Flying Drosophila show sex-specific attraction to fly-labelled food

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SUPPLEMENTAL MATERIAL

(Tables S1 & S2; Figures S1-S4)

Table S1: Nomemclature of the principal cuticular hydrocarbons and *c*VA detected on the fly cuticule. (The color code corresponds to that shown in Figure 3).

Abbreviation	Chemical name
C21	n-Heneicosane
***c*VA**	(*Z*)-11-Vaccenyl acetate
C22	n-Docosane
7.11TD	(*Z,Z*)-7,11-Tricosadiene
23Br	2-Methyldocosane
9T	(*Z*)-9-Tricosene
7T	(*Z*)-7-Tricosene
5T	(*Z*)-5-Tricosene
C23	n-Tricosane
9,13PD	(*Z,Z*)-9,13-Pentacosadiene
7,11PD	(*Z,Z*)-7,11-Pentacosadiene
5,9PD	(*Z,Z*)-5,9-Pentacosadiene
25Br	2-Methyltetracosane
7P	(*Z*)-7-Pentacosene
5P	(*Z*)-5-Pentacosene
C25	n-Pentacosane
9,13HD	(*Z,Z*)-9,13-Heptacosadiene
7,11HD	(*Z,Z*)-7,11-Heptacosadiene
5,9HD	(*Z,Z*)-5.9-Heptacosadiene
27Br	2-Methylhexacosane
7H	(*Z*)-7-Heptacosene
5H	(*Z*)-5-Heptacosene
C27	n-Heptacosane
9,13ND	(*Z,Z*)-9,13-Nonacosadiene
7,11ND	(*Z,Z*)-7,11-Nonacosadiene
29Br	2-Methyloctacosane
9N	(*Z*)-9-Nonacosene
7N	(*Z*)-7-Nonacosene
5N	(*Z*)-5-Nonacosene
C29	n-Nonacosane
31Br	2-Methyltriacontane

Table S2: Production of the principal cuticular hydrocarbons and *c*VA in individual flies of all lines used to label food (mean ± sem).

MALES	**Cs M**	**Antibiot. Cs M**	**Dechorion. Cs M**	**Desat 1 M**	**Z30 M**	**Oe- M**	***D. simulans* M**
C21	11 ± 1	16 ± 1	9 ± 1	24 ± 1	1 ± 0		4 ± 0
***c*VA**	45 ± 10	56 ± 11	6 ± 1	1 ± 0	23 ± 4	107 ± 66	95 ± 20
C22	8 ± 0	10 ± 0	6 ± 0	20 ± 1	1 ± 0		9 ± 1
23 Br	1 ± 0	9 ± 0	1 ± 0	72 ± 2	1 ± 0		1 ± 0
9T	33 ± 3	37 ± 2	34 ± 2	1 ± 0	12 ± 1		57 ± 3
7T	449 ± 21	549 ± 12	381 ± 15	29 ± 2	152 ± 6	2 ± 0	714 ± 30
5T	38 ± 1	50 ± 1	38 ± 1	4 ± 0	107 ± 5		40 ± 2
C23	186 ± 6	180 ± 5	176 ± 5	535 ± 12	42 ± 1	1 ± 0	169 ± 8
25Br	30 ± 3	29 ± 2	26 ± 1	41 ± 1	25 ± 2	1 ± 0	29 ± 2
9P	53 ± 3	51 ± 3	52 ± 1	167 ± 5	49 ± 3	1 ± 0	25 ± 4
7P	280 ± 16	235 ± 11	290 ± 11	11 ± 1	146 ± 8	1 ± 0	61 ± 3
5P	6 ± 1	6 ± 1	8 ± 1	69 ± 2	17 ± 1		1 ± 0
C25	30 ± 1	27 ± 1	29 ± 1	7 ± 1	20 ± 1	1 ± 0	34 ± 2
27 Br	114 ± 5	38 ± 1	124 ± 3	633 ± 9	82 ± 6	7 ± 0	153 ± 5
9H	0 ± 0	81 ± 2	0 ± 0	685 ± 21	1 ± 0	1 ± 0	0 ± 0
7H	8 ± 1	8 ± 1	9 ± 0	14 ± 1	6 ± 1	1 ± 0	1 ± 0
5H	0 ± 0	0 ± 0	0 ± 0	276 ± 6	1 ± 0		0 ± 0
C27	19 ± 1	25 ± 1	19 ± 1	7 ± 0	16 ± 1	1 ± 0	14 ± 2
29 Br	78 ± 3	94 ± 3	83 ± 3	8 ± 1	71 ± 4	18 ± 1	66 ± 7
7N	0 ± 0	0 ± 0	0 ± 0	180 ± 5	1 ± 0	2 ± 0	2 ± 1
C29	3 ± 0	7 ± 0	2 ± 0	14 ± 0	4 ± 0	1 ± 0	2 ± 0
31Br	7 ± 0	12 ± 1	9 ± 1	23 ± 1	10 ± 1	8 ± 0	4 ± 0
C31				2 ± 1			
∑ HCs	1354 ± 46	1466 ± 21	1296 ± 25	2823 ± 36	763 ± 21	46 ± 2	1387 ± 46
∑ Desaturated	867 ± 31	1018 ± 19	813 ± 20	135 ± 4	490 ± 13	7 ± 1	902 ± 34
∑ Ramified	231 ± 10	183 ± 4	242 ± 6	1135 ± 25	189 ± 10	34 ± 1	252 ± 10
∑ Linear	256 ± 8	265 ± 6	241 ± 6	1533 ± 21	84 ± 2	5 ± 0	232 ± 7

