Supplementary Information for "A nonspecific defensive compound evolves into a competition avoidance cue and a female sex pheromone"

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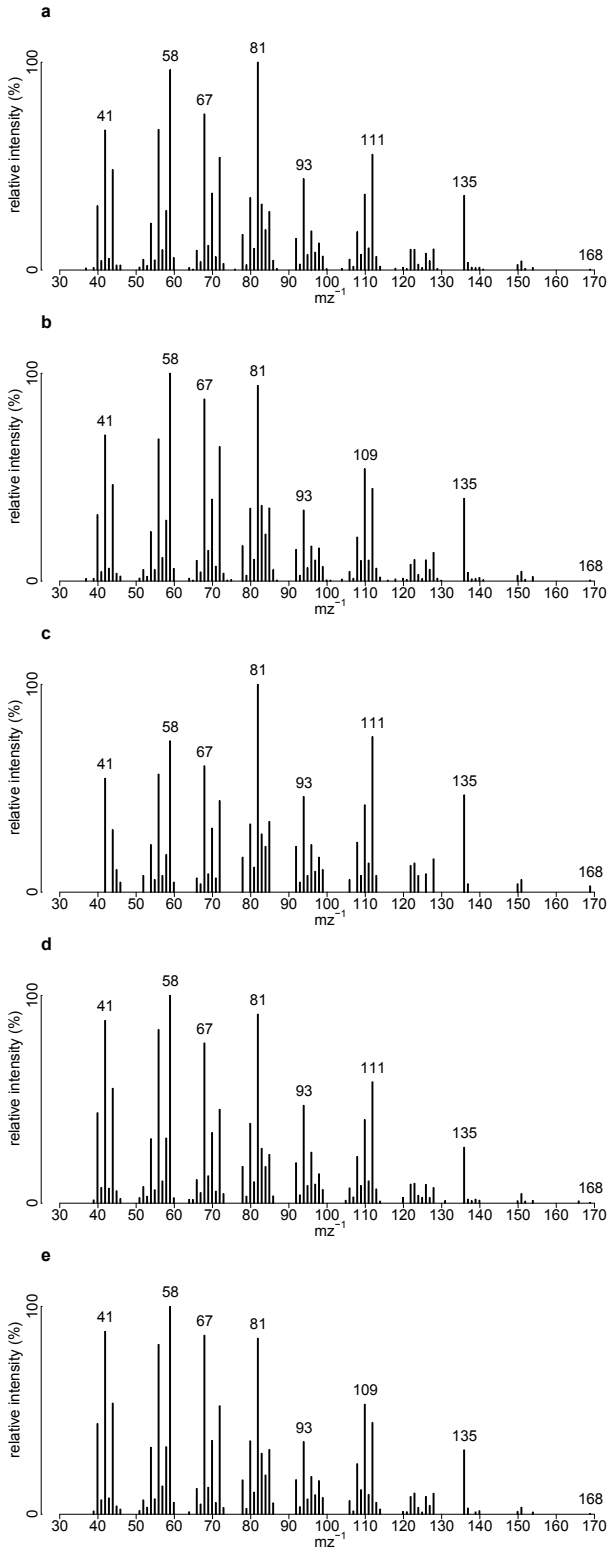
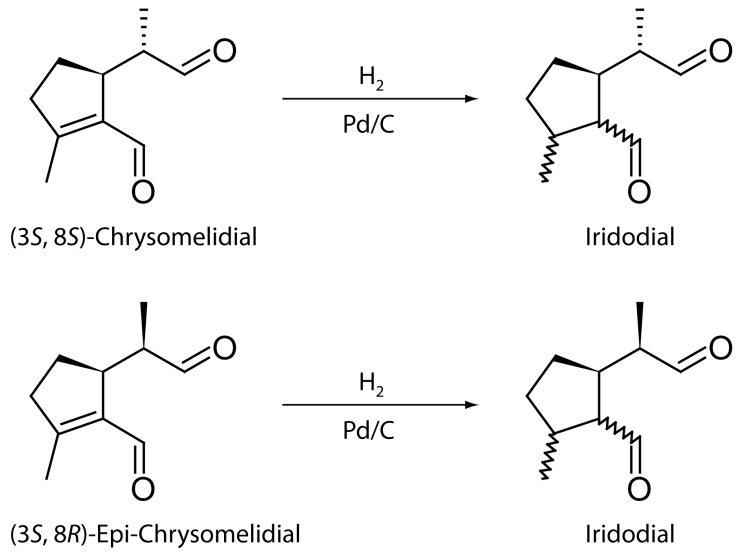
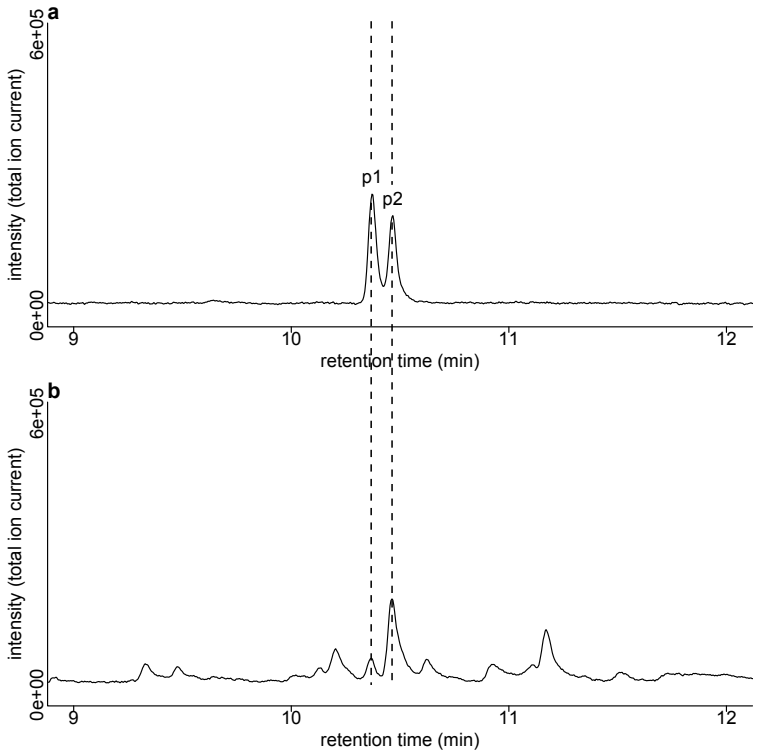


