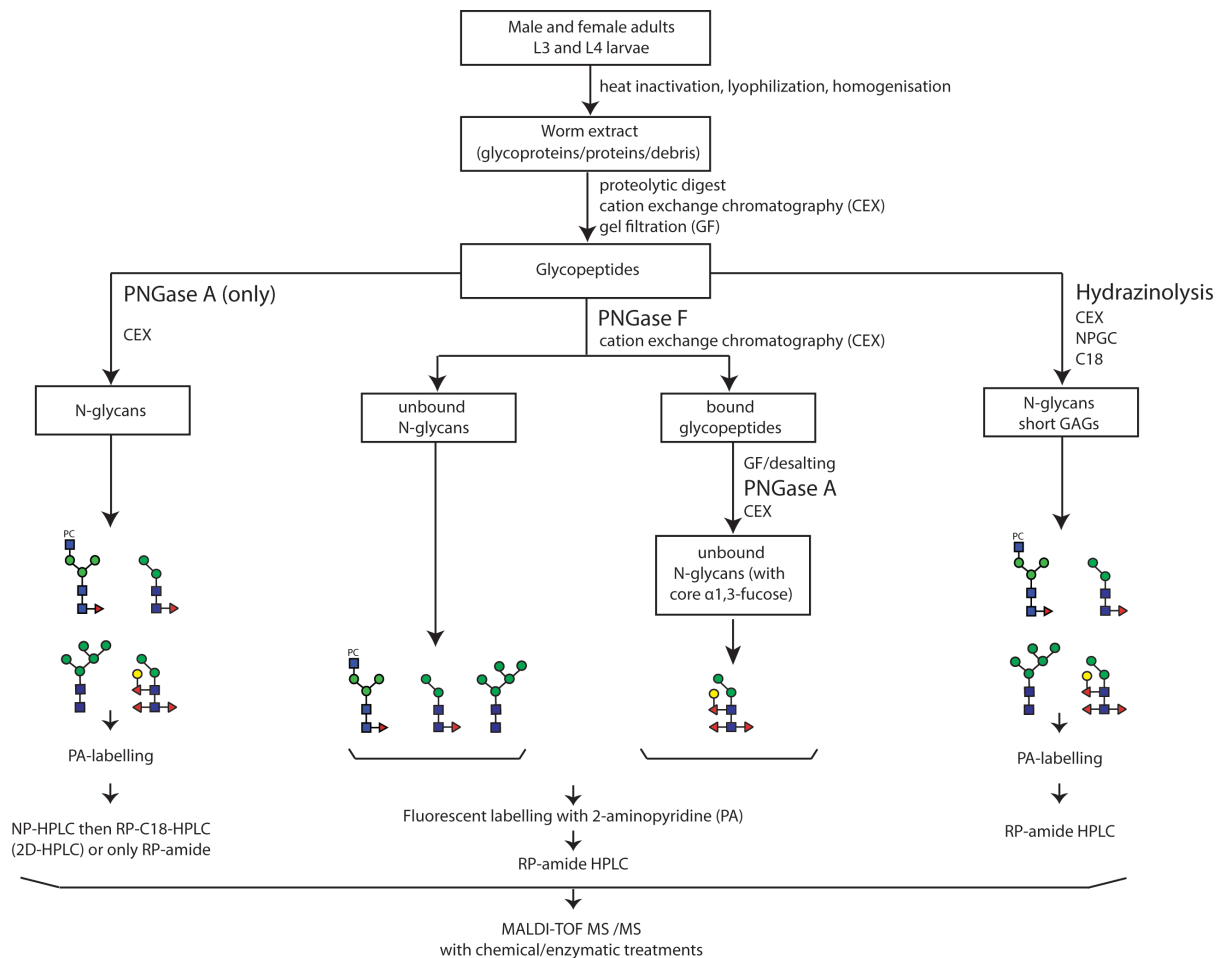


Gender and developmental specific N-glycomes of the porcine parasite *Oesophagostomum dentatum*

