A numerical phenotypic taxonomic study of the genus *Neisseria*

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A numerical phenotypic taxonomic study of 315 strains of *Neisseria* and some allied bacteria examined for 155 phenotypic tests showed 31 groups, most of which were reasonably distinct. These fell into four major areas. Areas A, B and C contained species of *Neisseria*, whereas area D contained the organisms known as 'false neisserias' together with *Branhamella*, *Moraxella* and *Kingella* species. Area A contained *N. gonorrhoeae* (which showed two subgroups), *N. meningitidis* (with two subgroups, and *N. cinerea* closely associated), *N. polysaccharea*, *N. elongata* subsp. *glycolytica* and *N. lactamica*. Area B contained mainly organisms from the human nasopharynx, and the nine groups were not very distinct: only three, *N. mucosa*, *N. perflava* and *N. sicca* could be recognized by the presence of type strains, and there was little relationship between taxonomic position and species epithets. Area C contained several groups from animals, *N. animalis*, *N. canis* and two phenons that may be justified as new species of *Neisseria*, one from lizards and the other from dental plaque of herbivores. Area C also contained *N. elongata*, *N. subflava* (with *N. flavescens*), type strain of *Morococcus cerebrosis* and the CDC groups M-5 (*N. weaveri*) and EF-4. Area D contained *Branhamella catarrhalis*, a combined group which consists of strains of the 'false neisserias' *N. caviae* and *N. cuniculi*, the 'false neisseria' *N. ovis*, and a group of *Moraxella* strains. A small group representing *Kingella kingae* is included in area D. Mean test error was 1.7%.

Keywords: *Neisseria*, numerical taxonomy, phenotypic taxonomy

INTRODUCTION

The genus *Neisseria* contains two important human pathogens, the meningococcus and the gonococcus, and some other species of significance in man and other animals. The taxonomy of the genus has been difficult to study because of the fastidious growth requirements of these organisms, the ease with which they die out or become contaminated in culture, and the high phenotypic similarity between species. There are relatively few phenotypic tests in the literature that clearly distinguish between species, and data on nucleic acids are available for only a few strains. Many strains in culture collections bear names whose application is uncertain, particularly those that were isolated from the human nasopharynx. Confusion has arisen, for example, from the grouping (Reyn, 1974) of saccharolytic strains previously classified (Branham & Pelczar, 1957) as *Neisseria perflava*, *Neisseria flava* and *Neisseria subflava* under the single species *Neisseria*

subflava. Furthermore, the commonly used name '*Neisseria pharyngis*' (Wilson & Smith, 1928), which is not validly published, has also been applied to partially characterized strains of these and other saccharolytic species from the nasopharynx.

The genus is closely allied to *Moraxella* and *Branhamella*, and there is a very similar group called the 'false neisserias': present knowledge is summarized by Bøvre (1984a, b), Vedros (1984) and Morse & Knapp (1992). A preliminary investigation (Le Monnier *et al.*, 1987) with rapid enzyme tests has been performed in our laboratory, and we here present our subsequent and wider findings on all these groups. Names that are not validly published are placed in inverted commas and the biovars of *N. subflava* are named (wherever possible) according to the classification of Branham & Pelczar (1957).

METHODS

Strains. A combination of partly identified and well characterized *Neisseria* and related species were received (listed in

Abbreviations: a, arylamidase reaction; FGN, fastidious Gram-negative; OTU, operational taxonomic unit.

Table 1. Strains studied and their clustering (using the S_G coefficient) in the same order as Fig. 1

Strain no.	Name as received	Sender and collection no.*	Comments*
Phenon 1: <i>Neisseria gonorrhoeae</i> a			
A9	<i>N. gonorrhoeae</i>	NCTC 11422	Urethra
A20	<i>N. gonorrhoeae</i>	LRI 287	Male urethra
A13	<i>N. gonorrhoeae</i>	LRI 261	Male urethra
A22	<i>N. gonorrhoeae</i>	LRI 273	Female urethra or cervix
A21	<i>N. gonorrhoeae</i>	LRI 289	Female urethra or cervix
A15	<i>N. gonorrhoeae</i>	LRI 292	Female urethra
A39	<i>N. gonorrhoeae</i>	NCTC 11148	Vagina
A28	<i>N. gonorrhoeae</i>	LRI 275	Female urethra or cervix
A36	<i>N. gonorrhoeae</i>	LRI 254	Male urethra, CEN
A59	<i>N. gonorrhoeae</i>	NCTC 8375 ^T	SNR, ATCC 19424
A59a	<i>N. gonorrhoeae</i>	NCTC 8375 ^T	Replicate of A59
A75	<i>N. gonorrhoeae</i>	LRI 1365	Female throat
A35	<i>N. gonorrhoeae</i>	LRI 253	Cervix
A37	<i>N. gonorrhoeae</i>	NCTC 11421	Urethra
A68	<i>N. gonorrhoeae</i>	LRI 2051	Female urethra
A29	<i>N. gonorrhoeae</i>	LRI 255	SNR
Phenon 2: <i>N. gonorrhoeae</i> b			
A11	<i>N. gonorrhoeae</i>	NCTC 10930	Cervix, ATCC 27630
A18	<i>N. gonorrhoeae</i>	LRI 288	Male urethra
A64	<i>N. gonorrhoeae</i>	LRI 261	Urine
A12	<i>N. gonorrhoeae</i>	LRI 291	Female urethra or cervix
A25	<i>N. gonorrhoeae</i>	LRI 293	Male urethra, CEN
A31	<i>N. gonorrhoeae</i>	LRI 3433	Cervix
A34	<i>N. gonorrhoeae</i>	LRI 3444	Male urethra
A74	<i>N. gonorrhoeae</i>	LRI 1208	Cervix
Satellites to phenons 1 and 2:			
A24	<i>N. gonorrhoeae</i>	LRI 276	Female urethra or cervix
A40	<i>N. gonorrhoeae</i>	NCTC 10022	Urethra
A50	' <i>N. gonorrhoeae</i> var. <i>kochii</i> '	Brooks 7	Conjunctiva, Egypt
Phenon 3: <i>Neisseria meningitidis</i> a			
A38	<i>N. gonorrhoeae</i>	LRI 1603	Male throat, later identified as <i>N. meningitidis</i>
B32	<i>N. meningitidis</i>	CCUG 11904	Pleural fluid, serovar B, asaccharolytic
B34	<i>N. meningitidis</i>	NCTC 8554	CSF, serovar C, Branham 1628, ATCC 13102
B35	<i>N. meningitidis</i>	LGH AD1	Throat
B57	<i>N. meningitidis</i>	HNCMB 122007	SNR, serovar Y, IEM NM19/68
B5	<i>N. meningitidis</i>	Ison 98	Throat, male homosexual
B25	<i>N. meningitidis</i>	NCTC 9714	CSF, Branham M158, ATCC 13113, serovar D
B28	<i>N. meningitidis</i>	LGH AD22	Throat, CEN
B30	<i>N. meningitidis</i>	LGH AD15	Throat
B19	<i>N. meningitidis</i>	LGH AD20	Throat
B20	<i>N. meningitidis</i>	LRI 1734	Female throat
B24	<i>N. meningitidis</i>	LRI 1773	Throat
B12	<i>N. meningitidis</i>	Ison 218	Throat, male homosexual
B14	<i>N. meningitidis</i>	Ison 170	Throat, male homosexual
B49	<i>N. meningitidis</i>	NHITCC770/376	SNR
B6	<i>N. meningitidis</i>	Ison 338	Throat, male homosexual
B21	<i>N. meningitidis</i>	LRI 1732	Female throat
B13	<i>N. meningitidis</i>	Ison 201	Throat, male homosexual
O11	<i>N. pharyngis</i>	LGH AD49	Throat, G455
B9	<i>N. meningitidis</i>	Ison 84842	Throat, male homosexual
B22	<i>N. meningitidis</i>	LRI 1703	Male throat
B16	<i>N. meningitidis</i>	NCTC 3372	SNR, serovar A
B11	<i>N. meningitidis</i>	Ison 222	Throat, male homosexual

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
B27	<i>N. meningitidis</i>	LGH AD4	Throat
B36	<i>N. meningitidis</i>	NCTC 3375	SNR, serovar A
B18	<i>N. meningitidis</i>	NCTC 10025 ^T	CSF, serovar A, Branham M1027, ATCC 13077
B18a	<i>N. meningitidis</i>	NCTC 10025 ^T	Replicate of B18
B41	<i>N. meningitidis</i>	Roberts M0942	SNR, serovar B type 15, Stroud M0942
B43	<i>N. meningitidis</i>	IID 850	B18 from different sender
B51	<i>N. meningitidis</i>	INS MA2511	SNR, serovar X
Phenon 4: <i>Neisseria cinerea</i>			
F1	<i>N. cinerea</i>	NCTC 10294 ^T	Nasopharynx, ATCC 14685, Berger 194, CEN
F1a	<i>N. cinerea</i>	NCTC 10294 ^T	Replicate of F1
F3	<i>N. cinerea</i>	LCDC 81-176	SNR
F10	<i>N. cinerea</i>	Vedros M601	Nasopharynx
Phenon 5: <i>Neisseria meningitidis</i> b			
B15	<i>N. meningitidis</i>	NCTC 8566	CSF meningitis, serovar C
B44	<i>N. meningitidis</i>	IID 854	SNR, serovar A, CIP 6764, CEN
B47	<i>N. meningitidis</i>	CN 8250	SNR, serovar X
B46	<i>N. meningitidis</i>	CN 8244	SNR, serovar 29E
B56	<i>N. meningitidis</i>	HNCMB 122006	SNR, serovar X
Satellite to Phenons 3–5			
B29	<i>N. meningitidis</i>	NCTC 8555	SNR, serovar C, Branham 2104
Satellite to Phenon 6			
B23	<i>N. meningitidis</i>	LRI 1777	Female throat
Phenon 6: <i>Neisseria polysaccharea</i>			
P1	<i>N. polysaccharea</i>	INS MA3008	Nasopharynx, CEN
P4	<i>N. polysaccharea</i>	CCUG 18031	Throat, child, Berger 25862
P7	<i>N. polysaccharea</i>	CCUG 24845	SNR, Riou N6817
P8	<i>N. polysaccharea</i>	CCUG 24846	SNR, Riou N6665
P6	<i>N. polysaccharea</i>	CCUG 18030 ^T	Replicate of P5
P5	<i>N. polysaccharea</i>	CCUG 18030 ^T	Throat, NCTC 11858, CIP 100113
P5a	<i>N. polysaccharea</i>	CCUG 18030 ^T	Replicate of P5
Satellites to Phenons 1–6			
B17	<i>N. meningitidis</i>	NCTC 8249	CSF, infant, serovar B
F6	<i>N. cinerea</i>	LCDC 86-368	SNR
F7	<i>N. cinerea</i>	CCUG 5746	Conjunctiva, infant
M7	<i>N. mucosa</i>	LCDC 85402	SNR
L21	<i>N. lactamica</i>	CCUG 17628	Nasopharynx
Phenon 7: <i>N. elongata</i> subsp. <i>glycolytica</i>			
I8	<i>N. elongata</i>	CCUG 4165A	Blood culture, Berger MOR 23, Bøvre 24
J2	<i>N. elongata</i> var. <i>glycolytica</i>	NCTC 11050 ^T	Pharyngitis, Henriksen 6171/75, ATCC 29315, CIP 82.85, CEN
J2a	<i>N. elongata</i> var. <i>glycolytica</i>	NCTC 11050 ^T	Replicate of J2, CEN
Satellite to phenons 1–7			
B45	<i>N. meningitidis</i>	IID 855	SNR, serovar C, CIP 52142
Phenon 8: <i>Neisseria lactamica</i>			
F2	<i>N. cinerea</i>	Bangor 13	Sputum
L19	<i>N. lactamica</i>	LCDC 845	SNR
L17	<i>N. lactamica</i>	LCDC 77-143	SNR
L11	<i>N. lactamica</i>	Ison 144	Throat, male homosexual
L12	<i>N. lactamica</i>	NCTC 10617 ^T	Nasopharynx, Hollis A7515, ATCC 23970
L12a	<i>N. lactamica</i>	NCTC 10617 ^T	Replicate of L12
L20	<i>N. lactamica</i>	CCUG 7852	Throat, CEN
L16	<i>N. lactamica</i>	INS MA1110	Nasopharynx
L18	<i>N. lactamica</i>	LCDC 80-111	SNR

