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Structures of and Allelic Diversity and Relationships among the Major Outer Membrane Protein (*ompA*) Genes of the Four Chlamydial Species†

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DNA sequences coding for 81% of the *ompA* gene from 24 chlamydial strains, representing all chlamydial species, were determined from DNA amplified by polymerase chain reactions. Chlamydial strains of serovars and strains with similar chromosomal restriction fragment length polymorphism had identical *ompA* DNA sequences. The *ompA* sequences were segregated into 23 different *ompA* alleles and aligned with each other, and phylogenetic relationships among them were inferred by neighbor-joining and maximum parsimony analyses. The neighbor-joining method produced a single phylogram which was rooted at the branch between two major clusters. One cluster included all *Chlamydia trachomatis ompA* alleles (trachoma group). The second cluster was composed of three major groups of *ompA* alleles: psittacosis group (alleles MN, 6BC, A22/M, B577, LW508, FEPN, and GPIC), pneumonia group (*Chlamydia pneumoniae* AR388 with the allele KOALA), and polyarthritis group (ruminant and porcine chlamydial alleles LW613, 66P130, L71, and 1710S with propensity for polyarthritis). These groups were distinguished through specific DNA sequence signatures. Maximum parsimony analysis yielded two equally most parsimonious phylograms with topologies similar to the *ompA* tree of neighbor joining. Two phylograms constructed from chlamydial genomic DNA distances had topologies identical to that of the *ompA* phylogram with respect to branching of the chlamydial species. Human serovars of *C. trachomatis* with essentially identical genomes represented a single taxonomic unit, while they were divergent in the *ompA* tree. Consistent with the *ompA* phylogeny, the porcine isolate S45, previously considered to be *Chlamydia psittaci*, was identified as *C. trachomatis* through biochemical characteristics. These data demonstrate that chlamydial *ompA* allelic relationships, except for human serovars of *C. trachomatis*, are cognate with chromosomal phylogenies.

Chlamydia represents the single genus of the family *Chlamydiaceae* and the order *Chlamydiales*. It is currently composed of four species, *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia pneumoniae*, and *Chlamydia trachomatis* (16, 18, 33). These bacteria depend on energy from eucaryotic cells and are characterized by their unique developmental cycle. The extracellular elementary bodies reorganize into fragile reticulate bodies upon entry into the host cell. These reticulate bodies divide within membrane-bound host cell compartments by binary fission and condense into a new generation of infectious elementary bodies.

Chlamydiae cause clinically inapparent infections and a variety of diseases in humans, marsupials and other mammals, and birds. In humans, the most notable are trachoma and urogenital infections due to *C. trachomatis*, respiratory infections due to *C. pneumoniae*, and psittacosis caused by *C. psittaci* (44). In animals, *C. psittaci* and *C. pecorum* are capable of inducing diverse disease syndromes like pneumonia, enteritis, abortion, urogenital infections, mastitis, polyarthritis, polyserositis, encephalitis, and conjunctivitis (52).

The four species can be readily distinguished. *C. trachomatis* is characterized through the presence of glycogen within oval, vacuolar intracellular inclusions and growth inhibition by sulfadiazine (33). Chlamydiae lacking these properties and possessing pleomorphic, dense inclusions are classified as *C. psittaci* and *C. pecorum* (16, 33). The only exception is *C. pneumoniae*, which follows the pattern of *C. psittaci* but exhibits unique seroreactivity and apparently pear-shaped elementary bodies (8). Serovars of *C. trachomatis* are well established. Isolates of *C. pneumoniae* appear to be antigenically uniform (6). Subspecies differentiation within the heterogeneous species *C. psittaci* and *C. pecorum* has been attempted on the basis of inclusion morphology and effect of cytoactive agents (49), plaque neutralization (45, 46), microimmunofluorescence (11, 36), and monoclonal antibodies (1, 14, 55) and by genetic approaches such as restriction fragment length polymorphism (RFLP) analysis of chlamydial chromosomal DNA (6, 29, 56), the presence and properties of plasmids (27, 30, 57), and DNA-DNA hybridization (8, 15).

Chlamydiae were proposed as a separate eubacterial division with a possible distant relationship to the genus *Planctomyces* (60). Phylogenetic relations within the genus are poorly understood. The currently established chlamydial species share less than 10% chromosomal DNA similarity (8).

The gene encoding the major outer membrane protein (MOMP), *ompA* (64), is about 68% identical between *C. psittaci* and *C. trachomatis* (67) and between *C. pneumoniae* and *C. trachomatis* and about 71% identical between *C. psittaci* and *C. pneumoniae* (32). MOMP functions as a redox-gated porin molecule and contributes through intra-

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TABLE 1. Chlamydial strains used in partial *ompA* DNA sequencing

Chlamydial strain	Sequence identical to *ompA* allele:	Biovar[a]	Serovar[b]	RFLP type[c]	Host	Host clinical condition(s)	Reference
C. psittaci							
BMZ1121	MN				Cattle	Mastitis	23
6BC				Avian 1c	Parakeet	Systemic infection	33
B577		1	1	Avian 2b	Sheep	Abortion	36
SV139	B577	1	1		Cattle	Seminal vesiculitis	36
wt parakeet	B577				Parakeet	Systemic infection	22
LW508		1	1		Cattle	Enteritis	36
FEPN		7	7		Cat	Live vaccine	36
FEPN Pring	FEPN				Cat	Conjunctivitis	62
FEPN Baker	FEPN			Feline 1	Cat	Pneumonia	3
KOALA					Koala	Keratoconjunctivitis	17
C. pecorum							
LW613		2	2		Cattle	Polyarthritis	36
LW623	LW613	2	2		Cattle	Encephalomyelitis, polyarthritis	36
FcStra	LW613	2	2		Cattle	Conjunctivitis, polyarthritis	36
IPA	LW613			Ruminant 1b	Sheep	Polyarthritis	35
E58	LW613			Ruminant 1c	Cattle	Encephalitis	31
L14	LW613		6		Cattle	Pneumonia	36
JP1751	LW613		9		Sheep	Intestinal infection	36
66P130		3	3		Cattle	Intestinal infection	36
L71		4	4		Swine	Polyarthritis	36
1710S		4	6		Swine	Abortion	36
1708	1710S	4	6		Swine	Pneumonia	36
C. pneumoniae							
AR388					Human	Respiratory infection	6
C. trachomatis							
MOPN		Mouse		Mouse	Mouse	Pneumonia	34
S45					Swine	Inapparent intestinal infection	36

[a] Biovars were determined by Spears and Storz (49).
[b] Serovars were determined by Perez-Martinez and Storz (36).
[c] RFLP types were determined by Fukushi and Hirai (15).

and intermolecular disulfide bonds to the structure of the chlamydial envelope (4). Four evenly spaced variable domains (VD), which determine the serovars of *C. trachomatis*, interrupt highly conserved regions (2, 50).

Macromolecules conserved throughout a genus such as MOMP can serve as molecular chronometers of evolutionary distances within bacterial genera (26, 63). Phylogenetic relationships between taxa can be inferred from variations in the aligned protein or DNA sequences of such chronometric proteins.

The *ompA* sequences of 24 chlamydial strains were determined from DNA amplified in polymerase chain reactions (PCR). These gene sequences were aligned with previously published sequences into 23 distinct *ompA* alleles. Neighbor-joining (NJ) and maximum parsimony analyses were used to ascertain the evolution of the chlamydial *ompA* locus and its relation to the chromosomal genealogy of chlamydial species.

(This paper contains parts of a dissertation presented by B.K. to the Graduate School of Louisiana State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.)

MATERIALS AND METHODS

Chlamydial strains. Twenty-one strains of *C. psittaci* and *C. pecorum* (representing at least five biovars, seven serovars, and five RFLP types), two strains of *C. trachomatis*, and one strain of *C. pneumoniae* were used to determine the partial DNA sequence of their *ompA* locus. The origins of these isolates, DNA sequence identity among them as found in this investigation, and other relevant information are summarized in Table 1.

All chlamydial strains except *C. pneumoniae* AR388 and *C. psittaci* KOALA were propagated in developing chicken embryos as described previously (36). Strain S45 was also cultivated in L-cell monolayer cultures and examined for the presence of glycogen within the inclusions (33).

Crude chlamydial DNA was extracted from infected-yolk-sac homogenates as described earlier (22). Purified DNAs from *C. pneumoniae* AR388 and from *C. psittaci* KOALA were kind gifts of L. A. Campbell, University of Washington, Seattle, and Adeeb A. Girjes, University of Queensland, Brisbane, Australia, respectively.

PCR amplification of chlamydial *ompA* DNA fragments. Chlamydial *ompA* DNA fragments were amplified from 10 to 100 ng of crude chlamydial DNA by PCR (42). A schematic representation of the *ompA* locus including the relative positions of oligonucleotide primers used in PCR and DNA sequencing is presented in Fig. 1, and the primers are listed in Table 2. Oligonucleotide primers were synthesized in our laboratory on a GenePlus Synthesizer (Pharmacia-LKB, Piscataway, N.J.) by phosphoramidite chemistry and used without further purification. With the exception of 5GPF and 3GPB, these oligonucleotide primers hybridized to chlamydial *ompA* DNA sequences conserved throughout the genus. Primers 5GPF and 3GPB were used to amplify the *ompA*

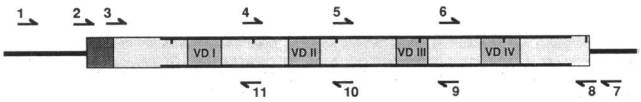

FIG. 1. Schematic representation of the chlamydial *ompA* locus indicating the relative location of the oligonucleotide primers used in PCR and DNA sequencing. The translated region of the *ompA* locus is boxed, and the leader peptide is darkly shadowed. Four VD are interspersed among highly conserved regions. Vertical bars mark every 200 nucleotides of the translated region. Numbered oligonucleotide primers are drawn to scale only with 3′ ends. The translated *ompA* region which was sequenced in all strains is indicated with bold lines. The *ompA* genes of strains BMZ1121, 6BC, B577, wt parakeet, SV139, LW508, FEPN, FEPN Pring, and FEPN Baker were amplified by PCR by using *C. psittaci* subspecies-specific primers 1 and 7 (5GPF and 3GPB). Other *ompA* genes were amplified with *Chlamydia* genus-specific primers 2 and 8 (−20CHOMP and CHOMP371). Plus-stranded DNA for sequencing was produced by PCR by using primers 1 and 2, respectively, and minus-stranded DNA was produced with primer 7 or 8. Genus-specific primers 3, 4, 5, and 6 (9CHOMP, 119CHOMP, 191CHOMP, and 277CHOMP, respectively) were employed for sequencing minus-stranded *ompA* DNA fragments, and genus-specific primers 8, 9, 10, and 11 (CHOMP371, CHOMP271, CHOMP185, and CHOMP111, respectively) served in confirmatory sequencing of positive-stranded *ompA* DNA.

genes of strains BMZ1121, 6BC, B577, wt parakeet, SV139, LW508, FEPN (feline pneumonitis), FEPN Pring, and FEPN Baker (22). Other chlamydial strains were amplified with the degenerate and inosine-containing oligonucleotide primers −20CHOMP and CHOMP371, which yielded a DNA fragment of approximately 1,200 bp.