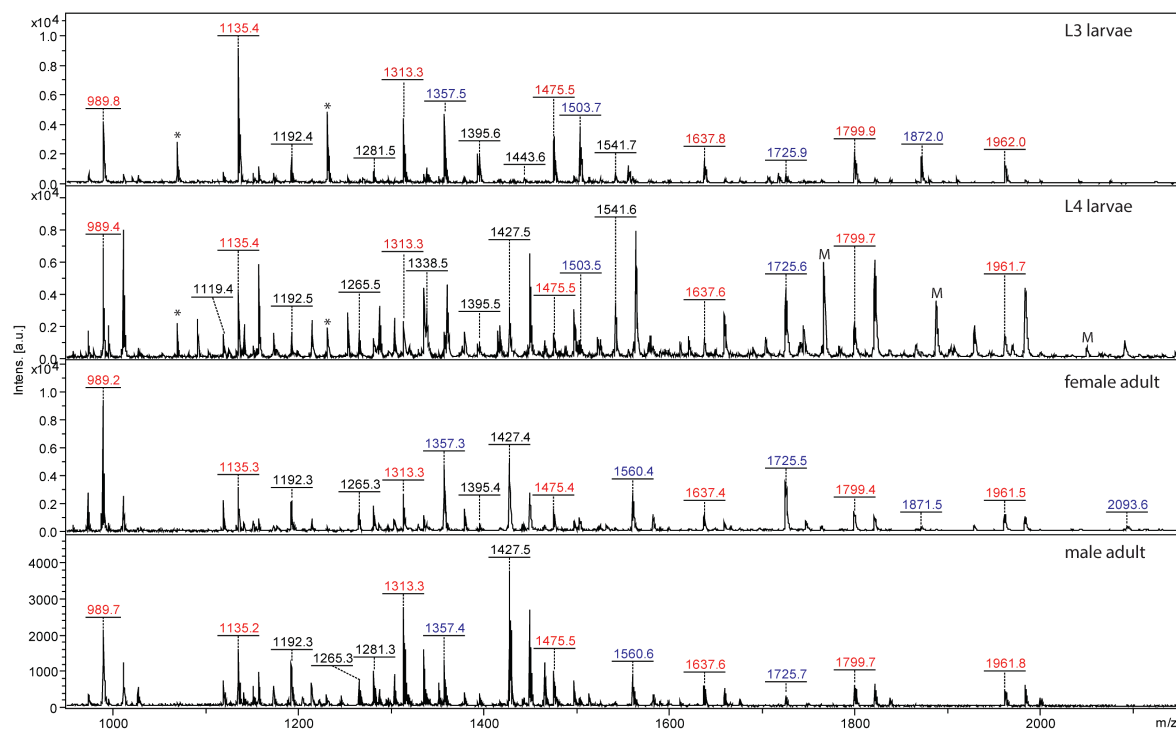
Carmen Jimenez-Castells, Jorick Vanbeselaere, Sonja Kohlhuber, Bärbel Ruttkowski,
Anja Joachim and Katharina Paschinger

Supplementary Data

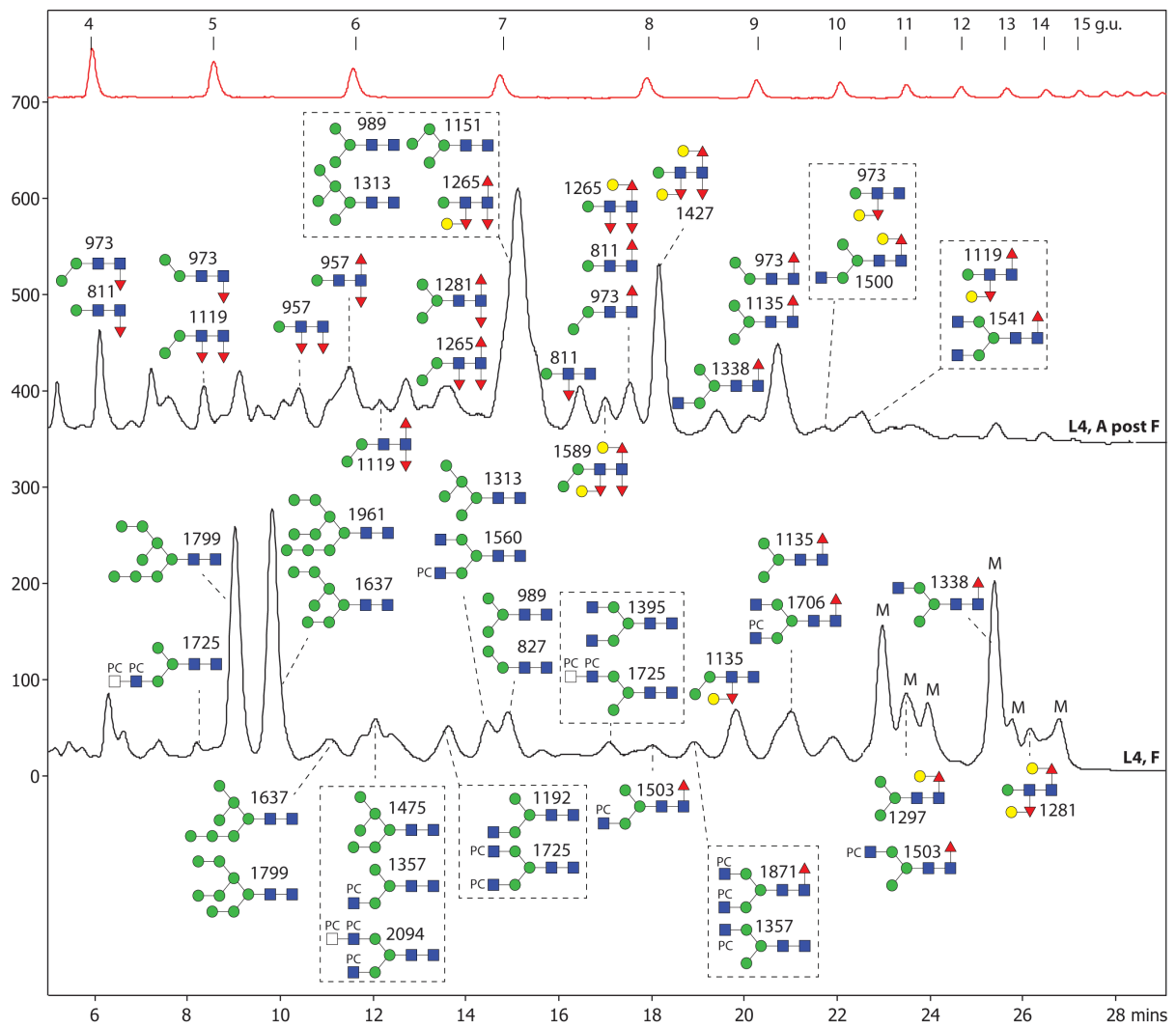
Scheme: Glycomic workflow for analysis of *O. dentatum* N-glycans. The basic approaches to isolate the N-glycans are shown, indicating that either serial PNGase F/A digestion, PNGase A alone or hydrazinolysis were employed. While the PNGase A only digests (for overall MALDI-TOF MS see Supplementary Figure 1) were subject to 2D-HPLC (NP-HPLC followed by RP-HPLC on C18; see Figure 1) as well as RP-amide HPLC, the other N-glycan pools were subject to 1D-HPLC (RP-amide only; see Figures 2 and 3 as well as Supplementary Figures 2 and 3). All RP-C18 and RP-amide HPLC fractions were subject to MALDI-TOF MS and MS/MS for which selected data before and after enzymatic/chemical digestions are shown in Figures 4-7.