FEMALES	**Cs F**	**Antibiot. Cs F**	**Dechorio. Cs F**	**Mated Cs F**	**Desat 1 F**	***D. simulans* F**
C21	5 ± 0	2 ± 0	6 ± 1	8 ± 1	31 ± 1	4 ± 0
*c*VA				96 ± 15		
C22	3 ± 0	4 ± 0	3 ± 0	4 ± 0	19 ± 1	6 ± 0
7.11TD	11 ± 0	10 ± 1	7 ± 0	6 ± 0	0 ± 0	
23Br	7 ± 0	5 ± 0	4 ± 0	5 ± 1	48 ± 1	1 ± 0
9T	4 ± 1	4 ± 0	3 ± 0	5 ± 0	1 ± 0	43 ± 1
7T	17 ± 1	17 ± 1	13 ± 1	86 ± 4	32 ± 1	889 ± 21
5T	2 ± 0	2 ± 0	1 ± 0	10 ± 1	4 ± 0	37 ± 1
C23	91 ± 2	108 ± 2	103 ± 5	132 ± 4	404 ± 9	148 ± 4
C24					22 ± 0	
9,13PD	11 ± 0	12 ± 1	9 ± 1	7 ± 0		
7,11PD	42 ± 1	40 ± 2	38 ± 2	39 ± 3	4 ± 0	
5,9PD	50 ± 2	35 ± 2	41 ± 2	45 ± 2	1 ± 0	
25Br	40 ± 1	46 ± 2	46 ± 2		147 ± 6	26 ± 1
9P				42 ± 2	11 ± 2	28 ± 2
7P	19 ± 1	21 ± 1	22 ± 2	75 ± 3	67 ± 3	38 ± 2
5P	2 ± 0	2 ± 0	2 ± 0	4 ± 0	4 ± 0	0 ± 0
C25	59 ± 2	65 ± 2	68 ± 3	73 ± 3	650 ± 15	31 ± 1
9,13HD	19 ± 1	21 ± 1	15 ± 1	11 ± 1		
7,11HD	593 ± 16	600 ± 11	577 ± 26	503 ± 21	49 ± 5	
5,9HD	10 ± 0	9 ± 0	9 ± 0	10 ± 0	6 ± 0	
27Br	244 ± 8	217 ± 4	206 ± 12	233 ± 8	642 ± 24	152 ± 4
9H						0 ± 0
7H	16 ± 2	33 ± 1	18 ± 2	14 ± 1	23 ± 1	0 ± 0
5H	2 ± 0	3 ± 0	1 ± 0	1 ± 0	2 ± 0	0 ± 0
C27	31 ± 2	50 ± 3	40 ± 2		369 ± 27	19 ± 1
28Br					9 ± 0	
C28				44 ± 2	9 ± 1	
9,13ND	7 ± 0	10 ± 1	6 ± 0	4 ± 0		
7,11ND	243 ± 9	285 ± 9	199 ± 12	169 ± 11	11 ± 1	
29Br	58 ± 2	83 ± 3	79 ± 3	79 ± 4	222 ± 6	78 ± 3
9N	0 ± 0	1 ± 0	0 ± 0	0 ± 0		
7N	1 ± 0	2 ± 0	1 ± 0	0 ± 0		0 ± 0
5N	0 ± 0	0 ± 0	0 ± 0	0 ± 0		
C29	3 ± 0	7 ± 0	5 ± 0	4 ± 0	37 ± 6	1 ± 0
31Br	7 ± 0	13 ± 1	14 ± 1	9 ± 1	74 ± 5	4 ± 0
∑ HCs	1599 ± 36	1708 ± 14	1537 ± 36	1624 ± 43	2901 ± 59	1505 ± 32
∑ Desaturated	1049 ± 23	1107 ± 13	963 ± 28	991 ± 29	216 ± 10	1035 ± 24
∑ Ramified	357 ± 10	365 ± 6	349 ± 14	369 ± 11	1142 ± 29	261 ± 5
∑ Linear	193 ± 5	237 ± 4	225 ± 8	265 ± 7	1542 ± 37	209 ± 6