PCR were performed with 2.5 U of *Taq* DNA polymerase (GIBCO-BRL, Gaithersburg, Md.) in 100-μl reaction mixtures containing 0.01% bovine serum albumin and 0.1% Tween 20 in 20 mM Tris-HCl (pH 8.3)–25 mM KCl–2 mM $MgCl_2$–200 μM (each) deoxynucleoside triphosphate and with each primer at 0.2 μM. The samples were overlaid with mineral oil and subjected to 30 cycles of 1 s at 96°C, 1 min at 59°C (primers 5GPF and 3GPB) or 1 min at 42°C (primers −20CHOMP and CHOMP371), and 1 min at 72°C in a programmable DNA Thermal Cycler (Eppendorf, Fremont, Calif.). A final incubation at 72°C for 7 min was performed, and the reaction mixtures were analyzed by 1.5% agarose gel electrophoresis.

Production of single-stranded DNA and dideoxy-DNA sequencing. Single-stranded DNA of the amplified *ompA* DNA fragments for DNA sequencing was produced in a modification of the asymmetric PCR (24). This method differed in that only one of the original primers was used at 0.4 μM, and incubation at 72°C was performed for 2 min. Ten-microliter aliquots of previous PCR containing the amplified *ompA* DNA fragment were used for the production of single-stranded DNA. The number of cycles varied depending on the primer: 18 cycles for CHOMP371, 20 cycles for 5GPF, 25 cycles for 3GPB, and 30 cycles for −20CHOMP. A final incubation was not performed.

Single-stranded DNA was purified by centrifugal ultrafiltration and sequenced with Sequenase 2.0 (United States Biochemical Corp., Cleveland, Ohio) as described earlier (24). The minus-sense, single-stranded DNA of all *ompA* fragments was sequenced, and positive-sense single-stranded DNAs served as sequencing controls.

Sequence alignment and dissimilarity matrices. In addition to partial *ompA* DNA sequences obtained in this investigation, we used for phylogenetic inference previously published sequences (38, 67) of *C. psittaci* MN (meningopneumonitis), A22/M (avian RFLP type), and GPIC (guinea pig inclusion conjunctivitis); *C. trachomatis* A, B, C, E, F, H, L1, and L2 (2, 19, 37, 39, 50, 66); and VD sequences of *C. trachomatis* Ba, D, G, I, J, K, and L3 (65). The *ompA* sequences were aligned by pairwise comparison on the peptide level to ensure correct alignment of functionally homologous residues. The University of Wisconsin Genetic Computer Group software package, version 6.1 (9), was used to align the deduced MOMP peptides, corresponding to nucleotides 184 to 1170 of *C. psittaci* MN (67). Gaps were introduced in the nucleotide sequences according to the aligned peptide sequences. The final alignment included 1,068 putatively homologous nucleotide positions (Fig. 2), translating into 356 amino acids (Fig. 3).

Dissimilarities between *ompA* DNA and MOMP peptide sequences were calculated as 100 minus 100 times the length of the aligned sequences reduced by the number of positions with gaps and mismatches divided by the average sequence length without gaps of the two sequences compared (9). The dissimilarity matrices of chlamydial chromosomal DNAs were compiled from the data of Cox et al. (8) and Fukushi and Hirai (15). Averages of the genomic DNA relations between human strains of *C. trachomatis* were calculated by using additional data (25, 61).

The deduced amino acid sequences were analyzed for hydrophilicity and amphipathicity in the software package

TABLE 2. Oligonucleotide primers used in PCR amplification and DNA sequencing of the chlamydial *ompA* genes

No.	Designation[a]	Sequence	Position[b]
1	5GPF	5′ ACGCATGCAAGACACTCCTCAAAGCC 3′	−146–−121
2	−20CHOMP	5′ TTAGAGGT(AG)AG(AT)ATGAA(AG)AA 3′	−12–8
3	9CHOMP	5′ GCI(CT)TGCCTGTIGGGAA(CT)CCIGCIGA(AG)CC 3′	64–92
4	119CHOMP	5′ TGGGATIGITT(TC)GAI(AG)TITT(CT)TG(CT)AC 3′	397–422
5	191CHOMP	5′ GCI(CT)TITGGGA(AG)TG(CT)GGITG(CT)GCIAC 3′	613–638
6	277CHOMP	5′ CCITA(CT)AT(ACT)GGIGTIAAITGG 3′	877–897
7	3GPB	5′ ACGAATTCCTAGGTTCTGATAGCGGGAC 3′	+60–+33
8	CHOMP371	5′ TTAGAAIC(GT)GAATTGIGC(AG)TTIA(TC)GTGIGCIGC 3′	+3–1177
9	CHOMP271	5′ CCAITTIACICC(AGT)AT(AG)TAIGG 3′	897–877
10	CHOMP185	5′ CCIA(AG)IGTIGC(AG)CAICC(AG)CATTCCCA 3′	644–619
11	CHOMP111	5′ GTICAGAAIA(TC)ITC(AG)AAICG(AG)TCCCA 3′	422–397

[a] OMP, outer membrane protein gene; CH, *Chlamydia*. The number of the primer designation indicates the codon of *C. psittaci* MN (67) at the 3′ end of the primer, and the position of the number left or right of the letters identifies sense or antisense priming, respectively.

[b] Position of the primer (5′ to 3′) on the *ompA* gene of *C. psittaci* MN. − and +, 5′ and 3′ nontranslated regions of the *ompA* locus, respectively.

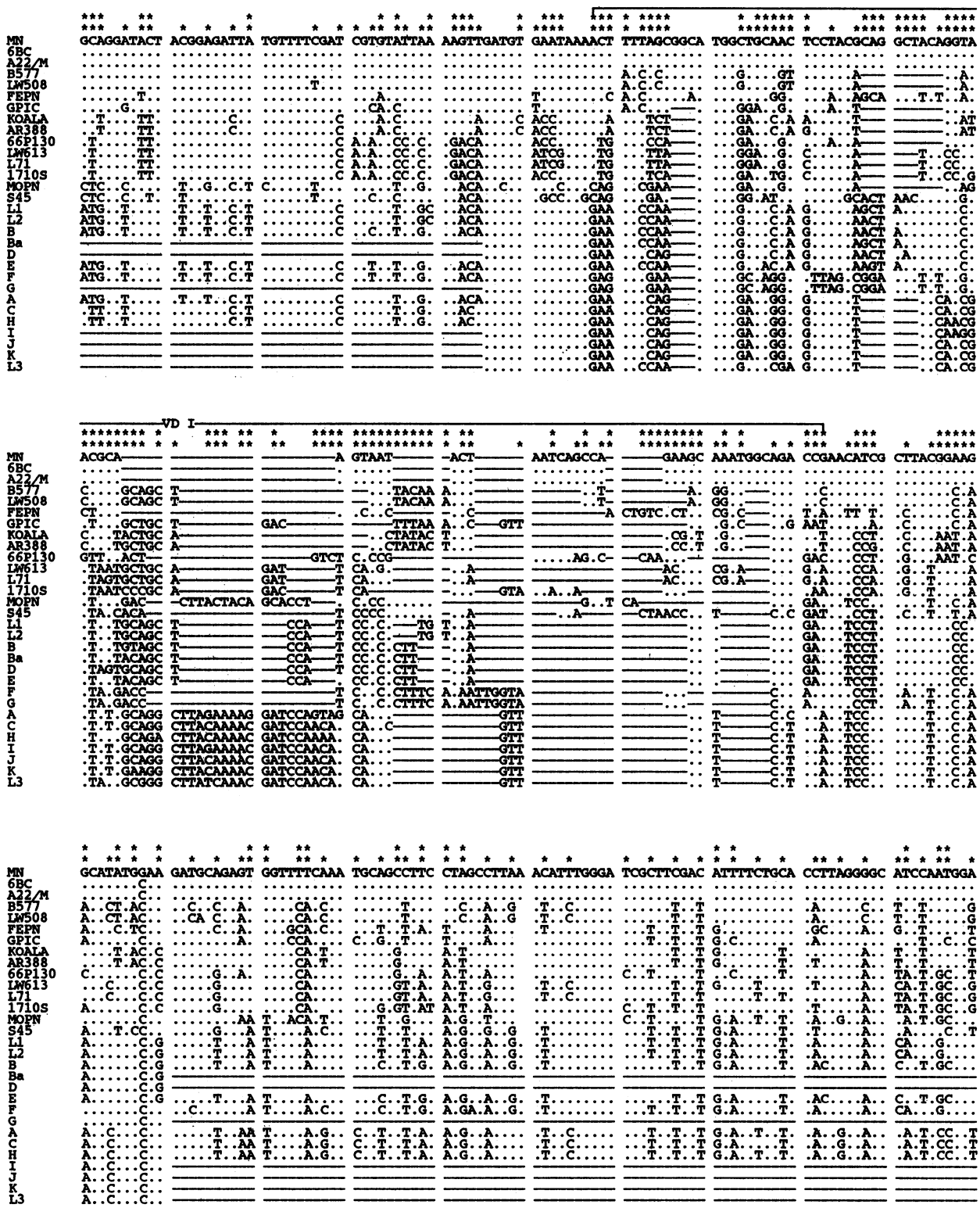

FIG. 2. Alignment of nucleotide sequences of the partial chlamydial *ompA* locus. Sequences were translated and pairwise aligned as peptide sequences by using the University of Wisconsin Genetic Computer Group software package. These alignments including gaps were transferred to the corresponding nucleotide sequences. Dots represent nucleotides of aligned sequences identical to the MN sequence; letters represent deviating nucleotides, and hyphens or solid lines indicate gaps. VD are marked by horizontal brackets above the MN sequence. Nonsynonymous, phylogenetically informative positions used in maximum parsimony analysis of the *ompA* locus are identified by two asterisks above the MN sequence, and synonymous informative sites are identified by a single asterisk.

