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Comparison of 17 Genome Types of Adenovirus Type 3 Identified among Strains Recovered from Six Continents

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Restriction endonucleases *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I were used to analyze 61 selected strains of adenovirus type 3 (Ad3) isolated from Africa, Asia, Australia, Europe, North America, and South America. It was noted that the use of *Bam*HI, *Bcl*I, *Bgl*II, *Hpa*I, *Sal*I, and *Sma*I was sufficient to distinguish 17 genome types; 13 of them were newly identified. All 17 Ad3 genome types could be divided into three genomic clusters. Genome types of Ad3 cluster 1 occurred in Africa, Europe, South America, and North America. Genomic cluster 2 was identified in Africa; genomic cluster 3 was identified in Africa, Asia, Australia, Europe (a few), and North America. This was of interest because 15 identified genome types of Ad7 could also be divided into three genomic clusters. The degree of genetic relatedness between the 17 Ad3 and the 15 Ad7 genome types was analyzed and was expressed in a three-dimensional model.

Since Rowe et al. (25) isolated the adenovirus 3 type (Ad3) prototype (Ad3p) in 1953, Ad3 has been demonstrated to have a worldwide distribution (34).

Ad3 isolates accounted for 13% of the 24,184 adenovirus strains reported to the World Health Organization in a survey of respiratory virus infections from 1967 to 1976 (27). The Ad3 strains were most frequently isolated from children (8, 21, 25, 31).

The impact of single-base substitutions for the generation of genetic diversity can be evaluated by DNA restriction analysis (32). Detailed characterizations of the genome of Ad3p have been performed (1, 4, 17, 30). In our analysis of the genetic variability of Ad3 strains, we have previously analyzed 121 isolates by use of restriction endonucleases (REs) *Bam*HI and *Sma*I and found the three genome types Ad3a, Ad3b, and Ad3c (33, 34, 36). Here we report results of an in-depth analysis of 59 strains of Ad3 representing a subset of 155 isolates from six continents and the two intermediate strains Ad3-7 (15) and Ad3-16 (6) by use of 12 REs: *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I. Altogether, 17 different genome types of Ad3 were detected that could be grouped into three genomic clusters. This is of considerable interest because the previously identified 15 genome types of Ad7 were also grouped into three genomic clusters (12). The genetic relatedness between all identified genome types of Ad3 and Ad7 was therefore established, with the aim of describing the genome type and serotype concepts.

MATERIALS AND METHODS

Basis for selection of Ad3 strains. The 59 strains of Ad3 and two intermediate strains of Ad3-7 and Ad3-16 were identified as Ad3 by the neutralization test. They represent a subset of 155 Ad3 isolates recovered from six continents. A total of 121 of these 155 isolates were previously analyzed with *Bam*HI and *Sma*I (33, 34). Of these 121 strains, 7 were isolated in South Africa, 14 in Japan and China, 5 in Australia, 62 in Holland and Sweden, 22 in Canada and the United States, and 11 in Brazil. Ad3p, Ad3a, Ad3b, and Ad3c were identified among these 121 strains isolated from six continents. We selected two to seven strains from each region that represented different genome types (Table 1). To observe the extent of the genetic variability among the Ad3 strains isolated in one limited area during a defined period, 32 of 124 strains isolated in the 302nd Hospital, Beijing, People's Republic of China, from 1962 to 1985 were analyzed in detail. Of the 32 strains, 24 were isolated from throat swab specimens, 7 from feces, and 1 from an autopsied lung.

Preparation of viral DNA. All strains were propagated in the A-549 cell line. The intracellular viral DNA was treated with phenol and precipitated with isopropanol as described previously (28).

DNA restriction. The REs *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst* EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I were purchased from Boehringer GmbH, (Mannheim, Federal Republic of Germany). All enzyme reactions were carried out as described by Maniatis et al. (14).

Agarose electrophoresis of DNA restriction fragments. The DNA fragments were separated by electrophoresis in 0.8 to 1.2% agarose (HGT; SeaKem) horizontal slab gels which were prepared and run in 89 mM Tris-borate buffer (pH 8.3) with 2.5 mM EDTA at 50 V. Gels were stained in ethidium bromide (0.5 μg/ml). Photography was performed with short-wave UV light with Polaroid film (4 by 5 Land Film, type 55 or 57; Polaroid).

