Supporting Information

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SI Materials and Methods

Gas Chromatography-Mass Spectrometry Analyses. Before analysis, samples were concentrated under a gentle flow of pure nitrogen to a final volume of 50 µL. One microliter was analyzed on a gas chromatograph (Hewlett Packard HP 6890 GC system, Agilent Technologies) equipped with an HP-5MS capillary column (30 m × 250 µm; d_f = 0.25 µm; carrier gas: helium; velocity: 30cm/s), an automatic injector (HP-7683), and coupled to a mass selective detector (HP 5973). The GC-MS was operated in electron impact mode (70 eV) and the injector was configured in splitless mode and maintained at 280 °C. The oven temperature was held at 40 °C for 2 min and rose at a rate of 8 °C/min up to 230 °C, 10 °C/min up to 280 °C, held for 10 min. For the time-course experiment and the incubation with FAME precursor mixtures, the gas chromatograph (Hewlett Packard HP 5890II GC system) was coupled to a mass selective detector (HP 5972) and equipped with a polar INNOWax column (100% polyethylene glycol, 30 m × 0.25 mm × 0.25 µm, Agilent Technologies). The GC-MS was operated in electron impact mode (70 eV) and the injector was configured in splitless mode at 220 °C with helium used as carrier gas (velocity: 30 cm/s). The oven temperature was maintained for 2 min at 50 °C and rose at a rate of 10 °C/min up to 220 °C, held for 20 min.

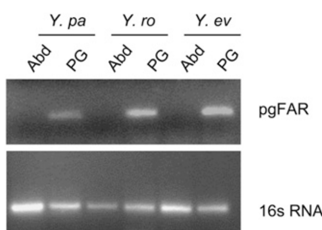

Fig. S1. Pheromone gland (PG) specific tissue expression of the *Yponomeuta padellus* (*Y. pa*), *Yponomeuta rorellus* (*Y. ro*), and *Yponomeuta evonymellus* (*Y. ev*) pgFAR mRNAs monitored by reverse-transcriptase PCR. RNAs were isolated from 15 female PGs and abdominal tissue (minus PG) (Abd) of each species and DNase-purified. Reactions were performed using 50 ng RNA under conditions as described in *Materials and Methods*. Amplicon sizes: pgFAR, 320 bp; 16s RNA, 397 bp.

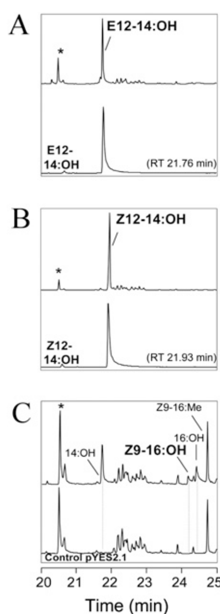

Fig. S2. GC-MS analyses of fatty-alcohol extracts from yeast transformed with pYES2.1-*Yev*-pgFAR, similarly as described in Fig. 6. In each panel, the upper chromatogram traces represent the total ion currents (TIC) from transformed yeast supplemented with 500 µM of (*A*) E12-14:Me, (*B*) Z12-14:Me, and (*C*) Z9-16:Me. The lower chromatogram traces represent the TICs of (*A*) the E12-14 and (*B*) the Z12-14:alcohol references (RT = retention time,) and (*C*) the pYES2.1 empty vector supplemented with Z9-16:Me. Asterisks (*) indicate the internal standard (150 ng Z11-13:OH). The *y* axes represent the relative abundance.

