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GENETIC VARIATION AMONG LASSA AND LASSA-RELATED ARENAVIRUSES ANALYSED BY T_1-OLIGONUCLEOTIDE MAPPING

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SUMMARY

Total RNA and small RNA species of several African arenavirus strains have been studied by T_1-oligonucleotide mapping. Genetic heterogeneity is observed and discussed on the basis of evolutionary biology of the Lassa complex.

KEY-WORDS: Arenavirus, Lassa virus, Genetics, RNA; Mapping, Evolution, Africa.

INTRODUCTION

We previously described structural and biological variations among African Lassa and Lassa-related virus strains [8, 9]. Furthermore, clinical, biological and physicochemical differences were demonstrated, but their significance had yet to be determined [4, 12]. In an attempt to increase understanding of the degree of relatedness amongst arenaviruses, we used T_1-oligonucleotide analysis of viral RNA to explore the genetic changes among viruses of the Lassa complex.

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MATERIALS AND METHODS

Virus strain.

The origin and characteristics of the Lassa (LAS), Mobala (MOB), Mopeia (MOP) and lymphocytic choriomeningitis (LCM) virus strains used in this study, have been previously described [2, 7, 16]: LAS « Josiah » (Sierra Leone), LAS Guinea (Guinea) and LAS Pinneo (Nigeria) are of human origin. LAS 1515 is a wild strain of virus (800 1515-SPB, Department of Viral Division, Centers for Disease Control, Atlanta) that was isolated from rodent blood (*Mastomys* sp. 36 chromosomes) from Sierra Leone [12]. MOP was isolated from *Mastomys natalensis* in Mozambique [16]. MOB 3200 was isolated from *Mastomys* sp. caught in the Central African Republic (CAR) [7]. Finally, LCM-ARM virus strain is an original cloned strain (E350) of LCM virus isolated from human blood in the USA [2]. All strains belong to the « Old World » arenavirus group.

Strains were either passaged or plaque-purified 3 times in Vero E6 cells as MOP Mp3, LAS Lp3 and MOB A11 strains [10, 12].

Growth and purification.

A confluent cell monolayer of Vero E6 cells was inoculated at a multiplicity of infection 0.01 PFU/cell, with a virus suspension in Eagle's minimal essential media. Cell culture supernatent was harvested on day 5 post-inoculation and virus purified by precipitation in polyethylene glycol followed by successive banding in potassium tartrate/glycerol and sucrose gradient as previously described [9].

Agarose gel electrophoresis.

RNA was extracted from purified virus by phenol/SDS [3]. RNA samples in 0.01 M sodium phosphate/0.15 M EDTA pH 7.4, were precipitated with 70 % ethanol at −20°C for 12 h. The RNA was then pelleted at 10,000 *g* for 30 min (Beckman GS rotor) then resuspended in 30 µl of running mixture containing 10 % sucrose in TNE buffer (150 mM NaCl/2 mM EDTA/10 mM Tris HCl pH 7.4) with 0.1 % SDS for agarose gel electrophoresis. Vertical gels containing low melting agarose (1.5 %) were run for 6 h at 100 V (constant voltage) in 0.25 M citrate buffer pH 3.5. RNA species were visualized under UV lamp after ethidium bromide staining. Gel slices were melted in TNE for 5 min at 70°C and RNA single species extracted by the phenol/SDS method [8, 9].

T_1-oligonucleotide mapping virion RNA.

T_1 mapping was done by the method previously used by Cox *et al.* [5, 6] with some modifications: RNA was labelled *in vitro* by ^{32}PATP (adenosine 5'-tri-phosphate, ^{32}P-tetra-triethylammonium salt, NEN-002A) with polynucleotide kinase (T4 polynucleotide kinase, NEN) 30 min at 37°C and reextracted with phenol. Digested RNA was then precipitated with cold 70 % ethanol (10 % sucrose, 0.02 % bromophenol blue and xylenecyanol markers). The oligonucleotides were resolved by two-dimensionnal polyacrylamide gel electrophoresis and the separated oligonucleotides were revealed by autoradiographic exposure X-ray film.

CAR = Central African Republic.
LAS = Lassa.
LCM = lymphocytic choriomeningitis.
LCMV = LCM virus.
MOB = Mobala.
MOP = Mopeia.
PATP = phospho-adenosine triphosphate.
PFU = plaque-forming unit.
SDS = sodium dodecyl sulphate.
SRNA = small ribonucleic acid.
TNE = Tris HCl/NaCl/EDTA (buffer).