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
L22	<i>N. lactamica</i>	CCUG 7757	Nasopharynx, child
L10	<i>N. lactamica</i>	Ison 132	Throat, male homosexual
L13	<i>N. lactamica</i>	NCTC 10618	Throat, Hollis A5906, ATCC 23971
Satellites of phenons 9-11			
B7	<i>N. meningitidis</i>	Ison 388	Throat, male homosexual
Z8	<i>Neisseria</i> sp.	CN 2457	SNR
Phenon 9: <i>Neisseria mucosa</i>			
M2	<i>N. mucosa</i>	Bangor 14	Sputum
M5	<i>N. mucosa</i>	Bangor 17	Sputum
M13	<i>N. mucosa</i>	Vedros M1801	Human, site not recorded
M3	<i>N. mucosa</i>	Bangor 15	Sputum
U23	<i>N. subflava</i>	CCUG 801	SNR, Berger 208
Q3	<i>N. sicca</i>	D226	SNR (teaching collection of strains), CEN
Q12	<i>N. sicca</i>	LCDC 89667	SNR
M15a	<i>N. mucosa</i>	CIP 59.51 ^T	Sputum, chronic bronchitis, replicate of M15
N4	' <i>N. mucosa</i> var. <i>heidelbergensis</i> '	NCTC 10776	Pharynx, Berger M1, ATCC 25998
Z12	<i>Neisseria</i> sp.	Hodge	Vagina, ATCC 43831, meningococcal biochemistry, gonococcal serology
Q13	<i>N. sicca</i>	LCDC R9742	SNR
Q14	<i>N. sicca</i>	LCDC R9888	SNR
Q10	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
W1	<i>N. macacae</i>	ATCC 33926 ^T	Oropharynx, rhesus monkey (<i>Macacus mulatta</i>), Vedros M-740
Phenon 10: <i>Neisseria perflava</i>			
Q2	<i>N. sicca</i>	Bristol SB2391	SNR
U41	<i>N. flava</i>	Bangor 10	Sputum
Q5	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
U15	<i>N. perflava</i>	CCUG 17915 ^T	SNR, Branham 7078, ATCC 10555
U15a	<i>N. perflava</i>	CCUG 17915 ^T	Replicate of U15
Q20	<i>N. sicca</i>	CCUG 431	SNR, Pelczar 1485
U32	<i>N. perflava</i>	NRL 36393	SNR, Berger 1a
U36	<i>N. perflava</i>	NRL 36390	SNR, Berger 10b
U34	<i>N. perflava</i>	NRL 36400	SNR, Berger 7b
U35	<i>N. perflava</i>	NRL 36392	SNR, Berger 9a
U39	<i>N. flava</i>	Bangor 8	Sputum, CEN
U12	<i>N. perflava</i>	INS MA1661	SNR
U10	<i>N. subflava</i>	NHITCC 843	SNR, CDC KC645
U21	<i>N. perflava</i>	CCUG 7826	Human urine, catalase negative
U26	<i>N. subflava</i>	IID 857	SNR, ATCC 11076
U28	<i>N. subflava</i>	Vedros M2085	CSF, human meningitis
Phenon 11: Pharyngeal 1			
O19	<i>N. pharyngis</i>	MUCOB 49	SNR
Q9	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
Q6	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
U14	<i>N. flava</i>	CCUG 4788	SNR, Berger H103, CEN
U22	<i>N. flava</i>	CCUG 4789	SNR, Berger 10d
Q11	<i>N. sicca</i> or <i>perflava</i>	Bangor	SNR
Q31	<i>N. sicca</i>	INS	SNR, Abrams AMC 14-3-1, ATCC 9913
Phenon 12: Pharyngeal 2			
M4	<i>N. mucosa</i>	Bangor 16	Sputum
O5	<i>N. pharyngis</i>	LGH AD60	Throat
Q25	<i>N. sicca</i>	NRL 36394	SNR, Berger 616
O14	<i>Neisseria</i> sp.		Isolated from a mixed culture
Q21	<i>N. sicca</i>	CN 3212	SNR

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
Q15	<i>N. sicca</i>	LCDC 81177	SNR
Q16	<i>N. sicca</i>	NHITCC 841	SNR, CDC C620, CEN
U42	<i>N. flava</i>	Bangor 11	Sputum
U1	<i>N. perflava</i>	LCDC 176	SNR
V12	<i>Neisseria</i> sp.	Dent TR/748	Dental plaque, tamarin (<i>Saguinus illigeri</i>)
Satellites of Phenons 9–12			
Q8	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
W1a	<i>N. macacae</i>	ATCC 33926 ^T	Replicate of W1
Phenon 13: <i>Neisseria sicca</i>			
M1	<i>N. mucosa</i>	NCTC 10774	Pharynx, Berger M26, ATCC 25996
M9	<i>N. mucosa</i>	CCUG 805	SNR, Berger M5
M10	<i>N. mucosa</i>	CCUG 804	SNR, Berger M33
M14	<i>N. mucosa</i>	Vedros M1066	Blowhole of dolphin
M16	<i>N. mucosa</i>	CCUG 17327	Wound, child, CEN
N1	' <i>N. mucosa</i> var. <i>heidelbergensis</i> '	NCTC 10777	Pharynx, Berger M3, ATCC 25999
N2	' <i>N. mucosa</i> var. <i>heidelbergensis</i> '	CCUG 10421	Pleural fluid
Q1	<i>N. sicca</i>	Bristol SB2391	SNR
Q29	<i>N. sicca</i>	NRL 30016 ^T	Pharynx, Berger 6b, ATCC 49276
Q29a	<i>N. sicca</i>	NRL 30016 ^T	Replicate of Q29
N3	' <i>N. mucosa</i> var. <i>heidelbergensis</i> '	CCUG 12390	Horse, site not recorded
Phenon 14: Pharyngeal 3			
Q26	<i>N. sicca</i>	NRL 36397	SNR, Berger 3a, CEN
Q27	<i>N. sicca</i>	NRL 36398	SNR, Berger 11
Q28	<i>N. sicca</i>	NRL 36395	SNR, Berger 8b
Phenon 15: Pharyngeal 4			
O2	<i>N. pharyngis</i>	Bristol SB2384	SNR
O4	<i>N. pharyngis</i> var. <i>flava</i>	NCTC 4590	Throat, Flemming 1
O10	<i>N. pharyngis</i>	LRI 1697	Sputum, CEN
U24	<i>N. subflava</i>	CCUG 800	SNR, Berger 158
O9	<i>N. pharyngis</i>	LRI 1783	Male conjunctiva
Phenon 16: Pharyngeal 5			
Q19	<i>N. sicca</i>	Surrey 321	Nose of dog
S13	<i>Branhamella catarrhalis</i>	LCDC 81016	SNR, CEN
S10	<i>Branhamella catarrhalis</i>	LGH 10543	Sputum
Phenon 17: Pharyngeal 6			
Q4	<i>N. sicca</i>	D926	SNR (teaching collection of strains), CEN
Q17	<i>N. sicca</i> or <i>perflava</i>	LCDC 82292	SNR
U11	<i>N. sicca</i> or <i>perflava</i>	LCDC 84193	SNR
Phenon 18: <i>Neisseria animalis</i>			
C1	<i>N. animalis</i>	NCTC 10212 ^T	Guinea pig nasopharynx, Berger Na10, CEN
C3	<i>N. animalis</i>	CIP 72.15	SNR, Pelczar Na1, ATCC 19573
C1a	<i>N. animalis</i>	NCTC 10212 ^T	Replicate of C1
V30	<i>Neisseria</i> sp.	Dent SH8/4084	Cow, dental plaque
V32	<i>Neisseria</i> sp.	Dent SH13/4494	Cow, dental plaque
C2	<i>N. animalis</i>	CCUG 809	SNR, Berger Na11
V2	<i>Neisseria</i> sp.	Dent OU/3301	Orang utan, dental plaque
H2	<i>N. denitrificans</i>	CCUG 17229	Gander, penis, API 110.09.84,557/1, 'atypical'
V5	<i>Neisseria</i> sp.	Dent GK/115	Dental plaque, Kangaroo (<i>Macropus kangaru</i>)
H1a	<i>N. denitrificans</i>	NCTC 10295 ^T	Replicate of H1 (see satellites of phenons 1–26)
V23	<i>Neisseria</i> sp.	Dent SH10/4328	Cow, dental plaque
V26	<i>Neisseria</i> sp.	Dent SH4/4008	Cow, dental plaque
Satellite to phenon 18			
X4	<i>Moraxella urethralis</i>	NHITCC SB81/0059	SNR

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
Phenon 19: <i>Neisseria canis</i>			
D1	<i>N. canis</i>	NCTC 10296 ^T	Dog mouth, Berger H6, ATCC 14687
D1a	<i>N. canis</i>	NCTC 10296 ^T	Replicate of D1
D2	<i>N. canis</i>	Vedros M637	Human, cat bite, CEN
G3	' <i>N. cuniculi</i> var. <i>gigantea</i> '	NRC 31008	Dog mouth, Berger H9, ATCC 14689; of uncertain origin: see Results
O13	<i>Neisseria</i> sp.	CCUG 14292	Human, dog bite, Hagen 802, CDC C2829
O17	<i>Neisseria</i> sp.	CCUG 13910B	Bite (no further details), API 107-6-82
Z5	<i>Neisseria</i> sp.	Surrey 334	Dog, site not recorded
V6	<i>Neisseria</i> sp.	Dent CA1/247	Cat, dental plaque
V22	<i>Neisseria</i> sp.	Dent PM3/2183	Patas monkey, dental plaque
V7	<i>Neisseria</i> sp.	Dent JRC/962	Cat, dental plaque
V17	<i>Neisseria</i> sp.	Dent PM5/3023	Patas monkey, dental plaque
Phenon 20: <i>Neisseria elongata</i>			
I1	<i>N. elongata</i>	NCTC 10660 ^T	Pharynx, Holten M2, ATCC 25295, CIP 72.27
I1a	<i>N. elongata</i>	NCTC 10660 ^T	Replicate of I1
U40	<i>N. flava</i>	Bangor 9	Sputum
I2	<i>N. elongata</i>	NHITCC 2376	SNR, New Zealand isolate, SB79/0263, CDC group M-6 (confirmed by CDC), CEN
I4	<i>N. elongata</i>	CCUG 2131	Pharynx, Bøvre 8553/4
I3	' <i>N. elongata</i> var. <i>intermedia</i> '	CCUG 4557	Pharynx, Berger 180
I5	' <i>N. elongata</i> var. <i>intermedia</i> '	CCUG 4554	Pharynx, Berger 156
Phenon 21: <i>Neisseria subflava</i>			
K1	<i>N. flavescens</i>	NCTC 8263 ^T	CSF meningitis, Branham N155, ATCC 13120
K3	<i>N. flavescens</i>	NCTC 3191	CSF meningitis, Branham N156, ATCC 13119
U37	<i>N. subflava</i>	NRL 30017 ^T	SNR, ATCC 49277
U31	<i>N. perflava</i>	NRC 31001	SNR
U37a	<i>N. subflava</i>	NRL 30017 ^T	Replicate of U37
K2	<i>N. flavescens</i>	NCTC 3656	SNR, Branham 409, CEN
K9	<i>N. flavescens</i>	IID 592 ^T	K1 from a different sender
K9a	<i>N. flavescens</i>	IID 592 ^T	Replicate of K9
K8	<i>N. flavescens</i>	LCDC 81189	SNR
K11	<i>N. flavescens</i>	NRC 31011 ^T	K1 from a different sender
K12	<i>N. flavescens</i>	CCUG 806	CSF, Branham N159, ATCC 13117
Satellites of Phenons 18–21			
V19	<i>Neisseria</i> sp.	Dent PM4/2219	Patas monkey, dental plaque
V21	<i>Neisseria</i> sp.	Dent PM4/2231	Patas monkey, dental plaque
Satellites of Phenons 9–21			
U6	<i>N. flava</i>	LCDC 83115	SNR
O12	<i>N. pharyngis</i>	LRI 1677	Sputum
Phenon 22: Iguana			
R1	<i>Neisseria</i> sp.	NVSL 85297	Common (green) iguana (<i>Iguana iguana</i>), tail abscess
R7	<i>Neisseria</i> sp.	NVSL 85728	Rhinoceros iguana (<i>Cyclura cornuta</i>), liver granuloma
R2	<i>Neisseria</i> sp.	NVSL 85738	Common iguana (<i>Iguana iguana</i>), tail abscess
R6	<i>Neisseria</i> sp.	NVSL 85737	Common iguana (<i>Iguana iguana</i>), tail abscess, CEN
R3	<i>Neisseria</i> sp.	NVSL 85739	Common iguana (<i>Iguana iguana</i>), tail abscess
Phenon 23: Cow 1			
V18	<i>Neisseria</i> sp.	Dent SH4/3992	Cow, dental plaque
V20	<i>Neisseria</i> sp.	Dent SH11/4359	Cow, dental plaque
V34	<i>Neisseria</i> sp.	Dent SH8/4231	Cow, dental plaque
V25	<i>Neisseria</i> sp.	Dent SH12/4390	Cow, dental plaque, CEN
V28	<i>Neisseria</i> sp.	Dent SH12/4399	Cow, dental plaque
V29	<i>Neisseria</i> sp.	Dent SH13/4484	Cow, dental plaque
V33	<i>Neisseria</i> sp.	Dent SH1/3848	Cow, dental plaque