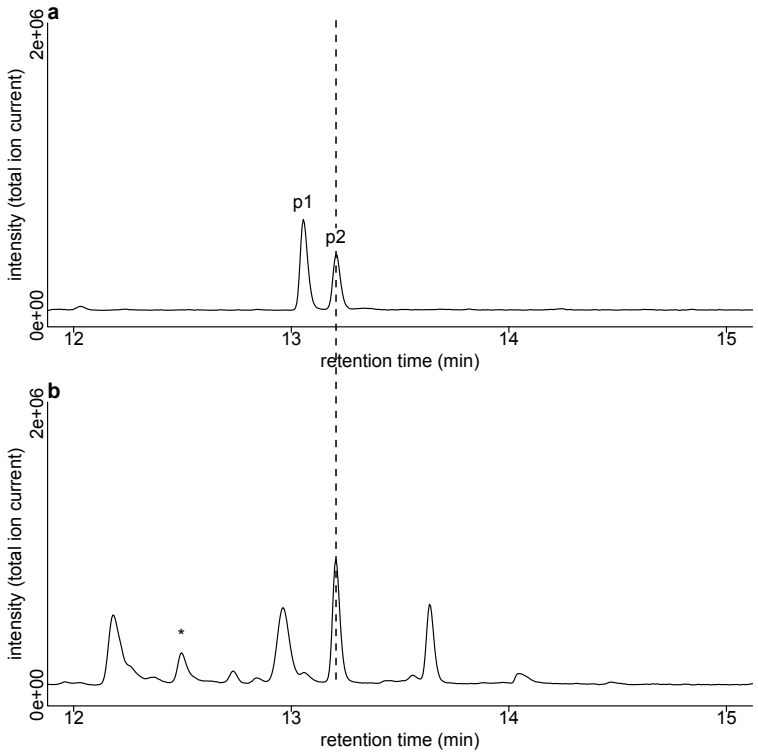
Supplementary Figure S1: Tentative identification of p1 and p2. Mass spectra (EI) of (**a**) compound p1 and (**b**) compound p2 from *L. heterotoma* females, (**c**) iridodial [25], and (**d**) and (**e**) iridodials derived from (epi-)/chrysomelidial.



