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Comparative Genomics Identifies the Genetic Islands That Distinguish *Neisseria meningitidis*, the Agent of Cerebrospinal Meningitis, from Other *Neisseria* Species

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Neisseria meningitidis **colonizes the nasopharynx and, unlike commensal *Neisseria* species, is capable of entering the bloodstream, crossing the blood-brain barrier, and invading the meninges. The other pathogenic *Neisseria* species, *Neisseria gonorrhoeae*, generally causes an infection which is localized to the genitourinary tract. In order to investigate the genetic basis of this difference in disease profiles, we used a strategy of genomic comparison. We used DNA arrays to compare the genome of *N. meningitidis* with those of *N. gonorrhoeae* and *Neisseria lactamica*, a commensal of the nasopharynx. We thus identified sequences conserved among a representative set of virulent strains which are either specific to *N. meningitidis* or shared with *N. gonorrhoeae* but absent from *N. lactamica*. Though these bacteria express dramatically different pathogenicities, these meningococcal sequences were limited and, in contrast to what has been found in other pathogenic bacterial species, they are not organized in large chromosomal islands.**

Cerebrospinal meningitis remains a devastating disease worldwide, with a morbidity of 1 to 3 per 100,000 in North America and Europe and considerably higher in poorer regions, such as Africa (4). The mechanisms by which the pathogen *Neisseria meningitidis* passes from the site of initial colonization, the nasopharyngeal mucosa, to invade the cerebrospinal fluid are not well understood. Two bacterial attributes are constantly present in clinical isolates and thus are identified as being essential in meningococcal pathogenesis, the capsular polysaccharide and type IV pili. The former is important for extracellular growth (39), while the latter have been shown to mediate interaction both with the nasopharyngeal epithelium and with the components of the blood-brain barrier (23, 30).

One approach to the search for new determinants of bacterial pathogenesis is comparison of the genetic contents of closely related species expressing different pathogenicities. *N. meningitidis* is very closely related to several other *Neisseria* species, such as *Neisseria gonorrhoeae* and *Neisseria lactamica* (11, 16). Despite this genetic similarity, the characteristic disease profiles of these bacteria are very different. *N. meningitidis*, a normal inhabitant of the human nasopharynx (29), has the ability, in a proportion of those colonized, to invade the epithelium, to disseminate within the bloodstream, and to cross the blood-brain barrier. *N. gonorrhoeae*, the gonococcus, colonizes and invades the urogenital epithelium to cause a localized inflammation, gonorrhea. Though it is able in some cases to invade the bloodstream, disseminated disease is unusual and meningitis is extremely rare, with 20 cases reported in the United States between 1922 and 1972 (12, 28). *N. lactamica* is one of the several *Neisseria* species which are harmless commensals of the nasopharynx and are not associated with invasive disease.

Though different pathogenic potentials may be due to subtle genetic or transcriptional differences, the determinants of pathogenicity in many medically important bacteria consist of strain-specific genes which are often grouped in large chromosomal regions, or pathogenicity islands (9, 10). These differences will therefore be identified by chromosome comparison techniques, such as subtractive hybridization and DNA array technology. Genes present only in *N. meningitidis* would be important for the specific aspects of meningococcal disease, i.e., bloodstream dissemination and crossing of the blood-brain barrier. Again, one would expect that regions of the meningococcal chromosome shared with *N. gonorrhoeae* but not with *N. lactamica* will be important in the stages of the diseases caused by the two bacteria which are similar, that is, primary colonization and invasion of the mucosa at the port of entry—the nasopharynx for the meningococcus and the urogenital tract for the gonococcus.

Preliminary work using subtractive hybridization (17, 25, 37) had identified several species-specific regions in the neisseriae. However, this technique is not easily applicable to extensive comparison of large sets of strains. The aim of this work was therefore to take advantage of DNA array technology to identify those species-specific regions conserved among a large set of representative virulent *N. meningitidis* strains.

MATERIALS AND METHODS

Bacterial strains. The strain on which the fabrication of the DNA arrays was based was *N. meningitidis* Z2491 (1), a serogroup A subtype IV-1 meningococcus (sequence type ST4 [19]) isolated from an African epidemic. This and other neisserial strains are listed in Table 1.

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TABLE 1. Strains used in this study

Strain	Serogroup	Epidemiological group	ST	Origin	Reference
N. meningitidis					
Z2491	A	Subgroup IV-1	ST4	The Gambia	1
Z5463	A	Subgroup IV-1	ST4	The Gambia	1
MC58	B	ET5 complex	ST74	United Kingdom	38
94N369	B	ET37 complex	ST11	Australia	5
FAM18	C	ET37 complex	ST11	USA	2
ROU	W135	ET37 complex	ST11	France	26
98068	C	ST41 complex	ST41	France	Gift of P. Nicholas
Z4673	B	ST41 complex	ST41	The Netherlands	Gift of M. Achtman
N. gonorrhoeae					
F62				United States	15
FA1090				United States	3
Ng MS11				United States	33
N. lactamica					
8064				France	Collection of X. Nassif
9764				France	Collection of X. Nassif

Computer-assisted genomic analysis. Chromosome sequence data were obtained from the following sites on the World Wide Web: http://www.sanger.ac.uk/Projects/N_meningitidis/(*N. meningitidis* Z2491), http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gnm (*N. meningitidis* MC58), http://www.sanger.ac.uk/Projects/N_meningitidis/seroC.shtml (*N. meningitidis* FAM18), and http://www.genome.ou.edu/gono.html (*N. gonorrhoeae* FA1090).

