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3 Chemical characterization of oligosaccharides in the milk of six species of  
4 New and Old world monkeys  
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45 Abstract  
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48 Human and great ape milks contain a diverse array of milk oligosaccharides,  
49 but little is known about the milk oligosaccharides of other primates, and  
50 how they differ among taxa. Neutral and acidic oligosaccharides were  
51 isolated from the milk of three species of Old World or catarrhine monkeys  
52 (Cercopithecidae: rhesus macaque (*Macaca mulatta*), toque macaque  
53 (*Macaca sinica*) and Hamadryas baboon (*Papio hamadryas*)) and three of  
54 New World or platyrrhine monkeys (Cebidae: tufted capuchin (*Cebus apella*)  
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3 and Bolivian squirrel monkey (*Saimiri boliviensis*); Atelidae: mantled  
4 howler (*Alouatta palliata*). The milks of these species contained 6-8% total  
5 sugar, most of which was lactose: the estimated ratio of oligosaccharides to  
6 lactose in Old World monkeys (1:4 to 1:6) was greater than in New World  
7 monkeys (1:12 to 1:23). The chemical structures of the oligosaccharides were  
8 determined mainly by <sup>1</sup>H-NMR spectroscopy. Oligosaccharides containing  
9 the type II unit (Gal(β1-4)GlcNAc) were found in the milk of the rhesus  
10 macaque, toque macaque, Hamadryas baboon and tufted capuchin, but  
11 oligosaccharides containing the type I unit (Gal(β1-3)GlcNAc), which have  
12 been found in human and many great ape milks, were absent from the milk  
13 of all species studied. Oligosaccharides containing Lewis x  
14 (Gal(β1-4)[Fuc(α1-3)]GlcNAc) and 3-fucosyl lactose (Gal(β1-4)[Fuc(α1-3)]Glc)  
15 were found in the milk of the three cercopithecoid monkey species, while  
16 2-fucosyl lactose (Fuc(α1-2)Gal(β1-4)Glc) was absent from all species studied.  
17 All of these milks contained acidic oligosaccharides that had  
18 N-acetylneuraminic acid as part of their structures, but did not contain  
19 oligosaccharides that had N-glycolylneuraminic acid, in contrast to the milk  
20 or colostrum of great apes which contain both types of acidic  
21 oligosaccharides. Two GalNAc-containing oligosaccharides, lactose  
22 3'-O-sulfate and lacto-N-novopentose I  
23 (Gal(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc) were found only in the milk  
24 of rhesus macaque, hamadryas baboon and tufted capuchin, respectively.  
25 Further research is needed to determine the extent to which the milk  
26 oligosaccharide patterns observed among these taxa represent wider  
27 phylogenetic trends among primates and how much variation occurs among  
28 individuals or species.  
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Keywords: old world monkey/new world monkey/milk oligosaccharide/rhesus  
macaque/toque macaque/baboon/capuchin/mantled howler/squirrel  
monkey/N-glycolylneuraminic acid

Introduction

1  
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3 Human colostrum and mature milk contain 22 ~ 24 g/L and 12 ~ 13  
4 g/L, respectively, of a large variety of oligosaccharides [1, 2], which  
5 collectively represent the third largest solid component of milk after lactose  
6 and lipids. To date the chemical structures of at least 115 human milk  
7 oligosaccharides have been characterized [3, 4, 5]. We have recently shown  
8 that the predominant oligosaccharides in human colostrum obtained during  
9 the first three days of lactation are 2'-FL, LNFP I, LNDFH I and LNT [6];  
10 see Table 1 for abbreviations and chemical formulae for oligosaccharides.  
11 These four oligosaccharides are also predominant in transitional and  
12 mature human milk [7, 8, 9]. It is noteworthy that LNFP I, LNDFH I and  
13 LNT all contain the type I unit (Gal( $\beta$ 1-3)GlcNAc, lacto-N-biose I) within  
14 their structures, in contrast to the oligosaccharides of the milk or colostrum  
15 of a wide variety of non human mammals, all of which have been shown to  
16 contain predominantly or, in most cases exclusively, the type II unit  
17 (Gal( $\beta$ 1-4)GlcNAc, N-acetyllactosamine) [3].

Table 1

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29 Recently, we studied the milk oligosaccharides of a hylobatid (the  
30 siamang) and of the great apes (chimpanzee, bonobo, gorilla and orangutan),  
31 which among living primates are the species most closely related to humans  
32 [10]. The milk or colostrum of chimpanzee, bonobo and orangutan contained  
33 both type I and type II oligosaccharides, but type II predominated over type I,  
34 while the milk or colostrum of gorilla and siamang contained only type II  
35 oligosaccharides. These observations suggested that the predominance of  
36 type I oligosaccharides in milk/colostrum may be a feature that is specific to  
37 humans. It is generally believed that human milk oligosaccharides act as  
38 soluble receptor analogs that inhibit the attachment of pathogenic bacteria,  
39 viruses, and bacterial toxins to the mucosa of the infant colon, and as  
40 prebiotics that stimulate the growth of beneficial bifidus bacterial flora [3].  
41 The biological significance of the predominance of type I oligosaccharides in  
42 human milk is unknown, but we believe that it may be advantageous to  
43 bifidus flora formation in the infant colon.

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3 to obtain further information on the phylogenetic distribution of milk  
4 oligosaccharides among primates.  
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## 7 8 Materials and methods 9

### 10 11 Sample and reference materials 12 13

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15 Milk samples were obtained via a number of collaborative research  
16 projects and shipped to the Nutrition Laboratory, Smithsonian National  
17 Zoological Park. They were kept frozen at  $-20^{\circ}\text{C}$  until thawed for analysis.  
18 The following samples were obtained:  
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23 (1) Rhesus macaque (Cercopithecidae: *Macaca mulatta*) milk (9.0 mL total)  
24 was collected at 91, 93, 95, 106, 113, 115, 119, 120 and 123 days postpartum  
25 from nine lactating females at the California National Primate Research  
26 Center, Davis, CA. All females were adult (mean age  $\pm$  SD was  $8.9 \pm 0.9$   
27 years), had previously reproduced (mean parity  $\pm$  SD was  $5.3 \pm 1.3$ ), and 4/9  
28 were rearing sons. Mothers were separated from their infants for 3.5-4 hours  
29 to prevent nursing. Following milk accumulation, mothers were sedated  
30 with ketamine hydrochloride (10 mg/kg) and administered exogenous  
31 oxytocin to induce milk let down [2 IU/kg (0.1 ml/kg) IM]. Milk was **obtained**  
32 **by hand-milking**.  
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36 (2) Toque macaque (Cercopithecidae: *Macaca sinica*) milk (10 mL) was  
37 collected at 2.4 months postpartum from two lactating females (2339 and  
38 2340) captured in dry evergreen forest at Polonnaruwa, Sri Lanka (see [11]  
39 for a description of the study site and study population). The females were  
40 baited into mesh holding traps with cooked rice, chemically immobilized  
41 with 4 mg ketamine hydrochloride [12] and injected IM with 0.20 cc (4 IU)  
42 oxytocin to induce milk **let down**. Milk was **obtained by hand-milking**.  
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46 (3) Hamadryas baboon (Cercopithecidae: *Papio hamadryas*) milk (5 mL) was  
47 collected at 157, 190 and 242 days postpartum from three lactating females  
48 at the Southwest Foundation for Biological Research, San Antonio, TX.  
49 The females in a large group living in an outdoor enclosure were captured  
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3 for routine veterinary examinations, immobilized with ketamine  
4 hydrochloride, injected IM with oxytocin, and milk was obtained by  
5 hand-milking. The females were 16.8, 17.5 and 6.1 years of age, weighed 17,  
6 23 and 16 kg, and had parities of 8, 12 and 1, respectively.  
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9 (4) Tufted capuchin (Cebidae: *Cebus apella*) milk (7.0 mL) was collected at  
10 43, 313 and 348 days postpartum from lactating females from a breeding  
11 colony maintained at Alpha Genesis, Inc. (Yemassee, SC) [13]. Mothers were  
12 given ketamine hydrochloride at 0.15ml/kg IM. Once mothers were sedated  
13 (approximately five minutes), they were given oxytocin (0.1 ml/kg IM).  
14 Fifteen to 30 minutes after oxytocin injection, milk was manually expressed.  
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17 (5) Bolivian Squirrel monkey (Cebidae: *Saimiri boliviensis*) milk (5.0 mL)  
18 was collected 152, 162 and 174 days postpartum from lactating females that  
19 were part of a breeding colony maintained at the University of South  
20 Alabama Center for Neotropical Primate Research and Resources [14]. The  
21 upper torso of each female was wrapped using a self-clinging bandage over  
22 gauze pads to prevent nursing or milk loss. Bandages were removed 4–4.5  
23 hr after animal capture. Milk was collected into 15-ml conical tubes by  
24 manual expression from the nipple and gentle massage of the underlying  
25 mammary gland. Milk was fully expressed by hand from the mammary  
26 glands. Oxytocin was not administered.  
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29 (6) Mantled howler (Atelidae: *Alouatta palliata*) milk (5.3 mL) was collected  
30 1 week, 1 month and 3 months postpartum from lactating females (76, 109,  
31 and 238) at Hacienda La Pacifica, Guanacaste Province, Costa Rica in  
32 August 1987 (see [15] for description of the study site and study population).  
33 Animals were darted in trees in their home range using the Pneu-Dart™  
34 system (Pneu-Dart, Inc., HC 31, Williamsport, PA 17701) at a dose rate of 25  
35 mg/kg of Telazol®, and captured in a large net when they fell from the trees  
36 into a large net. They were kept sedated with additional doses of 5mg/kg of  
37 Telazol® administered IM. Milk was collected by hand-milking after IM  
38 administration of oxytocin.  
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57 Oligosaccharides reference materials (see Table 1 for full names and  
58 formulas), LNFP III, LNnT, LNnH, LST c and 2'-FL were purchased from  
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3 Seikagaku Co. (Tokyo, Japan). 3'-NAc-SL and 6'-NAc-SL were from Sigma  
4 Co. (St. Louis, MO). Novo-LNP I was isolated from brown capuchin  
5 colostrum [16], while 3'-GL was purified from caprine colostrum [17].  
6 MSLNnH, GM2 tetra and lactose 3'-O-sulfate were isolated from siamang  
7 milk [10], bottlenose dolphin milk [18] and dog milk [19], respectively.  
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#### 10 11 12 Measurement of hexose content of whole milk and colostrum

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Milk samples were thawed prior to pooling and assayed for total sugar content by the phenol-sulfuric acid method [20, 21], using lactose monohydrate for preparation of a standard, with results expressed on a lactose equivalent basis.

#### Preparation of the oligosaccharides from milk/ colostrum

Milk samples were pooled within each species (rhesus macaque, toque macaque, Hamadryas baboon, tufted capuchin, Bolivian squirrel monkey and mantled howler) to give a sufficient amount (5-10 ml) for oligosaccharide analysis, and thus all results are for pooled samples. Subsamples for oligosaccharide analysis were thawed and extracted with four volumes of chloroform/ methanol (2:1, v/v). After agitation, the emulsion was centrifuged at  $5000 \times g$ , 4°C for 30 min, and the lower chloroform layer and the denatured protein were discarded. The methanol was evaporated from the upper layer, and the lyophilized residue was designated as the carbohydrate fraction.