RESULTS

Identification of 17 genome types of Ad3. Analysis of DNA extracted from 61 selected Ad3 strains with the 12 REs *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I revealed the occurrence of 17 Ad3 genome types. They were designated Ad3p, Ad3p1, Ad3p2, Ad3a, Ad3a1 to Ad3a8, Ad3b, Ad3c, Ad3d, Ad3e, and Ad3-7. Thirteen of them, Ad3p1, Ad3p2, Ad3a1 to Ad3a8, Ad3d, Ad3e, and Ad3-7, were newly discovered. Schematic presentations of the DNA restriction patterns of these 17 genome types are shown in Fig. 1 and 2. Each of the three REs *Bam*HI, *Bcl*I, and *Bgl*II recognized six different DNA restriction patterns among the Ad3 strains. *Bst*EII, which can distinguish six different genome types of Ad7 (12), only yielded one pattern upon analysis of the 61 Ad3 strains.

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TABLE 1. Origin of 61 Ad3, Ad3-7, and Ad3-16 strains identified as 17 different genome types

Genome type[a]	Representative strain	Place of isolation	Yr of isolation	No. of isolates
Ad3p	GB	United States	1953	1
Ad3p1	66-13887	Holland	1966–1980	2
	B17737	Brazil	1982	2
	295/73	South Africa	1973	2
	SC8(Ad3-16)	United States	1959	1
Ad3p2	484/67	South Africa	1967	1
Ad3a	5-0164	United States	1977–1981	2
	405/82	Canada	1982	2
	YC79-78	Japan	1979–1980	2
	79-8766	Holland	1979	1
Ad3a1	4076-75	Australia	1975	1
Ad3a2	BC8	People's Republic of China	1962–1985	26
	368-72	South Africa	1972–1973	2
Ad3a3	J1385	Japan	1980	1
Ad3a4	BC4020	People's Republic of China	1983	3
Ad3a5	BC4255	People's Republic of China	1983–1984	2
Ad3a6	BC4621	People's Republic of China	1984	1
Ad3a7	11670	Canada	1983	1
Ad3a8	11489	Canada	1983	1
Ad3b	61-6247	Holland	1961	1
Ad3c	TC3449	Japan	1983	1
Ad3d	8543	Australia	1982	2
Ad3e	440/71	South Africa	1971	2
Ad3-7	Takeuchi	Japan	1966	1

[a] The genome types were designated as described by Li and Wadell (12).

Six REs, *Bam*HI, *Bcl*I, *Bgl*II, *Hpa*I, *Sal*I, and *Sma*I, were used to distinguish all of the 17 genome types of Ad3.

Analysis of 17 genome types. Several genome types were very closely related in pairwise comparison (Fig. 1 and 2). Only one restriction site differed between the genomes of Ad3p and Ad3p1, Ad3p1 and Ad3b, Ad3a and Ad3a2, Ad3a and Ad3a3, Ad3a and Ad3a8, Ad3a and Ad3c, Ad3a2 and Ad3a6, and Ad3a6 and Ad3a5. For instance, the approximate 4,600- and 2,420-base-pair Ad3p *Bam*HI fragments were expected to form the 7,020-base-pair *Bam*HI fragment of Ad3b.

To estimate the genetic relationships between the 17 genome types of Ad3 and the 15 genome types of Ad7, pairwise analysis of comigrating DNA restriction fragments was performed. Between 158 and 172 fragments were compared for each pair of genome types (Table 2). The degree of pairwise comigrating restriction fragments (PCRFs) varied from 61 to 98% among the 17 Ad3 genome types and from 57 to 80% when the 17 Ad3 genome types were compared with the 15 Ad7 genome types.