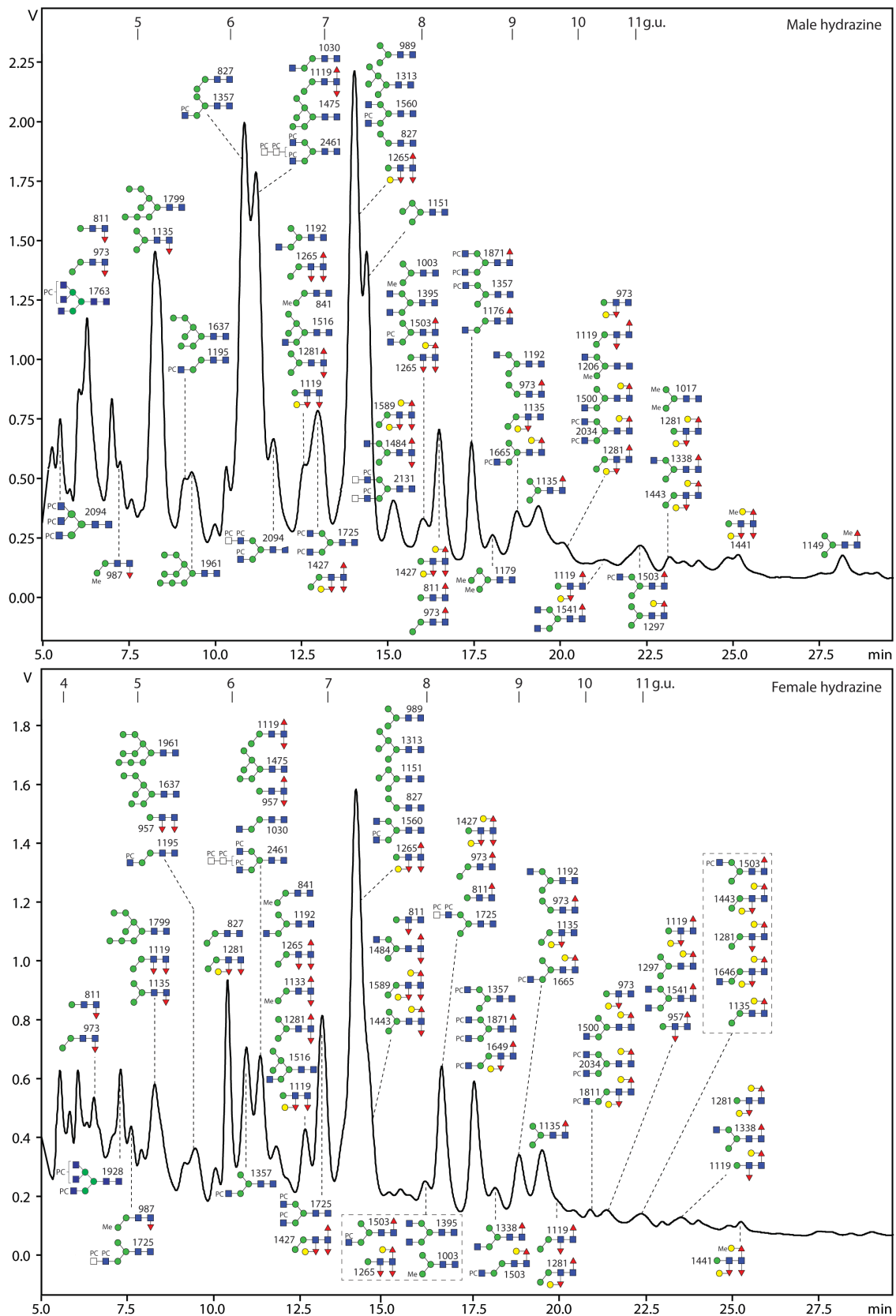
Supplementary Figure S1: MALDI-TOF MS profiling of pyridylaminated PNGase A-released N-glycans from *O. dentatum*. Positive mode MALDI-TOF mass spectra of the unfractionated PNGase A-released glycans from L3 and L4 larvae and male and female adults are shown with the major protonated pyridylaminated species annotated with the relevant m/z (red, pauci- and oligomannosidic; blue, phosphorylcholine-modified; black, fucosylated and hybrid/complex; asterisks indicate oligohexose contaminants of the larval preparations). The intensities are given in arbitrary units (a.u.); sodiated adducts are not annotated but are present particularly in the L4 preparation; as the L4 stage is maintained in porcine serum, some glycans of mammalian origin are also present (e.g., Hex₃HexNAc₅Fuc₁ and Hex₅₋₆HexNAc₄Fuc₁; annotated with 'M').



