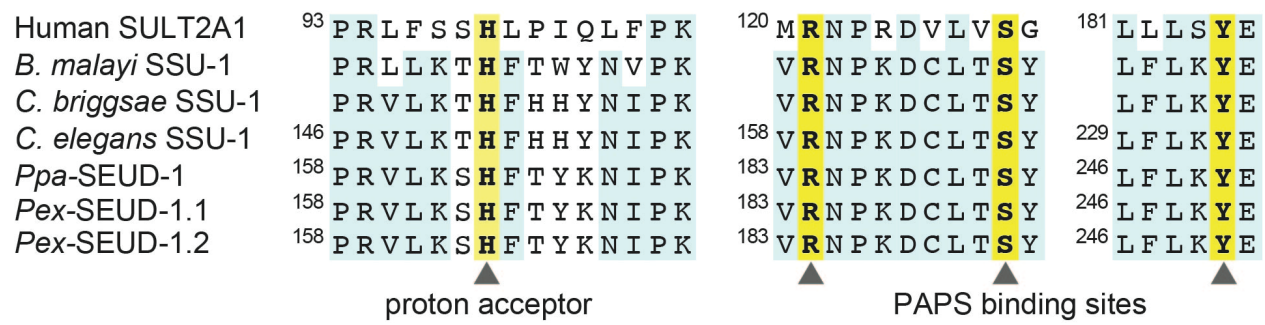
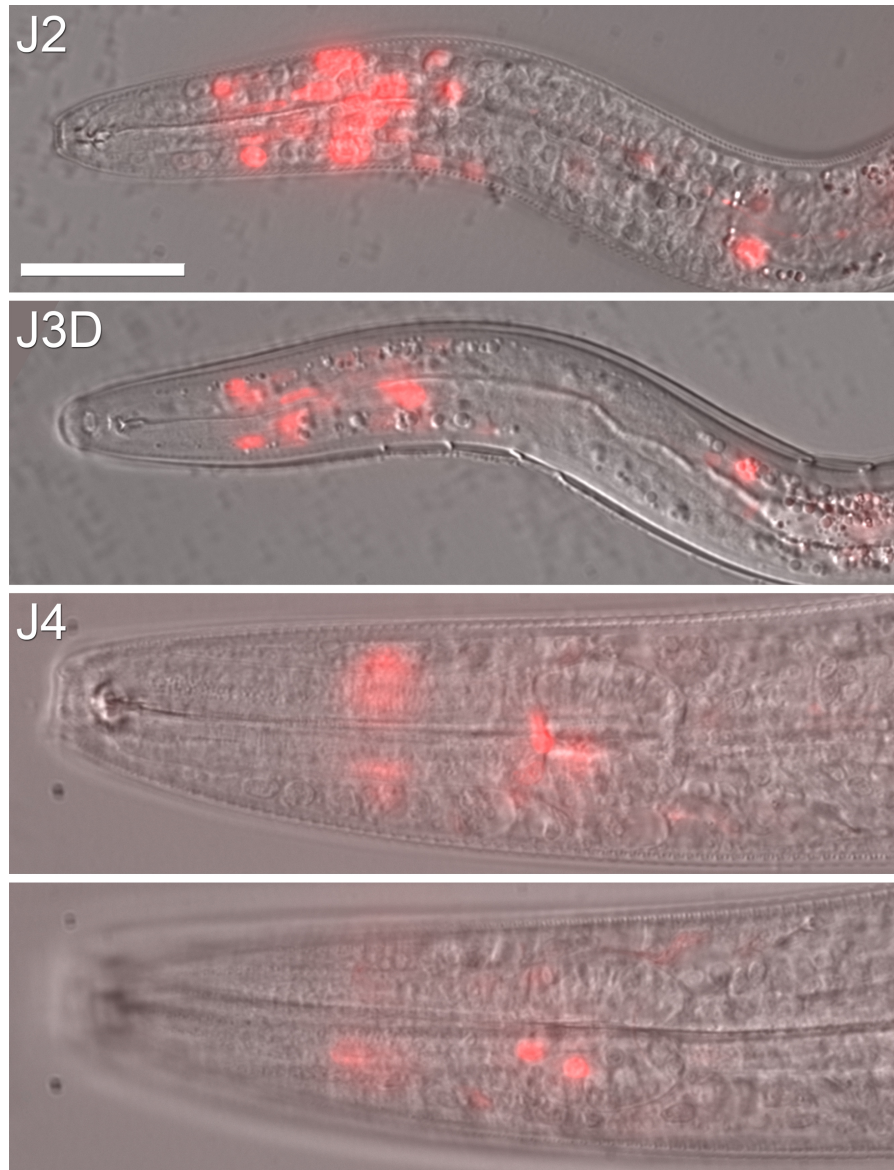


