Supplementary Material

Functional evolution of a bark beetle odorant receptor clade detecting monoterpenoids of different ecological origins

Xiao-Qing Hou[1], Jothi Kumar Yuvaraj[1], Rebecca E. Roberts[1], Dan-Dan Zhang[1], C. Rikard Unelius[2], Christer Löfstedt[1], Martin N. Andersson[1]*

[1] Department of Biology, Lund University, Lund, Sweden

[2] Faculty of Health and Life Sciences, Linnaeus University, Kalmar, Sweden

*Corresponding author: Martin N. Andersson

Department of Biology, Sölvegatan 37, 223 62 Lund, Sweden.

Email: martin_n.andersson@biol.lu.se

Phone: +46-(0)462229344

Table S1. List of compounds initially tested on all five ORs, with purity, source and examples of biological origin.

Class	Compound	Purity (%)	Source*	Biological origin
Oxygenated monoterpenoids homoterpenoids, and hemiterpenes	(1*S*,4*S*)-*cis*-Verbenol	95	Borregaard	Beetle
	(1*R*,4*R*)-(+)-*trans*-Verbenol	92	SCM	Beetle
	(1*S*,4*S*)-(–)-*trans*-Verbenol	97	SciTech Ltd., Prague	Beetle
	(1*S*)-(–)-*cis*-Verbenone	>99	Fluka	Beetle, fungi
	(±)-Ipsdienol	94	Bedoukian	Beetle
	(±)-Ipsenol	95	Synergy Semiochemicals	Beetle
	Amitinol	91	R. U.	Beetle
	(+)-*trans*-4-Thujanol	97	Sigma-Aldrich	Host, fungi
	(±)-Grandisol (grandlure I)	95	Bedoukian (E. W.)	Beetle
	Geranylacetone	>99	Fluka	Non-host, fungi
	(+)-Isopinocamphone	>99	R. U.	Host, fungi
	1,8-Cineole	99	Aldrich	Host
	α-Isophorone	>99	Acros	**
	Lanierone	>99	Synergy Semiochemicals	Beetle
	2-Methyl-3-buten-2-ol	>99	Acros	Beetle, fungi
Monoterpenes	(+)-α-Pinene	98	Janssen Chimica	Host
	Myrcene	95	Sigma-Aldrich	Host
	p-Cymene	>99	Acros	Host
	(+)-3-Carene	99	Aldrich	Host
Aliphatic alcohols and spiroacetals	(±)-3-Octanol	97	Sigma-Aldrich	Non-host, fungi
	(±)-1-Octen-3-ol	98	Janssen Chimica	Non-host, fungi
	1-Hexanol	>99	Fluka	Non-host, fungi
	Ethanol	99.5	Solveco	Host
	(5*S*,7*S*)-*trans*-Conophthorin	94	W. F.	Non-host, fungi
Aromatic compounds	2-Phenylethanol	>99	Sigma	Beetle, fungi
	Acetophenone	99	Acros	Beetle, fungi
	Styrene	>99	Fluka	Fungi
	4-Vinylanisole	97	Aldrich	Fungi
	Estragole (4-allylanisole)	>99	Aldrich	Host, fungi
	3,4-Dimethoxytoluene	98	Givaudan-Roure	Host, fungi
	Eugenol methyl ether	>99	Fluka	Host, fungi
	4-Ethylguaiacol	98	Sigma-Aldrich	Fungi

*R. U. = synthesized by Rikard Unelius (Linnaeus University, Kalmar, Sweden); W. F. = gift from Wittko Francke (University of Hamburg, Germany). **: The biological source of α-isophorone is unknown. Female *Ips typographus* produce tiny amounts of β-isophorone which is unstable, and we found that α-isophorone was more active than β-isophorone in SSR study (Kandasamy et al., in prep); we hence tested α-isophorone in this study.

Table S2. Additional OR-specific compounds, with purity, source, examples of biological origin and target ORs. For consistency, these compounds were also tested on non-target ORs showing no (or insignificant) activity.