Production and use of DNA arrays. Primers for PCR were designed according to the chromosomal sequence of *N. meningitidis* Z2491 (http://www.sanger.ac.uk/Projects/N_meningitidis/), published on the World Wide Web before annotation, to amplify contiguous stretches of DNA about 1 kb long covering the chromosome. Oligonucleotide primers were designed, avoiding previously described repetitive sequences (Correia and highly represented insertion sequences [24]). The PCR products produced from Z2491 chromosomal DNA (2,045 amplicons) were spotted robotically (MicroGrid; Biorobotics Ltd., Cambridge, United Kingdom) in duplicate onto nylon membranes and fixed by treatment with alkali. The DNA arrays were designed to search for genetic islands which distinguish the meningococcus from the gonococcus and commensal *Neisseria* species. The use of amplicons of a similar size (1 kb), rather than predicted genes, avoids the problem of comparison of very low hybridization intensities associated with short genes (16% of the open reading frames [ORFs] in the annotation of the Z2491 chromosome are <300 bases long, and 8% are <200 bases; the signal from the DNA arrays is roughly proportional to the length of the PCR product spotted [data not shown]). In addition, it avoids possible inaccuracies in gene prediction and is a better strategy for comparisons of genomic content between strains.

Membranes were hybridized with ^{33}P-labeled chromosomal DNAs of various *Neisseria* species and washed under standard Southern blotting conditions (6). Images were revealed with a STORM PhosphorImager (Molecular Dynamics) and interpreted with the software XDotsReader (COSE, Dugny, France), which quantitated the intensity of the signal associated with each spot.

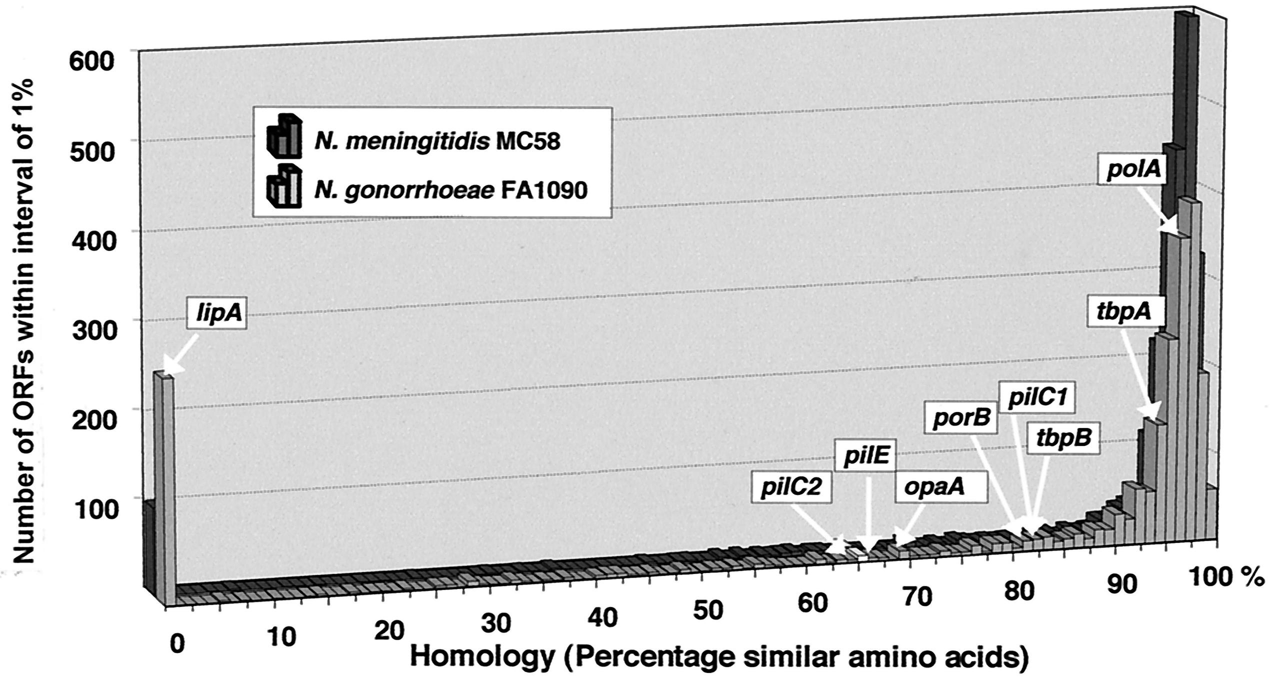
The extents of the strain- and species-specific regions were derived from the presence or absence of the amplicon sequences in each strain. In order to identify the genes included within these regions, the corresponding amplicon coordinates were compared with the gene coordinates (base numbers) given by the Z2491 chromosome annotation (http://www.sanger.ac.uk/Projects/N_meningitidis/). All results are available at Supplementary Material, together with details of the amplicons, PCR, and Southern hybridization conditions.