The degree of genetic relatedness was used to group the 15 genome types of Ad7 into three clusters (12). By use of the same approach, the 17 genome types of Ad3 could be grouped into three clusters designated as genomic cluster 1, containing Ad3p, Ad3p1, Ad3p2, and Ad3b; genomic cluster 2, containing one member only, Ad3e; and genomic cluster 3, containing Ad3a, Ad3a1 to Ad3a8, Ad3c, Ad3d, and Ad3-7. To ascertain the relevance of the suggested clusters, the data were analyzed by the χ^2 method (Table 3). The highly significant deviations confirmed the occurrence of the three genomic clusters of Ad3 and the three genomic clusters of Ad7 genome types.

Distribution of genome types of Ad3. (i) Africa. Both Ad3p1 and Ad3p2 of genomic cluster 1 and Ad3e, the only member of cluster 2, were isolated in South Africa from 1967 to 1973. Ad3p1 and Ad3p2 shared 95% PCRFs. Ad3a2 of cluster 3 was also detected in South Africa from 1972 to 1973.

(ii) Asia. The genomic cluster 3 of Ad3 predominated from 1962 to 1985. Ad3a, Ad3a3, Ad3c, and Ad3-7 were detected in Japan only. The Ad3-7 genome type was isolated in 1966. This strain was neutralized by both Ad3 and Ad7 antisera,

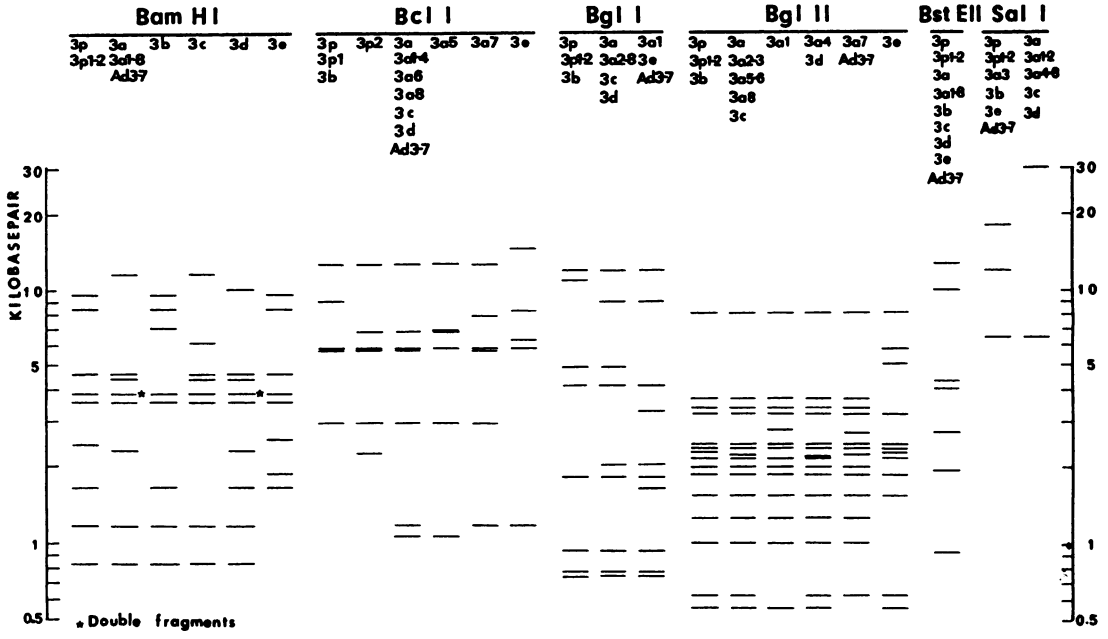


FIG. 1. Schematic presentation of restriction patterns obtained after cleavage of DNA from 17 genome types with *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, and *Sal*I.

