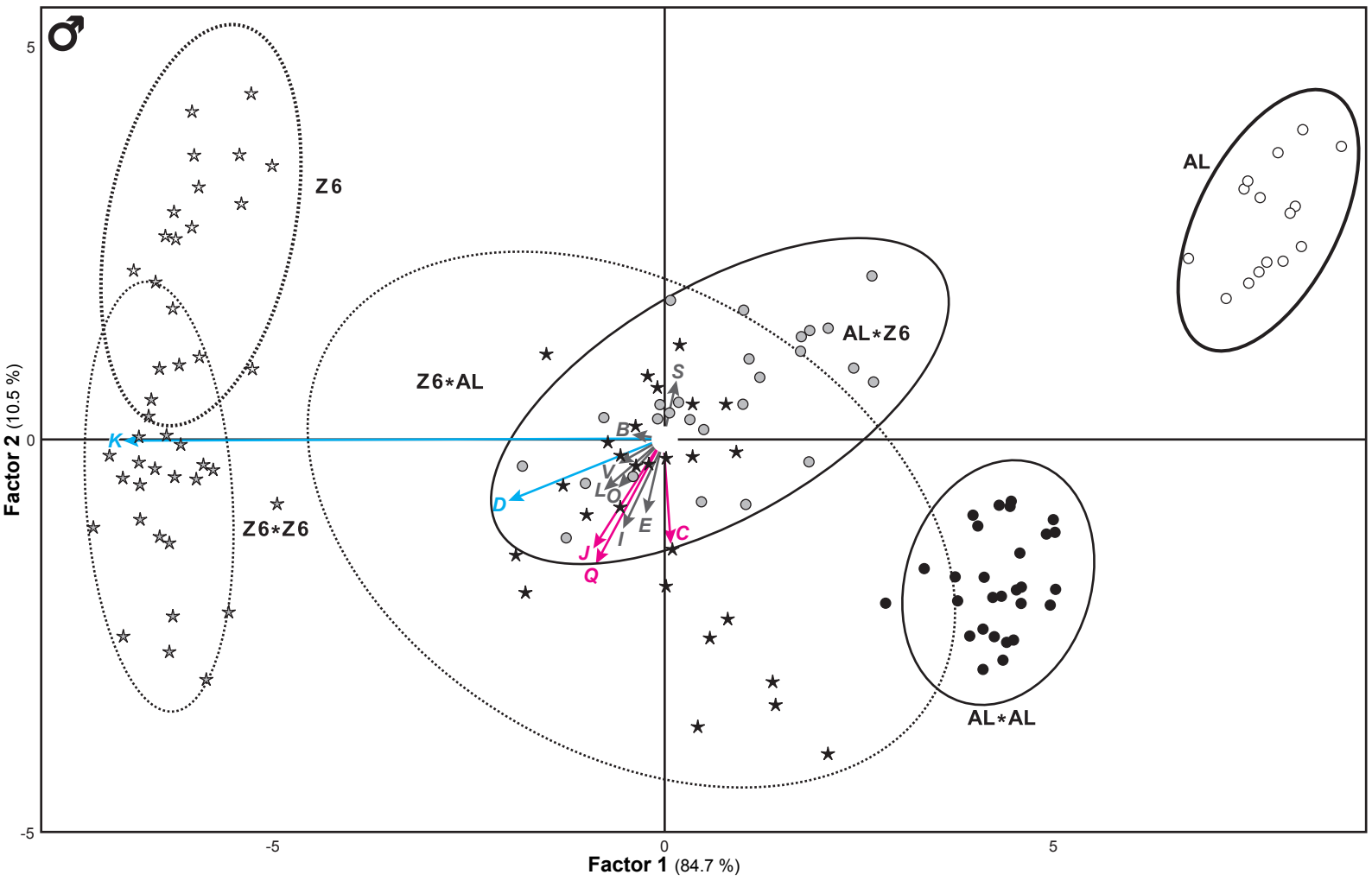
	Compounds	AL	Dij	Z 1	Z 2	Z 3	Z 4	Z 5	Z 6
A	7,11-TD	0.9 ± 0.1	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
B	23MeBr + 9-T	0.9 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	0.2 ± 0.1	1.5 ± 0.2	1.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
C	7-T	1.7 ± 0.1	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
D	5-T	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	1.3 ± 0.2	0.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
E	n-C23	7.7 ± 0.2	10.7 ± 0.5	6.7 ± 0.2	2.0 ± 0.2	5.1 ± 0.3	5.6 ± 0.1	2.6 ± 0.3	2.9 ± 0.1
F	9,13-PD	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
G	7,11-PD	5.5 ± 0.4	5.4 ± 0.4	1.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
H	5,9-PD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 1.0	23.7 ± 1.3	16.0 ± 0.8	5.6 ± 0.9	18.5 ± 0.8
I	9-P	6.9 ± 0.2	5.5 ± 0.4	6.3 ± 0.2	7.0 ± 1.7	1.1 ± 0.3	0.8 ± 0.3	2.7 ± 1.3	0.0 ± 0.0
J	7-P	1.7 ± 0.1	2.1 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	2.3 ± 0.2	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.1
K	5-P	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.1	2.0 ± 0.2	2.0 ± 0.0	2.5 ± 0.2	2.1 ± 0.1