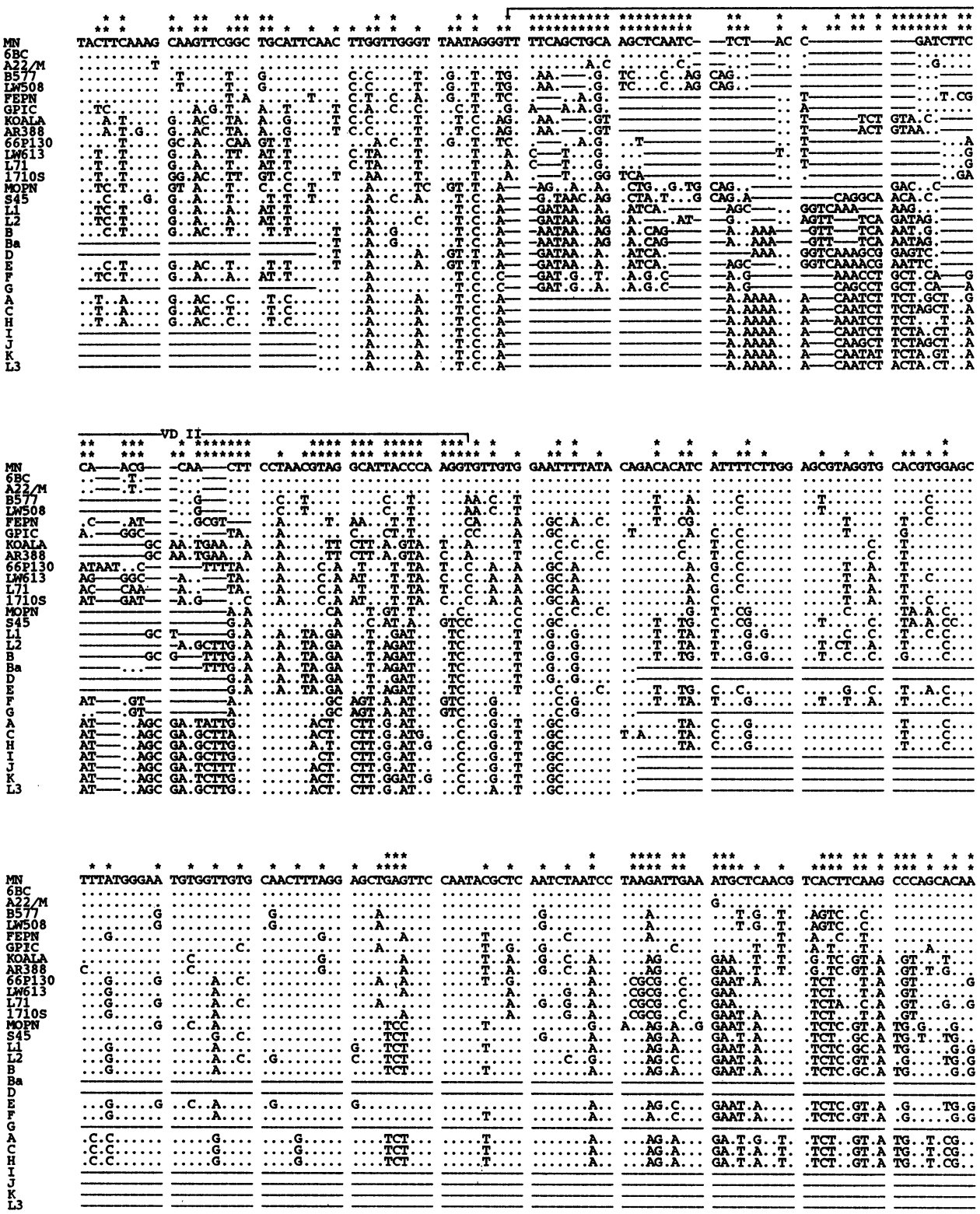

FIG. 2—*Continued.*

MacVector (International Biotechnologies, New Haven, Conn.).

Phylogenetic inference. In addition to partial *ompA* DNA sequences of *C. psittaci*, *C. pecorum*, *C. pneumoniae*, and *C. trachomatis* MOPN and S45, the complete known sequences of human *C. trachomatis* A, B, C, E, F, H, L1, and L2 were also used for the construction of phylograms. The chlamydial *ompA* and genomic DNA dissimilarity matrices

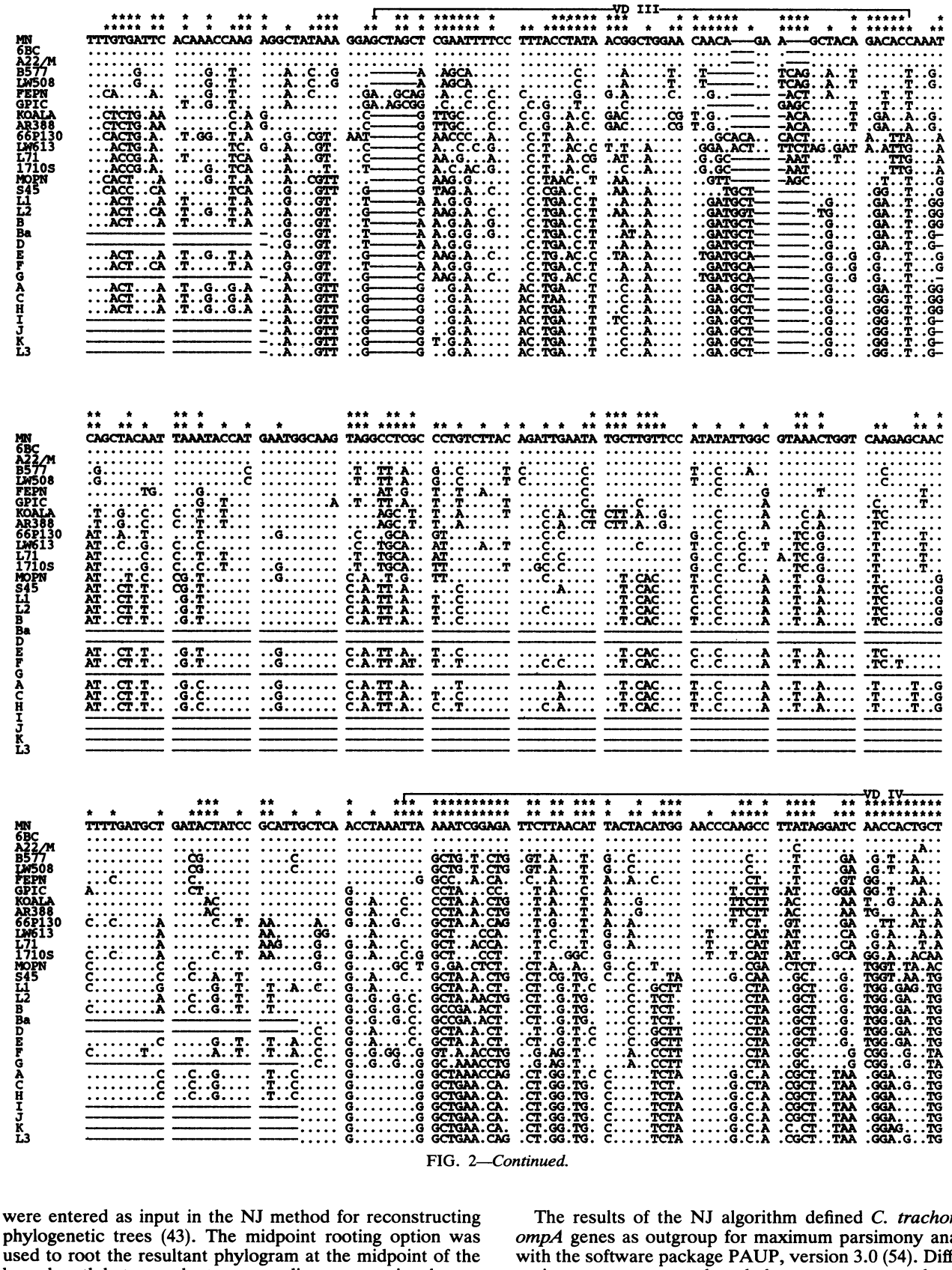

FIG. 2—*Continued.*

were entered as input in the NJ method for reconstructing phylogenetic trees (43). The midpoint rooting option was used to root the resultant phylogram at the midpoint of the branch path between the two most distant operational taxonomic units (OTU) (41).

The results of the NJ algorithm defined *C. trachomatis ompA* genes as outgroup for maximum parsimony analysis with the software package PAUP, version 3.0 (54). Different options to construct the phylograms were employed to minimize the chances of suboptimal results due to idiosyn-

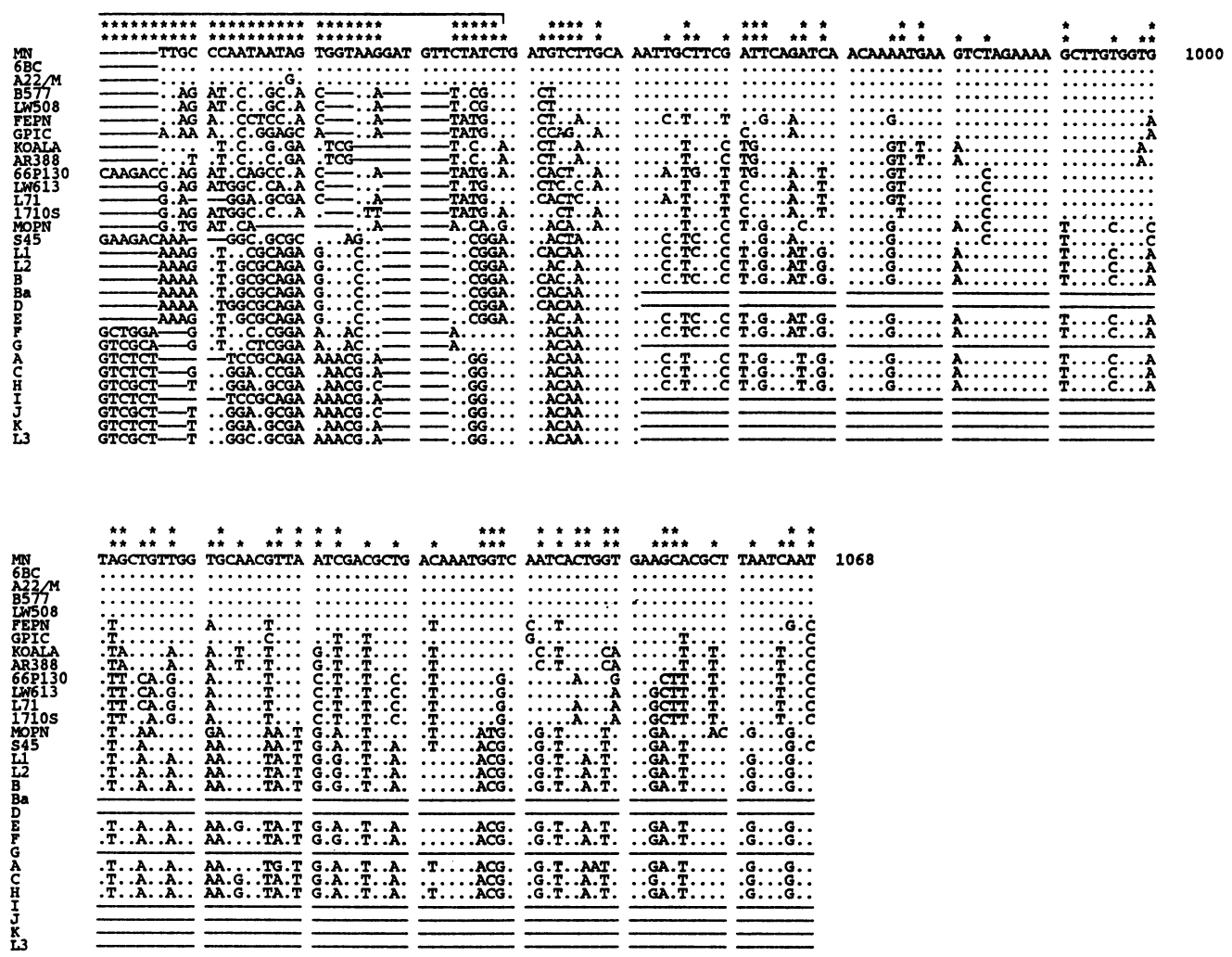

FIG. 2—*Continued.*

crasies of the algorithms. Phylograms were constructed by using 598 informative sites (variable sites where at least two OTU potentially share a derived nucleotide), 419 nonsynonymous character changes, or 179 synonymous changes.

