Review

Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants

Jörg Degenhardt [a,*], Tobias G. Köllner [a], Jonathan Gershenzon [b]

[a] Martin Luther University Halle-Wittenberg, Institute for Pharmacy, Hoher Weg 8, D-06120 Halle/Saale, Germany
[b] Max Planck Institute for Chemical Ecology, Hans-Knöll Strasse 8, D-07745 Jena, Germany

ARTICLE INFO

ABSTRACT

The multitude of terpene carbon skeletons in plants is formed by enzymes known as terpene synthases. This review covers the monoterpene and sesquiterpene synthases presenting an up-to-date list of enzymes reported and evidence for their ability to form multiple products. The reaction mechanisms of these enzyme classes are described, and information on how terpene synthase proteins mediate catalysis is summarized. Correlations between specific amino acid motifs and terpene synthase function are described, including an analysis of the relationships between active site sequence and cyclization type and a discussion of whether specific protein features might facilitate multiple product formation.

© 2009 Elsevier Ltd. All rights reserved.

Contents

1. Introduction

Terpenes are not only the largest group of plant natural products, comprising at least 30,000 compounds (Connolly and Hill, 1991), but also contain the widest assortment of structural types. Hundreds of different monoterpene (C10) (Dewick, 1999), sesquiterpene (C15) (Fraga, 2006), diterpene (C20) (Hanson, 2000) and triterpene (C30) (Connolly and Hill, 2005) carbon skeletons are known. Natural products chemists have long marveled at the structural diversity of terpenes and speculated on its biosynthetic basis.

The wealth of terpene carbon skeletons can be attributed to an enzyme class known as the terpene synthases. These catalysts convert the acyclic prenyl diphosphates and squalene into a multitude of cyclic and acyclic forms. The chief causes of terpene diversity are the large number of different terpene synthases and the fact that some terpene synthases produce multiple products. This review discusses the role of terpene synthases in creating terpene diversity. We first present a current list of reported terpene synthases and describe the evidence for their ability to form multiple products. Next, we summarize current knowledge of the reaction

Table 1
Monoterpene synthase genes isolated to date.

Gene bank accession no.	Main product(s)[a] (%)	Designation[b]	Species	Cyclization type[c]	References
AAB71084	Myrcene (~100)	Ag2	_Abies grandis_	None	Bohlmann et al. (1997)
AAB71085	(−)-β-Pinene (58)	Ag3	_Abies grandis_	1–6, 2–7	Bohlmann et al. (1997)
AAB70707	(−)-Camphene (54)	Ag6	_Abies grandis_	1–6, 3–7, H	Bohlmann et al. (1999)
AAF61453	(−)-(4S)-β-Phellandrene (52)	Ag8	_Abies grandis_	1–6, H	Bohlmann et al. (1999)
AAF61454	Terpinolene (42)	Ag9	_Abies grandis_	1–6, H	Bohlmann et al. (1999)
AAB70907	(−)-(4S)-Limonene (~70)	Ag10	_Abies grandis_	1–6	Bohlmann et al. (1997)
AAF61455	(−)-(4S)-Limonene (35)	Ag11	_Abies grandis_	1–6	Bohlmann et al. (1999)
AAK83564	nd	Similar to Ag3	_Abies grandis_		Trapp and Croteau (2001)
AAK83565	nd	Similar to Ag10	_Abies grandis_		Trapp and Croteau (2001)
AAL17636	(+)-(4R)-Limonene (~100)	ArLMS	_Agastache rugosa_	1–6	Maruyama et al. (2002)
AAO41727	Myrcene (100)	Ama1e20	_Antirrhinum majus_	None	Dudareva et al. (2003)
AAO41726	Myrcene (100)	Ama0C15	_Antirrhinum majus_	None	Dudareva et al. (2003)
AAO42614	(E)-β-Ocimene (97)	Ama0a23	_Antirrhinum majus_	None	Dudareva et al. (2003)
ABR24418	(+)-(3S)-Linalool (100) #	AmNES/LIS-2	_Antirrhinum majus_	None, $	Nagegowda et al. (2008)
AAO85533	(+)-(3S)-Linalool (100)	At1g61680	_Arabidopsis thaliana_	None, $	Chen et al. (2003)
NP_189209	β-Myrcene/(E)-β-Ocimene	At3g25810	_Arabidopsis thaliana_	None	Chen et al. (2003)
AAN65379	(E)-β-Ocimene (94)	AtTPS03	_Arabidopsis thaliana_	None	Fäldt et al. (2003b)
AAG09310	Myrcene (56)	AtTPS10	_Arabidopsis thaliana_	None	Bohlmann et al. (2000)
AAU01970	1,8-Cineole (52)	AtTPS-Cin	_Arabidopsis thaliana_	1–6, 3-O-7, $	Chen et al. (2004)
AAF13357	(−)-(3R)-Linalool (100)	QH1	_Artemisia annua_	None, $	Jia et al. (1999)
AAF13356	(−)-(3R)-Linalool (100)	QH5	_Artemisia annua_	None, $	Jia et al. (1999)
AAK58723	(−)-β-Pinene (94)	QH6	_Artemisia annua_	1–6, 2–7	Lu et al. (2002)
ABI21837	(−)-(4S)-Limonene (~90)	CsTPS1	_Cannabis sativa_	1–6	Günnewich et al. (2007)
ABI21838	(+)-α-Pinene (~85)	CsTPS2	_Cannabis sativa_	1–6, 2–7	Günnewich et al. (2007)
CAD29734	Geraniol (100)	CtGES	_Cinnamomum tenuipilum_	None, $	Yang et al. (2005)
AAM53945	(−)-β-Pinene (81.4)	Cl(−)βPINS	_Citrus limon_	1–6, 2–7	Lucker et al. (2002)
AAM53944	(+)-(4R)-Limonene (99.1)	Cl(+)LIMS1	_Citrus limon_	1–6	Lucker et al. (2002)
AAM53946	(+)-(4R)-Limonene (99.1)	Cl(+)LIMS2	_Citrus limon_	1–6	Lucker et al. (2002)
AAM53943	γ-Terpinene (71.4)	ClγTS	_Citrus limon_	1–6, H	Lucker et al. (2002)
BAD91045	1,8-Cineole (>97.2)	CitMTSL1	_Citrus unshiu_	1–6, 3-O-7, $	Shimada et al. (2005a,b)
BAD91046	(E)-β-Ocimene (>97.2)	CitMTSL4	_Citrus unshiu_	None	Shimada et al. (2005a,b)
BAD27260	β-Pinene (82.4)	CitMTSL62	_Citrus unshiu_	1–6, 2–7	Shimada et al. (2004)
BAD27256	(+)-(4R)-Limonene (97)	CitMTSE1	_Citrus unshiu_	1–6	Shimada et al. (2004)
BAD27257	(+)-(4R)-Limonene (100)	CitMTSE2	_Citrus unshiu_	1–6	Shimada et al. (2005a)
BAD27258	γ-Terpinene (85.4)	CitMTSL3	_Citrus unshiu_	1–6, H	Shimada et al. (2004)
BAD27259	γ-Terpinene (78.4)	CitMTSL61	_Citrus unshiu_	1–6, H	Shimada et al. (2004)
AAC49395	(+)-(3S)-Linalool (~100)°	LIS	_Clarkia breweri_	None, $	Dudareva et al. (1996)
AAD19840	nd	LIS2	_Clarkia breweri_		Cseke et al., 1998
AAD19839	nd	LIS	_Clarkia concinna_		Cseke et al. (1998)
CAD57081	(+)-(3S)-Linalool (100) #	FaNES1	_Fragaria ananassa_	None, $	Aharoni et al. (2004)
CAD57106	(+)-(3S)-Linalool (100) #	FaNES2	_Fragaria ananassa_	None, $	Aharoni et al. (2004)
CAD57092	α-Pinene (~80)	FvPINS	_Fragaria vesca_	1–6, 2–7	Aharoni et al. (2004)
ABB73044	(+)-(4R)-Limonene (~39)	LaLIMS	_Lavandula angustivolia_	1–6	Landmann et al. (2007)
ABB73045	(−)-(3R)-Linalool (100)	LaLINS	_Lavandula angustivolia_	None, $	Landmann et al. (2007)
AAT86042	(E)-β-Ocimene (98)	LjEβOS	_Lotus japonicus_	–	Arimura et al. (2004)
AAX69063	(−)-(3R)-Linalool (100)	LeMTS1	_Lycopersicon esculentum_	None, $	van Schie et al. (2007)
AAX69064	β-Myrcene (~50)	LeMTS2	_Lycopersicon esculentum_	None	van Schie et al. (2007)
ACC66282	α-Terpineol (100)	Mg17	_Magnolia grandiflora_	1–6, $	Lee and Chappell (2008)
AAP40638	Inactive	–	_Melaleuca alternifolia_		Shelton et al. (2004)
AAL99381	(−)-(3R)-Linalool (96)	–	_Mentha citrata_	None, $	Crowell et al. (2002)
AAC37366	(−)-(4S)-Limonene (94)	LC5.2	_Mentha spicata_	1–6	Colby et al. (1993)
ABP88782	1,8-Cineole (~50)	CIN	_Nicotiana suaveolens_	1–6, 3-O-7, $	Roeder et al. (2007)
AAR11765	Geraniol (100)	GES	_Ocimum basilicum_	None, $	Iijima et al. (2004a)
AAV63789	(−)-(3R)-Linalool (100)	LIS	_Ocimum basilicum_	None, $	Iijima et al. (2004b)
AAV63792	Terpinolene (~50)	TES	_Ocimum basilicum_	1–6, H	Iijima et al. (2004b)
AAV63790	Fenchol (~50)	FES	_Ocimum basilicum_	1–6, 2–7, H, $	Iijima et al. (2004b)
AAV63791	Myrcene (100)	MYS	_Ocimum basilicum_	None	Iijima et al. (2004b)
EU596453	(+)-(3S)-Linalool (100)	Os02g02930	_Oryza sativa_	None, $	Yuan et al. (2008)
AAY88965	Geraniol (100)	PcTps-C	_Perilla citriodora_	None, $	Ito and Honda (2007)
AAF65545	nd	–	_Perilla citriodora_		Ito et al. (2000)
AAG31438	(−)-(4S)-Limonene (~100)	PFLC1	_Perilla frutescens_	1–6	Yuba et al. (1996)
AAF76186	Myrcene (~54)	PTS-5526	_Perilla frutescens_	None	Hosoi et al. (2004)
ABB30218	Geraniol (100)	PfTPS-PL	_Perilla frutescens_	None	Ito and Honda (2007)
ABY65110	(E)-β-Ocimene (98)	PlOS	_Phaseolus lunatus_	None	Arimura et al. (2008b)
AAO73863	(+)-3-Carene (78)	PaJF67	_Picea abies_	1–6, 5–7	Fäldt et al. (2003)
AAS47694	(−)-(4S)-Limonene (88)	PaTPS-Lim	_Picea abies_	1–6	Martin et al. (2004)
AAS47696	Myrcene (100)	PaTPS-Myr	_Picea abies_	None	Martin et al. (2004)
AAS47693	(−)-(3R)-Linalool (97)	PaTPS-Lin	_Picea abies_	None, $	Martin et al. (2004)
AAS47692	(−)-β-Pinene (57)	PaTPS-Pin	_Picea abies_	1–6, 2–7	Martin et al. (2004)
AAP72020	(−)-α-Pinene (62.5)	PsTPS2	_Picea sitchensis_	1–6, 2–7	McKay et al. (2003)
ABA86248	(−)-(4S)-Limonene (100)	PsTPS-Lim	_Picea sitchensis_	1–6	Byun-McKay et al. (2006)
ABA86247	nd	PsTPS-Linl	_Picea sitchensis_		Byun-McKay et al. (2006)
AAO61225	(−)-α-Pinene (79)	Pt1	_Pinus taeda_	1–6, 2–7	Phillips et al. (2003)
AAO61227	α-Terpineol (57.3)	Pt10	_Pinus taeda_	1–6, $	Phillips et al. (2003)
AAO61228	(+)-α-Pinene (97)	Pt30	_Pinus taeda_	1–6, 2–7	Phillips et al. (2003)
AAO61229	Inactive	Pt42	_Pinus taeda_		Phillips et al. (2003)

Table 1 (*continued*)

Gene bank accession no.	Main product(s)[a] (%)	Designation[b]	Species	Cyclization type[c]	References
AAX07267	(−)-α-Pinene (~40)	PmeTPS1	*Pseudotsuga menziesii*	1–6, 2–7	Huber et al. (2005)
AAX07264	Terpinolene (~40)	PmeTPS2	*Pseudotsuga menziesii*	1–6, H	Huber et al. (2005)
CAC41012	Myrcene (~99)	MyrS	*Quercus ilex*	None	Fischbach et al. (2001)
ABH07677	1,8-Cineole (72)	Sf-CinS1	*Salvia fruticosa*	1–6, 3-O-7, $	Kampranis et al. (2007)
AAC26018	(+)-Sabinene (63)	SSS	*Salvia officinalis*	1–6, 2–6, H	Wise et al. (1998)
AAC26017	(+)-Bornyl diphosphate (75)	SBS	*Salvia officinalis*	1–6, 3–7, §	Wise et al. (1998)
AAC26016	1,8-Cineole (79)	SCS	*Salvia officinalis*	1–6, 3-O-7, $	Wise et al. (1998)
ABH07678	Sabinene (~98)	Sp-SabS1	*Salvia pornifera*	1–6, 2–6, H	Kampranis et al. (2007)
AAM89254	(+)-3-Carene (73)	–	*Salvia stenophylla*	1–6, 5–7	Hoelscher et al. (2003)
ACF24767	α-Terpineol (~44)	SamonoTPS1	*Santalum album*	1–6, $	Jones et al. (2008)
AAG01140	(+)-(4R)-Limonene (~75)	dLMS	*Schizonepeta tenuifolia*	1–6	Maruyama et al. (2001a)
AAS79351	(−)-α-Terpineol (50.1)	VvTPS1891	*Vitis vinifera*	1–6, $	Martin and Bohlmann (2004)
AAS79352	(−)-α-Terpineol (50.1)	VvTPS4568	*Vitis vinifera*	1–6, $	Martin and Bohlmann (2004)
AAL59230	(−)-α-Terpineol (~40)	STC1-B73	*Zea mays*	1–6, $	Lin et al. (2008)
ABR09292	(−)-α-Terpineol (~60)	TPS26-B73	*Zea mays*	1–6, $	Lin et al. (2008)

The symbols indicate the following steps in the reaction mechanism: $, carbocation capture by water; §, phosphorylation of carbocation; H, hydride shift; #, FaNES1, FaNES2, and AmNES/LIS2 also produce the sesquiterpene nerolidol; nd, the enzymes were not expressed and characterized; °, the enzyme was not expressed, but the purified protein was characterized by Pichersky et al. (1995).

[a] The percentage refers to the approximate amount of major product in the total blend of terpenes produced by the enzyme.
[b] The designation refers to the name in the original publication. These names have occasionally been changed in later publications.
[c] The numbers refer to the carbon atoms involved in ring formation using the nomenclature of the geranyl skeleton (Fig. 1).

mechanism of these enzymes. Later sections are devoted to the terpene synthase proteins and their role in mediating terpene skeleton formation.

To keep this review to a manageable size, we limit our coverage to monoterpene and sesquiterpene synthases. Triterpene synthases and the formation of the triterpene carbon skeleton have been very authoritatively reviewed in several recent articles (Xu et al., 2004; Phillips et al., 2006). Monoterpene and sesquiterpene biosynthesis in general were covered in comprehensive fashion in two outstanding chapters written in l999 (Wise and Croteau, 1999; Cane, 1999) and terpene synthases in three reviews (Davis and Croteau, 2000; Tholl, 2006; Christianson, 2006).

2. Monoterpene and sesquiterpene synthases in plants

The wide scope of plant terpene synthase activities was first revealed by studies with crude plant extracts and purified preparation of native enzymes (reviewed in Wise and Croteau, 1999). More recently the isolation of terpene synthase genes and their heterologous expression has provided the best evidence for the size of this enzyme family and the breadth of its catalytic prowess. A comprehensive list of all plant monoterpene and sesquiterpene synthases cloned up to and including 2008 is given in Tables 1 and 2, ordered by the genus of the taxon from which the gene was isolated. Also given are the major products of the enzyme and notes on the reaction type. Until now research has focused on a few species chosen because of their economic importance or designation as model taxa. Obviously many more terpene synthases remain to be described. The size of this enzyme class is a principal reason for terpene diversity.