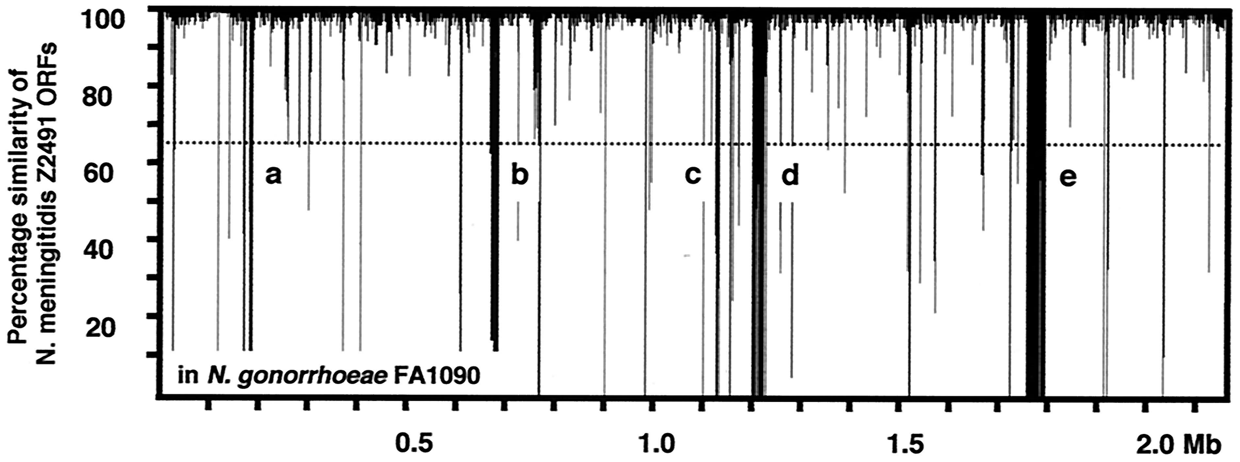
Performance of DNA arrays. The efficacy of the DNA arrays was assessed by comparison of their results with those of computer-assisted comparisons between the genome of the reference strain *N. meningitidis* Z2491 and those of the two other sequenced *Neisseria* strains, *N. gonorrhoeae* FA1090 (http://www.genome.ou.edu/gono.html) and *N. meningitidis* MC58 (34), as described in Supplementary Material. Comparison of these computer-assisted analyses with the DNA array results showed that 97% of the amplicons gave correct predictions of the presence or absence of the corresponding DNA sequence in the heterologous strains and of the presence or absence of the corresponding ORFs (3% [63 amplicons] gave false predictions and were rejected from the analysis). With respect to the ability of the DNA arrays to detect potential pathogenicity islands, further analysis of these results showed that the membranes identified 90% of strain-specific genes or regions >1.5 kb long, 96% of those >2 kb long, and all of those >3 kb long and therefore would identify genetic islands which might determine different pathogenicities.

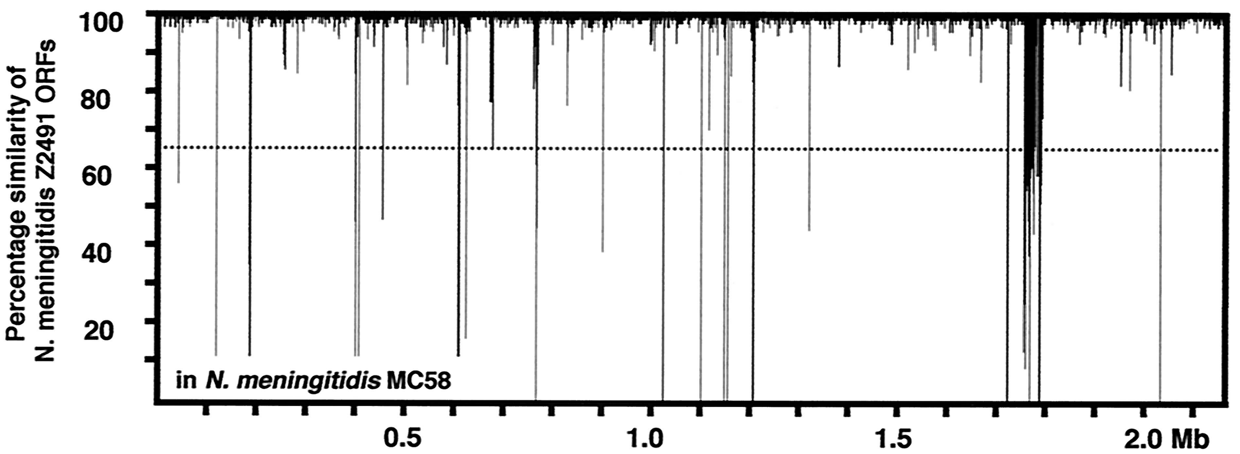
RESULTS

In silico comparisons of pathogenic *Neisseria* genomes. In order to identify sequences specific for *N. meningitidis*, we first took advantage of the completion of three genomic sequences

FIG. 1. Computer-assisted comparison of publicly available neisserial genome sequences. (A) TBlastN comparison of ORFs in Z2491 against the genomes of *N. gonorrhoeae* FA1090 and *N. meningitidis* MC58. The comparison used a minimum E value of 10^{-4} as the cutoff for reporting hits, which corresponds to ~20% amino acid similarity for an ORF of 100 bases and <10% for an ORF of 300 bases or larger. The percentage of homologous amino acids in a Z2491 ORF (abscissa) is plotted against the number of ORFs (ordinate) presenting that percentage of homology. The positions of selected genes are shown. *lipA* is a meningococcus-specific capsular biosynthesis gene. PolA is a DNA polymerase involved in chromosome replication. The protein PilC1 is an adhesin, PilE is the pilin subunit, and OpaA and PorB are surface antigens. TbpA and TbpB are the membrane transport and surface-exposed components, respectively, of a human transferrin binding and iron acquisition system. Hence, proteins known experimentally to have similar functions but varying sequences in strains of pathogenic neisseriae are found to have between 65 and 80% amino acid similarity and allow a choice of 65% homology as a cutoff to define the presence or absence of a functional homologue in a test strain. (B) Chromosomal distribution of genetic differences. The degree of homology of the *N. meningitidis* Z2491 ORFs to sequences in the FA1090 genome (percentage amino acid similarity of predicted proteins) is plotted along the length of the Z2491 chromosome. The larger islands of strain-specific DNA are as follows: a, part of the capsule locus NMA0184-NMA0185 and NMA0195-NMA0202; b, two-partner secretion family proteins NMA0687-NMA0698; c and d, phage-related proteins NMA1183-NMA1200 and NMA1298-NMA1324; and e, prophage NMA1820-NMA1883. (C) Chromosomal distribution of differences between *N. meningitidis* Z2491 and MC58. The homology of ORFs to sequences in the MC58 genome is plotted along the length of the Z2491 chromosome. Note that many of these differences correspond to those between Z2491 and *N. gonorrhoeae* FA1090 and are hence strain and not species specific.