Supplementary Figure S2: RP-amide chromatograms of larval (*O. dentatum* L4) PNGase A and PNGase F-released glycans; M indicates mammalian-like glycans probably originating from pig serum used during maintenance. The oligoglucose standard chromatogram is shown in red and annotated with glucose units. Structural variance is reduced as compared to *O. dentatum* adults. The fluorescence scale (excitation/emission 320/400 nm) is in millivolts.



Supplementary Figure S3: RP-amide chromatograms of hydrazine-released N-glycans from male and female adult *O. dentatum*. Peaks are annotated with proposed structures and retention times in terms of minutes and glucose units. The fluorescence scale (excitation/emission 320/400 nm) is in volts.



Supplementary Table

A summary of structures, elution times, occurrence in different preparations (male, female, L3 and L4 PNGase A only; male and female A post F; male and female F; male and female hydrazine-released N-glycans) as well as evidence for the structural annotations with reference to relevant figures in the main text.

The PNGase A post PNGase F ('A post F') digests lack many of the low abundance structures present in the previous PNGase F ('F') digests, whereas the PNGase A only ('A only') and hydrazine digests contain structures present in both the 'F' and 'A post F' digests; PNGase A and hydrazine, but not PNGase F, are capable of releasing N-glycans with a proximal (reducing terminal) core α 1,3-fucose.
















For the 2D-HPLC analyses (NP then RP-HPLC followed by MALDI-TOF MS of PNGase A only digests), percentage abundances are given based on integration of fluorescent intensities; for the 1D-HPLC analyses (RP-amide then MALDI-TOF MS of male and female A post F, female A only, male and female F and male and female hydrazine-released N-glycans) only qualitative information is shown (+, present; nd, not determined; trace, low amount).

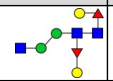

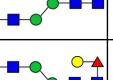
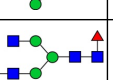
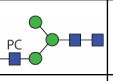
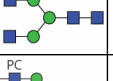
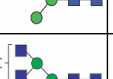
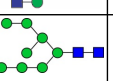
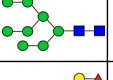
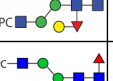

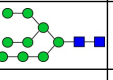
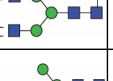


Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	811.35	4.3	nd	nd	0.21	nd	+	+	+	nd	nd	M, F	Key fragment: 446, 665 Sensitive to HF (-1F) then MS/MS frag. 300 (no proximal Fuc) coelution with Pristionchus and Haemonchus
	811.35	7.5	nd	nd	nd	nd	trace	trace	+	+	+	M, F	Key fragments: 300 (no proximal fucose), 665 coelution with Pristionchus and Haemonchus
	811.35	8.2	nd	nd	nd	nd	+	+	+	+	+	M, F	Key fragments: 446, 665 coelution with Pristionchus and Haemonchus
	827.34	6.0	2,92	1,35	0.08	nd	+	+	+	+	+	M, F	Key fragments: 300, 665 Sensitive to 3Man (-1H) coelution with Pristionchus and Haemonchus
	827.34	6.8	0.34	2.58	0.75	nd	+	+	+	+	+	M, F	Key fragments: 300, 665 Sensitive to 6Man (-1H) coelution with Pristionchus and Haemonchus
	841.36	6.6	nd	nd	nd	nd	+	+	+	+	+	M, F	Key fragments: 300, 665 Elutes after 827 isomer at 6.0 g.u.
	957.40	5.5	nd	0.48	0.04	nd	trace	+	+	nd	nd	M, F	Key fragments: 446, 811 (loss of 146) Sensitive to HF (-2F), then MS/MS frag. 300 (loss of distal and proximal Fuc)
	957.40	6.0	nd	nd	0.32	nd	nd	+	+	nd	nd	M, F	Key fragments: 446, 592, 811 Sensitive to HF (-1F) then MS/MS frag. 446 coelution with Pristionchus
	957.40	10.2	nd	nd	nd	nd	nd	nd	+	trace	trace	M, F	Key fragments: 446, 665, 811 Sensitive to bovine Fuc (-1F), then MS/MS frag. 300 (loss of proximal Fuc) Sensitive to HF (-1F), MS/MS still with 446
	973.40	4.3	0.73	1.47	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 827 Sensitive to 3Man (-1H) Sensitive to HF (-1F) then MS/MS frag. 300 (no core Fuc) coelution with Pristionchus and Haemonchus
	973.40	5.2	nd	nd	nd	nd	trace	trace	trace	nd	nd	nd	Key fragments: 446, 827 coelution with Pristionchus
	973.40	8.2	nd	nd	nd	nd	+	+	+	+	+	M, F	Key fragments: 446, 827 Sensitive to 3Man (-1H) coelution with Pristionchus and Haemonchus
	973.40	8.8	0.34	0.92	0.70	nd	+	+	+	+	+	M, F	Key fragments: 446, 827 Sensitive to 6Man (-1H), Resistant to 3Man coelution with Pristionchus and Haemonchus
	973.40	9.8	nd	0.75	0.18	nd	+	+	+	+	+	M, F	Key fragments: 300, 665, 811 (no proximal Fuc) Resistant to 3Man Sensitive to HF (-FH)
	973.40	11.5-12.5	nd	nd	nd	nd	nd	nd	+	+	+	nd	Key fragments: 608, 665
	987.41	4.8	0.49	1.62	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 841 (eluting after 973 isomer at 4.3 g.u.) Sensitive to HF (-1F) then MS/MS frag. 300 (loss of core Fuc) Resistant to bovFuc