We attempted to construct a phylogram of the species *C. trachomatis* based on the results of the phylogram of the genus. Data about *ompA* constant regions were missing for *C. trachomatis* serovars Ba, D, G, I, J, K, and L3. These regions were therefore excluded (positions not analyzed were 1 to 45, 211 to 327, 463 to 621, 700 to 825, and 952 to 1068). MOPN was specified as outgroup to polarize the character states.

Nucleotide sequence accession number. The GenBank accession numbers of 11 *ompA* sequences are M73033, M73034, M73035, M73036, M73037, M73038, M73039, M73040, M73042, M73043, and M73044.

RESULTS

Structure of the chlamydial *ompA* loci. Analysis of *ompA* DNA and deduced peptide sequences representing approximately 81% of the protein coding region revealed the presence of all salient features of the chlamydial *ompA* locus described previously (2, 7, 12, 19, 21, 32, 37–39, 50, 51, 65–67). Four VD interrupt highly conserved regions of the *ompA* genes at precisely the same locations as found earlier. We repositioned the 3′ end of VD III 12 nucleotides in the 3′ direction (positions 696 and 232 in Fig. 2 and Fig. 3, respectively) because of substantial dissimilarity of *ompA* alleles LW613 and 66P130 to other *ompA* alleles. Conserved regions between VD had identical lengths in all *ompA* genes, while VD differed in length. We sequenced the complete *ompA* coding region of strains BMZ1121, 6BC, B577, wt parakeet, and FEPN (data not shown) and found that the lengths of 5′ and 3′ terminal regions were conserved. Thus, the DNA sequence between the start codon and the first nucleotide in Fig. 2 was 183 bp long, and the sequence between nucleotide 1068 in Fig. 2 and the 3′ end of the protein coding region of the *ompA* locus including the stop codon was 39 bp long.

Cysteines at positions 93, 171, 173, and 332 (Fig. 3) were present in all chlamydial strains analyzed. The cluster of cysteines at positions 26, 29, and 33 in the *C. psittaci* MN *ompA* gene (67) was conserved in all *ompA* alleles (data not shown). A cysteine residue at position 196 (Fig. 3), previously identified only in *C. trachomatis*, was found in MOMP peptides of the *ompA* allele KOALA, in *C. pneumoniae* AR388, and in *C. trachomatis* MOPN and S45. Additional

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                                                  VD I
MN      AGYYGDYVFD RVLKVDVNKT FSGMAATPTQ ATGNA------ ---SN--T-- NQP--EANGR PNIAYGRHME DAEWFSNAAF LALNIWDRFD IFCTLGASNG   100
6BC     .......... .......... .......... ------.--- ---.--.--- ...--.---- .......Q.. .......... .......... ..........
A22/M   .......... .......... .......... ------.--- ---.--.--- ...--.---- .......Q.. .......... .......... ..........
B577    .......... .......... IT..G.V..- --.T.AA--- ------.YK- --.---TD-. ......K.LQ ....T..... .......... ..........
LW508   .......... .......... IT..G.V..- --.T.AA--- ------.YK- --.---TD-. ......K.LQ .T...T.... .......... ..........
FEPN    ..F....... .I........ I....A..A .S.T.------ ------.--- ---TVA.D-. S.F...K.LQ ....CT...Y .......... V.........
GPIC    .......... .I........ I.-.GTA..- --...AA--- D---FK.-V -------.D-. N....K..Q ....ST.... .......... V.........
KOALA   ..F....... .I....AP.. ..-.G.K..- --.S.TA--- ------.YT- ------AVD-. ..P..NK.LH .....T..G. I......... V.........
AR388   ..F....... .I....AP.. ..-.G.K..- --.S.AA--- ------.YT- ------AVD-. ..P..NK.LH .....T..G. I......... V.........
66P130  V.F....... ....T..P.M .P-.G.A..- --.SST---- ---V.P------ ..A-Q.---- D.P..NT..H .....T..GY I......... .......TS.
LW613   V.F....... ....T..S.M .L-.GTA..- -SP.NAA--- D---.S---.-- -------T.E-. A.P...K..H .....T..GY I......... V......TS.
L71     V.F....... ....T..S.M .L-.GTA..- -SP.SAA--- D---.---.-- -------T.E-. A.P...K..H .....T..GY I......... .......TS.
1710s   V.F....... ....T..P.M ..-.G.A..- -SPDNPA--- D---.----.-V K.------.-- Q.P...K..H .....T..GY I......... .......TS.
MOPN    L.....F... ....T....Q .E-.G.A..- ---.D.D-LTT AP-TP------ ---AS------ E.P...K..Q ...MYT.... M......... V......TS.
S45     L......... ....T..A.Q .D-.GD..ST N...TT----- ----.P---.-- -.---LT.--- D.P...K.FQ ...M.T.... M......... V......T..
L1      M.....F... ....QT...E .Q-.G.K..A T....AA--- -P-.T-C---.-- -------.--- E.P......Q ...M.T...Y M......... V......TS.
L2      M.....F... ....QT...E .Q-.G.K..T .....AA--- -P-.T-C---.-- -------.--- E.P......Q ...M.T...Y M......... V......TS.
B       M.....F... ....T....E .Q-.G.K..T T....VA--- -P-.TL--.-- -------.--- E.P......Q ...M.T...C M......... V.......S.
Ba      ********** ******....E .Q-.G.K..A T....TA--- -P-.TL--.-- -------.--- E.P......Q ********** ********** **********
D       ********** ******....E .Q-.G.K..T D...SAA--- -P-.TL--.-- -------.--- E.P......Q ********** ********** **********
E       M.....F... ....T....E .Q-.GDK..S T....TA--- -P-TTL--.-- -------.--- E.P......Q ...M.T...C M......... V.......S.
F       M.....F... ....T....E .E-.GEALAG .S..TT----- ----.TLSKLV -------.--- T.P...K..Q ...M.T...C MT........ V......TS.
G       ********** ******....E .E-.GEALAG .S..TT----- ----.TLSKLV -------.--- T.P...K..Q ********** ********** **********
A       M.....F... ....T....E .Q-.G.A..- -.SDVAGLEK DPVA.-----V -------.--- ..P...K..Q ...M.T...Y M......... V......TT.
C       V.....F... ....T....E .Q-.G.A..- -.SDVAGLQN DPTT.-----V -------.--- ..P...K..Q ...M.T...Y M......... V......TT.
H       V.....F... ....T....E .Q-.G.A..- -.ND.ADLQN DPKT.-----V -------.--- ..P...K..Q ...M.T...Y M......... V......TT.
I       ********** ******....E .Q-.G.A..- -.KDVAGLEN DPTT.-----V -------.--- ..P...K..Q ********** ********** **********
J       ********** ******....E .Q-.G.A..- -.SDVAGLQN DPTT.-----V -------.--- ..P...K..Q ********** ********** **********
K       ********** ******....E .Q-.G.A..- -.SDVEGLQN DPTT.-----V -------.--- ..P...K..Q ********** ********** **********
L3      ********** ******....E .Q-.G.E..- -.SDTAGLSN DPTT.-----V -------.--- ..P...K..Q ********** ********** **********

                                                  VD II
MN      YFKASSAAFN LVGLIGFSAA SSI-S-T---- -DLP-T-Q-L PNVGITQGVV EFYTDTSFSW SVGARGALWE CGCATLGAEF QYAQSNPKIE MLNVTSSPAQ   200
6BC     .......... .......... ..-.-.---- -...-M-.-. .......... .......... .......... .......... .......... .......... ..........
A22/M   ...S...... .........T ..T-.-.---- -E..-M-.-. .......... .......... .......... .......... .......... .......... V.........
B577    .......... ......VK-G ...AA----- -.------.-. .......I.. .....T.... .......... .......... .......... .....V.....
LW508   .......... ......VK-G ...AA----- -.------.-. .......I.. .....T.... .......... .......... .......... .....V.....
FEPN    .......D.. .....L.-.G ------.--- -.FA-N-.R- ...E.S..I. L....A.... .......... .......... .......... .....T.....
GPIC    .L..NA.... ....L.V-TG ------.--- -..Q-G-.-Y ...A.S..L. L....T.... .......... .......... .......... ....I...T.
KOALA   .I.GN.T... ....F.VK-G ------.--S VA-----ANE. ...SLSN... L......... .......... .......... .....K..V. E...ICNV..
AR388   .IRGN.T... ....F.VK-G ------.--T VN-----ANE. ...SLSN... L......... .......... .......... .....K..V. E...ICNVS.
66P130  ...GN.KS.. .I....L-.G ------.--- ---.NN.---FY ..AS.SN... L....T.... .......... .......... .....K.RVQ E...L.NV..
LW613   ...GN.SS.. .I....I-SG ------S--- ---.E-G-K-Y ..AN.SN... L....T.... .......... .......... .....K.RVQ E...L.NV..
L71     ...GN..S.. .I....I-SG ------.--- ---.D-Q-K-Y ..AS.SN... L....T.... .......... .......... .....K.RVQ E...L.NV..
1710s   ...GN.SS.. .I....I-SG ------.--- ---.N-D-K-. ..AN.SN... L....T.... .......... .......... .....K.RVQ E...L.NV..
MOPN    .L.GN..... ....F.-RDE TAVAA-----D. -------I ...SLS.A.. L....A.A. .....A.... .......S. .....K..V. E...LCNA.E
S45     .L.GN..S.. ....F.-ANE TT-AA---QA TA-------V ...SLS.S.. L....A.A. .....A.... .......S. .....K..V. E...LCNA.E
L1      .L.GN..S.. ....F.-DNE NQ---.-VK- K.-----A--V ..MSFD.S.. L....T.A. .....A.... .......S. .....K..V. E...LCNA.E
L2      .L.GN..S.. ....F.-DNE N-H-A..V-S DS------KLV ..MSLD.S.. L....T.A. .A...A.... .......S. .....K..V. E...LCNA.E
B       .L.GN..S.. ....F.-NNE NQ---TK-V-S NG-----A-FV ..MSLD.S.. L....A.A. .....A.... .......S. .....K..V. E...LCNA.E
Ba      *********. ....F.-NNE NQ---TK-V-S NS----.--FV ..MSLD.S.. L..***** ********** ********** ********** **********
D       *********. ....F.-DNE NQ---K.VKA ES-------V ..MSFD.S.. L..***** ********** ********** ********** **********
E       .L.GN..S.. ....F.-DNE NQ---.-.VKT NS-------V ..MSLD.S.. L....A... .....A.... .......S. .....K..V. E...LCNA.E
F       .L.GN..S.. ....F.-DGV NA--T----KP AA-D-S----I ...QLN.S.. L....T.A. .....A.... .......S. .....K.... E...LCNA.E
G       *********. ....F.-DGE NA--T----QP AA-T-S----I ...QLN.S.. L..***** ********** ********** ********** **********
A       .L.GN..S.. ....F.***** ******TK.-QS SGFD-.ANIV ..TALN.A.. L....T.A. .....A.... .......S. .....K..V. E...LCNASE
C       .L.GN..S.. ....F.***** ******TK.-QS SSFN-.AKLI ..TALNEA.. L.IN.T.A. .....A.... .......S. .....K..V. E...LCNASE
H       .L.GN..S.. ....F.***** ******TK.-KS S.FN-.AKLV ..IALNRA.. L....T.A. .....A.... .......S. .....K..V. E...LCNASE
I       *********. ....F.***** ******TK.-QS SNFN-.AKLV ..AALN.A.. L..***** ********** ********** ********** **********
J       *********. ....F.***** ******TK.-QA SSFN-.ANLF ..TALN.A.. L..***** ********** ********** ********** **********
K       *********. ....F.***** ******TK.-QY SKFN-.ANLV ..TALDRA.. L..***** ********** ********** ********** **********
L3      *********. ....F.***** ******TK.-QS TNFN-.AKLV ..TALN.A.. L..***** ********** ********** ********** **********