Terpene skeletal diversity arises not only from the number of terpene synthases, but also from the ability of these catalysts to form multiple products from a single substrate. In addition to their main product, nearly half of all characterized monoterpene and sesquiterpene synthases also form significant amounts of additional products (defined as at least 10% of the total) when the expressed protein is assayed *in vitro* (Tables 1 and 2). This property was first recognized during investigation of terpene synthases in plant extracts when it was realized that individual activities persisted in producing multiple products in the same consistent proportions during sequential purification steps (Gambliel and Croteau, 1984). As confirmation of these observations, Croteau and co-workers demonstrated that their preparations exhibited

isotopically sensitive branching when offered certain deuterium-labeled substrates (Croteau, 1987; Wagschal et al., 1991). A reduction in the rate of formation of one or more products due to deuterium substitution was correlated with an increase in the rate of formation of others, indicating that these enzyme products are derived from a common intermediate of a single enzyme (rather than different enzymes). The most definitive proof for the capacity of a single terpene synthase to produce multiple products comes from studies with terpene synthase genes. Expression in heterologous hosts has made it clear that a single terpene synthase protein has the ability to form several terpenes from a single substrate. For example, one of the first cloned monoterpene synthases, (+)-sabinene synthase from *Salvia officinalis*, produces 63% (+)-sabinene, but also 21% γ-terpinene, 7.0% terpinolene, 6.5% limonene and 2.5% myrcene in *in vitro* assays (Wise et al., 1998). These additional products or their immediate metabolites are also present in the monoterpene-rich essential oil of the plant.

3. Reaction mechanisms

3.1. Monoterpene synthases

Research over many years has established a common carbocationic reaction mechanism for all monoterpene synthases initiated by the divalent metal ion-dependent ionization of the substrate (Fig. 1A and B). The resulting cationic intermediate undergoes a series of cyclizations, hydride shifts or other rearrangements until the reaction is terminated by proton loss or the addition of a nucleophile. This mechanism was elucidated largely by Croteau and co-workers by studies with substrate analogs, inhibitors, intermediates and analogs and native enzymes (Croteau, 1987; Wise and Croteau, 1999). For example, the participation of carbocationic intermediates was established by the inhibitory effect of fluorinated (Croteau, 1986) and sulfonium (Croteau et al., 1986) substrate analogs coupled with previous investigations of model chemical reactions.

Of the characterized monoterpene synthases in Table 1, approximately one-third convert the substrate geranyl diphosphate (GPP) to acyclic products (Fig. 1B). The mechanism of these reactions proceeds by ionization to the extended geranyl cation followed by proton loss to form (E)-β-ocimene (Dudareva et al., 2003; Chen et al., 2003; Fäldt et al., 2003a; Shimada et al., 2005a; Arimura et al., 2004) and myrcene (Dudareva et al., 2003; Chen et al.,

Table 2
Sesquiterpene synthase genes isolated to date.

Gene bank accession no.	Main product(s)[a] (%)	Designation[b]	Species	Cyclization type[c]	References
AAC24192	(E)-α-Bisabolene (~100)	Ag1	*Abies grandis*	1–6	Bohlmann et al. (1998)
AAC05727	δ-Selinene (~25)°	Ag4	*Abies grandis*	1–10, 2–7, H, P	Steele et al. (1998)
AAC05728	γ-Humulene (~29)	Ag5	*Abies grandis*	1–11	Steele et al. (1998)
AAK83562	nd	(similar to Ag1)	*Abies grandis*		Trapp and Croteau (2001)
AAK83561	nd	(similar to Ag4)	*Abies grandis*		Trapp and Croteau (2001)
ABR24417	(3S)-(E)-Nerolidol (100)	AmNES/LIS-1	*Antirrhinum majus*	None, $	Nagegowda et al. (2008)
AAO85539	(−)-(E)-β-Caryophyllene (~80)	At5g23960	*Arabidopsis thaliana*	1–11, 2–10	Chen et al. (2003)
AAX59990	(+)-α-Barbatene (~27)	At5g44630	*Arabidopsis thaliana*	1–6, 7–11, 2–11,∗, H	Tholl et al. (2005) Wu et al. (2005)
NP_193064	(Z)-γ-Bisabolene (~90)	At4g13280	*Arabidopsis thaliana*	1–6	Ro et al. (2006)
NP_193066	(Z)-γ-Bisabolene (~90)	At4g13300	*Arabidopsis thaliana*	1–6	Ro et al. (2006)
CAB94691	Amorpha-4,11-diene	KCS12	*Artemisia annua*	1–10, 1–6, H	Chang et al. (2000)
AAF61439	Amorpha-4,11-diene (~89)	NAC	*Artemisia annua*	1–10, 1–6, H	Mercke et al. (2000)
AAF98444	Amorpha-4,11-diene (~89)	–	*Artemisia annua*	1–10, 1–6, H	Wallaart et al. (2001)
AAL79181	(E)-β-Caryophyllene	QHS1	*Artemisia annua*	1–11, 2–10	Cai et al. (2002)
CAC12732	nd	ASC34	*Artemisia annua*		van Geldre et al. (2000)
CAC12731	nd	ASC125	*Artemisia annua*		van Geldre et al. (2000)
AAF80333	8-epi-Cedrol (~94)	–	*Artemisia annua*	1–6, 6–10, 2–11, $, H	Hua and Matsuda (1999)
CAC08805	8-epi-Cedrol (~94)^	–	*Artemisia annua*	1–6, 6–10, 2–11, $, H	Mercke et al. (1999)
AAX39387	(E)-β-Farnesene (100)	β-FS	*Artemisia annua*	None	Picaud et al. (2005)
ABE03980	Germacrene A (100)	AaGAS	*Artemisia annua*	1–10	Bertea et al. (2006)
CAA06614	nd	PEAS1	*Capsicum annuum*		Zavala-Paramo et al. (2000)
AAC61260	5-epi-Aristolochene	PEAS	*Capsicum annuum*	1–10, 2–7, ∗, H, P	Back et al. (1998)
AAF21053	Inactive	CASC2	*Capsicum annuum*		Back et al. (2000)
AAM21658	(+)-Germacrene A (~100)	CiGASlo	*Cichorium intybus*	1–10	Bouwmeester et al. (2002)
AAM21659	(+)-Germacrene A (~100)	CiGASsh	*Cichorium intybus*	1–10	Bouwmeester et al. (2002)
AAK54279	(E)-β-Farnesene (~100)	CjFS	*Citrus junos*	None	Maruyama et al. (2001a)
AAQ04608	Valencene (~100)	CsTPS1	*Citrus sinensis*	1–10, 2–7, ∗, H, P	Sharon-Asa et al. (2003)
ABX83200	δ-Cadinene (~70)	CmTPSNY	*Cucumis melo*	1–10, 1–6, H	Portnoy et al. (2008)
ABX83201	α-Farnesene (100)	CmTPSDul	*Cucumis melo*	None	Portnoy et al. (2008)
AAU05951	(E,E)-α-Farnesene (~100)	CsαFS	*Cucumis sativus*	None	Mercke et al. (2004)
AAU05952	(E)-β-Caryophyllene (~100)	CsβCS	*Cucumis sativus*	1–11, 2–10	Mercke et al. (2004)
AAC31570	nd	–	*Elaeis oleifera*		Shah and Cha (2000)
AAA93064	(+)-δ-Cadinene (~100)	CAD1-C1	*Gossypium arboreum*	1–10, 1–6, H	Chen et al. (1995)
CAA76223	nd	CAD1-C2	*Gossypium arboreum*		Meng et al. (1999)
AAD51718	nd	CAD1-C3	*Gossypium arboreum*		Tan et al. (2000)
AAA93065	(+)-δ-Cadinene (~100)	CAD1-C14	*Gossypium arboreum*	1–10, 1–6, H	Chen et al. (1995)
CAA65289	(+)-δ-Cadinene (~100)	CAD1-A	*Gossypium arboreum*	1–10, 1–6, H	Chen et al. (1996)
AAC12784	(+)-δ-Cadinene	CDN1-C	*Gossypium hirsutum*	1–10, 1–6, H	Davis et al. (1996)
AAF74977	(+)-δ-Cadinene	CDN1-C4	*Gossypium hirsutum*	1–10, 1–6, H	Townsend et al. (2005)
AAA86337	Vetispiradiene (>93)	VS1	*Hyoscyamus muticus*	1–10, 2–7, ∗∗, P	Back and Chappell (1995)
AAL92481	Germacrene A	IdGAS	*Ixeris dentata*	1–10	Kim et al. (2005)
AAM11626	Germacrene A	LTC1	*Lactuca sativa*	1–10	Bennett et al. (2002)
AAM11627	Germacrene A	LTC2	*Lactuca sativa*	1–10	Bennett et al. (2002)
ABB73046	(E)-α-Bergamotene (~74)	LaBERS	*Lavandula angustivolia*	1–6, 2–7	Landmann et al. (2007)
AAG41889	δ-Elemene (~50) #	SSTLE1	*Lycopersicon esculentum*	1–10, H	van der Hoeven et al. (2000)
AAG41890	δ-Elemene (~75) #	SSTLE2	*Lycopersicon esculentum*	1–10, H	van der Hoeven et al. (2000)
AAC39432	Germacrene C (64)	–	*Lycopersicon esculentum*	1–10, H	Colby et al. (1998)
AAG41891	Germacrene B (~100)	SSTLH1	*Lycopersicon hirsutum*	1–10	van der Hoeven et al. (2000)
AAG41892	Germacrene D (~70)	SSTLH2	*Lycopersicon hirsutum*	1–10, H	van der Hoeven et al. (2000)
ACC66281	β-Cubebene (~24)	Mg25	*Magnolia grandiflora*	1–10, 1–6, 6–2, H	Lee and Chappell (2008)
	Inactive	Mg11	*Magnolia grandiflora*		Lee and Chappell (2008)
AAO22848	(E,E)-α-Farnesene (~98)	AFS1	*Malus x domestica*	None	Pechous and Whitaker (2004)
AAV36464	(E)-β-Caryophyllene (~92)	MtTPS1	*Medicago truncatula*	1–11, 2–10	Arimura et al. (2008a)
AAV36466	(3S)-(E)-Nerolidol (100)	MtTPS3	*Medicago truncatula*	None, $	Arimura et al. (2008a)
ABB01625	(−)-Cubebol (30)	MtTPS5	*Medicago truncatula*	1–10, 1–6, 2–6, H, $	Arimura et al. (2008a)
AAB95209	(E)-β-Farnesene (~85)	TSPA11	*Mentha x piperita*	None	Crock et al. (1997)
CAH10288	(Z)-Muurola-3,5-diene (45)	MxpSS1	*Mentha x piperita*	1–10, 1–6, H	Prosser et al. (2006)
CAH10289	Inactive	MxpSS2	*Mentha x piperita*		Prosser et al. (2006)
AAA19216	5-epi-Aristolochene (~79)	EAS3/EAS4	*Nicotiana tabacum*	1–10, 2–7, ∗, H, P	Facchini and Chappell (1992) Back et al. (1994) O'Maille et al. (2006)
AAP79448	5-epi-Aristolochene (100)	g110	*Nicotiana tabacum*	1–10, 2–7, ∗, H, P	Wu et al. (2005)
AAP05760	5-epi-Aristolochene (~100)	NaEAS12	*Nicotiana attenuata*	1–10, 2–7, ∗, H, P	Bohlmann et al. (2002)
AAP05761	5-epi-Aristolochene (~100)	NaEAS34	*Nicotiana attenuata*	1–10, 2–7, ∗, H, P	Bohlmann et al. (2002)
AAP05762	5-epi-Aristolochene (~100)	NaEAS37	*Nicotiana attenuata*	1–10, 2–7, ∗, H, P	Bohlmann et al. (2002)
AAV63787	γ-Cadinene (~30)	CDS	*Ocimum basilicum*	1–6, 1–10, H	Iijima et al. (2004a)
AAV63785	β-Selinene (~30)	SES	*Ocimum basilicum*	1–10, 2–7, P	Iijima et al. (2004a)
AAV63788	α-Zingiberene (~40)	ZIS	*Ocimum basilicum*	1–6, H	Iijima et al. (2004a)
AAV63786	Germacrene D (100)	GDS	*Ocimum basilicum*	1–10	Iijima et al. (2004a)
EU596452	Zingiberene (~25)	Os08g07100	*Oryza sativa*	1–6, H	Yuan et al. (2008)
EU596454	(E)-β-Caryophyllene (~47)	Os08g04500	*Oryza sativa*	1–11, 2–10	Yuan et al. (2008)
ABJ16553	(E)-β-Caryophyllene (~46)	OsTPS3	*Oryza sativa*	1–11, 2–10	Cheng et al. (2007)
ABJ16554	(E,E)-Farnesol (~84)	OsTPS13	*Oryza sativa*	None, $	Cheng et al. (2007)

Table 2 (*continued*)

Gene bank accession no.	Main product(s)[a] (%)	Designation[b]	Species	Cyclization type[c]	References
AAS47695	Longifolene (61)	PaTPS-Lon	*Picea abies*	1–11, 1–6, 3–7, **, H	Martin et al. (2004)
AAS47697	(E,E)-α-Farnesene (100)	PaTPS-Far	*Picea abies*	None	Martin et al. (2004)
AAS47689	(E)-α-Bisabolene (100)	PaTPS-Bis	*Picea abies*	1–6	Martin et al. (2004)
ABA86249	nd	PsTPS-Sell	*Picea sitchensis*		Byun-McKay et al. (2006)
AAO61226	(E,E)-α-Farnesene (~100)	Pt5	*Pinus taeda*	None	Phillips et al. (2003)
AAS86319	γ-Curcumene (~90)	PatTpsA	*Pogostemon cablin*	1–6, H	Deguerry et al. (2006)
AAS86320	(−)-Germacrene D (100)	PatTpsBF2	*Pogostemon cablin*	1–10, H	Deguerry et al. (2006)
AAS86321	(+)-Germacrene A (~90)	PatTpsCF2	*Pogostemon cablin*	1–10	Deguerry et al. (2006)
AAS86322	(−)-Germacrene D (~60)	PatTpsB15	*Pogostemon cablin*	1–10, H	Deguerry et al. (2006)
AAS86323	(−)-Patchoulol (~50)	PatTPS177	*Pogostemon cablin*	1–10, 7–11, 2–6, **, $, H	Deguerry et al. (2006)
AAR99061	(−)-Germacrene D (~79)	PtdTPS1	*Populus trichocarpa x deltoides*	1–10, H	Arimura et al. (2004)
AAX07266	(E)-γ-Bisabolene (100)	PmeTPS3	*Pseudotsuga menziesii*	1,6	Huber et al. (2005)
AAX07265	(E)-α-Farnesene (100)	PmeTPS4	*Pseudotsuga menziesii*	None	Huber et al. (2005)
–	Germacrene D (~100)	FC0592	*Rosa hybrida*	1–10, H	Guterman et al. (2002)
ACF24768	Germacrene D-4-ol (~39)	SasesquiTPS1	*Santalum album*	1–10, H, $	Jones et al. (2008)
BAA82109	nd	PVS3	*Solanum tuberosum*		Yoshioka et al. (1999)
BAA82108	nd	PVS2	*Solanum tuberosum*		Yoshioka et al. (1999)
BAA82029	nd	PVS1	*Solanum tuberosum*		Yoshioka et al. (1999)
AAR31144	(+)-Germacrene D (~90)	(+)GDS, Sc11	*Solidago canadensis*	1–10, H	Prosser et al. (2004)
AAR31145	(−)-Germacrene D (~90)	(−)GDS, Sc19	*Solidago canadensis*	1–10, H	Prosser et al. (2004)
CAC36896	(+)-Germacrene A (~98)	Sc1	*Solidago canadensis*	1–10	Prosser et al. (2002)
AAS66358	(+)-Valencene (50)	VvVal	*Vitis vinifera*	1–10, 2–7, *	Lucker et al. (2004)
AAS66357	(−)-Germacrene D (92)	VvGerD	*Vitis vinifera*	1–10, H	Lucker et al. (2004)
AAO18435	(E,E)-Farnesol (~45)	TPS1-B73	*Zea mays*	None; $	Schnee et al. (2002)
AAS88571	(S)-β-Bisabolene (~29)	TPS4-B73	*Zea mays*	1–6, 2–6	Köllner et al. (2004)
AAS88572	Inactive	TPS5-B73	*Zea mays*	None	Köllner et al. (2004)
AAS88574	Sesquithujene (~28)	TPS5-Del1	*Zea mays*	1–6, 2–6, H	Köllner et al. (2004)
AAS88575	Inactive	TPS5-Del2	*Zea mays*		Köllner et al. (2004)
AAX99146	(E)-β-Farnesene (~50)	TPS10-B73	*Zea mays*	None	Schnee et al. (2006)
AAS88576	(−)-β-Macrocarpene (~95)	TPS6-B73	*Zea mays*	1–6, 11–14, P	Köllner et al. (2008a)
AAT70085	(−)-β-Macrocarpene (~95)	TPS6-Ban	*Zea mays*	1–6, 11–14, P	Köllner et al. (2008a)
ACF58240	(−)-β-Macrocarpene (~95)	TPS11-B73	*Zea mays*	1–6, 11–14, P	Köllner et al. (2008a)
ABY79206	(E)-β-Caryophyllene (~90)	TPS23-Del1	*Zea mays*	1–11, 2–10	Köllner et al. (2008b)
ABY79209	(E)-β-Caryophyllene (~90)	TPS23-dip	*Zea diploperennis*	1–11, 2–10	Köllner et al. (2008b)
ABY79210	(E)-β-Caryophyllene (~90)	TPS23-hue	*Zea m. huehuetenangensis*	1–11, 2–10	Köllner et al. (2008b)
ABY79211	(E)-β-Caryophyllene (~90)	TPS23-lux	*Zea luxurians*	1–11, 2–10	Köllner et al. (2008b)
ABY79212	(E)-β-Caryophyllene (~90)	TPS23-mex	*Zea mays mexicana*	1–11, 2–10	Köllner et al. (2008b)
ABY79213	(E)-β-Caryophyllene (~90)	TPS23-par	*Zea mays parviglumis*	1–11, 2–10	Köllner et al. (2008b)
ABY79214	(E)-β-Caryophyllene (~90)	TPS23-per	*Zea perennis*	1–11, 2–10	Köllner et al. (2008b)
AAX40665	(+)-Germacrene D (~50)	–	*Zingiber officinale*	1–10, H	Picaud et al. (2006)
BAG12020	α-Humulene (~95)	ZSS1	*Zingiber zerumbet*	1–11	Yu et al. (2008a)
BAG12021	β-Eudesmol (63)	ZSS2	*Zingiber zerumbet*	1–10, 2–7, H	Yu et al. (2008b)

The symbols indicate the following steps in the reaction mechanism: *, methyl shift; **, ring contraction; $, carbocation capture by water; H, hydride shift; P, protonation of a reaction intermediate. #, δ-elemene could have been formed from germacrene C in the GC injector at the 250 °C temperature used; °, the purified enzyme produced germacrene B (33%) as main product (Little and Croteau, 2002); nd, the enzymes were not expressed and characterized; ^, identical to AAF80333.