The carbohydrate fraction from each sample was dissolved in 2 mL of water, and the solution passed through a BioGel P-2 (< 45  $\mu$ m, 2.0 X 100 cm, Bio-Rad, Hercules, CA) that had been calibrated with 2 mg of each of galactose (monosaccharide), lactose (disaccharide) and raffinose (trisaccharide). The gel was washed with 0.1 M NaOH and 0.1 M HCl before use. Elution was done with distilled water at a flow rate of 15 mL/ h, and 5 mL fractions were analyzed for hexose with the phenol-H<sub>2</sub>SO<sub>4</sub> method [20] and for sialic acid with the periodate-resorcinol method [22]

Fig. 1  
Fig. 2

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3 (chromatogram in Figure 1 and 2). The peak fractions were pooled and  
4 lyophilized.  
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6 The components in R-2, R-3, R-4, R-6 and R-7 from rhesus macaque  
7 milk (Figure 1a), T-4, T-8 and T-9 from toque macaque milk (Figure 1b), B-3,  
8 B-9 and B-10 from Hamadryas baboon milk (Figure 1c), C-2, C-3, C-4 and C-6  
9 from tufted capuchin milk (Figure 2a), S-5 from Bolivian squirrel monkey  
10 (Figure 2b) and M-8 from mantled howler milk (Figure 2c) were  
11 characterized by <sup>1</sup>H-NMR spectroscopy. The component R-4 was also  
12 characterized by <sup>13</sup>C-NMR and heteronuclear single quantum coherence  
13 (HSQC) spectrum.  
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20 Fractions R-1 (Figure 1a), T-1 (Figure 1b), B-1 (Figure 1c) and M-1  
21 (Figure 2c) from the milks of rhesus macaque, toque macaque, Hamadryas  
22 baboon, and mantled howler, respectively, were dissolved in 2 ml of 50 mM  
23 Tris-hydroxyaminomethane – HCl buffer (pH 8.7) and subjected to anion –  
24 exchange chromatography using a DEAE – Sephadex A-50 (GE Healthcare,  
25 Uppsala, Sweden) column (2.0 X 20 cm) equilibrated with the same buffer  
26 solution. The unadsorbed components were eluted with 250 mL of the same  
27 buffer solution and the adsorbed components were then eluted with a linear  
28 gradient of 0 – 0.5 M NaCl in the Tris buffer solution. Elution was done at a  
29 flow rate of 15 mL/h and fractions of 5 mL were collected. Aliquots (0.5 mL)  
30 of each fraction were analyzed for hexose using the phenol – H<sub>2</sub>SO<sub>4</sub> method.  
31 The fractions of R-1-2 (Figure 3a), T-1-2 (Figure 3b), B-1-1 (Figure 3c) and  
32 M-1-2 (Figure 3d) were each pooled, lyophilized, dissolved in 2 mL of water  
33 and passed through a column (2.0 X 35 cm) of BioGel P-2 to remove salts, as  
34 described above.  
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47 Each component in R-1-2 (Figure 3a), T-1-2 (Figure 3b), B-1-1 (Figure  
48 3c), C-1 (Figure 2a), M-1-2 (Figure 3d) and S-1 (Figure 2b) was further  
49 purified using HPLC on a TSK gel Amido-80 column (4.6 X 250 mm, pore  
50 size 80 Å, particle size 5 µm; Tosho, Tokyo, Japan) using a LC-10ATVP  
51 pump (Shimadzu, Tokyo, Japan) (see chromatograms in Fig. 4 and 5). The  
52 mobile phase was 50% and 80% (v/v) acetonitrile (CH<sub>3</sub>CN) in a 15 mM  
53 potassium phosphate buffer (pH 5.2). Elution was done using a linear  
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Fig. 3

Fig. 4

Fig. 5

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3 gradient of acetonitrile, 80 – 50% at 60°C at a flow rate of 1 mL/min. The  
4 eluates were monitored by measuring the absorbance at 195 nm. The peak  
5 fractions of oligosaccharides were pooled, concentrated by rotary  
6 evaporation, and characterized by <sup>1</sup>H-NMR.  
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## 10 11 <sup>1</sup>H, <sup>13</sup>C and HSQC NMR spectroscopy 12 13

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15 The NMR spectra were recorded in D<sub>2</sub>O (100.00 atom D%, Aldrich,  
16 Milwaukee, USA) at 500 or 600 MHz for <sup>1</sup>H-NMR with a JEOL-ECP-500  
17 FT-NMR or Varian INOVA 600 spectrometer, and at 125 MHz for <sup>13</sup>C-NMR  
18 with a JEOL ECP-500 FT-NMR spectrometer, operated at 293.1 K.  
19 Chemical shifts are expressed in ppm from down-field from internal  
20 3-(trimethylsilyl)-1-propane sulfonic acid sodium salt (TPS), but actually  
21 measured by reference to internal acetone ( $\delta=2.225$  for <sup>1</sup>H shift and 32.910  
22 for <sup>13</sup>C).  
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29 <sup>1</sup>H and <sup>13</sup>C chemical shifts were obtained from a heteronuclear single  
30 quantum coherence (HSQC) spectrum, and recorded over spectral widths of  
31 16384 Hz (<sup>1</sup>H) and 32768 Hz (<sup>13</sup>C), with 512  $t_1$  increments of 1024 data  
32 points and 16 scans per increment.  
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## 38 Results 39 40

41 The milks of the six primates contained 6-8% total sugar by the  
42 phenol-sulfuric acid method: rhesus macaque, 7.22%  $\pm$  0.44 s.d. (n=9); toque  
43 macaque, 8.09%  $\pm$  0.83 (n=2); Hamadryas baboon, 7.33%  $\pm$  0.58 (n=3); tufted  
44 capuchin, 7.54%  $\pm$  1.18 (n=3); Bolivian squirrel monkey, 6.92%  $\pm$  0.44 (n=3);  
45 Mantled howler, 6.05%  $\pm$  0.61 (n=3).  
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52 When subjected to size – exclusion chromatography on BioGel P-2, the  
53 carbohydrate fraction of each milk sample resolved into several peaks, each  
54 of which was designated as in Figures 1 and 2. Fractions R-1, T-1, B-1, C-1,  
55 S-1 and M-1 reacted positively to periodate – resorcinol, showing that they  
56 contained sialic acid. Fractions R-1, T-1, B-1 and M-1 were accordingly  
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3 subjected to anion – exchange chromatography on DEAE – Sephadex A-50;  
4 fractions C-1 and S-1 were too small for this procedure. During  
5 chromatography, the fractions R-1, T-1, B-1 and M-1 each separated into  
6 several peaks (Figure 3), of which the presumptive monosialyl  
7 oligosaccharides in fractions R-1-2, T-1-2, B-1-1 and M-1-2 were used for  
8 purification of each sialyl oligosaccharide. The oligosaccharides in each of  
9 R-1-2, T-1-2, B-1-1 and M-1-2 as well as in C-1 and S-1 (Figure 2) were  
10 further purified by HPLC as shown in Figures 4 and 5. The components in  
11 the predominant peaks of Figures 1, 2, 4 and 5 were characterized by  
12 <sup>1</sup>H-NMR or HSQC.  
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## 22 Rhesus macaque milk oligosaccharides

### 23 R-1-2-1 and R-1-2-3

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25 As the <sup>1</sup>H-NMR spectra (chemical shifts in [Table S1](#)) of the saccharides  
26 in R-1-2-1 and R-1-2-3 were essentially identical with those of 3'-NAc-SL  
27 and LST c, respectively, these were characterized to be  
28 Neu5Ac(α2-3)Gal(β1-4)Glc, and  
29 Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc, respectively.  
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### 38 R-1-2-2

39 The <sup>1</sup>H-NMR spectrum ([Table S1](#)) showed that R-1-2-2 contained two  
40 oligosaccharides, one major (R-1-2-2-1) and another minor (R-1-2-2-2). As  
41 the anomeric shifts and characteristic resonances of the major component  
42 were essentially similar to those of authentic 6'-NAc-SL, the major  
43 oligosaccharide (R-1-2-2-1) was considered to be identical with  
44 Neu5Ac(α2-6)Gal(β1-4)Glc.  
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50 The spectrum of the minor component had the anomeric shifts at δ  
51 4.734 and 4.533 of β(1-4) linked GalNAc and β(1-4) linked Gal, respectively;  
52 the characteristic H-3 and H-4 shifts of β(1-4) linked Gal at δ 4.152 and  
53 4.120, respectively; H-3 axial and equatorial shifts of α(2-3) linked Neu5Ac  
54 at δ 1.926 and 2.657, respectively; and NAc shifts of β(1-4) linked [GalNAc](#)  
55 and α(2-3) linked Neu5Ac at δ 2.014 and 2.030, respectively. As this pattern  
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3 was similar to the published data [18] for GM2 tetrasaccharide isolated  
4 from bottlenose dolphin milk, the minor saccharide (R-1-2-2-2) was  
5 identified as GalNAc( $\beta$ 1-4)[Neu5Ac( $\alpha$ 2-3)]Gal( $\beta$ 1-4)Glc.  
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10 It was confirmed that the components in the peaks eluted prior to  
11 R-1-2-1 in the HPLC were not saccharides, as shown by their  $^1\text{H-NMR}$ .  
12 Other minor peak components in Figure 4(a) were not characterized in this  
13 study because the amounts were too small.  
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18 R-7, R-6, R-3 and R-2.  
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20 As the  $^1\text{H-NMR}$  spectra (chemical shifts in **Table S2**) of the saccharides  
21 in R-7, R-6, R-3 and R-2 were essentially identical with those of lactose,  
22 3-FL, LNnT and LNFP III, these were characterized to be Gal( $\beta$ 1-4)Glc,  
23 Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]Glc, Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc, and  
24 Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc, respectively  
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31 R-4

32 The  $^1\text{H}$  spectrum (chemical shifts in **Table S2**) had the anomeric shifts  
33 of  $\alpha$ -Glc and  $\beta$ -Glc at  $\delta$  5.220 and 4.663, respectively, and  $\beta$ (1-4) linked Gal at  
34  $\delta$  4.439. In addition, the spectrum had the anomeric shift of  
35  $\beta$ -N-acetylhexosamine at  $\delta$  4.618 and 4.614, and its NAc shift at  $\delta$  2.035, and  
36 the characteristic doublet shift of H-4 of  $\beta$ (1-4) linked Gal residue at  $\delta$  4.159.  
37 This pattern is different from those of GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc [23] and  
38 GlcNAc( $\beta$ 1-6)Gal( $\beta$ 1-4)Glc [17], showing that the saccharide in R-4 differs  
39 from these two.  
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46 This saccharide was further characterized by  $^{13}\text{C-NMR}$  (chemical  
47 shifts in **Table S3**) and  $^1\text{H-}^{13}\text{C}$  HSQC spectrum analysis. The  $^1\text{H}$  shift of H-4  
48 of the  $\beta$ (1-4) linked Gal at  $\delta$  4.159 correlated with the  $^{13}\text{C}$  shift at  $\delta$  71.2; this  
49 showed that the OH-4 of this residue was not substituted. If this position  
50 were substituted, the H-4 shift of  $\beta$ (1-4) linked Gal would have correlated  
51 with the  $^{13}\text{C}$  shift at  $\sim$  80 ppm. The  $^{13}\text{C}$  NMR spectrum had the shift at  $\delta$   
52 84.9; this shift value was similar to that ( $\delta$  84.5) of C-3 of  $\beta$ (1-4) linked Gal  
53 residue of Gal( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc [24]. Therefore, it was thought that the  
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3 OH-3 of the  $\beta(1-4)$  linked Gal residue of the saccharide was substituted by a  
4  $\beta$ -hexosamine residue. The residual  $^{13}\text{C}$  shifts at  $\delta$  177.8, 106.0, 77.7, 73.4,  
5 70.4, 55.2 and 24.9 were assigned by comparison with the  $^{13}\text{C}$  shifts of free  
6  $\beta$ -GalNAc and the corresponding shifts of  $\beta$ -GalNAc of  
7 GalNAc( $\beta 1-4$ )[Neu5Ac( $\alpha 2-3$ )]Gal( $\beta 1-4$ )Glc [18]. As these chemical shifts were  
8 relatively close to the corresponding shifts of  $\beta$ -GalNAc of these saccharides,  
9 it was concluded that the saccharide in R-4 contained a  $\beta$ -GalNAc residue;  
10 the saccharide in R-4 was therefore characterized to be  
11 GalNAc( $\beta 1-3$ )Gal( $\beta 1-4$ )Glc.  
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#### 20 R-5