                                  VD III                                                                  VD IV
MN      FVIHKPRGYK GASSNFPLPI TAGTT-E-AT DTKSATIKYH EWQVGLALSY RLNMLVPYIG VNWSRATFDA DTIRIAQPKL KSEILNITTW NPSLIGSTTA   300
6BC     .......... .......... .....-.-.. .......... .......... .......... .......... .......... .......... ..........
A22/M   .......... .......... .....-.-.. .......... .......... .......... .......... .......... ....L.....T
B577    ..V....... .---TA....L .....-DQ.. .......... .......... .........S .......... .A........ AAAV..L... ..T.L.EA..
LW508   ..V....... .---TA....L .....-DQ.. .......... .......... .......... .......... .A........ AAAV..L... ..T.L.EA..
FEPN    .M........ .TAA.....V A...A---T.. ......V... .......... .......... .......... ......... A.A...L... ..T.L.VA.T
GPIC    .......... .TAA.....L .....---.S. .......... ...I...... .......... .......... .S........ PTA...L... ..T.L.EA.T
KOALA   .SVN..K... .---VA....T D..VA---T.. G.....N.. .....AS... ....S..... .Q........ .N....... PTAV..L.A. ....L.N..T
AR388   .SVN..K... .---VA....T D..VA---T.. G.....N.. .....AS... ....S..... .Q........ .N....... PTAV..L.A. ....L.NA..
66P130  .TV.R.K..V N---QPL.... ....STDT.. NL.N...N.. .....A.... .......... .Q........ ...Q...... ATAV..L... ..T.L.EI.S
LW613   .TV......V .---QTL...L S...ETDSSD KL.N...N.. .....A.... .......... .Q........ ...Q..E... A.P.F.L... ..T.L.QA.T
L71     .TV...Q..V .---QSL...T N...S---N.S .L.N...N.. .....A.... .......... IQ........ ...QV..... A.P.F.L... ..T.L.QA.S
1710s   .TV...Q..I .---QQL...T ....S---N.. .L.N...N.. .....A.... .......... .Q........ ...Q...... A.P.FRL... ..T.L.QA.Q
MOPN    .T.N..K..V .---QE...N. K...V---S.. ...D.S.D.. ...AS..... ....FT.... .K....S... ......... ETS..KM... ..TIS..GID
S45     .T.N..Q..V .---VE...DL K....A---.. G..D.S.D.. ...AS..... ....FT.... .K....S... ......... ATAVFDV..L ..TIA.AGKV
L1      .T.N..K..V .---KE...DL ....DA---.. G..D.S.D.. ...AS..... ....FT.... .K....S... ......... ATA.FDT..L ..TIA.AGEV
L2      .T.N..K..V .---QE...DL K...DG---V. G..D.S.D.. ...AS..... ....FT.... .K....S... .......S ATTVFDV..L ..TIA.AGDV
B       .T.N..K..V .---KEL..DL ....DA---.. G..D.S.D.. ...AS..... ....FT.... .K....S... .......S AET.FDV..L ..TIA.AGDV
Ba      ********.V .---KEL..DL .S..DA---.. G..******* ********** ********** ********** ******....S AET.FDV..L ..TIA.AGDV
D       ********.V .---KE...DL ....DA---.. G..******* ********** ********** ********** ******....S ATA.FDT..L ..TIA.AGDV
E       .T.N..K..V .---QE...AL I...DA---.. G..D.S.D.. ...AS..... ....FT.... .K....S... .......S ATA.FDT..L ..TIA.AGDV
F       .T.N..K..V .---KE...DL ....DA---.. G..D.S.D.. ...AS.S... ....FT.... .K....S... .......R VTPVVD...L ..TIA.CGSV
G       ********.V .---QE...AL ....DA---.. G..******* ********** ********** ********** ******..... AKPVVD...L ..TIA.CGSV
A       .T.N..K..V .---AE...D. ....EA---.. G..D.S.D.. ...AS..... ....FT.... .K...VS... ......... AKPV.DT..L ..TIA.KG.V
C       .T.N..K..V .---AE...N. ....EA---.. G..D.S.D.. ...AS..... ....FT.... .K...VS... ......... AEA..DV..L ..TIA.KGSV
H       .T.N..K..V .---AE...D. ....EA---.. G..D.S.D.. ...AS..... ....FT.... .K...VS... ......... AEA..DV..L ..TIA.KG.V
I       ********.V .---AE...D. I...EA---.. G..******* ********** ********** ********** ******..... AEA..DV..L ..TIA.KG.V
J       ********.V .---AE...D. ....EA---.. G..******* ********** ********** ********** ******..... AEA..DV..L ..TIA.KG.V
K       ********.V .---VE...D. ....EA---.. G..******* ********** ********** ********** ******..... AEA..DV..L ..TIT.KGAV
L3      ********.V .---AE...D. ....EA---.. G..******* ********** ********** ********** ******..... AEAV.DV..L ..TIA.KGSV
```

FIG. 3. Alignment of deduced amino acid sequences of partial chlamydial MOMP peptides. Dots represent amino acids of aligned sequences identical to the MN sequence, letters represent deviating amino acids, and hyphens or solid lines indicate gaps. VD are marked by horizontal brackets above the MN sequence.

