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# Trans-specific S-RNase and SFB alleles in *Prunus* self-incompatibility haplotypes

Bruce G. Sutherland · Kenneth R. Tobutt · Timothy P. Robbins

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Abstract Self-incompatibility in the genus *Prunus* is controlled by two genes at the S-locus, S-RNase and SFB. Both genes exhibit the high polymorphism and high sequence diversity characteristic of plant self-incompatibility systems. Deduced polypeptide sequences of three myrobalan and three domestic plum S-RNases showed over 97% identity with S-RNases from other Prunus species, including almond, sweet cherry, Japanese apricot and Japanese plum. The second intron, which is generally highly polymorphic between alleles was also remarkably well conserved within these S-allele pairs. Degenerate consensus primers were developed and used to amplify and sequence the co-adapted polymorphic SFB alleles. Sequence comparisons also indicated high degrees of polypeptide sequence identity between three myrobalan and the three domestic plum SFB alleles and the corresponding Prunus SFB alleles. We discuss these trans-specific allele identities in terms of S-allele function, evolution of new allele specificities and Prunus taxonomy and speciation.

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B. G. Sutherland (⊠) · K. R. Tobutt East Malling Research, New Road, East Malling, Kent ME19 6BJ, UK e-mail: bruce.sutherland@yahoo.co.uk; bruce.sutherland@emr.ac.uk

B. G. Sutherland · T. P. Robbins Plant Science Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK **Keywords** *Prunus* · Self-incompatibility · SFB · *S*-RNase · Trans-specific evolution

## Introduction

Self-incompatibility in *Prunus*, to which the stone fruits belong, is controlled by a multiallelic *S*-locus acting gamet-ophytically in pollen. A pollen grain is rejected if its *S*-allele matches one of those in the style, whereas pollen with an *S*-allele not matching either in the style is accepted (Crane and Lawrence 1929). The gametophytic *S*-locus is bipartite, containing an element expressing specificity in the style (stylar-*S*) and another element expressed in pollen (pollen-*S*) (Lewis 1949). The stylar-*S* component in *Prunus* encodes a polymorphic ribonuclease, *S*-*RNase* (Bošković and Tobutt 1996). A pollen-*S* gene for *Prunus* has been identified more recently, which encodes an *S*-linked F-box protein, or SFB (Ushijima et al. 2003).

To date, molecular techniques have detected some 19 distinct S-RNase alleles in sweet cherry (P. avium) (Sonneveld et al. 2001, 2003; De Cuyper et al. 2005), 29 alleles in almond (P. dulcis) (Ortega et al. 2005), nine alleles in Japanese apricot (P. mume) (Tao et al. 2002), 15 alleles in European apricot (P. armeniaca) (Halász et al. 2005) and 14 alleles in Japanese plum (P. salicina) (Beppu et al. 2003). The origin of S-locus allelic diversity has interested geneticists for many years. The S-locus is under balancing selection (Wright 1939), in which rare haplotypes have a selective advantage and are less likely to be lost by genetic drift. Thus the S-locus sustains a marked degree of allelic diversity, unlike the majority of plant genes, but akin to other self/nonself recognition systems such as plant pathogen resistance genes, fungal mating types (Wu et al. 1998) or the mammalian MHC complex (Klein 1987).

For most genes, new alleles can arise from the progressive accumulation of non-synonymous point mutations. Generation of new S-alleles is unlikely to be so straightforward, because of the co-adaptation of the two genes at the S-locus in S-RNase-based self-incompatibility (Lewis 1949). Correct function of the S-locus depends upon interaction of stylar-S and pollen-S gene products in pollen tubes. Thus the generation of a new S-allele specificity requires complementary mutations to occur in both stylar-S and pollen-S components. Mutation at only one or other of the components could cause breakdown of the self-incompatibility system leading to self-compatibility, as demonstrated by the chimeric S-RNase experiments of Zurek et al. (1997). Precisely how complementarity is maintained during S-allele evolution has not been satisfactorily explained.

Molecular techniques have allowed researchers to characterise S-RNases in several important and widely separated plant families, including the Rosaceae, Solanaceae and Scrophulariaceae. As a group, S-RNases share greater similarity with each other than with plant non-S RNases or fungal RNases (Despres et al. 1994; Ushijima et al. 1998; Steinbachs and Holsinger 2002), possibly indicating a single common origin of all plant S-RNases (Igic and Kohn 2001; Roalson and McCubbin 2003). Within plant families, S-RNases often show interspecific shared polymorphism, in which alleles from different species can be more similar than alleles in the same species (Ioerger et al. 1990). As a consequence of balancing selection, individual S-RNase lineages can persist for very long times in a population and in the subsequent species into which it may diverge. Thus very similar S-alleles can be shared by relatively divergent species (Richman and Kohn 1996).

Pollen-S gene sequence data from S-RNase based incompatibility systems is more restricted, though candidates have been identified in the Scrophulariaceae (Lai et al. 2002) and the Solanaceae (Sijacic et al. 2004). Each encodes an F-box protein expressed in pollen that may function by binding non-self S-RNases and catalysing their polyubiquitination, prior to proteolysis by the 26S proteasome (Ushijima et al. 2003). Proof of SFB function in pollen rejection was obtained by investigating several welldocumented self-compatible mutants. Thus the Sf allele in P. mume was caused by a 6.8 kb insertion into the SFB coding region (Ushijima et al. 2004); and Sonneveld et al. (2005) found the *P. avium* self-compatible mutant  $S_3'$  was found to be caused by a complete deletion of  $S_3$  SFB (Sonneveld et al. 2005) and a 4 bp frameshift in  $S_4'$ (Ushijima et al. 2004; Sonneveld et al. 2005). Like S-RNase, Pollen-S also shows patterns of trans-specific evolution (Ikeda et al. 2003; Qiao et al. 2004).

Our work on self-incompatibility has focused on *P. avium* and *P. dulcis*, by developing methodologies for identifying *S*-alleles (Sonneveld et al. 2001, 2003;

Sutherland et al. 2004a), genotyping Prunus cultivars (Tobutt et al. 2001; Ortega et al. 2005), phylogenetic studies (Sonneveld 2002), and characterising pollen-S in Prunus (Sonneveld et al. 2005; Ortega et al. 2006). These approaches have been recently extended to hexaploid domestic plum (P. domestica) and diploid myrobalan (P. cerasifera) (Sutherland 2005; Sutherland et al. 2004b, 2007). Six S-RNases were identified from the two plum species, which showed very high sequence identity with those of other *Prunus* species, far higher than is typically observed in interspecific comparisons. Their corresponding SFB alleles also revealed high sequence identities with those available in databases. These are outstanding examples of "interspecific shared polymorphism", or "transspecific identity", first identified in S-RNases by Ioerger et al. (1990), where sequence identities between alleles from different species exceed that found between alleles of the same species. This paper focuses on the deduced polypeptide sequences and introns of the trans-specific S-RNase alleles and on the deduced polypeptides of the associated SFB alleles, and discusses their possible evolutionary significance.

#### **Experimental procedures**

## Plant materials

The following accessions were used for amplifying and sequencing *S-RNase* and *SFB* sequences: Myrobalan (*P. cerasifera*) accessions M1 ( $S_3S_4$ ), M3 ( $S_3S_6$ ), P2944 ( $S_3S_4$ ) and C34-1 ( $S_9S_{10}$ ) were held at East Malling Research. Domestic plum (*P. domestica*) cultivars "Blue Rock" and "Verity" came from the National Fruit Collection at Brogdale, UK. Almond cultivar (*P. dulcis*) CEBAS-I ( $S_{13}S_{20}$ ) was provided by CEBAS-CSIC Murcia, Spain; "Gabaix" ( $S_{10}S_{24}$ ) and "Pestañeta" ( $S_{12}S_{23}$ ) were provided by SIA-SDA Zaragoza, Spain. Japanese plums (*P. salicina*) "Burmosa" ( $S_aS_b$ ), "Formosa" ( $S_bS_d$ ) and "Bonnie" ( $S_gS_h$ ) were provided by The National Institute of Fruit Tree Science, Tsukuba, Japan. Japanese apricot (*P. mume*) cultivar "Bungo" ( $S_7$ ) was also provided by the National Institute of Fruit Tree Science.

#### DNA extraction

Genomic DNA was extracted from ground buds or young leaves. A miniprep protocol of the CTAB extraction technique described by Doyle and Doyle (1987) was used, with the following modifications: 2% PVP 40 (polyvinyl pyrollidone) (Sigma, St Louis, USA) was added to the extraction buffer, and the final concentration of  $\beta$ -mercaptoethanol (Sigma, St Louis, USA) was raised to 1%.

Consensus primer PCR for characterising *Prunus* S-RNase coding regions and second introns

S-RNase alleles were amplified from genomic DNA of the following species and accessions: P. cerasifera M3 and C3-41; P. domestica "Blue Rock" and "Verity"; P. dulcis "Pestañeta"; P. mume "Bungo"; P. salicina "Bonnie". Degenerate consensus primers hybridising to the C1 and C5 conserved regions of Prunus S-RNases were used: forward primer EM-PC1consFD (5'-TTTCARTTTGTKCAACAR TGGC-3') and reverse primer EM-PC5consRD (Sutherland et al. 2004a). Reaction conditions were as described in Sutherland et al. 2004a. Samples were pooled and mixed with 4  $\mu$ l loading buffer, and separated by electrophoresis at 100 V on 1.5% TAE agarose gels. After the gels were stained in 0.5 µg/ml ethidium bromide, bands were excised and PCR products were purified using the Oiagen OIAEX II DNA Extraction Kit. Purified PCR products were cloned using the Invitrogen Original TA Cloning Kit (Invitrogen, Carlsbad, USA). E. coli colonies containing correctly sized inserts were identified with M13 primers and sequenced at Imperial College Advanced Biotechnology Centre, London, UK. Each allele was sequenced in triplicate using independent colonies.

Consensus primer PCR for characterising S-RNase first introns

First introns of *P. domestica*  $S_6$  ("Blue Rock") and *P. domestica*  $S_q$  ("Verity") were amplified using a modified version of the PCR technique described by Sonneveld et al. (2003). The four introns were chosen on the basis of their taxonomic distance: P. domestica and P. salicina are very closely related and are within the same subfamily (Prunus), whereas P. domestica and P. avium are taxonomically more distant. We were interested to see if there was a relationship between taxonomic distance of the species and sequence distance of the introns. PaIconsF was used as a consensus forward primer in conjunction with new allele-specific reverse primers hybridising with the variable regions of the S-RNase sequence:  $DpS_6rev$  (5'-CAGCTGAGTATTCGC CTGTAC-3') and DpSorev (5'-CATGTAACAGCTGAG TGCTCTTAGCT-3'). The PCR reaction contained 10-20 ng DNA,  $2 \mu l 10 \times PCR$  Buffer (Qiagen),  $2.5 \text{ mM MgCl}_2$ , 0.2 mM dNTPs, 0.5 U Taq polymerase (Qiagen) and 0.25 µM of forward and reverse primers, giving a final reaction volume of 20 µl. PCR reaction conditions were: initial denaturation at 94°C for 2 min, then 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1.5 min, and a final 10 min extension at 72°C. Amplified products were purified, cloned and sequenced as described above.