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
Phenon 24: Cow 2			
V10	<i>Neisseria</i> sp.	Dent GG1/2481	Gorilla (<i>Gorilla gorilla</i>), dental plaque, CEN
V35	<i>Neisseria</i> sp.	Dent SH7/4188	Cow, dental plaque
V37	<i>Neisseria</i> sp.	Dent SH12/4390	Replicate of V25
V36	<i>Neisseria</i> sp.	Dent SH12/4394	Cow, dental plaque
Satellite of Phenons 9–24			
Z11	<i>Morococcus cerebrosus</i>	NCTC 11393 ^T	Brain abscess
Phenon 25: 'Mucosa-flava'			
M6	<i>N. mucosa</i>	NHITCC 842	SNR, CDC C2186
M15	<i>N. mucosa</i>	CIP 59.51 ^T	Sputum, chronic bronchitis, Veron N16, ATCC 19696
U4	<i>N. flava</i>	LCDC 81187	SNR
U9	<i>N. flava</i>	NHITCC 844	SNR, CDC KC808, CEN
Satellite of Phenons 9–25			
Q7	<i>N. sicca</i> or <i>perflava</i>	Bangor	Sputum
Phenon 26: M-5, EF-4			
I10	' <i>N. parelongata</i> '	NHITCC 2759	SNR, SB81/0708, CDC group M-5 (confirmed by CDC)
I11	' <i>N. parelongata</i> '	CCUG 4007	Dog ear, CCUG reference strain of ' <i>N. parelongata</i> '
I15	' <i>N. parelongata</i> '	CCUG 9689	Dog mouth, Weaver B1674, CDC group M-5, CEN
I14	' <i>N. parelongata</i> '	CCUG 9693	Replicate of I13
I13	' <i>N. parelongata</i> '	CCUG 9693	Dog, upper respiratory tract, Bailie 712, CDC group M-5?
I17	CDC group EF4a	CCUG 1976	SNR
I18	CDC group EF4a	CCUG 12416	SNR, CDC D8251
O16	' <i>N. parelongata</i> '	CCUG 14293	Dog, site not recorded, Hagen 807, CDC C7866
Satellites to Phenons 1–26			
H1	<i>N. denitrificans</i>	NCTC 10295 ^T	Guinea pig mouth, Berger M37, ATCC14686, CIP 72.16
V13	<i>Neisseria</i> sp.	Dent LP/2082	Lesser red panda (<i>Ailurus fulgens</i>), dental plaque
L5	<i>N. lactamica</i>	Bristol SB2341	SNR
S8	<i>Branhamella catarrhalis</i>	LCDC 81195	SNR
Phenon 27: <i>Branhamella catarrhalis</i>			
A33	<i>Neisseria gonorrhoeae</i>	LRI 1608	Female throat, later identified as <i>B. catarrhalis</i>
O6	<i>N. pharyngis</i>	LRI 1794	Male conjunctiva
S1	<i>B. catarrhalis</i>	LGH AD13	Sputum, 1538
S5	<i>B. catarrhalis</i>	NCTC 3625	Throat, Fleming 3
S6	<i>B. catarrhalis</i>	LGH AD64	Conjunctiva, 1433/6
S11	<i>B. catarrhalis</i>	NCTC 3622	Throat, Fleming 0
S14	<i>B. catarrhalis</i>	NCTC 11020 ^T	SNR, Catlin Ne11, ATCC 25238, CIP 73.21, CEN
S14a	<i>B. catarrhalis</i>	NCTC 11020 ^T	Replicate of S14
S12	<i>B. catarrhalis</i>	LGH AD9	Sputum
S15	<i>B. catarrhalis</i>	CN 6801	SNR, from CDC
S2	<i>B. catarrhalis</i>	LGH AD3	Sputum
S7	<i>B. catarrhalis</i>	LGH AD65	Sputum
S3	<i>B. catarrhalis</i>	LGH AD12	Sputum
S4	<i>B. catarrhalis</i>	LGH AD39	Sputum
Satellites of Phenon 27			
M88	<i>N. mucosa</i>	LCDC 81079	SNR
O18	<i>Neisseria</i> sp.	NCTC 11049	Sputum, Papua, Henderson 522
D3	<i>N. canis</i>	Piotrowski H18R	SNR, Berger H18
S16	<i>Branhamella catarrhalis</i>	CN 118	SNR, from CDC
P2	<i>N. polysaccharea</i>	LCDC 85322	Throat, Berger 15883, 'atypical'

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
Satellites of Phenon 28			
B10	<i>N. meningitidis</i>	Ison 228	Throat, male homosexual
E6	<i>N. caviae</i>	CIP 52.185	Guinea pig pharynx, Pelczar GP16
P3	<i>N. polysacchara</i>	CCUG 18163	P2 from a different sender
Z9	<i>Neisseria</i> sp.	CN 1012	SNR
Phenon 28: 'caviae-cuniculi'			
E1	<i>N. caviae</i>	NCTC 10293 ^T	Guinea pig pharynx, Pelczar GP11, ATCC 14659, CIP 73.19
E3	<i>N. caviae</i>	Vedros M597	E1 from a different sender, CEN
E2	<i>N. caviae</i>	NCTC 10891	Guinea pig pharynx, Berger 1, CEN
E1a	<i>N. caviae</i>	NCTC 10293 ^T	Replicate of E1
Q24	<i>N. sicca</i>	NRC 31021	SNR
E5	<i>N. caviae</i>	NCTC 10892	Guinea pig pharynx, Berger 4
Q23	<i>N. sicca</i>	NRC 31022	SNR
E4	<i>N. caviae</i>	Vedros M1912	Northern fur seal, kidney
U38	<i>N. flava</i>	NRL 30008 ^T	SNR, ATCC 49275
U38a	<i>N. flava</i>	NRL 30008 ^T	Replicate of U38
G2	<i>N. cuniculi</i>	Vedros M1913	Northern fur seal, oropharynx
G1	<i>N. cuniculi</i>	NCTC 10297 ^T	Rabbit mouth, Berger K19, ATCC 14688, CIP 73.17
G1a	<i>N. cuniculi</i>	NCTC 10297 ^T	Replicate of G1
V3	<i>Neisseria</i> sp.	Dent AD/472	Axis deer, dental plaque
Phenon 29: <i>Neisseria ovis</i>			
X8	<i>N. ovis</i>	NRC 31020	SNR, CDC KC987
X14	<i>N. ovis</i>	Varga 1575/7	Healthy lamb, eye, CEN
X17	<i>N. ovis</i>	NCTC 11019	Sheep, conjunctivitis, Pelczar 2, ATCC 19575, CIP 73.20, Lindquist 1971
X16	<i>N. ovis</i>	NCTC 11018	SNR, Pelczar 1, ATCC 19576
X13	<i>N. ovis</i>	Varga 818	Healthy lamb, eye
X15	<i>N. ovis</i>	NCTC 11227 ^T	Sheep, keratoconjunctivitis, Bøvre 199/55, ATCC 33078
X15a	<i>N. ovis</i>	NCTC 11227 ^T	Replicate of X15
Phenon 30: <i>Moraxellas</i>			
X9	<i>N. ovis</i>	Vedros M2080	SNR, from CDC
X11	<i>N. ovis</i>	Varga Szeudro 1	Calf, keratoconjunctivitis
X12	<i>N. ovis</i>	Varga Martovasar 2135	Calf, keratoconjunctivitis
X2	<i>Moraxella osloensis</i>	NHITCC SB82/0809	SNR
X5	<i>M. bovis</i>	CN 7557	Cow, keratoconjunctivitis, CEN
X6	<i>M. bovis</i>	CN 7556	Cow, keratoconjunctivitis
X7	<i>M. bovis</i>	CN 7555	Cow, keratoconjunctivitis
X10	<i>M. lacunata</i>	NCTC 11011 ^T	Conjunctivitis, ATCC 17967, CIP A182
Satellites of Phenons 27–30			
V8	<i>Neisseria</i> sp.	Dent JRC/974	Cat, dental plaque
Z6	<i>Neisseria</i> sp.	Surrey 507	Pig nose
Phenon 31: <i>Kingella kingae</i>			
Y1	<i>K. kingae</i>	NHITCC 74/2257	SNR, CEN
Y5	<i>K. kingae</i>	NCTC 10529 ^T	Nose, Bøvre 4177/66, ATCC 23330, CEN
Satellites of Phenon 31			
Y3	<i>Kingella indologenes</i>	NHITCC SB83/0434	SNR
Z13	<i>Gemella haemolysans</i>	NCTC 10243	SNR, Berger H3
Satellites of Phenons 1–31			
V27	<i>Neisseria</i> sp.	Dent S10/4343	Cow, dental plaque
V14	<i>Neisseria</i> sp.	Dent AB1/3360	American black bear, dental plaque

Table 1 (cont.)

Strain no.	Name as received	Sender and collection no.*	Comments*
O8	<i>N. pharyngis</i>	LGH AD6	Throat
V4	<i>Neisseria</i> sp.	Dent CA1/365	Cat, dental plaque
V9	<i>Neisseria</i> sp.	Dent ST/918	Spiny tenrec (<i>Setifer setosus</i>), dental plaque

* Sources and abbreviations: Human isolates unless otherwise stated. ATCC, American Type Culture Collection, Rockville, MD, USA; Bangor, Dr A. J. Howard, Ysbyty Gwynedd, Bangor LL57 2PW, UK; Bristol, Gonococcus Reference Unit, PHL, Kingsdown, Bristol B52 8EL, UK; Brooks, Dr Geo F. Brooks, University of California San Francisco, School of Medicine, Dept. of Laboratory Medicine, San Francisco, CA 94143, USA; CCUG, Dr E. Falsen, CCUG, Dept. of Clinical Microbiology, University of Goteburg, Goteburg, Sweden; CEN, centrotyping of phenon; CIP, Mde Y. Cerisier, Collection de L'Institut Pasteur, Paris, France; CN, Dr S. Thorley, Wellcome Biotech, Culture Collection, Beckenham, Kent BR3 3BS, UK; CSF, cerebrospinal fluid; D, Teaching collection of strains, Department of Microbiology, University of Leicester, Leicester, LE1 9HN, UK; Dent, Dr V. Pratley (formerly Dr V. Dent), private collection, UK; HNCMB, Dr B. Lanyi, Hungarian National Collection of Medical Bacteria, National Institute of Hygiene, Budapest, Hungary; Hodge, Dr D. S. Hodge, Director, Public Health Laboratory, Orillia, Ontario, L3V 6KS, Canada; IID, Institute of Infectious Diseases, Department of Medical Science, Tokyo University, Japan; INS, Dr J. A. Saez-Nieto, Instituto Nacional de Sanidad, Centro Nacional de Microbiologia, Virologia & Immunologia Sanitarias, Majadahonda, Madrid, Spain; Ison, Dr C. A. Ison, Dept. of Medical Microbiology, St. Mary's Hospital Medical School, London W2 1PG, UK; LCDC, Laboratory Centre for Disease Control, Ottawa K1A 0L2, Canada; LGH, Microbiology Laboratory, Leicester General Hospital, Leicester LE5 4PW, UK; LRI, Public Health Laboratory, Leicester Royal Infirmary, Leicester LE1 5WW, UK; MUCOB, Dr N. Preston, Curator, Manchester University Collection of Bacteria (MUCOB), Dept. of Medical Microbiology, Medical School, Manchester M13 9PT, UK; NCTC, Dr L. R. Hill, National Collection of Type Cultures, London NW9 5EQ, UK; NHITCC, Dr K. A. Bettelheim, Curator, National Health Institute Type Culture Collection, Dept. Health, National Health Institute, Porira, Wellington, New Zealand; NRC, Dr R. K. Latta, NRCC, Division of Biological Sciences, Ottawa, Ontario K1A 0R6, Canada; NRL, Dr J. S. Knapp (Neisseria Reference Laboratory), Bacteriology Research Branch, Sexually Transmitted Diseases Program, Center for Infectious Diseases, Atlanta, GA 30333, USA; NVSL, Dr L. K. Schlater, Diagnostic Bacteriology Laboratory, USDA, National Veterinary Services Laboratories, Ames, Iowa, USA; Piotrowki, Dr H. D. Piotrowki, Dept. of Bacteriology, University of Heidelberg, 6900 Heidelberg 1, Germany; Roberts, Dr I. Roberts, Microbiology Department, Leicester University, Leicester LE1 9HN, UK; SNR, source not recorded; Surrey, University of Surrey Collection of Micro-organisms, Dept. of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK; T, Type strain; Varga, Prof. Dr J. Varga, Dept. of Epizootiology, University of Veterinary Medicine, Budapest XIV, Hungary; Vedros, Prof. N. Vedros, University of California Berkeley, School of Public Health, Dept. Biomedical and Environmental Health Sciences, Berkeley, CA 94720, USA.

Table 1). Purity-checked, overnight GCA subcultures were harvested and stored on beads at -70°C as thick suspensions in brain heart infusion (BHI) broth (Oxoid) containing 15% (v/v) glycerol (Feltham *et al.*, 1978).

Basal media. GC chocolate agar (GCA) consisted of double-strength GC agar base (Difco) containing post-autoclaving additions of (sterile) 5% (v/v) chocolate horse blood (Difco, defibrinated without preservative) and 1% (v/v) Vitox (Oxoid). Columbia chocolate agar (CCA) was Columbia blood agar base (Oxoid) containing 5% chocolate horse blood. Modified supplement peptone medium (MSPM) was an agar-free derivative of the medium of Stoakes *et al.* (1987), containing 1.5% (w/v) Proteose peptone no. 3 (Difco), 0.5% (w/v) NaCl, 1% (v/v) Andrade's indicator (prepared according to Cowan, 1974) and 1% Vitox (hydrated in distilled water, not the glucose-containing diluent supplied). The pH was adjusted to 8.1 prior to autoclaving. BMA and BMB contained 0.1% (w/v) soluble starch, 0.4% (w/v) K_2HPO_4 , 0.1% (w/v) KH_2PO_4 and 1% Vitox as additives to BHI agar (BMA) or broth (BMB).

Inocula. Unless stated otherwise, strains were recovered from beads by overnight incubation on GCA. Subsequent 18–24 h subcultures on GCA were re-checked for purity (Gram stain, oxidase reaction) prior to use. All solid media were streaked with a moderately charged loop.

Standard culture conditions. Except where stated to the contrary, cultures were incubated for the given times at 35°C

under controlled (saturation) humidity in air containing 5% (v/v) CO_2 .

Details specific to API galleries. All galleries were incubated under unmodified air. Tests named in subsequent sections with Zym, FGN or 50CH suffixes were performed in API galleries.

API Zym galleries were inoculated using previously frozen (-70°C) GCA-harvested cells suspended in 0.85% NaCl to point 6–7 on the McFarland scale. Reagent addition and subsequent colour development with a 1000 W light source were performed as described in the manufacturer's instructions after 4 h incubation. Colour intensity was scored on a scale of 0–5 using the colour chart supplied.