**A sulfotransferase dosage-dependently regulates mouthpart polyphenism in the
nematode *Pristionchus pacificus***

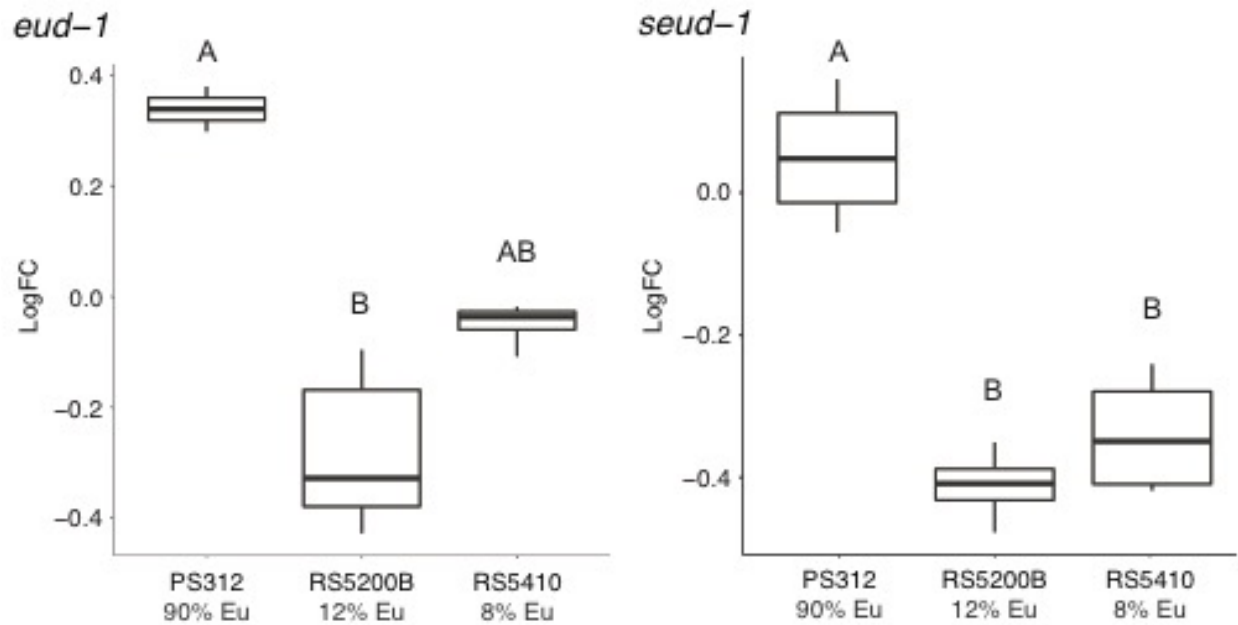
Bui, Ivers & Ragsdale

Supplementary Information





Supplementary Figure 2. Expression of *seud-1* in *Pristionchus pacificus* juveniles. Expression of a nucleus-localised *seud-1*^{promoter}::*TurboRFP* is shown for J2, dauer (J3D), and J4 larvae. The cells that build and make-up dimorphic mouthparts were not all observed to express *seud-1* at all times, including among individuals of a given life-stage, although collectively expression was observed in the same set of cells identified in the text. Pictured J2 shows expression in pharyngeal myoepithelial (pm) and epithelial (mc) cells, as well as in arcade (ac) and epidermal (hyp) cells; dauers reported expression in similar cells, suggesting an overlapping function for *seud-1* in direct-developing and facultative diapause larvae; J4, shown in two focal planes (above, left of sagittal; below, right of sagittal), highlights expression in pm1, pm3, the pharyngeal neuron I1, and possibly I2, another neuron known to innervate teeth-producing (pm1) cells, as well as weak expression in hyp and arcade cells. Scale bar (all images), 20 μ m.