RESULTS

Four RNA species were observed: two large (L) RNA and small (S) RNA of viral origin and two others of cellular ribosomal origin (28-S and 18-S RNA) (fig. 1). Preliminary experiments showed that contamination by these ribosomal RNA was absent in the total RNA T_1-oligonucleotide maps. Vezza *et al.* noticed variations in the ribosomal RNA content of Pichinde virus that depended on growth conditions and virus strain [15]. Also, « finger prints » of Vero cell 18-S and 28-S RNA allowed us to identify any contaminating oligonucleotides of cellular origin with respect to the viral RNA.

In all of these experiments, the plaque-purified LAS Josiah Lp3 strain from Sierra Leone served as our reference.

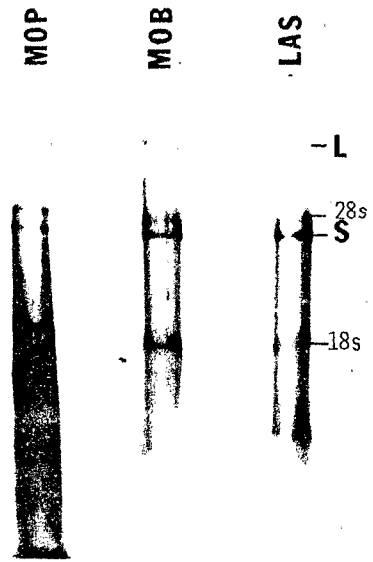


FIG. 1. — *RNA species resolution by agarose gel electrophoresis.*

Low melting agarose at 1.5 % concentration was used for RNA-extract electrophoresis in a slab gel system (6 h at 100 V constant voltage). Ethydium bromide staining, observed under UV light.

Total RNA of analysed strains showed variable homology (table I and fig. 2). For strains of human origin, LAS Guinea presented less homology to the reference strain than expected. However, only 6 unique oligonucleotides were observed, favouring limited base substitutions, compared to rodent strains which had three times more unique oligonucleotides.

To assess the validity of the total RNA T_1-oligonucleotide maps for comparative purposes, we also produced T_1 maps from S-RNA of the plaque-purified strains used in the original comparison (fig. 3). RNA from 4 low-passage strains and 3 plaque-purified strains were analysed. We also produced a map of LCM virus RNA for further comparison.

Rodent strain homology was analysed regarding total RNA and S-RNA. Analysis of S-RNA showed less homology than that of total RNA.

As already observed with the total RNA T_1 mapping, MOB virus and the reference strain of LAS virus demonstrated more homology between them than the latter with MOP virus. Additionally, among respectively the 21 and

TABLE I. — **RNA T_1-oligonucleotide analysis of several arenavirus strains from the «Old World».**

Strain	Percentage homology with reference strain (*)	Number of unique oligonucleotides (**)
LAS Josiah	100	0
LAS Guinea	76	5
MOP Mp3	76	30
MOB A11	87	15
MOB 3200	83	36
LCMV	33	33

(*) Reference strain = LAS Josiah (63 oligonucleotides counted).
(**) Number of common oligonucleotides.