ATB FGN (fastidious Gram negative) PROTOTYPE microcupules were inoculated with 55 μl aliquots of McFarland point 4 turbidity suspensions of overnight CCA grown cells in FGN medium (0.85% NaCl). Development, reading and interpretation of individual tests as described later, were performed after 4 h incubation according to protocols supplied by Dr D. Monget [Laboratoire de biochimie (API), La Balme Les Grottes, Montalieu-Vercieu, France]. Colour intensities were scored on a scale of 0–3 10 min after any reagent additions. The ATB FGN test names used in this study are those applied by the manufacturer according to the main enzyme which each test was designed to detect. Whilst it may have been more accurate to name specific substrates (since other related enzymes with similar specificities and lesser activities could potentially con-

tribute to substrate degradation) this was not possible for commercial reasons.

API 50CH galleries were inoculated with GCA grown cell suspensions approximating to McFarland point 1 turbidity in MSPM medium. Cupule sugar concentration was 2% (w/v). After incubation for 17, 24 and 48 h, negative, weak and strong (pink colour) acid reactions in cupule or tube were scored 0, 1 and 2 respectively. The 24 h data were used in subsequent computation of similarities. Cupules with suspect or unusual reactions were checked for contamination. Berger (1963) has shown that strains of *N. flava* and *N. subflava* produce ammonia from peptone. It is considered that other saccharolytic *Neisseria* species may well also possess this capability given the weak acid reactions often reported for these bacteria. The latter could be explained by the neutralization of acid (from the breakdown of carbohydrate) by ammonia released from peptone degradation. Thus, in this study, for the majority of strains, growth was confirmed in cupules after 48 h incubation by the addition of one drop of Nessler's reagent (BDH). Immediate yellow (deeper than the control) to deep orange colouration was indicative of ammonia production from the peptone contained within the medium.

Gram reaction and micromorphology. Heat-fixed smears of 18 h GCA grown colonies were stained using Hucker's modification (Cowan, 1974), prior to microscopic examination.

Catalases. Standard catalase was performed by emulsifying 18 h GCA grown colonies in 3% (v/v) hydrogen peroxide and recording effervescence within 1 min as positive. Additionally, catalase FGN was tested for in the FGN sugar acidification control well with reagent and timing details as above. Superoxol was determined in the same manner as the original catalase except that 30% H_2O_2 was used (Young *et al.*, 1984) with instant vigorous (within 1 s), equivocal (strong but delayed for up to 5 s or instant weak) and negative effervescent reactions scored as 2, 1 and 0 respectively.

Carbohydrate degradation. All carbohydrate acidification tests except dextrin FGN (rapid, with phenol red as indicator) were determined using API 50CH galleries as described above. Other API systems (Zym and FGN galleries) were used for specific carbohydrases. Starch hydrolysis was determined after 2 d incubation using BMA streak plates incorporating 1% soluble starch, flooded with Lugol's iodine prior to examination for clearing (positive).

Amidases and proteolytic enzymes. All arylamidase reactions (those with .a suffixes) except additional valine.a, cystine.a and leucine.a (all three from the Zym gallery), were read in FGN galleries after the addition of fast blue (FB) reagent to develop the orange coloured (positive) end-product. Trypsin (Zym) and chymotrypsin (Zym) were also determined. Gelatin hydrolysis was observed after 3 d incubation of BMA streak plates containing 1.5% (w/v) gelatin. These plates were cooled to 5 °C prior to examining for (positive) clearing.

Lipases and esterases. Butyrate (C_4), caprylate (C_8) esterases and myristate (C_{14}) lipase activities were determined in Zym galleries. BMA streak plates with 0.016% (w/v) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.5% (v/v) Tween were observed after 3 d for opacity indicative of Tween hydrolysis if growth had occurred. Tributyrinase and phospholipase C reactions were studied by placing the appropriate diagnostic tablet (Rosco Diagnostica) in 0.25 ml of a McFarland 4 turbidity suspension in 0.85% NaCl and incubating for 21 h prior to reading according to manu-

facturer's instructions (Casals & Pringler, 1987). Readings at earlier times of 3 and 6 h were not used in the cluster analysis.

Activity towards nitrogenous substances. Nitrate and nitrite reduction were determined by method 1 of Cowan (1974) in BMB containing 0.1% (w/v) KNO_3 and 0.0005% (w/v) NaNO_2 respectively. At least 5 min were allowed for colour development after addition of nitrate reagents. Nitrate reduction FGN was also determined employing similar reagents. Urea hydrolysis, phenylalanine deaminase and ornithine decarboxylase were also performed in FGN galleries.

Antibiotic interactions. Penicillinase FGN relied upon the acidimetric method with phenol red as indicator. Colistin sensitivity was determined using GCA spread plates carefully prepared using 0.1 ml aliquots of McFarland 4 suspensions in 0.85% NaCl. After 1 d of incubation any zones of inhibition around a centrally placed 10 µg Colistin disk (API Biomerieux) were inspected and the distances between growth and disk measured. Distances were coded 0 for no inhibition zone (resistance), 1 for growth within 5 mm (generally 2–3 mm) and 2 for very sensitive strains producing growth at only 5–10 mm.

Growth on special media. Streak plates of Nutrient agar (Oxoid), GCA containing 0.5% (w/v) sodium taurocholate and modified New York City medium (GCA with 1% (v/v) VCAT (Oxoid) antibiotic supplement) were examined after 1 and 2 d incubation. Presence of growth at 1 d, at 2 d and the absence of growth were coded 2, 1 and 0 respectively. Streak plates used to determine hydrolysis of Tweens 20, 40, 60, 80 and 85 (see under Lipases and esterases) were initially examined for the presence of growth. Growth under unmodified air and under 5% CO_2 in the presence of 0, 100, 250, 500 and 1000 µg acetazolamide ml^{-1} was assessed after 3 d incubation of BMA streak plates incorporating the appropriate concentrations of this carbonic anhydrase inhibitor. Results were coded on scale 0 (for growth with 0 µg ml^{-1}) to 5 (for growth with 1000 µg ml^{-1}). CO_2 results were not used in the computation of similarities.

Phosphatases. Activities towards two different acid (FGN and Zym) and four different alkaline (unnumbered one from Zym, phosphatases 1–3 from FGN) phosphatase substrates were examined. FGN acid and alkaline (phosphatase 1 only) phosphatases required addition of FB reagent prior to reading.

Miscellaneous biochemical tests. The reduction of pink resorufin to a colourless end product was determined in FGN galleries, as was production of indole, indicated by the development of a red colour with James reagent. Hippurate hydrolysis FGN was read after the addition of ninhydrin (NIN) reagent that produced deep violet positive reactions. The oxidase reaction was tested by the method of Kovacs (1956) using 18 h GCA growth. Dark purple colouration formed within 10 s was scored positive. The production of DNase was determined using streak plates of Oxoid DNase agar with 1% proteose peptone no. 3 (Difco) and 1% Vitox (Oxoid) that were flooded with 1 M HCl after 2 or 5 d incubation. Clearing within the opaque medium adjacent to growth was positive for DNase and any pigmentation was enhanced to a bright yellow colour on the acidified medium (DNase 2 and 5 d results were identical). Tetrathionate reductase was determined using the appropriate diagnostic tablet (Rosco Diagnostica) in the manner previously described for tributyrinase and phospholipase C (see under Lipases and esterases). Streak plates of Columbia blood agar base (Oxoid) containing 1% Vitox and 8% non-chocolatized horse or sheep blood were observed at 1 and 2 d for alpha- and beta-haemolysis. Polysaccharide synthesis was recorded as

positive where BMA streak plates incubated for 2 d with 3% sucrose developed blackening of colonies and adjacent medium on flooding with Lugol's iodine.

Numerical taxonomy. Computing was done on the University of Leicester's VAX computer using the TAXPAK suite of programs written by M. J. Sackin and associates of the Microbiology and Immunology Department, Leicester University.

The $1 \times n$ matrix consisted of 315 strains scored for 155 characters (see Table 3 and constant tests in Results). Binary characters were scored 0 for negative and 1 for positive. Multistate characters were mainly those performed in API strips and these were scored as detailed above.

The similarity coefficients used were the Gower coefficient including negative matches (S_G), the simple matching coefficient (S_{SM}) and the pattern difference (D_{P2}) coefficient of Sackin (1981).

Single linkage and average linkage (UPGMA) cluster analyses were done, and the vigour of cultures was calculated using the V_2 statistic (Sackin, 1981). Details of these methods other than the V_2 statistic are given in Sneath & Sokal (1973). For S_{SM} and D_{P2} the multistate characters were converted to binary ones.

Test reproducibility was estimated by duplication of 21 of the strains. These duplicate strains were carried through as independent strains, and their identities are shown in Table 1. Because the number of multistate tests was large the S_G values were used to estimate test error by formulae 1 and 4 in Sneath & Johnson (1972).

Other calculations (including identification statistics) were carried out by the methods given in Sneath & Stevens (1985). The programs MATIDEN, CHARSEP and OVERMAT are respectively from Sneath (1979a), Sneath (1979b) and Sneath (1980).

RESULTS

Test reproducibility

The mean S_G between 21 replicated pairs of strains was 96.7%, and the mean test error, p , was therefore low, about 1.7%. The mean difference in vigour between these replicates was 1.4%.

Reduction of nitrite was the least reproducible test ($p = 0.21$), but was retained because of its historical importance; a number of arylamidase (.a) tests were over $p = 0.1$ (ornithine.a FGN, serine.a FGN, tyrosine.a FGN, aspartyl-alanine.a FGN, phenylalanyl-proline.a FGN, prolyl-arginine.a FGN and seryl-methionine.a FGN). Catalase FGN was also not very reproducible ($p = 0.13$) but differentiated the phenons with very high catalase activity.

Constant tests

The following tests were all negative or all positive in all strains (with rare exceptions shown in parentheses).

The following were negative: indole production (O8, V4, V9, Y3), acid phosphatase FGN, α -arabinosidase FGN, β -fucosidase FGN, ornithine decarboxylase (V9, V14),

histidine.a (V8, Z9), pyroglutamic acid.a (Z13), threonine.a, glycyl-tryptophan.a, lysyl-serine.a, valyl-tyrosyl-serine.a, acid from dextrin, erythritol, D-arabinose, L-arabinose, D-xylose (V4, V9), L-xylose, adonitol, sorbose, rhamnose (U6), dulcitol, inositol, sorbitol (V4), methyl-D-mannoside, amygdalin, aesculin, cellobiose, inulin, melezitose, D-raffinose, amidon, glycogen, xylitol, D-turanose, D-lyxose (O8, V4, V9), D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate, Gram reaction, mulberry clumps (Z11), gelatin hydrolysis (M8), L-valine.a Zym, L-cystine.a Zym, chymotrypsin, α -galactosidase Zym, β -glucuronidase Zym, β -glucosidase Zym, N-acetyl- β -glucosaminidase Zym, α -mannosidase Zym, α -fucosidase Zym.

The following were positive: alanine.a (Z13), oxidase.

The exceptional strains listed above were mostly satellites near the bottom of the phenogram (Fig. 5).

Main cluster analysis

The UPGMA analysis with S_G (Figs 1–5) is used as the basis for the systematic treatment. We chose S_G because it reflects the strength of quantitative reactions, which is desirable in studying these phenotypically very similar

Fig. 1. Simplified UPGMA phenogram based on S_G . The cophenetic correlation was 0.784. Further detail is shown in Figs 2–5.

Fig. 2. Area A from Fig. 1.

Fig. 3. Area B from Fig. 1.

Fig. 4. Area C from Fig. 1.

Fig. 5. Area D from Fig. 1.

organisms. The cophenetic correlation was 0.784. Thirty-one groups of strains were considered sufficiently distinct for descriptive purposes. The distinctness of phenons 9 to 17 is less than would normally be accepted in phenotypic analyses. However, if these are pooled into one het-

erogeneous phenon a number of significant points are obscured: the differentiation of all the phenons is near the limits of the methods employed, because of the very high similarity between all the neisserias. As a consequence of these factors there are a number of strains of well-founded species that are misplaced because of somewhat atypical test results.

The phenogram is conveniently broken into four major areas (Fig. 1); the detail within each is shown in Figs 2–5. The areas are: **A**, the gonococci, meningococci, and a few other species (phenons 1–8); **B**, strains that are commensals in the human nasopharynx, conveniently termed the pharyngeal strains (phenons 9–17); **C**, species found mainly in animals other than humans (phenons 18–26); and **D**, the moraxellas, branhamellas and so-called ‘false neisserias’, with a few other organisms which were studied for comparative purposes (phenons 27–31).

Description of phenons

Constituent strains of phenons are given in Table 1. The parameters of the phenons, with intercentroid distances, are shown in Table 2. The percentages of positive test reactions are given in Table 3.

Area A: phenons 1–8 (Fig. 2). Strains of these phenons tended to attack few carbohydrates, and have low vigour scores. None grew in the presence of Tween 20 and, except for phenon 7, all grew on modified New York City medium. Apart from phenon 6, strains of area A did not grow on nutrient agar. There are two groups of gonococci, phenon 1 (*gonococcus a*) and phenon 2 (*gonococcus b*). Both replicates of the type strain of *Neisseria gonorrhoeae* (A59, A59a) are in phenon 1. Almost all these strains are of urethral or cervical origin, but A75 was isolated from the throat, though it appears quite typical of gonococci. There is no reason to doubt that members of phenon 2 are also gonococci; several are from urethra or cervix. Three other strains are attached to these phenons. One of these, A50, was isolated from the human conjunctiva in Egypt (Mazloum *et al.*, 1986). To elucidate the relationships of strains of phenons 1 and 2 and their satellites further, a principal coordinates analysis was made which confirmed the separation of phenons 1 and 2, and showed that the three satellites fell close to one or other phenon. Phenons 1 and 2 shared a few distinctive properties that are not common among other neisserias: they were colistin resistant, positive in the catalase FGN test and were positive for superoxol and negative for maltose acidification. They differed in glycine.a and lysine.a with phenon 1 positive for both, and the latter (unlike phenon 2) contained some penicillinase producing strains.