21 The identity of this small peak (Figure 1a) was not determined.  
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#### 25 Toque macaque oligosaccharides

#### 26 T-9, T-8, T-4 and T-1-2-1

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29 As the  $^1\text{H-NMR}$  spectra (chemical shifts in [Table S4](#)) of the saccharides  
30 in T-9, T-8, T-4 and T-1-2-1 were essentially identical with those of lactose,  
31 3-FL, LNFP III and 3'-NAc-SL, respectively, these were characterized to be  
32 Gal( $\beta 1-4$ )Glc, Gal( $\beta 1-4$ )[Fuc( $\alpha 1-3$ )]Glc,  
33 Gal( $\beta 1-4$ )[Fuc( $\alpha 1-3$ )]GlcNAc( $\beta 1-3$ )Gal( $\beta 1-4$ )Glc and  
34 Neu5Ac( $\alpha 2-3$ )Gal( $\beta 1-4$ )Glc, respectively. The  $^1\text{H-NMR}$  of T-1-2-1 contained  
35 another minor sialyl oligosaccharide, which contained  $\alpha$  (2-6) linked  
36 Neu5Ac, because the  $^1\text{H-NMR}$  spectrum had the resonances of H-3 axial and  
37 equatorial at  $\delta$  1.724 and 2.710, respectively.  
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48 It was confirmed that the components in the peaks eluted prior to  
49 T-1-2-1 in the HPLC (Figure 4 b) were not saccharides, as shown by their  
50  $^1\text{H-NMR}$ .  
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#### 55 T-2, T-3, T-5, T-6 and T-7

56 The identities of these small peaks (Figure 1b) were not determined.  
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3 Hamadryas baboon oligosaccharides  
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6 B-10, B-1-1-1, B-1-1-2 and B-1-1-3  
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8 As the  $^1\text{H-NMR}$  spectra (chemical shifts in [Table S5](#)) of the saccharides  
9 in B-10, B-1-1-1, B-1-1-2 and B-1-1-3 were essentially identical with those of  
10 lactose, 3'-NAc-SL, 6'-NAc-SL and LST c, respectively, these were  
11 characterized to be Gal( $\beta$ 1-4)Glc, Neu5Ac( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc,  
12 Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)Glc and  
13 Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc, respectively.  
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20 B-9  
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22 The  $^1\text{H-NMR}$  spectrum (chemical shifts in [Table S6](#)) showed that B-9  
23 contained two oligosaccharides, B-9-1 and B-9-2. As their spectra of two  
24 oligosaccharides were essentially identical with those of 3-FL and 3'-GL,  
25 B-9-1 and B-9-2 were characterized to be Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]Glc and  
26 Gal( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc, respectively  
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32 It was confirmed that the components in the peaks eluted prior to  
33 B-1-1 in the HPLC were not saccharides, as shown by their  $^1\text{H-NMR}$ . Other  
34 minor peak components in Figure 4(c) were not characterized in this study  
35 because the amounts were too small.  
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41 B-3  
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43 The  $^1\text{H-NMR}$  spectrum (chemical shifts in [Table S6](#)) showed that B-3  
44 contained two oligosaccharides, B-3-1 and B-3-2. The spectrum had the  
45 anomeric shifts of  $\alpha$ -Glc, two of  $\alpha$ (1-3) linked Fuc, of  $\beta$ (1-3) linked GlcNAc, of  
46  $\beta$ -Glc, and of  $\beta$ (1-6) linked GlcNAc, and three of  $\beta$ (1-4) linked Gal at  $\delta$  5.218,  
47 5.126 and 5.105, 4.708, 4.665, 4.637, and 4.466, 4.452 and 4.424, respectively.  
48 It had the characteristic shifts of H-4 of  $\beta$ (1-4) linked Gal, which was  
49 substituted at OH-3 by a  $\beta$ -linked GlcNAc, at  $\delta$  4.142, of NAc of  $\beta$ (1-6) and  
50  $\beta$ (1-3) linked GlcNAc at  $\delta$  2.049 and 2.020, respectively, and of H-6 of  $\alpha$ (1-3)  
51 linked Fuc at  $\delta$  1.174. The shifts at  $\delta$  5.126 and 5.105 of H-1 of  $\alpha$ (1-3) linked  
52 Fuc showed the presence of two Lewis x (Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc) units.  
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3 As this pattern was essentially similar to the published data [25] for  
4 DFLNnH and also to fraction O-2 separated from orangutan colostrum [10],  
5 one of the oligosaccharides in B-3-1 was characterized to be the  
6 Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-3){Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-6)}Gal( $\beta$ 1-  
7 4)Glc.  
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11 The spectrum had other anomeric shifts at  $\delta$  5.224 and 4.569, a  
12 characteristic doublet doublet shift at  $\delta$  4.343, and a doublet shift at  $\delta$  4.295.  
13 As these shifts were essentially similar to anomeric shifts of  $\alpha$ -Glc and  $\beta$ (1-4)  
14 linked Gal, and H-3 and H-4 of  $\beta$ (1-4) linked Gal of 3'-O-lactose sulfate,  
15 respectively [19], the other oligosaccharide in B-3 (B-3-2) was characterized  
16 to be Gal( $\beta$ 1-4)Glc-3'-O-sulfate.  
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24 B-2, B-4, B-5, B-6, B-7 and B-8

25 The identities of these small peaks (Figure 1c) were not determined.  
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29 Tufted capuchin oligosaccharides  
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32 C-6, C-4, C-2, C-1-1 and C-1-2  
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34 As the  $^1\text{H-NMR}$  spectra (chemical shifts in [Table S7 and S8](#)) of the  
35 saccharides in C-6, C-4, C-2, C-1-1 and C-1-2 were essentially identical with  
36 those of lactose, LNnT, LNnH, 3'-NAc-SL and 6'-NAc-SL, respectively, these  
37 were characterized to be Gal( $\beta$ 1-4)Glc, Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc,  
38 Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)[Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-6)]Gal( $\beta$ 1-4)Glc,  
39 Neu5Ac( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc and Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)Glc, respectively.  
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47 C-1-3

48 It was shown that the component in C-1-3 was not a sialyl  
49 oligosaccharide by its  $^1\text{H-NMR}$  and it could not be characterized in this  
50 study, because the spectrum was unusual.  
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56 C-1-4

57 The spectrum (chemical shifts in [Table S7](#)) of the saccharide in [C-1-4](#)  
58 had the anomeric shifts of  $\alpha$ -Glc,  $\beta$ (1-3) linked GlcNAc,  $\beta$ -Glc,  $\beta$ (1-6) linked  
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GlcNAc and three  $\beta(1-4)$  linked Gal at  $\delta$  5.220, 4.726, 4.669, 4.647 and 4.640, and 4.472, 4.455 and 4.433, respectively. The spectrum had the characteristic H-3 axial, equatorial, and NAc of  $\alpha(2-6)$  linked Neu5Ac and  $\beta(1-6)$  linked GlcNAc at  $\delta$  2.052 and 2.061, respectively, and H-4 of  $\beta(1-4)$  linked Gal, which was substituted at OH-3 by  $\beta$ -GlcNAc, at  $\delta$  4.149. As this pattern was essentially similar to the published data [26] for MSLN<sub>n</sub>H and also to that of S-1-4 separated from siamang milk [10], the oligosaccharide in **C-1-4** was characterized to be Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)[Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-6)]Gal( $\beta$ 1-4)Glc.

It was confirmed that the components in the peaks eluted prior to C-1-1 in the HPLC were not saccharides, as shown by their <sup>1</sup>H-NMR. Other minor peak components in Figure 5(a) were not characterized in this study because the amounts were too small.

#### C-3

The <sup>1</sup>H-NMR spectrum (chemical shifts in **Table S8**) of the saccharide in C-3 had the anomeric shifts of  $\alpha$ -Glc,  $\beta$ -Glc,  $\beta(1-6)$  linked GlcNAc,  $\beta(1-3)$  linked Gal and two of  $\beta(1-4)$  linked Gal at  $\delta$  5.224, 4.669, 4.644, 4.610, and 4.500 and 4.472, respectively, and NAc of  $\beta(1-6)$  linked GlcNAc at  $\delta$  2.062. As this pattern was essentially similar to the published data [16] for novo-LNP I separated from tufted (or brown) capuchin colostrum, the oligosaccharide in C-3 was characterized to be Gal( $\beta$ 1-3)[Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-6)]Gal( $\beta$ 1-4)Glc.

#### C-5

The identity of this small peak (Figure 2a) was not determined.

#### Squirrel monkey oligosaccharides

##### S-5, S-1-1 and S-1-2

As the <sup>1</sup>H-NMR spectra (chemical shifts in **Table S9**) of the saccharides in S-5, S-1-1 and S-1-2 were essentially identical with those of

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3 lactose, 3'-NAc-SL and 6'-NAc-SL, respectively, these were characterized to  
4 be Gal( $\beta$ 1-4)Glc, Neu5Ac( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc and Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)Glc,  
5 respectively.  
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10 S-2, S-3 and S-4

11 The identities of these small peaks (Figure 2b) were not determined.  
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14  
15 Mantled howler oligosaccharides

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18 M-8, M-1-2-1 and M-1-2-2

19 As the  $^1\text{H-NMR}$  spectra (chemical shifts in Table S10) of the  
20 saccharides in M-8, M-1-2-1 and M-1-2-2 were essentially identical with  
21 those of lactose, 3'-NAc-SL and 6'-NAc-SL, respectively, these were  
22 characterized to be Gal( $\beta$ 1-4)Glc, Neu5Ac( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc and  
23 Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)Glc, respectively.  
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31 M-1-2-3

32 M-1-2-3 was found to have a unique structure, studies on which are  
33 the subject of a separate paper.  
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38 M-1-2-4

39 The saccharide in M-1-2-4 could not be characterized by its  $^1\text{H-NMR}$  in  
40 this study, because the spectrum was unusual.  
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45 It was confirmed that the components in the peaks eluted prior to  
46 M-1-2-1 in the HPLC were not saccharides, as shown by their  $^1\text{H-NMR}$ .  
47 Other minor peak components in Figure 5(c) were not characterized in this  
48 study because the amounts were too small.  
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54 M-2, M-3, M-4, M-5, M-6 and M-7

55 The identities of these small peaks (Figure 2c) were not determined.  
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59 Discussion  
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Table 2