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	989.39	7.2	14.52	18.44	10.92	1.65	+	+	+	+	+	M, F	Key fragments: 300, 827 Sensitive to 3Man (-1H), Sensitive to JBMan (-2H) coelution with Pristionchus and Haemonchus
	1003.41	7.6	0.60	nd	nd	nd	+	trace	trace	+	trace	M, F	Key fragments: 300, 841 (Figure 6B) Resistant to 3Man but sensitive to JBMan (-1H)
	1017.42	11.5-12.5	0.72	nd	nd	nd	+	trace	trace	+	trace	M	Key fragments: 300, 717, 841 (Figure 6C)
	1030.42	6.2	1.58	0.15	nd	nd	+	+	+	+	+	M, F	Key fragments: 365, 665 Sensitive to FDL (-1N) coelution with Pristionchus
	1103.46	7.2	nd	nd	nd	nd	trace	trace	trace	nd	nd	trace	Key fragments: 446, 592, 811 (-2 x 146)
	1119.46	5.2	0.11	0.27	0.01	nd	+	+	+	nd	nd	F	Key fragments: 446, 827, 973 Sensitive to 3Man (-1H) Sensitive to HF (-2F; Figure 4A/B) then MS/MS frag. 300 (loss of core Fuc) coelution with Pristionchus
	1119.46	6.2	0.58	1.42	1.44	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 592, 973 Sensitive to 3Man (-1H) Sensitive to HF (-1F) then MS/MS frag. 446 coelution with Pristionchus and Haemonchus
	1119.46	6.6	nd	nd	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 665, 811 (loss of 308), 957, 973 Sensitive to HF (-F/FH), then MS/MS frag. 300 Resistant to 3Man
	1119.46	9.6	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 446, 827, 973 (loss of 146) Sensitive to 3Man (-1H)
	1119.46	10.2	0.24	0.48	0.15	nd	trace	+	+	+	+	M, F	Key fragments: 446, 665, 811 (loss of 308), 957, 973 Resistant to 3Man, but sensitive to HF (-FH) then MS/MS frag. 446, 665 Sensitive to bov Fuc (-1F), then MS/MS frag. 300 (loss of core Fuc)
	1119.46	11.5-12.5	nd	nd	nd	nd	nd	nd	+	+	+	trace	Key fragments: 608, 665, 811, 973 (loss of 146)
	1133.47	6.7	nd	nd	nd	nd	+	+	+	nd	nd	trace	Key fragments: 446, 592, 841, 987 Elution after 1119 isomer at 6.2 g.u.
	1135.45	5.2	0.34	0.27	0.01	1.03	+	+	+	nd	nd	trace	Key fragments: 446, 827, 989 (Figure 4A/B) Sensitive to 3Man (-1H) Sensitive to HF (-1F) then MS/MS frag. 300 (loss of core Fuc) coelution with Pristionchus and Haemonchus
	1135.45	8.8	0.31	1.03	0.28	nd	+	+	+	+	+	trace	Key fragments: 300 (no proximal Fuc), 827 (loss of 308) Sensitive to 3Man (-1H) Sensitive to HF (-FH; Figure 4C/D) coelution with Haemonchus
	1135.45	9.3	2.93	4.03	6.98	17.49	+	+	+	+	+	M, F	Key fragments: 446, 973, 989 (Figure 4E/F) Sensitive to 3Man (-1H) Sensitive to JBMan (-2H) Sensitive to BovFuc (-1F) then MS/MS 300 (no core Fuc) coelution with Pristionchus and Haemonchus
	1135.45	11.0	nd	nd	nd	nd	trace	trace	+	+	+	M, F	Key fragments: 608, 827 (Figure 4G/H) Sensitive to β4Gal (-1H), then MS/MS frag. 446 Resistant to bovine Fuc