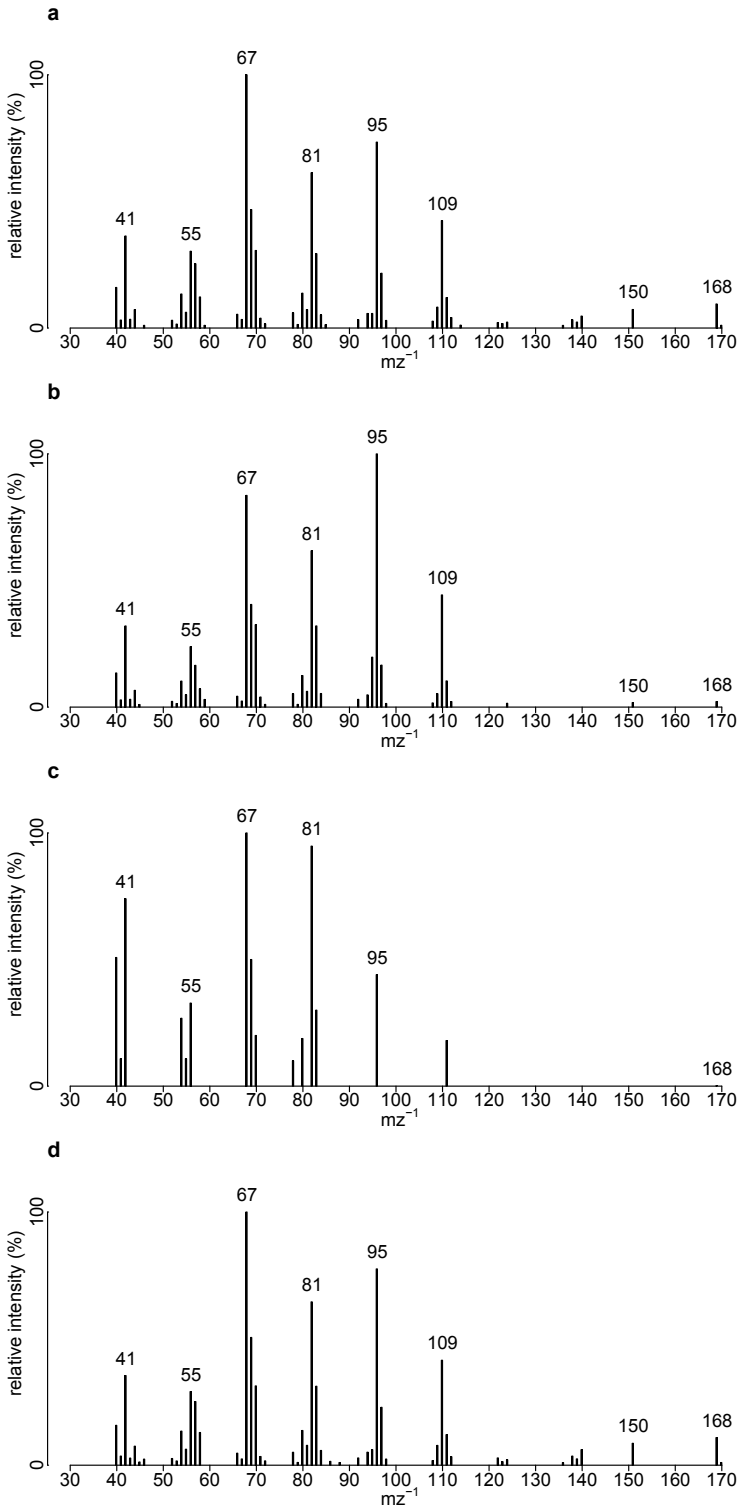
Supplementary Figure S2: Hydrogenation of (epi-)/ chrysomelidial. Catalytic hydrogenation of (epi-)/chrysomelidial yields multiple stereoisomers of iridodial through the addition of hydrogen at the carbon-carbon double bond.



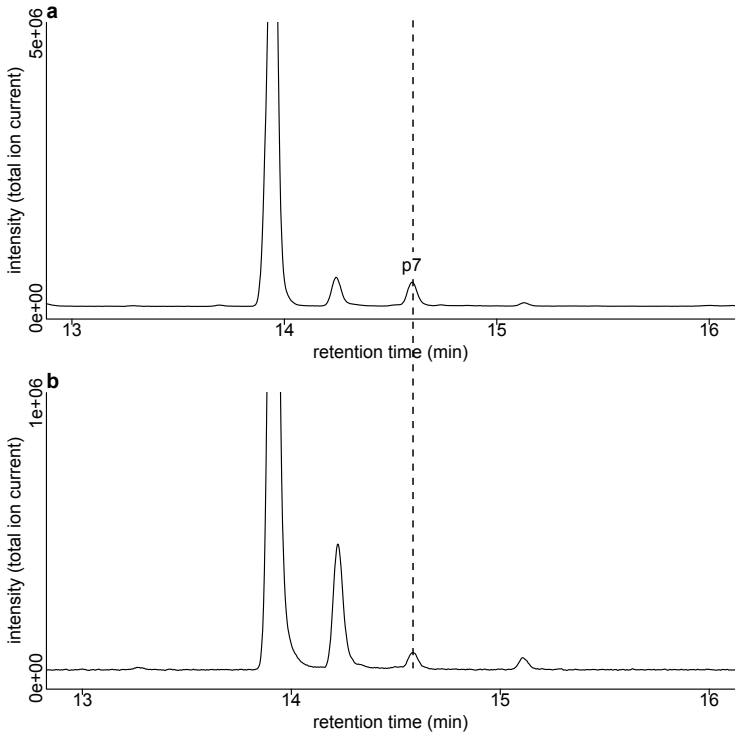
Supplementary Figure S3: Tentative identification of p1 and p2. Total ion current chromatograms on a non-polar column of (**a**) the female sex pheromone of *L. heterotoma* and (**b**) the iridoidials derived from (epi-)/chrysomelidial.