SUPPLEMENTAL FIGURES

Figure S1: Cumulated frequency for the latency to to take upwind flight and for flight duration in control CS flies to food labelled by CS flies.

The cumulated frequencies represent the latency at which flies take upwind flight (top diagrams) and the flight duration (equivalent to landing latency; bottom) in CS males (left) and females (right). The data correspond to those shown on Figure 2. No statistical difference was detected between sexes and LFs. Curves were compared using Kaplan-Meier procedure with a subsequent Log-rank test. : (a) males upwind flight: $Khi^2_{(5df)} = 9.7$, $p= 0.08$, (b) females upwind flight : $Khi^2_{(5df)} = 5.7$, $p= 0.33$, (c) male overall landing = $Khi^2_{(5df)} = 3.3$, $p= 0.65$, (d) females overall landing $Khi^2_{(5df)} = 3$, $p= 0.70$

Figure S2: Labelled food preference in a Y-maze olfactometer.

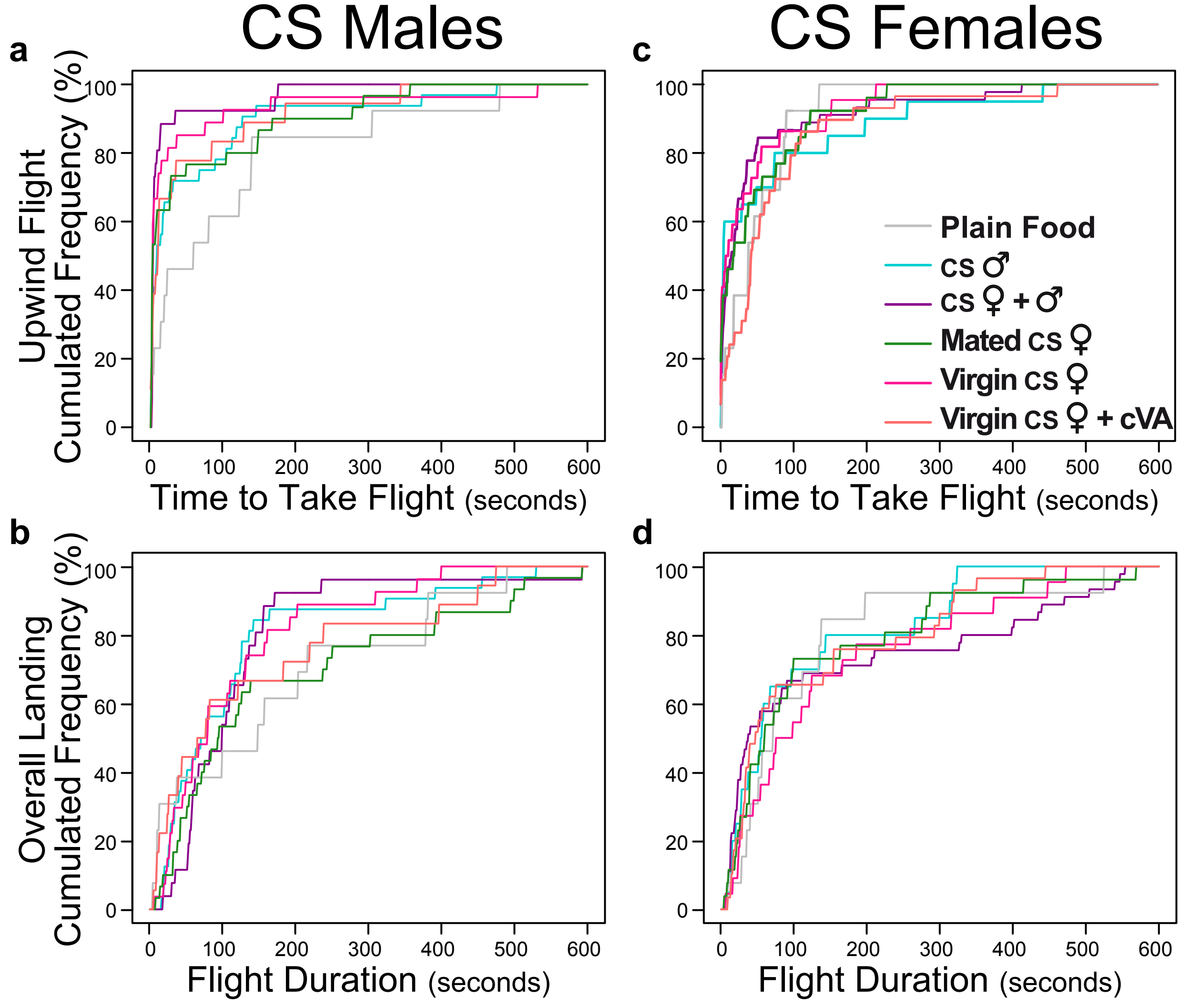
The response of individual CS male (blue colour, left histograms and blue curve) and female flies (right bars and pink coloured bars and curve) was tested in a Y-maze olfactometer with a binary choice either consisting of two plain food (PF/PF, left choice for each sex) or PF with food labelled by mix sexes (LF; right choice). (**a**) Histograms represent the frequency of flies making a choice, after 240 min (the sample size is indicated at the base of each bar). (**b**) Representation of the food choice inside the Y-maze olfactometer. (**c**) Cumulated frequencies of flies responding during 240 min. The proportion of responding flies was tested with a Wilks G^2 likelihood ratio test completed with a computation of significance by cell (Fisher's exact test; significant differences at level α =0.05 are indicated by different letters above the bars). Food preference was tested with a *z*-test, and the corresponding choice frequencies were compared for both sexes and between the different choices using a Marascuilo procedure. The level of significance for preference is represented (or not) by stars (**: α <0.01; ***: α <0.001; no star: not significant). (**a**): $G^2_{(3df)}=22.7$, $p<10^{-4}$; (**b**): $Khi^2_{(5df)}=21.8$, $p<10^{-4}$.

Figure S3: Cumulated frequency for the latency to take upwind flight and for flight duration in control CS flies tested to food labelled by various genotypes.