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4 All species were found to contain 6-8% total sugar in their milks, and  
5 values are typical for primate milks in general and comparable to those in  
6 previous papers [13, 14, 27, 28, 29]. In addition, these values are rather  
7 similar to those of hylobatid and great apes (5~9%) [10] and humans (6~7%).  
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11 The ratios of milk oligosaccharides to lactose in the monkey milks  
12 could be estimated from the peak areas of the gel chromatograms shown in  
13 Figures 1 and 2. The results were as follows: rhesus macaque milk 1:6;  
14 toque macaque milk 1:4; Hamadryas baboon milk 1:4; tufted capuchin milk  
15 1:13; mantled howler milk 1:12; Bolivian squirrel monkey milk 1:23. The  
16 ratios observed in the Old World monkey milks were rather similar to those  
17 found with chimpanzee (1:4), bonobo (1:5) and siamang (1:3) milks [10], but  
18 those of the New World cebid (squirrel monkey, capuchin) and atelid  
19 (howler) milks were much lower. In mature human milk the ratio estimated  
20 by this method is about 1:2.7 [10]. Thus although oligosaccharide to lactose  
21 ratios in Old World monkey and great ape milks approach that of human  
22 milk, they are somewhat smaller. We conclude that that the milks of all  
23 catarrhine primates that have been studied (including cercopithecoid  
24 monkeys, hylobatids, great apes and humans) contain higher concentrations  
25 of oligosaccharides than do New World or platyrrhine monkey milks.  
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28 This suggests that the oligosaccharide:lactose ratio and total  
29 oligosaccharide content have increased following the divergence of  
30 catarrhines from platyrrhines, which occurred about 32-40 MYA from fossil  
31 evidence or even earlier according to some molecular estimates [30,31, 32,  
32 33, 34]. By contrast the divergence between cercopithecoids (such as  
33 macaques and baboons) and hominoids (such as great apes) at about 21-29  
34 MYA, and the divergence of *Homo* from chimps and bonobos at about 4-8  
35 MYA [30, 31, 35, 36] resulted in little if any further increase in total  
36 oligosaccharides or oligosaccharide ratios. This assumes that the lower  
37 oligosaccharide concentrations and oligosaccharide:lactose ratios in the New  
38 World platyrrhine monkeys (atelid and cebids) represents the ancestral  
39 condition, rather than a secondary reduction.  
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42 In this study zero to three neutral and two to three acidic  
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2 oligosaccharides were identified in each of the New World platyrrhine  
3 monkey milks, and two to five neutral and one to three acidic  
4 oligosaccharides in each of the Old World catarrhine monkey milks (Table 2).  
5  
6 By comparison, three to six neutral and two to six acidic oligosaccharides  
7 were found in each of 4 species of great apes [10]. This suggests a trend for  
8 the diversity of oligosaccharides to increase from New World to Old World  
9 monkeys, and from cercopithecoid monkeys to great apes. However, in each  
10 species one to several minor oligosaccharides that were evident in the  
11 chromatograms of milk carbohydrates (Figures 1-5) could not be  
12 characterized due to the small amounts. Further research is needed on  
13 larger sample volumes and on additional species to determine if the  
14 difference in total milk oligosaccharide content between New World  
15 primates and Old World primates is in fact matched by a comparable  
16 increase in the diversity of milk oligosaccharides. Certainly no non-human  
17 primate studied to date comes close to the extraordinary diversity in milk  
18 oligosaccharides observed in human milk [3,4,5].  
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22 As described in the Introduction, type I oligosaccharides predominate  
23 over type II in human milk, but not in the milk of the great apes, in which  
24 type II predominate over type I. In this study, type II oligosaccharides were  
25 found in the milks of the cercopithecids and in milk of the tufted capuchin,  
26 but type I oligosaccharides were not detected. In mantled howler and  
27 Bolivian squirrel monkey milks lactose was the only neutral saccharide that  
28 was identified, and acidic oligosaccharides had lactose as the core unit, but  
29 other oligosaccharides present at very low concentrations (Figure 2) could  
30 not be identified. Our data suggest that type I milk oligosaccharides were  
31 acquired by the common ancestor of apes and humans after divergence from  
32 cercopithecoid monkeys. At present, the particular biological significance of  
33 the predominance of type I oligosaccharides in human milk is uncertain, but  
34 we hypothesize that it may be relevant to bifidus flora formation in the  
35 neonatal infant colon, because a specific metabolic pathway for type I but  
36 not type II oligosaccharides has been found in certain bifidobacterial strains  
37 such as *Bifidobacterium bifidum* [5, 37, 38].  
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40 It is noteworthy that oligosaccharides containing Lewis x as well as  
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3 3-FL were found in the milks of the three species of cercopithecoid monkeys.  
4 In our previous study [10], these oligosaccharides were found in milk or  
5 colostrum of chimpanzee, bonobo and orangutan, but not in those of gorilla  
6 and siamang. This type of milk oligosaccharide appears to have existed in  
7 the common catarrhine ancestor of Old World monkeys, apes and humans,  
8 and has subsequently been lost from the milk/colostrum of a few ape species  
9 such as gorilla and siamang. However, we cannot rule out the possibility  
10 that other individual gorillas or siamangs may contain these  
11 oligosaccharides at low concentrations, and that we happened to study  
12 individuals in which these could not be measured; inter-individual variation  
13 in milk oligosaccharides has been observed in humans. It is noteworthy that  
14 Lewis x and 3-FL were not identified in the milks of the howler or cebid  
15 monkeys, even though 3-FL had been found in tufted capuchin colostrum in  
16 a previous study [16]. It is possible 3-FL was present in capuchin milk and  
17 other New World monkey milks but at such low concentration (e.g., peaks  
18 C-5, S-4, M-7 in Figure 2) that we could not identify it due to the small  
19 amounts of milk (5 - 7 ml) available for study.

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22 Although Fuc( $\alpha$ 1-2)Gal( $\beta$ 1-4)Glc (2'-FL) is the most prominent  
23 oligosaccharide in human milk/colostrum [6] and was found in the milk or  
24 colostrum of chimpanzee, bonobo and gorilla [10], this trisaccharide was not  
25 found in the milks of any of the monkeys in this study, nor was it detected in  
26 tufted capuchin colostrum in our previous study [16]. This trisaccharide  
27 reduces the incidence of diarrhea caused by *Campylobacter jejuni* in breast  
28 fed human infants [39]; it is possible that milk 2'-FL was acquired by the  
29 ape/human lineage following its divergence from other primates.

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32 Oligosaccharides containing N-glycolylneuraminic acid (Neu5Gc) were  
33 not found in the milks of the monkeys in this study. In our previous study,  
34 Neu5Gc( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc was detected in milk or colostrum of chimpanzee,  
35 bonobo, gorilla and orangutan, but not in siamang milk [10].  
36 Glycoconjugates containing Neu5Gc are absent from the tissues or body  
37 fluids, including milk/colostrum, of healthy humans because of the absence  
38 of the hydroxylase enzyme that converts CMP-Neu5Ac to CMP-Neu5Gc [40].  
39 It is worth noting that among primate species studied to date, only great  
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3 ape milk or colostrum has been found to contain this type of oligosaccharide.  
4 It is possible, however, that Neu5Gc-containing glycoconjugates could be  
5 present in other tissues or body fluids of primates.  
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8 It is interesting that among the monkeys studied, only rhesus  
9 macaque milk had oligosaccharides containing GalNAc such as  
10 GalNAc(β1-3)Gal(β1-4)Glc and GM2 tetrasaccharide. This type of saccharide  
11 has previously been found only in rhesus macaque milk and not in any other  
12 primates, including apes and humans. Further studies will clarify whether  
13 GalNAc-containing saccharides are found in the milk/colostrum of other  
14 primate species. GM2 tetrasaccharide has previously been found only in  
15 bottlenose dolphin milk [18].  
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18 It was surprising that 3'-O-lactose sulfate was found in baboon milk,  
19 but only baboon milk. This disaccharide or oligosaccharides containing  
20 sulfate have been found in the milks of the dog [19], bearded seal [41,42]  
21 and humans [43]. Further study is needed to clarify if sulfated saccharides  
22 occur in the milk or colostrum of other primates.  
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25 Lacto-N-novopentaose I was found only in tufted capuchin milk and  
26 had previously been detected in tufted capuchin colostrum [10]. To date the  
27 tufted capuchin is the only primate in which milk/colostrum contains this  
28 pentasaccharide. Among non-primates it has been found in milk or  
29 colostrum of the tammar wallaby [44], cow [45] and horse [46], too.  
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32 This study found common features among the milk oligosaccharides of  
33 monkeys of three families (Atelidae, Cebidae, Cercopithecidae) as well as  
34 apparent differences among these taxa and apes and humans. It also found  
35 that there are specific milk oligosaccharides in certain monkey species that  
36 appear to be absent from the milks of other primates that have been studied.  
37 From our previous and present studies, we conclude that human  
38 oligosaccharides are unique insofar as they consist of many varieties of  
39 oligosaccharides of both types I and II, with type I predominating, and  
40 that the ratio of oligosaccharides to lactose is somewhat higher than that  
41 found in other primates. The question of how these human-specific features  
42 may relate to elements of human evolution such as expansion of brain size,  
43 change in locomotor pattern to bipedalism, production of relatively  
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3 immature neonates and transition to larger social groups is open to  
4 speculation.  
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6 Although the results of HPLC-Chip/TOF-MS analysis [47] have  
7 suggested the presence of almost 200 oligosaccharides in human  
8 milk/colostrum, it has been found that the predominant oligosaccharides are  
9 2'-FL, LNFP I , LNDFH I and LNT [3]. It is thought that 2'-FL can act as a  
10 receptor analogue that inhibits the attachment of *Campylobacter jejuni* to  
11 the infant colonic mucosa [48], while LNT, LNFP I and LNDFH I can be  
12 utilized as prebiotics that simulate the growth of Bifidobacteria, including  
13 *Bifidobacterium bifidum*, within the infant colon [37, 38, 49, 50]. Our results  
14 indicate that these oligosaccharides are all either absent or present in only  
15 trace amounts in the milk of these New and Old World monkeys; this  
16 difference between humans and monkeys may have biological significance.  
17 In human milk/colostrum, the dominant acidic oligosaccharides are LST c,  
18 6'-SL, DSLNT (Neu5Ac(  $\alpha$  2-3)Gal(  $\beta$  1-4)[Neu5Ac(  $\alpha$  2-6)]GlcNAc(  $\beta$   
19 1-3)Gal(  $\beta$  1-4)Glc) and 3'-SL in this order, but in the milk of the monkeys  
20 3'-SL predominates over 6'-SL. The biological significance of each of the  
21 major milk oligosaccharides should be easier to assess following accurate  
22 quantitation.  
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36 Since this study is based on a limited number of individuals per  
37 species, and only a handful of species, further studies using larger milk  
38 samples and additional individuals and species are required to confirm  
39 suggested phylogenetic patterns.  
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15  
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17  
18 and Use authorizations of these organizations and/or the universities of the coauthors.

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Fig. 1. Gel chromatograms of the carbohydrate fraction from milk of (A) rhesus macaque, (B) toque macaque and (C) Hamadryas baboon. Elution from a Bio Gel P-2 column (2.6  $\times$  100 cm) was done with distilled water at a flow rate of 15 mL/ h, and of 5.0 mL fractions were collected. Each fraction was monitored by the phenol-H<sub>2</sub>SO<sub>4</sub> method at 490 nm (as shown in solid line) and the periodate-resorcinol method at 630 nm (as shown in dot line).