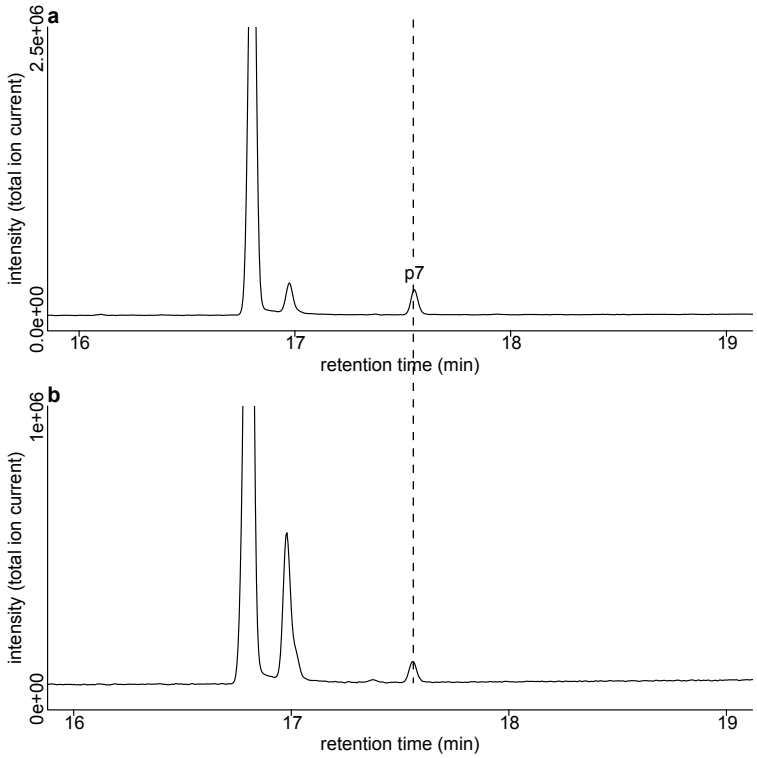
Supplementary Figure S4: Tentative identification of p1 and p2. Total ion current chromatograms on a cyclodextrin (Beta DEX 225) column of (**a**) the female sex pheromone of *L. heterotoma* and (**b**) the iridoidials derived from (epi-)/chrysomelidial. The asterisk denotes the compound that coeluted with p1 on the non-polar column.



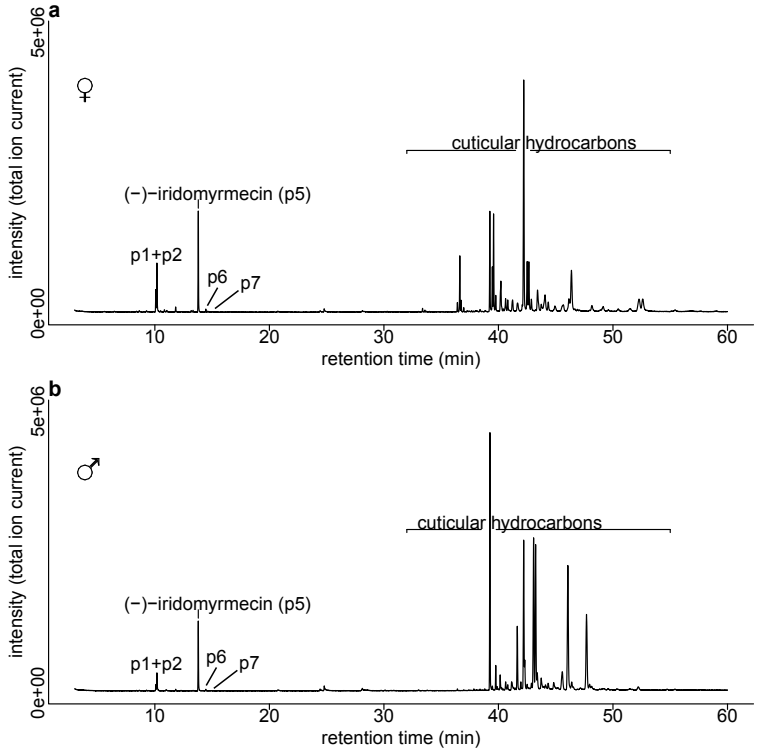
Supplementary Figure S5: Tentative identification of p7. Mass spectra (EI) of (**a**) compound p7 from *L. heterotoma* females, (**b**) synth. (-)-iridomyrmecin, (**c**) a trans-fused iridomyrmecin [26] and (**d**) the minor product in the synthesis of (-)-iridomyrmecin.



