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Supplemental Data

The Survival Advantage of Olfaction in a Competitive Environment

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Supplemental Experimental Procedures

Experimental animals

Flies (*Drosophila melanogaster*) were raised on standard medium at 25 degrees Celsius on a 12 hr light:12 hr dark cycle, with lights on at 9AM. Genotypes of strains used in this work are as follows:

Common name	Genotype
Or83b$^{-/-}$	*w*^{1118}; +; *Or83b*^{1}/*Or83b*^{2}
Or83b functional	*w*^{1118}; *Or83b-Gal4/+; Or83b*^{2}*/ Or83b*^{1}*, UAS-Or83b*
Or42a functional	*w*^{1118}; *Or42a-Gal4/+; Or83b*^{2}*/ Or83b*^{1}*, UAS-Or83b*

Transgenic and knockout strains were generated on the same *w*^{1118} background.

Embryo collection and behavioral arena

Adult flies were introduced into a population cage with a 100 mm grape fruit agar plate in the evening of the day before collection of embryos, defined as 0 days after egg-laying (AEL). Yeast paste was deposited in the center of the plate to stimulate egg-laying, and the cage was placed at in the dark at 25 degrees Celsius, 70% relative humidity overnight. At 1 day AEL, embryos laid on the plate (12-15 hr old) were collected.

Behavioral experiments used an arena comprising a 150 mm × 15 mm Petri dish (Falcon) coated with a layer of 25 ml of 2.5% agarose and covered with a lid. For the survival and competition assays, a 3-4 mm diameter hole was punched into the lid and covered with fine mesh and used as a port to inject CO_2 into the arena and anesthetize newly emerged flies for collection and counting. The first food source was 100 mg of standard fly medium dispensed into a plastic screw cap (12mm diameter, Sarstedt,

Germany). The second food source was identical to the first, but supplemented with 70 mg of live yeast paste. Unless stated otherwise, all experiments were carried out in the dark at 25 degrees Celsius, 70% relative humidity.

Survival Assay

Either 10 or 50 embryos were introduced on the first food, which was placed at the center of the arena. At 3 days AEL, the second food source was introduced 70 mm from the center of the arena in the experimental group. The control group received an empty screw cap at the same position. Starting at 10 days AEL, newly emerged adults were collected and counted daily. The experiment was terminated on 20 days AEL, by which time most adults had emerged. There was no significant difference in the survival of control transgenic *Or83b* functional animals and a wild type strain (w^{1118}) in the survival assay (data not shown; Student's t-test with Bonferroni correction, $P>0.05$). To control for effects of Gal4/UAS and genetic background we therefore used *Or83b* functional as the control strain throughout the paper.

To measure the spatial distribution of larvae from 4 to 8 days AEL (Figure 1C-D,), 5 arenas with 50 embryos were prepared in parallel. At 3 days AEL, the second food source was introduced. Starting at 4 days AEL, the number of larvae in either the first or second food source was counted by washing the food cups with buffer to liberate the larvae. The number of larvae and pupae in the arena outside of either food source was also counted, including any animals that were located on the side of the plate or on the underside of the lid that covered the arena.

Competition Assay

The competition assay was carried out exactly as the survival assay, except that an equal number of embryos from two strains to be competed were simultaneously introduced into the first food source at the center of the arena. The number of embryos was either 5 each (10 total), 25 each (50 total) or 40 each (80 total). Initially, experiments were carried out with intermittent illumination since the arenas were not covered. Later, experiments were carried out in the dark. We did not observe

statistically significant difference between the results from the two conditions (data not shown), and thus the data were pooled.

For the three competition pairings (*Or83b* functional vs. *Or83b* mutant, *Or83b* mutant vs. *Or42a* functional, and *Or42a* functional vs. *Or83b* functional), genotype was determined by diagnostic PCR analysis of individual flies. Genomic DNA was prepared by mechanical disruption of individual flies in buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, 25 mM NaCl, 200 µg/ml proteinase K) in a 96 well PCR plate. Primer sets were as follows:

A. *Or83b* knockout insertion (600 bp)
primer 1: TGT GAA GGC GTA GAC ATT CAC TCC
primer 2: GCA ATC GCA GTT CCT ATA GA

B. *UAS-Or83b* (500 bp)
primer 1: TGT GAA GGC GTA GAC ATT CAC TCC
primer 2: TTC GCC TGC GAG CAG CTG CAG

C. *Or42a-Gal4* (600 bp)
primer 1: GTG AAG CGT TAT CCA ATG GG
primer 2: TAA GTC GGC AAA TAT CGC ATG

They were applied as follows:
Primers A and B: *Or83b* functional (2 bands) vs. *Or83b* mutant (1 band)
Primers A and B: *Or83b* mutant (1 band) vs. *Or42a* functional (2 bands)
Primers B and C: *Or42a* functional (2 bands) vs. *Or83b* functional (1 band)

Data Analysis

For survival and competition assays, cumulative eclosion rates on N days AEL were calculated as follows:

$$\frac{\text{(number of adults eclosed by N days AEL)}}{\text{(number of embryos initially introduced)}}$$

Statistical analysis was performed with Microsoft Excel and XLSTAT software (Addinsoft). The Bonferroni correction was applied to adjust tests for multiple comparisons, maintaining significance at $P<0.05$.