Consensus primer PCR for characterising *Prunus SFB* alleles

DNA sequences of eight published Prunus SFB alleles were aligned using ClustalX (DNAStar, Madison, USA). The sequences were: P. dulcis  $S_1$  (AB092966),  $S_5$ (AB096858), S<sub>7</sub> (AB092967), S<sub>8</sub> (AB079776) (Ushijima et al. 2003), *P. mume S*<sub>1</sub> (AB081648), *S*<sub>7</sub> (AB101440), *S*<sub>9</sub> (AB101441) (Entani et al. 2003) and P. avium  $S_4$ (AB092646). Degenerate consensus primers were designed to anneal with the conserved regions, spanning approximately 85% of the coding region as identified by Ushijima et al. (2003): EM-SFB-cds-for 5'-YGACATC CTAGYAAGACTDMCWG-3' and EM-SFB-cds-rev 5'-ACYTGYTTRGATTCRTAATYMCMCAA-3'. SFB sequences were amplified from the following species and accessions; P. cerasifera M3 and C3-41; P. domestica "Blue Rock" and "Verity"; P. dulcis "CEBAS-I", "Gabaix" and "Pestañeta"; P. mume "Bungo"; P. salicina "Bonnie", "Burmosa" and "Formosa". PCR reactions contained 10–20 ng DNA, 2  $\mu$ l 10 × PCR buffer (Qiagen), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.5 U Taq polymerase (Qiagen) and 0.25 µM forward and reverse primers, giving a final reaction volume of 20 µl. For cloning and sequencing, reaction volumes were pooled to a final volume of 160 µl. Purification, cloning and sequencing was carried out as described above.

Database comparisons and alignments

The following *S-RNase* sequences which were already available in the EMBL database, and which gave close matches to myrobalan and domestic plum in initial searches, were used for more detailed comparisons: *P. dulcis*  $S_k$  (AF267511),  $S_{10}$  (AF454003) and  $S_{13}$  (AJ617484); *P. salicina*  $S_a$  (AB026981),  $S_d$  (AB094103) and  $S_g$ (AB093131); *P. avium*  $S_4$  (AJ635287); *P. mume*  $S_7$ (AF432850). The EMBL database has two distinct sequence classes for *P. mume*  $S_7$ . This paper refers at all times to the "Bungo" sequence (AF432850).

Genomic S-RNase sequences were divided into exons and introns and were compared with published sequences in the EMBL database using the FASTA algorithm. Exon sequences showing exceptional similarity with published *Prunus S-RNase* sequences were translated and pairwise alignments were prepared for each trans-specific pair using the MegAlign (DNAStar, Madison, USA). Deduced SFB sequences were treated in the same manner. *S-RNase* intron DNA sequences were used directly for pairwise alignment using MegAlign, and were then adjusted by eye.

# Results

# Comparison of Prunus S-RNase sequences

A total of six new *S-RNase* sequences were obtained from *P. cerasifera* and *P. domestica* (Table 1). Genomic sequence information was obtained for the first time for *P. dulcis*  $S_{12}$ , and additional coding region sequence was obtained for *P. mume*  $S_7$  and *P. salicina*  $S_g$ . Comparison of the new *S-RNase* DNA sequences with public databases revealed that each of the three *P. cerasifera S-RNases* ( $S_3$ ,  $S_9$  and  $S_{10}$ ) and three *P. domestica S-RNases* ( $S_5$ ,  $S_6$  and  $S_9$ ) had exceptionally high similarity with one or two previously reported *Prunus S-RNases*.

Polypeptide identities for the special group of six new S-RNases and their database pair allele were exceptionally

high and in a narrow range from 96 to 100% (Table 2). Identities of the six new S-RNase polypeptides with other alleles were markedly lower, and were distributed in a broader range from 71 to 85%, values typical for S-RNase comparisons. There is a large discontinuity of 11% separating the identities of the two groups, a discontinuity which separated the six new alleles from the rest. On this basis the six new alleles were termed as "trans-specific", since they each held such high identity with one (or in some cases two) S-RNases from another *Prunus* species. Figure 1 (a–f) presents an alignment of each new trans-specific S-RNase polypeptide from *P. cerasifera* and *P. domestica* with its database pair allele.

*Prunus cerasifera* accession M3 gave two *S-RNase* sequences,  $S_3$  and  $S_6$ , and the deduced polypeptide of one of them held 97.6% identity with a putative polypeptide of  $S_{12}$ 

<b>Table 1</b> Description of sixtrans-specific S-haplotypes	Species	Allele	Cultivar/accession	EMBL (S-RNase)	EMBL (SFB)
sequenced from <i>P. cerasifera</i>	P. cerasifera	S <sub>3</sub>	M3	AM746943	AM746952
specific pair alleles in other	P. dulcis	$S_{12}$	Pestañeta	AM746949	AM746959
species of <i>Prunus</i>	P. cerasifera	$S_9$	C34–1	AM746944	AM746953
	P. salicina	$S_d$	Formosa <sup>a</sup>	AB094103 <sup>b</sup>	AM746962
	P. dulcis	S <sub>13</sub>	CEBAS-I <sup>a</sup>	AJ617484 <sup>b</sup>	AM746960
	P. cerasifera	$S_{10}$	C34-1	AM746945	AM746954
	P. salicina	$S_{g}$	Bonnie	AM746950	AM746963
	P. mume	$S_7$	Bungo <sup>a</sup>	AM746951	AM746964
	P. domestica	$S_5$	Verity	AM746946	AM746955
	P. dulcis	$S_{10}$	Gabaix <sup>a</sup>	AF454003 <sup>b</sup>	AM746958
<sup>a</sup> Indicates cultivars used only	P. domestica	$S_6$	Blue Rock	AM746947	AM746956
for SFB sequencing	P. salicina	$S_a$	Burmosa <sup>a</sup>	AB026981 <sup>b</sup>	AM746961
<sup>b</sup> Indicates an <i>S-RNase</i> or <i>SFB</i>	P. domestica	$S_9$	Verity	AM746948	AM746957
sequence available previously in the databases	P. avium	$S_4$		AJ092644 <sup>b</sup>	AY649872 <sup>b</sup>

Table 2 Percentage amino acid identities for trans-specific Prunus S-RNase and SFB deduced polypeptides

	Pcer $S_3$	$Pcer S_9$	Pcer S <sub>10</sub>	$Pdom S_5$	$Pdom S_6$	$Pdom S_9$	$Psal S_a$	$Psal S_d$	$Psal S_g$	$Pmu S_7$	$Pdul S_{10}$	$Pdul S_{12}$	$Pdul S_{13}$	Pav S <sub>4</sub>
Pcer $S_3$	_	80.9	77.6	76.6	80.5	81.8	81.2	80.6	77.6	78.5	77.2	97.9	80.5	79.3
Pcer $S_9$	71.3	_	77.6	75.2	77.6	79.6	77.9	99.1	77.6	77.3	75.5	80.6	97.9	77.0
Pcer $S_{10}$	73.7	77.8	-	77.6	77.3	77.2	78.2	78.5	<b>98.8</b>	90.3	78.2	78.8	78.2	74.5
$Pdom S_5$	78.2	77.6	79.4	-	77.0	77.8	77.3	76.1	77.9	77.3	97.0	77.5	76.0	76.8
Pdom $S_6$	77.1	79.5	77.1	77.0	-	78.1	97.6	77.9	77.3	78.2	77.5	80.9	77.8	76.8
$Pdom S_9$	77.2	83.3	78.4	80.6	77.7	-	78.7	79.9	78.1	77.8	79.0	81.8	80.2	93.3
Psal $S_a$	77.1	79.5	77.1	77.0	100	79.6	_	78.2	78.2	78.5	77.6	81.5	77.9	76.1
$Psal S_d$	74.1	98.1	77.8	78.2	80.1	84.0	80.1	_	78.5	77.0	76.4	80.9	98.5	78.4
Psal $S_g$	74.5	76.4	100	79.4	77.1	78.4	77.1	77.8	_	91.2	79.1	78.8	78.2	74.8
$Pmu S_7$	73.9	75.8	98.8	78.8	76.5	77.8	77.0	75.8	98.8	-	78.5	79.2	77.3	74.3
Pdul S <sub>10</sub>	78.2	79.4	78.2	96.4	77.6	80.6	77.6	80.0	78.2	77.6	-	78.1	76.3	77.4
Pdul $S_{12}$	97.6	71.3	74.3	77.8	76.6	74.9	76.6	71.9	74.3	73.7	78.8	-	81.5	79.6
Pdul S <sub>13</sub>	75.3	96.9	79.0	80.9	82.7	85.2	82.7	97.5	79.0	78.4	81.5	75.3	-	77.8
Pav $S_4$	77.8	82.7	77.8	81.5	79.9	98.8	79.0	83.3	77.8	77.2	80.0	74.3	84.0	-

Values for SFBs are in the upper right half and for S-RNases in the lower left. Trans-specific percentages are marked in bold