[a] The approximate percentage refers to the amount of the major product in the total blend of the terpenes produced by the enzyme.
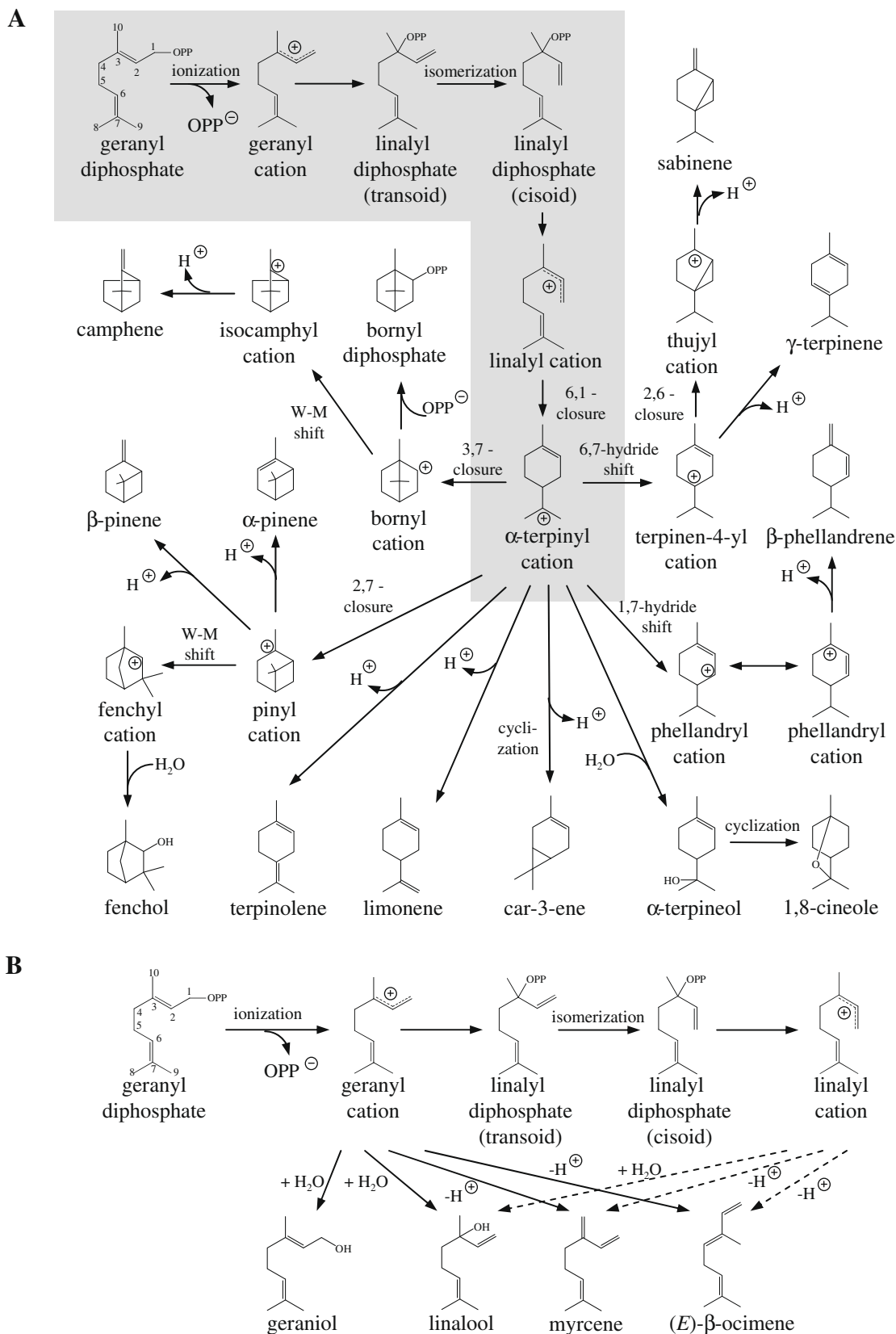
[b] The designation refers to the name in the original publication. These names have occasionally been changed in later publications.

[c] The numbers refer to the carbon atoms involved in ring formation using nomenclature of the farnesyl skeleton (Fig. 2).

2003; Fischbach et al., 2001; Martin et al., 2004; Hosoi et al., 2004; Iijima et al., 2004a; Bohlmann et al., 1997, 2000) or addition of water to form geraniol (Yang et al., 2005; Iijima et al., 2004b) or linalool (Chen et al., 2003; Martin et al., 2004; Iijima et al., 2004a; Jia et al., 1999; Dudareva et al., 1996; Aharoni et al., 2004; Crowell et al., 2002) (Fig. 1B). It is also conceivable that linalool, myrcene and (E)-β-ocimene are derived from the linalyl cation that is the result of a previous isomerization (Fig. 1B). The formation of cyclic products is impeded by the (E)-geometry of the 2,3-double bond of the geranyl cation. However, preliminary conversion of the geranyl cation to the tertiary linalyl cation facilitates cyclization to a six-membered ring. Studies with LPP as an alternative substrate in combination with GPP indicated the formation of a linalyl diphosphate (LPP) (Fig. 1A) as an intermediate in terpene synthase catalysis (Croteau et al., 1980). These observations were supported by investigations with tritium-labeled GPP (Croteau and Felton, 1981) and non-cyclizable GPP analogs (Wheeler and Croteau, 1986, 1987). The elucidation of the three-dimensional structure of *Mentha* limonene synthase cocrystalized with the substrate analog 2-fluorogeranyl diphosphate and 2-fluorolinalyl diphosphate provided further evidence for the

intermediacy of LPP in the isomerization reaction (Hyatt et al., 2007).

From the cisoid, anti-endo conformer of the linalyl cation, electrophilic attack of C1 on the C6–C7 double bond gives the cyclic α-terpinyl cation, a critical branchpoint intermediate in the formation of all cyclic monoterpenes. From the α-terpinyl cation, terpene synthase-catalyzed proton loss leads to limonene (Rajaonarivony et al., 1992; Martin et al., 2004; Bohlmann et al., 1997, 1999; Maruyama et al., 2001b, 2002; Lucker et al., 2002; Shimada et al., 2004, 2005b; Colby et al., 1993; Yuba et al., 1996; Byun-McKay et al., 2006), or terpinolene (Iijima et al., 2004a; Bohlmann et al., 1999; Huber et al., 2005) while water capture gives α-terpineol (Croteau et al., 1994; Martin and Bohlmann, 2004). Terpene synthase-mediated 1,2- or 1,3-hydride shifts of the α-terpinyl cation followed by proton losses yield the isomeric terpinene and phellandrene products (LaFever and Croteau, 1993; Shimada et al., 2004; Bohlmann et al., 1999). Formation of 1,8-cineole is also thought to proceed from the α-terpinyl cation via an α-terpineol intermediate which undergoes internal additional cyclization of the alcoholic oxygen (Croteau et al., 1994; Wise et al., 1998; Chen et al., 2004).
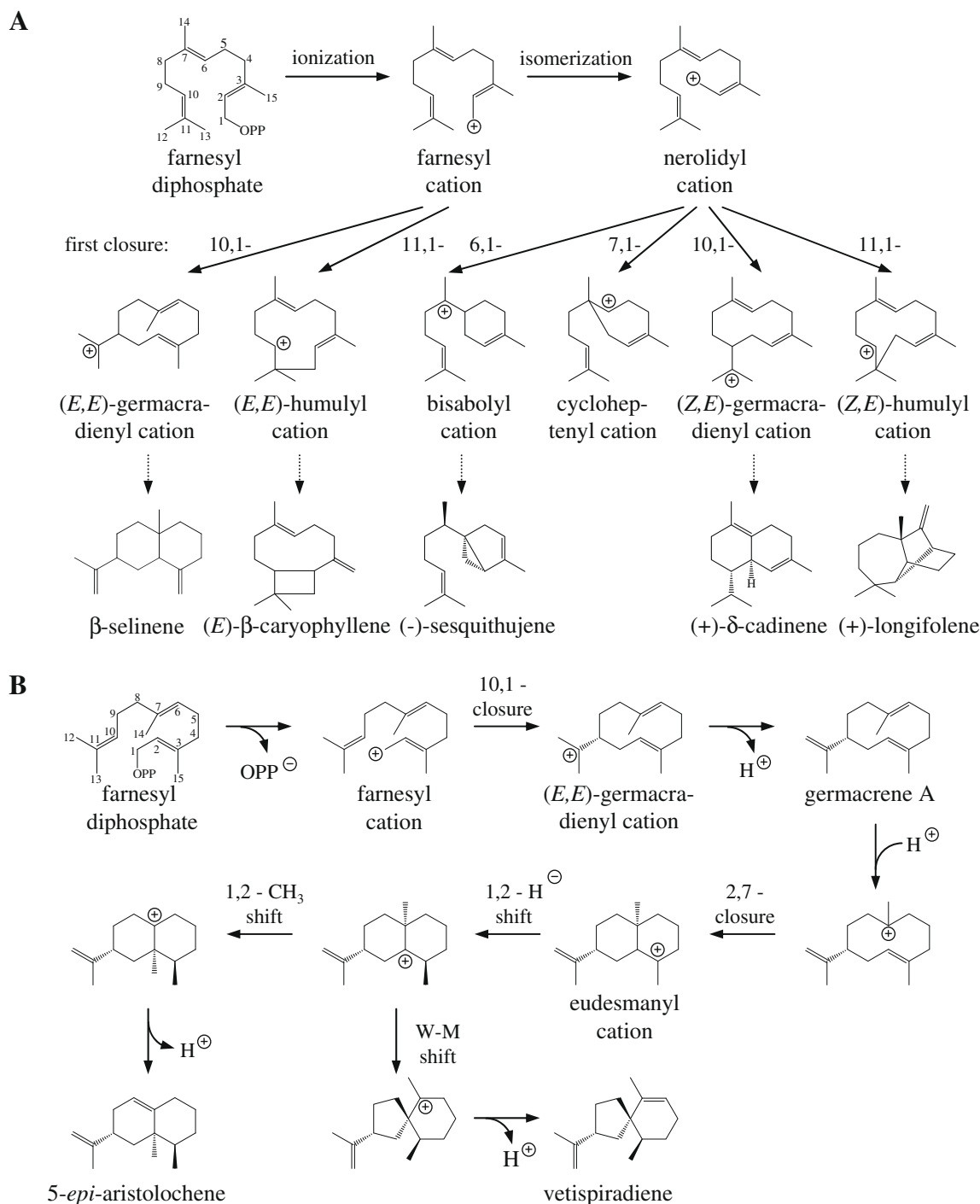
A

B

Fig. 1. The reaction mechanisms of all monoterpene synthases start with the ionization of the geranyl diphosphate substrate. The resulting carbocation can undergo a range of cyclizations, hydride shifts and rearrangements before reaction is terminated by deprotonation or water capture. The mechanisms of cyclic (A) and acyclic (B) monoterpene synthases are depicted separately. The formation of cyclic monoterpenes requires the preliminary isomerization of the geranyl cation to a linalyl intermediate capable of cyclization. The production of the initial cyclic species, the α-terpinyl cation, opens the door to secondary cyclizations. The route to the central α-terpinyl intermediate is highlighted in grey and the reaction pathways to the major products of all cloned monoterpene synthases are shown. The formation of the acyclic monoterpenes linalool, myrcene and (E)-β-ocimene might proceed either via the geranyl cation or via the linalyl cation. The numbering of carbon atoms of intermediates and products refers to that for GPP.

A

B

Fig. 2. The reaction mechanism of all sesquiterpene synthases start with the ionization of FPP. The resulting carbocation undergoes a range of cyclizations, some of which are preceded by isomerization to a nerolidyl intermediate (A). Steps following the initial cyclization, which include secondary cyclization, deprotonation to a neutral intermediate, hydride shifts, methyl shifts and Wagner–Meerwein rearrangements, are not shown except for the 5-*epi*-aristolochene synthase of *Nicotiana tabacum*. The reaction mechanism of this enzyme proceeds via a neutral intermediate, germacrene A, that is re-protonated to form the bicyclic reaction products (B). The numbering of carbon atoms of intermediates and products refers to that for FPP.

The α-terpinyl cation can also undergo additional cyclizations resulting from electrophilic attack of the carbocationic center on one of the carbon atoms of the remaining double bond. A Markovnikov addition (2,7-cyclization) generates the pinyl cation, which can undergo proton loss to yield α-pinene (Croteau et al., 1989; Aharoni et al., 2004; McKay et al., 2003) or β-pinene (Croteau et al., 1989; Martin et al., 2004; Bohlmann et al., 1997; Lucker et al., 2002; Shimada et al., 2004; Lu et al., 2002), the main prod-

ucts of many terpene synthases especially in conifers. Anti-Markovnikov addition (3,7-cyclization) generates the bornyl cation (Croteau et al., 1990). The major monoterpenes with a bornyl carbon skeleton, borneol and camphor, are formed via a bornyl diphosphate intermediate (Croteau and Karp, 1977). In this process, a terpene synthase catalyzes bornyl diphosphate formation from the bornyl cation via internal return of the diphosphate moiety from the original geranyl diphosphate substrate (Whittington

et al., 2002a). The product is then hydrolyzed and oxidized to borneol and camphor, respectively (Croteau and Karp, 1979; Dehal and Croteau, 1987). The formation of two other bicyclic monoterpene skeletons are thought to involve terpene synthase-mediated Wagner–Meerwein rearrangements forming the fenchyl skeleton (precursor of fenchol) from the pinyl cation (Croteau et al., 1989; Iijima et al., 2004a) and the isocamphyl skeleton (precursor of camphene) from the bornyl cation (Croteau et al., 1990; Bohlmann et al., 1999). Another cyclization from the α-terpinyl cation is 5,7-closure to form a cyclopropyl ring leading to 3-carene (Savage and Croteau, 1993; Hoelscher et al., 2003). Terpene synthases form bicyclic monoterpenes with a cyclopropyl ring via another route in which a 1,2-hydride shift of the α-terpinyl cation is followed by 2,6-closure to give the sabinyl cation, precursor of sabinene and related monoterpenes (Hallahan and Croteau, 1989; Wise et al., 1998).

The highly reticulate general reaction mechanism of monoterpene synthases suggests a rationale for the ability of these enzymes to make multiple products. Given that an individual intermediate may have multiple fates, the enzyme may simply exploit this property. For example, a terpene synthase could allow one carbocation to be converted to a mixture of others by facilitating various cyclizations, hydride shifts or rearrangements (Lucker et al., 2002; Shimada et al., 2004; Huber et al., 2005; Martin and Bohlmann, 2004). Or it could mediate different types of termination reactions (deprotonation, water capture) on a single cation (McKay et al., 2003; Peters and Croteau, 2003). In some cases, the minor products of the enzyme have fewer cyclizations than the main product, suggesting that the minor products are formed from reaction intermediates subjected to premature termination (Iijima et al., 2004a). Whatever the explanation for multiple product formation, the occurrence of a broad spectrum of products has often proved valuable in tracing the reaction mechanism.

3.2. Sesquiterpene synthases

The formation of sesquiterpenes from farnesyl diphosphate catalyzed by sesquiterpene synthases employs similar carbocationic-based reaction mechanisms as those of monoterpene synthases. However, the larger carbon skeleton of farnesyl diphosphate (FPP) and the presence of three, instead of two, double bonds greatly increases structural diversity of the products. Thus we have space only to describe the major steps of these mechanisms. As for monoterpene synthases, the basic principles of sesquiterpene synthase mechanisms were derived from studies with native enzymes, although microbial enzymes played a much larger role here than they did for monoterpene synthases. Since this review is dedicated to plant terpene synthases, only few of the thorough studies on the reaction mechanisms of fungal and bacterial terpene synthases can be mentioned here.