A
N. meningitidis MC58
N. gonorrhoeae FA1090
Number of ORFs within interval of 1%
600
500
400
300
200
100
lipA
polA
tbpA
pilC1
porB
tbpB
pilE
pilC2
opaA
0
10
20
30
40
50
60
70
80
90
100 %
Homology (Percentage similar amino acids)


B
Percentage similarity of N. meningitidis Z2491 ORFs
100
80
60
40
20
a
b
c
d
e
in N. gonorrhoeae FA1090
0.5
1.0
1.5
2.0 Mb


C
Percentage similarity of N. meningitidis Z2491 ORFs
100
80
60
40
20
in N. meningitidis MC58
0.5
1.0
1.5
2.0 Mb

of pathogenic *Neisseria* strains: *N. gonorrhoeae* strain FA1090 (http://www.genome.ou.edu/gono.html), *N. meningitidis* strain MC58 (34), and *N. meningitidis* strain Z2491 (1, 24). Based on the annotation of the Z2491 genome, we performed TBlastN comparisons (protein versus translated DNA) against the chromosome sequences of the other two strains. This computer-assisted comparison demonstrated that in general the genes were either very homologous among the strains or completely absent (Fig. 1A). Seventy-five percent of the *N. meningitidis* Z2491 genes had homologues with >80% amino acid similarity in *N. gonorrhoeae* FA1090, and 12% of the genes showed no significant homology. Ninety-four percent of the Z2491 ORFs had >80% homology in the meningococcus MC58, while 5% had no homologue. The chromosomal distribution of these genetic differences is shown in Fig. 1B and C. Though some large regions of the Z2491 chromosomal sequence (5 to 40 kb) were absent from the gonococcus FA1090 and could therefore represent meningococcus-specific islands, a significant proportion of this DNA is also absent from *N. meningitidis* MC58. Full data from these comparisons are available in the Supplementary Material.

This analysis demonstrates chromosomal differences between the meningococcus and the gonococcus and also between the two meningococcal strains investigated. Therefore, in order to identify regions which are both specific to *N. meningitidis* and conserved among disease isolates, we undertook a larger-scale comparison, using DNA arrays and a defined panel of virulent strains of meningococcus. In addition, such a strategy permits a comparison of the meningococcal genome with that of the nonpathogenic *N. lactamica*, whose genomic sequence is not available.

Dramatic differences in pathogenic potential result from small genetic changes. DNA arrays were produced as described in Materials and Methods. A panel of virulent meningococci was chosen to represent the major phylogenetic groups presently causing disease. Epidemiological studies have shown that the majority of meningococcal diseases worldwide are caused by a relatively restricted number of clonal groups (4), now defined as sequence types (STs) (19). Thus, most meningococcal disease in Europe is caused by meningococci of STs 11, 32, and 41 (ET37, ET5, and ST41 complexes, respectively), while ST4 (serogroup A) causes severe epidemics in Africa. We used disease isolates from each of these clonal groups, so that genes common to and specific for these strains would be likely to be involved in properties important for the lifestyle of the meningococcus and, hence, the differential pathogenesis of meningococcal disease. The DNA arrays were therefore reacted with genomic DNAs extracted from these meningococcal strains, from three strains of *N. gonorrhoeae*, and from two strains of *N. lactamica* (Table 1). The majority of the amplicons on the DNA array reacted with sequences in each of the chromosomes tested (Fig. 2; note the large peak around unity). In addition to this peak of homologous sequences, in each case the tested strains showed genomic differences. These correspond to the amplicons of lower hybridization ratios, hence sequences absent from the strains, and represent between 3 and 8% of the total for *N. meningitidis*, about 10% for *N. gonorrhoeae*, and between 15 and 20% for *N. lactamica*, in agreement with earlier analyses (16). It is notable, in addition, that these values are very similar to those obtained from the above-mentioned in silico comparison between *N. meningitidis* strain Z2491 and *N. meningitidis* strain MC58 or *N. gonorrhoeae* strain FA1090.

To determine the extent of the genetic differences between the species, these differences were mapped along a linear representation of the meningococcal (Z2491) chromosome (Fig. 3). Comparison of the meningococci alone showed that 89% of the chromosome of Z2491 was shared with all other strains, while strain-specific differences characterized the remaining 11% (Fig. 3A). In contrast, comparison with *N. gonorrhoeae* and *N. lactamica* (Fig. 3B) demonstrated species-specific chromosomal regions—most of the regions absent from one gonococcus were also absent from the others, and similarly for *N. lactamica*.

In order to obtain new insights into meningococcal pathogenesis, our analysis focused on the large majority of the genome of Z2491 that was shared by all virulent strains of *N. meningitidis*. Most of these sequences (78% of the chromosome; 1.7 Mb) were present in all isolates of the three species *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica* and thus correspond to the core neisserial genome. Of the rest, 46 kb (2.2%) are strictly meningococcus specific, that is, present in all strains of invasive meningococci and absent from all the *N. gonorrhoeae* and *N. lactamica* strains. Seventy-three kilobases (3.3%) of the Z2491 genome is pathogen specific, i.e., shared with the gonococcus and absent from *N. lactamica*. Twelve kilobases (0.6%) is shared with *N. lactamica* but absent from the gonococcus. The genes corresponding to these sequences are reported in Table 2. Their analysis reveals that they correspond in general to single genes or small groups of genes scattered around the genome, since only one genetic island >10 kb in length was found (NMA0687-NMA0698) (Table 2). It should be noted that the capsular polysaccharide synthesis gene cluster (~20 kb) is known to be meningococcus specific; the technique identified the genes encoding the conserved enzymes which attach the lipid anchor to the polysaccharide chain, while genes encoding polysaccharide biosynthesis and secretion show interstrain variation.