A Pcer-S3 : Pdul-S12 :	***         ***         #           QFVQQWPPTNCRVRIKQPC           QFVQQWPPTNCRVRIKQPC           C1	* ******* * # SNPRPLQYFTIHGLWPSNYSNPT SNPRPLQY <u>FTIHGLWPS</u> NYSNPT C2	* # ##### ## * # * KPSNCNGSKFEAKKLSPEMRTKLKK KPSNCNGS <u>KFEAMKLSPEMT</u> KLKK RHV	**** SWPDVESGNDTKFWAGEWNKHGKCS SWPDVESGNDTKFWAG <u>EWNKHGKCP</u> C3
Pcer-S3 : Pdul-S12 :	#**** * **#* EQTLNQMQYFERSFAMWKS EQTLNQMQYFERSFAMWKS	ANITEILKNASIVPSATKTWKYS NITEILKNASIVPSATGTWKYS RC4	** #** # * ** DIVSPIKAVTKTTPLLRCKYDLSHP DIVSPIKAVTKTTPLLRCKYDLSHP	## ** ** NKPELLHEVV NK <u>PELLHEVV</u> C5
B Pcer-S9 Psal-Sd Pdul-S13	• • • • • • • • • • • • • • • • •	RPLQNFTIHGLWPSNYSNPTMI RPLQNFTIHGLWPSNYSNPTMI RPLQN <u>FTIHGLWPS</u> NYSNPT <u>M</u> I C2	* # ##### ##* # * PSKCTGSRFKKENVYPQLRSKMKIS PSKCTGSRFKKENVYPQLRSKMKIS PSKCTGS <u>RFKKENVYPQLRS</u> KMKIS <b>RHV</b>	**** #** #** *** *** WPDVESGNDTRFWESEWNKHGTCS WPDV©SGNDTRFWESEWNKHGTCS WPDVESGNDTRFWES <u>EWNKHGTCS</u> C3
Pcer-S9 Psal-Sd Pdul-S13	#**** * *** : EGTLNQVQYFQRSHAMWRS : EGTLNQVQYFQRSHAMWRS : EDTLNQVQYFQRSHAMWRS	HNVTEILRNASIAPHPTQTWS HNVTEILRNASIMPHPTQTWS HNVTEILRNASIMPHPTQTWS RH4	** #** # * ** KSDIVSPIKTATKRTPLIRCKYDKK SDIVSPIKTATKRTPLIRCKYDKK SDIVSPIKTATKRTPLERCKYDKK	tv tv TQLLHEVV TQLLHEVV TQLLHEVV C5
C Pcer-S10 Psal-Sg Pmum-S7	<pre>*** *#* # * VQQWPPTTCRVRKRPCTKS * VQQWPPTTCRVRKRPCTKS * VQQWPPTTCRVRKRPCTKS C1</pre>	RPLQIFTIHGLWPSNYSNPWKI RPLQIFTIHGLWPSNYSNPWKI RPLQI <u>FTIHGLWPS</u> NYS <b>D</b> PWKI C2	* # ###### ##* # * PSNCSGSQFKDGKVYPQLRSKLKKS PSNCSGSQFKDGKVYPQLRSKLKKS PSNCSGS <u>QFKDGKVYPQLRS</u> KLKKS RHV	**** #** *** *** WPDVESGNDTKFWEGEWNKHGTCS WPDVESGNDTKFWEG <u>EWNKHGTCS</u> WPDVESGNDTKFWEG <u>EWNKHGTCS</u> C3
Pcer-S10 Psal-Sg Pmum-S7	#**** * * **#* : EEKLNQMQYFERSHNMWRS : EEKLNQMQYFERSHNMWRS : EEKLNQMQYFERSHNMWRS	YNITEVLKNASIVPHPTQTWSY YNITEVLKNASIVPHPTQTWSY NITEVLKNASIVPHPTQTWSY RC4	** #** # * ** (SDIVSPIKTATGRTPTLRCRTDPA (SDIVSPIKTATGRTPTLRCRTDPA (SDIVSPIKTATGRTPTLRCRTDPA	# ** ** HPNVELLHEVV HPNVELLHEVV C5
D Pdom-S5 Pdul-S10	*** *#* # : VQQNPPTNCRVRLKRPCSK : <u>VQQNPP</u> TNCRVRLKRPCSK C1	PRPLQNFTIHGLWPSNFSNPTK PRPLQN <u>FTIHGLWPS</u> N¥SNPTK C2	* # ##### ##* # * PSNCNGSKYEDRKVYPKLRSKLKRS PSNCNG <mark>A</mark> KYEDRKVYPKLRSKLKRS RHV	**** #** *** *** WPDVESGNDTRFWEGEWNKHGRCS WPDVESGNDTRFWEG <u>EWNKHGRCS</u> C3
Pdom-S5 Pdul-S10	#**** * * **#* : EQILNQMQYFEVSHDMWLS : EQTLNQMQYFEVSHDMWLS	YNITEILRNASIVPHPTQTWTY YNITEILRNASIVPHPTQTWTY RC4	** #** # * ** SDIVSPIKAATKRTPLLRCKIDTAT SDIVSPIKAATKRTPLERCKIDTAT	# ** ** NTELLHEVV NTQLLHEVV C5
<b>E</b> Pdom-S6 : Psal-Sa :	FQFVQQWPPTNCRVRVKRPC: FQFVQQWPPTNCRVRVKRPC: C1	* ******* * # SNPRPLQYFTIHGLWPSNYSNPR SNPRPLQY <u>FTIHGLWPS</u> NYSNPR C2	* # ##### ##* # * MPSNCTGSQFKKQNLYPYMQSKLKI MPSNCTGSQFKKQNLYPYMQSKLKI RHV	#** #** *** *** SWPDVESGNDTKFWEGEWNKHGTCS SWPDVESGNDTKFWEG <u>EWNKHGTCS</u> C3
Pdom-S6 : Psal-Sa :	#**** * * **#* ERTLNLMQYFQRSHAMWKSH ERTLNLMQYFQRSHAMWKSH	<pre>** ** * # # * MITEILKNASIVPHPTQTWKYSD NITEILKNASIVPHPTQTWKYSD RC4</pre>	* #** # * ** # IESPIKRATKRTPVLRCKRDPVQAN IESPIKRATKRTPVLRCKRDPVQAN	TQLLHEVVFC TQLLHEVVFC C5
F Pdom-S9 : Pavi-S4 :	FQFVQQWPPTNCRVRNKPCT <u>FQFVQQWPP</u> TNCRVRNKPCT <u>C1</u>	********** KPRPLQNFTIHGLWPSNYSNPRN KPRPLQN <u>FTIHGLWPS</u> NYSNPRN C2	* # ##### ##* # * APSKCTGSLFNFRKVYPQLRSDLKI APSKCTGS <u>LFNFRKVYPQLRS</u> DLKI RHV	#** #** *** *** SWPDVESGNDTRFWESEWNKHGRCS SWPDVESGNDTRFWES <u>EWNKHGRCS</u> C3
Pdom-S9 : Pavi-S4 :	#**** * **#* EDSLNQMQYFERSHAMWISY EASLNQMQYFERSHAMWISY	** ** * * # # NITEILKNASIVPSATQNWTYSI <u>NITEILKNASIVP</u> SATKNWTYSI RC4	* * * * * * * * ** DIVSPIKRATKRTPLLRCKYDKSTQ DIVSPIKRATKRTPLLRCKYDKS <u>TQ</u>	LLHEVVFC LLHEVVFC C5

**Fig. 1** Alignments of deduced polypeptides of trans-specific S-RNases in *Prunus*. **a** *P. cerasifera*  $S_3$  and *P. dulcis*  $S_{12}$ . **b** *P. cerasifera*  $S_9$ , *P. salicina*  $S_d$  and *P. dulcis*  $S_{13}$ . **c** *P. cerasifera*  $S_{10}$ , *P. salicina*  $S_g$  and *P. mume*  $S_7$ . **d** *P. domestica*  $S_5$  and *P. dulcis*  $S_{10}$ . **e** *P. domestica*  $S_6$  and *P. salicina*  $S_d$ . **f** *P. domestica*  $S_9$  and *P. avium*  $S_4$ . The five conserved regions (C1 to C5) and the hypervariable regions (RHV) are *labelled* and *underlined* (Ushijima et al. 1998). Residues differing

from *P. dulcis* cultivar "Pestañeta" (Certal et al. 2002), with three non-conserved and one conserved amino acid differences occurring out of 169 residues in the available deduced polypeptide chain (Fig. 1a).

*Prunus cerasifera* C34-1 produced two *S*-RNase sequences, provisionally named  $S_9$  and  $S_{10}$ . The deduced polypeptide of the  $S_9$  allele showed 96.9% identity with *P. dulcis*  $S_{13}$  with five amino acid differences in a 164-residue

between polypeptides are shaded in *grey*. The amino acid sites identified as being most variable in rosaceous S-RNases are marked with #, and sites which are conserved are marked with \* (Ushijima et al. 1998). The alignment was prepared using the Clustal method and the PAM250 residue weight table in DNAStar, as defined by Dayhoff (1979). Similarity groups are C, STAPG, MILV, HRK, NDEQ, FYW

polypeptide, four being non-conservative replacements; and a 98.1% identity with *P. salicina*  $S_d$  with three non-conservative replacements in a 167-residue deduced polypeptide (Fig. 1b).

The deduced polypeptide for  $S_{10}$  had 98.8% identity with *P. mume*  $S_7$  with two conservative replacements differing between the two, and shared 100% identity with *P. salicina*  $S_g$ . (Fig. 1c).

Cloning and sequencing from *P. domestica* "Verity" identified two distinct *S-RNase* sequences,  $S_5$  and  $S_9$ . The deduced polypeptide of  $S_5$  shared 96.4% identity with *P. dulcis*  $S_{10}$ , with five conservative replacements and one non-conservative amino acid replacements out of 165 in the deduced polypeptide sequence (see Fig. 1d) The  $S_9$  deduced polypeptide held 98.8% identity with *P. avium*  $S_4$ , with only two non-conservative amino acid differences out of 167 in the available sequence (Fig. 1e). An *S-RNase* cloned from *P. domestica* "Blue Rock",  $S_6$ , was completely identical with *P. salicina*  $S_a$  when translated into a polypeptide (Fig. 1f).

# Comparison of Prunus S-RNase second intron sequences

DNA sequence alignments were made with the polymorphic second intron of the *S-RNase* alleles. Second introns are generally very difficult to align due to their extreme length and sequence polymorphism. In spite of this, the sequences and intron lengths of the trans-specific pairs were often very close (Table 3). Alignments can be viewed in Fig. 4 in the electronic version.

Two groups were discernible in the second intron comparisons: introns which showed very large divergence with other introns in the subset presented here; and those which showed high similarity with one or more introns. Percentage identities for the divergent group were always below 57.9%. Identities for the second group were much higher, from 74.1to 99.9%. Almost complete similarity was found between second introns from *P. cerasifera*  $S_9$  and *P. salicina*  $S_d$ , from *P. cerasifera*  $S_{10}$  and *P. salicina*  $S_g$  and from *P. domestica*  $S_6$  and *P. salicina*  $S_a$ . Introns from other transspecific pairs showed some divergence, caused primarily by large indels, though identities were always much higher than those found between other *Prunus S-RNase* second introns.

## S-RNase first introns

Allele-specific and consensus primers were used in combination to amplify and sequence first introns from two *P. domestica S-RNase* alleles,  $S_6$  and  $S_9$ . The new introns were aligned with their trans-specific counterparts, *P. salicina*  $S_a$ and *P. avium*  $S_4$  respectively (Fig 5 in the electronic version). Almost perfect identity was seen between the first introns of *P. domestica*  $S_6$  and *P. salicina*  $S_a$ , 99.3%. The only notable difference was a 5 bp indel. Greater differences were evident between *P. domestica*  $S_9$  and *P. avium*  $S_4$ , a 2 bp and a 47 bp indel, and five nucleotide substitutions.

## Comparison of Prunus SFB deduced polypeptides

The new consensus primers described in the "Experimental procedures" were successful in amplifying *SFB* alleles from *Prunus*, giving a total of 13 new *SFB* sequences; three from *P. cerasifera*, three from *P. domestica*, three from *P. dulcis*, three from *P. salicina* and one from *P. mume* (Table 1). Polypeptide alignments are shown sequentially in Fig. 2. Percentage identities of the new SFB polypeptides are given in Table 2. As the *S*-genotype of the material used for cloning *SFB* alleles was already known, it was possible to correlate each *SFB* sequence class as a putative *S*-allele. Exceptionally high identities were apparent between the three *P. cerasifera* and three *P. domestica* SFB alleles and their trans-specific counterparts from other *Prunus* species, ranging from 90.3 to 99.1%. Identities of non trans-specific pairs ranged from 74.3 to 81.8%.