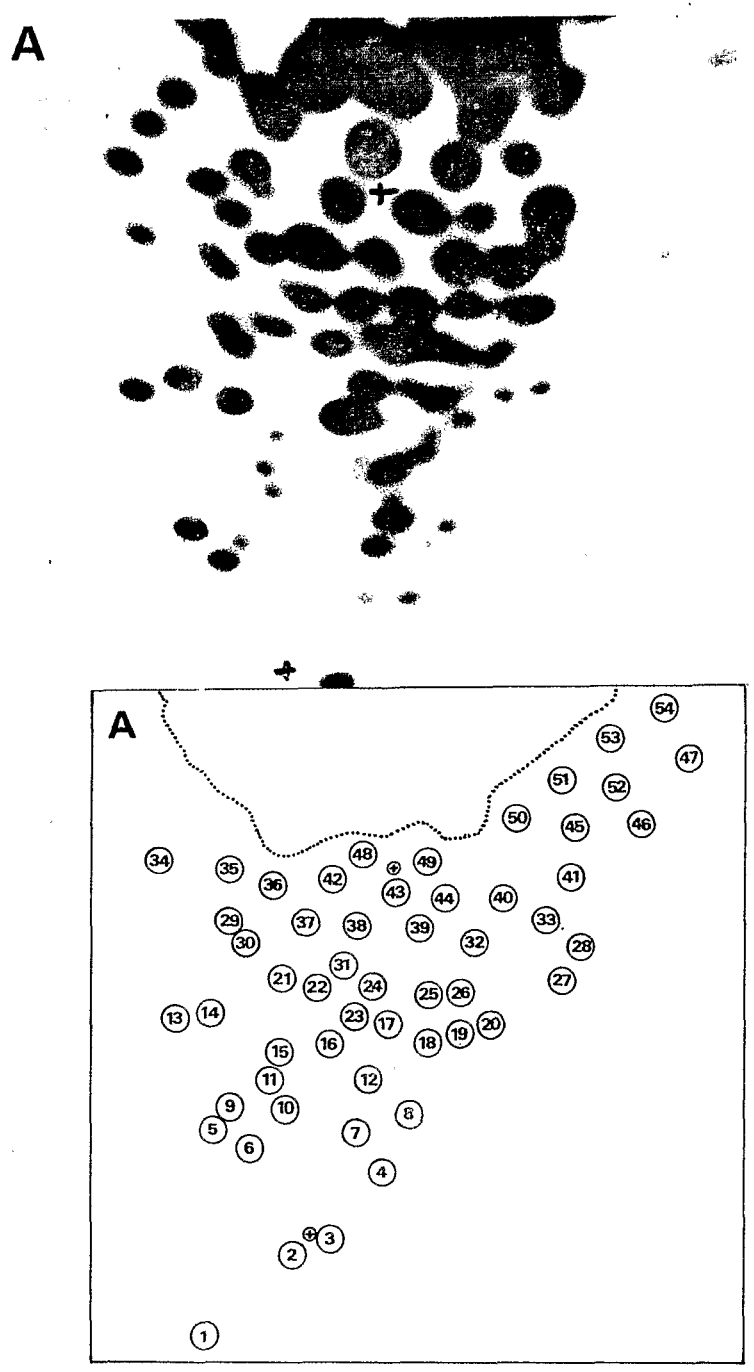
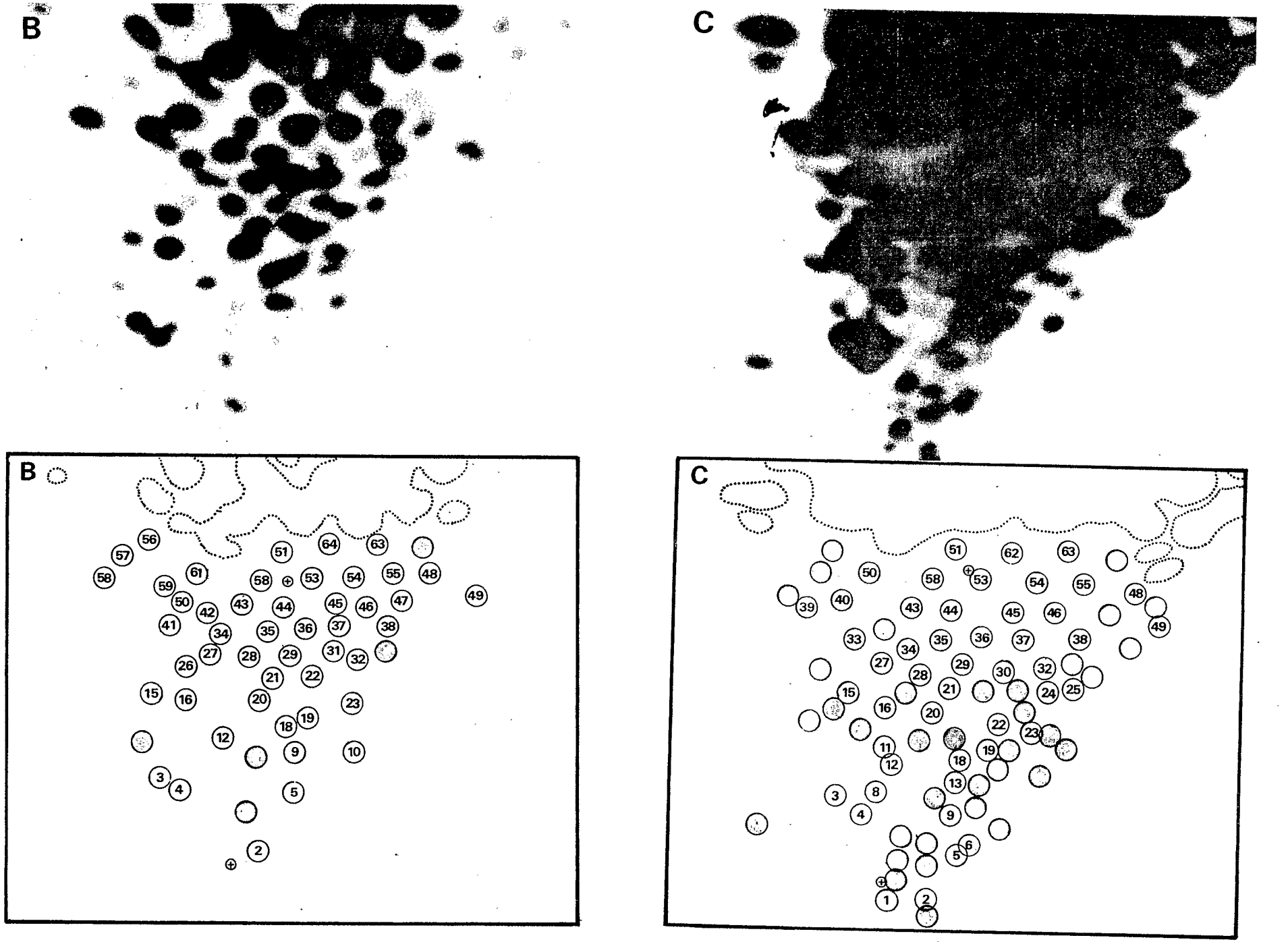