Among the *N. meningitidis*-specific sequences, only seven were >2 kb long. As mentioned above and as expected, one of these regions corresponds to the *cps* locus specifying the production of capsular polysaccharide, which is known to be necessary for meningococcal virulence (27). Two other meningococcus-specific regions (Table 2) encode the production and secretion of the protein Frp of the RTX toxin family (35, 36). The largest of the five regions showed homology to proteins of the newly described family of two-partner virulence factor secretion systems (14) and would elaborate an ~200-kDa protein with homology to the filamentous hemagglutinin of *Bordetella pertussis*. This region, along with three others encoding a group of metabolic enzymes, a putative type I secretion system, and a disulfide oxidoreductase involved in the correct folding of secreted proteins, respectively, has been described previously (17)

As for meningococcus-specific regions, pathogen-specific sequences (i.e., common to *N. meningitidis* and *N. gonorrhoeae* but absent from *N. lactamica*) are scattered as small islands around the chromosome. They were present in larger numbers than the meningococcus-specific regions, but nevertheless, only three of these genes have been shown to play roles in

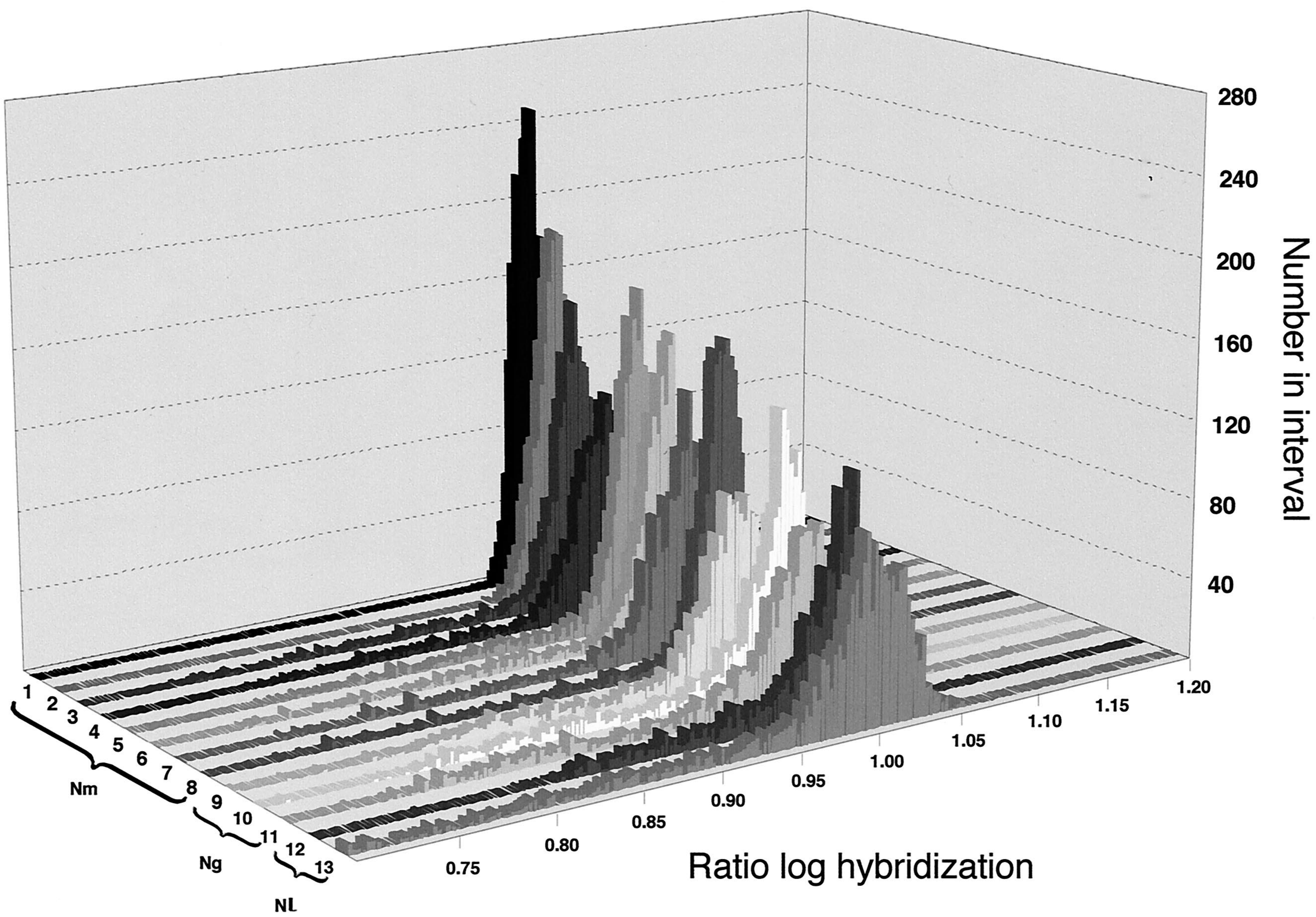


FIG. 2. Distribution of reactivities with different *Neisseria* strains. The histogram shows the distribution of reactivities of amplicons for each of the strains. Related strains are grouped on the basis of previous epidemiological studies (Table 1). Lanes: 1, *N. meningitidis* Z2491 (serogroup A); 2, Z5463 (serogroup A); 3, MC58 (serogroup B); 4, 94N369 (serogroup B); 5, FAM18 (serogroup C); 6, ROU (serogroup W135); 7, 98068 (serogroup C); 8, Z4673 (serogroup B); 9, *N. gonorrhoeae* F62; 10, MS11; 11, FA1090; 12, *N. lactamica* 8064; 13, 9764.