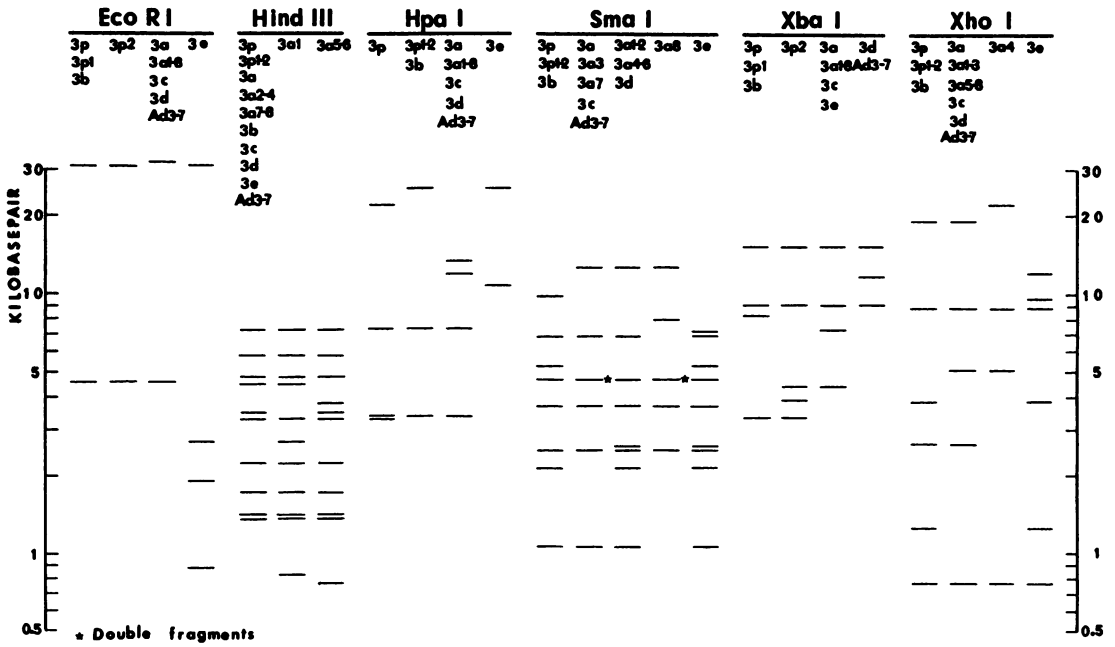


FIG. 2. Schematic presentation of restriction patterns obtained after cleavage of DNA from 17 genome types with *Eco*RI, *Hin*dIII, *Hpa*I, *Sma*I, *Xba*I, and *Xho*I.

was identified as type 3 by the hemagglutination inhibition test, and was regarded as a serological intermediate (15, 20, 24). In the present analysis Ad3-7 was identified as a distinct genome type that could be definitely grouped into cluster 3 of Ad3. Ad3-7 was closely related to Ad3a3. They displayed 93% PCRFs.

Ad3a2 predominated in the People's Republic of China for 24 years (1962 to 1985). Ad3a4, Ad3a5, and Ad3a6 have occurred in parallel with Ad3a2 since 1983, when an outbreak of Ad3 infections with characteristic symptoms, upper respiratory tract infection, pharyngoconjunctival fever, pneumonia, and diarrhea, took place in the Beijing area. One child who suffered from measles died from adenovirus pneumonia associated with Ad3a2. The Ad3a2, Ad3a4, Ad3a5, and Ad3a6 genome types were very closely related and shared 95 to 98% PCRFs.

(iii) Australia. Ad3a1 and Ad3d of genomic cluster 3 were isolated in Australia. Ad3a1 appeared in 1975, while Ad3d was isolated in 1982. Five restriction sites differed between the Ad3a1 and Ad3d genome types (Fig. 1 and 2).

(iv) Europe. During the period 1961 to 1980, genomic cluster 1 predominated. It was represented by Ad3p1 and Ad3b. In addition, a few strains of Ad3a were isolated. Ad3b appeared in 1961, whereas Ad3p1 was first detected in 1966. Ad3p1 and Ad3b were very closely related and displayed 98% PCRFs.

(v) North America. Ad3p and Ad3p1 of genomic cluster 1 were isolated in the United States. Ad3p is the prototype of Ad3 and was first isolated in 1953. The intermediate type Ad3-16 was typed as Ad3 by the neutralization test and as Ad16 in the hemagglutination inhibition assay (6). The Ad3-16 dodecons could be distinguished from both Ad3 and Ad16 (18). The RE analysis revealed that Ad3-16 was identified as Ad3p1 of the Ad3 cluster 1. Ad3p and Ad3p1 were very closely related and displayed 98% PCRFs.