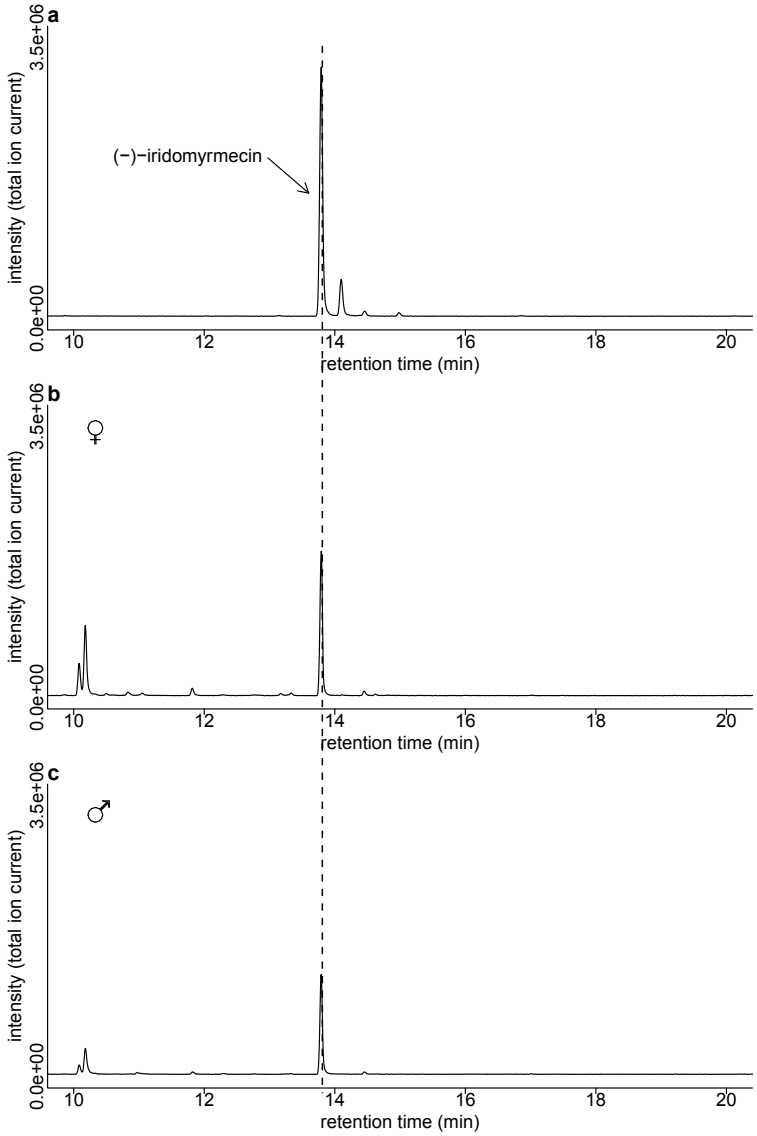
Supplementary Figure S6: Tentative identification of p7. Total ion current chromatograms on a non-polar column of (**a**) an extract of virgin *L. heterotoma* females and (**b**) the synthetic sample of (-)-iridomyrmecin with minor compounds.



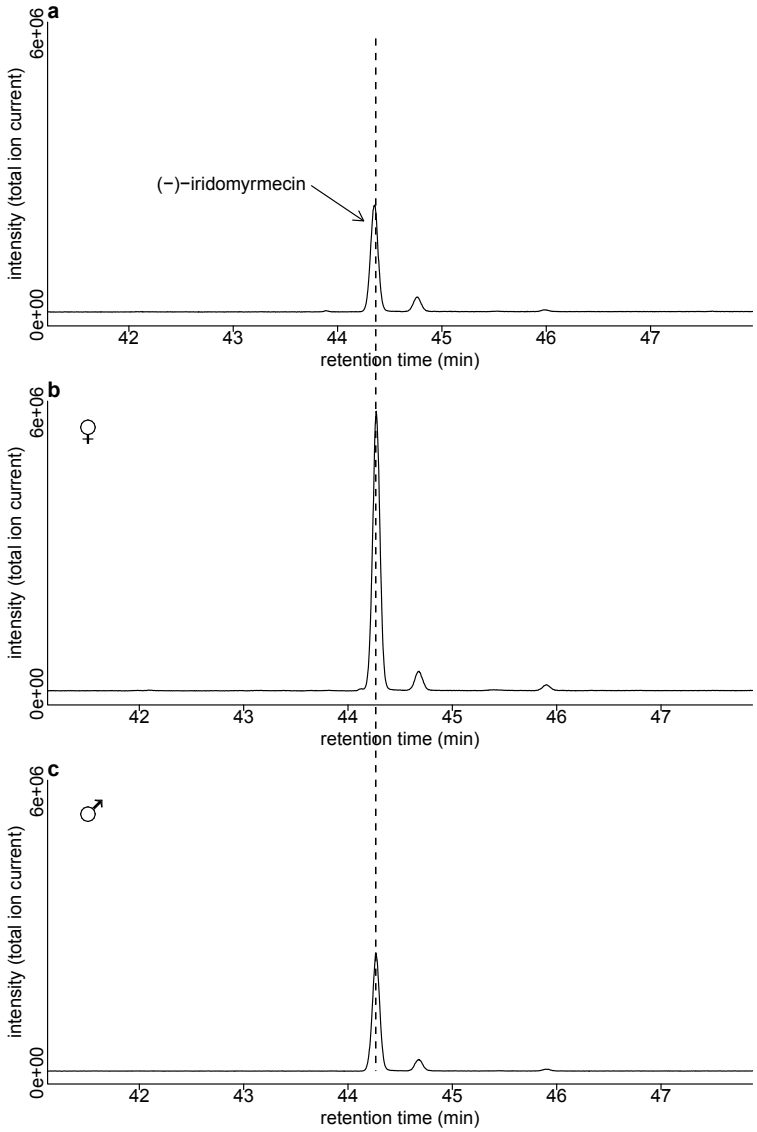
Supplementary Figure S7: Tentative identification of p7. Total ion current chromatograms on a cyclodextrin (Gamma DEX 120) column of (**a**) an extract of virgin *L. heterotoma* females and (**b**) the synthetic sample of (-)-iridomyrmecin with minor compounds.