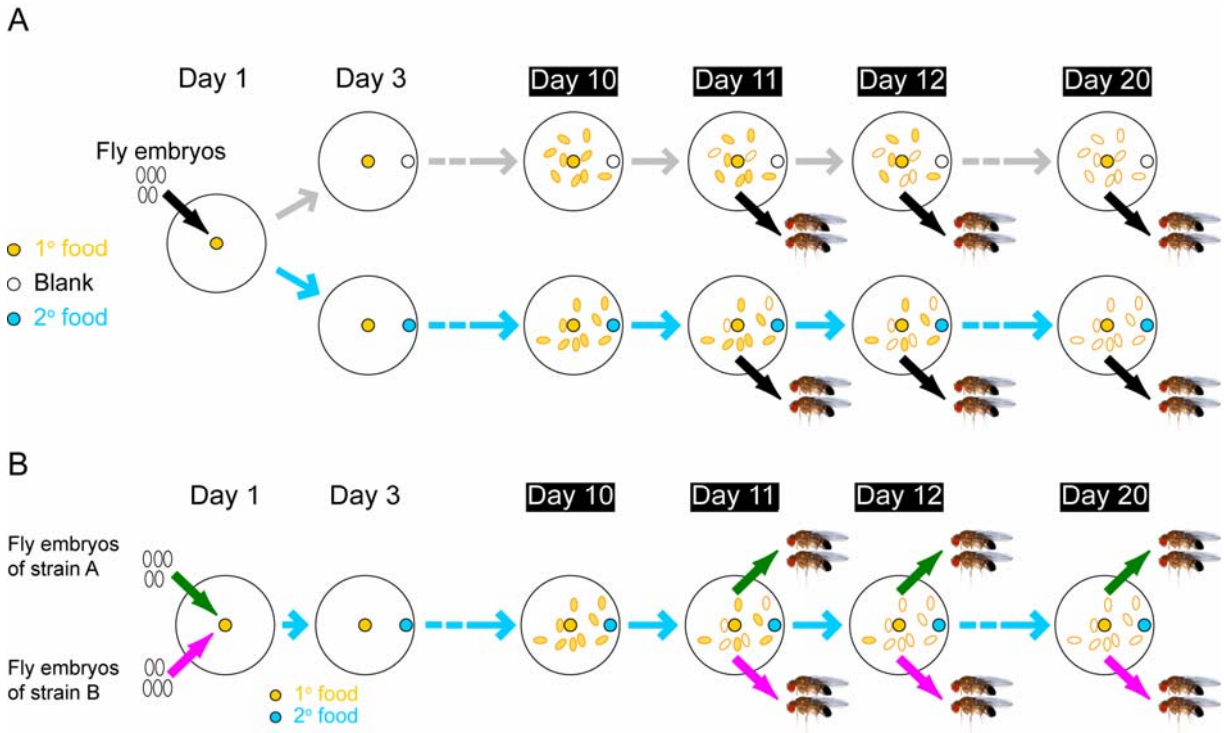

Figure S1. Schematic of survival and competition assays

(A-B) Schematic of survival (A) and competition (B) assays. Beginning on 10 days AEL, pupae are schematized as filled orange ovals and empty pupal cases as open orange ovals.

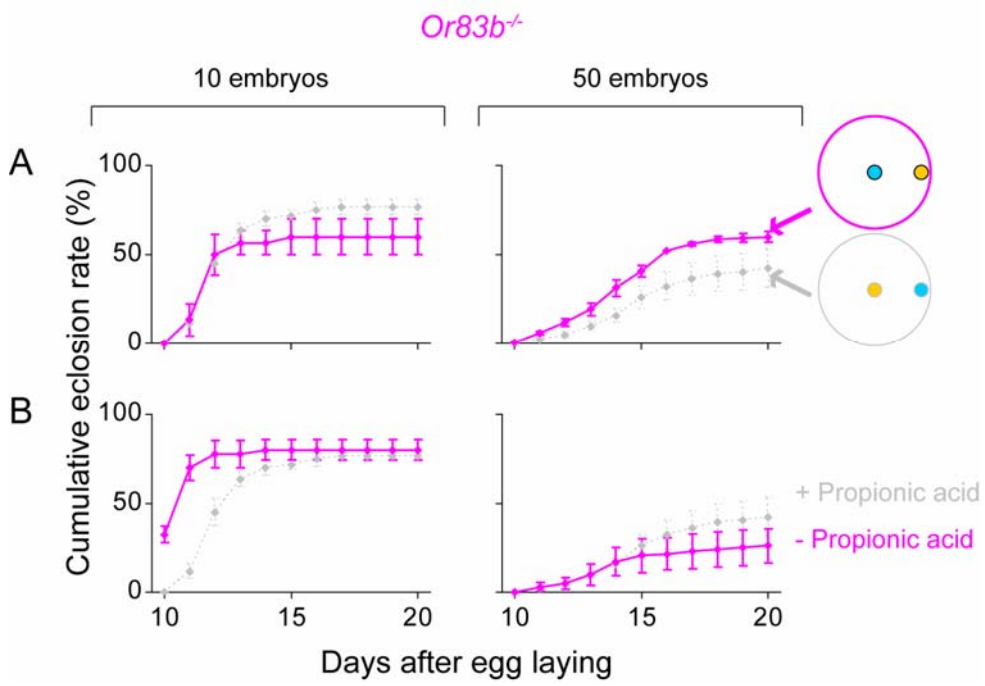

Figure S2. Control experiments altering the position of the second food source or including propionic acid in the food

(A) Cumulative eclosion rates of *Or83b* null mutants (n=3) (magenta) when the location of the first food and the second food were reversed. Values from standard food positions from Figure 1A-B are re-plotted in grey. Values of cumulative eclosion do not differ statistically across treatments at 20 days AEL (Student's t-test; P>0.05).

(B) Cumulative eclosion rate of *Or83b* null mutants is plotted against days AEL when food without propionic acid was used for survival assay (magenta) (n=4). Values from Figure 1A-B from standard fly food containing propionic acid are re-plotted in grey. Values of cumulative eclosion do not differ statistically across treatments at 20 days AEL (Student's t-test; P>0.05).