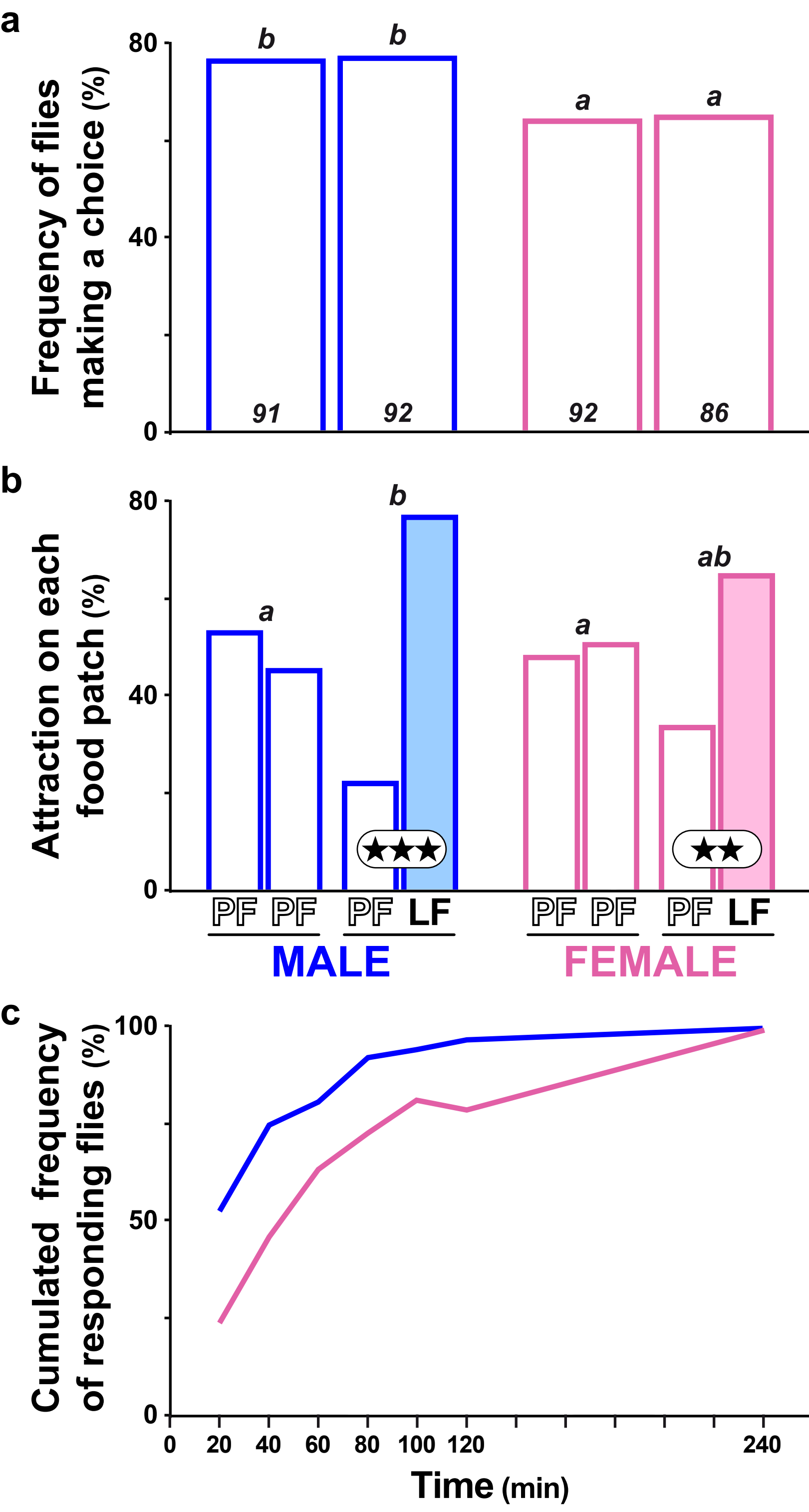
The data correspond to those shown on Figure 4. Curves were analysed using Kaplan-Meier procedure with a subsequent Log-rank test. (a) Males upwind flight: $Khi^2_{(6df)} = 8.8$, $p = 0.18$, (b) females upwind flight: $Khi^2_{(6df)} = 10.5$, $p = 0.106$, (c) males overall landing $Khi^2_{(6df)} = 5.6$, $p = 0.47$, (d) females overall landing: $Khi^2_{(6df)} = 10.5$, $p = 0.104$. For more information, please refer to Fig. S1.