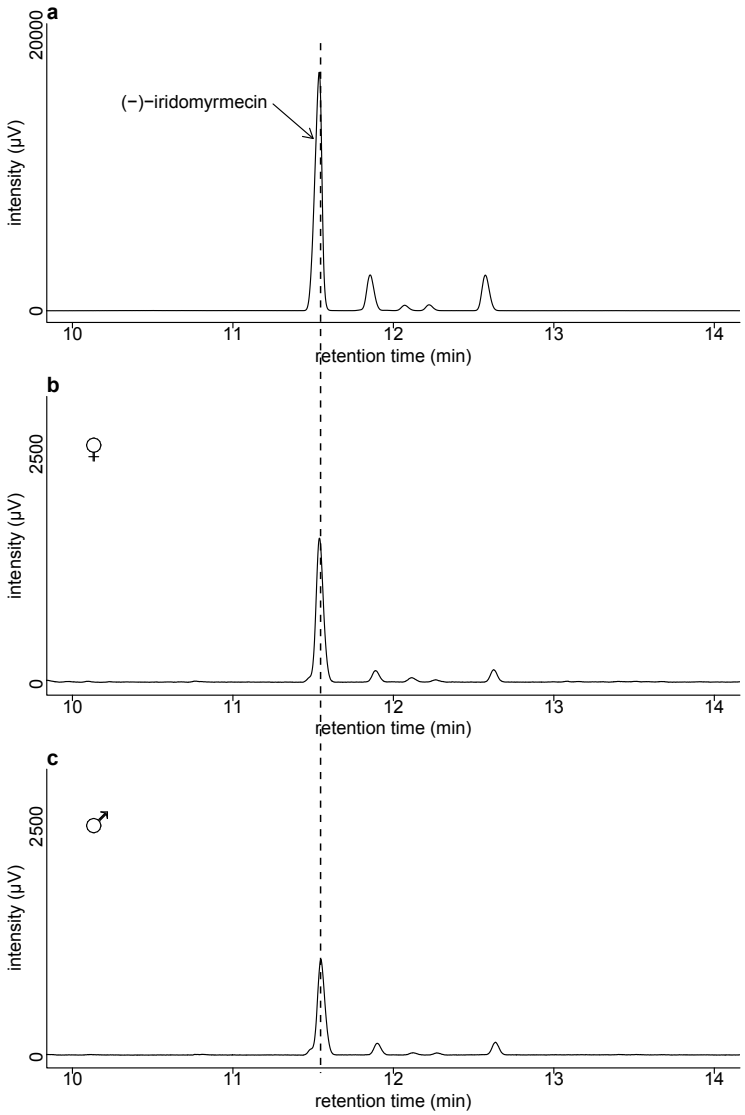
Supplementary Figure S8: Chemical compounds produced by *L. boulardi*. Total ion current chromatograms of an extract of (**a**) virgin *L. boulardi* females and (**b**) *L. boulardi* males.



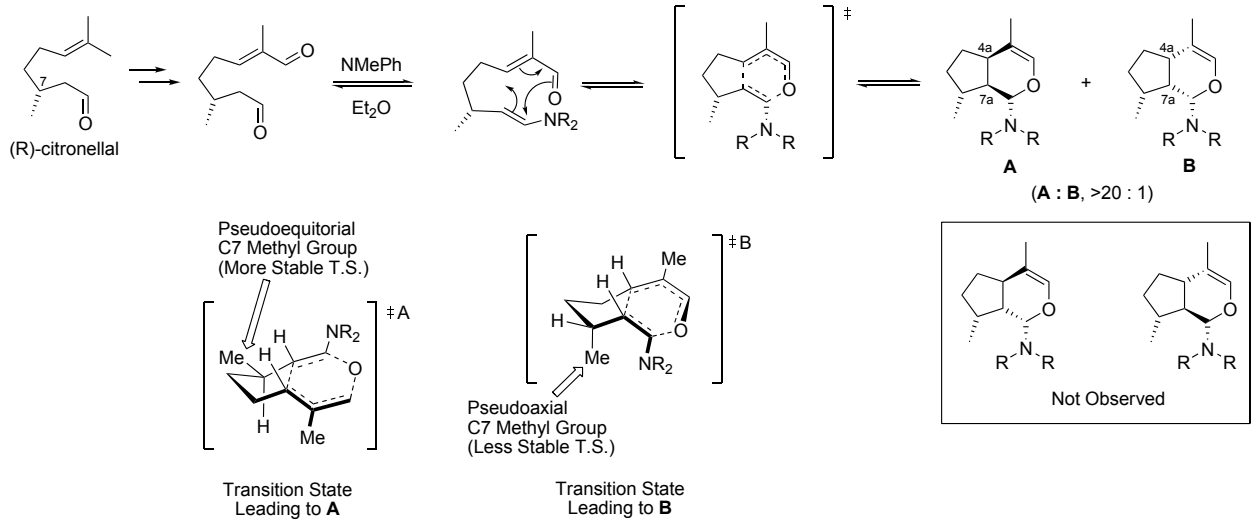
Supplementary Figure S9: Identification of (-)-iridomyrmecin in *L. boulardi*. Total ion current chromatogram on a non-polar column of (**a**) synthetic (-)-iridomyrmecin, (**b**) the iridoid fraction of *L. boulardi* female extract and (**c**) the iridoid fraction of *L. boulardi* male extract.