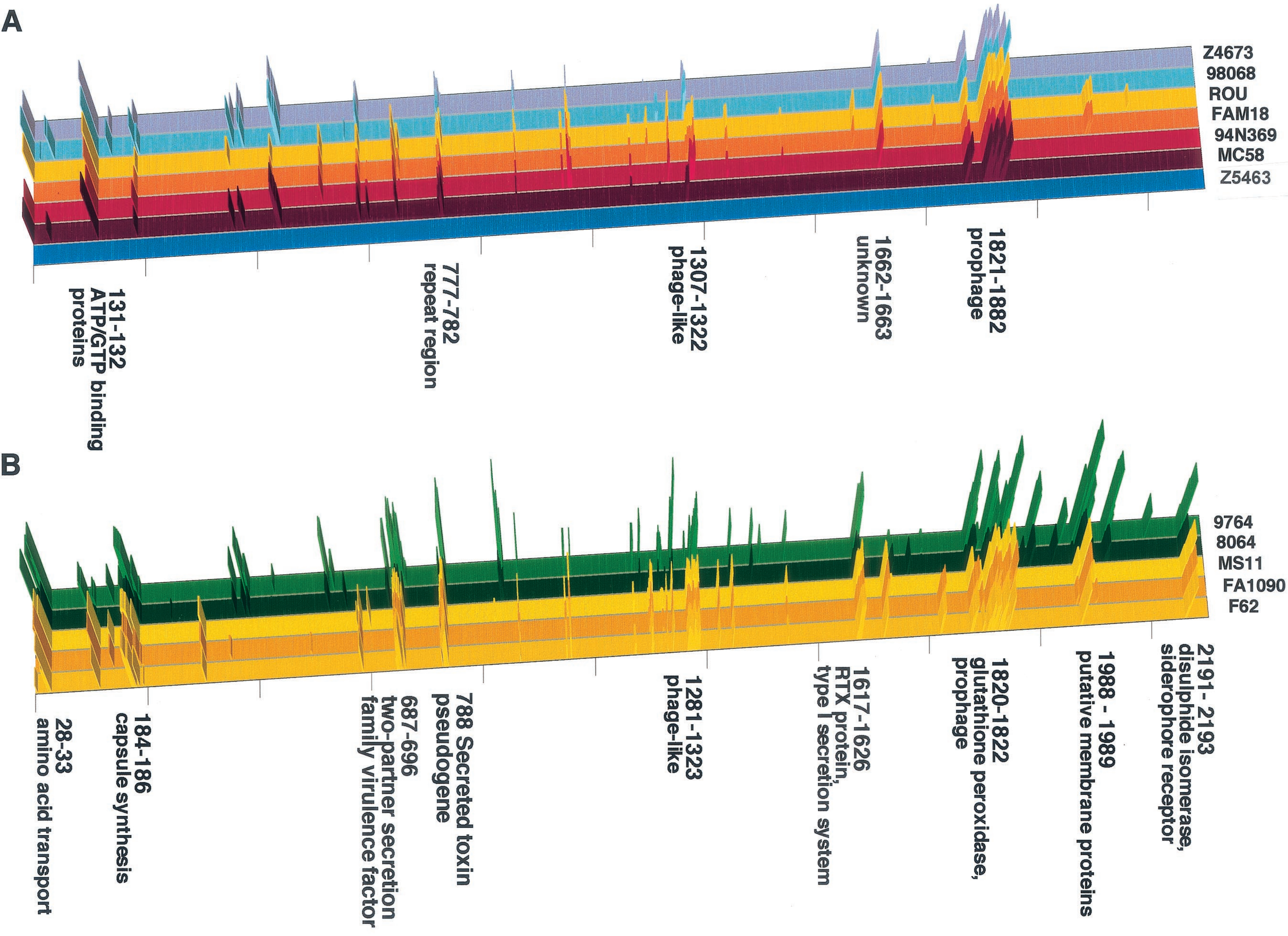


FIG. 3. Distribution of strain-specific sequences. Distribution along the chromosome of Z2491 of amplicons absent from each of the strains of *N. meningitidis* (A) and from each of the strains of *N. gonorrhoeae* and *N. lactamica* (B). The strains are arranged as in Table 1 and Fig. 2. The peak height represents the difference between the value for an amplicon and the cutoff value, where the sequence is absent from the strain. Only groups of at least two contiguous nonreacting amplicons are shown. Selected genes or groups of genes, meningococcus serogroup A specific (A) and meningococcus specific (B), are named. Strain Z5463 (A) is an isolate from the same epidemic as the reference strain, Z2491, and bears witness to their high sequence homology.