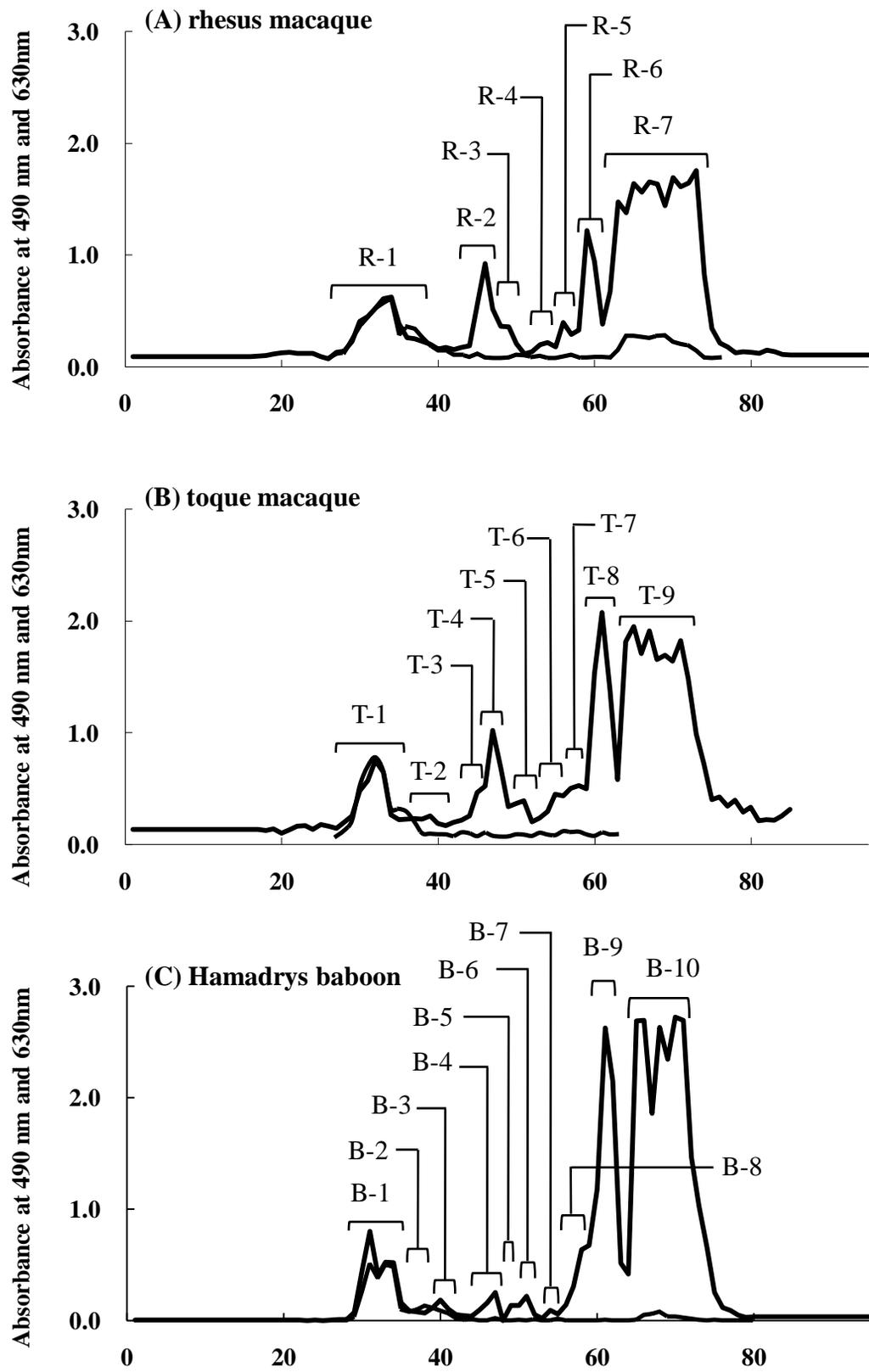
Fig. 2. Gel chromatograms of the carbohydrate fraction from milk of (A) tufted capuchin, (B) mantled howler and (C) Bolivian squirrel monkey. The gel chromatographies were done as in Figure 1.