Since 1977, genomic cluster 3 made up of Ad3a, Ad3a7, and Ad3a8 has been detected in North America. They shared 96 to 98% PCRFs.

(vi) South America. The Ad3p1 genome type was isolated in Brazil in 1982.

The degree of genetic relatedness between all identified genome types of Ad3 and Ad7. To express the relationships between the 17 genome types of Ad3 and the 15 genome types of Ad7, a coefficient of the estimated genetic difference between the two members in each analyzed pair of genome types was calculated by the following formula: $K = ([100/x] - 1) \times 100$, where the coefficient of genetic difference between two genome types K is the inverse ratio of the percentage (x) of PCRFs. When x reaches 100, i.e., identity, K, which expresses the difference, should be 0. The formula is consequently expressed as follows: $K = (100/x) - 1$. The K was amplified 100 times, as expressed in Table 2.

Subsequently, a three-dimensional model of the relationship between all identified genome types of Ad3 and Ad7 was constructed (Fig. 3).

A compilation of information on the first identified appearance and persistence of each of the identified Ad3 and Ad7 genome types revealed that the members of the genomic clusters also displayed a relation with regard to the original date of identification. The prototype strains were not identified any longer, but certain genome types persisted during most of (Ad3p1, Ad3a2, and Ad7a1) or the entire (Ad7b) observation period (Fig. 4).

DISCUSSION

Ad3 and Ad7 are two closely related serotypes, both of which cause a whole array of respiratory symptoms with or without conjunctivitis (2, 3, 9–11, 13, 16, 19, 21–23, 31) and which sometimes cause urinary or enteric tract infection (5, 37). The infections may have a severe or even fatal outcome (7, 10, 29).

Infections by Ad3 and Ad7 cannot be clinically distinguished, although there seems to be more reports of fatal infections caused by Ad7. Both serotypes Ad3 and Ad7 are widely distributed and account for 13 and 19.7%, respec-

TABLE 2. Comigration analysis of 17 Ad3 and 15 Ad7 genome types[a]