Table 3 Percentage nucleotide identities for pairwise comparisons of trans-specific Prunus S-RNase second introns

	$PcerS_3$	$Pcer S_9$	Pcer S <sub>10</sub>	$PdomS_5$	$Pdom \ S_6$	$Pdom S_9$	$Psal S_a$	$Psal\ S_d$	$Psal\ S_g$	$Pmu S_7$	$Pdul S_{10}$	$Pdul S_{12}$	$Pdul S_{13}$	Pav $S_4$
Pcer $S_3$	_													
Pcer $S_9$	50.3	-												
Pcer S <sub>10</sub>	46.8	54.0	-											
Pdom $S_5$	48.9	54.8	53.8	-										
Pdom $S_6$	47.9	60.6	58.1	56.4	-									
Pdom $S_9$	51.0	50.3	56.3	48.9	51.0	-								
Psal $S_a$	48.3	61.6	58.1	56.4	99.9	50.3	_							
$Psal S_d$	50.0	99.6	54.2	54.1	60.6	50.3	61.3	_						
Psal $S_g$	46.9	53.9	99.9	53.5	57.8	56.1	57.8	54.1	_					
$Pmu S_7$	48.1	53.2	96.3	51.1	58.0	57.1	58.0	52.5	96.3	_				
Pdul S <sub>10</sub>	49.6	30.1	50.6	94.5	56.6	51.4	57.3	55.8	50.4	51.3	-			
Pdul S <sub>12</sub>	96.7	49.0	49.2	46.9	49.0	55.7	47.9	50.0	49.2	49.6	45.6	_		
Pdul S <sub>13</sub>	48.9	92.9	55.3	54.6	57.95	46.1	58.6	93.1	55.2	57.6	52.1	51.3	-	
Pav $S_4$														-