L	n-C25	4.5 ± 0.1	6.5 ± 0.3	7.0 ± 0.1	8.3 ± 0.3	6.8 ± 0.2	6.4 ± 0.2	6.9 ± 0.5	4.6 ± 0.2
M	9,13-HD	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0
N	7,11-HD	32.5 ± 0.4	31.5 ± 0.9	24.5 ± 0.4	7.9 ± 0.4	0.0 ± 0.0	7.1 ± 0.2	3.7 ± 0.2	1.0 ± 0.0
O	27MeBr	13.0 ± 0.3	11.4 ± 0.2	13.7 ± 0.2	8.2 ± 0.2	6.8 ± 0.4	7.6 ± 0.1	9.5 ± 0.3	5.9 ± 0.3
P	5,9-HD	3.5 ± 0.1	2.9 ± 0.1	5.3 ± 0.1	40.7 ± 0.7	36.1 ± 1.6	32.4 ± 0.4	47.3 ± 1.5	47.1 ± 0.9
Q	7-H	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
R	5-H	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.0
S	n-C27	2.2 ± 0.1	3.3 ± 0.2	3.6 ± 0.1	3.7 ± 0.2	2.6 ± 0.1	3.9 ± 0.1	3.3 ± 0.2	2.6 ± 0.1
T	9,13-ND	0.3 ± 0.1	0.2 ± 0.1	1.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.8 ± 0.1
U	7,11-ND	10.3 ± 0.5	8.1 ± 0.5	18.5 ± 0.5	5.0 ± 0.8	2.9 ± 0.2	5.3 ± 0.2	2.8 ± 0.5	3.4 ± 0.2
V	29MeBr	7.1 ± 0.2	5.9 ± 0.2	6.5 ± 0.1	4.9 ± 0.2	4.5 ± 0.2	5.5 ± 0.1	6.7 ± 1.0	7.3 ± 0.5
W	5,9-ND	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	1.5 ± 0.2	0.9 ± 0.1
X	n-C29	0.1 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.7 ± 0.3	0.4 ± 0.2
	C5/C7 ratio	0.07 ± 0.00	0.06 ± 0.00	0.12 ± 0.00	3.85 ± 0.25	10.42 ± 0.51	3.78 ± 0.11	8.66 ± 0.75	13.05 ± 0.52