The initial cyclization reactions can be divided into two types (Fig. 2A and B). One type involves cyclization of the initially-formed farnesyl cation to yield 10-membered ((E,E)-germacradienyl cation) or 11-membered ((E)-humulyl cation) rings. Given the large size of these rings, the (E)-geometry of the C2–C3 double bond is no barrier to cyclization. The second type of cyclization proceeds after preliminary isomerization of the C2–C3 double bond to the tertiary nerolidyl cation, in direct analogy with the isomerization of GPP to the linalyl cation in the mechanism of the monoterpene synthases. Evidence for the intermediacy of the nerolidyl intermediate in the sesquiterpene synthase mechanism comes from detailed studies with multiply-labeled FPP and nerolidyl diphosphate substrates (Cane and Iyengar, 1979; Cane et al., 1981; Cane and Yang, 1994; Cane and Tandon, 1995; Alchanati et al., 1998). The cisoid conformer of the nerolidyl cation can undergo cyclization to either the central or distal double bond form-

ing 1,6-(bisabolyl cation), 1,7-(cycloheptanyl cation), 1,10-((Z,E)-germacradienyl cation) or 1,11-((Z)-humulyl cation) products.

Subsequent cyclizations can involve carbocationic attack on either of the two remaining double bonds giving a wide range of different carbon skeletons. With so many possibilities, it is often difficult to determine the sequence of ring closures. For example, δ-cadinene which has a naphthalene-type ring skeleton could arise from initial formation of a 10-membered ring followed by partitioning into two six-membered rings, or from sequential formation of six-membered rings. Characterization of a cotton δ-cadinene synthase suggests that 1,10-cyclization is the first step followed by 1,6-closure to give ring partitioning (Chen et al., 1995; Alchanati et al., 1998; Benedict et al., 2001). However, the sequence of cyclizations may differ for sesquiterpene synthases forming other naphthalene-type skeletons. Another example of ring closure sequence is that catalyzed by 2-epi-cedrol synthases from Artemisia annua in which initial 1,6-ring formation is followed by 6,10- and then 2,11-closure (Hua and Matsuda, 1999; Mercke et al., 1999). In addition to cyclization, sesquiterpene synthase mechanisms include a range of different reaction types similar to that found in monoterpene synthase mechanisms, including 1,2-, 1,3- and 1,4-hydride shifts. Skeletal rearrangements observed include ring contractions (Martin et al., 2004; Back and Chappell, 1995) and other Wagner–Meerwein rearrangements as well as 1,2-methyl shifts (Tholl et al., 2005).

Unique to the formation of certain sesquiterpenes is the intermediacy of neutral sesquiterpene species. For example, the mechanism of 5-epi-aristolochene synthase proceeds via an initial 1,10-cyclization forming the (E,E)-germacradienyl cation to germacrene A. This neutral olefin is then protonated on the C6–C7 double bond in Markovnikov orientation to reform a cation, which then undergoes 2,7-cyclization to form a eudesmane cation (Rising et al., 2000). An analogous protonation of a neutral germacrene intermediate is part of the mechanistic sequence of vetispiradiene synthase (Back and Chappell, 1995), valencene synthase (Sharon-Asa et al., 2003) and β-selinene synthase (Iijima et al., 2004a). A different neutral intermediate, (S)-β-bisabolene, is involved in the biosynthesis of (S)-β-macrocarpene by the maize terpene synthases TPS6 and TPS11. In both enzymes, the proton required for the reprotonation of the (S)-β-bisabolene intermediate is abstracted from water (Köllner et al., 2008b).

4. Structure–function correlations in terpene synthases

Despite our detailed knowledge of monoterpene and sesquiterpene synthase mechanisms, comparatively little information is available about how various structural features of the proteins themselves mediate substrate binding and catalysis. Even though there is a substantial degree of amino acid sequence similarity among plant monoterpene and sesquiterpene synthases, similarity is based more on taxonomic affinities of the plant species from which the gene was isolated rather than the type of products formed (Bohlmann et al., 1998b). Nevertheless a number of common structural elements has been recognized.

4.1. General sequence and domains

In considering the entire protein, plant monoterpene synthases with 600–650 amino acids are longer than sesquiterpene synthases (550–580 amino acids) due to an N-terminal signal peptide that targets the initial translation product towards the plastids (Turner et al., 1999). These signal peptides characteristically contain a high frequency of serine and threonine residues and low amounts of acidic amino acids, but no common sequence elements have been identified (Bohlmann et al., 1997). Analysis of the

three-dimensional structures of four plant terpene synthases, a sesquiterpene synthase from *Nicotiana tabacum* (Starks et al., 1997) and three monoterpene synthases, from *Salvia officinalis* (Whittington et al., 2002b), *Mentha spicata* (Hyatt et al., 2007) and *Salvia fruticosa* (Kampranis et al., 2007), respectively, shows extensive similarities despite differences in reaction mechanism. These proteins all have a tertiary structure consisting entirely of α-helices and short connecting loops and turns and are organized into two structural domains, of which the C-terminal domain contains the active site. This domain possesses a structure similar to those of fungal and bacterial terpene synthases (Caruthers et al., 2000; Rynkiewicz et al., 2001; Shishova et al., 2007; Lesburg et al., 1997; Christianson, 2006) despite the lack of sequence similarity, suggesting the existence of a general 'terpene synthase fold' (Lesburg et al., 1997). The enzyme active site is a hydrophobic pocket of the C-terminal domain that is formed by six α-helices and closed off towards the outside by two loops which are located on the protein surface. Substrate binding in the active site of tobacco 5-*epi*-aristolochene synthase (TEAS), as determined by co-crystallization with the non-hydrolyzable FPP analogs, farnesyl hydroxyphosphonate and trifluorofarnesyl diphosphate, demonstrated that the hydrophobic moiety of FPP reaches into the active cavity while the diphosphate function interacts with magnesium ions at the entrance of the active site.

The N-terminal domain of plant terpene synthases has structural similarity with some glycosylhydrolases (Dudareva et al., 1996) but few functional elements have been identified. Evidence from mutational analyses indicates that this domain acts as a scaffold facilitating proper folding of the catalytically active C-terminal domain (Köllner et al., 2004). Experiments in which domains were exchanged between the sesquiterpene synthases TEAS and vetispiradiene synthase from *Hyoscyamus muticus* demonstrated that vetispiradiene synthase was fully functional with the N-terminal domain of TEAS, but the reverse combination resulted in a hybrid with strongly reduced activity (Back and Chappell, 1996). An unusual element of approximately 210 amino acid residues in the N-terminal domain appears in a sesquiterpene synthase, the (*E*)-α-bisabolene synthase from *Abies grandis* (Bohlmann et al., 1998a), a monoterpene synthase, the (*S*)-linalool synthase of *Clarkia breweri* (Dudareva et al., 1996), and in most isolated diterpene synthases (Bohlmann et al., 1998b). No function has yet been described for this element, although it may constitute an ancestral feature of plant terpene synthases that points to the common origin of this enzyme class (Bohlmann et al., 1998b).

The best known structural motif of the terpene synthase family is an aspartate-rich region, DDxxD, found in virtually all isolated plant terpene synthases as well as in isoprenyl diphosphate synthases and microbial terpene synthases. Site-directed mutagenesis as well as X-ray structural analysis revealed that this region is involved in binding divalent metal ions which in turn interact with the diphosphate moiety of the substrate (Starks et al., 1997; Lesburg et al., 1997; Tarshis et al., 1994, 1996; Cane et al., 1996a,b). The location of the DDxxD motif at the entrance of the catalytic site appears to be critical in positioning the substrate for catalysis. Mutations in this region frequently lead to decreased catalytic activity and the appearance of abnormal products which can be attributed to altered substrate binding (Cane et al., 1996a,b; Rynkiewicz et al., 2002; Seemann et al., 2002; Little and Croteau, 2002; Prosser et al., 2004). However, a naturally occuring variant of the DDxxD motif, a NDxxD sequence in the fully active (+) germacrene D synthase from goldenrod, has no impact on catalytic activity demonstrating that the highly conserved DDxxD motif is not as necessary for catalytic activity in farnesyl diphosphate cyclization as previously assumed (Prosser et al., 2004). X-ray structural analysis of terpene synthases revealed an additional metal cofactor binding motif located on the opposite site of the active site entry

(Christianson, 2006). This motif, designated NSE/DTE motif, has apparently evolved from a second aspartate-rich motif conserved in prenyl transferases to form a consensus sequence of (L,V)(V,L,A)-(N,D)D(L,I,V)x(S,T)xxxE (Cane and Kang, 2000; Christianson, 2006). Both the DDxxD motif and the NSE/DTE motif bind a trinuclear magnesium cluster involved in fixation of the pyrophosphate substrate. Whereas the DDxxD motif is highly conserved throughout almost all plant terpene synthases, the NSE/DTE motif appears to be less well conserved. Recently, mutational investigation of the NSE/DTE motif in abietadiene synthase from *Abies grandis* led to the speculation that a water molecule could substitute for the hydroxyl side chain of the central serine/threonine in terpene synthases when a glycine is located at this position (Zhou and Peters, 2009). In some sesquiterpene synthases, the NSE/DTE motif is replaced by a second DDxxD motif (Steele et al., 1998) which was also shown to be involved in catalysis (Little and Croteau, 2002).

Several terpene synthases from gymnosperms (Bohlmann et al., 1998b) as well as an apple α-farnesene synthase (Green et al., 2007) require potassium for enzyme activity. Recently, the H-α1 loop, which is located in direct proximity to the NSE/DTE motif, was identified as the potassium binding region in these enzymes. It is assumed that potassium ions stabilize this loop region for optimal substrate binding (Green et al., 2009).

About 35 amino acids upstream of the DDxxD motif is a highly conserved RxR motif that was implicated in the complexation of the diphosphate function after ionization of the substrate preventing nucleophilic attack on any of the carbocationic intermediates (Starks et al., 1997).

A second arginine-rich motif found approximately 60 residues from the N-terminus of many monoterpene synthases is a tandem arginine (RR). Deletion studies on the limonene synthase of *Mentha spicata* demonstrated that all amino acids N-terminal to this point were dispensable for enzyme activity (Williams et al., 1998). However, deletion of the tandem arginine motif rendered the limonene synthase unable to accept geranyl diphosphate as a substrate. Since the enzyme was still able to convert linalyl diphosphate to limonene, this suggested that the RR motif might participate in the isomerization of GPP to a cyclizable intermediate, such as the linalyl cation (Williams et al., 1998). In keeping with this suggestion, the RR motif can be absent in monoterpene synthases producing only acyclic compounds, which do not require isomerization.

Additional amino acids have been implicated in the catalytic function of specific terpene synthases. For example, in TEAS the formation of 5-*epi*-aristolochene proceeds via the enzyme-bound, neutral intermediate, germacrene A (Cane, 1990). The protonation of this intermediate at C6 is catalyzed by a triad of amino acids consisting of Y520, D444 and D52565 as demonstrated by mutational analysis of the tyrosine residue (Rising et al., 2000). The N-terminus of the enzyme folds over the entrance of the active site after substrate binding and so might contribute to shielding the active site from the outer aqueous medium (Starks et al., 1997). However, N-terminal deletions of TEAS65 or the limonene synthase from *M. spicata* (Davis and Croteau, 2000) did not result in an increased proportion of hydroxylated products as might be expected after increased exposure of carbocationic intermediates to water.

4.2. Other structure–function correlations

A variety of structure–function correlations have been reported for other terpene synthases. In a monoterpene synthase, (−)-α-pinene synthase from *A. grandis*, the amino acids serine 485 and cysteine 480 were implicated as terminal proton acceptors in the final deprotonation of the pinyl cation to form α- and β-pinene, while cysteine 372 and phenylalanine 579 were found to influence the ratio of α- to β-pinene (Hyatt and Croteau, 2005). Domain

swapping experiments indicated that the product specificity of this α-pinene synthase and a related (−)-limonene synthase from *A. grandis* are determined by amino acids in the helices D–F which contribute to the active site cavity (Katoh et al., 2004). A similar region was found to be catalytically important in domain swapping experiments with monoterpene synthases from *Citrus limon* (El Tamer et al., 2003). Differences in termination chemistry between a 1,8-cineole synthase and sabinene synthase from *S. officinalis* were traced to a 73 amino acid region including helices C and D of the C-terminal domains (Peters and Croteau, 2003). Structural elucidation of a 1,8-cineole synthase from *S. fruticosa* identified five amino acid residues involved in the final step of the reaction, the addition of water to an α-terpinyl intermediate. Conversion of these five residues to those found in sabinene synthases of the closely related species *S. officinalis* and *Salvia pomifera* abolished the formation of 1,8-cineole in favor of sabinene (Kampranis et al., 2007).

Among sesquiterpene synthases, a mutational analysis of a germacrene A synthase from *Ixeris dentata* which has a reaction mechanism similar to the early steps of the TEAS mechanism revealed that a threonine in position 401 is important for intermediate stabilization and the formation of the eudesmane skeleton (Chang et al., 2005). Comparison of two closely related sesquiterpene synthases in maize, TPS4 and TPS5, identified a four amino acid motif that determines the stereospecificity of these multi-product enzymes (Köllner et al., 2004). The motif is located on helix G which divides the active site into two pockets with different catalytic functions (Köllner et al., 2006). In a δ-cadinene synthase from cotton, the alteration of two amino acids in the active site affected the cyclization and termination reactions resulting in the formation of a germacrene alcohol as main product (Yoshikuni et al., 2006a).

Sesquiterpene synthases have also been the subject of several large-scale structure–function studies. For example, saturating mutagenesis of four amino acid residues in the active center of γ-humulene synthase from *A. grandis* (Yoshikuni et al., 2006b) showed that the effect of mutations in these residues was additive. Seven enzymes were engineered with different combinations of mutations each of which produced a different terpene blend. Another systematic approach to identify the residues responsible for terpene synthase product specificity compared the closely related sesquiterpene synthases, TEAS from *N. tabacum* and vetispiradiene synthase from *H. muticus*. A mutational analysis of amino acid differences with different distances from the active center demonstrated that the nine amino acid residues most important in determining product specificity include residues lining the active site, and also those forming points of contact with the surrounding alpha helices. These latter residues seem more likely to function by altering the shape of the active site cavity rather than by providing functional groups for catalysis (Greenhagen et al., 2006). Study of a library of all possible combinations of these nine residues in TEAS revealed that no one single residue is more important than the others in controlling product distribution and that successive mutations usually had additive effects (O'Maille et al., 2008). Mutation of TEAS usually increased the number of minor products suggesting that extant terpene synthases have evolved from more promiscuous ancestors.

4.3. Correlation of active site residues and mechanistic features- a meta-analysis

Many of the mutational studies cited above demonstrate that the product specificity of terpene synthases is both dependent on the amino acid residues forming the active site cavity and the special constraints of the active site. Hence it is conceivable that the active site residues are conserved in enzymes with similar reaction mechanisms. The compilations of terpene synthases in Tables 1 and 2 of this review provide a simple means for an approximate

test of this hypothesis. First, we identified the residues forming the active sites of bornyl diphosphate synthase (for monoterpene synthases) and TEAS (for sesquiterpene synthases) using the structural information available for these enzymes (Back and Chappell, 1996; Greenhagen et al., 2006; O'Maille et al., 2008; Rising et al., 2000; Starks et al., 1997; Whittington et al., 2002a,b). Then, we carried out an alignment of all monoterpene synthases or sesquiterpene synthases with the program package DNAstar utilizing a Clustal W algorithm (matrix: PAM250, gap penalty: 10, gap length: 0.2, delay divergent sequence: 20, DNA transition weight: 0.5) to identify the amino acids most likely to form the active sites in each enzyme based on its correspondence with the active site sequences of bornyl diphosphate synthase or TEAS. Finally, we created dendrograms comparing the deduced active site amino acid residues of each of the enzymes to search for correlations with specific properties of the reactions they catalyze. These graphs were made using the TREECON 1.3b software package (Van de Peer and De Wachter, 1994) employing a neighbor-joining algorithm with bootstrap values from 1000 trials (Figs. 3 and 4).

For monoterpene synthases, the resulting dendrogram of active site residues shows some correlation with reaction type. Based on similarities among the 47 residues identified as part of the active site, several enzyme clusters are evident whose members lack any cyclization mechanism, that is they make only acyclic products. For example, in the middle of the dendrogram there is a well-defined clade containing six enzymes from *Fragaria ananassa*, *Arabidopsis thaliana* and *Antirrhinum majus* that produce (E)-β-ocimene, myrcene and linalool. Immediately above it is a subclade of three (E)-β-ocimene synthases from *A. thaliana*, *Lotus japonicus* and *Citrus unshiu*, while just below is a subclade of three enzymes, two of which (from *Ocimum basilicum*) produce the acyclic products, geraniol and linalool. The existence of these clusters suggests that the production of acyclic monoterpenes may involve certain sets of conserved features in the active site. The remaining areas of the dendrogram contain principally monoterpene synthases producing cyclic products. Since all of these cyclases mediate 1,6-cyclizations to form the α-terpinyl cation, there appear to be some conserved features in the active site for this component of the mechanism.