Trans-specific identities are marked in bold

Part - 61 2       PITUNE PART - 100 00000000000000000000000000000000	Α		
Pdu1-612       DILVALPAASILAR/ECTOX         Pdu1-612       VI         Pdu1-612       VIX         Pdu1-613       DIX         Pdu1-613       DIX         Pdu1-614	Pcer-S3	DILVRLPAKSLLRFLCTCKSWSDLIGSSSFVSTHLHR	NVRKHAHVYLLCLHHPNVRRQVDPDDPYVKQEFQWSLFSNETFEE
F-box     VI       Point - 512     YTKLENDLOG FKYTYLIGGEN KLYCLIGEN K	Pdul-S12	DILVRLPAKSLLRFLCTCKSWSDLIGSSSFVSMHLHR	NVRKHAHVYLLCLHHPNV <u>RRQVDPDDPYVKQE</u> FQWSLFSNETFEE
Part - 612       I VALUATION ON ALVOID DI LANDON ALVOID ALVOIDALVOID ALVOID ALVOID ALVOIDALVOID ALVOID ALVOID		F-box	V1
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Part - 63       Internet bit with a second with second with a second with a second with a second with			
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Peter-53 : CIDVIENDIC_LLPRYYCOUDDAANDUWVIENERWEDUC/PIERDEGO GOSTIONUL_LERADJONCUL_LERADJONUL_LERADJ	Paul-Siz	: KINKMALAVEVISLKIDSWKMIEAIPPWLKCIWQHLK	GTFFGGVSIHIIQKGPIFSIVSFDSGSEEFEEFIAPDAICKPFAL
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Pdul-513 :       DILÜRLP MKSLVRFLCTCKSNSDLIGSSSLVSTHLURRNYTKNDHVYLLCLHHSNPELGADPDDPYVKÖFFÖVSLPSNÖTFEÖC         Fbox       V1         Pcs-59 :       SKLSNPLGSTHYVIVGSSNGLVCIADBILNPDTIYINNPSVKKLRTLPISTNINIKFSHVALQ7GPHPVVNDXKAVKMMKT         Pdul-513 :       SKLSNPLGSTHYVIVGSSNGLVCIADBILNPDTIYINNPSVKKLRTLPISTNINIKFSHVALQ7GPHPVVNDXKAVKMMKT         Pdul-513 :       SKLSNPLGSTHYVIVGSSNGLVCIADBILNPDTIYINNPSVKKLRTLPISTNINIKFSHVALQ7GPHPVVNDXKAVKMMKT         Pdul-513 :       SKLSNPLGSTHYVIVGSSNGLVCIADBILNPDTIYINNPSVKKLRTLPISTNINIKFSHVALQ7GPHPVVNDXKAVKMMKT         Pdul-513 :       NKNPLAVEVYSLRTDSKKMIEVIPPMLKCTWKNHKGTFLNGVAVMIOGKPIFSIVSPDSGSREFORFIAPDAICNPCELVRI         Pdul-513 :       NKNPLAVEVYSLRTDSKKMIEVIPPMLKCTWKNHKGTFLNGVAVMIOGKPIFSIVSPDSGSREFORFIAPDAICNPCELVRI         Pdul-513 :       NKNPLAVEVYSLRTDSKMMIEVIPPMLKCTWKNHKGTPLNGVAVMIOGKPIFSIVSPDSGSREFORFIAPDAICNPCELVRI         Pdul-513 :       DVYKKQICLLCSLYPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCOGTIGISIDMELLMEKADPDRGIANVHLCMYSSKQ         Pdul-513 :       DVYKKQICLLCSLYPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCOGTIGISIDMELLMEKADPDRGIANVHLCMYSSKQ         Pdul-513 :       DVYKKQICLLCSLYPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCOGTIGISIDMELLMEKADPDRGIANVHLCMYSSKQ         VV       VV          Pdu-513 :       DVYKKQICLLCSLYPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCOGTIGISIDMELMEKADPDRGIANVHLCMYSSKQ         Pdu-513 :       DVYKKQICLLCSLYPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCOGTIGISIDMELMEKADPDRGIANVHLCMYSSKQ	Pcer-S9 : Psal-Sd :	: DILVRLPVKSLVRFLCTCKSWSDLIGSSSLVSTHLHRN : DILARLPVKSLVRFLCTCKSWSDLIGSSSLVSTHLHRN	N V T K H D H V Y L L C L H H S N F E L Q A D P D D P Y V K Q E F Q W S L F S N Q T F E Q C N V T K H D H V Y L L C L H H S N F E L Q A D P D D P Y V K Q E F Q W S L F S N O T F E O C
F-box     V1       Pcer-59     : SKLSRHLGSTEHVVIVGSNGLVCISDEILNDTDTIVINDSVRLLTLPISTNNIKFSNVALOGGENPVVNOTXAVRMNRT       Pdul-513     : SKLSRHLGSTEHVVIVGSSNGLVCISDEILNDTDTIVINDSVRLLRLLDISTNNIKFSNVALOGGENPVVNOTXAVRMNRT       Pcer-59     : NNNPLAVEVYSLRTDSWKNIEVIPPNLKCTNKNHKGTFLNGVAVINTOKGEIPSIVSPDSGEERPOERIAPDAICNPCELVRI       Pcer-59     : NNNPLAVEVYSLRTDSWKNIEVIPPNLKCTNKHHKGTFLNGVAVINTOKGEIPSIVSPDSGEERPOERIAPDAICNPCELVRI       Pcer-59     : NNNPLAVEVYSLRTDSWKNIEVIPPNLKCTNKHHKGTFLNGVAVINTOKGEIPSIVSPDSGEERPOERIAPDAICNPCELVRI       Pcer-59     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Psal-64     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Psal-54     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Psal-54     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Psal-54     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Psal-54     : DVVKSOICLLCSLVPSSEDGMGKNOLWVLQEKONGOLCPFVVPLOCDGTIGISINNELLMEKRDPDRGIANVHLCUTSKO       Pcar-510     : DILARLPAKSLVAPICCTCKSNADLIGSSSPVSTHLNNRATKNAHLYLLCLHHSNERGADPDDPVVKQEPONSIPSNETFEEC       Pcar-510     : DILARLPAKSLVAPICCTCKSNADLIGSSSPVSTHLNRATKNAHLYLLCLHHSNERGADPDDPVVKQEPONSIPSNETFEEC       Pcar-510     : DILARLPAKSLVAPICCTCKSNADLIGSSPVSTHLNRATKNAHLYLLCLHHSNERGADPODPVVKQEPONSIPSNETFEEC       Pcar-510     : DILARLPAKSLVAPICTCKSNASDLIGSSSPVSTHLNRATKNAHLYLLCLHHSNERGADPODPVVKQEPONSIPSNETFEEC	Pdul-S13 :	DILVRLPAKSLVRFLCTCKSWSDLIGSSSLVSTHLHRN	NVTKHDHVYLLCLHHSNFELQADPDDPYVKQEFQWSLFSNQTFEQC
Pcsr-59       Skishplasteryvirgssnadverisdeitund trivirungsverkleripistninnikgshvalopgen pevnoykavennet         Pcsr-59       Skishplasteryvirgssnadverisdeitund trivirungsverkleripistninnikgshvalopgen pevnoykavennet         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVENLETIPISTNINNIKGSBERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVENLETIPISTNINNIKGSBERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVENLIGKER PSIVSEDSSEERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVENLIGKER PSIVSEDSSEERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVENLIGKER PDEDETISTDSELMERRDPDRGTANNH (CDVELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVEN (CDVERTSKOSSEERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVEN (CDVERTSKOSSEERGERIAPDALCONCELVRI         Pcsr-59       INN PLAVENTSLRTDSWKNIEVIPMLECTHKNH KGTFLNGVAVEN (CDVERTSKOSSEERGERIAPDALCONCELVANE)         Pcsr-510       INTRACKSERGERIAPSENDERGEN (CONCELCHENKENCELCPFUYPLOCOGTIGISIDNELLMERRDPDRGTANNH (CMERSKO         Pcsr-510       ILARITAKSUVAPICTERSKSBULIGSSEFVSTHENRNATKRAHEVLICCHHSNERGAD PDDPVKKOBFONSTERSKTEREC         Pcsr-510       SKLSRPLGSTEPVVIGSSHGLVCISDEINNDSSTHENRNATKRAHEVLICCHRSNERGAD PDDPVKKOBFONSTERSKTEREC         Pcsr-510       SKLSRPLGSTEPVVIGSSHGLVCISDEINNDSSTHENRNATKRAHEVLICCHRSNERGAD PDDPVKKOBFONSKARRMERT         Pcsr-510 <th></th> <td>F-box</td> <td>V1</td>		F-box	V1
PCer-S1 : SKLSHPLGSTERHVITUSSKULVCISDELLAPDTFITURNPSVRALRTIPISTNIHIKSENVALQFGFHFVVNDTKAVRHART Pdul-S1 : SKLSHPLGSTERHVITUSSKULVCISDELLAPDTFITURNPSVRALRTIPISTNIHIKSENVALQFGFHFVVNDTKAVRHART Pcer-S1 : NKNPLAVEVYSLRDSKKHIVITOSSKULVCISDELLAPDTFITURNPSVRALRTIPISTNIHIKSENVALQFGFHFVVNDTKAVRHART Pcer-S1 : NKNPLAVEVYSLRDSKKHIVITOSSKULVCISDELLAPDTFITURNPSVRALRTIPISTNIHIKSENVALQFGFHFVVNDTKAVRHART Pcer-S1 : NKNPLAVEVYSLRDSKKHIVITOSSKULVCISDELLAPDTFITURNPSVRALRTIPISTNIHIKSENVALQFGFHFVVNDTKAVRHART Pcer-S1 : NKNPLAVEVYSLRDSKKHIVITOSSKULVCISDELLAPDTFITURNSTKALRT Pcer-S1 : DVYKEQICLLCSLYPSSEDGKKNDLWVLOKKNKGTPLNGVATHMIGKGPITSIVSFDSGSBEFGFFIAPDAICNPCELVKI Pcer-S1 : DVYKEQICLLCSLYPSSEDGKKNDLWVLOKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGIANVHLCDYSKO Psal-S2 : DVYKEQICLLCSLYPSSEDGKKNDLWVLQKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGIANVHLCHYESKO Pdul-S1 : DVYKEQICLLCSLYPSSEDGKKNDLWVLQKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGIANVHLCHYESKO Pdul-S1 : DVYKEQICLLCSLYPSSEDGKKNDLWVLQKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGIANVHLCHYESKO Pdul-S1 : DVYKEQICLLCSLYPSSEDGKKNDLWVLQKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGIANVHLCHYESKO Psal-S2 : DVYKEQICLLCSLYPSSEDGKKNDLWVLQKKNKGLCPFVYPLOCDGTIGISIDNELLMEKRDFDRGADPDDPVKGEFONSLFSSNETFEC Pmm-S7 : DILARLPAKSLVRFLCTCKSNSDLIGSSFYSTHLNRNAKKNAHLYLLCLHHSNFRROADPDDPVKGEFONSLFSSNETFEC Pmm-S7 : SKLSHPLGSTFPVVIYGSSHGLVCISDEINNFDSSTHLNRNAKKNAHLYLLCLHHSNFFROADPDDPVKGEFONSLFSSNETFEC Pmm-S7 : SKLSHPLGSTFPVVIYGSSHGLVCISDEINNFDSSTHLNNPSVKKKTLPISSNNIHKFSHVALQFFHCOKNOKAIRMIRT Pcer-S10 : SKLSHPLGSTFPVVIYGSSHGLVCISDEINNFDSSTHLNNPSVKKKTLPISSNNIHKFSHVALQFFHCOKNOKAIRMIRT Paun-S7 : SKLSHPLGSTFPVVIYGSSHGLVCISDEINNFDSSTHLWNPSVKKKTLPISSNNIHKFSHVALQFFHCOKNOKAIRMIRT Paun-S7 : NKKALMVEVYSLRADSHMIKAIPPMLKTNOHHOGTFFNGVAYHHIEKGPTFSISINGSSESFEFFMAPDAICSSWRCHTH Paun-S7 : NKKALMVEVYSLRADSHKMIKAIPPMLKTNOHHOGTFFNGVAYHHIEKGPTSSINIKKSHKALQFFHAPDAICSSWRCH Pun-S7 : NKKALMVEVYSLRADSHKMIKAIPPMLKTNOHHOGTFFNGVAYHHIEKGPTSSINDELLMGKKGKAGVADLELMINSKG Pun-S7 : VYKEQICLFFGFYCC-EKGMKKIDLWGLQKKKWKLCPTIYDPHDYCYTHIGSSINNELLMGKKGKAGVADLELMINSKG PUN-S7 : VYK	D	* * * #**************	***** ** ** *# * * #* * **** # **** ****
Pdul-S13 : SKLSHPLGSTEHYVIYGSSNGLVCISDEILNPDTPTHIWNPSVRKLRALPISTNINIKFSHVALQFGFHFVVNDYKAVRMMRT  Pcer-S9 : DVYKEQICLLSLYPSBDGMGKMDLWVLQKKMKKLCFFINGVAYHMIQKGPIFSIVSFDSGSBEFQEFIAPDAICNPCELVRI Pdul-S13 : NKNPLAVEVYSLRADSWKMIEVIPPWLKCTWKHHKGTFLNGVAYHMIQKGPIFSIVSFDSGSBEFQEFIAPDAICNPCELVRI Pdul-S13 : NKNPLAVEVYSLRADSWKMIEVIPPWLKCTWKHHKGTFLNGVAYHMIQKGPIFSIVSFDSGSBEFQEFIAPDAICNPCELVRI Pcer-S9 : DVYKEQICLLSLYPSSBDGMGKMDLWVLQKKMKKQLCPFVYPLDCDGTIGISIDWELLMEKKDPPRGIANVHLCDVESKQ Pdul-S13 : DVYKEQICLLSLYPSSBDGMGKMDLWVLQKKMKQLCPFVYPLDCDGTIGISIDWELLMEKKDPPRGIANVHLCDVESKQ Pdul-S13 : DVYKEQICLLCSLYPSSBDGMGKMDLWVLQKKMKQLCPFVYPLDCDGTIGISIDWELLMEKKDPPRGIANVHLCDVESKQ Pdul-S13 : DVYKEQICLLCSLYPSSBDGMGKMDLWVLQKKMKQLCPFVYPLDCDGTIGISIDWELLMEKKDPPRGIANVHLCOVESKQ Pdul-S13 : DVXKEQICLLCSLYPSSBDGMGKMDLWVLQKKMKQLCPFVYPLDCDGTIGISIDWELLMEKKDPPRGIANVHLCOVESKQ Pdul-S13 : DILARLYAKSLVRPLCTCKSNSDLIGSSFYSTHLNRNATKHAHLYLLCLHHSNPFRQADPDDPYVQEFQWSLFSNKTPFEC Pmmn-S7 : DILARLYAKSLVRPLCTCKSNSDLIGSSFYSTHLNRNATKHAHLYLLCLHHSNPFRQADPDDPYVQEFQWSLFSNKTPFEC Pmmn-S7 : SKLSHPLGSTEPYVIJGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQFGPHFGVNDYKAIRMLRT Psal-Sg : SKLSHPLGSTEPYVIJGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQFGPHFGVNDYKAIRMLRT Psal-SG : NKNALVVEVYSLRADSWMIKAIPPHKKTUPHSTNANPSVKKFKTLPISTNINIKPSHVALQFGPHFGVNDYKAIRMLRT Psal-SG : NKNALVVEVYSLRADSWMIKAIPPHKKTUPHSTNANPSVKKFKTLPISTNINIKPSHVALQFGPHFGVNDYKAIRMLRT Psal-SG : NKNALVVEVYSLRADSWMIKAIPPHKKTUPHSTNGVAYHIEKGPIFSISFDSGSSEFFEFMAPDAICSSWKCUTH Psal-SG : NKKALVVEVYSLRADSWKMIKAIPPHKKTUPHSTNGVAYHIEKGPIFSISFDSGSSEFFEFMAPDAICSSWKCUTH Pmm-S7 : NKKALMVEVYSLRADSWKMIKAIPPHKKTUPHSTNGVAYHIEKGPIFSISISPDSGSSEFFEFMAPDAICSSWKCUTH Pmm-S7 : NKKALMVEVYSLRADSWK	Psal-Sd :	SKLSHPLGSTEHIVIIGSSNGLVCISDEILNFDTPIII SKLSHPLGSTEHIVIIGSSNGLVCISDEILNFDTPIII	IWNPSVRKLRTLPISTNINIKFSHVALQFGFHPVVNDIKAVRMMRT IWNPSVRKLRTLPISTNINIKFSHVALQFGFHPVVNDYKAVRMMRT
PCEI-S9 :: NKNPLAVEVYSLKTDSMKMIEVIPPMLKCTMKHHKGTPLNGVAYHMIQKOPIPSIVSPDSGSEEPQEPIAPDAICNPCELVRI PSAL-S4 :: NKNPLAVEVYSLKTDSMKMIEVIPPMLKCTMKHHKGTPLNGVAYHMIQKOPIPSIVSPDSGSEEPQEPIAPDAICNPCELVRI PCEI-S9 :: DVYKEQICLLCSLYPSSEDGMGKNDLWVLQEKOMKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ PSAL-S4 :: DVYKEQICLLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVYKEQICLLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVYKEQICLLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVYKEQICLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVYKEQICLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVXKEQICLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCDGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVXKEQICLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVXKEQICLCSLYPSSEDGMGKNDLWVLQEK MKQLCPPVYPLDCGTIGISIDNELLMKKRDPDRGIANVHLCOVESKQ Pdul-S13 :: DVXKEQICLCSLSPFSTHINKNDLKALVLCLHHSNPERQADPDDPVYKQEPQNSLFSNETFEEC Pmum-S7 :: DILARLPAKSLVKPLCTCKSMSDLIGSSSFVSTHINKNATKHAHVLLLCLHHSNPERQADPDDPVYKQEPQNSLFSNETFEEC F-box V1 Pcer-S10 :: SKLSNPLGSTEPYVIYGSSNGLVCISSEINMFDSSIMMPDSSIMMPDSSIKKKKTLDISININIKFSNALQPGFNFGVNDYKAIRMLKT Psal-S2 :: SKLSNPLGSTEPYVIYGSSNGLVCISSEINMFDSSIMMPDSSIKKKKTLDISININIKFSNALQPGFNFGVNDYKAIRMLKT Pmum-S7 :: SKLSNPLGSTEPYVIYGSSNGLVCISSENDENFFFFQ Pmum-S7 :: SKLSNPLGSTEPYVIYGSSNGLVCISSENDENFFFQ Pmum-S7 :: SKLSNPLGSTEPYVIYGSSNGLVCISSENTAPPOKKKTMCHDHOGTFFNGVAYHILEKGPIFSIISFDSGSEBFREFFAAPDAICSSNRLCIH Pcer-S10 :: VKKALVVEVYSLKADSMKMIEAIPPHKKTMCHNOGTFFNGVAYHILEKGPIFSIISFDSGSEBFREFFAAPDAICSSNRLCIH Pmum-S7 :: NKKALVVEVYSLKADSMKMIEAIPPHKKTMCHNOGTFFNGVAYHILEKGPIFSIISFDSGSEBFREFFAAPADAICSSNRLCIH Pmum-S7 :: NKKALVVEVYSLKADSMKMIEAIPPHKKTMCHNOGTFFNGVAYHILEKGPIFSIISFDSGSEBFREFFAAPADAICSSNRLCIH Pmum-S7 :: VKKSQICLFPFGTYGC-EBGMEKIDLWGQKKKWKKLCPFIYDPDDVCXIIGISIDNELLMGREGFAKGVADLELMNYBSKQ Pmum-S7 :: VKKSQICLFPFGTYGC-EBGMEKID	Pdu1-513 :	SKLSHPLGSTEHYVIYGSSNGLVCISDEILNFDTPI <b>H</b> I	IWN PSVRKLRALPISTNINIKFSHVALQFGFH PVVN DYKAVRMMRT
Pcer-S9       :       NKNPLAVEVYSLRTDSWKNIEVIPPWLKCTWKHRKGTFLNGVAYHNIGKGPIPSIVSPDGSSEPGUFIAPDAICOPCELVRI         Pdul-S13       :       NKNPLAVEVYSLRTDSWKNIEVIPPWLKCTWKHRKGTFLNGVAYHNIGKGPIPSIVSPDGSSEPGUFIAPDAICOPCELVRI         Pcer-S9       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         Psal-Sd       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         Pdul-S13       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         Pdul-S13       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         Pdul-S13       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         Pdul-S13       :       DVYKEGICLLCSLYPSSEDGMGKNDLWVLGEKONKGLCPFVYPLDCOGTIGISIDMELLMEKROPPDRGIANVHLCMYESKG         V2       HVa       HVb         C			
PCer-S10 : DILARLPAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHSNFERQADPDDPVKQBFQWSLFSNETFEC Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKGWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKKWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKKWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKKWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKKWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DVYKEQICLLCSLVPSSEDGMGKNDLWVLQEKKWKQLCPFVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCUVESKQ Pdul-S13 : DILARLPAKSLVFFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHSNFERQADPDDPVKQEFGWSLFSNETFEC Pmum-S7 : DILARLFAKSLVFFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHSNFERQADPDDPVKQEFGWSLFSNETFEC Fbal-Sg : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQPGPHPGVNDYKAIRMLRT Pcer-S10 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSFIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSFIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSFIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNPDSFIHIWNPSVKKFKTLPISTNINIKPSHVALQPGFHPGVNDYKAIRMLRT Pmum-S7 : NKKALWVEVYSLRADSWMMIEAIPPHKKTMQHHDGTFFNGVAYHITEKGFIFSISPDSGSBEFEFFMAPDAICSSWRLCIH Pmum-S7 : NKKALWVEVSLRADSWMMIEAIPPHKKTMQHHDGTFFNGVAYHITEKGFIFSISDPSGSBEFFEFFAPADAICSSWRLCIH Pmum-S7 : NKKALWVEVSLRADSWMMIEAIPPHKKTMQHHDGTFFNGVAYHITEKGFIFSISDNELLMGREGFAKGVADLELUNYESKQ Pmum-S7 : VYKEQICLFFGFYGC-ESGMEKIDLWGLQKKKWKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELUNYESKQ Pmum-S7 : YYKEQICLFFGFYGC-ESGMEKIDLWGLQKKKWKLCPFIYDPLDYCYRIIGISIDNELLMG	B	* #* ******* * * ** ******* * * *	* ** ****** ******** ******* * **