Supplementary Figure 3. Expression levels of *eud-1* and *seud-1* in *Pristionchus pacificus* polyphenism variants. Expression was quantified for wild isolates with different morph-ratios under standardised environmental cues. Mean expression levels of *eud-1* and *seud-1* were calculated as log fold change (LogFC) normalized to two reference genes (*tth-4* and Y45F10D). We found significant differences among strains in *eud-1* expression ($P = 0.03$, Tukey's test). Specifically, the Eu-biased California strain (PS312) had significantly higher expression of *eud-1* than the St-biased strain RS5200B ($P_{\text{adj}} = 0.03$, Tukey's test) and had a non-significant trend for higher expression than the St-biased strain RS5410 ($P_{\text{adj}} = 0.10$, Tukey's test). Additionally, we found significant differences among strains in *seud-1* expression ($P = 0.02$, Tukey's test). Here, the reference strain had a significantly higher level of *seud-1* expression than two of the other strains (RS5200B and RS5410; $P = 0.02$ and $P = 0.03$, respectively, Tukey's test). % Eu, mean proportion of eurytomatic nematodes under standardised laboratory conditions, as previously reported¹. Box plots show $\Delta\Delta\text{Ct}$ values (centre line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range).

Supplementary Table 1. Mouth-morph phenotypes for reference and mutant strains of *Pristionchus pacificus*.

Genotype	% Eu	<i>n</i>
PS312	90	250
<i>eud-1(tu445)</i>	0	250
<i>seud-1(iub7); eud-1(tu445)</i>	100	260
<i>seud-1(iub8); eud-1(tu445)</i>	100	120

Supplementary Table 2. Summary of mutant lesions of *seud-1* alleles.

Allele	Lesion	Genome position	Location in gene	Effect
<i>iub7</i>	G to A	Contig20:1004979	Exon 8	STOP
<i>iub8</i>	C to T	Contig20:1009019	Exon 1	STOP

Genome position is given with respect to the “Hybrid1” assembly for *Pristionchus pacificus*.

Supplementary Table 3. Primers used to confirm *seud-1* and *eud-1* genotypes.

Gene	Forward primer	Reverse primer	Amplicon length (bp)
<i>seud-1</i>	GAAAGAGAATGCGCTCTTCG	GGATGGAAAGGTGGGTTTCT	338
<i>eud-1</i>	TCACTACCGGAGGGTGCTAC	TTCTACGTTTCGCTCTGCAA	361

Amplicons distinguished between mutant (*iub7*, *tu445*) and wild-type genotypes following outcrossing and generation of double- and triple-mutant lines of *Pristionchus pacificus*.

Supplementary Table 4. Mouth-morph phenotypes for rescued strains and epistasis tests.

Genotype	% Eu	<i>n</i>
Ex[<i>seud-1</i>]	28	212
<i>nhr-40(tu505)</i>	100	250
<i>nhr-40(tu505); Ex[seud-1]</i>	100	262
Ex[<i>nhr-40</i>]	1	139
<i>seud-1(iub7); Ex[nhr-40]</i>	100	61

Supplementary Table 5. Primers used for generating transgenic *seud-1* constructs.

Region	Forward primer	Reverse primer	Amplicon length (bp)
Promoter	TCTGAAGGGGTACGGTAGG	TCACGGATCCAACCTTTTCGA	4,223
Full gene	CTTTATCAACGAATTAGTAGGAA	CAGCAACTTTCACGTGTCTG	10,983

Supplementary Table 6. Crosses performed to manipulate gene dosage in *Pristionchus pacificus*.