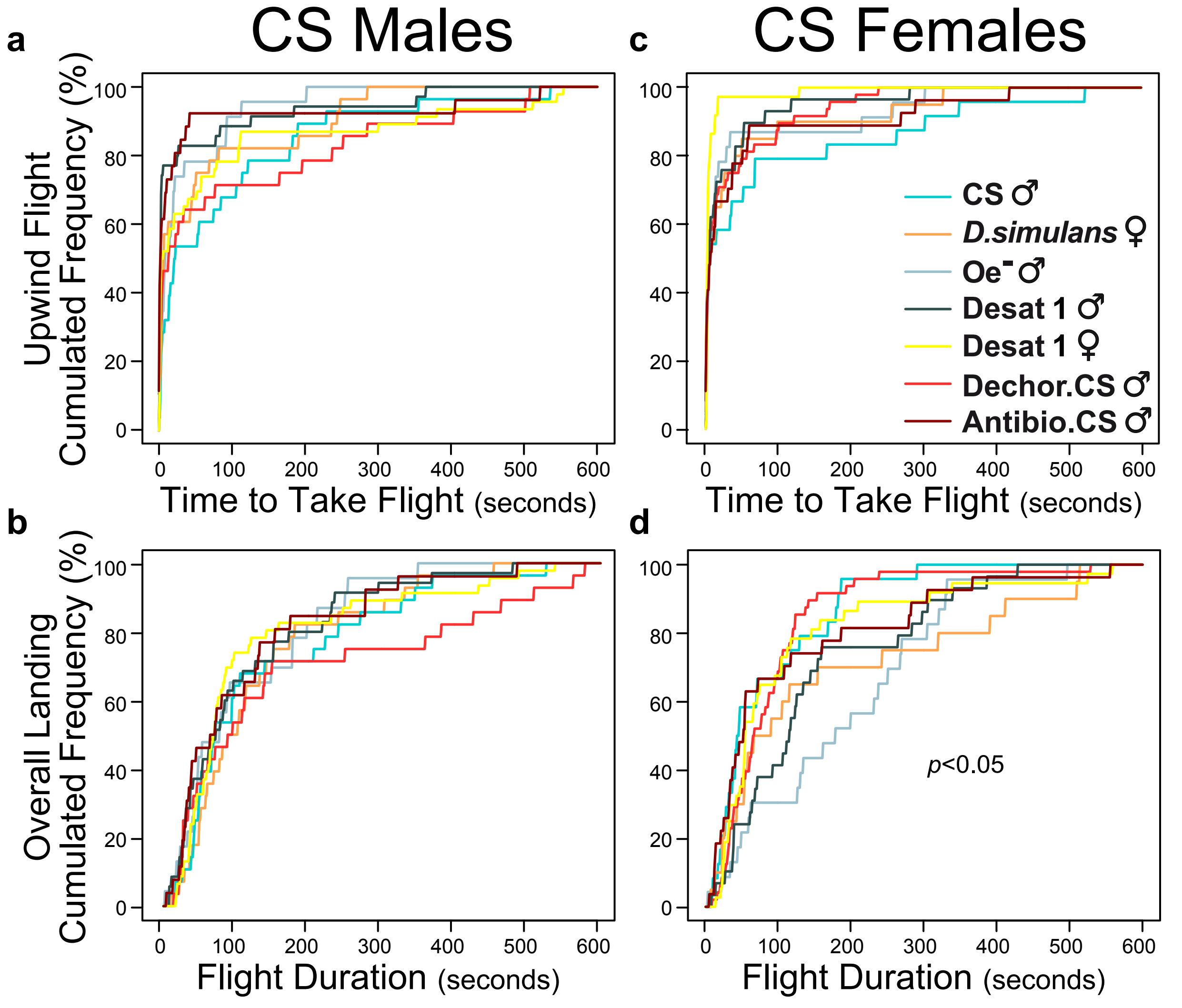
Figure S4: Cumulated frequency for the latency to take upwind flight and for flight duration in various male flies tested to food labelled by CS males.