Pdul-s13 :       NKNPLAVEVYSLRTDSWKMIEVLPPWLKCTWKHHKGTFLNGVAYHMIQKGPIPSIVSFDSGSEEFQEFIAPDAICNPCELVRI         Pcer-S9 :       DVYKEQICLLCSLYPSEDGMGKNDLWVLQEKNKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCDYESKQ         Pdul-s13 :       DVYKEQICLLCSLYPSEDGMGKNDLWVLQEKNKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCDYESKQ         V2       HVa         V2       HVa         V2       HVa         V2       HVa         V2       HVb         Pcer-S10 :       DILARLPAKSLVRPLCTCKSWSDLIGSSSFVSTHLNRNARKHAHLYLLCHHSNPERQADPDDPYVKQEFQWSLFSNETFEEC         Pmum-S7 :       DILARLPAKSLVRPLCTCKSWSDLIGSSSFVSTHLNRNARKHAHLYLLCHHSNPERQADPDDPYVKQEFQWSLFSNETFEEC         F-box       V1         Pcer-S10 :       SKLSHPLGSTEPYVIYGSSHGLVCISDEINNFDSFHLNNNMKKHAHLYLLCHHSNPERQADPDDPYVKQEFQWSLFSNETFEEC         Fbox       V1         Pcer-S10 :       SKLSHPLGSTEPYVIYGSSHGLVCISDEINNFDSFHLNNNNKKHAHLYLLCHHSNPERVALQFGFHPGVNDVKAIRMLRT         Pmum-S7 :       SKLSHPLGSTEPYVIYGSSHGLVCISDEINNFDSFHLNNNSVKKFKILPISTNINIKFSHVALQFGFHPGVNDVKAIRMLRT         Pmum-S7 :       NKHALVVEVYSLRADSWMITAIPPHLKTWQHHDGTFFNGVAYHIIEKGPIFSILSPDSGSEEFTEFMAPDAICSSWRLCH         Pcer-S10 :       NKHALVVEVYSLRADSWMITAIPPHLKTWQHHDGTFFNGVAYHIIEKGPIFSILSPDSGSEEFTEFMAPDAICSSWRLCH         Pmum-S7 :       NKHALVVEVYSLRADSWMITAIPPHLKTWQHHDGTFFNGVAYHIIEKGPIFSILSPDSGSEEFTEFMAPDAICSSWRLCH         Pmum-S7 :       NKHALVVEVYSLRADSWMITAIPPHLKTWQHHDGTFFN	Psal-Sd :	NKNPLAVEVISLRTDSWKMIEVIPPWLKCTWKHHKGTP: NKNPLAVEVYSLRTDSWKMIEVIPPWLKCTWKHHKGTP	FLNGVAYHMIQKGPIFSIVSFDSGSEEFQEFIAPDAICNPCELVRI FLNGVAYHMIQKGPIFSIVSFDSGSEEFQEFIAPDAICNPCELVRI
Pcer-S9       EDVYREQICLLCSLYPSSEDGMGKNDLWVLQEKOMKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCDYESKQ         Pdul-S13       DVYREQICLLCSLYPSSEDGMGKNDLWVLQEKOMKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCDYESKQ         V2       HVa         V1       HVb         V1       HVa         HVA       HVA <t< td=""><th>Pdul-513 :</th><td>NKNPLAVEVYSLRTDSWKMIEVLPPWLKCTWKHHKGT</td><td>FLNGVAYHMIQKGPIFSIVSFDSGSEEFQEFIAPDAICNPCELVRI</td></t<>	Pdul-513 :	NKNPLAVEVYSLRTDSWKMIEVLPPWLKCTWKHHKGT	FLNGVAYHMIQKGPIFSIVSFDSGSEEFQEFIAPDAICNPCELVRI
Pcer-sio : bvkegicLicSivpsSebGMGKNDLWvLQEKHWKQLCPFVVPLDCDGTIGTSIDNELLMEKKDFDRGTANVHLCUNVESKQ Pdul-si3 : DvykEqicLicSivpSSebGMGKNDLWvLQEKHWKQLCPFVVPLDCDGTIGTSIDNELLMEKKDFDRGTANVHLCUNVESKQ V2 V2 HVa HVa HVb C C Pcer-sio : DILARLPAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHHSNFERQADPDDPVVKQEFQWSLFSNETFEEC Pmum-s7 : DILARLTAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHHSNFERQADPDDPVVKQEFQWSLFSNETFEEC F-box V1 Pcer-sio : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNFSVKKKKTLPISTNINIKFSHVALQFGFHFGVNDVKAIRMLRT Pmum-s7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNFSVKKKKTLPISTNINIKFSHVALQFGFHFGVNDVKAIRMLRT Psal-sg : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNFSVKKKKTLPISTNINIKFSHVALQFGFHFGVNDVKAIRMLRT Pcer-sio : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNFSVKKKKTLPISTNINIKFSHVALQFGFHFGVNDVKAIRMLRT Psal-sg : NKNALVVEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALVVEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALVVEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSNMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSWMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : NKKALWEVYSLRADSWMMIEAIPPWLKTWQHHDGTFFNGVAYHTIEKGPIFSIISFDSGSEEFEEFMAPDATCSSWRLCIH Pmum-s7 : VYKEQICLFFGFYGC-BEGMEKKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum-s7 : VYKEQICLFFGFYGC-BEGMEKKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum-s7 : VYKEQICLFFGFYGC-BEGMEKKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum-s7 : VYKEQICLFFGFYGC-BEGMEKKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ			
Pcsal-sd       DVYKEQICLLCSLIPSSEDGMGKNDLMVLQEK MYKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCNISSKQ         Pdul-si3       DVYKEQICLLCSLIPSSEDGMGKNDLMVLQEK MYKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCNISSKQ         V2       HVa         V2       HVa         Pdul-si3       DIVKEQICLCSSIPSSEDGMGKNDLMVLQEK MYKQLCPPVYPLDCDGTIGISIDNELLMEKRDPDRGIANVHLCNISSKQ         V2       HVa         V2       HVa         Psal-sg       DILARLTAKSLVRFLCTCKSMSDLIGSSSFVSTHLNRNATKHAHLVLLCHHSNFERQADPDDPVKQEFQMSLFSNETFEEC         Fbox       V1         Pcer-s10       SKLSHPLGSTEPVVIGSSHGLVCISDEIMNPDSVKKFKTLPISTNINIKFSHVALQFGHPGVNDYKAIRMLRT         Pcer-s10       SKLSHPLGSTEPVVIGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGHPGVNDYKAIRMLRT         Pmum-s7       SKLSHPLGSTEPVVIGSSHGLVCISDEIMNPDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGHPGVNDYKAIRMLRT         Pcer-s10       NNNALVVEVYSLRADSWMMIEAIPPWLKTWQHNDGTFFNGVAYHIIEKGPITSIISFDSGSEBFEEFMAPDAICSSWRLCH         Prai-sg       NNNALVVEVYSLRADSWMMIEAIPPWLKTWQHNDGTFFNGVAYHIIEKGPITSIISFDSGSEBFEEFMAPDAICSSWRLCH         Prum-s7       NKKALWEVYSLRADSWMMIEAIPPWLKTWQHNDGTFFNGVAYHIIEKGPITSIISPDSGSEBFEEFMAPDAICSSWRLCH         Prum-s7       VYKEQICLFFFGFYGC-BEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMERGFAKGVADLELWNYSKQ         Prum-s7       VYKEQICLFFFGFYGC-BEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMERGFAKGVADLELWNYSKQ         Prum-s7       VYKEQICLFFFGFYGC-BEGMEKIDLWGLQKKRWKKLCPFIYDP	Baar - 69		
Pdul-sl3 :       DVYKEQICLLCSLYPS BEGGMGKNDLWVLQEK NKKQLCPFVYPLDCDGTIGISIDNELLMEKRDFDRGIANVHLCNYESKQ         V2       HVa       HVb         C	Psal-Sd :	DVIKEQICLLCSLYPSSEDGMGKNDLWVLQEKRWKQLC	CPFVIPLDCDGTIGISIDNELLMEKRDFDRGIANVHLCDIESKQ
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C Pres-S10 : DILARLPAKSLVRFLCTCKSNSDLIGSSSFVSTHINRNATKHAHLYLLCLHHSNFERQADPDDPYVKQEFQNSLFSNETFEEC Pmum-S7 : DILARLTAKSLVRFLCTCKSNSDLIGSSSFVSTHINRNATKHAHLYLLCLHHSNFERQADPDDPYVKQEFQNSLFSNETFEEC F-box V1 Pres-S10 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Psal-Sg : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Pmum-S7 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Psal-Sg : NKNALVVEVYSLRADSWMMIEAIPPWLKRTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEFEEFFMAPDAICSSWRLCIH Pres-S10 : NKNALVVEVYSLRADSWMMIEAIPPWLKRTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEFEEFFMAPDAICSSWRLCIH Praum-S7 : NKKALWVEVYSLRADSWMMIEAIPPWLKRTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEFEEFFMAPDAICSSWRLCIH Praum-S7 : NKKALWVEVYSLRADSWMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEFEEFFMAPDAICSSWRLCIH Praum-S7 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum-S7 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ PMUM-S7 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ PMUM-S7 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ PMUM-S7 : VYKEQICLFFGFYGCFEFFGFFGAPADIC		V2	HVa HVD
C PC er - 510 : DILARLIPAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHHSNPERQADPDDPYVKQEFQWSLFSNETFEEC Pnum - 57 : DILARLIPAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHHSNPERQADPDDPYVKQEFQWSLFSNETFEEC F-box V1 Pcer - 510 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Psal - 57 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Pnum - 57 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Pmum - 57 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Pmum - 57 : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT Procer-s10 : NKNALVVEVYSLRADSHMMIEAIPPWLKRTHQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFMAPDAICSSWRLCIH Psal - 57 : NKNALVVEVYSLRADSHMMIEAIPPWLKRTHQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFMAPDAICSSWRLCIH Procer-s10 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum - 57 : NKKALAVEVYSLRADSHMMIEAIPPWLKRTHQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFMAPDAICSSWRLCIH Procer-s10 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum - 57 : NKKALAVEVYSLRADSHMMIEAIPPWLKRTHQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFFAPDAICSSWRLCIH Procer-s10 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum - 57 : NKKALAVEVYSLRADSHMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFFAPDAICSSWRLCIH Procer-s10 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDVCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum - 57 : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKLCPFIYDPLDVCYRIIGISIDNELLMGREGFAKGVADLELWNYES			
Pcer-S10     :     <	C		
Psal-Sg       DILARLITAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRNATKHAHLYLLCLHHSNFERQADPDDPYVKQEFQWSLFSNETFEEC         Pmum-S7       DILARLITAKSLVRFLCTCKSWSDLIGSSSFVSTHLNSNVKKHAHVYLLCLHHSNFERQADPDDPYVKQEFQWSLFSNETFEEC         V1         Fbox         V1         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Psal-Sg         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Psal-Sg         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Pmum-S7         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Pmum-S7         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Pmum-S7         SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHWNPSVKKFKTLPISTNINIKFSHVALQFGFHAPDAICSSWRLCIH         Psal-Sg         NKNALVVEVYSLRADSWMMIEAIPPWLKRTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIH         Psal-Sg         NKNALVVEVYSLRADSWMMIEAIPPWLKTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIH         Psal-Sg         NKKALWVEVYSLRADSWMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIH	<b>U</b>	*** *** ****** *** * * * * **** * * *	* * * * ** ##* *** # **** #
Pmum-S7       :       DILUMRLTAKSLVRFLCTCCKSWSDLIGSSSFVSINLNSNVKKHAH VYLLCLHHPNFERIADPDDPVVKQGFQWSLFSNETFEEC         F-box       V1         *	Psal-Sg :	DILARLPARSLVRFLCTCRSWSDLIGSSSFVSTHLNRF DILARLTAKSLVRFLCTCKSWSDLIGSSSFVSTHLNRF	NATKHAHLILLCLHHSNFERQADPDDPIVKQEFQWSLFSNETFEEC NATKHAHLYLLCLHHSNFERQADPDDPYVKQEFQWSLFSNETFEEC
F-box     V1       Pcer-S10     :     * #***********************************	Pmum-S7 :	DILVRLTAKSLVRFLCTCKSWSDLIGSSSFVSINLNS	NVKKHAHVYLLCLHHPNFERLADPDDPYVKQGFQWSLFSNETFEEC
* * * * * * * * * * * * * * * * * * *		F-box	V1
Psal-Sg       : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         Pmum-S7       : SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHIWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDYKAIRMLRT         *       ************************************	Pcer-S10	* * * #*******************************	***** ** ** *#* * * #* * **** # **** ****
Pmum-S7       :       SKLSHPLGSMEPYVIYGSSNGLVCISDEILNFDSPIHIWNPSVSKLRTTPISTNITIKFSHVALQFGFHPGVNDYKAIRMLRT         * #* ******       * ** ********************************	Psal-Sg :	SKLSHPLGSTEPYVIYGSSHGLVCISDEIMNFDSPIHI	IWNPSVKKFKTLPISTNINIKFSHVALQFGFHPGVNDIKAIRMLRT
Pcer-S10       :       NKNALVVEVYSLRADSWMMIEAIPPWLKRTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFMAPDAICSSWRLCIH         Psal-Sg       :       NKNALVVEVYSLRADSWMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFIAPDAICSSWRLCIH         Pmum-S7       :       NKKALAVEVYSLRADSWMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFIAPDAICSSWRLCIH         Pcer-S10       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Psal-Sg       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       :       VYKEQICLFFGFYGC-EEGMEKIDLWGQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       :       VYKEQICLFFGFYGC-EEGMEKIDLWGQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ	Pmum-S7 :	SKLSHPLGSREPYVIYGSSNGLVCISDEI NFDSPIHI	IWNPSV <b>SKLRTT</b> PISTNI <b>T</b> IKFSHVALQFGFHPGVNDYKAIRMLRT
** #* ********************************			
Psal-Sg       : NKNALVVEVYSLRADSWMMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIH         Pmum-S7       : NKKALAVEVYSLRADSWKMIEAIPPWLKCTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIR         ****       # **# # *## ****** * ** * #### # ** # * # # *## *#* *****         Pcer-S10       : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Psal-Sg       : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGC # EEGMEKIDLWGMQDKRWKQLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGC # EEGMEKIDLWGMQDKRWKQLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ	Pcer-S10	* #* ******* * * ** ****** * * * * NKNALVVEVYSLRADSWMMTEATPPWLKRTWOHHDGTF	* ** ****** ******* ****** * *# FFNGVAYHIIEKGPIFSIISFDSGSEEFEEFMAPDAICSSWRLCTH
Pmum-S7       : NKKALAVEVYSLRADSWKMIEAIPPWLKGTWQHHDGTFFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSLWRLCIR         * * **       # * # # *## *****         * * **       # * # # *## *****         Pcer-S10       : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Psal-Sg       : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGCEEEGMEKIDLWGMQDKRWKKQLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGCEEEGMEKIDLWGMQDKRWKQLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       : VYKEQICLFFGFYGCEEEGMEKIDLWGMQDKRWKQLCPFIYDPDDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ	Psal-Sg :	NKNALVVEVYSLRADSWMMIEAIPPWLKCTWQHHDGT	FFNGVAYHIIEKGPIFSIISFDSGSEEFEEFTAPDAICSSWRLCIH
* * **       # # * ## *****       * ** * # # * # # *## *#* *****         Pcer-S10       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Psal-Sg       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       :       VYKEQICLFFGFYGCEEGMEKIDLWGMQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       :       VYKEQICLFFGFYGCEEGMEKIDLWGMQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLEDLWDYESKQ         PMum-S7       :       VYKEQICLFFGFYGCEEGMEKIDLWGMQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLEDLWDYESKQ	Pmum-S7 :	: NKKALAVEVYSLRADSWKMIEAIPPWLKCTWQHHDGTF	FFNGVAYHIIEKGPIFSIISFDSGSEEFEEF <b>T</b> APDAICS <b>L</b> WRLCI <b>R</b>
* * **       # * *#       * * **       * * **       * * * # # *#       * ** * *         Pcer-S10       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Psal-Sg       :       VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         Pmum-S7       :       VYKEQICLFFGFYGCEEGMEKIDLWGMQDKRWKQLCPFIYDPEDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ         V2       HVa       HVb			
Psal-Sg : VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCPFIYDPFDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ Pmum-S7 : VYKEQICLFFGFYGCEEEGMEKIDLWGMQDKRWKQLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLDLWDYESKQ V2 HVa HVb	Pcer-S10 :	* * * * # # * *# #* ***** * ** * : VYKEQICLFFGFYGC-EEGMEKIDLWGLOKKRWKKLCF	* *### # ** # * # # # ### *#* ***** PFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKO
Pmum-s/ : vikeQicLFFGFYGCEEEGMEKIDLWGMQDKRWKQLCPFIYDPLDYCYRIIGISIDNELLMGREGFAKGVADLDLWDYESKQ V2 ΗVa	Psal-Sg	VYKEQICLFFGFYGC-EEGMEKIDLWGLQKKRWKKLCF	PFIYDP <b>F</b> DYCYRIIGISIDNELLMGREGFAKGVADLELWNYESKQ
	rmum-S7 :	VIKEQICLFFGFIGCEEEGMEKIDLWGMQDKRWKQLCE	HVa HVb