Beyond these trends, little correlation can be noted between active site sequences of monoterpene synthases and cyclization type. And, there is no relationship between the incidence of multiple product formation and active site sequence. Instead, the major split in the dendrogram is between a clade of angiosperm monoterpene synthases (Fig. 3) and a clade of gymnosperm monoterpene synthases (below). Such sharp differences between the sequences of angiosperms and gymnosperm terpene synthases genes were previously noted (Bohlmann et al., 1998b), suggesting independent evolution of these two groups. The fact that there is a large overlap between angiosperm and gymnosperm monoterpene synthase products [e.g., myrcene, (−)-linalool, (−)-limonene, terpinolene, (−)-β-pinene, 3-carene] illustrates convergence of enzyme function using different active site constructions. Enzyme specificity may be determined to a large degree by the spatial proportions of the active center defined by the overall structure of the enzyme rather than the precise residues present (Greenhagen et al., 2006). At lower taxonomic levels, terpene synthases from the same genus often show a close similarity in active site sequence despite functional differences. However, the monoterpene synthases of *Citrus* sp. and *A. thaliana* appear in three distant subclades within the angiosperms. Clearly, different evolutionary lines of monoterpene synthases exist within a single species, a trend which may become much more evident once more terpene synthase sequences are available.

Analysis of the 45 residues forming the active site of sesquiterpene synthases also showed some correlations between enzyme
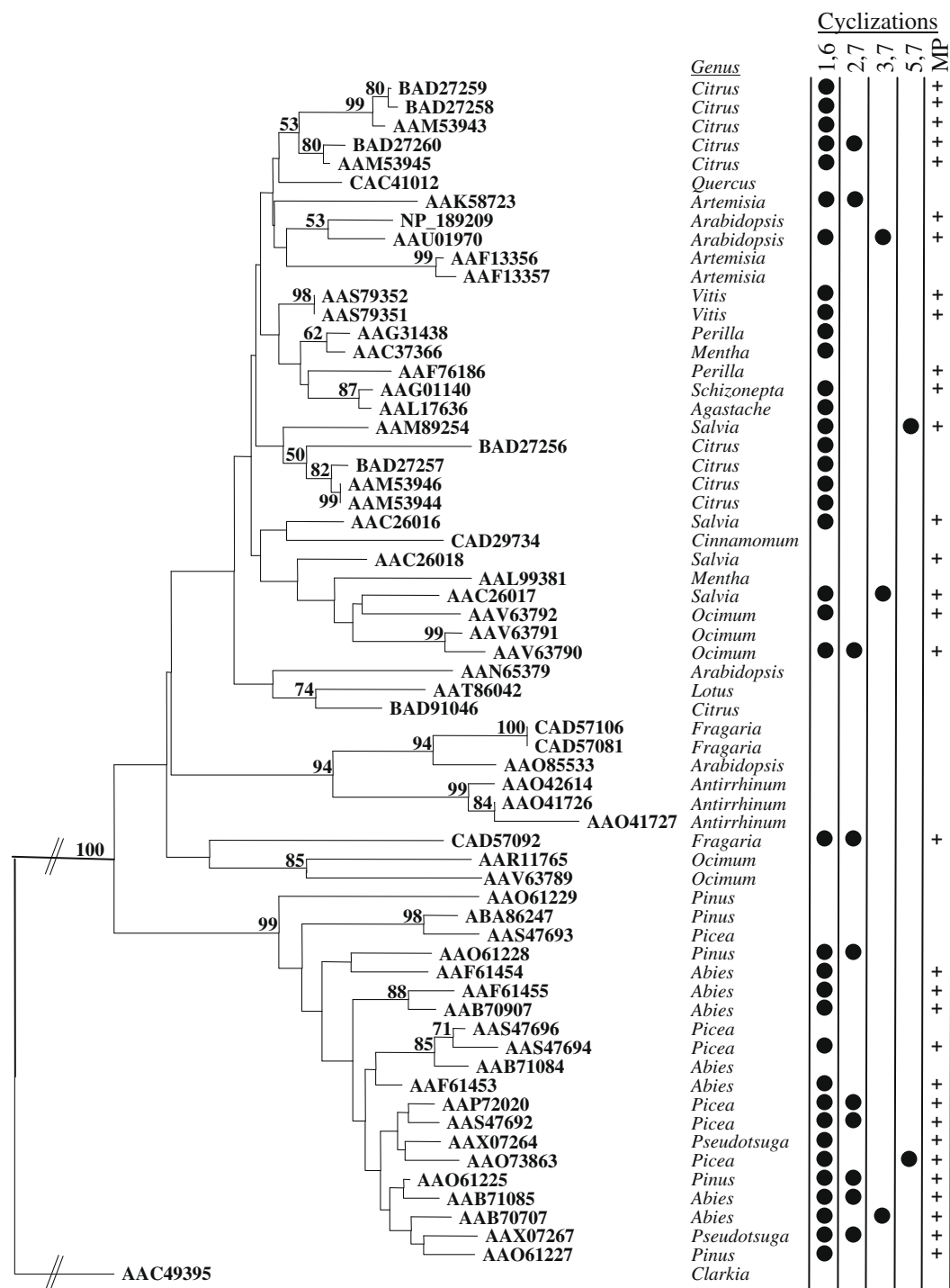
Fig. 3. Correlation of monoterpene synthase active site sequences and features of the reaction mechanism. The 47 amino acids that make up the active site of each isolated monoterpene synthase were identified by threading the sequence onto the structure of bornyl diphosphate synthase and then subjected to dendrogram analysis (Neighbor joining method with a bootstrap sample of 1000). Bootstrap values for all branches >50% are listed. Some mechanistic features of each enzyme are given in the middle columns, including the types of cyclizations present and the incidence of multiple product formation (MP).

structure and reaction mechanism. Nearly all of the top 28 sequences in the dendrogram (Fig. 4) employ a 1,10-cyclization mechanism. Included in this group are both direct 1,10-cyclizations of FPP forming the (E,E)-germacradienyl cation and 1,10-cyclizations following isomerization to the nerolidyl cation which form the (Z,E)-germacradienyl cation. The presence of both mechanism types in this sequence group indicates that similar protein

features may mediate these cyclizations, regardless of stereochemistry.

Acyclic sesquiterpene synthases are scattered over the dendrogram suggesting multiple evolutionary origins for this enzyme class. A clade of 13 sequences in the lower middle part of the dendrogram (Fig. 4) has a high proportion of enzymes making acyclic products, but also a subclade of enzymes from Asteraceae taxa
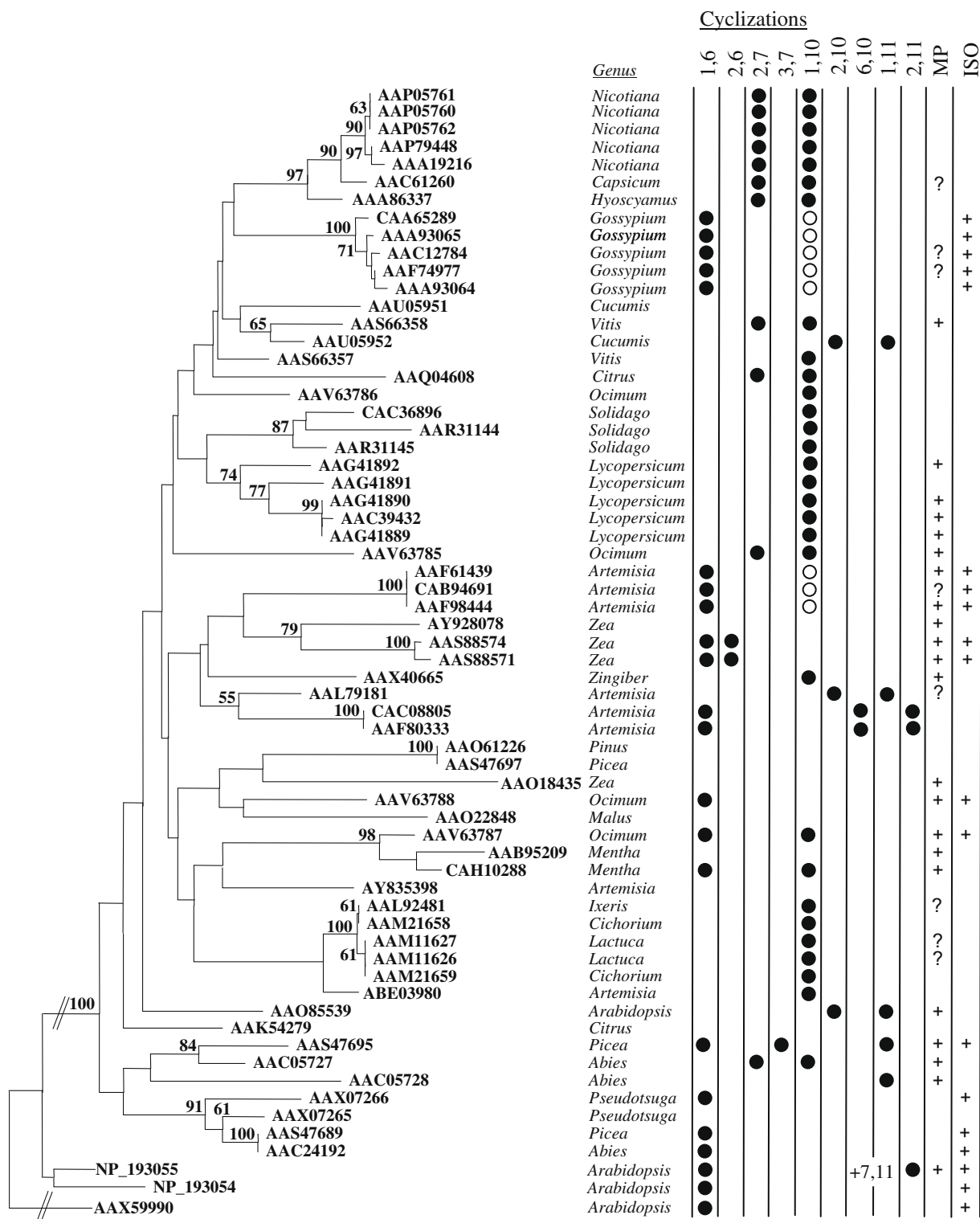
Cyclizations

Accession	Genus	1,6	2,6	2,7	3,7	1,10	2,10	6,10	1,11	2,11	MP	ISO
AAP05761	Nicotiana			●		●						
AAP05760	Nicotiana			●		●						
AAP05762	Nicotiana			●		●						
AAP79448	Nicotiana			●		●						
AAA19216	Nicotiana			●		●						
AAC61260	Capsicum			●		●					?	
AAA86337	Hyoscyamus			●		●						
CAA65289	Gossypium	●				○						+
AAA93065	Gossypium	●				○						+
AAC12784	Gossypium	●				○					?	+
AAF74977	Gossypium	●				○					?	+
AAA93064	Gossypium	●				○						+
AAU05951	Cucumis											
AAS66358	Vitis				●	●					+	
AAU05952	Cucumis						●		●			
AAS66357	Vitis											
AAQ04608	Citrus			●		●						
AAV63786	Ocimum					●						
CAC36896	Solidago					●						
AAR31144	Solidago					●						
AAR31145	Solidago					●						
AAG41892	Lycopersicum					●					+	
AAG41891	Lycopersicum					●						
AAG41890	Lycopersicum					●					+	
AAC39432	Lycopersicum					●					+	
AAG41889	Lycopersicum					●					+	
AAV63785	Ocimum			●		●					+	
AAF61439	Artemisia	●				○					+	+
CAB94691	Artemisia	●				○					?	+
AAF98444	Artemisia	●				○					+	+
AY928078	Zea	●									+	+
AAS88574	Zea	●	●								+	+
AAS88571	Zea	●	●								+	+
AAX40665	Zingiber					●					?	
AAL79181	Artemisia						●		●			
CAC08805	Artemisia	●						●		●		
AAF80333	Artemisia	●						●		●		
AAO61226	Pinus											
AAS47697	Picea											
AAO18435	Zea										+	
AAV63788	Ocimum	●									+	+
AAO22848	Malus											
AAV63787	Ocimum	●				●					+	+
AAB95209	Mentha										+	
CAH10288	Mentha	●				●					+	
AY835398	Artemisia											
AAL92481	Ixeris					●					?	
AAM21658	Cichorium					●						
AAM11627	Lactuca					●					?	
AAM11626	Lactuca					●					?	
AAM21659	Cichorium					●						
ABE03980	Artemisia					●						
AAO85539	Arabidopsis						●		●		+	
AAK54279	Citrus											
AAS47695	Picea	●			●						+	+
AAC05727	Abies		●		●						+	
AAC05728	Abies								●		+	
AAX07266	Pseudotsuga	●										+
AAX07265	Pseudotsuga											
AAS47689	Picea	●										+
AAC24192	Abies	●										+
NP_193055	Arabidopsis	●								+7,11 ●	+	+
NP_193054	Arabidopsis	●										+
AAX59990	Arabidopsis	●										+

Fig. 4. Correlation of sesquiterpene synthase active site sequences and features of the reaction mechanism. The 45 amino acids that make up the active site were identified by threading the sequence onto the structure of 5-*epi*-aristolochene synthase of *Nicotiana tabacum* and then subjected to dendrogram analysis (Neighbor joining method with a bootstrap sample of 1000). Bootstrap values for all branches >50% are listed. Some mechanistic features of each enzyme are given in the middle column, including the types of cyclizations present, the incidence of multiple product formation (MP), and the catalysis of (*E*,*E*)-FPP to (*E*,*Z*)-FPP.

employing a 1,10-cyclization in their reaction mechanisms. Over the entire figure, there is no relationship between the sequence of the active site and the tendency to form multiple products, suggesting no universal feature of terpene synthases exists that distinguishes multiple- from single-product enzymes.

These conclusions about sesquiterpene synthase active site structure and mechanism are necessarily premature since the enzymes characterized to date represent only a small portion of those present in nature. Since most recent sesquiterpene synthase genes have been isolated by homology-based PCR approaches, according

to their similarity to known gene sequences, divergent types of genes might have been missed. In addition, the analysis might be refined by considering not just active site residues, but other residues adjacent to the catalytic center.

The dendrogram of sesquiterpene synthase active site sequences, unlike that for monoterpene synthases, does not give an absolute split between gymnosperm and angiosperm enzymes. In addition to the tight cluster of 7 gymnosperm sequences at the bottom of the dendrogram, two other gymnosperm sequences making acyclic products cluster with angiosperm enzymes. This may indicate that more than one type of sesquiterpene synthase existed before the evolutionary divide between gymnosperms and angiosperms. At lower taxonomic levels, there is a strong tendency for sequences from the same genus to cluster together. However, as with monoterpene synthases, sequences for certain genera are widely scattered in the dendrogram, highlighting the diversity of evolutionary lines within sesquiterpene synthases. As the terpene synthase families of genera besides *Arabidopsis* (Aubourg et al., 2002) become better known, the outline of sesquiterpene synthase evolution should become clearer, and the structural elements required for specific reactions should be better revealed.

4.4. Terpene synthase structure and multiple products

The ability of terpene synthases to convert a prenyl diphosphate substrate to diverse products during different reaction cycles is one of the most unique traits of this enzyme class. As described above, this property is found in nearly half of all characterized monoterpene and sesquiterpene synthases and may be attributed to the fact that the various reactive carbocationic intermediates can be stabilized in more than one way. However, since half of all monoterpene and sesquiterpene synthases channel their carbocationic-based catalysis into a single product, it is clear that some feature of the protein, rather than the mechanism itself, is responsible for multiple product formation. However, our simplistic analysis of the active site sequences of all currently-known enzymes (Figs. 3 and 4) did not reveal any such general feature.

Individual terpene synthases may have specific ways of generating multiple products. For example, the γ-humulene synthase of *A. grandis*, which generates 52 different sesquiterpenes, has two DDxxD motifs located on opposite sides of the active site cleft, which led to the suggestion that substrate binding in two different conformations results in different sets of products (Steele et al., 1998). Indeed, inactivation of one of the DDxxD motifs by mutagenesis resulted in enzymes that produce a lower number of products (Steele et al., 1998). The formation of multiple products might also be enhanced by the NSE/DTE motif that is situated at the position of the second DDxxD motif in some terpene synthases.

The relationship between active site architecture and multiple product formation has been studied with the terpene synthase TPS4 of *Zea mays* which forms the sesquiterpenes 7-*epi*-sesquithujene, β-bisabolene and 12 other olefins in minor amounts (Köllner et al., 2004). To understand the complex reaction mechanism of TPS4, the active site cavity was modeled and docking simulations with the substrate farnesyl diphosphate, with several predicted carbocation intermediates and with the final reaction products were conducted (Köllner et al., 2006). The results suggested that discrete steps of the reaction sequence occur in two different active site pockets, with a conformational change in the bisabolyl cation intermediate causing a shift from one pocket to the other. Site-directed mutagenesis and measurements of mutant enzyme activity with both (*E,E*)- and (*Z,E*)-farnesyl diphosphate were employed to support this model. Amino acid alterations in pocket I indicated that early steps of the catalytic process are localized in this compartment up to the formation of the monocyclic bisabolyl cation. Mutations in pocket II primarily inhibited the formation of bicyclic

compounds, suggesting that secondary cyclizations of the bisabolyl cation are catalyzed in pocket II (Köllner et al., 2006).