```
                 ┌──────────┐
MN       —LPNNSGKD VLSDVLQIAS IQINKMKSRK ACGVAVGATL IDADKWSITG EARLIN  356
6BC      —........ .......... .......... .......... .......... ......
A22/M    —....G... .......... .......... .......... .......... ......
B577     —.DTSN-.- -FA.F..... .......... .......... .......... ......
LW508    —.DTSN-.- -FA.F..... .......... .......... .......... ......
FEPN     —.DTSN-.- -YA.FM..V. M......... ...I...... .......... .....D
GPIC     —INTGA-.- -YA.Q..... L......... ...I...... .......... ......
KOALA    —..TSDS— -F..FM..V. C....F.... ....T..... V......L.A ......
AR388    —.STTDS— -F..FM..V. C....F.... ....T..... V......L.A ......
66P130   QD.DTAN-.- -YA.T...V. C....L.... ....SM.... L.....A.N. .L....
LW613    —VDGTN-.- -FA.S...V. L....L.... ....SM.... L.....A... .L....
L71      —V-GSD-.- -YA.T...V. L....L.... ....SM.... L.....A.N. .L....
1710S    —VDGTN-I- -YA.A...V. L....L.... ....SM.... L.....A.N. .L....
MOPN     —VDT——.- -IT.T...V. L.L....... S..L.I.T.I V....YAV.V .T...D
S45      EDK-GSA.E- -.G.TM..V. M......... S..L...T.I V....YAV.V .T...D
L1       —KA.AE.Q- -.G.TM..V. L.L....... S..I...T.I V....YAV.V .T...D
L2       —KASAE.Q- -.G.TM..V. L.L....... S..I...T.I V....YAV.V .T...D
B        —KTSAE.Q- -.G.TM..V. L.L....... S..I...T.I V....YAV.V .T...D
Ba       —KTSAE.Q- -.G.TM.—— ———————— ———————— ———————— ——————
D        —KTGAE.Q- -.G.TM.—— ———————— ———————— ———————— ——————
E        —KASAE.Q- -.G.TM..V. L.L....... S..I...T.I V....YAV.V .T...D
F        AG-A.TE.Q- -I..TM..V. L.L....... S..I...T.I V....YAV.V .T...D
G        VA-A.SE.Q- -I..TM.—— ———————— ———————— ———————— ——————
A        VS—SAENE- -.A.TM..V. L.L....... S..I...T.V V....YAV.I .T...D
C        VS-AGTDNE- -.A.TM..V. L.L....... S..I...T.I V....YAV.V .....D
H        VA-SGSDND- -.A.TM..V. L.L....... S..I...T.I V....YAV.V .T...D
I        VS—SAENE- -.A.TM.—— ———————— ———————— ———————— ——————
J        VA-SGSEND- -.A.TM.—— ———————— ———————— ———————— ——————
K        VS-SGSDNE- -.A.TM.—— ———————— ———————— ———————— ——————
L3       VA-SGSENE- -.A.TM.—— ———————— ———————— ———————— ——————
```

FIG. 3—*Continued.*

cysteines at position 321 were found in *ompA* alleles 66P130, KOALA, and AR388 and at position 75 in FEPN.

A hydrophobic nonapeptide within VD IV was present in all MOMP peptide sequences. VD I, II, and IV in all MOMPs contained linear regions of significant hydrophilicity (data not shown), rendering them potential B-cell epitopes. Such regions might function as antigenic determinants defining the serovar status of the *ompA* alleles (2, 50). A T-helper-cell epitope has been defined in *C. trachomatis* MOMP (53). This peptide, ALNIWDRFDVF (positions 82 to 92 in Fig. 3), was conserved in *ompA* alleles GPIC, FEPN, LW613, KOALA, AR388, S45, and MOPN, and a single substitution of valine to isoleucine was present in MN, 6BC, A22/M, B577, LW508, 66P130, L71, and 1710S. This conservative substitution did not change the overall amphipathicity of the T-cell epitope and is likely to result in an equally effective T-helper-cell epitope.

Sequence dissimilarities among chlamydial *ompA* loci. We first compared chlamydial *ompA* DNA sequences by direct alignment without introducing gaps. The complete *ompA* locus of *C. psittaci* wt parakeet was identical to that of B577, and that of BMZ1121 was identical to that of MN. Chlamydial strains with identity of the partial *ompA* genes were SV139 and B577; FEPN, FEPN Pring, and FEPN Baker; 1710S and 1708; and LW613, LW623, FcStra, IPA, E58, L14, and JP1751. The complete *ompA* DNA sequences of strains 6BC and A22/M deviated by 2 and 11 bases, respectively, from that of MN. The 6BC *ompA* DNA sequence was identical to the one recently published by Everett et al. (12). The partial *ompA* locus of LW508, a mammalian serovar 1 strain of *C. psittaci* (36), differed in only three nucleotide positions (two amino acid residues) within the conserved regions from the serovar 1 isolate B577. The complete *ompA* locus of strain B577 had a single base difference (synonymous substitution at position 793, Fig. 2) from that of *C. psittaci* S26/3, which is, like B577, an isolate from ovine abortion (21) and which was treated as *ompA* allele B577. The partial *ompA* DNA sequence of *C. pneumoniae* AR388 was identical to the sequences of two other strains of *C. pneumoniae* (7, 32). The KOALA *ompA* DNA sequence was surprisingly similar to that of *C. pneumoniae* AR388 and differed by only 20 nucleotides and 9 amino acids. Generally, chlamydial strains of the same biovar, serovar, or RFLP type (15, 36) had identical or closely related *ompA* alleles. We selected one strain of each group of identical chlamydial *ompA* loci as the representative (*ompA* allele) for further analysis.

Similarities between partial *ompA* DNA sequences of chlamydial strains were less pronounced when calculated after the introduction of gaps during multiple pairwise alignments with the University of Wisconsin Genetic Computer Group software. Chlamydial *ompA* alleles, listed in Table 3, were aligned as peptide sequences (Fig. 3). This approach yielded a more consistent alignment of functionally homologous residues than did alignment of nucleotides.

Dissimilarities between partial *ompA* DNA sequences are presented in the lower triangle of Table 4. They extend from 0.2 for MN and 6BC to a maximum of 38.4 for the relation of *C. pecorum ompA* allele 66P130 to *C. trachomatis* H. The dissimilarities between MOMP peptide sequences were also analyzed (data not shown). DNA sequence dissimilarities below 10% correlated well with peptide sequence dissimilarities, which were not more than 2.5% lower than the observed DNA sequence dissimilarities. However, the peptide dissimilarity was on average 10% lower than the DNA sequence dissimilarity for *ompA* loci with more than 10% DNA differences. These differences ranged from a minimum of 8.5% peptide dissimilarity and 14.2% nucleotide dissimilarity between 1710S and LW613 to a maximum difference of 21.5% peptide dissimilarity and 36.5% nucleotide dissimilarity between *C. pecorum* L71 and *C. trachomatis* A. Alternative codon usage accounted for these differences (5), and we interpreted them as an indication of correct sequence alignment.

Molecular evolution. The 24 partial chlamydial *ompA* DNA sequences obtained in our study combined with previously published sequences provided a data set for deriving a phylogeny of the *ompA* locus.

A single chlamydial *ompA* phylogenetic hypothesis illustrated in Fig. 4 was identified by using the *ompA* dissimilarity data of Table 4 in the NJ algorithm (41). Mantel test statistics (28) indicated a very good fit (cophenetic correlation, 0.93) and a low error probability of the phylogram in relation to the original *ompA* dissimilarity matrix (the observed Z value was greater than the Z values found in 250 random permutations of one matrix order). The midpoint rooting algorithm of the NJ method provided a possible solution to the problem of the unknown ancestral chlamydiae. The root of this phylogenetic tree was placed at the branch between the cluster of *C. trachomatis ompA* alleles

TABLE 3. Chlamydial *ompA* representatives used in phylogenetic inference

ompA allele[a]	Biovar	Serovar(s)[b]	RFLP type(s)	Host(s)	Host clinical condition(s)	Reference(s)[c]
C. psittaci						
MN			Avian 1b	Birds, cattle, ferret/human[d]	Respiratory infection, mastitis	13, 67
6BC			Avian 1c	Birds, human	Respiratory and systemic infections	
A22/M[e]			Avian	Unknown	Unknown	38
B577	1	1	Avian 2b	Ruminants, parakeet, human	Abortion, seminal vesiculitis, systemic infection	20, 21
LW508	1	1		Cattle	Enteritis	
FEPN	7	7	Feline 1	Cat, muskrat, human	Conjunctivitis, pneumonia, systemic infection	11, 15, 47
GPIC	8	8		Guinea pig	Conjunctivitis	67
KOALA				Koala	Keratoconjunctivitis	
C. pecorum						
66P130	3	3		Cattle	Inapparent intestinal infection	
LW613	2	2, 6, 9[f]	Ruminant 1a, 1b, 1c[g]	Ruminants	Polyarthritis, encephalitis, pneumonia, conjunctivitis	
L71	4	4		Swine	Polyarthritis	
1710S	4	6		Swine	Pneumonia, abortion	
C. pneumoniae						
AR388				Human	Respiratory infection	
C. trachomatis						
MOPN	Mouse		Mouse	Mouse	Pneumonia	
S45				Swine	Intestinal infection	
A	Trachoma	A		Human	Trachoma	2
B	Trachoma	B		Human	Trachoma	2, 50
Ba	Trachoma	Ba		Human	Trachoma	65
C	Trachoma	C		Human	Trachoma	2, 50
D	Trachoma	D		Human	Urogenital infection	65
E	Trachoma	E	Human	Human	Urogenital infection	37
F	Trachoma	F		Human	Urogenital infection	66
G	Trachoma	G		Human	Urogenital infection	65
H	Trachoma	H		Human	Urogenital infection	19
I	Trachoma	I		Human	Urogenital infection	65
J	Trachoma	J		Human	Urogenital infection	65
K	Trachoma	K		Human	Urogenital infection	65
L1	LGV[h]	L1		Human	LGV	39
L2	LGV	L2	Human	Human	LGV	2, 50
L3	LGV	L3		Human	LGV	65

[a] Serovars, RFLP types, hosts, and clinical conditions associated with a group of chlamydial strains with identical partial *ompA* loci are listed with the representative *ompA* allele.
[b] Serovars of *C. trachomatis* were determined by Wang et al. (58, 59).
[c] Sources of *ompA* DNA sequences are listed. Fukushi and Hirai (15) provided information for grouping chlamydial isolates into *ompA* allele MN; Herring et al. (20, 21) provided information for grouping isolates into *ompA* allele B577; and Eb et al. (11), Fukushi and Hirai (15), and Schachter et al. (47) provided information for grouping isolates into *ompA* allele FEPN.