**Fig. 2** Alignments of deduced polypeptides of trans-specific SFBs in *Prunus.* **a** *P. cerasifera*  $S_3$  and *P. dulcis*  $S_{12}$ . **b** *P. cerasifera*  $S_9$ , *P. salicina*  $S_d$  and *P. dulcis*  $S_{13}$ . **c** *P. cerasifera*  $S_{10}$ , *P. salicina*  $S_g$  and *P. mume*  $S_7$ . **d** *P. domestica*  $S_5$  and *P. dulcis*  $S_{10}$ . **e** *P. domestica*  $S_6$  and *P. salicina*  $S_d$ . **f** *P. domestica*  $S_9$  and *P. avium*  $S_4$ . The alignment was prepared using the Clustal method and the PAM250 residue weight table in DNAStar, as defined by Dayhoff (1979). Similarity groups are C,

*Prunus cerasifera* M3 ( $S_3S_4$ ) and *P. dulcis* "Pestañeta" ( $S_{12}S_{23}$ ) produced two *SFB* sequences each; including a single trans-specific pair of with 97.9% deduced polypeptide identity with five non-conservative and one conservative

STAPG, MILV, HRK, NDEQ, FYW. Annotations for conserved and variable regions and individual positions are according to Ikeda et al. (2003). The conserved F-box motif and the four variable regions (V1, V2, HVa, HVb) are *labelled* and *underlined*. Residues differing between polypeptides are shaded in *grey*. Sites marked with \* are conserved, and sites marked with # are hypervariable

replacement. These alleles were assigned to *P. cerasifera*  $S_3$  and *P. dulcis*  $S_{12}$ .

*Prunus cerasifera* C34-1 produced two *SFB* sequences, one of which  $(S_9)$  shared 97.9% identity with the deduced

les and their deduced polypeptides showed exceptionally close identity with other Prunus SFB alleles: P. domes*tica*  $S_5$  had 97.0% identity with *P. dulcis*  $S_{10}$  from "Gabaix" with eight non-conservative and two conservative replacements; P. domestica  $S_6$  had 97.6% identity with P. salicina  $S_a$  from "Burmosa" with two non-conservative and five conservative replacements: and P. domestica  $S_9$  showed a lower identity of 93.3% with P. avium  $S_4$  SFB with eight non-conservative and two conservative replacements.

V2 HVa HVb Fig. 2 continued polypeptide of  $S_{13}$  SFB from *P. dulcis* CEBAS-I ( $S_{13}S_{20}$ ) and Prunus domestica "Verity" produced three SFB alle-

had four non-conservative and one conservative replacement.  $S_9$  also had 99.1% identity with the  $S_d$  SFB from *P. salicina* "Formosa"  $(S_b S_d)$ , with just two non-conservative and one conservative replacement. The second SFB sequence from C34-1, S10, shared 99.1% polypeptide identity with the  $S_g$  SFB from *P. salicina* "Bonnie" and had two non-conservative and one conservative change.  $S_{10}$  had a noticeably lower polypeptide identity of 90.3% with the  $S_7$ allele from P. mume "Bungo", and 21 of the 28 replacements were non-conservative (Fig. 3 in the electronic version).

D Pdom-S5

Pdom-s5       :       DILARLPAKSLVRFFCTCKSWSDLIDNSSFVSIHVNRNVTKHAHVYLLCLHHPNFERQNDNDDPNDIEELQWSLFSNETFEQF         Pdul-s10       :       DILARLTAKSLVRFVCTCKSWSDLIGSSSFVSIHVNRNVTKHAHVYLLCLHHPNFERQNDNDDPYDIEELQWSLFSNETFQQF         F-box         V1
* * * #******************** ** ***** **
* #* ******* * * ** ******* * * * * * *
* * ** # # * *# #* ****** * ** * * *## # ** # * # # *## *#* **** Pdom-S5 : IYKERICLLLDFYPCEEECMEKFDFWVLQEKRWKQLSPFIYSSDYCFRTIGISIDNELLLARRGFDQGIADLCLCNYESK Pdul-S10 : IYKERICLLEDFYPCEEECMEKFDFWVLQEKRWKQLGPFIYSSDYCFRTIGISIDNELLLARRGFDQGIADLC V2 HVa HVb
E *** *** ******** *** * * * * * * * *
* * * #*******************************
* #* ******* * * ** ******* * * * ** ******
* * ** # # * *# #* ***** * ** * * *### # ** # * # # # *## *#* **** Pdom-S6 : IYKEQICLLFRCYGCEEEGMNKVDLWVLQEKRWKQLCPFIFPLDEWSGAIGISMDDELLMAITDFDKGVADLYLGNYESK Psal-Sa : IYKEQIC <u>LLFRCYGCEEEGMNKV</u> DLWVLQEKRWKQLCP <u>FIFPLDEWSGA</u> IGIS <b>I</b> DDELL <u>MAITDFDKGVADLY</u> LWDYESK V2 HVa HVb
F       *** *** *** *** ** ** ** ** ** ** ** **
* * # #************* ** ******** ** ***** **
* #* ******* * * ** ****** * * * * * *
*# * * * # # * *# # * *# #* ***** * * * * ### # ** # * # # # # ### *#* **** Pdom-S9 : CIDLYKEQICLLSSFYSCEEKGMRKIDFWVLQEKWKQLCPFIYPSHYYGTLGISIDNELLMEKRDFSRGIGDLHLGNYESK Pavi-S4 · WIDLYKEQICLLSSFYSCEEKGMPKIDFWVLQEKBWKOLCPFIYPSHYYGTLGISENDNELLMEKRDFSRGIGDLHLGNYESK

#### Discussion

# S-RNases

In this study, six new myrobalan and domestic plum *S*-RNases were found to have exceptionally close identity with eight *S*-RNases of other *Prunus* species. Minimal variation was seen in the deduced peptide sequences of the trans-specific *S*-RNase pairs, with just two to five single amino acid substitutions. Very few of the substitutions were in the hypervariable region, and only a minority were at the hypervariable residue positions. Ushijima et al. (1998) have suggested these positions may encode specificity, and changes at these sites are more likely to generate new specificities.