Phenons 3, 4 and 5 show unexpected taxonomic relationships. Phenon 3 is *Neisseria meningitidis*, containing the type strain NCTC 10025 (B18, B18a, B43), and phenon 5 also consists of meningococci (see Discussion). Phenon 4, containing the type strain of *N. cinerea*, is embedded within the meningococci in the dendrogram (Fig. 2). A principal coordinates analysis, however, showed that strains of phenon 4 were within the region composed by phenons 3 and 5 (Fig. 6), and strains of the latter were not well separated. The *N. cinerea* strains are somewhat peripheral in Fig. 6 but do not seem to form a distinct group despite being inactive to carbohydrates, unlike meningococci. Overlap between these three phenons is relatively high, with nominal overlap between 3 and 4, and 3 and 5 approaching 1% (see Discussion). However,

phenon 4 is usually colistin sensitive, whereas phenons 3 and 5 are resistant. Phenons 3 and 5 are positive for γ -glutamyl.a unlike the gonococcal and cinerea phenons. These findings are in agreement with Knapp (1988). The satellites B29 and B23 (Fig. 2) appear to be meningococci that gave rather aberrant test results.

Phenon 6 is *N. polysaccharea* (Riou & Guibourdenche, 1987), a compact group, though some strains received under this name do not cluster here (see Discussion). Distinctive properties are the ability to synthesize polysaccharide and to hydrolyse starch. Some strains produced yellow pigment.

A group of satellites follow phenon 6. They were received under various names, and may be strains which gave rather atypical test reactions. Two were received as *Neisseria cinerea*.

Phenon 7 is one of three containing strains received under the epithets ‘elongata’ or ‘parelongata’ (the others are phenons 20 and 26). The strains are distinctive in being positive in the tests for superoxol, hydroxyproline.a and phenylalanyl-proline.a. It corresponds to *Neisseria elongata* subsp. *glycolytica*. The satellite that follows, B45, is a misplaced meningococcus.

Phenon 8 is *Neisseria lactamica* and is well defined, characterized by being β -galactosidase positive and often acidifying lactose, neither of which properties are seen in other phenons.

Area B: the human pharyngeal area (phenons 9–17, Fig. 3). The strains of area B are predominantly from the nasopharynx, and despite a number of species epithets they are all phenotypically very similar. Collectively these phenons tend to acidify fructose and sucrose, to grow on nutrient agar often giving yellow pigment, often reduce nitrite and sometimes nitrate, sometimes hydrolyse starch and synthesize polysaccharide, and give a positive reaction with leucine.a Zym. Some of these properties are shared with phenon 25. There are few properties that distinguish the phenons. Some phenons show tetrad formation, some are usually haemolytic, some do not synthesize polysaccharide, but the phenons seem to form a continuum, with rather large overlap values for adjacent phenons (commonly 0.01%, which is relatively large, and probably the overlap is underestimated because of the small number of variable tests in which the phenons differ, see Discussion).

The position was only partly clarified by principal coordinates analysis. The epithets under which strains were received were not grouped in a sharp manner on the first three axes. Some broad trends were seen. Strains received as ‘mucosa’ tended to score high on axis I. Although ‘sicca’ might seem the antithesis of ‘mucosa’ the ‘sicca’ strains were also high on axis I (and on axis III). Strains with epithets ‘flava’, ‘subflava’ and ‘perflava’, presumably given because of yellow pigmentation on first isolation, had low scores on axis I. However, their

Table 2. Parameters of phenons and intercentroid distances

The entries in the body of the table are the intercentroid Euclidean distances, from which Fig. 7 was derived. The mean vigour is the mean proportion of characters with positive states modified by the D_{p_2} formula of Sackin (1981), and sd is the standard deviation of the vigour of a phenon to their own centroid is d_j , calculated as $\sqrt{(\sum d_{ij}^2 / (t_j - 1))}$, where d_{ij}^2 is the squared distance $(1 - \delta_{ij})$ of a strain to the centroid of phenon j , and t_j is the number of strains in j . The Euclidean distances referred to above are $\sqrt{155}$ times $\sqrt{(1 - \delta_{ij})}$ because there are 155 characters

Phenon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
2	1560																														
3	2201	2585																													
4	2463	2954	2118																												
5	2849	3052	2347	2596																											
6	2995	3392	2347	2615	2834																										
7	2644	2879	2906	2293	3281	3307																									
8	2692	3054	2357	2636	2681	2808	3144																								
9	4753	4432	3682	4132	4044	3483	3825	3924																							
10	4753	4432	3682	4132	4044	3483	3825	3924	1651																						
11	3758	4053	3311	3449	3650	3325	3177	3268	1853	1874																					
12	4179	4370	3646	3914	3926	3498	3685	3505	1853	1985	1874																				
13	3931	4146	3392	3752	3653	3282	3595	3474	1832	2170	1875	1935																			
14	4574	4827	4102	4357	4355	3895	3902	3892	2449	2879	2567	2666	2486																		
15	3828	4172	3398	3331	3646	2877	3180	3517	2202	1858	2246	2286	2511	3178	2986																
16	3812	4116	3374	3493	3292	3701	3745	3460	2762	2885	2599	2801	2453	3452	2951	2951															
17	3882	4162	3680	3960	3906	3484	3484	3525	2812	2762	2892	2798	2774	2496	2489	3551	3685														
18	3923	4129	3246	3173	3554	3427	2804	3802	3103	3097	2597	2688	2283	3414	3182	3511	3685	2841													
19	3821	4036	3188	3040	3718	3667	2753	3462	3106	3039	2805	2202	2057	3129	3105	3175	3175	2841	2228												
20	3821	4036	3188	3040	3718	3667	2753	3462	3106	3039	2805	2202	2057	3129	3105	3175	3175	2841	2228	2208											
21	3595	3838	3188	3040	3718	3667	2753	3462	3106	3039	2805	2202	2057	3129	3105	3175	3175	2841	2228	2208	2208										
22	4370	4559	3867	4279	4110	4451	4241	4518	3804	3394	3594	3612	3436	4448	3970	4007	4350	3983	3855	3455											
23	3764	3629	3347	3964	3615	3913	3634	3797	3242	3121	3140	2458	3618	3500	3202	3865	2993	3202	369	3454	3078										
24	4001	4230	3462	4220	4120	4161	3946	4066	4038	2768	3265	2941	3071	2331	3399	3490	3153	3799	2655	3728	3257	3558	2720								
25	4610	4876	474	4201	4408	4116	4104	4278	2562	2627	2411	4033	2820	2957	2986	3268	3478	3451	3490	3647	3151	4154	3905	3692							
26	3683	4031	3178	3658	4143	4074	3211	3854	3471	3394	3251	3484	4674	4638	4329	4677	3681	3406	2969	2895	4084	3761	3638	3584	3665						
27	4228	4495	4309	4366	4595	4394	4487	4554	4565	4346	4594	4470	4674	4638	4329	4677	3681	3406	2969	2895	4084	3761	3638	3584	3665	3045					
28	4228	4495	4309	4366	4595	4394	4487	4554	4565	4346	4594	4470	4674	4638	4329	4677	3681	3406	2969	2895	4084	3761	3638	3584	3665	3045	3045				
29	4835	5097	4749	4790	5145	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	5082	3045	3045			
30	4134	4264	4025	4017	4459	4597	4707	4768	4436	4252	4113	4127	4165	4165	4165	4165	4165	4165	4165	4165	4165	4165	4165	4165	4165	4165	3045	3045			
31	4501	4717	4158	4313	4349	4264	4587	4408	4928	4628	4675	4586	4718	4919	4468	4919	4726	4306	4770	4556	4638	4811	4860	5044	4940	4456	4857	4468	5120	4418	
Phenon of	16	8	30	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
No.																															
Mean	0.107	0.093	0.102	0.081	0.099	0.123	0.087	0.135	0.202	0.189	0.165	0.193	0.187	0.208	0.165	0.165	0.191	0.143	0.159	0.125	0.156	0.184	0.153	0.210	0.220	0.191	0.217	0.189	0.213	0.182	
Vigour	0.011	0.012	0.016	0.017	0.015	0.008	0.018	0.023	0.014	0.021	0.021	0.018	0.022	0.020	0.012	0.014	0.008	0.011	0.024	0.033	0.015	0.016	0.022	0.016	0.022	0.016	0.026	0.023	0.024	0.014	
SD	0.152	0.153	0.178	0.161	0.188	0.173	0.208	0.208	0.185	0.185	0.185	0.192	0.208	0.210	0.208	0.195	0.200	0.229	0.208	0.210	0.211	0.178	0.172	0.218	0.220	0.211	0.216	0.221	0.184	0.231	

Table 3. Characteristics of phenons (percentage of strains positive)

No. Character	Phenon...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Micromorphology																																	
1. Diplococci		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	43	100	100	100	100	100	38	100	100	100	38	0	
2. Diplo rods		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57	0	0	0	0	0	63	0	0	0	50	100	
3. Long filaments		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	
4. Tetrads		6	13	7	0	0	0	33	25	7	31	100	20	55	67	0	0	100	17	0	0	0	100	14	25	75	0	0	21	29	0	0	
Catalases																																	
5. Catalase		100	100	100	100	100	100	100	92	92	100	100	100	100	100	100	100	100	100	100	43	100	100	100	100	100	100	100	100	100	100	100	
6. Catalase FGN		100	100	10	0	0	0	67	33	0	0	0	0	9	33	0	33	33	25	100	0	36	100	100	100	0	75	86	100	71	100	0	
7. Superoxol		100	100	0	0	0	0	100	0	8	7	0	11	36	0	0	0	67	8	64	0	9	0	14	75	0	88	64	23	0	0	0	
Acid from carbohydrates																																	
8. Arbutin		0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
9. D-fructose		0	0	0	0	0	0	0	0	100	94	71	100	100	100	100	100	100	8	9	0	9	0	86	100	100	13	0	0	0	0	0	
10. Galactose		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	13	0	
11. β-Gentiobiose		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	
12. Gluconate		0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	8	0	0	0	100	100	50	25	0	0	0	0	0	0	
13. 5-keto- Gluconate		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	
14. D-Glucose		69	75	60	0	100	57	33	83	100	100	86	90	91	100	100	67	100	25	18	0	9	80	100	100	100	38	0	7	0	0	100	
15. Glycerol		0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
16. Lactose		0	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17. Maltose		0	0	63	0	100	86	0	92	100	100	71	100	100	100	100	100	100	0	0	0	0	29	0	100	0	0	0	0	0	0	100	
18. Mannitol		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	
19. D-Mannose		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	
20. Melibiose		0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	
21. α-Methyl-D-glucoside		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	
22. β-Methylxyloside		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	
23. N-acetyl-glucosamine		0	0	0	0	0	0	0	7	6	0	0	18	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24. Ribose		0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
25. Salicine		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	25	0	0	0	0	0	13	0	
26. Sucrose		0	0	0	0	0	0	0	93	94	86	100	100	100	80	100	100	42	9	0	0	100	100	100	75	0	0	0	0	0	13	0	
27. Trehalose		0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	50	25	0	0	0	0	0	0	
Carbohydrases																																	
28. β-Galactosidase Zym		0	0	0	0	0	0	75	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
29. β-Galactosidase 1 FGN		0	0	0	0	0	0	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
30. α-Glucosidase Zym		0	0	3	0	0	0	0	7	0	0	20	0	0	0	0	0	0	0	0	0	0	0	25	25	0	0	0	0	0	0	0	
31. α-Glucosidase FGN		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	
32. Starch hydrolysis		0	0	0	0	0	100	0	17	64	33	0	20	18	67	60	0	100	9	0	57	64	0	29	50	0	0	8	0	0	0	NT	
Amidases and proteolytic enzymes (FGN tests unless stated otherwise)																																	
33. Alanyl-phenylalanyl-proline.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	13	0		
34. Alanyl-phenylalanyl-prolyl-alanine.a		0	0	0	0	0	0	0	43	31	14	10	0	0	40	0	0	0	0	0	14	18	0	0	0	100	13	100	0	0	75	0	
35. Aspartyl-alanine.a		100	88	57	0	60	43	0	58	0	19	0	10	0	0	0	0	33	8	0	0	27	20	14	25	25	38	86	21	71	75	100	
36. Cystine.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0		
37. α-Glutamyl-α-glutamic acid.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	21	100	63	100		
38. γ-Glutamyl.a2		0	0	100	0	100	0	0	0	0	0	0	9	0	0	0	0	42	0	0	0	100	71	25	0	0	0	0	0	0	0	0	
39. Glycine.a		100	0	93	100	80	100	67	92	100	100	100	90	100	100	100	100	100	100	100	43	100	100	29	100	100	100	100	93	100	100	100	
40. 2-Glycyl-glycyl-arginine.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	
41. Glycyl-phenylalanine.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	71	0	0	38	50		
42. Glycyl-proline.a		0	0	20	0	0	71	33	8	93	94	100	90	45	100	100	67	100	42	55	57	100	0	25	100	38	93	36	29	13	50		
43. Histidyl-phenylalanine.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0		
44. Histidyl-serine.a		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0		
45. Hydroxyproline.a		44	13	3	50	0	0	100	75	100	100	100	90	27	100	100	33	100	0	18	100	100	0	25	100	100	0	0	0	0	0	0	
46. Leucine.a Zym		0	25	33	0	40	0	33	83	86	25	57	100	91	67	20	67	67	75	73	57	73	60	71	75	25	88	86	93	100	88	0	
47. Lysine.a		100	0	97	100	80	100	67	92	100	100	100	90	100	100	100	100	100	92	100	43	100	100	43	100	100	100	100	100	100	100	100	
48. Ornithine.a		0	0	10	25	0	14	0	8	21	38	29	10	0	0	0	0	0	9	14	55	40	0	0	100	75	57	0	0	25	100		
49. Phenylalanine.a		0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	9	14	0	0	0	0	100	0	57	0	0	25	0		
50. Phenylalanyl-arginine.a		0	0	0	0	0	0	0	43	6	0	20	18	67	0	0	100	0	0	9	20	0	0	50	38	14	0	100	0	0	0		
51. Phenylalanyl-proline.a		0	0																														

Table 3 (cont.)