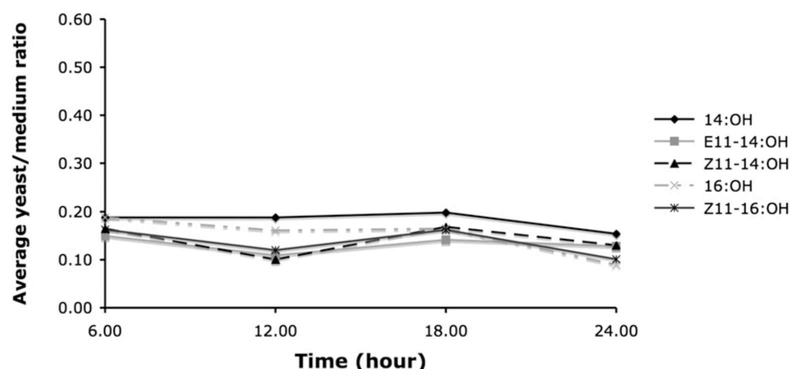

Fig. S3. Average yeast/medium ratio of fatty-alcohol extracts obtained at different time points over a 24-h experimental period ($n_{\text{time_point}} = 3$). The transformed yeasts expressing the pYES2.1-*Ypa*-pgFAR were supplemented with a mixture of Z11-16:Me (50 μM) and Z- and E11-14:Me (5 μM each). The yeast pellet and the incubation medium were extracted separately with 1 mL *n*-hexane spiked with 150 ng of Z11-13:OH as an internal standard and analyzed by GC-MS as described in *SI Materials and Methods*. The 14:OH and 16:OH are concomitantly converted from the inherent yeast fatty-acyl pool (Fig. S2*C*). There is no correlation between the yeast/medium ratio and the incubation time (Spearman's rank correlation; $P \geq 0.09$ for all alcohol products considered individually). Also, the yeast/medium ratio does not differ significantly between the different fatty alcohols (Kruskal-Wallis test; all time-points considered; $P = 0.12$). In other words, the composition in fatty alcohols extracted from the yeast cells and those recovered from the incubation medium is identical, independent of incubation times, and the yeast fatty-alcohol content accurately reflects the total fatty-alcohol production.

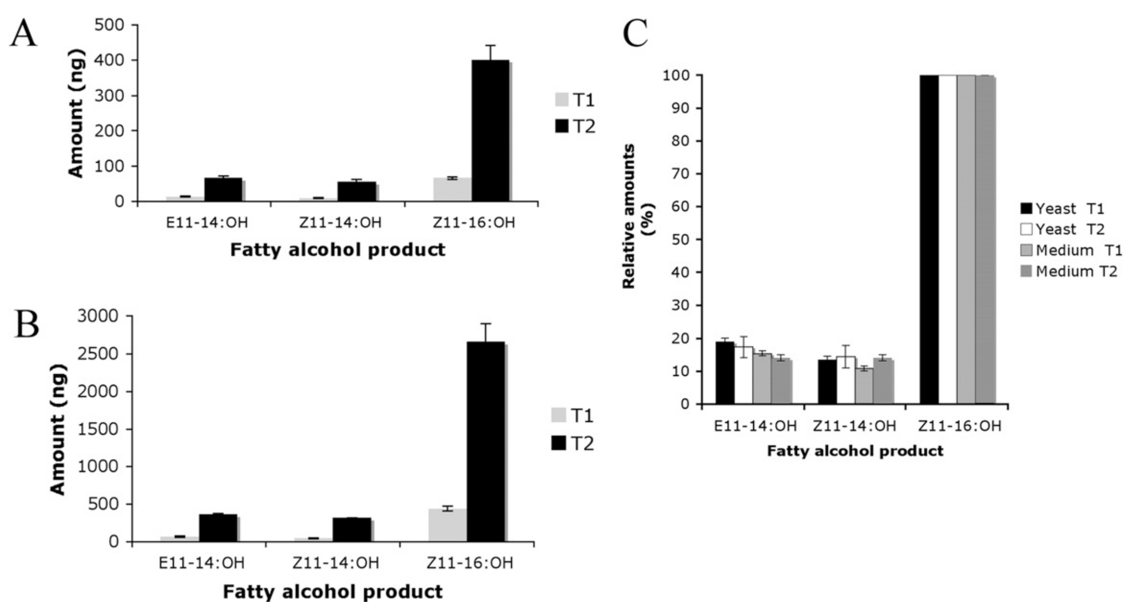

Fig. S4. Fatty-alcohol products extracted after a 24-h incubation period from the *InvSc1* yeast expressing the pYES2-1-*Ypa*-pgFAR supplemented with a mixture of E11-14:Me, Z11-14:Me and Z11-16:Me in a 1:1:100 ratio, which corresponds to the relative abundance of each pheromone precursor in the insect gland (1). The functional assay procedure followed the same protocol as described under *Materials and Methods*. The precursor concentrations in treatment 1 (T1) were 0.5 μM:0.5 μM:50 μM and in treatment 2 (T2), 5 μM:5 μM:500 μM, respectively. The yeast pellet (*A*) and the incubation medium (*B*) were extracted separately with 1 mL *n*-hexane spiked with 150 ng of Z11-13:OH as an internal standard and analyzed by GC-MS as described in *SI Materials and Methods*. Fatty alcohols are found both in the culture medium and the yeast cells with an identical composition. The total fatty-alcohol amounts are proportional to the initial fatty-acyl precursor amounts but the observed ratio between the three fatty alcohol products is constant (15:15:100) (*C*) and corresponds well to the 1:1:5 ratio found between the three sex pheromone components in *Y. padellus.*

1. Löfstedt C, Herrebout W, Menken S (1991) Sex pheromones and their potential role in the evolution of sex reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology* 2(1):20–28.