The formation of multiple products could also be a consequence of more conformational flexibility in the active center allowing the formation of more reaction intermediates and thus more products. However, the short reaction times required by cationic cyclizations might not provide sufficient time for larger conformational changes of the intermediates (Vedula et al., 2005). Also, structural elucidation of complexes consisting of trichodiene synthase and an analog of the intermediate suggested the intermediates are present in thermodynamically preferred conformations rather than those expected from the course of the reaction mechanism (Christianson, 2006). Therefore, terpene synthase reactions are more likely to be controlled by kinetic rather than thermodynamic processes (Vedula et al., 2005). From this perspective, the formation of multiple products is dependent on differences in substrate conformation occurring at the very beginning of the reaction.

Further studies of terpene synthase structure and function are clearly necessary to understand how these enzymes catalyze the formation of multiple products. The availability of additional terpene synthase structures will no doubt speed progress in this area and facilitate further correlations between structural elements and properties of the reaction. In this regard, identification of the amino acid residues that interact with the termination steps of the cyclic cascade would be particularly helpful. The increased appreciation of theoretical methods would also be a welcome development. Knowledge of the free energies of reaction intermediates, for example, should provide new insights on multiple product formation.

The literature review for this article was completed in 2008. However, in 2009 two reports appeared that require revision of some basic assumptions about the universality of geranyl diphosphate (GPP) as a substrate for monoterpene synthases and the universality of farnesyl diphosphate (FPP = E,E-FPP) as a substrate for sesquiterpene synthases. A tomato monoterpene synthase was described that uses neryl diphosphate, the Z-isomer of GPP, as a substrate instead of GPP (Schilmiller AL, Schauvinhold I, Larson M, Xu R, Charbonneau AL, Schmidt A, Wilkerson C, Last R, Pichersky E (2009) Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proc. Natl. Acad. Sci. USA 106, 10865–10870), while a tomato sesquiterpene synthase was reported that uses Z,Z-FPP instead of the usual E,E-FPP (Sallaud C, Ronstein D, Onillon S, Jabès F, Duffé, Giagalone C, Thoraval S, Escoffer C, Herbette G, Leonhardt N, Causse M, Tissier A (2009) A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl pyrophosphate in the wild tomato Solanum habrochaites. Plant Cell 21, 301-317). These and other isomeric prenyl diphosphates may prove to be more widespread substrates for the terpene synthase class.

Acknowledgements

References

Aharoni, A., Giri, A.P., Verstappen, F.W.A., Bertea, C.M., Sevenier, R., Sun, Z.K., Jongsma, M.A., Schwab, W., Bouwmeester, H.J., 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. Plant Cell 16, 3110–3131.
Alchanati, I., Patel, J.A.A., Liu, J.G., Benedict, C.R., Stipanovic, R.D., Bell, A.A., Cui, Y.X., Magill, C.W., 1998. The enzymatic cyclization of nerolidyl diphosphate by delta-

cadinene synthase from cotton stele tissue infected with *Verticillium dahliae*. Phytochemistry 47, 961–967.

Arimura, G., Ozawa, R., Kugimiya, S., Takabayashi, J., Bohlmann, J., 2004. Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (E)-beta-ocimene and transcript accumulation of (E)-beta-ocimene synthase in *Lotus japonicus*. Plant Physiol. 135, 1976–1983.

Arimura, G.I., Garms, S., Maffei, M., Bossi, S., Schulze, B., Leitner, M., Mithoefer, A., Boland, W., 2008a. Herbivore-induced terpenoid emission in Medicago truncatula: concerted action of jasmonate, ethylene and calcium signaling. Planta 227, 453–464.

Arimura, G.I., Köpke, S., Kunert, M., Volpe, V., David, A., Brand, P., Dabrowska, P., Maffei, M.E., Boland, W., 2008b. Effects of feeding *Spodoptera littoralis* on lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. Plant Physiol. 146, 965–973.

Aubourg, S., Lecharny, A., Bohlmann, J., 2002. Genomic analysis of the terpenoid synthase (AtTPS) gene family of *Arabidopsis thaliana*. Mol. Genet. Genomics 267, 730–745.

Back, K.W., Yin, S.H., Chappell, J., 1994. Expression of a plant sesquiterpene cyclase gene in *Escherichia coli*. Arch. Biochem. Biophys. 315, 527–532.

Back, K., Chappell, J., 1995. Cloning and bacterial expression of a sesquiterpene cyclase from *Hyoscyamus muticus* and its molecular comparison to related terpene cyclases. J. Biol. Chem. 270, 7375–7381.

Back, K.W., Chappell, J., 1996. Identifying functional domains within terpene cyclases using a domain-swapping strategy. Proc. Natl. Acad. Sci. USA 93, 6841–6845.

Back, K.W., He, S.L., Kim, K.U., Shin, D.H., 1998. Cloning and bacterial expression of sesquiterpene cyclase, a key branch point enzyme for the synthesis of sesquiterpenoid phytoalexin capsidiol in UV-challenged leaves of *Capsicum annuum*. Plant Cell Physiol. 39, 899–904.

Back, K., Nah, J., Lee, S.B., Song, J.H., Shin, D.H., Kim, H.Y., 2000. Cloning of a sesquiterpene cyclase and its functional expression by domain swapping strategy. Mol. Cells 10, 220–225.

Benedict, C.R., Lu, J.L., Pettigrew, D.W., Liu, J.G., Stipanovic, R.D., Williams, H.J., 2001. The cyclization of farnesyl diphosphate and nerolidyl diphosphate by a purified recombinant delta-cadinene synthase. Plant Physiol. 125, 1754–1765.

Bennett, M.H., Mansfield, J.W., Lewis, M.J., Beale, M.H., 2002. Cloning and expression of sesquiterpene synthase genes from lettuce (*Lactuca sativa* L.). Phytochemistry 60, 255–261.

Bertea, C.M., Voster, A., Verstappen, F.W.A., Maffei, M., Beekwilder, J., Bouwmeester, H.J., 2006. Isoprenoid biosynthesis in *Artemisia annua*: cloning and heterologous expression of a germacrene A synthase from a glandular trichome cDNA library. Arch. Biochem. Biophys. 448, 3–12.

Bohlmann, J., Steele, C.L., Croteau, R., 1997. Monoterpene synthases from Grand fir (*Abies grandis*) – cDNA isolation, characterization, and functional expression of myrcene synthase, (−)(4S)-limonene synthase, and (−)-(1S, 5S)-pinene synthase. J. Biol. Chem. 272, 21784–21792.

Bohlmann, J., Crock, J., Jetter, R., Croteau, R., 1998a. Terpenoid-based defenses in conifers: cDNA cloning, characterization, and functional expression of wound-inducible (E)-alpha-bisabolene synthase from grand fir (*Abies grandis*). Proc. Natl. Acad. Sci. USA 95, 6756–6761.

Bohlmann, J., Meyer-Gauen, G., Croteau, R., 1998b. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. Proc. Natl. Acad. Sci. USA 95, 4126–4133.

Bohlmann, J., Phillips, M., Ramachandiran, V., Katoh, S., Croteau, R., 1999. CDNA cloning, characterization, and functional expression of four new monoterpene synthase members of the Tpsd gene family from grand fir (*Abies grandis*). Arch. Biochem. Biophys. 368, 232–243.

Bohlmann, J., Martin, D., Oldham, N.J., Gershenzon, J., 2000. Terpenoid secondary metabolism in Arabidopsis thaliana: cDNA cloning, characterization, and functional expression of a myrcene/(E)-beta-ocimene synthase. Arch. Biochem. Biophys. 375, 261–269.

Bohlmann, J., Stauber, E.J., Krock, B., Oldham, N.J., Gershenzon, J., Baldwin, I.T., 2002. Gene expression of 5-epi-aristolochene synthase and formation of capsidiol in roots of *Nicotiana* attenuata and *N. Sylvestris*. Phytochemistry 60, 109–116.

Bouwmeester, H.J., Kodde, J., Verstappen, F.W.A., Altug, I.G., de Kraker, J.W., Wallaart, T.E., 2002. Isolation and characterization of two germacrene A synthase cDNA clones from chicory. Plant Physiol. 129, 134–144.

Byun-McKay, A., Godard, K.A., Toudefallah, M., Martin, D.M., Alfaro, R., King, J., Bohlmann, J., Plant, A.L., 2006. Wound-induced terpene synthase gene expression in sitka spruce that exhibit resistance or susceptibility to attack by the white pine weevil. Plant Physiol. 140, 1009–1021.

Cai, Y., Jia, J.W., Crock, J., Lin, Z.X., Chen, X.Y., Croteau, R., 2002. A cDNA clone for beta-caryophyllene synthase from *Artemisia annua*. Phytochemistry 61, 523–529.

Cane, D.E., Iyengar, R., 1979. Enzymatic conversion of farnesyl to nerolidyl pyrophosphate – role of the pyrophosphate moiety. J. Am. Chem. Soc. 101, 3385–3388.

Cane, D.E., Swanson, S., Murthy, P.P.N., 1981. Trichodiene biosynthesis and the enzymatic cyclization of farnesyl pyrophosphate. J. Am. Chem. Soc. 103, 2136–2138.

Cane, D.E., 1990. Enzymatic formation of sesquiterpenes. Chem. Rev. 90, 1089–1103.

Cane, D.E., Yang, G.H., 1994. Trichodiene synthase – stereochemical studies of the cryptic allylic diphosphate isomerase activity using an anomalous substrate. J. Org. Chem. 59, 5794–5798.

Cane, D.E., Tandon, M., 1995. Epicubenol synthase and the stereochemistry of the enzymatic cyclization of farnesyl and nerolidyl diphosphate. J. Am. Chem. Soc. 117, 5602–5603.

Cane, D.E., Xue, Q., Fitzsimons, B.C., 1996a. Trichodiene synthase. Probing the role of the highly conserved aspartate-rich region by site-directed mutagenesis. Biochemistry 35, 12369–12376.

Cane, D.E., Xue, Q., VanEpp, J.E., 1996b. Enzymatic formation of isochamigrene, a novel sesquiterpene, by alteration of the aspartate-rich region of trichodiene synthase. J. Am. Chem. Soc. 118, 8499–8500.

Cane, D.E., 1999. Comprehensive Natural Products Chemistry, Isoprenoids Including Caroteinoids and Steroids. vol. 2. Elsevier, Amsterdam. pp. 155–200.

Cane, D.E., Kang, I., 2000. Aristolochene synthase: Purification, molecular cloning, high-level expression in *Escherichia coli*, and characterization of the *Aspergillus terreus* cyclase. Arch. Biochem. Biophys. 376, 354–364.

Caruthers, J.M., Kang, I., Rynkiewicz, M.J., Cane, D.E., Christianson, D.W., 2000. Crystal structure determination of aristolochene synthase from the blue cheese mold, Penicillium roqueforti. J. Biol. Chem. 275, 25533–25539.

Chang, Y.J., Song, S.H., Park, S.H., Kim, S.U., 2000. Amorpha-4, 11-diene synthase of *Artemisia annua*: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis. Arch. Biochem. Biophys. 383, 178–184.

Chang, Y.J., Jin, J., Nam, H.Y., Kim, S.U., 2005. Point mutation of (+)-germacrene A synthase from *Ixeris dentata*. Biotechn. Lett. 27, 285–288.

Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E., Gershenzon, J., 2003. Biosynthesis and emission of terpenoid volatiles from Arabidopsis flowers. Plant Cell 15, 481–494.

Chen, F., Ro, D.K., Petri, J., Gershenzon, J., Bohlmann, J., Pichersky, E., Tholl, D., 2004. Characterization of a root-specific Arabidopsis terpene synthase responsible for the formation of the volatile monoterpene 1, 8-cineole. Plant Physiol. 135, 1956–1966.

Chen, X.Y., Chen, Y., Heinstein, P., Davisson, V.J., 1995. Cloning, expression, and characterization of (+)-delta-cadinene synthase: A catalyst for cotton phytoalexin biosynthesis. Arch. Biochem. Biophys. 324, 255–266.

Chen, X.Y., Wang, M.S., Chen, Y., Davisson, V.J., Heinstein, P., 1996. Cloning and heterologous expression of a second (+)-delta-cadinene synthase from *Gossypium arboreum*. J. Nat. Prod. 59, 944–951.

Cheng, A.X., Xiang, C.Y., Li, J.X., Yang, C.Q., Hu, W.L., Wang, L.J., Lou, Y.G., Chen, X.Y., 2007. The rice (E)-beta-caryophyllene synthase (OsTPS3) accounts for the major inducible volatile sesquiterpenes. Phytochemistry 68, 1632–1641.

Christianson, D.W., 2006. Structural biology and chemistry of the terpenoid cyclases. Chem. Rev. 106, 3412–3442.

Colby, S.M., Alonso, W.R., Katahira, E.J., McGarvey, D.J., Croteau, R., 1993. (4S)-Limonene synthase from the oil glands of Spearmint (*Mentha spicata*) – cDNA isolation, characterization, and bacterial expression of the catalytically active monoterpene cyclase. J. Biol. Chem. 268, 23016–23024.

Colby, S.M., Crock, J., Dowdle-Rizzo, B., Lemaux, P.G., Croteau, R., 1998. Germacrene C synthase from *Lycopersicon esculentum* cv. VFNT Cherry tomato: cDNA isolation, characterization, and bacterial expression of the multiple product sesquiterpene cyclase. Proc. Natl. Acad. Sci. USA 95, 2216–2221.

Connolly, J.D., Hill, R.A., 1991. Dictionary of Terpenoids. Chapman and Hall, London.

Connolly, J.D., Hill, R.A., 2005. Triterpenoids. Nat. Prod. Rep. 22, 487–503.

Crock, J., Wildung, M., Croteau, R., 1997. Isolation and bacterial expression of a sesquiterpene synthase cDNA clone from peppermint (Mentha x piperita. L.) that produces the aphid alarm pheromone (E)-beta-farnesene. Proc. Natl. Acad. Sci. USA 94, 12833–12838.

Croteau, R., Karp, F., 1977. Demonstration of a cyclic pyrophosphate intermediate in enzymatic conversion of neryl pyrophosphate to borneol. Arch. Biochem. Biophys. 184, 77–86.

Croteau, R., Karp, F., 1979. Biosynthesis of monoterpenes – hydrolysis of bornyl pyrophosphate, an essential step in camphor biosynthesis, and hydrolysis of geranyl pyrophosphate, the acyclic precursor of camphor, by enzymes from sage (*Salvia officinalis*). Arch. Biochem. Biophys. 198, 523–532.

Croteau, R., Felton, M., Ronald, R.C., 1980. Biosynthesis of monoterpenes – conversion of the acyclic precursors geranyl pyrophosphate and neryl pyrophosphate to the rearranged monoterpenes fenchol and fenchone by a soluble enzyme preparation from fennel (Foeniculum vulgare). Arch. Biochem. Biophys. 200, 524–533.

Croteau, R., Felton, M., 1981. Conversion of [1-H-3(2) G-C-14]geranyl pyrophosphate to cyclic monoterpenes without loss of tritium. Arch. Biochem. Biophys. 207, 460–464.

Croteau, R., 1986. Evidence for the ionization steps in monoterpene cyclization reactions using 2-Fluorogeranyl and 2-Fluorolinalyl pyrophosphates as substrates. Arch. Biochem. Biophys. 251, 777–782.

Croteau, R., Wheeler, C.J., Aksela, R., Oehlschlager, A.C., 1986. Inhibition of monoterpene cyclases by sulfonium analogs of presumptive carbocationic intermediates of the cyclization reaction. J. Biol. Chem. 261, 7257–7263.

Croteau, R., 1987. Biosynthesis and catabolism of monoterpenoids. Chem. Rev. 87, 929–954.

Croteau, R., Satterwhite, D.M., Wheeler, C.J., Felton, N.M., 1989. Biosynthesis of monoterpenes – stereochemistry of the enzymatic cyclizations of geranyl pyrophosphate to (+)-alpha-pinene and (−)-beta-pinene. J. Biol. Chem. 264, 2075–2080.

Croteau, R., Gershenzon, J., Wheeler, C.J., Satterwhite, D.M., 1990. Biosynthesis of monoterpenes – stereochemistry of the coupled isomerization and cyclization of geranyl pyrophosphate to camphane and isocamphane monoterpenes. Arch. Biochem. Biophys. 277, 374–381.

Croteau, R., Alonso, W.R., Koepp, A.E., Johnson, M.A., 1994. Biosynthesis of monoterpenes – partial-purification, characterization, and mechanism of action of 1, 8-cineole synthase. Arch. Biochem. Biophys. 309, 184–192.

Crowell, A.L., Williams, D.C., Davis, E.M., Wildung, M.R., Croteau, R., 2002. Molecular cloning and characterization of a new linalool synthase. Arch. Biochem. Biophys. 405, 112–121.