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence	
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine		
	1149.47	>12	nd	nd	nd	nd	nd	nd	nd	nd	trace	nd	M	Key fragments: 460, 989 (loss of 160; Figure 6D)
	1151.45	7.2	1.72	1.75	1.16	0.05	+	+	+	+	+	+	M, F	Key fragments: 300, 827, 989 Sensitive to 3Man (-1H) and sensitive to JbMan (-3H) coelution with Pristionchus and Haemonchus
	1176.48	8.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	M	Key fragments: 446, 811, 1030
	1179.48	8.5	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	M	Key fragments: 177, 841, 1003 Elution after 1151 at 7.2 g.u.
	1192.47	6.6	2.36	0.59	4.78	2.33	+	+	+	+	+	+	M, F	Key fragment: 1030 Resistant to 3Man coelution with Pristionchus and Haemonchus
	1192.47	8.8	3.65	3.85	1.82	1.19	+	+	+	+	+	+	M, F	Key fragments: 365, 989 Sensitive to 3Man (-1H) coelution with Pristionchus and Haemonchus
	1195.48	5.5	nd	nd	nd	nd	+	+	+	+	+	+	M, F	Key fragments: 369, 531 coelution with Pristionchus (elution before 1030 at 6.2 g.u.)
	1195.48	7.8	nd	nd	nd	nd	+	+	+	+	+	+	nd	Key fragments: 369, 531
	1206.49	9.7	trace	trace	nd	nd	trace	nd	+	trace	trace	trace	M, F	Key fragments: 841, 1003 Elution after 1192 isomer at 8.8 g.u. Resistant to FDL
	1265.51	6.6	0.42	0.30	0.13	1.69	+	+	+	nd	nd	nd	M, F	Key fragments: 446, 592, 827, 973, 1119 (Figure 4I) Sensitive to 3Man (-1H) Sensitive to HF (-2F) then MS/MS frag. 446 (loss of one proximal Fuc) coelution with Pristionchus and Haemonchus
	1265.51	7.2	1.64	1.37	1.22	nd	+	+	+	nd	nd	nd	M, F	Key fragments: 446, 592, 665, 957 (loss of 308), 1103, 1119 (Figure 4J) Resistant to 3Man Sensitive to HF (-F/FH) then MS/MS frag. 446 (loss of one proximal Fuc)
	1265.51	7.8	0.79	0.80	1.51	nd	+	+	+	nd	nd	nd	M, F	Key fragments: 608, 665, 754, 957, 1119 (loss of 146; Figure 4K) Sensitive to β4Gal (-1H) then MS/MS frag. 446, 592 Sensitive to HF (-2F) then MS/MS frag. 608, 665 (loss of proximal Fuc)
	1279.53	10.2	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	F	Key fragments: 446, 592, 1119 (loss of 160), 1133 (Figure 6E) Sensitive to HF (-F/FMe), then MS/MS frag. 446 (loss of one proximal Fuc) Sensitive to 3 Man (-1H) and bov Fuc (-1F) then MS/MS 446 (loss of one proximal Fuc)
	1281.51	6.0	1.29	2.19	nd	nd	nd	+	+	nd	nd	nd	F	Key fragments: 446, 827, 973 (loss of 308), 1119, 1135 (Figure 4L) Sensitive to 3Man (-H) then MS/MS frag. 446, 811 (loss of 308) Sensitive to HF (-F/FH) then MS/MS frag. 300 (no core Fuc) coelution with Haemonchus
	1281.51	6.6	0.28	0.20	0.51	0.42	+	+	+	nd	nd	nd	M, F	Key fragments: 446, 592, 1119, 1135 (Figure 4M) Sensitive to 3Man (-1H) Sensitive to HF (-1F) then MS/MS frag. 446 (loss of one proximal Fuc) coelution with Pristionchus and Haemonchus
	1281.51	9.3	nd	nd	nd	1.19	nd	nd	+	+	+	+	trace	Key fragments: 446, 973 (loss of 308), 1119, 1135 (Figure 4N) Sensitive to HF (-FH) then MS/MS still with 446 (no loss of proximal Fuc) Sensitive to 3Man (-1H) coelution with Haemonchus

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	1281.51	10.5-11.5	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 608, 827, 1135 (Figure 4O) Sensitive to 3Man (-1H) Corresponds to one glycan in hex-2;hex-3 mutant
	1281.51	11.5-12.5	nd	nd	0.49	nd	+	nd	+	+	+	M, F	Key fragments: 608, 665, 973 (loss of 308), 1119 (Figure 4P) Resistant to 3Man Sensitive to HF (-FH) then MS/MS frag. 608, 665
	1295.52	6.6	nd	0.56	nd	nd	nd	+	+	nd	nd	trace	Key fragments: 446, 841, 987 (loss of 308), 1133, 1149 (Figure 6A) Sensitive to HF (-FH/F) then MS/MS frag. 300 (no core Fuc) Elution after 1281 isomer at 6.0 g.u.
	1295.52	>14	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	Key fragments: 621, 665, 973, 987 (loss of 308)
	1297.50	10-11	nd	nd	0.18	nd	+	+	+	+	+	M, F	Key fragments: 608, 827, 989 Sensitive to β 4Gal (-1H; Figure 4G/H), then MS/MS frag. 446 Sensitive to 3Man (-1H); coelution with Pristionchus
	1313.50	7.0	8.00	9.33	8.19	8.72	+	+	+	+	+	M, F	Key fragment: 827 coelution with Pristionchus and Haemonchus
	1338.53	8.5	nd	nd	2.71	0.92	+	trace	+	+	+	M, F	Key fragments: 446, 1176 Sensitive to FDL (-1N) coelution with Pristionchus and Haemonchus
	1338.53	11.5-12	nd	nd	0.28	1.46	trace	nd	+	+	+	M, F	Key fragments: 446, 973, 1135 coelution with Pristionchus and Haemonchus
	1341.53	7.7	nd	nd	nd	nd	trace	nd	trace	trace	trace	nd	Key fragments: 369, loss of 446; elution before 1176 at 8.3 g.u. coelution with Pristionchus
	1357.53	6.0	3.56	2.28	4.13	7.28	+	+	+	+	+	M, F	Key fragments: 369, 531, 1195 (loss of 162) Sensitive to HF (-1PC) then MS/MS frags. 300, 1030 afterwards sensitive to FDL (-1N) coelution with Pristionchus and Haemonchus
	1357.53	8.5	1.55	0.25	0.83	nd	+	+	+	+	+	M, F	Key fragments: 369, 531 Elution before 1192 isomer at 8.8 g.u. coelution with Pristionchus
	1395.55	7.8	0.47	0.25	3.83	0.65	+	+	+	+	+	M, F	Key fragments: 365, 1030, 1192 coelution with Pristionchus and Haemonchus
	1427.57	6.8	3.37	9.50	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 592, 827, 1119 (loss of 308), 1265, 1281 (Figure 5A-D) Sensitive to 3Man (-1H) Sensitive to HF (-F/FH) then MS/MS frag. 446 coelution with Haemonchus
	1427.57	8.0	4.82	7.50	3.86	0.44	+	+	+	nd	nd	M, F	Key fragments: 608, 665, 754, 973, 1119 (loss of 308; Figure 5E-I) Sensitive to β 4Gal (-1H) then MS/MS frags. 446, 592 Sensitive to HF (-F/FH) then MS/MS frags. 300, 608, 665 Resistant to 3Man; coelution with Pristionchus
	1441.58	7.2	0.28	0.73	nd	nd	nd	nd	+	nd	nd	trace	Key fragments: 446, 592, 841, 987, 1133 (loss of 308), 1295, 1279 (Figure 6F) Sensitive to HF (-F/FH) then MS/MS frag. 446
	1441.58	10.2	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	Key fragments: 446, 606, 1281 (loss of 160; Figure 6G) Sensitive to 3Man (-1H) and bovine Fuc (-1F; then 460 fragment) Sensitive to HF (-FMe/-FH), then MS/MS frag. 446