No.	Character	Phenon...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
67.	Tween 60 hydrolysis		0	0	0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	77	100	100	NT	
68.	Tween 80 hydrolysis		0	0	0	0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	
69.	Tween 85 hydrolysis		0	0	0	0	0	0	0	17	0	0	0	0	9	0	0	0	0	0	0	0	0	0	17	0	0	0	54	64	86	63	0	
Activity towards																																		
nitrogenous substances																																		
70.	Hippurate hydrolysis		0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	29	0	0	
71.	Nitrite reduction		46	43	76	75	50	100	33	83	100	100	86	89	91	100	100	100	100	78	80	100	82	100	67	100	75	100	100	64	0	25	NT	
72.	Nitrate reduction		0	0	3	25	0	0	0	0	29	0	29	22	45	0	20	50	0	22	70	0	9	60	0	75	50	25	54	57	100	63	NT	
73.	Nitrate reduction FGN		0	0	0	0	0	0	0	0	0	0	14	10	18	0	0	0	0	8	64	0	0	0	0	100	25	13	43	0	86	38	0	
74.	Phenylalanine deaminase		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	
75.	Urea hydrolysis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	13	0	
Antibiotic interactions																																		
76.	Colistin sensitivity		0	0	3	75	0	0	100	11	100	100	100	100	100	67	100	100	33	100	100	100	100	100	100	100	100	100	100	93	100	100	100	
77.	Penicillinase		13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	29	0	0	
Growth on special media																																		
78.	Growth acetazolamide (air)		0	0	5	0	0	0	0	17	8	29	0	20	11	33	20	67	33	18	18	50	0	20	33	25	0	0	83	93	33	63	0	
79.	Growth modified New York City medium		100	100	100	100	100	100	0	100	0	0	0	0	11	0	0	67	67	0	0	0	0	0	0	0	0	0	92	0	0	0	0	
80.	Growth nutrient agar		6	0	3	0	0	71	33	42	100	100	100	100	100	100	100	100	100	83	90	43	100	0	100	100	75	100	50	79	100	88	0	
81.	Growth 0.5% sodium taurocholate		0	0	3	33	80	0	0	8	86	63	29	30	27	0	80	100	0	75	70	29	0	100	29	75	50	100	31	36	0	0	0	
82.	Growth Tween 20		7	0	4	0	0	0	0	17	100	88	86	50	91	100	0	67	0	100	91	100	64	100	100	75	100	38	77	100	100	50	0	
83.	Growth Tween 40		7	25	30	0	40	0	0	0	100	100	86	80	100	100	20	100	0	100	100	86	91	100	100	100	100	100	100	100	100	100	0	
84.	Growth Tween 60		47	50	52	0	100	29	67	17	100	88	100	90	100	100	60	100	67	100	100	100	91	100	100	100	100	63	92	93	100	100	0	
85.	Growth Tween 80		100	100	89	33	0	71	33	83	100	100	100	100	100	100	80	33	33	100	100	100	100	100	100	100	100	100	92	100	100	100	50	
86.	Growth Tween 85		93	100	93	33	20	71	33	42	100	100	100	100	100	33	100	100	67	100	100	100	100	100	100	100	100	100	100	100	100	100	50	
Phosphatases																																		
87.	Acid phosphatase Zym		0	0	23	0	40	14	0	67	93	44	45	90	91	100	0	100	67	83	18	57	36	20	57	100	50	75	0	29	14	0	100	
88.	Alkaline phosphatase		0	0	0	0	0	0	0	8	14	6	0	20	27	100	0	0	0	17	0	0	0	20	14	75	0	0	29	64	14	13	100	
89.	Alkaline phosphatase 1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	9	0	0	0	0	0	0	13	7	79	0	0	100	
90.	Alkaline phosphatase 2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	100	0	0	0	0	0	64	0	0	100	
91.	Alkaline phosphatase 3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	
92.	Naphthol-AS-BI-phosphatase Zym		0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0	25	0	0	14	25	50	
Miscellaneous biochemical tests																																		
93.	DNase 2 d		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	40	0	0	0	14	0	0	75	86	0	14	0	0	
94.	DNase 5 d		27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	50	0	0	0	14	0	0	100	93	7	100	50	0	
95.	Haemolysis (horse blood)		0	0	3	0	0	0	0	0	7	80	0	100	0	0	20	0	0	0	9	0	0	0	100	0	0	0	14	83	80	100	100	
96.	Haemolysis (sheep blood)		0	0	0	0	0	0	0	8	43	100	17	100	0	0	20	0	50	0	0	0	0	100	14	0	0	50	0	0	100	40	0	
97.	Polysaccharide synthesis		0	0	0	0	0	100	0	0	93	94	14	80	100	0	80	0	100	82	27	43	82	100	71	75	50	0	0	0	0	0	0	
98.	Resorufin reduction		56	88	0	0	0	0	0	8	0	0	0	10	0	0	20	0	33	8	0	0	0	0	14	0	0	13	14	7	14	0	100	
99.	Tetrathionate reductase		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	
100.	Yellow pigment production		0	0	0	0	0	86	0	8	91	23	60	22	36	67	0	0	33	0	45	0	80	0	0	100	33	0	0	0	0	0	0	0

NT, Not tested.

pigment production in our test was very poorly correlated with these epithets. Strains labelled 'pharyngis' were not associated with any axis. Strains with less metabolic activity (low vigour scores) tended to score low on axes II and III. There was some correlation between phenons and tetrad formation (Table 3).

Phenon 9 is preceded by two satellites, B7 and Z8. The first was isolated from the throat of a male homosexual as *Neisseria meningitidis*, but phenotypically it is atypical (see Discussion). The latter is of uncertain origin. Phenon 9 itself is very tentatively equated with *Neisseria mucosa* because it contains one replicate, M15a, of the type strain, which consistently behaved differently from M15. The latter gave stronger reactions in arylamidase tests and appears in the loose phenon 25 close to the moraxellas. One replicate, W1, of *Neisseria macacae* also occurs here (the other is a satellite of phenons 9 to 12).

Phenon 10 is identified with *Neisseria perflava* because it

contains the type strain (both replicates, U15 and U15a). One strain, U28, was from a case of meningitis.

Phenons 11 and 12 contain no type strains, and are referred to as pharyngeal groups 1 and 2 respectively. One strain, V12, is from monkey dental plaque. Two satellites follow, one being the replicate of W1.

Phenon 13 is identified with *Neisseria sicca* because it contains the type strain (Q29, Q29a) but other epithets occur (see Discussion). Not all strains are from humans (Table 1).

Phenons 14 to 17 contained no type strains, so they are referred to as pharyngeal groups 3 to 6 respectively.

Area C: human and animal strains (phenons 18–26, Fig. 4). This area shows some phenotypic overlap with area B, but is separated to assist high level description. Many strains are from animals. Phenon 18 is *Neisseria animalis*

Fig. 6. Principal coordinate analysis of meningococci (groups a and b) and *Neisseria cinerea*. The first two principal axes are shown. The respective eigenvalues are 0.171 and 0.134, corresponding to 23% of the total variation and $n' = 15.7$. The type strains and their replicates are indicated by T. Group a (●) is phenon 3, group b (○) is phenon 5 and *cinerea* (▲) is phenon 4.

(type strain C1, C1a), but also contains one replicate, H1a, of the type strain of *N. denitrificans*. The phenon has few distinctive properties except that it is proline.a negative, like phenons 22, 23 and the moraxellas, whereas most 'true neisserias' are positive. A strain received as *Moraxella urethralis* (X4) follows: its position is unexpected, but this species has often been considered *incertae sedis* (Bøvre, 1984b).

Phenon 19 is *Neisseria canis* (type strain D1, D1a); most strains are from carnivores, or humans bitten by carnivores, though two are from monkeys. It is notable for being positive for catalase FGN and negative for maltose; most are positive for nitrate reduction. It also contains a strain received as '*N. cuniculi* var. *gigantea*', but we are unsure of the status of this strain since it has been suggested (Professor U. Berger, personal communication) that strains bearing this name result from a confusion; unfortunately the organism of Berger (1962) has died and no longer exists in any collection (ATCC no longer list this strain). Also we did not observe, under the conditions of this study, that the cells were particularly large as originally described by Berger (1962).

Phenon 20 is *Neisseria elongata* subsp. *elongata* (type strain I1, I1a). It is characteristically inactive toward carbohydrates, and often exhibits diplobacillary morphology. Strain I2, CDC confirmed as group M-6, also clustered here (see Discussion).

Phenon 21 has some puzzling features. It contains the type strain of *Neisseria flavescens* (K1, K9, K9a, K11) which has been recorded as a rare cause of human meningitis, and which is generally regarded as a well defined species (Vedros, 1984). The type strain of *N. subflava* (U37, U37a) is also included. There is no indication of two subphenons, so the earlier name, *N. subflava* has been used here. The phenon is inactive toward carbohydrates and most strains show yellow pigment.

Several satellite strains follow phenon 21; two from dental plaque V19 and V21 are haemolytic.

Phenons 22 to 24 appear to represent undescribed species from animals. They are unusual for *Neisseria* in being mainly gluconate and sucrose acidification positive, and positive in catalase FGN. Phenon 22 contains strains from sepsis in iguana lizards (Plowman *et al.*, 1987). They are characteristically positive in alkaline phosphatase 2 and γ -glutamyl.a. They are strongly α -haemolytic and show prominent tetrad morphology. Although phenon 22 appears in Fig. 4 as within the phenon complex 22 to 24, a principal coordinate diagram showed that the iguana strains were well separated from the others, and that the distinction between phenons 23 and 24 was weak.

Phenons 23 and 24 might be described as a new species. All strains were from dental plaque of dairy cows except one from gorilla plaque (Dent, 1982). They differ from phenon 22 in that they are fructose positive, grow on nutrient agar, seldom exhibit tetrads and are negative for alkaline phosphatase 2 and haemolysis. Phenon 23 does not reduce nitrate whereas phenon 24 does, and only phenon 24 displayed pronounced yellow pigmentation. One of the duplicates of V25 was in phenon 23, the other in phenon 24 (labelled V37).

A satellite, Z11, follows which was received as *Morococcus cerebrosus* (type strain). It was unique among our strains in forming prominent 'mulberry clumps' of cocci in wet mounts as described by Long *et al.* (1981).

Phenon 25, referred to as the 'mucosa-flava' phenon, is a small rather diffuse group, with somewhat unusual test results in that the strains are usually positive in ornithine.a, phenylalanine.a and tyrosine.a and, like most moraxellas, are positive in prolyl-arginine.a and alanyl-phenylalanyl-prolyl-alanine.a. Although it contains one of the replicates of the type strain of *Neisseria mucosa* this behaved in an unusual manner, so we believe that this phenon does not represent that species. A satellite, Q7, follows.

Phenon 26 corresponds to the CDC M-5 and EF-4 groups (Tatum *et al.*, 1974; Holmes & Ahmed, 1981), which have been called '*Neisseria parelongata*', though the name is not validly published. The two groups were separated within phenon 26, though not very distinctly: the M-5 group has now been validly named *Neisseria weaveri* (Holmes *et al.*, 1993; Anderson *et al.*, 1993a, b). Most strains are from dogs and grow as short diplococci. They are characteristically DNase positive and grow on taurocholate

media. Four satellites follow which may be strains of other species in which test reproducibility was poor and caused them to stray from their expected phenons. One strain is a replicate of the type strain of *Neisseria denitrificans* but it behaved unusually in several tests (e.g. it did not reduce nitrate) and the other replicate is in phenon 18.

Area D: phenons 27–31, moraxellas and allies (Fig. 5).

The branhamellas, 'false neisserias' (*Neisseria caviae*, *N. cuniculi*, *N. ovis*) and moraxellas show several phenotypic features that are unusual in the 'true neisserias'. They are usually positive in α -glutamyl- α -glutamic acid.a, alanyl-phenylalanyl-prolyl-alanine.a and prolyl-arginine.a, but are proline.a negative, and they do not attack carbohydrates.

Phenon 27 is *Branhamella catarrhalis*, and contains the type strain (S14, S14a). We believe that A33 and O6 belong to this species and have earlier been misidentified or mislabelled. A number of satellites follow: M8 was unusual in being weakly positive for gelatin hydrolysis, and Z9 was histidine.a positive.

Phenon 28 contains the type strains of both *Neisseria caviae* (E1, E1a) and *Neisseria cuniculi* (G1, G1a) and, surprisingly, the type strain of *Neisseria flava* (U38, U38a); the implications are considered in the Discussion. Many strains are from rodents. Strains received as *N. caviae* produced a wide zone of opacity around the clearing on Tween 85 agar plates, but strains received as *N. cuniculi* and the *N. flava* type strain did not.

Phenon 29 is *Neisseria ovis* (*Moraxella ovis*) characterized by being positive for glycyl-glycyl-arginine.a, phenylalanyl-arginine.a, and α -glutamyl- α -glutamic acid.a and like phenon 30 it hydrolyses several of the Tweens and caprylate (C_8 esterase). Most strains produced a response on Tween 85 similar to that of *N. caviae* and they produced β -haemolysis near the colony edge only.