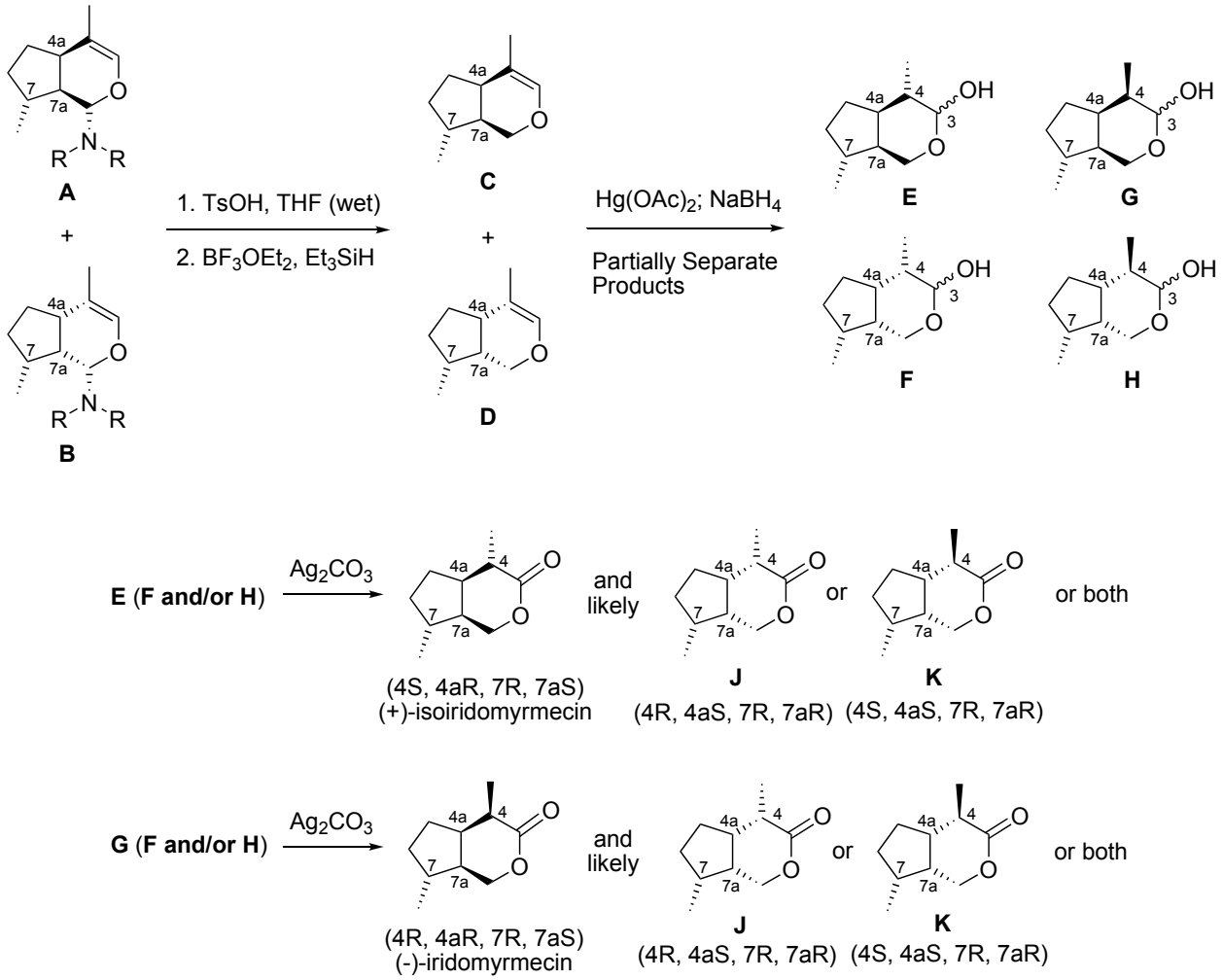
Supplementary Figure S10: Identification of (-)-iridomyrmecin in *L. boulardi*. Total ion current chromatogram on a polar (RH-WAX) column of (**a**) synthetic (-)-iridomyrmecin, (**b**) the iridoid fraction of *L. boulardi* female extract and (**c**) the iridoid fraction of *L. boulardi* male extract, both (**b**) and (**c**) coinjected with synthetic (-)-iridomyrmecin.