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	1441.58	14	0.37	0.44	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 446, 622, 768, 1133 (loss of 308), 1279, 1295 (Figure 6H) Sensitive to HF (-F/FH) then MS/MS frag. 622, 665 Resistant to β4Gal
	1443.56	7.5	nd	nd	nd	nd	trace	trace	+	nd	nd	trace	Key fragments: 608, 754, 1281, 1297 Sensitive to HF (-1F), then MS/MS frag. 608
	1443.56	10.5-11.5	nd	nd	nd	1.47	trace	+	+	+	+	M, F	Key fragments: 608, 827, 1135 (loss of 308), 1281 Sensitive to HF (-FH) then MS/MS frag. 608, 827 Sensitive to 3Man (-1H) then MS/MS 608, 973
	1475.56	6,2	1.01	0.67	0.36	1.09	+	+	+	+	+	M, F	Key fragment: 827 coelution with Pristionchus and Haemonchus
	1475.56	6,4	8.17	5.83	4.66	12.55	+	+	+	+	+	M, F	Key fragment: 989 Sensitive to 2/3Man (-2H), then MS/MS frag. 665 coelution with Pristionchus
	1484.59	7.6	nd	nd	nd	nd	nd	+	+	nd	nd	M, F	Key fragments: 446, 592, 1135, 1338 Resistant to FDL; sensitive to HF (-1F, then frag. 446) and to 3Man (-1H) Elution after 1281 at 6.6 g.u.
	1500.58	9,6	nd	nd	nd	nd	+	+	+	+	+	M, F	Key fragments: 608, 1030, 1192, 1338 Sensitive to β4Gal (-1H, then frag. 446) and to FDL (-1N)
	1503.59	7.7	nd	nd	1.59	4.71	+	+	+	+	+	M, F	Key fragments: 369, 531, 1057 (loss of 445), 1341 (loss of 162) Sensitive to HF (-1PC, then frag. 446) and then to FDL (-1N) coelution with Pristionchus
	1503.59	8.7	nd	nd	nd	nd	nd	nd	trace	+	+	trace	Key fragments: 369, 531, 895 (loss of 607), 1195 (loss of 308; Figure 7A)
	1503.59	11-11.5	nd	nd	nd	1.19	trace	trace	+	+	+	M, F	Key fragments: 369, 531, 1058 (loss of 445), 1357 (loss of 146; Figure 7B) Elution before 1338 isomer at 11.5 g.u.; coelution with Pristionchus
	1516.58	6.7	0.48	nd	0.78	0.56	nd	nd	trace	+	trace	trace	Key fragments: 1030, 1313
	1541.61	10-11	nd	nd	0.18	nd	+	+	+	+	+	M, F	Key fragments: 446, 1176, 1338 Sensitive to bov Fuc (-1F), then MS/MS frag. 300 (loss of core Fuc) coelution with Pristionchus and Haemonchus
	1560.61	7.0	3.11	4.09	1.94	0.81	+	+	+	+	+	M, F	Key fragments: 369, 531, 1195 (loss of 365), 1357 (loss of 203) Elution before 1395 at 7.8 g.u. coelution with Pristionchus
	1589.62	7.7	0.60	0.30	nd	nd	+	+	+	nd	nd	M, F	Key fragments: 607, 754, 827, 1135, 1281 (loss of 308) Sensitive to HF (-F/FH) then MS/MS frag.608 Sensitive to 3Man (-1H)
	1637.60	5.5	4.42	1.72	4.02	5.07	+	+	+	+	+	M, F	Key fragment: 989 coelution with Pristionchus and Haemonchus
	1637.60	6.0	2.89	0.86	1.10	1.07	nd	nd	+	+	+	trace	Key fragment: 1151 coelution with Pristionchus and Haemonchus