Phenon 30 contains classical species of *Moraxella*: there was some internal differentiation in the phenogram into species, but there were too few strains to justify separating these in Fig. 5. These strains were often diplobacillary and had activity toward Tweens 40, 60, 80 and tributyrin similar to phenon 29. They did not produce the opacity in Tween 85 agar observed with *N. caviae*.

A number of satellites are seen at the end of the phenogram, most of which are related to the other strains at relatively low similarity levels. Only the two strains of *Kingella kingae* have been placed into a described phenon (phenon 31), chiefly to record their properties in Table 3 for comparative purposes. They show diplobacillary morphology, are strongly positive for alkaline phosphatase, α -glutamyl- α -glutamic acid.a, ornithine.a and resorufin reduction; they haemolyse horse blood but are not lipolytic towards tributyrin or caprylate.

The satellites O8, V4 and V9 form a group that is unusual in being positive for indole production and acidifying D-

lyxose; the latter two also acidified D-xylose and V4 acidified sorbitol. Other unusual test results were as follows: V8 was histidine.a positive; V14 was positive for ornithine decarboxylase; Y3, *Kingella indologenes*, now transferred to a new genus as *Suttonella indologenes* (Dewhirst *et al.*, 1990) was (as expected) indole positive; and Z13, *Gemella haemolysans*, was haemolytic, positive for pyroglutamic acid.a but negative for alanine.a.

Distinctness of phenons and their relationships in phenetic space

Compactness and overlap of phenons. The compactness of phenons is shown by d_j in Table 2 and ranges from 0.15 for phenons 1 and 2 to over 0.24 for phenons 24 and 31 (the large value for phenon 31 is due to the small number of degrees of freedom for only two strains). These values of d_j are quite typical for bacterial species (e.g. Bridge & Sneath, 1983; Sneath & Stevens, 1985). The overlap statistics were reasonably satisfactory: only three pairs of phenons (phenons 3 and 4, 3 and 5, and 10 and 12) showed nominal overlap V_G of more than 0.1% and none were over 1%. However, because the similarity values are so high, the numbers of test differences are small relative to many studies, and this reduces the overlap when calculated by using the full number of tests (Bridge & Sneath, 1983). The main effect of reducing the number of differences between a pair of phenons (as occurs when the average interphenon similarity is high) is to increase proportionately the variance on the axis joining the centroids of the phenons. Because the overlap statistics are here applied to binary data instead of continuous variables the statistics are not straightforward and have not been worked out in detail. Applying the correction suggested by Bridge & Sneath (1983), to replace the number of characters n by $m = 2n(1 - S)$ where S is the mean interphenon similarity, leads to reducing the standard deviations on the q axis to about half for the closer pairs. This results in some pairs exhibiting a nominal overlap close to the 8% level where clusters are continuous. This is in reasonable agreement with Fig. 7 which is described below. The most overlapping phenon pairs are then 1 and 2, 3 and 4, 4 and 5, 9 and 10, and 18 and 19. The conclusion (which must be only tentative in view of these statistical limitations) is that most clusters are satisfactorily distinct except for the gonococcal phenons, the meningococcal-cinerea phenons, some of the pharyngeal phenons and the animalis-canis and branhamella-moraxella phenons.

Principal coordinate analysis. A general view of the major relationships of the phenons is shown in Fig. 7. Such an ordination does not exactly reflect the phenogram but discrepancies are small. There are a few overlaps as mentioned above.

The main areas are seen: area A is toward the back, area B is toward the left, area C is in the centre and area D toward the right (except for *Kingella kingae* which is high up and somewhat nearer the neisserias than area D). The variance of 50% accounted for by the first three axes is

Fig. 7. Principal coordinate analysis of the distances between centroids of the 31 phenons. The phenon numbering is the same as in Figs 2–5. The intercentroid distances and radii of the phenons are from Table 2. The radii were scaled by multiplying d_i by $\sqrt{(3/155)}$, so as to take account of the reduction from 155 to 3 dimensions. The first principal axes are shown with the baseplate lying at -2 on the vertical axis, III. The respective eigenvalues are 45.4, 38.5 and 18.0, corresponding to 50.2% of the total variation and n' of 8.9.

similar to that usually found in such analyses (Bridge & Sneath, 1983; Sneath & Stevens, 1985). Phenons with high metabolic activity lie toward the front and right as indicated by vigour values (Table 2). Low values on axis I are associated with phenons that are weakly positive for glucose, fructose, maltose and sucrose acidification and polysaccharide synthesis. Low values on axis II are associated with hydrolysis of Tween 60.

Single linkage clustering with S_G . This showed less structure than UPGMA, but broadly the same phenons were seen.

Vigour and pattern coefficients. The vigour of the phenons (which largely reflects the range of metabolic activity) is shown in Table 2, and varies from the less active gonococci to the more active moraxellas. The UPGMA phenogram from the pattern coefficient D_{P2} (Sackin, 1981) with cophenetic correlation of 0.74, was very similar to that from S_G (Figs 1–5). Phenons 1 and 2, however, were combined. Phenon 5 was still separated

from phenon 3, but the strains of phenon 4 were scattered among the pharyngeal strains. The pharyngeal phenons were less well separated and strains were sometimes mingled in diffuse groups. Phenons 23 and 24 were mingled.

The main difference was in the treatment of the 'elongata' phenons 7 and 26 which were partly mingled.

Cophenetic discrepancies. Most of the distortion between a similarity matrix and a phenogram is generally due to a few of the OTUs (Sneath and Sokal, 1973; p. 282), and these poorly-fitting members are commonly satellite strains or members of the most peripheral clusters (Sneath *et al.*, 1981). This behaviour was also noted in this study, and as observed earlier strains with high distortion Δ_j often showed very high or very low vigour. Distortion values were also high in phenons of area D.

Identification statistics. The percentages in Table 3 when treated as an identification matrix gave reasonably

good results when evaluated by computer programs (see Methods). The CHARSEP program showed that the following tests, many of them not traditional ones, were particularly useful for discriminating between all phenons: acidity from glucose, maltose, sucrose and fructose, the arylamidase tests proline.a, hydroxyproline.a and glycylproline.a (seryl-methionine.a, serine.a, phenylalanine.a and catalase FGN were also useful but less reproducible), growth on Tween 20 and modified New York City medium, polysaccharide synthesis, colistin sensitivity, caprylate, superoxol, haemolysis, alkaline and acid phosphatases (Zym), tetrads and DNase (5 d). The MATIDEN and OVERMAT programs showed satisfactory separation of all the phenons, except some pairs of phenons in the pharyngeal area (phenons 9–17) which have been discussed earlier.

DISCUSSION

Test reproducibility

Test reproducibility from the replicated strains was satisfactory. Replicate strains were usually close and even the most distant (H1 and H1a) showed S_G of 86 %. The properties of the better known species were in fairly close agreement with those summarized by Bøvre (1984b), Vedros (1984) and Morse & Knapp (1992). We confirmed the value of proline.a and γ -glutamyl.a for separating species of *Neisseria* (Eriquez & Hodinka, 1983). The sensitivity of the carbohydrate fermentations in API 50CH galleries seemed lower than conventional tests for strains of gonococci and meningococci (see Table 3), but for all strains there were a few instances of positive fermentations where none were anticipated.

Although the remaining 100 tests have given a satisfactory identification matrix, these are too numerous for practical identification. Nevertheless we consider that a good many more tests will be required for accurate diagnosis than the twenty or so tests of the tables in Vedros (1984) and Morse & Knapp (1992). Some promising additional tests are mentioned above.

Phenons

Area A: phenons 1–8. Marelló & Bochner (1989) have reported two subgroups within *Neisseria gonorrhoeae*, but we have not been able to collate our subgroups with theirs due to lack of information on their strains. The single strain of '*N. gonorrhoeae* var. *kochii*' (A50) was a satellite of these phenons and it is possible that it represents another subgroup of the species, though we did not find any prominent features that distinguished it from the other gonococci. In the principal coordinate analysis it was in the larger group containing phenon 1.

One strain of phenon 3, O11, seems to be mislabelled, and is from the throat; it is well known that correct identification of throat strains may be difficult. This has been investigated by Ison *et al.* (1982) who found that γ -glutamyl aminopeptidase activity or the ability to acidify

maltose was expressed by a small percentage of gonococci that had been transformed *in vitro* by DNA from strains of meningococci or *Neisseria lactamica*, respectively. They also observed that, although the majority of their meningococcal throat isolates were typical of meningococci (in that, like meningococci from cerebrospinal fluid or blood, they were positive for γ -glutamyl aminopeptidase and negative for hydroxyproline aminopeptidase), about a third displayed weak hydroxyproline aminopeptidase activity and a few strains were similar to *N. gonorrhoeae* in being negative for γ -glutamyl aminopeptidase and strongly positive for hydroxyproline aminopeptidase. Seven strains of meningococci from the throats of male homosexuals were sent to us by Dr Ison in case they showed phenotypic properties intermediate between meningococci and gonococci, but these seemed to be typical meningococci. All of these (except strains B7 and B10) clustered within phenon 3 which was predominantly composed of throat isolates but with at least three strains from cerebrospinal fluid. Phenon 5 included a cerebrospinal fluid isolate and four strains for which, unfortunately, we have no isolation details. Of the other strains from homosexuals, two seem typical of *Neisseria lactamica* and one (B7) is a satellite of phenons 9–11 (and may belong to the pharyngeal area). Only strain B10 appears unusual but it is in region D with branhamellas, moraxellas and 'false neisserias'. The isolate of Hodge *et al.* (1987) clustered with the pharyngeal isolates of phenon 9. We have, therefore, no firm evidence that any are intermediate between gonococci and meningococci, but this may be difficult to determine when these two species exhibit so few phenotypic differences, even with a large number of tests. Knapp (1988) has commented that these suspected intermediates are in any case very rare.

Phenon 5 also seems to represent the meningococcus. Serological groupings show no strong relations to phenons 3 and 5; thus serovars A and C occur in both, and Fig. 6 suggests that these phenons run together. The anomalous position of strains labelled *Neisseria cinerea*, which appear so close to the meningococci, and yet differ in fermentation of glucose and maltose and colonial appearance (Vedros, 1984), indicates the need for closer study of this species. The species is serologically heterogeneous, but reactions with anti-glutamate hydrogenase indicate that it is close to *N. meningitidis* (Berger, 1984).

Most strains received as *Neisseria polysaccharea* were in phenon 6, but replicates of an atypical strain (P2, P3) seem to be more like moraxellas (see Fig. 5). There may be grounds for separating *N. elongata* subsp. *glycolytica* (phenon 7) from *N. elongata* subsp. *elongata* (phenon 20) and raising it to species rank if more strains can be studied. Phenon 8, *N. lactamica*, is one of the better defined species; there is DNA and serological evidence that it is close to *N. meningitidis* (Berger, 1984; Riou & Guibourdenche, 1987).

Area B: phenons 9–17; the pharyngeal area. This is the most problematic area in the genus. The phenons are

borne on quite short stems (Fig. 3), and the principal coordinate diagram showed no evidence of clusters. The first three axes in the latter accounted for only 25 % of the variation, and the effective dimensionality n' was very high at 22.6. Most of the phenons had low diagnostic scores in the DIACHAR program, and the OVERMAT program showed that most of the larger overlaps were in this area. All this suggests that there is little structure in the area. None of the traditional features for separating the named species (e.g. pigmentation, mucoid growth) seemed particularly associated with species names; indeed mucoid and dry growth as expressed in epithets *mucosa* and *sicca*, seem to occur in all parts of the area '*Neisseria mucosa* var. *heidelbergensis*' does not seem distinctive, and *N. macacae* appears to belong to the area. The evidence from serology, summarized by Berger (1984) is also difficult to interpret; some pairs of species, such as *N. mucosa* and *N. sicca*, and *N. subflava* and *N. flava*, seem identical, but minor cross-reactions occur widely. We consider that on present knowledge few species of human commensals can be well defined.

Area C: phenons 18–26. This area is not sharply separated from area B (Fig. 1), and some phenons (e.g. phenon 21) are evidently close to the pharyngeal phenons (Fig. 7). Phenon 18, *Neisseria animalis*, also contains one replicate of *N. denitrificans*, H1a. The two species are evidently very similar, as noted by Vedros (1984), and *animalis* is the earlier epithet. There is insufficient evidence to justify separate species. Phenon 19 is of interest because some strains were isolated by Dr V. E. Dent from dental plaque, and other strains from this source are found in phenons 23 and 24. This may be of significance in the production of plaque and caries because of the highly aerobic metabolism of neisserias and, though they do not produce large amounts of acid from carbohydrates, they could reduce oxygen tension very rapidly, which may be a factor in plaque formation.

There seems insufficient evidence to separate the variety '*intermedia*' from the rest of *Neisseria elongata* subsp. *elongata* (phenon 20). Only a single strain designated as CDC group M-6 (*Neisseria elongata* strain I2) was included in this study and this clustered with *Neisseria elongata* subsp. *elongata* strains in phenon 20. Isolates of the M-6 group originate from the throat, sputum, or from blood, where they are often associated with endocarditis (Grant *et al.*, 1990). These authors have shown that all the M-6 strains they studied have high DNA relatedness to the *Neisseria elongata* subsp. *elongata* type strain and are distant in this respect to CDC groups M-5 (*N. weaveri*) and EF-4. They also showed that group M-6 strains differ from those of *Neisseria elongata* subsp. *elongata* in being positive for nitrate reduction and they sometimes weakly acidify glucose (group M-6 strains differ from *Neisseria elongata* subsp. *glycolytica* in being positive for nitrate reduction and catalase negative). Grant *et al.* (1990) have proposed the M-6 group as a subspecies *Neisseria elongata* subsp. *nitroreducens* because of its clinical significance and the ease with which it can be differentiated from *Neisseria elongata* subsp. *elongata*.