Exceptionally close identities between *S-RNase* alleles have been reported previously in the Rosaceae: *P. dulcis*  $S_6$ and  $S_{11}$  showed 98 and 100% DNA sequence identity with *MRSN-2* from *P. mume* and  $S_1$  from *P. avium* respectively (Ortega et al. 2006). The allele  $S_8$  from the dwarf almond *P. tenella* had 100% polypeptide identity with *P. avium*  $S_1$ and differed by one amino acid with  $S_{11}$  in *P. dulcis* (Šurbanovski et al. 2007). Ishimizu et al. (1998) found alleles  $S_3$ and  $S_5$  in *Pyrus serotina* to be very similar.

Trans-specific S-RNases have been identified in the Solanaceae: between  $S_3$  and  $S_{26}$  in *Physalis cinerascens* and *Physalis longifolia* respectively (Lu 2001). Other work documents high identity between S-RNases from the same species. Saba-El-Leil et al. (1994) reported 95% identity between  $S_{11}$  and  $S_{13}$  in S. chacoense, and controlled crosses confirmed them to be functionally distinct. Matton et al. (1999) showed that four amino acid changes could convert specificity of  $S_{11}$  to  $S_{13}$ , and just three amino acid changes resulted in dual specificity. Transformants carrying the chimeric  $S_{11}/S_{13}$  S-RNase rejected  $S_{11}$  and  $S_{13}$  pollen.

Minor differences are evident in the *Prunus S*-RNase and in the SFB sequences of the trans-specific pairs, and it is unclear whether these differences are sufficient to produce a new *S*-allele specificity. Ideally, the function and specificity of the trans-specific *S*-haplotypes reported here in *Prunus* should be examined in controlled crosses.

*Physalis cerasifera*, *P. dulcis* and *P. salicina* can be intercrossed, so there is no interspecific barrier to the experiment in those cases. If the trans-specific haplotypes were functionally distinct, then crosses should be fully compatible, and the seedlings will sort into four *S*-genotype classes, which could be detected by PCR. However, if the transspecific haplotypes are functionally equivalent, a cross should show semi-compatibility and would produce only two *S*-genotype classes. Controlled crosses could be attempted with *P. domestica*, though it is difficult to predict the effect polyploidy might have on the outcome of any crosses made. Further, a cross between *P. avium* and *P. domestica* 

for  $S_4$  and  $S_9$  may be too wide and is likely to fail. Pseudocompatibility could also arise, as the minor differences may lead to a weakened incompatibility reaction.

# Second introns

Second introns in *Prunus S-RNases* are notable for their length and sequence polymorphism (Tao et al. 1999; Sonneveld et al. 2003). As non-coding regions, they can accumulate mutations at random and with few constraints, unlike the neighbouring coding regions. Consequently, these trans-specific second introns may give an indication of species divergence times.

Conceivably, the 99–100% match found between the introns of *P. cerasifera*  $S_9$  and *P. salicina*  $S_d$ , between *P. cerasifera*  $S_{10}$  and *P. salicina*  $S_g$ , and between *P. domestica*  $S_6$  and *P. salicina*  $S_a$ , may indicate a relatively recent divergence of these alleles. The poorer intron match between the other trans-specific alleles (~90% to 95%) may point to an earlier divergence. Introns in non-transspecific *S-RNases* are too variable and divergent to be compared in this way.

Alternatively, a shared S-allele may indicate an introgression event in the recent past, occurring after species divergence but prior to their geographical spread. P. dulcis and P. cerasifera share a common geographical centre of origin in the Middle East (Clapham et al. 1987; Ladizinsky 1999), and hybrids can be raised between the two species (Lecouls et al. 2004), so there is the possibility of genetic exchange between the two. Eryomine (1990) suggested that P. salicina may have contributed via introgression to the origin of P. cerasifera, which in turn may have been a parent species of *P. domestica*, in which case the occurrence of S-alleles from P. salicina in P. cerasifera and in P. domestica is a reflection of this relationship. However, the centre of origin of P. salicina is in eastern China (Faust and Surányi 1999), very distant from the centres of origin of either P. cerasifera or P. domestica. Geography would also exclude the introgression of P. mume S-alleles into P. cerasifera or P. dulcis.

# First introns

*Prunus S-RNase* alleles have a variable length intron immediately upstream of the start codon (Tao et al. 1999; Igic and Kohn, 2001). It is known that intron presence/absence can act as a taxonomic marker between closely related species. Their absence from Maloideae *S*-RNases agrees with the marked differences between Maloideae and Prunoideae *S*-RNases noted by Ushijima et al. (1998).

Sequence polymorphism and length variability generally prevents *S*-RNase second introns from being used in phylogeny construction, though *Prunus* first introns tend to be smaller and better conserved than second introns and could be used in phylogenetic analyses. It is noteworthy that the two trans-specific *S*-alleles from the two closest species (*P. domestica* and *P. salicina*) shared an almost identical first intron, while those from *P. domestica* and *P. avium* were less similar.

## SFB alleles

Pollen-*S* has been identified more recently than *S*-RNases; consequently there are far fewer sequences available in the databases and there is no record of trans-specific pollen-*S* sequences in the Rosaceae.

For each trans-specific S-RNase identified here, an accompanying trans-specific SFB was recovered. Differences between the trans-specific pairs were again small, though the degree of variation was greater than was present among the S-RNases, from a minimum of three to a maximum of 28 amino acid substitutions out of  $\sim$ 330 residues, or from 90.3 to 99.1% in overall identity. Variant residues did not cluster in or near the four hypervariable regions, or the numerous hypervariable residues identified by Ikeda et al. (2004), but were distributed uniformly through the polypeptide chain. Given that S-RNases and SFB polypeptides interact in a very particular and specific way, the expectation would be for identity and polypeptide structure to be highly conserved.

One point of note is the co-variation in *P. domestica*  $S_6$  and *P. salicina*  $S_a$ . The *S*-RNase amino acid sequence for these two alleles was identical, whereas their co-adapted SFB sequences displayed seven amino acid changes. Uyenoyama and Newbigin (2000) have suggested that new *S*-allele specificities arise initially through mutations in the pollen component; mutant pollen phenotypes will be less likely to meet stylar inhibition and will spread in the population and eventually the pollen-*S* mutation will be complemented by a corresponding mutation at stylar-*S*, and full self-incompatibility will be restored.

Another point of interest is the large difference between *P. mume*  $S_7$  and its supposed trans-specific pairs *P. cerasifera*  $S_{10}$  and *P. salicina*  $S_g$ . Percentage identities were 90.3 and 91.2% respectively, far lower than is seen among other trans-specific pairs. Two SFB alleles were recovered from the *P. mume* accession used, and the second allele showed only 78–80% identity with *P. salicina*  $S_{10}$ , so it seems unlikely that the incorrect allele has been used in the analyses. Of the 28 residue changes between the two alleles, only five are within the variable regions and only three are at hypervariable sites. Why the SFB polypeptide sequences are so divergent is not clear, especially as the corresponding *S*-RNase and second intron sequences are so highly conserved between species. It may be that SFB is more plastic, and can tolerate a greater degree of variation

without causing self-incompatibility to break down. It would be interesting to discover the conformation of the two mature proteins, to examine whether the residue differences impact on their tertiary structures.

Another difference between trans-specific *S*-allele haplotypes is the variation in length of the intergenic region between *S*-*RNase* and *SFB*. A 5 kb length difference was found in the intergenic region between *P. cerasifera*  $S_3$  and *P. dulcis*  $S_{12}$  (data not shown). Šurbanovski et al. (2007) found a ~0.7 kb difference in the intergenic region by sequencing trans-specific *S*-haplotypes of *P. avium*  $S_1$  and *P. tenella*  $S_8$ , which was caused almost entirely by a single 709 bp indel.

# S-allele evolution

The S-locus poses a series of questions to geneticists concerning the source of its allelic diversity, the generation of new alleles and the maintenance of functional integrity between its co-adapted elements. The S-locus does not easily fit conventional models of gene evolution. With most genes, random point mutations in DNA lead to harmful, neutral or beneficial changes in phenotype, changes upon which natural selection can then act. Mutations at the S-locus can break down the self-incompatibility system and confer self-compatibility. For the system to be maintained, both stylar and pollen components must somehow shift simultaneously to a new functional phenotype. Matton et al. (2000) have suggested a series of intermediate steps to a new S-allele specificity, without loss of self-incompatibility. An S-allele may be able to support a small number of amino acid changes in the S-RNase or SFB without loss of specificity. Indeed, minor changes may broaden specificity and permit a degree of change in the cognate component. These incremental changes could in time give rise to a completely new specificity.

Intermediate allele forms could therefore be identified in a single species after an extensive sequencing effort. The *Prunus* trans-specific *S*-RNases presented here are consistent with the Matton et al. (2000) model, albeit isolated in different species. It seems reasonable to propose that these trans-specific pairs have arisen in common ancestor species from common ancestor alleles, and have acquired a series of changes without apparent loss of function. They could be useful study material for functional studies of protein-protein interactions between *S*-RNase and SFB.

#### Phylogeny of Prunus

According to Rehder (1940), the genus *Prunus* can be divided into five subgenera: *Prunus*, *Amygdalus*, *Cerasus*, *Laurocerasus* and *Padus*. The plum species *P. cerasifera*, *P. domestica* and *P. salicina* are placed with the apricot

*P. mume* in subgenus *Prunus*. The almond *P. dulcis* is at a greater distance from the plums in subgenus *Amygdalus*, and sweet cherry *P. avium* is placed further still from the plums in subgenus *Cerasus*. Modern phylogenies constructed from ITS and *trnL-trnF* sequences have supported these classifications (Bortiri et al. 2001). The close relationship between the various species of plum is suggested by the trans-specific *S*-RNase data reported here, as the closest identities were always between *S*-RNases of *P. cerasifera*, *P. domestica* and *P. salicina*.

# Final comments

It is now possible to study allelic diversity in both components of the *Prunus S*-locus. This paper presents an exemplary set of co-adapted *S*-RNases and SFB alleles that show convincing trans-specific evolution for both genes, for coding regions and for introns. There is now scope for examining the determining factors of allele specificity and allele generation at the *Prunus S*-locus, by controlled pollination and by *in silico* predictions of protein tertiary structure.

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