FIG. 2. — *RNase T_1 fingerprint of total RNA of several ^{32}P-labelled strains of arenaviruses.*

A = LAS Josiah Lp3, human strain from Sierra Leone; B = LAS Guinea, human strain from Guinea; C = MOP Mp3, rodent strain from Mozambique; D = MOB A11, rodent strain from CAR; E = MOB 3200, rodent strain from CAR; F = LCMV, human strain from USA. The position of bromophenol blue and/or xylene cyanol dyes are indicated by small crossed circle. Grayish circles stand for unique oligonucleotides.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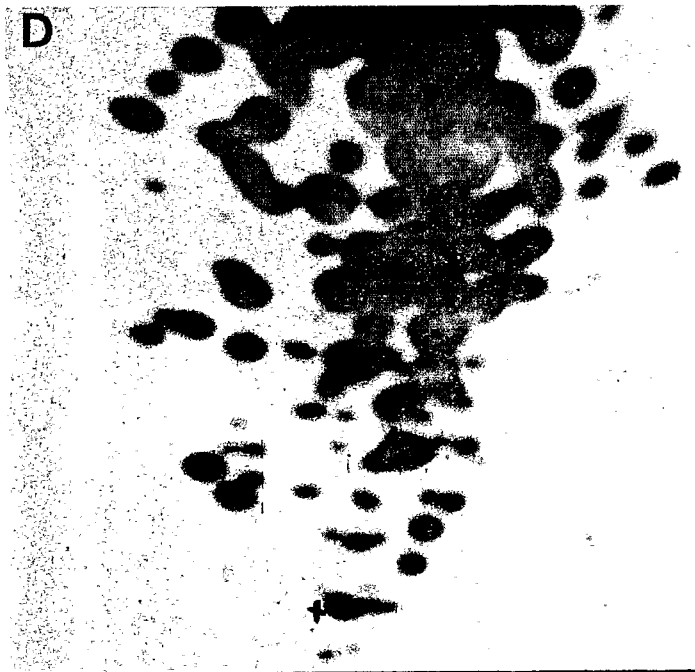
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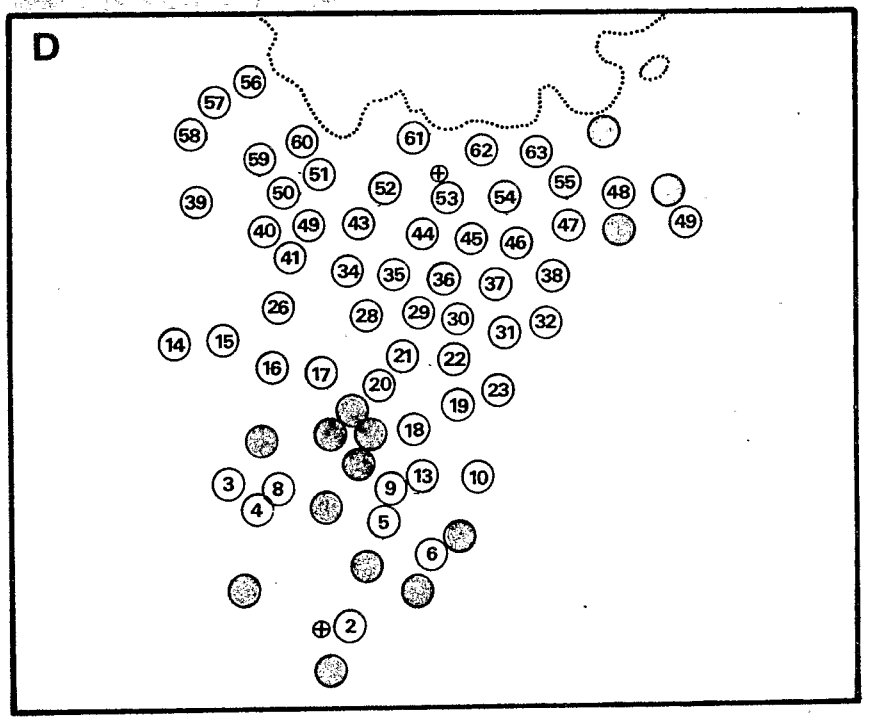