[d] *C. psittaci* MN was isolated via ferret passage from a human influenza patient (13). It is now agreed upon that this patient suffered from psittacosis.
[e] The exact origin of *ompA* allele A22/M is unknown, and it is now considered an avian RFLP type of *C. psittaci* (21).
[f] Elementary bodies of strains L14 (assigned to serovar 6) and JP1751 (designated serovar 9) exhibited 100% cross-reactivity with LW623 (*C. pecorum ompA* allele LW613) antiserum (36).
[g] *C. pecorum* Bo/Maeda of the ruminant 1a RFLP type exhibited 0 and 12% DNA dissimilarity to *C. pecorum ompA* allele LW613 IPA (ruminant 1b) and E58 (ruminant 1c), respectively (15).
[h] LGV, lymphogranuloma venereum.

(termed trachoma group) and all other chlamydial *ompA* alleles.

Murine isolate MOPN represented the most ancestral *C. trachomatis ompA* allele, followed by the porcine allele S45. Human *ompA* alleles of *C. trachomatis* sorted out by serogroups, as expected for a phylogeny based on the genetic determinants of serovars. They were differentiated in two clades, with serogroup C *ompA* alleles A, C, and H composing one clade and serogroup B alleles B, E, F, L1, and L2 clustering as the other one. Serovar F, related to serogroup B, was ancestral to the other serogroup B alleles within this second clade.

The second cluster of related chlamydial *ompA* alleles was composed of three groups of *C. psittaci*, *C. pecorum*, and *C. pneumoniae* alleles. *C. pecorum* alleles 66P130, LW613, L71, and 1710S of ruminant or porcine origin with propensity for polyarthritis formed one group (polyarthritis group). *C. pneumoniae* AR388 and the KOALA *ompA* allele represented the pneumonia group. *C. psittaci* alleles GPIC, FEPN, B577, LW508, MN, 6BC, and A22/M formed the psittacosis group of *ompA* alleles. These groups were differentiated by distinct DNA sequence signatures: nucleotides 562 to 564 (Fig. 2) were unique in the polyarthritis group of *ompA* alleles, 700 to 714 were unique in the psittacosis group, 760 to 768 were unique in the pneumonia group, and 1037 to 1056 were unique in the trachoma group.

Maximum parsimony analyses of the chlamydial *ompA* locus were performed with several algorithms within the software package PAUP (54). On the basis of the results of the NJ method, all *C. trachomatis ompA* alleles including

TABLE 4. Chlamydial genomic DNA and partial *ompA* locus dissimilarities[a,b]

Allele	*C. psittaci*								*C. pneumoniae*	*C. pecorum*				*C. trachomatis*									
	MN	6BC	A22/M	B577[c]	LW508	FEPN[d]	GPIC	KOALA	AR388[e]	66P130	LW613[f]	L71	1710S	MOPN	S45	L1	L2[g]	B	E	F	A	C	H
MN		3.5		34.0		77.5	70.0		94.5								95.0						
		10.5		*63.5*		*68.0*					*90.0*			*88.5*			*72.2*						
6BC	0.2			16.5		70.5	74.0		94.2								95.0						
				40.5		*69.0*					*90.0*			*96.0*			*85.0*						
A22/M	1.1	0.9																					
B577	20.3	20.2	21.0			69.5	67.5		92.0								94.5						
						76.5					*99.0*			*96.0*			*90.0*						
LW508	20.4	20.3	21.1	0.3																			
FEPN	24.1	24.0	24.5	21.8	21.9		68.5		92.7								91.5						
											89.5			*94.5*			*78.0*						
GPIC	24.2	24.1	24.4	21.9	22.0	21.5			93.5								95.0						
KOALA	32.2	32.1	32.0	30.3	30.4	30.4	30.7																
AR388	32.4	32.3	32.4	30.4	30.5	30.5	30.9	2.1									94.7						
66P130	34.7	34.7	35.0	34.2	34.3	31.8	31.5	31.9	32.5														
LW613	33.8	33.7	33.9	32.9	33.0	31.7	30.1	31.3	31.3	20.6													
														97.5			*84.7*						
L71	33.8	33.7	33.8	32.8	32.9	32.6	30.2	30.7	30.9	18.0	8.8												
1710S	34.4	34.3	34.5	34.4	34.5	33.4	30.7	31.3	31.3	18.3	14.2	14.0											
MOPN	36.0	35.9	36.3	34.4	34.3	35.6	35.9	37.4	37.4	34.4	36.7	36.0	35.3										
																	68.7						
S45	37.1	37.0	37.2	35.7	35.6	35.9	35.6	35.7	36.1	35.4	35.8	34.5	35.4	25.9									
L1	36.3	36.2	36.5	36.1	36.2	35.8	36.6	36.3	36.0	36.2	35.4	36.0	36.6	25.3	21.6		4.0[h]	4.0	4.0	4.0	4.0	4.0	4.0
L2	37.2	37.1	37.3	35.7	35.8	35.6	36.4	35.8	35.6	36.6	35.3	35.2	36.3	25.7	21.5	7.5		4.0	4.0	4.0	4.0	4.0	4.0
B	36.9	36.8	37.0	35.9	36.0	36.5	36.5	36.4	36.3	37.4	36.1	37.0	37.2	26.2	21.4	6.9	7.5		4.0	4.0	4.0	4.0	4.0
E	37.4	37.3	37.6	35.9	36.1	35.9	36.7	35.5	35.4	36.5	35.9	36.1	36.6	25.8	21.7	8.0	9.4	9.1		4.0	4.0	4.0	4.0
F	36.5	36.4	36.6	38.2	38.3	36.9	37.8	37.6	37.8	35.9	36.0	36.5	36.3	26.8	24.8	15.9	17.5	18.0	18.1		4.0	4.0	4.0
A	38.3	38.3	38.3	37.5	37.6	36.4	35.6	37.4	37.0	38.2	36.1	36.5	36.7	27.2	26.1	20.6	20.9	20.8	22.3	22.8		4.0	4.0
C	37.7	37.7	37.7	36.9	37.0	35.5	35.3	36.9	36.2	38.2	36.3	36.3	35.7	27.4	26.4	21.6	21.2	21.1	22.7	23.7	4.9		4.0
H	37.1	37.1	37.2	36.7	36.8	35.8	35.2	36.9	36.5	38.4	36.3	36.0	36.1	27.3	25.6	21.2	21.2	21.1	22.6	23.3	4.6	3.0	

[a] Percent chlamydial genomic DNA dissimilarities are displayed in the upper-right-hand triangle; percent dissimilarities between chlamydial *ompA* loci are displayed in the lower-left-hand triangle.

[b] DNA dissimilarity values compiled from the data published by Fukushi and Hirai (15) are shown in italic under the respective values from Cox et al. (8).

[c] DNA dissimilarity data published by Cox et al. (8) are derived from an ovine abortion isolate of *C. psittaci*, which is termed "ABORTION" in phylogram A of Fig. 6.

[d] DNA dissimilarity data for *ompA* allele FEPN are derived from strain FEPN Baker, termed Fe/Pn-1 by Fukushi and Hirai (15).

[e] DNA dissimilarity data for strain AR388 are derived from *C. pneumoniae* TW183, AR39, LR65, and AR458 and were used as "*C. pneumoniae*" for the construction of phylogram A in Fig. 6. All strains of *C. pneumoniae* examined exhibit identical restriction patterns and have less than 6% mutual DNA dissimilarity (6, 8).

[f] DNA dissimilarity data for *ompA* allele LW613 are derived from *C. pecorum* Bo/Maeda. Bo/Maeda is RFLP type ruminant 1a and is 0 and 12% dissimilar to *C. pecorum* IPA (ruminant 1b) and E58 (ruminant 1c), respectively (15, 16). These strains are of the LW613 *ompA* allele.

[g] DNA dissimilarities between human serovars of *C. trachomatis* and other chlamydiae are summarized under the *ompA* allele L2 and were used as "human *C. trachomatis*" for the construction of the phylograms in Fig. 6.

[h] All human serovars of *C. trachomatis* examined exhibit DNA dissimilarities among each other between 0 and 8% (8, 15, 24, 61).

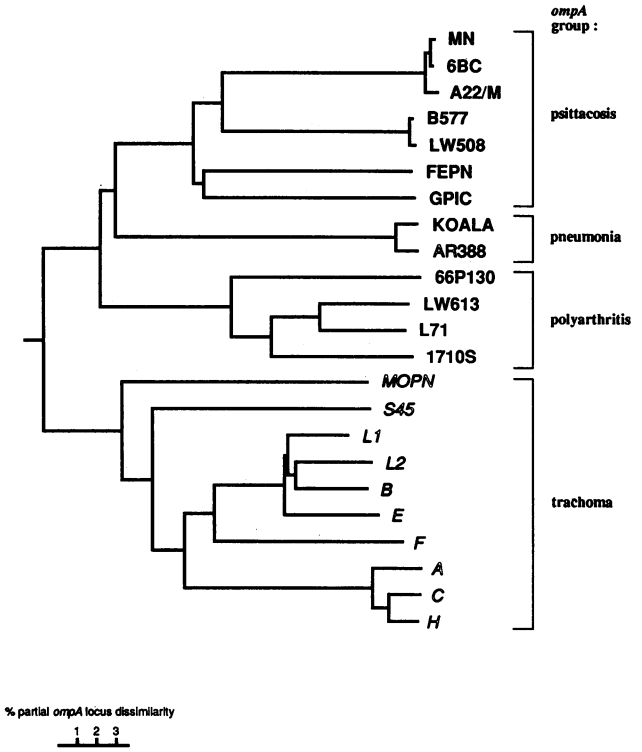

FIG. 4. Phylogram of the partial chlamydial *ompA* locus inferred by the NJ method. A single phylogenetic tree (phylogram) was constructed from the chlamydial *ompA* dissimilarity data (Table 4). The root was placed at the midpoint of the branch path between the two most distant OTU. Phylogenetically related groups of chlamydial *ompA* alleles are indicated by brackets. Boldface type, *C. psittaci* and *C. pecorum*; open type, *C. pneumoniae*; open italic type, *C. trachomatis*.

S45 were specified as outgroup. Two equally most parsimonious phylograms were found for the set of nonsynonymous, informative character changes (419 sites), each with a total length of 1,274 evolutionary steps and a consistency index of 0.568 (Fig. 5). One thousand random permutations of the data set resulted in an average tree length of 2,646 steps and a highly skewed length distribution of these trees, indicating with high probability that the informative sites in these trees exhibited nonrandom character changes.

Both unrooted phylograms had a topology quite similar to that of the phylogram obtained by the NJ method. The main difference was the clustering of AR388 and KOALA *ompA* alleles with the polyarthritis group of *ompA* alleles. Also, *ompA* alleles L1, L2, B, and E of *C. trachomatis* serocomplex B separated in a way slightly different from that of the NJ phylogram. The *ompA* alleles MN and 6BC, and B577 and LW508, respectively, lacked differences in nonsynonymous characters and were not distinguished. Branching of GPIC and FEPN was the only topological difference between the two equally parsimonious phylograms. GPIC was ancestral to FEPN in tree 2, while they were congeneric in tree 1 (Fig. 5).

We also performed maximum parsimony analyses with the complete *ompA* data and with synonymous character changes. The two phylograms resulting for each data set had essentially the same topologies as the trees inferred from the nonsynonymous character changes. The only differences were in branching of S45 and human serovars of *C. trachomatis*. We rejected these hypotheses because the consistency indices were lower (0.542 and 0.457, respectively) than those of the nonsynonymous *ompA* phylograms.

On the basis of the phylogeny of the genus, we attempted to infer a most parsimonious phylogram of the *ompA* alleles of the species *C. trachomatis*. MOPN was specified as outgroup, and the data set consisted of the nonsynonymous sites of the VD region of *ompA* DNA sequences from all serovars of *C. trachomatis*. Fourteen equally most parsimonious phylograms which varied considerably in their topologies were found (data not shown). Serovars B, Ba, D, E, L1, and L2 composing serogroup B and serovars A, C, H, I, and J composing serogroup C always clustered. Serovars F and G related to the B complex were a separate group but associated with the serogroup B cluster, and intermediate serovars K and L3 always were grouped with the C complex.