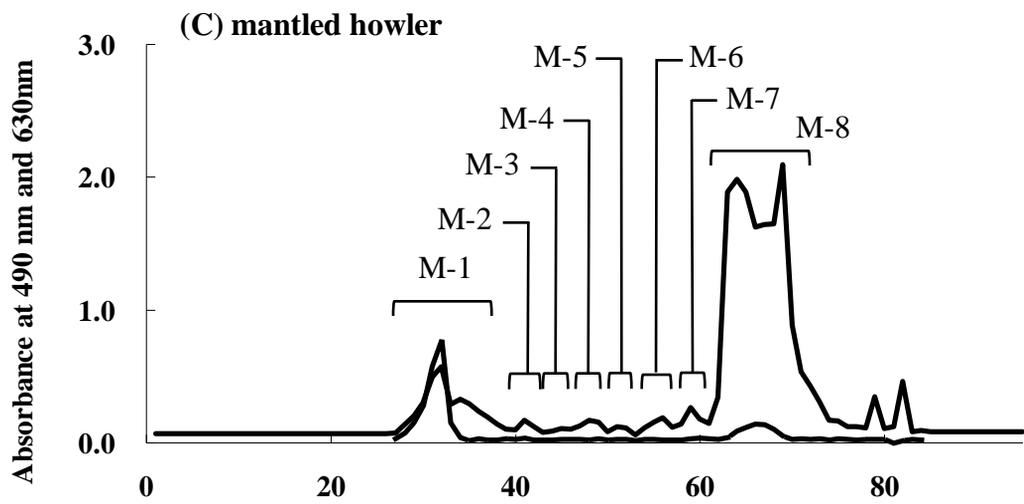
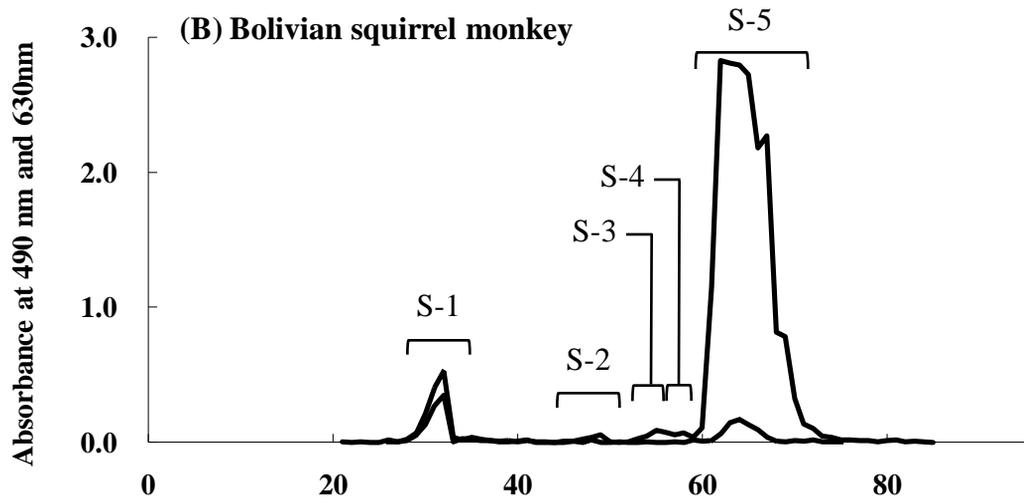
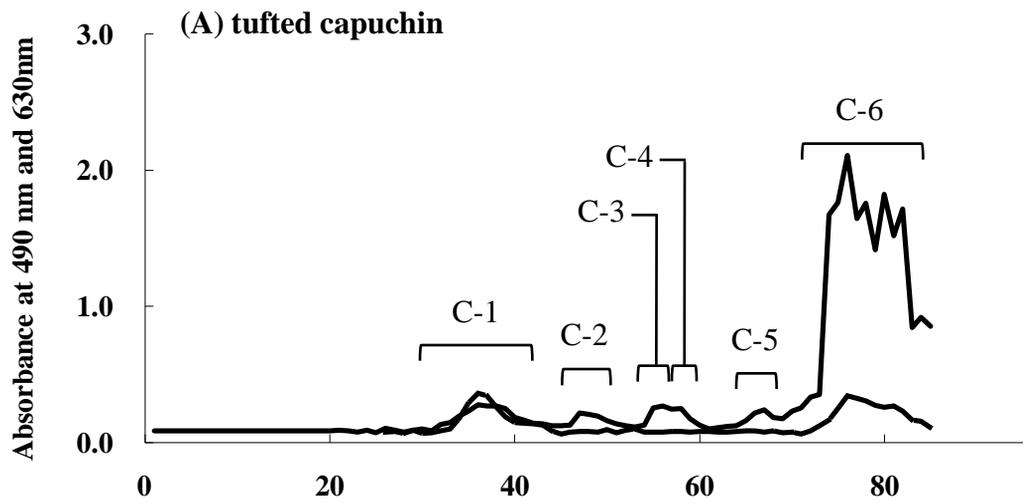
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4 Fig. 3. Anion exchange chromatograms of (A) R-1, (B) T-1, (C) B-1 and (D)  
5 M-1 separated from rhesus macaque, toque macaque, Hamadryas baboon  
6 and mantled howler milk, respectively, by gel chromatography on Bio Gel  
7 P-2. A DEAE-Sephadex A-50 column ( $1.5 \times 20$  cm) equilibrated with 50 mM  
8 tris-HCl buffer solution (pH 8.7) was used. Elution was done first with 250  
9 mL of the same solution, and then with a linear gradient of the same  
10 containing NaCl from 0 to 0.5 M. The flow rate was 15 mL/ h and 5 mL  
11 fractions were collected. Each fraction was monitored by the phenol-H<sub>2</sub>SO<sub>4</sub>  
12 method.  
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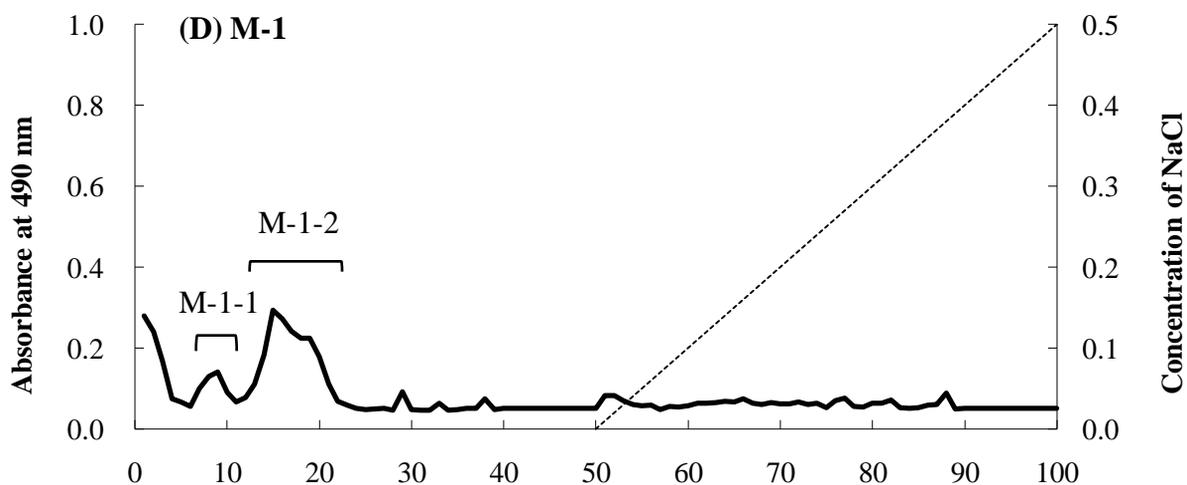
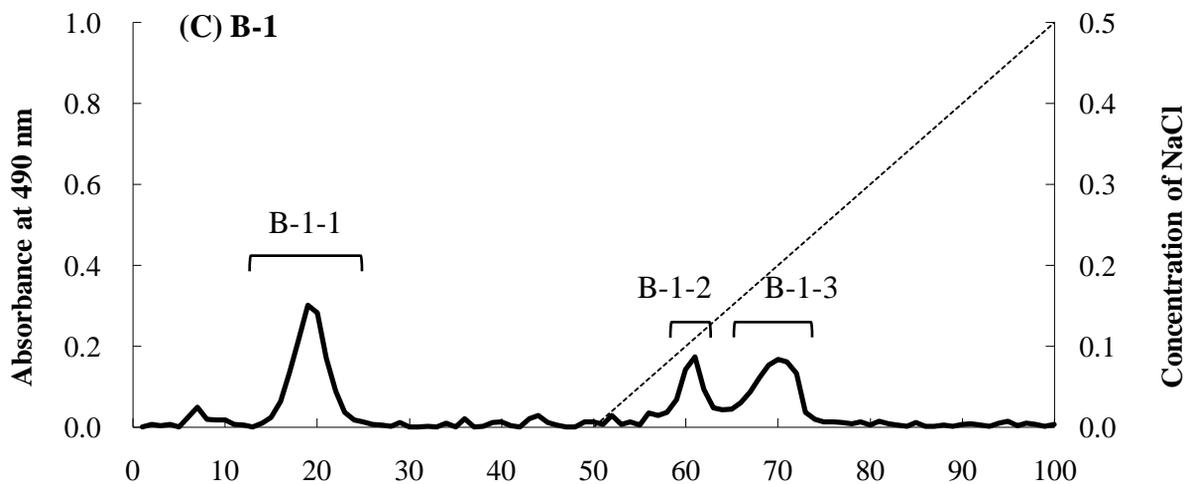
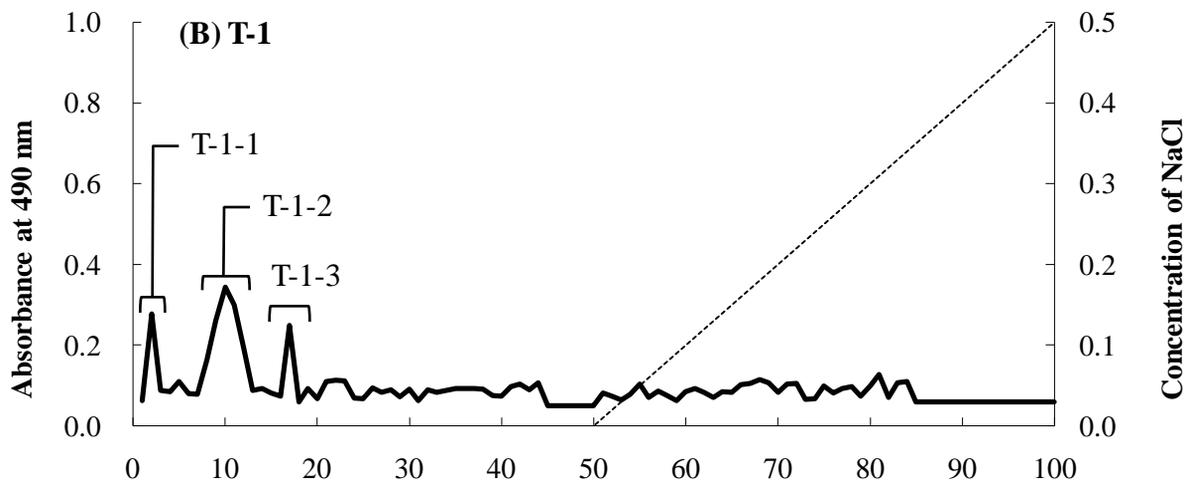
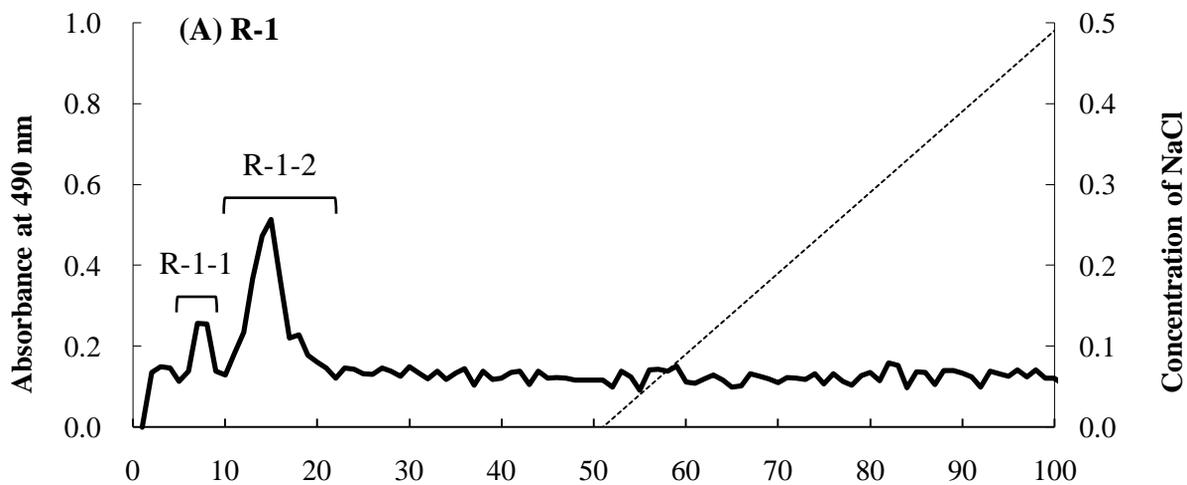
22 Fig. 4. HPLC of (A) R-1-2, (B) T-1-2 and (C) B-1-1 separated from rhesus  
23 macaque, toque macaque and Hamadryas baboon milk, respectively. HPLC  
24 was done using a Shimadzu LC-10 AT VP pump on a TSK-gel Amido-80  
25 column ( $4.6 \times 250$  mm, pore size 80Å, particle size 5µm). The mobile phase  
26 was 80% and 50% acetonitrile in 15 mM potassium phosphate buffer  
27 solution, denoted buffer A and buffer B. Elution was done using a linear  
28 gradient of 0% to 50% of B buffer for 15 min, followed by 50% to 100% of B  
29 buffer for 65 min at 60°C at a flow rate of 1 mL/ min. Detection of peaks was  
30 done by UV absorption at 195 nm.  
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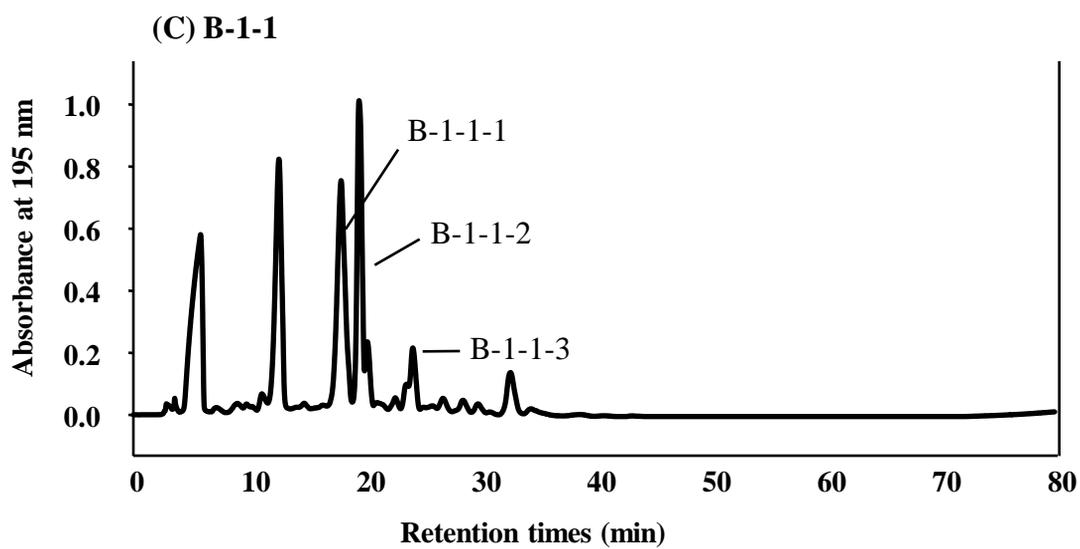
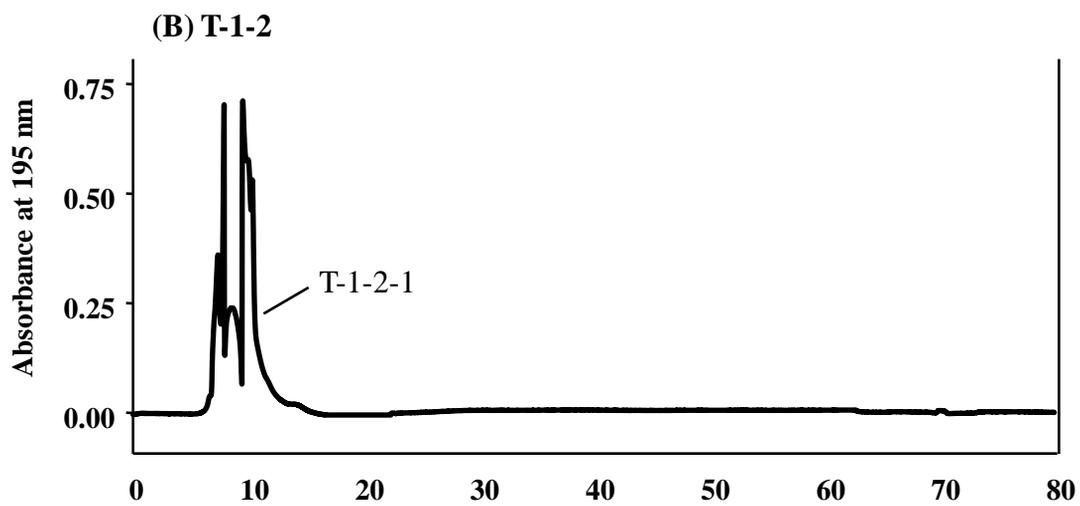
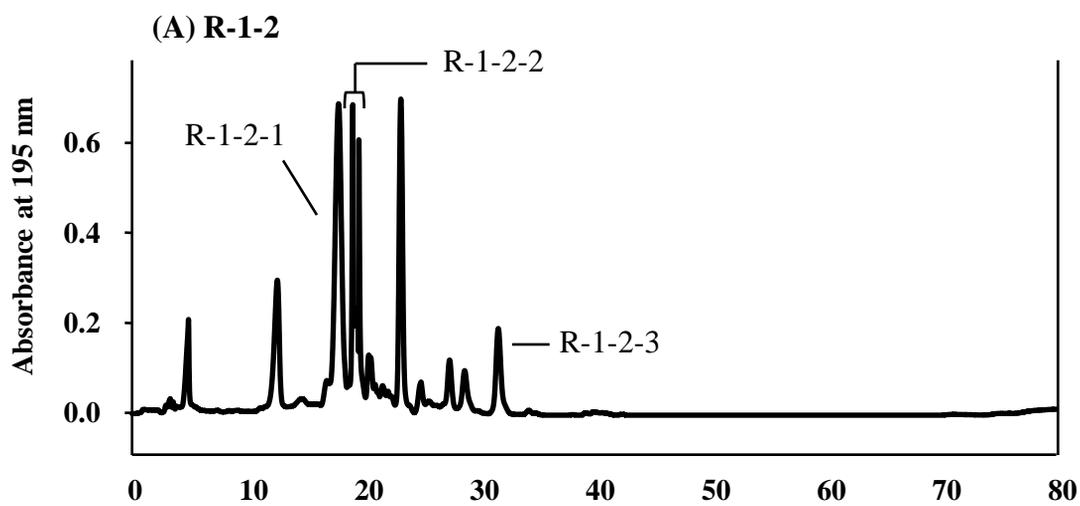
40 Fig. 5. HPLC of (A) C-1, (B) M-1-2 and (C) S-1 separated from tufted  
41 capuchin, mantled howler and Bolivian squirrel monkey milk, respectively.  
42 The chromatographies were performed as described in Fig. 4.  
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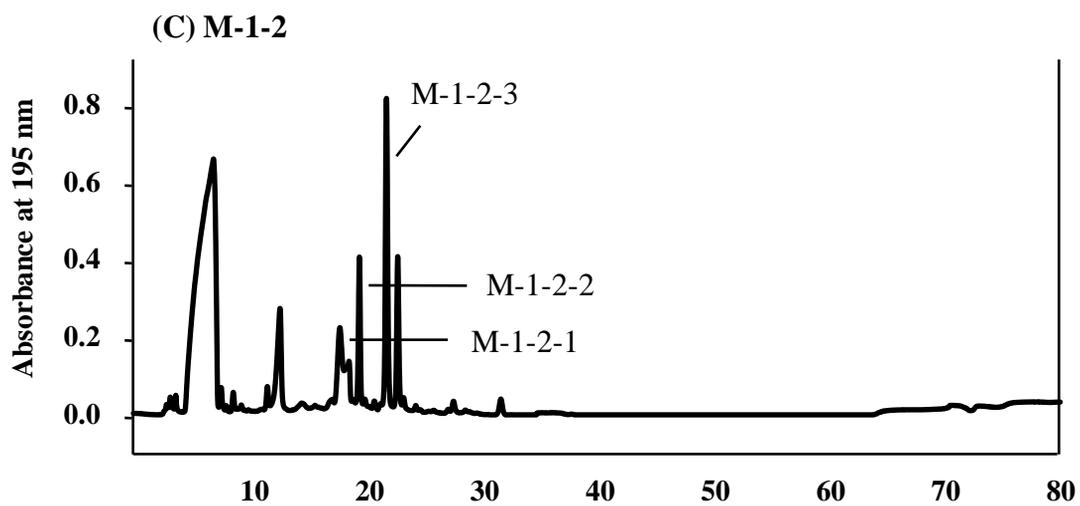
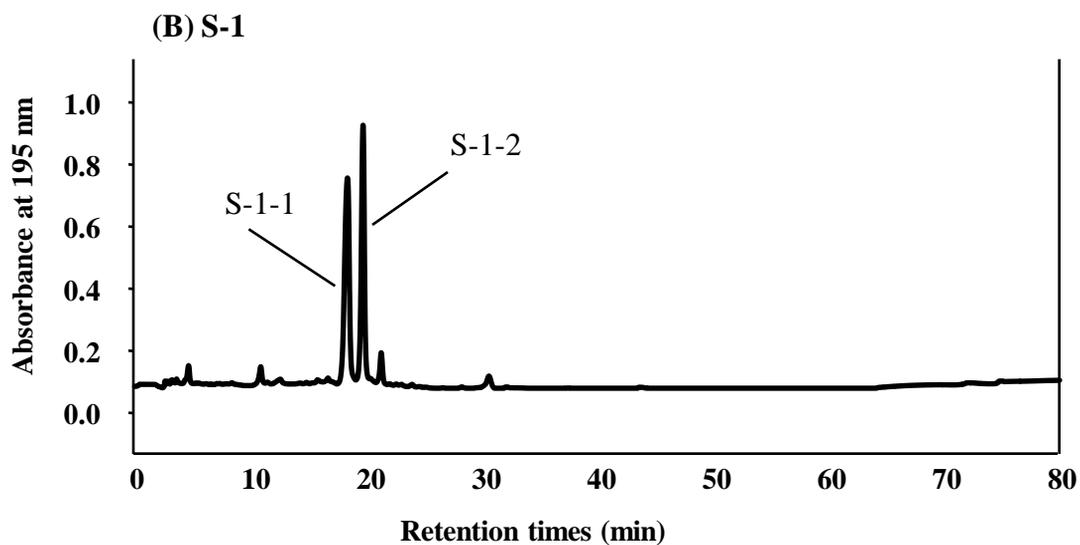
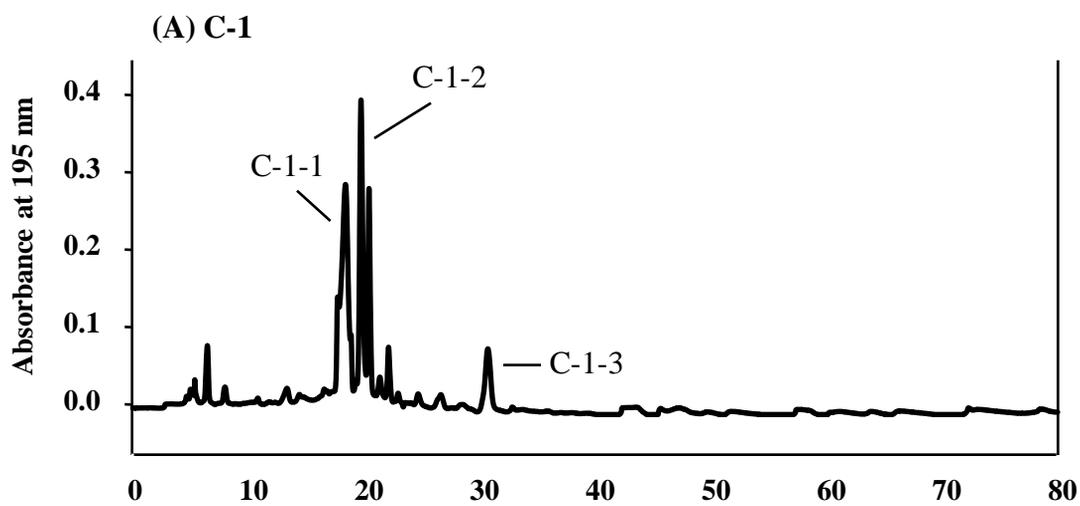
**Figure**  
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**Table**[Click here to download Table: Table.doc](#)