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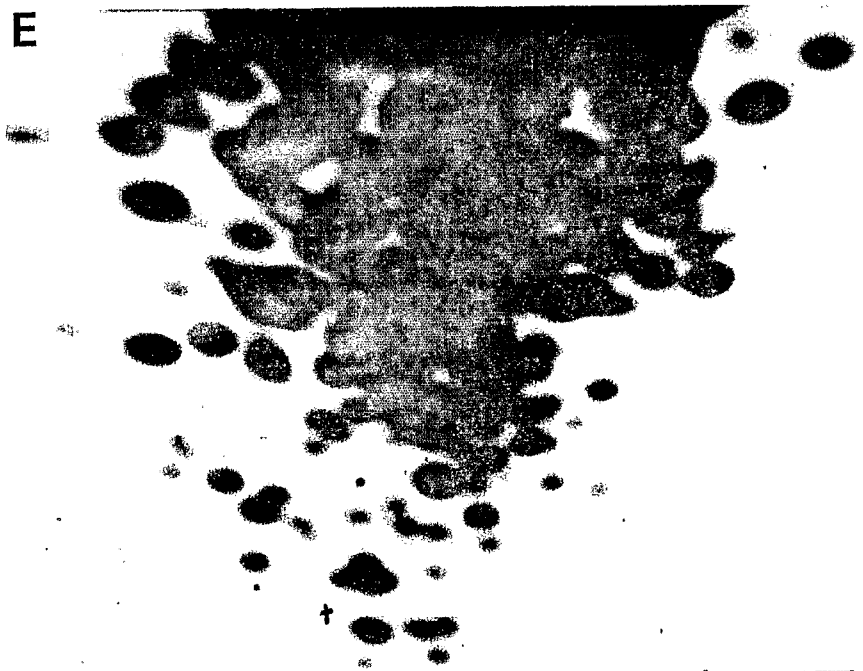
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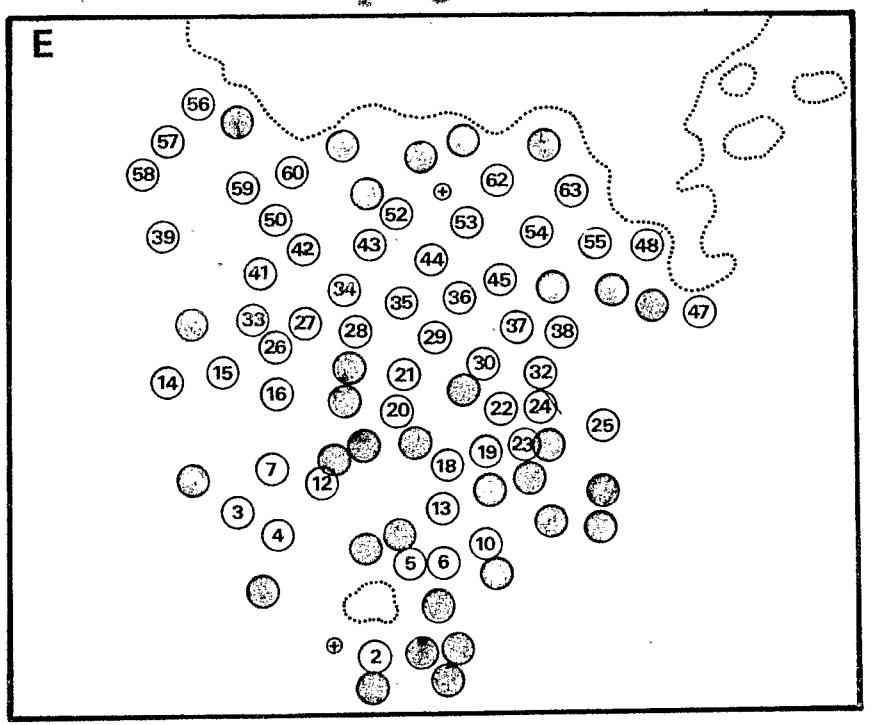
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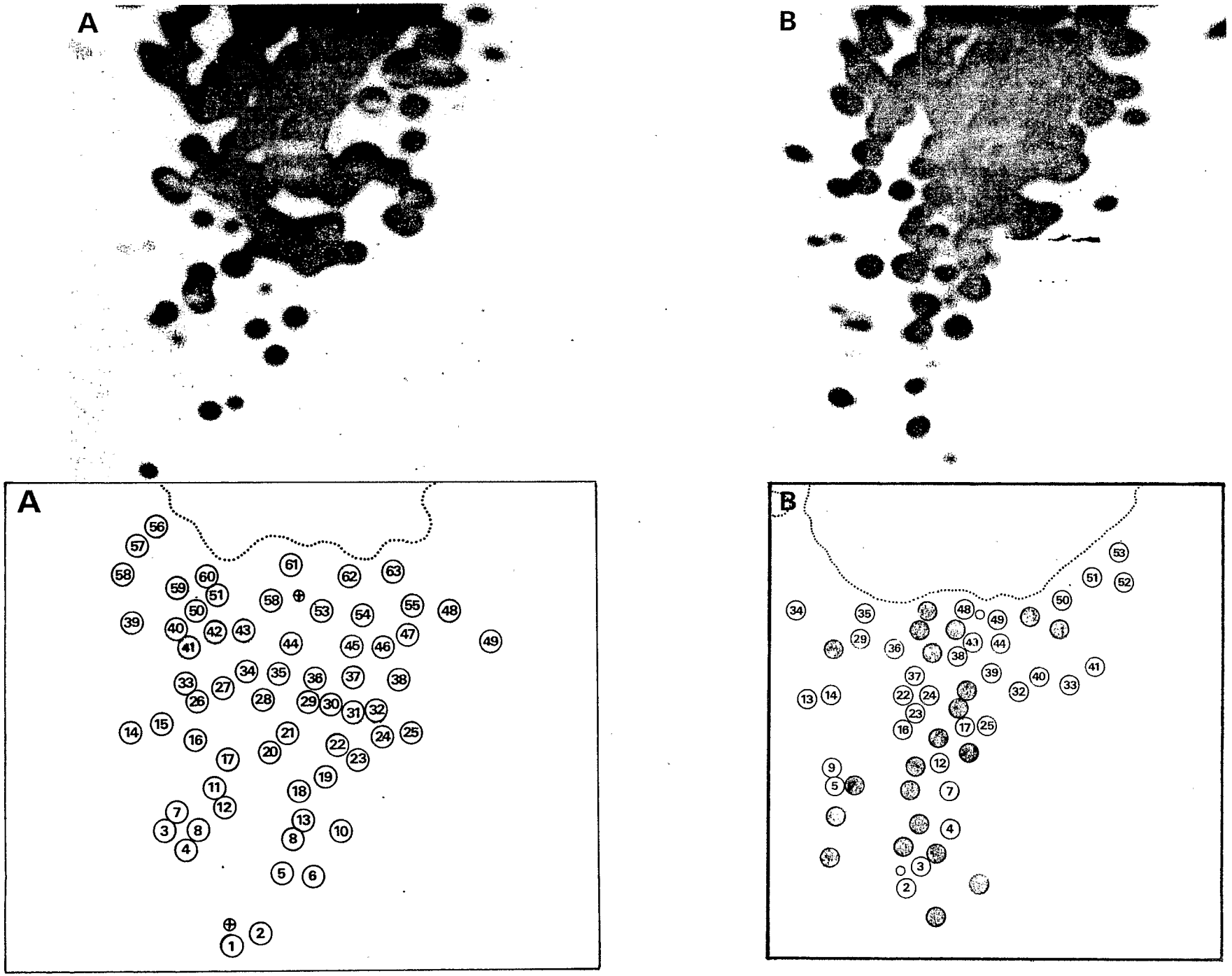
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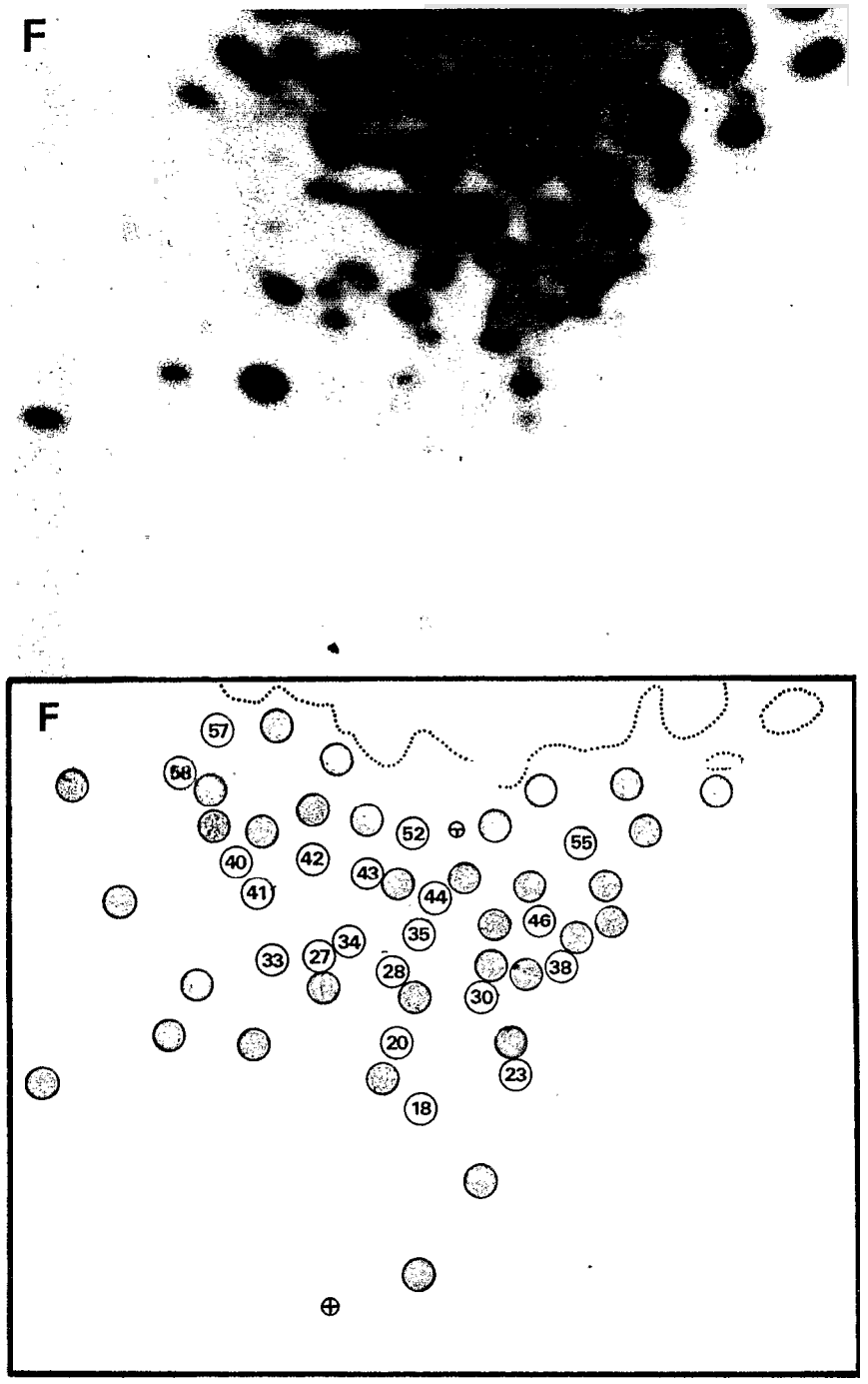