Table S1. *Bombyx mori* (Bmo) gene accession numbers used for NJ tree construction

Sequence name	Gene accession number*
Bmo-swdb1	BGIBMGA010457
Bmo-pgFAR	BAC79426[†]
Bmo-swdb2	BGIBMGA010553[‡§]
Bmo-swdb3	BGIBMGA011217_2[¶‡]
Bmo-swdb4	BGIBMGA011217_1[¶‡]
Bmo-swdb5	BGIBMGA011149[¶]
Bmo-swdb6	BGIBMGA011147+48[¶]
Bmo-swdb7	BGIBMGA006569[¶]
Bmo-swdb8	BGIBMGA000659
Bmo-swdb9	BGIBMGA011207
Bmo-swdb10	BGIBMGA011145[¶]
Bmo-swdb11	BGIBMGA011398[¶]
Bmo-swdb12	BGIBMGA011395[¶‡]
Bmo-swdb13	BGIBMGA011129[¶‡§]
Bmo-swdb14	BGIBMGA011138[‡]
Bmo-swdb15	BGIBMGA014047
Bmo-swdb16	BGIBMGA011126
Bmo-swdb17	BGIBMGA011140
Bmo-swdb18	BGIBMGA011164
Bmo-swdb19	BGIBMGA002160
Bmo-swdb20	BGIBMGA011116[¶]
Bmo-swdb21	BGIBMGA010511[‡§]

*Sequences retrieved from the Silkworm Genome Database (swdb), otherwise indicated.
[†]Sequence retrieved from GenBank.
[‡]Manually corrected.
[§]Partial sequence.
[¶]Also present in EST databases.

Table S2. Oligonucleotide primer sets

Primer name	Primer sequence (5′–3′)	Amplicon size (bp)
5′and 3′ cDNA RACE (5R or 3R) and RT-PCR (s + as)		
Yev-FARI-5R-as	TGACGACATATTCGATGACCCGGTGTTC	330*
Yev-FARI-3R-s	CGCTCCCTCACTTGTGACAGGTGACAGT	
Yev-FARII-5R-as[†]	GCGTAAGGATGACCCTTCGGCGCTCAA	320*
Yev-FARII-3R-s	TTAGCGGCGCCGGGGGAGGGTAGACA	
Yev-FARIII-5R-as	CGTGCCCCAACGTAGGCTGCCTGTATC	333*
Yev-FARIII-3R-s	TGTGCGGCAAGGAGTCGCAAATGTTC	
16SRNA-s	TGAAGGGCTGCAGTATTTTG	397
16SRNA-as	TCGAGGTCGCAAACTCTTTT	
Quantitative PCR		
Yev-pgFARs	TGACTTGACAATGCCTAACC	179
Yev-pgFARas	TTTCCAGACGATGACAAAGG	
16SRNA-s	GACCTCGATGTTGGATTAAG	95
16SRNA-as	GGTTTGAACTCAGATCATGTAAG	
ORF amplification for functional assay		
Yev-pgFAR-s[‡§]	aaaATGgTTCAGTTGAAAGAAGATTCTG	1,504
Yev-pgFAR-as[‡¶]	CGACTCTCTCTAGGCCAGCTTTTC	
Yev-FARI-s	aaaATGgCAACAGAAACAGTTGACGT	1,749
Yev-FARI-as	CCGCATAAATTCTCAAAATACACTCG	
Yev-FARIII-s	aacATGgTGGCACGCAGGTTGTCG	1,586
Yev-FARIII-as	AGCAAATCCTACGCAGCAGGCAGCAA	

*RT-PCR amplicons.
[†]Yev-FARII corresponds to Yev-pgFAR.
[‡]Primer also used to amplify Ypa-pgFAR and Yro-pgFAR ORFs.
[§]The start and stop codons are underlined, the Kozak sequence is indicated in lowercase letters.
[¶]Primer designed based on the 3′UTR sequence information.