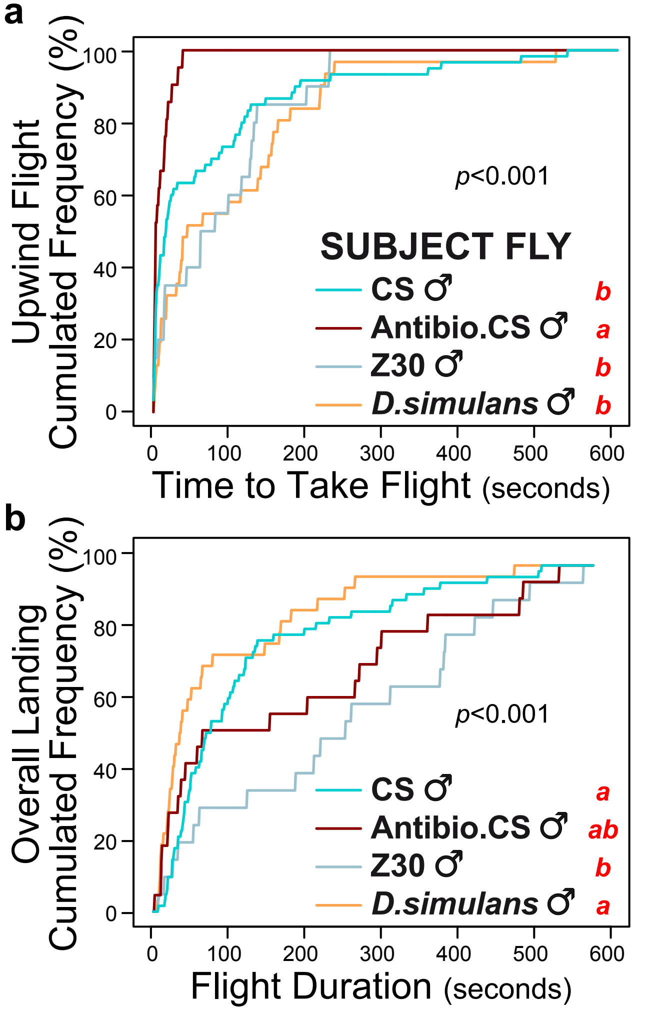
The data correspond to those shown on Figure 6. The curves were analysed using Kaplan-Meier procedure with a subsequent Log-rank test and a post-hoc multiple pairs comparison (α=0.05 with Bonferroni correction); different letters beside the genotypes indicated in the legend reveals some significative differences between tester males. (a) upwind flight: $Khi^2_{(3df)} = 23.2$, $p<10^{-4}$, (b) overall landing : $Khi^2_{(3df)} = 16.5$, $p<10^{-4}$. For more information, please refer to Fig. S1.



CS Males
CS Females
a
b
c
d
Upwind Flight Cumulated Frequency (%)
Overall Landing Cumulated Frequency (%)
Time to Take Flight (seconds)
Flight Duration (seconds)
0
20
40
60
80
100
0
100
200
300
400
500
600
Plain Food
CS ♂
CS ♀ + ♂
Mated CS ♀
Virgin CS ♀
Virgin CS ♀ + cVA


a
Frequency of flies making a choice (%)
80
40
0
b
b
a
a
91
92
92
86
b
Attraction on each food patch (%)
80
40
0
a
b
a
ab
★★★
★★
PF PF PF LF
PF PF PF LF
MALE
FEMALE
c
Cumulated frequency of responding flies (%)
100
50
0
0 20 40 60 80 100 120 240
Time (min)


CS Males
CS Females
a
b
c
d
Upwind Flight
Cumulated Frequency (%)
Overall Landing
Cumulated Frequency (%)
Time to Take Flight (seconds)
Flight Duration (seconds)
CS ♂
D.simulans ♀
Oe⁻ ♂
Desat 1 ♂
Desat 1 ♀
Dechor.CS ♂
Antibio.CS ♂
p<0.05
0
20
40
60
80
100
0
100
200
300
400
500
600


a
Upwind Flight Cumulated Frequency (%)
Time to Take Flight (seconds)
p<0.001
SUBJECT FLY
CS ♂ b
Antibio.CS ♂ a
Z30 ♂ b
D.simulans ♂ b
b
Overall Landing Cumulated Frequency (%)
Flight Duration (seconds)
p<0.001
CS ♂ a
Antibio.CS ♂ ab
Z30 ♂ b
D.simulans ♂ a