33 unique oligonucleotides of MOB and MOP compared to the reference strain (LAS), 11 were common to the 2 viruses strains. This does not significantly increase their genetic homology and gives a total of 63 % homology of MOP virus when compared to either LAS or MOB strains.

DISCUSSION

The degree of oligonucleotide homology between the different experimental strains compared to the reference strain ranged from 70 to 87 %. The related LCM virus showed only 33 % homology. Considering the whole viral genome, analysis of the T_1-oligonucleotide map permits exploration of only 10 to 15 % of the complete genome and oligonucleotides with the longest sequences are therefore the least frequently analysed. Aaronson *et al.* estimated the sensitivity of the method by computer simulation, which, for all strains studied excluding LCM, gave a 99.8 to 99.5 % absolute base homology [1].

The apparent heterogeneity between the 2 human strains from Sierra Leone and Guinea, mentioned above, reflects a limited change in the genome and favours a high degree of relatedness between these two so geographically close strains.

Previous findings using T_1 mapping of the same reference strain, consistently showed that a human strain from Nigeria was related more to the reference strain than to a rodent strain from Sierra Leone [8].

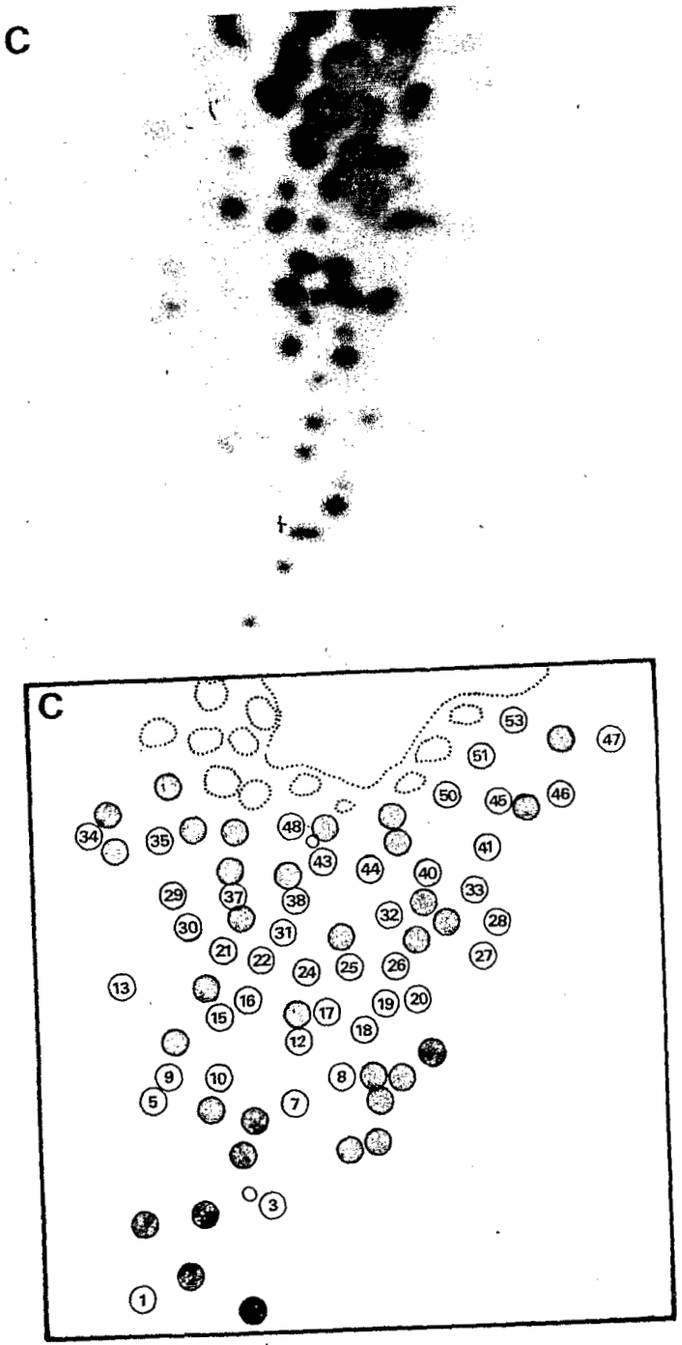
TABLE II. — **RNA T_1-oligonucleotide homology of S-RNA from plaque-purified African arenaviruses.**

Strain	Geographic origin	Percentage homology (*)	Number of single oligonucleotides
LAS	Sierra Leone	100	0
MOP	Mozambique	63	21
MOB	CAR	72	33

(*) Common oligonucleotides *vs.* the 54 analysed from the reference strain, cloned S-RNA of LAS Josiah Lp3.

FIG. 3. — *RNase T_1-oligonucleotide fingerprint of the ^{32}P-labelled S-RNA of three arenaviruses isolates from Africa.*

A = S-RNA from LAS Josiah Lp3 human cloned strain; B = S-RNA from the MOP Mp3 rodent cloned strain; C = S-RNA from the MOB A11 rodent cloned strain.



Even though heterogeneity between genomes of the strains studied was observed, with S-RNA having a larger variability than L-RNA, from the point of view of genetics, differences are few. However, study of S-RNA was done because of its implication in the synthesis of structural proteins, *i.e.,* some of the differences between human and rodent arenavirus strains are found in the antigenic molecular determinants **[13, 14]**.