Cross type	Crosses (N)	F1 genotypes	% Eu	n
PS312 ♂ x <i>pdl-2</i> (-) ♀	28	<i>seud-1</i> (+/+); <i>eud-1</i> (+/+)	24	160
		<i>seud-1</i> (+/+); <i>eud-1</i> (+/0)	91	198
<i>seud-1</i> (<i>iub7</i>); <i>eud-1</i> (<i>tu445</i>) ♂ x <i>pdl-2</i> (-); <i>eud-1</i> (<i>tu445</i>) ♀	30	<i>seud-1</i> (+/-); <i>eud-1</i> (-/-)	0	248
		<i>seud-1</i> (+/-); <i>eud-1</i> (-/0) #2	0	708
PS312 ♂ x <i>pdl-2</i> (-); <i>eud-1</i> (<i>tu445</i>) ♀	45	<i>seud-1</i> (+/+); <i>eud-1</i> (-/+)	22	148
		<i>seud-1</i> (+/+); <i>eud-1</i> (-/0)	0	25
<i>seud-1</i> (<i>iub7</i>) ♂ x <i>pdl-2</i> (-); <i>eud-1</i> (<i>tu445</i>) ♀	90	<i>seud-1</i> (+/-); <i>eud-1</i> (-/+)	90	1231
		<i>seud-1</i> (+/-); <i>eud-1</i> (-/0) #1	0	1988
<i>seud-1</i> (<i>iub7</i>); <i>eud-1</i> (<i>tu445</i>) ♂ x <i>pdl-2</i> (-) ♀	27	<i>seud-1</i> (+/-); <i>eud-1</i> (+/-)	90	202
		<i>seud-1</i> (+/-); <i>eud-1</i> (+/0) #1	90	556
<i>seud-1</i> (<i>iub7</i>) ♂ x <i>pdl-2</i> (-) ♀	26	<i>seud-1</i> (+/-); <i>eud-1</i> (+/+)	99	406
		<i>seud-1</i> (+/-); <i>eud-1</i> (+/0) #2	91	289
<i>seud-1</i> (<i>iub7</i>); <i>eud-1</i> (<i>tu445</i>) ♂ x <i>seud-1</i> (<i>iub7</i>); <i>pdl-2</i> (-) ♀	14	<i>seud-1</i> (-/-); <i>eud-1</i> (+/-)	100	127
		<i>seud-1</i> (-/-); <i>eud-1</i> (+/0) #1	100	298
<i>seud-1</i> (<i>iub7</i>) ♂ x <i>seud-1</i> (<i>iub7</i>); <i>pdl-2</i> (-) ♀	26	<i>seud-1</i> (-/-); <i>eud-1</i> (+/+)	100	260
		<i>seud-1</i> (-/-); <i>eud-1</i> (+/0) #2	100	437
<i>seud-1</i> (<i>iub7</i>); <i>eud-1</i> (<i>tu445</i>) ♂ x <i>seud-1</i> (<i>iub7</i>); <i>eud-1</i> (<i>tu445</i>) ♀	7	<i>seud-1</i> (-/-); <i>eud-1</i> (-/-)	100	509
		<i>seud-1</i> (-/-); <i>eud-1</i> (-/0)	100	146

Supplementary Table 7. *ssu-1* homologues extracted from published genome sequences.

Species	Gene or putative gene	Sequence identifier	Transcript
<i>Ancylostoma ceylanicum</i>	<i>ssu-1-A</i>	ANCCEY_10587	Predicted
<i>Ancylostoma ceylanicum</i>	<i>ssu-1-B</i>	EYC21177	Predicted
<i>Ancylostoma ceylanicum</i>	<i>ssu-1-C</i>	EYC21178	Predicted
<i>Ancylostoma ceylanicum</i>	<i>ssu-1-D</i>	EYC21179.1	Predicted
<i>Ancylostoma duodenale</i>	<i>ssu-1</i>	ANCDUO_03922	Predicted
<i>Brugia malayi</i>	<i>ssu-1</i>	Bm7300a	Partially confirmed
<i>Caenorhabditis brenneri</i>	<i>ssu-1</i>	CBN06215	Predicted
<i>Caenorhabditis briggsae</i>	<i>ssu-1</i>	CBG12932	Confirmed
<i>Caenorhabditis elegans</i>	<i>ssu-1</i>	CELE_Y113G7A.11	Partially confirmed
<i>Caenorhabditis japonica</i>	<i>ssu-1</i>	CJA13369n	Predicted
<i>Caenorhabditis remanei</i>	<i>ssu-1</i>	CRE21191	Predicted
<i>Dictyocaulus viviparus</i>	<i>ssu-1</i>	DICVIV07955	Predicted
<i>Haemonchus contortus</i>	<i>ssu-1</i>	HCOI_00221200	Predicted
<i>Loa loa</i>	<i>ssu-1</i>	LOAG_07209	Predicted
<i>Necator americanus</i>	<i>ssu-1</i>	NECAME_16579	Predicted
<i>Oesophagostomum denatum</i>	<i>ssu-1</i>	OESDEN_03346	Predicted
<i>Pristionchus borbonicus</i>	<i>ssu-1</i>	scaffold4422_c61143	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1.1</i>	scaffold2136-pex_aug2013-10260	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1.2</i>	scaffold86-pex_aug2013-27432	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 980	scaffold1056-pex_aug2013-980	Predicted
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 1471	scaffold1098-pex_aug2013-1471	Predicted
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 9567	scaffold201-pex_aug2013-9567	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 10260	scaffold2136-pex_aug2013-10260	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 20599	scaffold494-pex_aug2013-20599	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 23887	scaffold64-pex_aug2013-23887	Confirmed
<i>Pristionchus exspectatus</i>	<i>ssu-1</i> homologue 27432	scaffold86-pex_aug2013-27432	Confirmed
<i>Pristionchus pacificus</i>	<i>ssu-1</i>	Contig20-aug8366.t1	Confirmed
<i>Pristionchus pacificus</i>	<i>ssu-1</i> homologue 8323	Contig20-aug8323.t1	Confirmed
<i>Pristionchus pacificus</i>	<i>ssu-1</i> homologue 18291	Contig80-aug18291.t1	Predicted
<i>Pristionchus pacificus</i>	<i>ssu-1</i> homologue 20924	Contig113-aug20924.t1	Partially confirmed
<i>Pristionchus pacificus</i>	<i>ssu-1</i> homologue 21057	Contig116-aug21057.t1	Confirmed
<i>Strongyloides ratti</i>	<i>ssu-1-A</i>	SRAE_0000037000	Partially confirmed
<i>Strongyloides ratti</i>	<i>ssu-1-B</i>	SRAE_2000472200	Predicted
<i>Strongyloides ratti</i>	<i>ssu-1-C</i>	SRAE_1000077400	Predicted
<i>Strongyloides ratti</i>	<i>ssu-1-D</i>	SRAE_2000454300	Predicted
<i>Strongyloides ratti</i>	<i>ssu-1-E</i>	SRAE_2000472300	Predicted
<i>Strongyloides ratti</i>	<i>ssu-1-F</i>	SRAE_2000472100	Predicted
<i>Toxocara canis</i>	<i>ssu-1</i>	Tcan_04678	Predicted
<i>Wucheria bancrofti</i>	<i>ssu-1</i>	WUBG_09659	Predicted