Class		Purity (%)*	Source**	Biological origin	Target OR
Oxygenated monoterpenoids	(*S*)-(+)-Ipsdienol	99 (98% ee)	A. M.	Beetle	ItypOR28
	(*R*)-(–)-Ipsdienol	99 (98% ee)	A. M.	Beetle	ItypOR28
	(–)-Terpinene-4-ol	99	Acros	Host, fungi	ItypOR23
	(±)-Camphor	97	Aldrich	Host, fungi	ItypOR29
	(–)-Pinocarvone	99	Y. N.	Beetle, fungi	ItypOR29
	(+)-Pinocamphone	84 (16% IPC)	R. U.	Host, fungi	ItypOR29
	(–)-Pinocamphone	81 (19% IPC)	R. U.	Host, fungi	ItypOR29
	(–)-Isopinocamphone	99	R. U.	Host, fungi	ItypOR29
Monoterpene	γ-Terpinene	97	Aldrich	Host	ItypOR27

Abbrevations: ee = Enantiomeric excess; IPC= isopinocamphone.

** R. U. = synthesized by Rikard Unelius (Linnaeus University, Kalmar, Sweden); A. M. = synthesized by Aleš Machara (Academy of Sciences of the Czech Republic, Prague); Y. N. = gift from Yoko Nakamura (Max Planck Institute for Chemical Ecology, Jena, Germany).

Table S3. Three compounds only tested on ItypOR28, with purity, source, and examples of biological origin.

Class	Compound	Purity (%)	Source*	Biological origin
Oxygenated monoterpenoids	*E*-Myrcenol	>99	Fytofarm	Beetle
	(±)-Myrtenol	96	G. B.	Beetle, fungi
Bicyclic acetal	(±)-Frontalin	>99	Synergy Semiochemicals	Beetle

* G. B. = gift from Gunnar Bergström (University of Gothenburg, Sweden).

Table S4. Primers used in this study.

Genes	Primer sequence (5'-3')
ItypOR23_F	CGCGGATCCGCCACCATGGCCGTGTATCCAAAATCAG
ItypOR23_R	GCTCTAGATTAAGTTCTTTTGTATGCTAGAGTAATATAAGAAT
ItypOR25_F	CGCGGATCCGCCACC ATGAAGATTTACCCTGACACAAAGT
ItypOR25_R	GCTCTAGA CTATCGAAACATGATAGTAATATAGGTGTAGG
ItypOR27_F	CGCGGATCCGCCACC ATGAGAGTGTATCCGGACATAGAA
ItypOR27_R	GCTCTAGATTAGTTGTTTCGGACTACCACG
ItypOR28_F	CGCGGATCCGCCACC ATGGGATTGTATCCAGCAAGTAGA
ItypOR28_R	GCTCTAGATTAATTTCTAAGAATTACCGATATGTAGGTGT
ItypOR29_F	CGCGGATCCGCCACCATGGCTGCTTATCCACAATGC
ItypOR29_R	GCTCTAGACTATTGCCTAAAAATAATACTCACATAGGAATAA
Sp6	ATTTAG GTGACACTATAG
EBV-rev	GTGGTTTGTCCAAACTCATC

Amino acid sequences of the five ItypORs

>ItypOR23
MAVYPKSEHLKVPAIYCSTIGIFPWKFMFQDNKNLQTIYRCYSIVMLAWCIGFVVTDYIQLVILLTSKTLDMQEISFN
TCITLLFTCIGLRAVIVYFSPNSANLIQSIIDSEKVTYLDDAECMKLEKEHLRSVRLISHCYFIFIIFSTTSRCVYFFSKE
PDFIQNGNETEIVKEHMLSIWFPFNQEKYYLTVYNIELLDSFLGTFFVAYVDIYTFNMISYPKGQLKKLQHIMKHFH
NYKAKYSSETNEENDFIVFKDLVQRHKQIIQHINAFNELMEFVAIFEFVQSSAQIACGLTQTSLENLTIGSFLFVMSF
LISMLVRLFLYYYAANDVTVESTKLAQCIWESNWYEESQKIKLSMLMVIIRAQKPLIFKIGGFGTMSVQSIVTILKATY
SYITLAYKRT