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	1646.64	10.5-11.5	nd	nd	nd	nd	trace	trace	trace	+	+	trace	Key fragments: 608, 1030, 1338 (loss of 308) Corresponds to one glycan in hex-2,hex-3 mutant
	1649.64	8.5	nd	nd	nd	nd	nd	nd	trace	trace	trace	trace	Key fragments: 369, 531, 1204 (loss of 445), 1341 (loss of 308)
	1665.64	8.8	nd	nd	nd	nd	nd	nd	+	+	+	trace	Key fragments: 369, 531, 1058 (loss of 607), 1503 (loss of H) Sensitive to HF (-1PC) then MS/MS frag. 608 Elution before 1500 at 9.6 g.u.
	1665.64	12.5-13.5	nd	nd	nd	nd	nd	nd	+	+	+	nd	Key fragments: 369, 531, 1058 (loss of 607), 1357 (loss of 308)
	1706.67	9.5	nd	nd	nd	1.33	nd	nd	+	+	+	trace	Key fragments: 369, 531, 1260 (loss of 445) Elution before 1541 at 10-11 g.u. coelution with Pristionchus
	1725.66	4.8	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 737, 900, 1563 (loss of 162; Figure 7C) Sensitive to HF (-2PC), then MS/MS frags. 407 and 1233) After HF, sensitive to chitinase (-1N), then MS/MS frags. 827, 1030
	1725.66	6.8	0.54	nd	nd	nd	+	+	+	+	+	M, F	Key fragments: 369, 531, 1195 (loss of 530), 1357 (loss of 368; Figure 7D) Sensitive to HF (-2PC) then MS/MS frag. 1030 coelution with Pristionchus/Haemonchus (before 1395/1560 at 7.8/7.0 g.u.)
	1725.66	8.0	nd	nd	nd	nd	nd	nd	+	+	+	trace	Key fragments: 369, 737
	1763.66	4.3	nd	nd	nd	nd	nd	nd	+	+	+	trace	Key fragments: 369, 531, 734, 896, 1561 (loss of N)
	1799.66	5.2	11.10	3.95	16.20	11.95	+	+	+	+	+	M, F	Key fragment: 1151 coelution with Pristionchus and Haemonchus
	1799.66	5.8	0.23	0.16	0.59	0.52	nd	nd	+	+	+	trace	Key fragment: 989 coelution with Pristionchus and Haemonchus
	1811.70	9.8	nd	nd	nd	nd	nd	nd	nd	+	+	F	Key fragments: 369, 531, 1204 (loss of 607), 1503 (loss of 308)
	1871.72	8.5	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 531, 1426 (loss of 445), 1503 (loss of 368) coelution with Pristionchus; elution before 1706 at 9.5 g.u.
	1928.74	4.8	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 531, 734, 1560, 1725 (loss of N)
	1961.71	5.5	5.64	4.69	9.53	11.01	+	+	+	+	+	M, F	Key fragment: 1151 coelution with Pristionchus and Haemonchus
	2033.77	9.8	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 531, 1426 (loss of 607), 1503 (loss of 531) Sensitive to β4Gal (-1H), then MS/MS frag. 1426 (loss of 445)
	2093.80	3.8	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	Key fragments: 369, 737, 1105 Sensitive to HF (-3PC), then MS/MS frag. 407, 610

Structure	m/z	RP-amide g.u.	% in RP (2D-HPLC, A only)				RP-amide (1D) occurrence						Evidence
			male	female	L4	L3	male A post F	female A post F	female A only	male F	female F	Hydrazine	
	2093.80	4.2	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 899, 1725 (loss of 368; Figure 7E)
	2093.80	6.4	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 737, 899, 1725 (loss of 368; Figure 7F) Sensitive to HF (-3PC), then MS/MS frag, 407, 1233
	2093.80	7.6	nd	nd	nd	nd	nd	nd	+	+	+	M, F	Key fragments: 369, 737, 1105, 1357 (loss of 736), 1725 (loss of 368) Sensitive to HF (-3PC, then 407 and 610), then chitinase (-2N; Figure 7K-P)
	2109.82	11.0	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	Key fragments: 568, 730, 608, 1233
	2123.76	6.6	nd	nd	nd	nd	nd	nd	nd	trace	+	+	Key fragment: 1313 coelution with Pristionchus
	2131.82	7.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	M	Key fragments: 369, 572, 734, 1195, 1560 (loss of 571; Figure 7G) Sensitive to HEX-4 (-2N) and HF (-2PC, then MS/MS with 407 and 1233)
	2239.86	5.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	M, F	Key fragments: 369, 531, 899, 1794 (loss of 445), 1871 (loss of 368; Figure 7H) Sensitive to HF (-3PC)
	2239.86	7.8	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	Key fragments: 369, 737, 899, 1794 (loss of 445), 1871 (loss of 368) Sensitive to HF (-3PC)
	2274.88	7.6	nd	nd	nd	nd	nd	nd	nd	nd	+	F	Key fragments: 369, 531, 734, 896, 1667 (loss of 607), 1966 (loss of 308)
	2461.93	6.3	nd	nd	nd	nd	nd	nd	nd	nd	+	M, F	Key fragments: 369, 737, 1106, 1725 (loss of 737), 2094 (loss of 368; Figure 7I)
	2666.01	3.5	nd	nd	nd	nd	nd	nd	+	+	+	nd	Key fragments: 369, 737, 1105, 1635, 1928 (loss of 736), 2298 (loss of 368; Figure 7J)