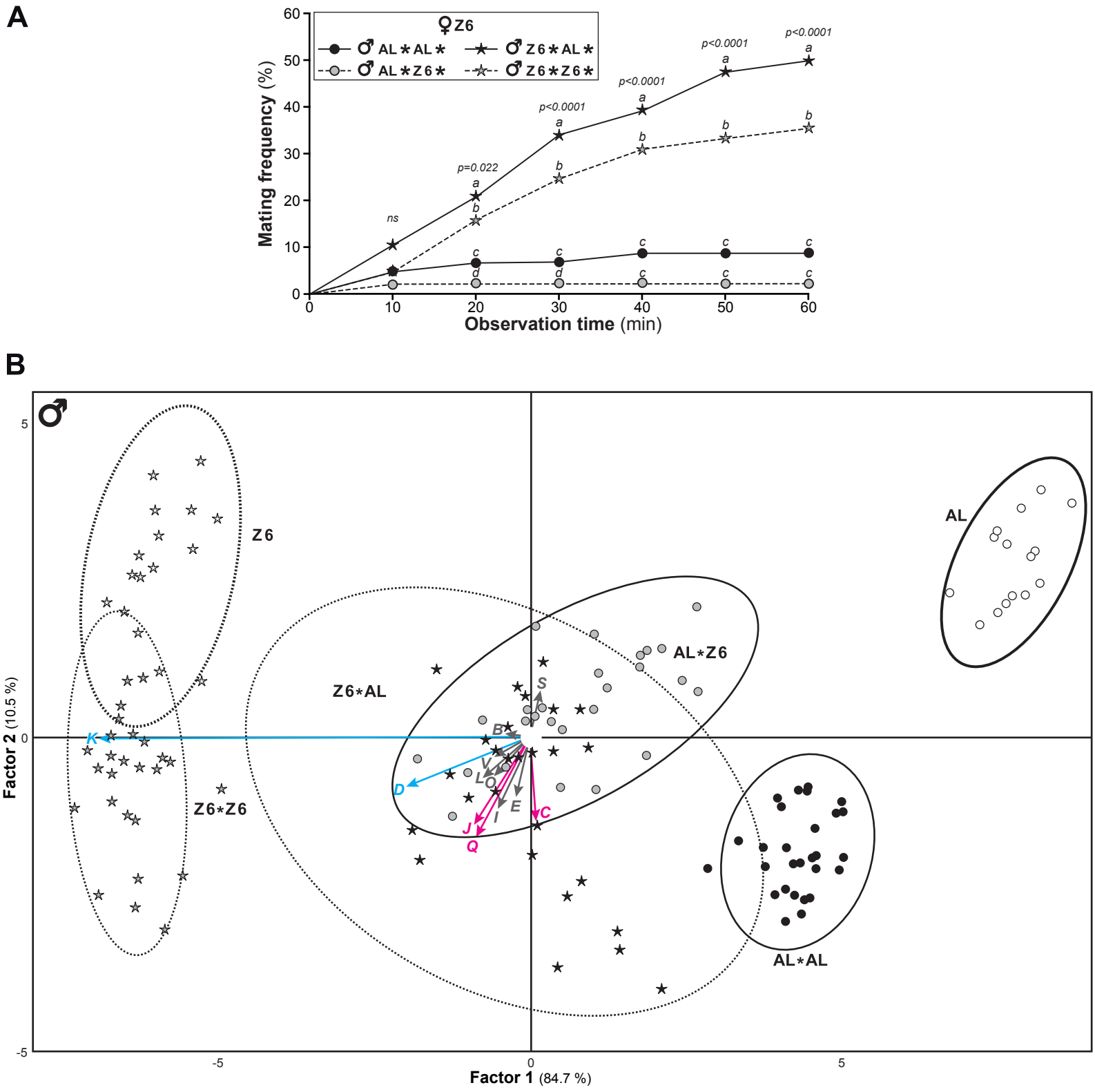
Mean (±SE) percentage of cuticular hydrocarbons and C5/C7 ratio in AL, Dij and Z1-6 males and females. (*n*=15)

A


♀Z6
♂ AL∗AL∗
♂ Z6∗AL∗
♂ AL∗Z6∗
♂ Z6∗Z6∗
Mating frequency (%)
Observation time (min)
0
10
20
30
40
50
60
ns
p=0.022
p<0.0001
p<0.0001
p<0.0001
p<0.0001
a
b
c
d

B


♂
Z6
Z6∗Z6
Z6∗AL
AL∗Z6
AL
AL∗AL
K
D
B
S
V
L
Q
E
I
J
C
Q
Factor 2 (10.5 %)
Factor 1 (84.7 %)
5
0
-5
-5
0
5


A
♀Z6
♂ AL∗AL∗
♂ Z6∗AL∗
♂ AL∗Z6∗
♂ Z6∗Z6∗
Mating frequency (%)
Observation time (min)
ns
p=0.022
p<0.0001
p<0.0001
p<0.0001
B
♂
Z6
Z6∗AL
AL∗Z6
AL
Z6∗Z6
AL∗AL
K
D
S
B
V
L
Q
E
I
J
C
Q
Factor 2 (10.5 %)
Factor 1 (84.7 %)

Supp. Figure