TABLE 2. Genes present within the regions defined as *N. meningitidis* or pathogen specific or common to *N. meningitidis* and *N. lactamica*[a]

Gene(s)[b]	Description	No. of ORFs	No. of kb specific[c]	% GC	No. of subtractive clones[d]
N. meningitidis specific					
Virulence associated					
NMA0184 to NMA0186	Capsule synthesis	3	**5.3**	55	2
NMA0788	Secreted toxin (Frp)	1	**2.1**	46	1
NAM1617 to NMA1626	Superoxide dismutase, probable RTX family exotoxin (Frp), and others	10	**6.9**	44	
NMA2191 and NMA2193	DsbA, disulfide isomerase; TonB-dependent receptor protein	2	**2.8**	49, 54	2
Possible virulence associated					
NMA0687-NMA0696	Filamentous hemagglutinin FhaB homologue (*B. pertussis*)	13	**17.3**	45	9
NMA1994 to NMA1996	TolC and Hly4; possible hemolysin secretion system (*Escherichia coli*)	3	**3.0**	54	1
Metabolic					
NMA0028 to NMA0033	Amino acid metabolism and transposase	6	**5.6**	55	1
NMA1820 (to NMA1884)	GpxA; glutathione peroxidase[e]	1 (53)	0.5 (**40**)	48 (53)	9
Others					
NMA0093	Probable integral membrane protein	1	0.8	54	
NMA1636 to NMA1637	No homology	2	1.1	53	
NMA1988 to NMA1989	Possible integral membrane proteins	2	1.7	34	
NMA2035	Conserved hypothetical protein	1	0.8	35	
Insertion sequences; bacteriophages					
NMA1814	Insertion element IS*1655* transposase	1	1.5	51	
Pathogen specific					
Virulence associated					
NMA0609	PilC; pilin-associated adhesin[f]	1/2 (3′)	1.5	49	4
NMA0905	Immunoglobulin A1 protease	1	**5.3**	47	3
NMA1925	HmbR; hemoglobin receptor	2	**3.2**	52	
Possible virulence associated					
NMA0478	Possible outer membrane peptidase; Ssa1 (*Pasteurella haemolytica*)[f]	1/2 (5′)	1.7	60	2
NMA0575 to NMA0578	Probable ferric siderophore receptors; ORF without homology	2	**5.7**	61	
NMA1642	PorA; class I outer membrane porin[g]	1	1.2	52	2
NMA1676	Variant of opacity protein	1	0.6	48	
NMA1725	Possible virulence-associated protein; VirG (*Shigella flexneri*)	1	1.9	41	1
NMA1900	Possible hemolysin Hly3 (*Bacillus cereus*)	1	0.6	51	1
Metabolic					
NMA1255	Ggt; probable gamma-glutamyltranspeptidase[g]	1	1.8	47	
NMA2011, NMA2013	BioF; biotin synthesis	2	1.8	42, 44, 51	
NMA2015 to NMA2017	SpeA and -B; polyamine metabolism	3	**4.5**		4
NMA2050, NMA2052, and NMA2054 to NMA2055	AckA2, acetate kinase, and AcnA, aconitase; PrpC, citrate synthase, and PrpB, possible carboxyphosphoenolpyruvate phosphomutase	4	**4.6**		3
Others					
NMA0020 to NMA0021	Probable integral membrane protein	2	**2.1**	48	
NMA0091	Probable amino acid transporter	1	1.4	54	
NMA0109 to NMA0114	Preprotein translocase; ribosomal proteins	6	**3.4**	37, 42	
NMA0305A	Probable transposase	1	0.6	47	
NMA0431	Possible inner membrane protein	1	1.3	49	
NMA0586	Possible lipoprotein	1	0.8	54	
NMA0668 to NMA0670	Two-component sensor/kinase and transporter pseudogene	1	**3.0**	44	1
NMA0678 to NMA0683	Possible lipoprotein, secretion protein pseudogene, and ORFs without homology	5	**3.0**	44	1
NMA0700	Possible ribonuclease	1	1.2	57	
NMA1101 to NMA1102	Possible integral membrane protein and ORF without homology	2	1.0	43	
NMA1777	Possible integral membrane protein	1	0.9	54	
NMA1797 to NMA1799	TspB; *Neisseria*-specific antigen and other ORFs without homology	3	**3.4**	47	2
NMA2069	Probable integral membrane protein	1	1.6	48	
Insertion sequences; bacteriophages					
NMA1167 to NMA1171	Possible phage-related proteins	5	1.6	45	
NMA1212	Transposase	1	1.1	44	
NMA1600	Possible transposase	1	0.4	37	

Continued on following page

TABLE 2—*Continued*

Gene(s)[b]	Description	No. of ORFs	No. of kb specific[c]	% GC	No. of subtractive clones[d]
No homology					
NMA0171A to NMA0173	No homology	3	1.1	37	
NMA0565 to NMA0568	No homology	3	1.1	46	
NMA1067 to NMA1068	No homology	2	1.0	33	
NMA1218 to NMA1220	No homology	3	0.8	53	
NMA1424	No homology	1	1	52	
NMA1438	No homology	1	1.4	52	
NMA1913	No homology	1	0.8	48	
NMA1997 to NMA1999	No homology	2	1.5	46	
Common to *N. meningitidis* and *N. lectamica*					
NMA1035 to NMA1036	Restriction modification system	2	**2.1**	33	1
NMA1200	Probable surface fibril protein; Hsf	1	1.8	49	
NMA1283 to NMA1286	Phage-related protein	4	**3.4**	51	
NMA1365 to NMA1366	Sulfate metabolism	2	**2.5**	56	

[a] Comparisons were made between the genetic complement of the eight virulent meningococcal strains and three *N. gonorrhoeae* and two *N. lactamica* strains. The presence or absence of amplicons in a test strain was translated into that of the corresponding genes, based on the coordinates (base numbers) of the amplicons and genes on the meningococcal chromosome (Z2491). Genes corresponding to one or more amplicons absent in the test strain were considered to be absent; genes which overlapped two amplicons one of which was present and one absent could not be designated clearly and are not tabulated.

[b] ORF nomenclature of Parkhill and colleagues (24).

[c] Regions over 2 kb are in boldface type.

[d] Regions previously brought to light by subtractive hybridization methods and the number of corresponding subtractive clones (1).

[e] This region is a 40-kb bacteriophage in group A subgroup IV strains (17, 24).