Supplementary Figure S11: Identification of (-)-iridomyrmecin in *L. boulardi*. Total ion current chromatogram on a cyclodextrin (Beta DEX 225) column of (**a**) synthetic (-)-iridomyrmecin, (**b**) the iridoid fraction of *L. boulardi* female extract and (**c**) the iridoid fraction of *L. boulardi* male extract, both (**b**) and (**c**) coinjected with synthetic (-)-iridomyrmecin.



Supplementary Figure S12: Stereochemical analysis of the Diels-Alder reaction. The putative mechanism of the Diels-Alder cycloaddition employed in the synthesis of (-)-iridomyrmecin and (+)-isoiridomyrmecin suggests that the formation of two cycloadducts, **A** (major) and **B** (minor), is likely.



Supplementary Figure S13: J and K are likely impurities in the authentic standard of (-)-iridomyrmecin. The nonselective introduction of the C4 stereocenter late in the synthesis of the authentic standard would ultimately provide two possible side products, **J** and **K**, either or both of these products could be a minor component of the authentic standard of (-)-iridomyrmecin.

Supplementary Table S1: Species specifity of mate recognition. Test statistics for pairwise Mann-Whitney U-tests of wing fanning duration from the mate recognition experiment (Fig. 6b). P-values (rounded to third decimal) were corrected using the Bonferroni-Holm method. Uncorrected P-values (rounded to third decimal) are given in parentheses. For each experiment n = 12.

	L. heterotoma iridoids (DCM fraction)	*L. boulardi* iridoids (DCM fraction)	synth. (-)-iridomyrmecin	DCM
L. heterotoma iridoids (DCM fraction)	-	U=80	U=63.5	U=28.5
L. boulardi iridoids (DCM fraction)	P=0.005 (P=0.001)	-	U=176.5	U=103
synth. (-)-iridomyrmecin	P=0.001 (P<0.001)	P=0.529 (P=0.529)	-	U=112
DCM	P<0.001 (P<0.001)	P=0.016 (P=0.005)	P=0.021 (P=0.011)	-

Supplementary Table S2: Behavioural assays. y-tube experiments conducted to identify the competition avoidance agent (1.A-1.I) and the female sex pheromone (2.A-2.I and 2S.A-2S.E) in *L. heterotoma*.

experiment	test arm	control arm	responding individual
Identification of the competition avoidance agent.			
1.A	host patch	empty Erlenmeyer flask	*L. heterotoma* mated female
1.B	host patch with 5 mated *L. heterotoma* females	host patch	*L. heterotoma* mated female
1.C	host patch with 5 mated *L. boulardi* females	host patch	*L. heterotoma* mated female
1.D	female extract	solvent	*L. heterotoma* mated female
1.E	host patch plus extract of virgin *L. heterotoma* females	host patch plus solvent	*L. heterotoma* mated female
1.F	host patch plus CHC fraction of female extract	host patch plus solvent	*L. heterotoma* mated female
1.G	host patch plus iridoid fraction of female extract	host patch plus solvent	*L. heterotoma* mated female
1.H	host patch plus synthetic (-)-iridomyrmecin	host patch plus solvent	*L. heterotoma* mated female
1.I	host patch plus synthetic (+)-isoiridomyrmecin	host patch plus solvent	*L. heterotoma* mated female
Identification of the female sex pheromone.			
2.A	10 virgin *L. heterotoma* females in Erlenmeyer flask	empty Erlenmeyer flask	*L. heterotoma* virgin male
2.B	extract of virgin *L. heterotoma* females	solvent	*L. heterotoma* virgin male
2.C	CHC fraction of female extract	solvent	*L. heterotoma* virgin male
2.D	iridoid fraction of female extract	solvent	*L. heterotoma* virgin male
2.E	synthetic (-)-iridomyrmecin and synth. (+)-isoiridomyrmecin	solvent	*L. heterotoma* virgin male
2.F	iridoid fraction of female extract, p5 (= (-)-iridomyrmecin) removed	solvent	*L. heterotoma* virgin male
2.G	iridoid fraction of female extract, p5 replaced with synthetic (-)-iridomyrmecin	solvent	*L. heterotoma* virgin male
2.H	iridoid fraction of female extract, p5 replaced with synthetic (+)-iridomyrmecin	solvent	*L. heterotoma* virgin male
2.I	extract of virgin *L. boulardi* females	solvent	*L. heterotoma* virgin male
2S.A	iridoid fraction of female extract. p4 removed	solvent	*L. heterotoma* virgin male
2S.B	iridoid fraction of female extract. p1, p2, p3 and p4 removed	solvent	*L. heterotoma* virgin male
2S.C	iridoid fraction of female extract, p6 (= (+)-isoiridomyrmecin) and p4 removed	solvent	*L. heterotoma* virgin male
2S.D	iridoid fraction of female extract, p7 and p6 removed	solvent	*L. heterotoma* virgin male
2S.E	iridoid fraction of female extract, p3 and p4 removed	solvent	*L. heterotoma* virgin male