Correlation of the chlamydial *ompA* phylogeny with the chromosomal phylogeny. Two approaches to estimate the degree of congruence of the chlamydial *ompA* phylogeny with the chromosomal phylogeny were explored. The sulfadiazine-sensitive chlamydial strain S45 had previously been considered to be *C. psittaci* (49). This strain should belong to the species *C. trachomatis* if the *ompA* tree correctly reflected chlamydial genomic evolution. Iodine staining of S45-infected L-cell monolayers demonstrated the presence of glycogen within the inclusions. Therefore, according to current taxonomic criteria for chlamydiae, we classified strain S45 as *C. trachomatis*.

Cox et al. (8) and Fukushi and Hirai (15) presented data on the chromosomal DNA-DNA relationships between chlamydiae. We constructed two chromosomal chlamydial phylograms from these dissimilarity data sets (Table 4, upper-right triangle) with the NJ algorithm. The chromosomal DNA dissimilarities between human serovars of *C. trachomatis* and between strains of *C. pneumoniae* are less than 8%, and these chlamydiae appear essentially identical within the precision limits of solution DNA hybridization. Averages of the dissimilarity data of these strains were therefore compiled into single OTU as "human *C. trachomatis*" and "*C. pneumoniae*," respectively.

Both data sets yielded single, unrooted phylograms with the NJ method (Fig. 6). Midpoint rooting was impossible in both trees because of statistically equivocal maximum branch path lengths between several OTU. Both trees fit well to the data matrix (0.94 cophenetic correlation for tree A from the data of Cox et al. [8] and 0.84 for tree B from the data of Fukushi and Hirai [15]).

Tree A displayed three groups of highly dissimilar taxa: human *C. trachomatis*, *C. pneumoniae*, and the group of *C. psittaci* MN, 6BC, ABORTION, GPIC, and FEPN. Similarly, three clusters of chlamydiae with unresolved phylogenetic relationships were present in tree B: human *C. trachomatis* with MOPN, polyarthritis *ompA* allele LW613, and the cluster of *C. psittaci* MN, 6BC, B577, and FEPN.

The topology of these phylograms—without considering the unresolved basal branching—is in agreement with the *ompA*-based trees. The single genomic OTU of human serovars of *C. trachomatis* exhibits considerable *ompA* dissimilarities compared with the distance span between *ompA* alleles MN and GPIC with a divergent chromosome (Fig. 4).

DISCUSSION

We sequenced a major part of the *ompA* locus of 24 chlamydial strains and used these data for phylogenetic

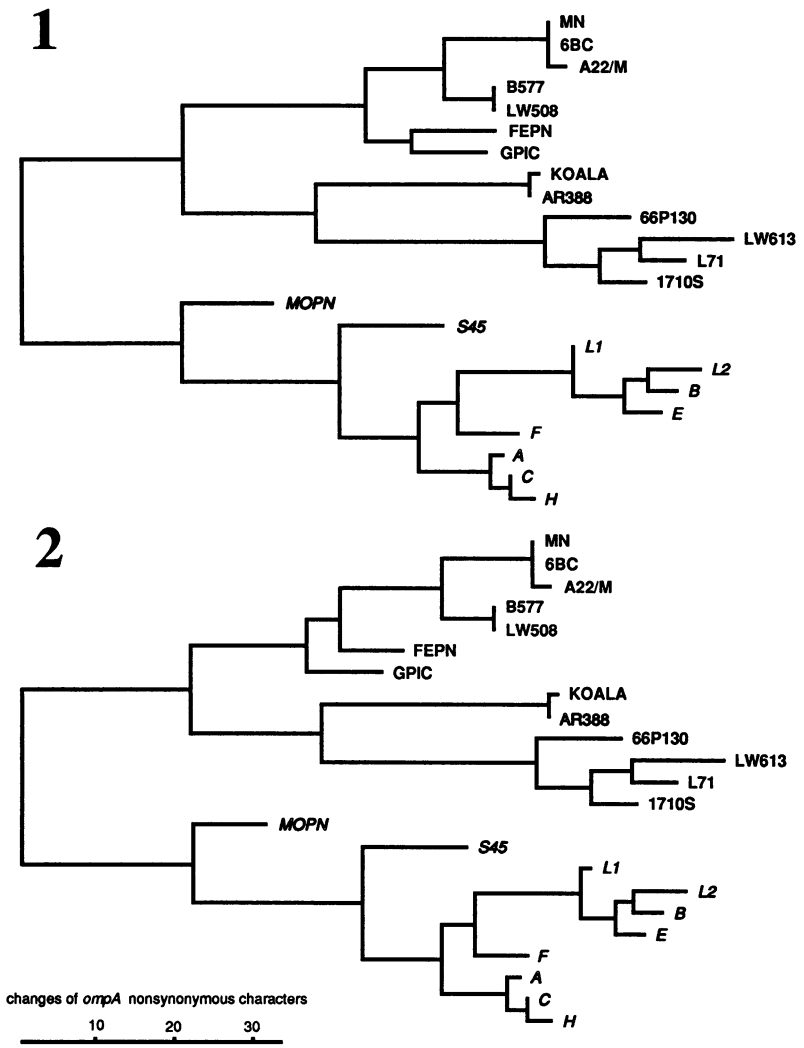

FIG. 5. Phylograms of the partial chlamydial *ompA* locus inferred by maximum parsimony analysis. Two equally most parsimonious hypotheses (phylograms) resulted from analysis of nonsynonymous *ompA* character changes (Fig. 2) with PAUP. *C. trachomatis ompA* alleles were specified as outgroup. Both trees consist of 1,274 evolutionary steps with a consistency index of 0.568. The topology of trees 1 and 2 differs in branching between FEPN and GPIC. The branch lengths of the phylograms are not entirely proportional to the number of inferred nonsynonymous character changes because of missing data (gaps) at different positions in pairs of *ompA* DNA sequences. Boldface type, *C. psittaci* and *C. pecorum*; open type, *C. pneumoniae*; open italic type, *C. trachomatis*.

inference. A phylogenetic tree constructed for a genetic locus does not necessarily represent the evolutionary pathway of the taxa involved. Construction of phylograms from several loci increases the probability of identifying a gene tree that is congruent with the species tree (10). In this report, we present several lines of evidence that the chlamydial *ompA* relationships reflect chromosomal DNA differentiation of three chlamydial species but not of the human serovars of *C. trachomatis*.

Does the *ompA* locus meet the criteria for a useful chronometer of evolutionary divergence with selective neutrality and random nucleotide change (63)? We analyzed by maximum parsimony whether selective forces, which most likely act on the chlamydial *ompA* locus, have resulted in different phylogenies of synonymous and nonsynonymous *ompA* sites. Both data sets yielded two equally most parsimonious phylograms with virtually identical topologies. Moreover, these phylograms were highly similar to the one derived by the NJ method from the *ompA* distances and to the hypothesis put forward by Carter et al. (7). These results suggest that the chlamydial *ompA* locus, despite presumably strong selective constraints, may be used for accurate phylogenetic reconstruction.

The lack of an outgroup sequence for *Chlamydia* spp. precluded testing of monophyly in the phylograms constructed and left some ambiguity about the correctness of the root as found by a numerical method (Fig. 4). We used the midpoint method to root the *ompA* phylogram generated by the NJ method. This method is most appropriately applied to phylogenies with constant evolution rates. Such an assumption cannot be tested for a phylogeny consisting only of extant chlamydial *ompA* alleles. The placement of the root in the middle of a relatively long branch most likely indicates correct rooting. Furthermore, a phylogram of the chlamydial *ompA* locus inferred by different algorithms from a smaller data set was rooted at a similar position (7). The phylograms

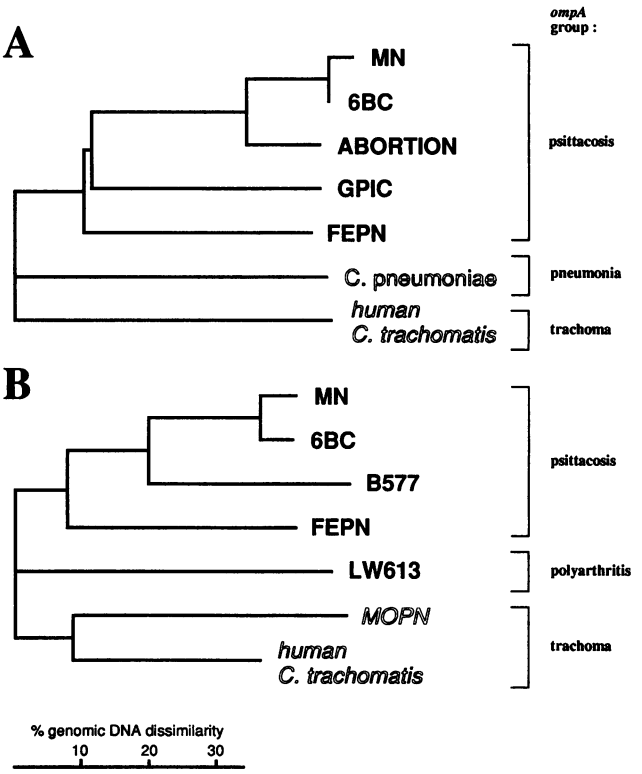

FIG. 6. Phylograms inferred from chlamydial chromosomal DNA distances by the NJ method. Single phylogenetic trees were constructed from chlamydial genomic DNA dissimilarities (Table 4) established by Cox et al. (8) (tree A) and Fukushi and Hirai (15) (tree B). Data for "human *C. trachomatis*" were compiled under *ompA* allele L2 in Table 4, data for "*C. pneumoniae*" were compiled under AR388, and data for strain "ABORTION" were compiled under allele B577. Both trees are unrooted because of statistically equivocal maximum branch path lengths between the most distant OTU (tree A, human *C. trachomatis*, *C. pneumoniae*, MN; tree B, MOPN, LW613, B577). Boldface type, *C. psittaci* and *C. pecorum*; open type, *C. pneumoniae*; open italic type, *C. trachomatis*.

derived from chlamydial chromosomal DNA distances (Fig. 6) suggest rooting at one of the branches among the four major groups of chlamydial *ompA* alleles (psittacosis, polyarthritis, pneumonia, and trachoma).

We assessed the agreement of the chlamydial *ompA* phylogeny with the phylogeny derived from chromosomal distances. Supporting evidence for the concordance of *ompA* and chromosomal phylogeny was provided by the biochemical identification of *ompA* allele S45 as *C. trachomatis*. This finding implies that species variation of chlamydial *ompA* alleles has evolved by mutational drift without significant recombination. In contrast, by recent analysis of nucleotide sequence variations in *Salmonella* phase 1 flagellin genes it was inferred that lateral transfer and intragenic recombination events of these genes generate new *Salmonella* serovars (48). Thus, *Salmonella* strains expressing the same phase 1 antigens may be distantly related in overall chromosomal genotype while strains exhibiting different phase 1 antigens may be nearly identical in genotype.

The congruence of chlamydial species and *ompA* trees was tested by reconstructing phylograms by using the NJ method and two independent sets of chlamydial chromosomal DNA distance data (Fig. 6). These chromosomal phylogenies were concordant with the *ompA* trees with respect to the separation of the four major groups of *ompA* alleles.

Human serovars of *C. trachomatis* have virtually identical genomes but highly divergent *ompA* loci. Our failure to identify by maximum parsimony analysis an unambiguous *ompA* tree for the species *C. trachomatis* can indicate significant levels of intragenic recombination. Immunological selection might promote such a process, as has been proposed earlier (50). Lateral transfer of gene segments is difficult to envision for *Chlamydia* spp. because it requires in all likelihood the close proximity of replicating forms (reticulate bodies) of two chlamydial variants within a single intracellular inclusion. This phenomenon has been demonstrated through the presence of organisms of two *C. trachomatis* serovars in inclusions which fused after superinfection of HeLa cells (40).

Strains with the *ompA* LW613 allele display significant heterogeneity in serological reactivity, genomic RFLP, and chromosomal DNA. Lateral transfer of the *ompA* locus might have conferred a selective advantage on these strains.

The intriguing finding that the *ompA* allele KOALA is closely related to *C. pneumoniae* might be explained by a similar transfer of the *ompA* locus. Alternatives would be mutational drift of the human *C. pneumoniae ompA* allele in the heterologous host, *Phascolarctos cinereus* (koala), or a common ancestry for both alleles.

The *ompA* sequences determined in this study also represent an important data base. Improved nucleic acid- and peptide-based methods for detection, differentiation, and serodiagnosis of chlamydial infections can be developed with these sequences.

The results presented in this report together with earlier investigations promise avenues for advancing the taxonomy of *Chlamydia* spp. Further analyses of chromosomal divergence and of genetic loci other than *ompA* should provide additional data for comprehensive reclassification of the order *Chlamydiales*.

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