[f] The genes *pilC* and *ssa1* are both represented by two amplicons which divide them into a 5′ half and a 3′ half.

[g] Both PorA and Ggt, previously described as *N. meningitidis*-specific attributes, are represented as pseudogenes in *N. gonorrhoeae* FA1090.

pathogenesis: those for the immunoglobulin A protease (13), the PilC adhesin (20, 26), and the hemoglobin receptor (31). As expected, there were also a more limited number of sequences which were shared between *N. meningitidis* and *N. lactamica*. Since these two species inhabit the same anatomical site, some of these genes (e.g., that for the fibrilar protein NMA1200) may play a role in the initial colonization of the nasopharyngeal mucosa.

These data demonstrate that, despite the dramatically different pathogenic potentials of the bacteria within the genus *Neisseria*, the chromosomal differences which could be responsible for these differences remain small. In addition the small sizes of these differential regions are in contrast with what is observed in other closely related bacteria expressing different pathogenicities, in which large pathogen-specific chromosomal regions, or pathogenicity islands, between 20 and 200 kb in extent (10) are a characteristic feature.

DISCUSSION

We have compared the genomic contents of three species of the genus *Neisseria:* the meningococcus *N. meningitidis*, the gonococcus *N. gonorrhoeae*, and a commensal of the nasopharynx, *N. lactamica*. Our analysis demonstrates the presence of species- and strain-specific differences corresponding to sequences of about 1 to 40 kb of chromosomal DNA. The association of these sequences with a range of virulent meningococcal, or gonococcal, isolates of diverse epidemiological groups isolated at different times in different countries suggests that the products of the species-specific genes play roles in the different lifestyles of the bacteria.

Interpreting the roles of these sequences in light of the differences in pathogenesis would imply that those present only in virulent meningococci are responsible for specific aspects of meningococcal pathogenesis. It is therefore possible that a specific interaction of *N. meningitidis* with the blood-brain barrier is mediated by one of the meningococcus-specific genes, and in fact, some of these genes show homology to known bacterial virulence factors, e.g., NMA0688, a filamentous hemagglutinin homologue (Table 2). However, to date, no phenotype has been associated with this gene (17), underlining our incomplete understanding of meningococcal genetics and physiology. Indeed, all of the larger *N. meningitidis*-specific regions have previously been investigated, and only two (the capsule locus [32] and the DsbA region [17]) have been implicated in pathogenesis. Mutations in these regions produce defects associated with the level of bacteremia in an infant rat model, the most dramatic effect being seen after deletion of the capsule locus. However, to invade the meninges from the bloodstream, *N. meningitidis* must cross the blood-brain barrier, probably by a transcellular route through brain endothelial cells. Surprisingly, none of the meningococcus-specific sequences have been shown to be involved in the interaction of the bacteria with endothelial cells. Though the interactions of *Neisseria* with human cellular barriers are complex processes, and present models of the process may not reveal some of their more subtle facets, these data suggest that the specificity of meningococcal pathogenesis depends on the ability of the bacteria to survive in the bloodstream, as has been shown for another cause of meningitis, *Haemophilus influenzae* (22). Moreover, this is in agreement with studies of human susceptibility to meningococcal infection (7), which demonstrate a correlation between serum bactericidal activity and resistance to disease. The ability to adhere to and invade endothelial cells is a property of both *N. meningitidis* and *N. gonorrhoeae*,

whereas *N. lactamica* interacts inefficiently with human cells, does not invade, and induces no intracellular cytoskeletal rearrangements as do the pathogenic species. This suggests that in *N. meningitidis* the genes important for the crossing of the blood-brain barrier are shared with *N. gonorrhoeae* but absent from *N. lactamica*. In support of this hypothesis, the only gene which has so far been associated with the crossing of the blood-brain barrier in vivo is that for PilC1 (26). This protein, which transforms the type IV pili into an adhesive structure, is found in both of the pathogenic *Neisseria* species but not in *N. lactamica*.

Usually, the pathogenesis of bacteria belonging to related species and responsible for different diseases is determined by large (20- to 200-kb), horizontally acquired pathogenicity islands (10) inserted into the core genome which may specify successive steps in infection (8). Our comparative genomic analysis did not reveal any such pathogenicity islands responsible for the dramatic difference in pathogenesis between *N. meningitidis* and the other closely related members of the genus, but rather sequences of relatively small extent scattered about the genome. Physical explanations (the size of transforming DNA [21] and frequent genomic rearrangements) may not be sufficient to explain this difference, since large islands (NMA1820 to NMA1884; apparently a prophage [Table 2]) do exist, and cotranscribed or coregulated genes (e.g., the capsular gene cluster) would tend to remain physically linked (18). The situation may be analyzed in terms of the lifestyles of the bacteria, and in this regard it is notable that the usual meningococcus-host interaction is one of asymptomatic carriage. Meningococcal infection is a deadly disease which moreover does not favor transmission. In this light, a pathogenicity island would provide no selective advantage to its host meningococcus, accounting for the absence of such large, complex islands. This genomic organization therefore strongly supports the idea that *N. meningitidis* is essentially a commensal species. Besides the anatomical site, the main difference between *N. meningitidis* and *N. gonorrhoeae* is the mode of transmission. *N. gonorrhoeae* is transmitted by direct contact, whereas *N. meningitidis* is spread from person to person by respiratory droplets, and some of the meningococcus-specific sequences presumably serve to optimize this mode of transmission. In addition, the meningococcal sequences important for interaction with the blood-brain barrier are likely to have been initially selected for to promote interaction with the nasopharyngeal cells, thus leading to the asymptomatic carriage which also involves an intracellular lifestyle (29). Meningococcal pathogenesis may therefore result from the expression of sequences necessary for bacterial transmission and pharyngeal colonization.

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