Cseke, L., Dudareva, N., Pichersky, E., 1998. Structure and evolution of linalool synthase. Mol. Biol. Evol. 15, 1491–1498.

Davis, E.M., Tsuji, J., Davis, G.D., Pierce, M.L., Essenberg, M., 1996. Purification of (+)-delta-cadinene synthase, a sesquiterpene cyclase from bacteria-inoculated cotton foliar tissue. Phytochemistry 41, 1047–1055.

Davis, E.M., Croteau, R., 2000. Biosynthesis: Aromatic Polyketides, Isoprenoids, Alkaloids, Cyclization Enzymes in the Biosynthesis of Monoterpenes, Sesquiterpenes, and Diterpenes., vol. 209. Springer, Berlin Heidelberg. pp. 53–95.

Deguerry, F., Pastore, L., Wu, S.Q., Clark, A., Chappell, J., Schalk, M., 2006. The diverse sesquiterpene profile of patchouli, Pogostemon cablin, is correlated with a limited number of sesquiterpene synthases. Arch. Biochem. Biophys. 454, 123–136.

Dehal, S.S., Croteau, R., 1987. Metabolism of monoterpenes – specificity of the dehydrogenases responsible for the biosynthesis of camphor, 3-thujone, and 3-isothujone. Arch. Biochem. Biophys. 258, 287–291.

Dewick, P.M., 1999. The biosynthesis of C-5-C-25 terpenoid compounds. Nat. Prod. Rep. 16, 97–130.

Dudareva, N., Cseke, L., Blanc, V.M., Pichersky, E., 1996. Evolution of floral scent in Clarkia: Novel patterns of (S)-linalool synthase gene expression in the C. Breweri flower. Plant Cell 8, 1137–1148.

Dudareva, N., Martin, D., Kish, C.M., Kolosova, N., Gorenstein, N., Fäldt, J., Miller, B., Bohlmann, J., 2003. (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: Function and expression of three terpene synthase genes of a new terpene synthase subfamily. Plant Cell 15, 1227–1241.

El Tamer, M.K., Lucker, J., Bosch, D., Verhoeven, H.A., Verstappen, F.W.A., Schwab, W., van Tunen, A.J., Voragen, A.G.J., de Maagd, R.A., Bouwmeester, H.J., 2003. Domain swapping of Citrus limon monoterpene synthases: impact on enzymatic activity and product specificity. Arch. Biochem. Biophys. 411, 196–203.

Facchini, P.J., Chappell, J., 1992. Gene family for an elicitor-induced sesquiterpene cyclase in Tobacco. Proc. Natl. Acad. Sci. USA 89, 11088–11092.

Fäldt, J., Arimura, G., Gershenzon, J., Takabayashi, J., Bohlmann, J., 2003a. Functional identification of AtTPS03 as (E)-beta-ocimene synthase: a monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in Arabidopsis thaliana. Planta 216, 745–751.

Fäldt, J., Martin, D., Miller, B., Rawat, S., Bohlmann, J., 2003b. Traumatic resin defense in Norway spruce (Picea abies): Methyl jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. Plant Mol. Biol. 51, 119–133.

Fischbach, R.J., Zimmer, W., Schnitzler, J.P., 2001. Isolation and functional analysis of a cDNA encoding a myrcene synthase from holm oak (Quercus ilex L.). Eur. J. Biochem 268, 5633–5638.

Fraga, B.M., 2006. Natural sesquiterpenoids. Nat. Prod. Rep. 23, 943–972.

Gambliel, H., Croteau, R., 1984. Pinene cyclase-I and cyclase-II – two enzymes from Sage (Salvia officinalis) which catalyze stereospecific cyclizations of geranyl pyrophosphate to monoterpene olefins of opposite configuration. J. Biol. Chem. 259, 740–748.

Green, S., Friel, E.N., Matich, A., Beuning, L.L., Cooney, J.M., Rowan, D.D., MacRae, E., 2007. Unusual features of a recombinant apple alpha-farnesene synthase. Phytochemistry 68, 176–188.

Green, S., Squire, C.J., Nieuwenhuizen, N.J., Baker, E.N., Laing, W., 2009. Defining the potassium binding region in an apple terpene synthase. J. Biol. Chem. 284, 8652–8660.

Greenhagen, B.T., O'Maille, P.E., Noel, J.P., Chappell, J., 2006. Identifying and manipulating structural determinates linking catalytic specificities in terpene synthases. Proc. Natl. Acad. Sci. USA 103, 9826–9831.

Günnewich, N., Page, J.E., Köllner, T.G., Degenhardt, J., Kutchan, T.M., 2007. Functional expression and characterization of trichome-specific (−)-limonene synthase and (+)-α-pinene synthase from Cannabis sativa. Nat. Prod. Commun. 2, 223–232.

Guterman, I., Shalit, M., Menda, N., Piestun, D., Dafny-Yelin, M., Shalev, G., Bar, E., Davydov, O., Ovadis, M., Emanuel, M., Wang, J., Adam, Z., Pichersky, E., Lewinsohn, E., Zamir, D., Vainstein, A., Weiss, D., 2002. Rose scent: Genomics approach to discovering novel floral fragrance-related genes. Plant Cell 14, 2325–2338.

Hallahan, T.W., Croteau, R., 1989. Monoterpene biosynthesis – mechanism and stereochemistry of the enzymatic cyclization of geranyl pyrophosphate to (+)-cis-sabinene and (+)-trans-sabinene hydrate. Arch. Biochem. Biophys. 269, 313–326.

Hanson, J.R., 2000. Diterpenoids. Nat. Prod. Rep. 17, 165–174.

Hoelscher, D.J., Williams, D.C., Wildung, M.R., Croteau, R., 2003. A cDNA clone for 3-carene synthase from Salvia stenophylla. Phytochemistry 62, 1081–1086.

Hosoi, M., Ito, M., Yagura, T., Adams, R.P., Honda, G., 2004. CDNA isolation and functional expression of myrcene synthase from Perilla frutescens. Biol. Pharm. Bull. 27, 1979–1985.

Hua, L., Matsuda, S.P.T., 1999. The molecular cloning of 8-epi-cedrol synthase from Artemisia annua. Arch. Biochem. Biophys. 369, 208–212.

Huber, D.P.W., Philippe, R.N., Godard, K.A., Sturrock, R.N., Bohlmann, J., 2005. Characterization of four terpene synthase cDNAs from methyl jasmonate-induced Douglas-fir, Pseudotsuga menziesii. Phytochemistry 66, 1427–1439.

Hyatt, D.C., Croteau, R., 2005. Mutational analysis of a monoterpene synthase reaction: Altered catalysis through directed mutagenesis of (−)-pinene synthase from Abies grandis. Arch. Biochem. Biophys. 439, 222–233.

Hyatt, D.C., Youn, B., Zhao, Y., Santhamma, B., Coates, R.M., Croteau, R.B., Khang, C.H., 2007. Structure of limonene synthase, a simple model for terpenoid cyclase catalysis. Proc. Natl. Acad. Sci. USA 104, 5360–5365.

Iijima, Y., Davidovich-Rikanati, R., Fridman, E., Gang, D.R., Bar, E., Lewinsohn, E., Pichersky, E., 2004a. The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. Plant Physiol. 136, 3724–3736.

Iijima, Y., Gang, D.R., Fridman, E., Lewinsohn, E., Pichersky, E., 2004b. Characterization of geraniol synthase from the peltate glands of sweet basil. Plant Physiol. 134, 370–379.

Ito, M., Kiuchi, F., Yang, L.L., Honda, G., 2000. Perilla citriodora from Taiwan and its phytochemical characteristics. Biol. Pharm. Bull. 23, 359–362.

Ito, M., Honda, G., 2007. Geraniol synthases from Perilla and their taxonomical significance. Phytochemistry 68, 446–453.

Jia, J.W., Crock, J., Lu, S., Croteau, R., Chen, X.Y., 1999. (3R)-linalool synthase from Artemisia annua L.: cDNA isolation, characterization, and wound induction. Arch. Biochem. Biophys. 372, 143–149.

Jones, C.G., Keeling, C.I., Ghisalberti, E.L., Barbour, E.L., Plummer, J.A., Bohlmann, J., 2008. Isolation of cDNAs and functional characterisation of two multi-product terpene synthase enzymes from sandalwood, Santalum album L. Arch. Biochem. Biophys. 477, 121–130.

Kampranis, S.C., Ioannidis, D., Purvis, A., Mahrez, W., Ninga, E., Katerelos, N.A., Anssour, S., Dunwell, J.M., Degenhardt, J., Makris, A.M., Goodenough, P.W., Johnson, C.B., 2007. Rational conversion of substrate and product specificity in a Salvia monoterpene synthase: Structural insights into the evolution of terpene synthase function. Plant Cell 19, 1994–2005.

Katoh, S., Hyatt, D., Croteau, R., 2004. Altering product outcome in Abies grandis (−)-limonene synthase and (−)-limonene/(−)-alpha-pinene synthase by domain swapping and directed mutagenesis. Arch. Biochem. Biophys. 425, 65–76.

Kim, M.Y., Chang, Y.J., Bang, M.H., Baek, N.I., Jin, J., Lee, C.H., Kim, S.U., 2005. CDNA isolation and characterization of (+)-germacrene A synthase from Ixeris dentata form. Albiflora Hara. J. Plant Biol. 48, 178–186.

Köllner, T.G., Schnee, C., Gershenzon, J., Degenhardt, J., 2004. The variability of sesquiterpenes cultivars is controlled by allelic emitted from two Zea mays variation of two terpene synthase genes encoding stereoselective multiple product enzymes. Plant Cell 16, 1115–1131.

Köllner, T.G., O'Maille, P.E., Gatto, N., Boland, W., Gershenzon, J., Degenhardt, J., 2006. Two pockets in the active site of maize sesquiterpene synthase TPS4 carry out sequential parts of the reaction scheme resulting in multiple products. Arch. Biochem. Biophys. 448, 83–92.

Köllner, T.G., Held, M., Lenk, C., Hiltpold, I., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2008a. A maize (E)-β-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most american maize varieties. Plant Cell 20, 482–494.

Köllner, T.G., Schnee, C., Li, S., Svatos, A., Schneider, B., Gershenzon, J., Degenhardt, J., 2008b. Protonation of a neutral (S)-β-bisabolene intermediate is involved in (S)-β-macrocarpene formation by the maize sesquiterpene synthases TPS6 and TPS11. J. Biol. Chem. 283, 20779–20788.

Landmann, C., Fink, B., Festner, M., Dregus, M., Engel, K.H., Schwab, W., 2007. Cloning and functional characterization of three terpene synthases from lavender (Lavandula angustifolia). Arch. Biochem. Biophys. 465, 417–429.

LaFever, R.E., Croteau, R., 1993. Hydride shifts in the biosynthesis of the p-menthane monoterpenes alpha-terpinene, gamma-terpinene, and beta-phellandrene. Arch. Biochem. Biophys. 301, 361–366.

Lee, S., Chappell, J., 2008. Biochemical and genomic characterization of terpene synthases in Magnolia grandiflora. Plant Physiol. 147, 1017–1033.

Lesburg, C.A., Zhai, G.Z., Cane, D.E., Christianson, D.W., 1997. Crystal structure of pentalenene synthase: Mechanistic insights on terpenoid cyclization reactions in biology. Science 277, 1820–1824.

Lin, C.F., Shen, B.Z., Xu, Z.N., Köllner, T.G., Degenhardt, J., Dooner, H.K., 2008. Characterization of the monoterpene synthase gene tps26, the ortholog of a gene induced by insect herbivory in maize. Plant Physiol. 146, 940–951.

Little, D.B., Croteau, R.B., 2002. Alteration of product formation by directed mutagenesis and truncation of the multiple-product sesquiterpene synthases delta-selinene synthase and gamma-humulene synthase. Arch. Biochem. Biophys. 402, 120–135.

Lu, S., Xu, R., Jia, J.W., Pang, J.H., Matsuda, S.P.T., Chen, X.Y., 2002. Cloning and functional characterization of a beta-pinene synthase from Artemisia annua that shows a circadian pattern of expression. Plant Physiol. 130, 477–486.

Lucker, J., El Tamer, M.K., Schwab, W., Verstappen, F.W.A., van der Plas, L.H.W., Bouwmeester, H.J., Verhoeven, H.A., 2002. Monoterpene biosynthesis in lemon (Citrus limon) – cDNA isolation and functional analysis of four monoterpene synthases. Eur. J. Biochem. 269, 3160–3171.

Lucker, J., Bowen, P., Bohlmann, J., 2004. Vitis vinifera terpenoid cyclases: functional identification of two sesquiterpene synthase cDNAs encoding (+)-valencene synthase and (−)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. Phytochemistry 65, 2649–2659.

Martin, D.M., Bohlmann, J., 2004. Identification of Vitis vinifera (−)-alpha-terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. Phytochemistry 65, 1223–1229.

Martin, D.M., Fäldt, J., Bohlmann, J., 2004. Functional characterization of nine Norway spruce TPS genes and evolution of gymnosperm terpene synthases of the TPS-d subfamily. Plant Physiol. 135, 1908–1927.

Maruyama, T., Ito, M., Honda, G., 2001a. Molecular cloning, functional expression and characterization of (E)-beta-farnesene synthase from Citrus junos. Biol. Pharm. Bull. 24, 1171–1175.

Maruyama, T., Ito, M., Kiuchi, F., Honda, G., 2001b. Molecular cloning, functional expression and characterization of d-limonene synthase from Schizonepeta tenuifolia. Biol. Pharm. Bull. 24, 373–377.

Maruyama, T., Saeki, D., Ito, M., Honda, G., 2002. Molecular cloning, functional expression and characterization of d-limonene synthase from Agastache rugosa. Biol. Pharm. Bull. 25, 661–665.

McKay, S.A.B., Hunter, W.L., Godard, K.A., Wang, S.X., Martin, D.M., Bohlmann, J., Plant, A.L., 2003. Insect attack and wounding induce traumatic resin duct development and gene expression of (−)-pinene synthase in Sitka spruce. Plant Physiol. 133, 368–378.

Meng, Y.L., Jia, J.W., Liu, C.J., Liang, W.Q., Heinstein, P., Chen, X.Y., 1999. Coordinated accumulation of (+)-delta-cadinene synthase mRNAs and gossypol in developing seeds of Gossypium hirsutum and a new member of the cad1 family from G. arboreum. J. Nat. Prod. 62, 248–252.

Mercke, P., Crock, J., Croteau, R., Brodelius, P.E., 1999. Cloning, expression, and characterization of epi-cedrol synthase, a sesquiterpene cyclase from Artemisia annua L. Arch. Biochem. Biophys. 369, 213–222.

Mercke, P., Bengtsson, M., Bouwmeester, H.J., Posthumus, M.A., Brodelius, P.E., 2000. Molecular cloning, expression, and characterization of amorpha-4, 11-diene synthase, a key enzyme of artemisinin biosynthesis in Artemisia annua L. Arch. Biochem. Biophys. 381, 173–180.

Mercke, P., Kappers, I.F., Verstappen, F.W.A., Vorst, O., Dicke, M., Bouwmeester, H.J., 2004. Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. Plant Physiol. 135, 2012–2024.

Nagegowda, D.A., Gutensohn, M., Wilkerson, C.G., Dudareva, N., 2008. Two nearly identical terpene synthases catalyze the formation of nerolidol and linalool in snapdragon flowers. Plant J. 55, 224–239.

O'Maille, P.E., Chappell, J., Noel, J.P., 2006. Biosynthetic potential of sesquiterpene synthases: Alternative products of tobacco 5-epi-aristolochene synthase. Arch. Biochem. Biophys. 448, 73–82.

O'Maille, P.E., Malone, A., Dellas, N., Hess, B.A., Smentek, L., Sheehan, I., Greenhagen, B.T., Chappell, J., Manning, G., Noel, J.P., 2008. Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. Nat. Chem. Biol. 4, 617–623.

Pechous, S.W., Whitaker, B.D., 2004. Cloning and functional expression of an (E, E)-alpha-farnesene synthase cDNA from peel tissue of apple fruit. Planta 219, 84–94.

Peters, R.J., Croteau, R.B., 2003. Alternative termination chemistries utilized by monoterpene cyclases: chimeric analysis of bornyl diphosphate, 1, 8-cineole, and sabinene synthases. Arch. Biochem. Biophys. 417, 203–211.

Phillips, M.A., Wildung, M.R., Williams, D.C., Hyatt, D.C., Croteau, R., 2003. CDNA isolation, functional expression, and characterization of (+)-alpha-pinene synthase and (−)-alpha-pinene synthase from loblolly pine (Pinus taeda): stereocontrol in pinene biosynthesis. Arch. Biochem. Biophys. 411, 267–276.

Phillips, D.R., Rasbery, J.M., Bartel, B., Matsuda, S.P.T., 2006. Biosynthetic diversity in plant triterpene cyclization. Curr. Opin. Plant Biol. 9, 305–314.

Picaud, S., Brodelius, M., Brodelius, P.E., 2005. Expression, purification and characterization of recombinant (E)-beta-farnesene synthase from Artemisia annua. Phytochemistry 66, 961–967.