Table 1. Milk oligosaccharides characterized in this study

Oligosaccharides (abbreviation)	Chemical structure
3-Fucosyllactose (3-FL)	Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]Glc
3'-Galactosyllactose (3'-GL)	Gal( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc
3'-N-acetylgalactosaminyllactose (3'-GalNAcL)	GalNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc
Lacto-N-neotetraose (LNnT)	Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc
Lacto-N-fucopentaose III (LNFP III)	Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc
Lacto-N- novopentaose I (novo-LNP I)	Gal( $\beta$ 1-3)[Gal( $\beta$ 1-4) GlcNAc( $\beta$ 1-6)]Gal( $\beta$ 1-4)Glc
Lacto-N-neohexaose (LNnH)	Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)[Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-6)] Gal( $\beta$ 1-4)Glc
Difucosyl-lacto-N-neohexaose (DFLNnH)	Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-3){ Gal( $\beta$ 1-4)[Fuc( $\alpha$ 1-3)]GlcNAc( $\beta$ 1-6)]- -Gal( $\beta$ 1-4)Glc
3'-N-acetylneuraminyllactose (3'-NAc-SL)	Neu5Ac( $\alpha$ 2-3)Gal( $\beta$ 1-4)Glc
6'-N-acetylneuraminyllactose (6'-NAc-SL)	Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)Glc
GM <sub>2</sub> tetrasaccharide (GM <sub>2</sub> tetra)	GalNAc( $\beta$ 1-4)[Neu5Ac( $\alpha$ 2-3)]Gal( $\beta$ 1-4)Glc
Sialyl-lacto-N-tetraose c (LSTc)	Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc
Monosialyl-lacto-N-neohexaose (MSLNnH)	Neu5Ac( $\alpha$ 2-6)Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-3)[Gal( $\beta$ 1-4)GlcNAc( $\beta$ 1-6)]Gal( $\beta$ 1-4)Glc
3'-o-lactose sulfate (L-3'-s)	Gal( $\beta$ 1-4)Glc-3'-o-sulfate

Table 2. Comparison of oligosaccharides in the milk of monkeys

Type of oligosaccharide		Species of primates					
		rhesus macaque	toque macaque	Hamadryas baboon	tufted capuchin	mantled howler	Bolivian squirrel monkey
Neutral	tri	3-FL 6'-GL 3'-GalNAcL	3-FL	3-FL			
	tetra	LNnT			LNnT		
	penta	LNFP III	LNFP III				
	hexa				novo LNP I LNnH		
	octa			DFLNnH			
	di			L-3'-S			
	tri	3'-NAc-SL 6'-NAc-SL	3'-NAc-SL	3'-NAc-SL 6'-NAc-SL	3'-NAc-SL 6'-NAc-SL	3'-NAc-SL 6'-NAc-SL	3'-NAc-SL 6'-NAc-SL
	tetra	GM <sub>2</sub> tetra					
Acidic	penta	LSTc		LSTc			
	hepta				MSLNnH		

Table S1. <sup>1</sup>H-NMR chemical shifts of R-1-2-1, 1-2-2-1, 1-2-2-2 and R1-2-3 separated from rhesus macaque milk

Reporter group	Residue	R-1-2-1	R-1-2-2-1	R-1-2-2-2	R-1-2-3
H-1	Glc α	5.223(4.0)	5.221(4.0)	5.221(4.0)	5.220(3.8)
	Glc β	4.663(8.0)	4.669(7.9)	4.669(7.9)	4.665(7.9)
	Gal(β1-4)	4.531(7.7)	4.427(7.9)	4.533(7.9)	4.456(7.6) 4.443(7.9)
	GlcNAc(β1-3)	-	-	-	4.728(7.6)
	GalNAc(β1-4)	-	-	4.734(8.5)	-
H-3	Gal(β1-4)	4.120(3.4 <sup>a</sup> )		4.152	
H-3ax	Neu5Ac(α2-3)	1.800 (12.0 <sup>b</sup> , -12.6 <sup>c</sup> )	-	1.926 (12.0 <sup>b</sup> , -12.0 <sup>c</sup> )	-
	Neu5Ac(α2-6)	-	1.747 (12.0 <sup>b</sup> , -12.3 <sup>c</sup> )	-	1.724 (12.3 <sup>b</sup> , -12.0 <sup>c</sup> )
H-3eq	Neu5Ac(α2-3)	2.757(5.7 <sup>d</sup> )	-	2.657(8.2 <sup>d</sup> )	-
	Neu5Ac(α2-6)	-	2.715(4.6 <sup>d</sup> )	-	2.670(4.5 <sup>d</sup> )
H-4	Gal(β1-4)			4.120	4.160(2.6 <sup>e</sup> )
NAc	GlcNAc(β1-3)	-	-	-	2.054
	GalNAc(β1-4)	-	-	2.014	-
	Neu5Ac(α2-3)	2.030	-	2.030	-
	Neu5Ac(α2-6)	-	2.028	-	2.027
		3'-NAc-SL	6'-NAc-SL	GM <sub>2</sub> tetra	LSTc

<sup>a</sup>J<sub>3,4</sub>; <sup>b</sup>J<sub>3ax,4</sub>; <sup>c</sup>J<sub>3ax,3eq</sub>; <sup>d</sup>J<sub>3eq,4</sub>; <sup>e</sup>J<sub>4,3</sub>

Table S2. <sup>1</sup>H-NMR chemical shifts of R-2, R-3, R-4, R-6 and R-7 separated from rhesus macaque milk

Reporter group	Residue	R-2	R-3	R-4	R-6	R-7
H-1	Glc α	5.219(3.4)	5.219(4.0)	5.220(3.4)	5.181(4.0)	5.223(4.0)
	Glc β	4.662(8.0)	4.662(7.4)	4.663(8.0)	4.653(8.0)	4.664(8.0)
	Gal(β1-4)	4.434(8.0)	4.437(7.4)	4.439 (8.0)	4.432(8.0)	4.451(7.4)
		4.462(7.4)	4.479(7.4)	-	-	-
	GlcNAc(β1-3)	4.709(8.6)	4.703(8.6)	-	-	-
	GalNAc(β1-3)	-	-	4.618(8.0)	-	-
		-	-	4.614(8.6)	-	-
	Fuc(α1-3)	5.127(4.0)	-	-	5.441(4.0)	-
					5.384(4.0)	-
	H-4	Gal(β1-4)	4.157(3.4 <sup>a</sup> )	4.156(3.4 <sup>a</sup> )	4.159(3.4 <sup>a</sup> )	
H-6	Fuc(α1-3)	1.174(6.9 <sup>b</sup> )	-	-	1.188(6.3 <sup>b</sup> )	-
					1.183(6.9 <sup>b</sup> )	-
NAc	GlcNAc(β1-3)	2.022	2.034	-	-	-
	GalNAc(β1-3)	-	-	2.035	-	-
		LNFP I	LNnT	3'GalNAcL	3-FL	Lactose

<sup>a</sup>J<sub>4,3</sub>; <sup>b</sup>J<sub>6,5</sub>

Table S3.  $^{13}\text{C}$ -NMR chemical shifts of R-4 separated from rhesus macaque milk

Reporter group	R-4		
	3'GalNAcL		
	[GalNAc( $\beta$ 1-3)Gal( $\beta$ 1-4)Glc]		
	III	II	I
C-1	$\alpha$ 94.4		$\beta$ 98.4
C-2	$\alpha$ 76.5		$\beta$ 76.5
C-3	$\alpha$ 74.1		$\beta$ 77.0
C-4	$\alpha$ 80.9		$\beta$ 80.9
C-5	$\alpha$ 72.7		$\beta$ 77.5
C-6	$\alpha$ 62.8		$\beta$ 62.8
C-1	105.6		
C-2	72.8		
C-3	84.9		
C-4	71.2		
C-5	77.7		
C-6	63.1		
C-1	106.0		
C-2	55.2		
C-3	73.4		
C-4	70.4		
C-5	77.7		
C-6	63.7		
CH3	24.9		
CO	177.8		

Table S4. <sup>1</sup>H-NMR chemical shifts of T-1-2-1, T-4, T-8 and T-9 separated from toque macaque milk