Nevertheless, such differences may reflect a greater antibody pressure on surface proteins compared to the need for conservation in the replication factors associated with L-RNA that code for the non-structural proteins. On one hand, heterogeneity appears to affect antigenic sites present on structural proteins, as determined by the use of monoclonal antibodies, and on the other hand, certain biological characteristics (*i.e.* plaque morphology, experimental pathogenicity, host specificity) **[10]**.

Therefore, a genetic gradient of divergence in S-RNA between West and Southern African strains, without assuming the direction, may be observed; it appears that MOB virus from Central Africa is situated genetically in an intermediate position between LAS virus from West Africa and MOP virus from Austral Africa **[11]**. These conclusions are based on oligopeptide maps of glycoprotein-2 which showed a greater identity between MOP and MOB than between them and LAS virus **[8]**. Observations such as pathogenicity for suckling mice, with a mortality rate varying from 0 to 70 %, depending on virus strain, appear to favour the existence of such variations **[7, 8]**. Evolutionary changes such as this could have been generated by migration of the rodent host and isolation of the strain in host-specific rodents inhabiting a new ecological niche **[11]**.

In the future, it will be largely the sequence homology of genomes, including arenaviruses of America and LCM virus strains, which should give a better idea of the phylogeny of arenaviruses in relation to their rodent-host evolution.

RÉSUMÉ

VARIATIONS GÉNÉTIQUES DE SOUCHES D'ARÉNAVIRUS DU COMPLEXE LASSA ANALYSÉES PAR «FINGERPRINT»

Les ARN totaux et SARN (small RNA) de différentes souches d'arenavirus d'Afrique (complexe Lassa) et d'une souche du virus de la choriméningite lymphocytaire de la souris, ont été comparés à l'aide de la technique de migration bidimentionnelle des oligonucléotides de restriction de la ribonucléase T_1. Ces souches présentent une homologie de 76 à 86 % du genome exploré. Un essai d'interprétation est proposé sur la base de la biologie évolutive de ces souches appartenant au complexe Lassa.

MOTS-CLÉS: Lassa, Arénavirus, Génétique, ARN; Carte, Evolution, Afrique.

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