Supplementary Table 8. Primers to quantify *eud-1* and *seud-1* expression in *Pristionchus exspectatus* inbred lines.

Gene	Forward primer	Reverse primer	Amplicon length (bp)
<i>eud-1</i>	ATGTCATCATACTCATGGTGGAC	CTCCGTCAGTACTGGTTTCTTCAT	210
<i>seud-1A</i>	CTCGTGGTTGGAGGGCATCGAAA	CCGCCTAAGAACGAGGCAATTTGA	114
<i>seud-1B</i>	ATCAGTTTGGATACGAACAATCCT	TATGAAGACACTTCACTGGTTGGT	154
<i>tbb-4</i> (reference)*	CTCGGAGGAGGAACTGGATC	GACCGTGTCAGAGACCTTAG	123

*Primer sequence published previously².

Supplementary Table 9. Genotypes and phenotypes of interspecific hybrids.

Cross type	F1 genotype	% Eu	Crosses (N)	n
RS5522B ♂ x <i>pdl-2(-); eud-1(tu445)</i> ♀	<i>eud-1(-/+); seud-1(+ /++)</i>	1	14	114
<i>seud-1(iub7); eud-1(tu445)</i> ♂ x RS5522B ♀	<i>eud-1(+/-); seud-1(++/-)</i>	8	19	72
PS312 ♂ x RS5522B ♀	<i>eud-1(+ /+); seud-1(++ /+)</i>	70	15	153
RS5522B ♂ x <i>pdl-2(-)</i> ♀	<i>eud-1(+ /+); seud-1(+ /++)</i>	68	21	47
<i>seud-1(iub7)</i> ♂ x RS5522B ♀	<i>eud-1(+ /+); seud-1(++ /-)</i>	89	54	404
RS5522B ♂ x <i>pdl-2(-); seud-1(iub7)</i> ♀	<i>eud-1(+ /+); seud-1(++ /-)</i>	87	34	227

PS312, wild-type *P. pacificus*; RS5522B, *P. exspectatus*.

Supplementary Table 10. Primers to quantify *seud-1* and *eud-1* expression in *Pristionchus pacificus* isolates.

Gene	Forward primer	Reverse primer	Amplicon length (bp)
<i>eud-1</i>	GGCACATTCGACCTCATGTCGA	TGCCGCCTGGACACATCTGCT	157
<i>seud-1</i>	AAGTACATTTTTGCAGTGAGAA	TGAACAACCTTGAAGAACACATC	121
<i>tbb-4</i> (reference)*	CTCGGAGGAGGAACTGGATC	GACCGTGTCTCAGAGACCTTAG	123
Y45F10D (reference)*	GATCAGAGTCGACGATAATG	CTTCTCAGCCCATTTCGATG	130

*Primer sequences published previously².

Supplementary references

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