Reporter group	Residue	T-1-2-1	T-4	T-8	T-9
H-1	Glc α	5.223(2.3)	5.219(3.4)	5.181(4.0)	5.223(4.0)
	Glc β	4.665(10.9)	4.662(8.0)	4.653(8.0)	4.665(8.0)
	Gal(β1-4)	4.531(7.2)	4.434(8.0)	4.432(8.0)	4.451(7.4)
		-	4.462(7.4)	-	-
	GlcNAc(β1-3)	-	4.710(8.6)	-	-
	Fuc(α1-3)	-	5.127(4.0)	5.441(4.0)	-
				5.384(4.0)	
H-3	Gal(β1-4)	4.116			
H-3ax	Neu5Ac(α2-3)	1.800 (12.0 <sup>a</sup> , -12.0 <sup>b</sup> )	-	-	-
H-3eq	Neu5Ac(α2-3)	2.757(3.4 <sup>c</sup> )	-	-	-
H-4	Gal(β1-4)		4.158(2.9 <sup>d</sup> )		
H-6	Fuc(α1-3)	-	1.174(6.9 <sup>e</sup> )	1.189(6.9 <sup>e</sup> )	-
				1.183(6.9 <sup>e</sup> )	-
NAc	GlcNAc(β1-3)	-	2.022	-	-
	Neu5Ac(α2-3)	2.029	-	-	-
		3'-NAc-SL	LNFP III	3-FL	Lactose

<sup>a</sup> J<sub>3ax,4</sub> ; <sup>b</sup> J<sub>3ax,3eq</sub> ; <sup>c</sup> J<sub>3eq,4</sub> ; <sup>d</sup> J<sub>4,3</sub> ; <sup>e</sup> J<sub>6,5</sub>

Table S5. <sup>1</sup>H-NMR chemical shifts of B-1-1-1, B-1-1-2 and B-1-1-3 separated from Hamadryas baboon milk

Reporter group	Residue	B-1-1-1	B-1-1-2	B-1-1-3
H-1	Glc α	5.221(3.5)	5.224(3.8)	5.220(4.1)
	Glc β	4.663(7.9)	4.669(7.9)	4.665(7.9)
	Gal(β1-4)	4.532(7.9)	4.427(7.9)	4.455(7.6)
				4.442(7.9)
	GlcNAc(β1-3)	-	-	4.729
H-3	Gal(β1-4)	4.116		
H-3ax	Neu5Ac(α2-3)	1.800 (12.3 <sup>a</sup> , -12.0 <sup>b</sup> )	-	-
	Neu5Ac(α2-6)	-	1.745 (12.3 <sup>a</sup> , -12.3 <sup>b</sup> )	1.724 (12.3 <sup>a</sup> , -12.0 <sup>b</sup> )
H-3eq	Neu5Ac(α2-3)	2.757(4.7 <sup>c</sup> )	-	-
	Neu5Ac(α2-6)	-	2.712(4.7 <sup>c</sup> )	2.669(4.7 <sup>c</sup> )
H-4	Gal(β1-4)			4.161
NAc	GlcNAc(β1-3)	-	-	2.054
	Neu5Ac(α2-3)	2.030	-	-
	Neu5Ac(α2-6)	-	2.028	2.027
		3'-NAc-SL	6'-NAc-SL	LSTc

<sup>a</sup> J<sub>3ax, 4</sub> ; <sup>b</sup> J<sub>3ax, 3eq</sub> ; <sup>c</sup> J<sub>3eq, 4</sub>

Table S6. <sup>1</sup>H-NMR chemical shifts of B-3-1, B-3-2, B-9-1, B-9-2 and B-10 separated from hamadryas baboon milk

Reporter group	Residue	B-3-1	B-3-2	B-9-1	B-9-2	B-10
H-1	Glc α	5.218(3.5)	5.224(3.5)	5.181(4.0)	5.225(3.4)	5.223(4.0)
	Glc β	4.665(7.9)	4.665(7.9)	4.653(8.0)	4.668(6.9)	4.665(8.0)
	Gal(β1-3)	-	-	-	4.612(8.0)	-
	Gal(β1-4)	4.424(7.9)	4.569(7.9)	4.432(8.0)	4.511(8.0)	4.450(8.0)
		4.452(8.2)	-	-	-	-
		4.466(8.2)	-	-	-	-
	GlcNAc(β1-3)	4.708(6.2)	-	-	-	-
	GlcNAc(β1-6)	4.637(7.6)	-	-	-	-
	Fuc(α1-3)	5.105(3.8)	-	5.441(4.0)	-	-
		5.126(4.1)	-	5.384(4.0)	-	-
H-3	Gal(β1-4)		4.343(2.9 <sup>a</sup> )			
H-4	Gal(β1-4)	4.142(3.2 <sup>b</sup> )	4.295(3.2 <sup>b</sup> )		4.199(3.4 <sup>b</sup> )	
H-6	Fuc(α1-3)	1.174(6.5 <sup>c</sup> )	-	1.189(6.9 <sup>c</sup> )	-	-
		-	-	1.183(6.9 <sup>c</sup> )	-	-
NAc	GlcNAc(β1-3)	2.020	-	-	-	-
	GlcNAc(β1-6)	2.049	-	-	-	-
		DFLNnH	Gal(β1-4)Glc-3' -o-sulphate	3-FL	3'GL	Lactose

<sup>a</sup> J<sub>3,4</sub>; <sup>b</sup> J<sub>4,3</sub>; <sup>c</sup> J<sub>6,5</sub>

Table S7. <sup>1</sup>H-NMR chemical shifts of C-1-1, C-1-2 and C-1-4 separated from tufted capuchin milk

Reporter group	Residue	C-1-1	C-1-2	C-1-4
H-1	Glc α	5.223(4.0)	5.224(3.4)	5.220(3.5)
	Glc β	4.664(8.0)	4.668(8.0)	4.669(7.9)
	Gal(β1-4)	4.530(7.4)	4.427(7.4)	4.433(7.9)
		-	-	4.455(8.1)
		-	-	4.472(7.9)
	GlcNAc(β1-3)	-	-	4.726(7.3)
	GlcNAc(β1-6)	-	-	4.647(7.9)
			4.640(7.9)	
H-3	Gal(β1-4)	4.114(3.2 <sup>a</sup> )		
H-3ax	Neu5Ac(α2-3)	1.800 (12.0 <sup>b</sup> , -11.5 <sup>c</sup> )	-	-
	Neu5Ac(α2-6)	-	1.745 (12.6 <sup>b</sup> , -12.0 <sup>c</sup> )	1.724 (12.3 <sup>b</sup> , -12.0 <sup>c</sup> )
H-3eq	Neu5Ac(α2-3)	2.756(4.6 <sup>d</sup> )	-	-
	Neu5Ac(α2-6)	-	2.712(4.3 <sup>d</sup> )	2.668(4.7 <sup>d</sup> )
H-4	Gal(β1-4)			4.149(3.5 <sup>e</sup> )
NAc	GlcNAc(β1-3)	-	-	2.052
	GlcNAc(β1-6)	-	-	2.061
	Neu5Ac(α2-3)	2.030	-	-
	Neu5Ac(α2-6)	-	2.028	2.027
		3'-NAc-SL	6'-NAc-SL	MSLNnH

<sup>a</sup> J<sub>3,4</sub>; <sup>b</sup> J<sub>3ax,4</sub>; <sup>c</sup> J<sub>3ax,3eq</sub>; <sup>d</sup> J<sub>3eq,4</sub>; <sup>e</sup> J<sub>4,3</sub>

Table S8. <sup>1</sup>H-NMR chemical shifts of C-2, C-3, C-4 and C-6 separated from tufted capuchin milk

Reporter group	Residue	C-2	C-3	C-4	C-6
H-1	Glc $\alpha$	5.219(3.4)	5.224(3.4)	5.220(3.4)	5.223(4.0)
	Glc $\beta$	4.665(8.0)	4.669(7.4)	4.663(8.0)	4.665(8.0)
	Gal( $\beta$ 1-3)	-	4.610(8.0)	-	-
	Gal( $\beta$ 1-4)	4.427(8.0)	4.472(8.0)	4.638(2.3)	4.451(8.0)
		4.468(4.6)	4.500(8.0)	4.478(8.0)	-
		4.483(4.4)	-	-	-
	GlcNAc( $\beta$ 1-3)	4.698(8.6)	-	4.705(8.6)	-
				4.701(8.0)	
	GlcNAc( $\beta$ 1-6)	4.644(8.0)	4.644(6.9)	-	-
		4.637(7.4)			
H-4	Gal( $\beta$ 1-4)	4.147(3.4 <sup>a</sup> )		4.157(3.4 <sup>a</sup> )	
NAc	GlcNAc( $\beta$ 1-3)	2.036	-	2.034	-
	GlcNAc( $\beta$ 1-6)	2.060	2.062	-	-
		LNnH	<i>novo</i> -LNP I	LNnT	Lactose

<sup>a</sup>  
J<sub>4,3</sub>

Table S9. <sup>1</sup>H-NMR chemical shifts of S-1-1, S-1-2 and S-5 separated from squirrel monkey milk

Reporter group	Residue	S-1-1	S-1-2	S-5
H-1	Glc α	5.223(4.0)	5.225(3.5)	5.223(4.0)
	Glc β	4.664(8.0)	4.669(7.9)	4.665(8.0)
	Gal(β1-4)	4.531(7.4)	4.428(7.6)	4.451(7.4)
H-3	Gal(β1-4)	4.115(3.2 <sup>a</sup> )		
H-3ax	Neu5Ac(α2-3)	1.800 (12.0 <sup>b</sup> , -12.3 <sup>c</sup> )	-	-
	Neu5Ac(α2-6)	-	1.744 (12.3 <sup>b</sup> , -12.3 <sup>c</sup> )	-
H-3eq	Neu5Ac(α2-3)	2.755(4.0 <sup>d</sup> )	-	-
	Neu5Ac(α2-6)	-	2.712(4.7 <sup>d</sup> )	-
NAC	Neu5Ac(α2-3)	2.030	-	-
	Neu5Ac(α2-6)	-	2.028	-
		3'-NAc-SL	6'-NAc-SL	Lactose

<sup>a</sup> J<sub>3,4</sub>; <sup>b</sup> J<sub>3ax,4</sub>; <sup>c</sup> J<sub>3ax,3eq</sub>; <sup>d</sup> J<sub>3eq,4</sub>

Table S10. <sup>1</sup>H-NMR chemical shifts of M-1-2-1, M1-2-2 and M-8 separated from mantled howler milk

Reporter group	Residue	M-1-2-1	M-1-2-2	M-8
H-1	Glc $\alpha$	5.223(4.0)	5.224(3.4)	5.223(4.0)
	Glc $\beta$	4.664(8.0)	4.668(8.0)	4.665(8.0)
	Gal( $\beta$ 1-4)	4.531(7.4)	4.426(8.0)	4.451(7.4)
H-3	Gal( $\beta$ 1-4)	4.116(2.3) <sup>a</sup>		
H-3ax	Neu5Ac( $\alpha$ 2-3)	1.800 (12.0 <sup>b</sup> , -11.5 <sup>c</sup> )	-	-
	Neu5Ac( $\alpha$ 2-6)	-	1.745 (12.0 <sup>b</sup> , -12.0 <sup>c</sup> )	-
H-3eq	Neu5Ac( $\alpha$ 2-3)	2.756(4.6) <sup>d</sup>	-	-
	Neu5Ac( $\alpha$ 2-6)	-	2.712(4.6) <sup>d</sup>	-
NAc	Neu5Ac( $\alpha$ 2-3)	2.030	-	-
	Neu5Ac( $\alpha$ 2-6)	-	2.028	-
		3'-NAc-SL	6'-NAc-SL	Lactose

<sup>a</sup> J<sub>3,4</sub>; <sup>b</sup> J<sub>3ax,4</sub>; <sup>c</sup> J<sub>3ax,3eq</sub>; <sup>d</sup> J<sub>3eq,4</sub>