Picaud, S., Olsson, M.E., Brodelius, M., Brodelius, P.E., 2006. Cloning, expression, purification and characterization of recombinant (+)-germacrene D synthase from Zingiber officinale. Arch. Biochem. Biophys. 452, 17–28.

Pichersky, E., Lewinsohn, E., Croteau, R., 1995. Purification and characterization of S-linalool synthase, an enzyme involved in the production of floral scent in Clarkia breweri. Arch. Biochem. Biophys. 316, 803–807.

Portnoy, V., Benyamini, Y., Bar, E., Harel-Beja, R., Gepstein, S., Giovannoni, J.J., Schaffer, A.A., Burger, J., Tadmor, Y., Lewinsohn, E., Katzir, N., 2008. The molecular and biochemical basis for varietal variation in sesquiterpene content in melon (Cucumis melo L.) rinds. Plant Mol. Biol 66, 647–661.

Prosser, I., Phillips, A.L., Gittings, S., Lewis, M.J., Hooper, A.M., Pickett, J.A., Beale, M.H., 2002. (+)-(10R)-germacrene A synthase from goldenrod, Solidago canadensis; cDNA isolation, bacterial expression and functional analysis. Phytochemistry 60, 691–702.

Prosser, I.M., Adams, R.J., Beale, M.H., Hawkins, N.D., Phillips, A.L., Pickett, J.A., Field, L.M., 2006. Cloning and functional characterisation of a cis-muuroladiene synthase from black peppermint (Mentha x piperita) and direct evidence for a chemotype unable to synthesise farnesene. Phytochemistry 67, 1564–1571.

Prosser, I., Altug, I.G., Phillips, A.L., Konig, W.A., Bouwmeester, H.J., Beale, M.H., 2004. Enantiospecific (+)- and (−)-germacrene D synthases, cloned from goldenrod, reveal a functionally active variant of the universal isoprenoid-biosynthesis aspartate-rich motif. Arch. Biochem. Biophys. 432, 136–144.

Rajaonarivony, J.I.M., Gershenzon, J., Croteau, R., 1992. Characterization and mechanism of (4S)-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (Mentha x piperita). Arch. Biochem. Biophys. 296, 49–57.

Rising, K.A., Starks, C.M., Noel, J.P., Chappell, J., 2000. Demonstration of germacrene A as an intermediate in 5-epi-aristolochene synthase catalysis. J. Am. Chem. Soc. 122, 1861–1866.

Ro, D.K., Ehlting, J., Keeling, C.I., Lin, R., Mattheus, N., Bohlmann, J., 2006. Microarray expression profiling and functional characterization of AtTPS genes: Duplicated Arabidopsis thaliana sesquiterpene synthase genes At4g13280 and At4g13300 encode root-specific and wound-inducible (Z)-gamma-bisabolene synthases. Arch. Biochem. Biophys. 448, 104–116.

Roeder, S., Hartmann, A.M., Effmert, U., Piechulla, B., 2007. Regulation of simultaneous synthesis of floral scent terpenoids by the 1, 8-cineole synthase of Nicotiana suaveolens. Plant Mol. Biol. 65, 107–124.

Rynkiewicz, M.J., Cane, D.E., Christianson, D.W., 2001. Structure of trichodiene synthase from Fusarium sporotrichioides provides mechanistic inferences on the terpene cyclization cascade. Proc. Natl. Acad. Sci. USA 98, 13543–13548.

Rynkiewicz, M.J., Cane, D.E., Christianson, D.W., 2002. X-ray crystal structures of D100E trichodiene synthase and its pyrophosphate complex reveal the basis for terpene product diversity. Biochemistry 41, 1732–1741.

Savage, T.J., Croteau, R., 1993. Biosynthesis of monoterpenes – regiochemistry and stereochemistry of (+)-3-carene biosynthesis. Arch. Biochem. Biophys. 305, 581–587.

Schnee, C., Köllner, T.G., Gershenzon, J., Degenhardt, J., 2002. The maize gene terpene synthase 1 encodes a sesquiterpene synthase catalyzing the formation of (E)-beta-farnesene, (E)-nerolidol, and (E, E)-farnesol after herbivore damage. Plant Physiol. 130, 2049–2060.

Schnee, C., Köllner, T.G., Held, M., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc. Natl. Acad. Sci. U. S. A. 103, 1129–1134.

Seemann, M., Zhai, G.Z., de Kraker, J.W., Paschall, C.M., Christianson, D.W., Cane, D.E., 2002. Pentalenene synthase. Analysis of active site residues by site-directed mutagenesis. J. Am. Chem. Soc. 124, 7681–7689.

Shah, F.H., Cha, T.S., 2000. A mesocarp-and species-specific cDNA clone from oil palm encodes for sesquiterpene synthase. Plant Sci. 154, 153–160.

Sharon-Asa, L., Shalit, M., Frydman, A., Bar, E., Holland, D., Or, E., Lavi, U., Lewinsohn, E., Eyal, Y., 2003. Citrus fruit flavor and aroma biosynthesis: isolation, functional characterization, and developmental regulation of Cstps1, a key gene in the production of the sesquiterpene aroma compound valencene. Plant J. 36, 664–674.

Shimada, T., Endo, T., Fujii, H., Hara, M., Ueda, T., Kita, M., Omura, M., 2004. Molecular cloning and functional characterization of four monoterpene synthase genes from Citrus unshiu Marc. Plant Sci. 166, 49–58.

Shimada, T., Endo, T., Fujii, H., Hara, M., Omura, M., 2005a. Isolation and characterization of (E)-beta-ocimene and 1, 8 cineole synthases in Citrus unshiu Marc. Plant Sci. 168, 987–995.

Shimada, T., Endo, T., Fujii, H., Omura, M., 2005b. Isolation and characterization of a new d-limonene synthase gene with a different expression pattern in Citrus unshiu Marc. Sci. Hortic. 105, 507–512.

Shelton, D., Zabaras, D., Chohan, S., Wyllie, S.G., Baverstock, P., Leach, D., Henry, R., 2004. Isolation and partial characterisation of a putative monoterpene synthase from Melaleuca alternifolia. Plant Physiol. Biochem. 42, 875–882.

Shishova, E.Y., Di Costanzo, L., Cane, D.E., Christianson, D.W., 2007. X-ray crystal structure of aristolochene synthase from Aspergillus terreus and evolution of templates for the cyclization of farnesyl diphosphate. Biochemistry 46, 1941–1951.

Starks, C.M., Back, K., Chappell, J., Noel, J.P., 1997. Structural basis for cyclic terpene biosynthesis by tobacco 5-epi-aristolochene synthase. Science 277, 1815–1820.

Steele, C.L., Crock, J., Bohlmann, J., Croteau, R., 1998. Sesquiterpene synthases from grand fir (Abies grandis) – Comparison of constitutive and wound-induced activities, and cDNA isolation, characterization and bacterial expression of delta-selinene synthase and gamma-humulene synthase. J. Biol. Chem. 273, 2078–2089.

Tan, X.P., Liang, W.Q., Liu, C.J., Luo, P., Heinstein, P., Chen, X.Y., 2000. Expression pattern of (+)-delta-cadinene synthase genes and biosynthesis of sesquiterpene aldehydes in plants of Gossypium arboreum L. Planta 210, 644–651.

Tarshis, L.C., Yan, M.J., Poulter, C.D., Sacchettini, J.C., 1994. Crystal-structure of recombinant farnesyl diphosphate synthase at 2.6-Angstrom resolution. Biochemistry 33, 10871–10877.

Tarshis, L.C., Proteau, P.J., Kellogg, B.A., Sacchettini, J.C., Poulter, C.D., 1996. Regulation of product chain length by isoprenyl diphosphate synthases. Proc. Natl. Acad. Sci. USA 93, 15018–15023.

Tholl, D., Chen, F., Petri, J., Gershenzon, J., Pichersky, E., 2005. Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from Arabidopsis flowers. Plant J. 42, 757–771.

Tholl, D., 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Curr. Opin. Plant Biol. 9, 297–304.

Townsend, B.J., Poole, A., Blake, C.J., Llewellyn, D.J., 2005. Antisense suppression of a (+)-delta-cadinene synthase gene in cotton prevents the induction of this defense response gene during bacterial blight infection but not its constitutive expression. Plant Physiol. 138, 516–528.

Trapp, S.C., Croteau, R.B., 2001. Genomic organization of plant terpene synthases and molecular evolutionary implications. Genetics 158, 811–832.

Turner, G., Gershenzon, J., Nielson, E.E., Froehlich, J.E., Croteau, R., 1999. Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. Plant Physiol. 120, 879–886.

Van de Peer, Y., De Wachter, Y., 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. 10, 569–570.

Van der Hoeven, R.S., Monforte, A.J., Breeden, D., Tanksley, S.D., Steffens, J.C., 2000. Genetic control and evolution of sesquiterpene biosynthesis in *Lycopersicon esculentum* and *L. Hirsutum*. Plant Cell 12, 2283–2294.

Van Geldre, E., De Pauw, I., Inze, D., Van Montagu, M., Van den Eeckhout, E., 2000. Cloning and molecular analysis of two new sesquiterpene cyclases from *Artemisia annua* L.. Plant Sci. 158, 163–171.

Van Schie, C.C.N., Haring, M.A., Schuurink, R.C., 2007. Tomato linalool synthase is induced in trichomes by jasmonic acid. Plant Mol. Biol. 64, 251–263.

Vedula, L.S., Rynkiewicz, M.J., Pyun, H.J., Coates, R.M., Cane, D.E., Christianson, D.W., 2005. Molecular recognition of the substrate diphosphate group governs product diversity in trichodiene synthase mutants. Biochemistry 44, 6153–6163.

Wagschal, K., Savage, T.J., Croteau, R., 1991. Isotopically sensitive branching as a tool for evaluating multiple product formation by monoterpene cyclases. Tetrahedron 47, 5933–5944.

Wallaart, T.E., Bouwmeester, H.J., Hille, J., Poppinga, L., Maijers, N.C.A., 2001. Amorpha-4, 11-diene synthase: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin. Planta 212, 460–465.

Wheeler, C.J., Croteau, R., 1986. Monoterpene cyclases – use of the noncyclizable substrate-analog 6, 7-dihydrogeranyl pyrophosphate to uncouple the isomerization step of the coupled isomerization cyclization reaction. Arch. Biochem. Biophys. 246, 733–742.

Wheeler, C.J., Croteau, R., 1987. Direct demonstration of the isomerization component of the monoterpene cyclase reaction using a cyclopropylcarbinyl pyrophosphate substrate-analog. Proc. Natl. Acad. Sci. USA 84, 4856–4859.

Whittington, D.A., Wise, M.L., Croteau, R., Christianson, D.W., 2002a. Insights into monoterpene cyclization reactions in biology: crystal structure of (+)-bornyl diphosphate synthase. Biochemistry 41, 8973.

Whittington, D.A., Wise, M.L., Urbansky, M., Coates, R.M., Croteau, R.B., Christianson, D.W., 2002b. Bornyl diphosphate synthase: Structure and strategy for carbocation manipulation by a terpenoid cyclase. Proc. Natl. Acad. Sci. USA 99, 15375–15380.

Williams, D.C., McGarvey, D.J., Katahira, E.J., Croteau, R., 1998. Truncation of limonene synthase preprotein provides a fully active 'Pseudomature' form of this monoterpene cyclase and reveals the function of the amino-terminal arginine pair. Biochemistry 37, 12213–12220.

Wise, M.L., Savage, T.J., Katahira, E., Croteau, R., 1998. Monoterpene synthases from common sage (*Salvia officinalis*) – cDNA isolation, characterization, and functional expression of (+)-sabinene synthase, 1, 8-cineole synthase, and (+)-bornyl diphosphate synthase. J. Biol. Chem. 273, 14891–14899.

Wise, M.L., Croteau, R., 1999. Comprehensive Natural Products Chemistry, Isoprenoids Including Caroteinoids and Steroids, vol. 2. Elsevier, Amsterdam. pp. 97–135.

Wu, S.Q., Schoenbeck, M.A., Greenhagen, B.T., Takahashi, S., Lee, S.B., Coates, R.M., Chappell, J., 2005. Surrogate splicing for functional analysis of sesquiterpene synthase genes. Plant Physiol. 138, 1322–1333.

Xu, R., Fazio, G.C., Matsuda, S.P.T., 2004. On the origins of triterpenoid skeletal diversity. Phytochemistry 65, 261–291.

Yang, T., Li, J., Wang, H.X., Zeng, Y., 2005. A geraniol-synthase gene from *Cinnamomum tenuipilum*. Phytochemistry 66, 285–293.

Yoshikuni, Y., Martin, V.J.J., Ferrin, T.E., Keasling, J.D., 2006a. Engineering cotton (+)-delta-cadinene synthase to an altered function: Germacrene D-4-ol synthase. Chem. Biol. 13, 91–98.

Yoshikuni, Y., Ferrin, T.E., Keasling, J.D., 2006b. Designed divergent evolution of enzyme function. Nature 440, 1078–1082.

Yoshioka, H., Yamada, N., Doke, N., 1999. CDNA cloning of sesquiterpene cyclase and squalene synthase, and expression of the genes in potato tuber infected with *Phytophthora infestans*. Plant Cell Physiol. 40, 993–998.

Yu, F.N., Okamto, S., Nakasone, K., Adachi, K., Matsuda, S., Harada, H., Misawa, N., Utsumi, R., 2008a. Molecular cloning and functional characterization of alpha-humulene synthase, a possible key enzyme of zerumbone biosynthesis in shampoo ginger (*Zingiber zerumbet* Smith). Planta 227, 1291–1299.

Yu, F., Harada, H., Yamasaki, K., Okamoto, S., Hirase, S., Tanaka, Y., Misawa, N., Utsumi, R., 2008b. Isolation and functional characterization of a beta-eudesmol synthase, a new sesquiterpene synthase from *Zingiber zerumbet* Smith. FEBS Lett. 582, 565–572.

Yuan, J.S., Köllner, T.G., Wiggins, G., Grant, J., Degenhardt, J., Chen, F., 2008. Molecular and genomic basis of volatile-mediated indirect defense against insects in rice. Plant J. 55, 491–503.

Yuba, A., Yazaki, K., Tabata, M., Honda, G., Croteau, R., 1996. CDNA cloning, characterization, and functional expression of 4S-(−)-limonene synthase from *Perilla frutescens*. Arch. Biochem. Biophys. 332, 280–287.

Zavala-Paramo, G., Chavez-Moctezuma, M.P., Garcia-Pineda, E., Yin, S., Chappell, J., Lozoya-Gloria, E., 2000. Isolation of an elicitor-stimulated 5-epi-aristolochene synthase gene (gPEAS1) from chili pepper (*Capsicum annuum*). Physiol. Plant. 110, 410–418.

Zhou, K., Peters, R.J., 2009. Investigating the conservation pattern of a putative second terpene synthase divalent metal binding motif in plants. Phytochemistry 70, 366–369.

Jörg Degenhardt is professor at Martin Luther University Halle-Wittenberg. He studied biology at Ruhr-University Bochum and pursued his Ph. D. in plant molecular biology under the supervision of Dr. Elaine Tobin at University of California at Los Angeles, USA. His studies on phytochrome regulation of genes were supported by a fellowship of the German Academic Exchange Service (DAAD). Jörg Degenhardt continued his education in plant molecular biology, biochemistry, and molecular genetics as a postdoctoral fellow at Cornell University, USA, under the guidance of Dr. Leon Kochian and Dr. Steve Howell. He started his work on plant terpene biosynthesis at the Max Planck Institute for Chemical Ecology in Jena, Germany, in the Department of Dr. Jonathan Gershenzon. With the help of a personal grant by the Claussen-Simon foundation, he acquired his *venia legendi* at Friedrich Schiller University in Jena in 2007. He was appointed full professor of Pharmaceutical Biology at Halle University in 2008. His research interests are the biosynthesis of plant terpenes, their biological functions and pharmaceutical values.

Born in Eisenach, Germany, in 1971, **Tobias G. Köllner** studied biochemistry and molecular biology at Friedrich-Schiller-University Jena. He earned his PhD degree in plant biochemistry with Professor J. Gershenzon at Max-Planck-Institute for Chemical Ecology in 2004, and did postdoctoral work in the same laboratory. In 2008, he took a position as scientist in the Department of Pharmaceutical Biotechnology at Martin-Luther-University Halle-Wittenberg. His research focuses on structure-function relationships and mechanisms of enzymes involved in terpene biosynthesis.

Jonathan Gershenzon is the Director of the Department of Biochemistry at the Max Planck Institute for Chemical Ecology in Jena, Germany and Honorary Professor in the Faculty of Biology and Pharmacy at Friedrich-Schiller University. He was born and raised in the United States, completing his Ph.D. at the University of Texas at Austin with Tom Mabry on the terpenoid chemistry of *Helianthus*. He was a post-doctoral researcher with Rod Croteau at Washington State University, Pullman, where he was first introduced to the magic and mysteries of terpene synthases. His current research interests include studying the roles of terpene synthase products and other plant metabolites in defense against enemies and investigating the biosynthesis and regulation of plant defense compounds.