Phenon 21 presents a problem that we cannot resolve: whether *Neisseria subflava* and *N. flavescens* should be regarded as different species. The type strains of both occur in this phenon, but we are reluctant to reduce them to synonymy (*N. subflava* has priority) because *N. flavescens* is classically associated with meningitis, and *N. subflava* is only rarely (see however Johnson, 1983). Furthermore, the latter is reported to ferment glucose and maltose unlike *N. flavescens*. *N. flavescens* appears to be serologically distinct from *N. subflava*, but there is evidence from anti-glutamate dehydrogenase that it is close to *N. meningitidis* (Berger, 1984). In our hands neither replicate of the type strain of *N. subflava* (U38, U38a) fermented any sugars (50CH or rapid sugars). *N. flavescens* caused a limited epidemic of meningitis in Chicago (Branham, 1930), and Cowan (1974) noted 'At first none of the strains isolated in Chicago attacked any sugars but after many years in artificial culture our strains (originally received from Dr Branham), and those kept by Dr Branham herself, developed the ability to produce acid from glucose, maltose and sucrose'.

It may therefore be that carbohydrate metabolism is repressed in this species but that genetic changes allow its expression. Available evidence (Holten, 1974a, b) suggests that glucokinase, glucose 6-phosphate and 6-phosphogluconate dehydrogenase are present in all 'true' neisserias, including *N. flavescens*, *N. cinerea* and *N. elongata*. However, catabolite repression effects on enzymes involved in carbohydrate metabolism have been observed in *N. flavescens* and some other neisserias with elevated glucose (Holten, 1974a, b). Variability in neisserial sugar acidification reactions might, therefore, be minimized by the use of media with lower concentrations of sugars than would normally be appropriate for other bacteria.

The attack on sucrose by *N. flavescens* in this study suggests affinities with the pharyngeal commensals. The rarity of *N. flavescens* has been noted (Knapp, 1988) and, given the unpredictability of carbohydrate metabolism suggested for both *N. flavescens* and *N. subflava* together with their high phenetic similarity as indicated in the present study, it is possible that it occurs more frequently than has been realised. Cowan believed that *N. flavescens* had not with certainty been isolated since the Chicago outbreak, but recent isolations supported by serology have been discussed by Johnson (1983) and Berger (1984).

Phenons 22–24 appear more typical of *Neisseria* than *Moraxella*. Phenon 22 is from lizards (Plowman *et al.*, 1987) and has been proposed elsewhere as a new species, *Neisseria ignanae* (Barrett *et al.*, 1994). As noted in Results we consider that phenons 23 and 24 should be combined, and these might represent a second new species.

The acidification of trehalose and salicin, as observed in phenons 22 and 23/24 respectively, has been recorded previously for an unidentifiable species of *Neisseria* causing epidemic venereal disease in geese (Szép *et al.*, 1978; Pataky, 1974). This organism also acidified glucose, sucrose, and maltose but was not haemolytic. This

occurrence of a *Neisseria* from geese is interesting because veterinary microbiologists have often considered avian body temperature to be too high for neisserias. It is possible that these strains were variants of *N. denitrificans*. The only avian isolate in our study was *N. denitrificans* strain H2 and this was from the penis of a gander. This strain clustered with *N. animalis* (phenon 18). Unfortunately, the Hungarian strains are no longer available for study (Dr I. Szép, personal communication).

The satellite strain Z11, the type strain of *Morococcus cerebrosis*, has a distinctive cellular morphology, with mulberry clumps. The clustering of this type strain here supports the findings of Long *et al.* (1981) and their proposed inclusion of the genus *Morococcus* as a new genus within the *Neisseriaceae*.

The succeeding phenon 25 is too diffuse to recognize as a species though these strains give notably strong arylamidase reactions. Some of these arylamidase reactions appeared more indicative of *Moraxella* than *Neisseria* and phenon 25 might possibly represent neisserias that have been transformed by strains of *Moraxella* species. Whilst it is known that the 'true neisserias' are closely related genetically and are distinct from the 'false neisserias' and *Branhamella* (Catlin & Cunningham, 1961; Bøvre, 1965; Hoke & Vedros, 1982), their genetic relations with *Moraxella* do not appear to have been examined in any detail and this may be worth further study.

There are some discrepancies between our results on phenon 26 and those of Holmes & Ahmed (1981) on CDC EF-4a strains, notably that we did not observe gelatin hydrolysis (although this may simply imply less sensitivity in our method). Holmes & Ahmed also reported fermentative reactions with carbohydrates. Unlike those of EF-4a, strains of a similarly named CDC group, EF-4b, do not usually digest gelatin and do not ferment glucose or hydrolyse arginine. These groups therefore deserve closer study.

Area D: phenons 27–31; false neisserias and moraxellas. This area is reasonably distinct. Phenon 27 (*Branhamella catarrhalis*) requires no comment other than that some mislabelled strains belong to this species; its separation from the 'false neisserias' (phenons 28 and 29) on the phenogram does appear to be significant and is in agreement with the findings of Bøvre (1965) and also those of Hoke & Vedros (1982) which were based on DNA base composition, genetic transformation and DNA hybridization. The satellites that follow it may have been strains of well known species that behaved aberrantly in our tests, rather than strains resulting from genetic transformation between *Neisseria* and *Branhamella* species which, on present evidence (Catlin & Cunningham, 1961; Siddiqui & Goldberg, 1975; Hoke & Vedros, 1982), would perhaps be possible but is likely to be an extremely rare occurrence.

Phenon 28 contains the type strains of both *Neisseria caviae* and *N. cuniculi*, but they were partly separated in the

phenogram and further study may show better distinction between them, so it seems best not to reduce them to synonymy. The two species show low DNA–DNA pairing values (Véron *et al.*, 1993). Strains of *N. caviae* were stronger in alkaline phosphatase, positive for Tween 40, Tween 80 and tributyrin hydrolysis and nitrite reduction, and only *N. caviae* gave a zone of opacity around the positive clearing on Tween 85 agar. A surprising finding was the occurrence here of both replicates (U38, U38a) of the type strain of *N. flava*, and we suspect that mislabelling may have occurred, as it showed no pronounced pigmentation in our hands, and did not acidify glucose as would be expected.

The study suggests that *Neisseria ovis* (phenon 29) is better placed in the genus *Moraxella* despite the fact that *N. ovis*, like strains of phenons 1–26 (*Neisseria* spp.) and 31 (*K. kingae*), had MIC values for acetazolamide that were very much lower when grown in air compared to 5% CO₂. Strains belonging to phenons 27 (*B. catarrhalis*), 28 (*N. caviae* and *N. cuniculi*) and 30 (moraxellas including some strains received as *N. ovis*) and *M. urethralis* were unaffected by CO₂ concentration. This is in good agreement with the study of Vaneechoutte *et al.* (1988) and is also harmonious with the findings of Berger & Issi (1971) which showed saccharolytic neisserias (and *N. canis*) to be sensitive to acetazolamide in air, unlike the 'false neisserias' and *Branhamella catarrhalis* which do not produce carbonic anhydrase (Berger & Piotrowski, 1974). Acetazolamide is a potent carbonic anhydrase inhibitor that affects the growth of carbonic-anhydrase-producing *Neisseria* most significantly, presumably because of reduced formation of bicarbonate; the degree of inhibition increases with decreasing ambient CO₂ concentration (Forkman, 1968).

Phenon 30 contains the classical moraxellas and it may be noted that strains X9, X11, X12 in phenon 30 labelled *N. ovis* are, when the source is recorded, from cattle, and they seem distinct from the sheep strains in phenon 29. We do not have enough evidence to determine whether X9, X11 and X12 should be included within *Moraxella bovis*. It may be significant that strains of phenons 29 and 30 seem to be usually isolated from human or animal conjunctiva with or without conjunctival inflammation.

Phenon 31, *Kingella kingae*, is clearly distinct in both Fig. 5 and Fig. 7. The single strain of *K. indologenes* groups near it but not very closely; this is in keeping with the considerable difference in 16S rRNA sequence which led Dewhirst *et al.* (1990) to transfer *K. indologenes* to a new genus *Suttonella*. It is not surprising that the strain Z13 of *Gemella haemolysans* is also near here. It is also noteworthy that it is the only strain that was alanyl.a negative, which appears characteristic of Gram-positive organisms (Carlone *et al.*, 1983). The pair V4 and V9 from dental plaque may represent an undescribed species: they are unique in being indole positive (except for the nearby strains Y3 and O8 which are also indole positive). The pair is also characterized by attacking ribose, D-xylose, galactose, mannose, mannitol and N-acetylglucosamine,

unlike any other strains. They differ from phenon 30 in being cocci, and are haemolytic on both horse and sheep blood, but resemble phenon 30 in showing a strong alkaline phosphatase reaction.

In general the grouping of these highly similar organisms is reasonably satisfactory. Host species, site of isolation and host trophic group (carnivore, herbivore or omnivore) are remarkably consistent within individual phenons (see Table 1), and this may also indicate a high level of naturalness for the proposed taxonomy. Evidence from serology and DNA supports the grouping of species into area A (Berger, 1984; Riou & Guiboudenché, 1987). Riou & Guiboudenché (1987) reported that *N. polysaccharea* shows DNA–DNA pairing values of over 69% to *N. gonorrhoeae*, *N. meningitidis* and *N. lactamica*, and low values of 29–40% with *N. flava*, *N. subflava*, *N. perflava* and *N. sicca*. This supports our finding that *N. polysaccharea* lies in area A. They report that *N. cinerea* showed 50–59% pairing to *N. gonorrhoeae* and to *N. polysaccharea*, which is in keeping with our placement of *N. cinerea* in area A. The analysis of electrophoretic banding patterns by Chun *et al.* (1985) also indicated that *N. cinerea* was close to *N. gonorrhoeae* and *N. meningitidis*, which were highly similar. Hoke & Vedros (1982) found that *N. gonorrhoeae* and *N. meningitidis* had 93% similarity by DNA–DNA pairing, but Rossau *et al.* (1989) found a lower value of 74%. Rossau *et al.* (1989) also found that the DNA–DNA pairing of *N. flavescens* and *N. perflava* (*N. subflava* in their paper) was 71%, but *N. flavescens* showed lower values of 31–36% when compared with *N. meningitidis*, *N. lactamica* and *N. mucosa*. This suggests that *N. flavescens* should be in the pharyngeal area B, and is misplaced in our study in area C. This is further supported by their DNA–rRNA diagrams and by the 16S rRNA sequence data of Anderson *et al.* (1993a), which shows *N. flavescens* linked to a group comprising *N. gonorrhoeae*, *N. polysaccharea* and *N. macacae*.

The rRNA–DNA data of Rossau *et al.* (1989) show three regions that correspond largely to our major areas. One region comprises organisms of our areas A and B, containing *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. mucosa*, *N. perflava* (referred to there as *N. subflava*), *N. flavescens* and *N. sicca*. Their second region contains organisms from our area C, including *N. animalis*, *N. canis*, *N. elongata* subsp. *elongata*, group EF-4 and *N. denitrificans*, but also *N. elongata* subsp. *glycolytica*, which is in area A in our study. This subspecies may be misplaced in area A, and this is supported by the finding of Grant *et al.* (1990) that the subspecies of *N. elongata* show high mutual DNA–DNA pairing values of 75–79%. *N. canis*, *N. animalis* and M-5 formed a cluster in the 16S rRNA diagram of Anderson *et al.* (1993a). The third region contained the moraxellas and false neisserias of our area D (*Moraxella bovis*, *N. ovis*, *N. cuniculi*, *N. caviae* and *Branhamella catarrhalis*).

The relationships among the true neisserias, false neisserias and moraxellas is also broadly similar to those from electrophoretic banding patterns (Chun *et al.*, 1985), genetic and DNA studies (Catlin & Cuningham, 1961;

Bøvre & Hagen, 1981; Hoke & Vedros, 1982) and from fatty acid profiles (Véron *et al.*, 1993).

The electrophoretic patterns studied by Chun *et al.* (1985) showed that species in our pharyngeal area formed two loose clusters together with *N. meningitidis* and *N. gonorrhoeae*, but *N. canis* and *N. animalis* were a third cluster. *N. caviae*, *N. ovis* and *Branhamella catarrhalis* formed a fourth cluster. There was, however, a strain (which we did not study) of *N. cuniculi* among the true neisserias. Rossau *et al.* (1991) found that the DNA–DNA pairing between strains of *Moraxella bovis*, *N. caviae*, *N. ovis*, *N. cuniculi* and *Branhamella catarrhalis* were in the range of 75–80%, which is in keeping with the close phenotypic similarities we observed.

Most classical species have emerged except in the doubtful pharyngeal region. The number of unclustered satellites is not very great. In a group where strain contamination readily occurs there is also the risk of mixed cultures, but the behaviour of the replicates that were made and the laboratory investigations of unusual test results suggests that contamination was not a major problem.

The distinctness between the 'true neisserias' on the one hand and the 'false neisserias', branhamellas and moraxellas on the other is well supported (Fig. 1, Fig. 7). The present study does not suggest a major difference between the branhamellas and moraxellas, though *Kingella kingae* is very different from the rest. These phenotypic relationships are in good accord with the nucleic acid evidence of Rossau *et al.* (1989, 1991).

We have noted that it is urgent to check carefully the type strains of *Neisseria* species. The *Neisseria* subcommittee of the International Committee on Systematic Bacteriology for this genus might be encouraged to take action here.

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