>ItypOR25
MKIYPDTKFFDVTAKFGAIVGLYPWQFMFPDNNTCRQIYRWYSYIVLLSFIVLLLPMYVELIILLRNEETSKDELGSN
LSITIVFSSAGLRALFLRRGSNLINLIQNVMDEEKKQLFVDCKKVQLLEDKCLKVVRKLSYIYAVIVVVAASQKSVTA
LLQTPTSSTGTPSRDLIISAWFPFDKQEYYWQAYCIQIYHTIIGASYLSYMDIFMFNLLSYPIGQFKKLQFIIKNMEVQ
HYSYNDSENKSIDDGVRSIIERHQYIIQYVDFYNKSMGTFALFDFLQSSLQIATVLLQFSPTVGTIIFMLIFFALMLLR
LFLYYYTANEVSVQSEKVKMAVWESKWYEQPPKIKYALLRIMTRAAKPSKYIIGAFGGMSTYSIIQILKATYTYITIM
FR

>ItypOR27
MRVYPDIENFKITAIYSSTIGLFPWKFMFQDNQVLQQTYRYYSYFIYGSFVIFIITAYVELIIMLNGDVLKMDAICSNIC
LTLAFTCSALRATVMRVGPNLLKIIEQVMHAEKNPASIEDQTSFNLERKSIKTMRKLSHLYAVAITMIASSKCALAPF
EKGEIVHIGNTTIIDRPLIMSAWVPFNKNTHYWAAYIIQIYFAALGAWHVAYVDMFMFNMLGYPIGQLKKLHYYIKNI
TTLTRNDDSLEEFKNVIRQHQQIISYVKFYNDSMGTFAIFEFLQSSVQIASIFIQTSPSDMNLGQFGFIGGFFIGMLF
RLFLYYYTANEVMTESEKVGVSVWESDWYEQPTNLKMALLTVMMRGQRPLYYKIGGFGLMSVQSIVAILKATYT
YLTVVVRNN

>ItypOR28
MGLYPASRYFKNPIMWSSILGAFPWQMIFQENAKLQQVYRWYSNFMLTWYFGMVTTEYIQLYHILNANVIQMDE
VCENVCMSLVFTCTGLRVWVMRRTNGLSEIIQTVVDAEREADGLDDEKTRQYEDIHVKHMEKVSFIYAAFVFMSV
TNGCLATLYADTKSVIIGNSTIVEKPLIISTWFPFDKNEHYWVAYGLQVFDGYMAALTVACTDILMFNMISYPIGQLT
KLQHLVRNMAVYKTHFEAFPTFTKIVQRHKHVIKYVELFNQSMGTFAIFEFVQSSVQIASVLVQTSPDDLTLMSFC
FIVLFFTSMLTRLFMYYYSANEVIIQSINLGDSVWESSWYHQPHQLKQAMLMVLVRAQKPVSYKIGGFGIMSMQSI
VAILKATYTYISVILRN

>ItypOR29
MAAYPQCKNLRVAIIYSSIIGVFPWQFMFQHNHLRQTLYRWYSVFLHFWFSGFIITEYIELYLQCTADELKLDEICA
NICVVMVFTSTAVRQLVMRFNKMVNDLIQSIIDEEKHNDFLEDDKTREIEDKFIKSSDSISNWYAAPVYITLFQYVLF
PMMSKPDIIQIGNTTQALRPLIVDSWFPFDKMEYYWIVYVLQLFDLLIGALYVTYLHILMFNMYRYPVAQLKKLQHV
LRNFGRYKVEYMRQSNCNEYISALVVFRECIKKHKKIIQYVDGINECMSTYTVFDFLQSSFQIAALLVQTSPNDMTF
ISFLTVFTFITTVMIRLFVYYHSGNELIFESVNISMAIWESNWHEQSPQIKSMMLLVMRRAQKPLCYTIGGFGVMSL
QSVIAILKATYSYVSIIFRQ