	3p	3p1	3p2	3b	3e	3a	3a1	3a2	3a3	3a4	3a5	3a6	3a7	3a8	3c	3d	3-7	7p	7p1	7g	7a	7a1	7a2	7a3	7a4	7a5	7b	7c	7d	7d1	7e	7f
3p		98	93	96	70	75	72	76	77	74	73	74	73	73	75	77	74	73	71	73	68	66	64	65	66	66	66	66	64	62	68	65
3p1	2		95	98	72	75	72	76	77	74	74	74	74	73	76	77	74	73	72	73	69	66	65	65	66	67	67	67	65	63	68	65
3p2	7	5		93	71	77	73	78	79	76	75	76	74	75	77	78	74	76	72	75	69	68	65	66	68	68	68	68	66	64	69	67
3b	4	2	7		71	75	71	75	77	73	73	74	73	73	75	77	74	73	71	74	68	65	64	65	65	66	67	66	65	63	67	64
3e	42	39	41	40		63	65	65	65	66	63	64	62	61	64	64	63	65	65	69	64	62	59	60	62	61	62	62	60	57	64	63
3a	34	33	30	34	58		93	98	98	95	94	96	96	98	98	93	93	70	68	78	64	67	62	65	67	65	70	68	70	65	66	66
3a1	40	39	36	41	54	8		95	91	93	91	93	89	91	91	91	89	68	66	79	65	68	65	66	68	66	71	68	70	65	67	67
3a2	32	31	29	33	53	2	6		96	97	97	98	94	96	96	95	91	71	69	80	66	69	64	67	69	67	70	72	70	72	67	68
3a3	30	29	27	31	53	2	10	4		93	93	95	94	97	96	91	94	72	70	80	66	69	64	67	69	67	72	70	72	67	68	68
3a4	36	35	32	36	52	5	8	3	7		93	95	91	93	93	94	88	69	67	78	64	66	62	65	66	64	69	67	68	64	66	66
3a5	37	36	33	37	59	6	10	4	8	7		98	91	93	93	91	87	68	66	76	63	67	62	65	67	66	70	67	69	66	65	65
3a6	35	34	32	36	57	4	8	2	6	5	2		93	94	94	93	89	69	67	78	64	68	63	66	68	66	71	68	70	65	66	66
3a7	36	36	35	37	62	4	13	6	6	10	10	8		94	94	89	93	68	66	76	63	67	63	65	67	66	70	67	69	66	65	64
3a8	37	36	34	38	63	2	10	4	4	7	8	6	6		96	91	91	68	66	76	62	65	60	63	65	63	68	66	67	63	64	64
3c	33	32	29	33	57	2	10	4	4	7	8	6	6	4		91	91	70	68	78	64	68	63	66	68	66	71	68	70	65	66	66
3d	30	29	29	31	56	7	10	5	9	6	9	7	12	9	9		90	69	67	77	63	66	63	65	66	64	69	66	69	63	65	65
3-7	36	35	34	36	58	8	12	10	6	14	15	12	8	10	10	12		70	68	80	67	71	67	70	71	69	74	71	73	69	69	70
7p	37	36	31	38	53	43	47	42	39	46	46	45	46	47	42	44	43		93	74	78	75	71	73	75	73	75	75	75	69	75	72
7p1	41	40	39	41	35	47	52	46	43	50	51	49	51	52	46	48	47	7		72	75	72	68	70	72	70	71	71	72	66	78	74
7g	37	36	34	36	45	29	26	25	25	29	31	28	31	32	28	29	25	35	39		76	77	73	75	77	75	81	76	79	75	78	76
7a	46	46	45	47	56	57	53	52	52	57	58	56	58	62	56	58	48	29	34	31		91	87	89	89	89	88	90	87	83	92	88
7a1	52	51	47	53	62	48	47	44	44	51	49	47	49	53	47	52	41	33	39	30	10		95	98	99	98	96	98	95	89	92	90
7a2	56	55	54	57	70	61	54	56	56	61	62	60	59	66	60	59	49	40	46	37	15	5		97	94	95	91	93	90	85	89	86
7a3	54	53	52	55	67	53	52	48	48	56	54	52	54	58	52	54	42	36	42	33	13	2	3		97	96	94	96	93	87	91	88
7a4	52	51	47	53	62	48	47	44	44	51	49	47	49	53	47	52	41	33	39	30	12	1	6	3		97	95	97	94	87	91	88
7a5	51	50	46	52	64	53	52	48	48	56	51	52	51	58	52	57	45	36	42	33	13	2	6	4	3		94	96	93	90	91	88
7b	51	50	46	49	61	42	42	38	38	45	43	42	43	46	41	46	35	34	40	23	14	4	10	6	6	6		95	98	92	89	93
7c	51	50	46	52	61	47	46	43	43	50	48	46	48	52	46	51	40	34	40	31	11	2	7	4	3	4	5		95	88	92	89
7d	56	55	51	54	66	44	43	40	40	46	45	43	45	48	43	47	37	33	39	26	15	5	11	7	6	7	2	6		93	89	91
7d1	61	60	56	59	76	54	53	49	49	57	52	53	52	59	53	58	46	44	51	34	21	13	18	15	14	11	9	14	7		82	85
7e	47	46	46	48	57	52	48	47	47	52	53	51	53	57	51	53	44	34	29	28	8	8	13	10	10	10	12	9	13	22		90
7f	55	54	50	56	58	52	48	47	47	52	53	51	53	57	51	53	44	39	36	31	14	11	16	14	13	14	8	12	10	17	11	

[a] A total of 158 to 172 restriction fragments were analyzed for each pair of genome types after genomes were cleaved with 12 REs. Values in the upper right indicate the percentage of PCRFs; values in the lower left indicate the coefficient of space between the two genome types.

tively, of all adenovirus isolates reported to the World Health Organization (27).

There are certain differences in the frequency of isolation of the Ad3 and Ad7 strains in different countries. In Japan Ad3 accounts for one half of all adenovirus isolates (35), whereas Ad7 is rarely isolated. During a period of 24 years (1962 to 1986), 124 strains of Ad3 and 57 strains of Ad7 were isolated at the 302nd Hospital, Beijing. In Europe Ad7 isolates are 2.5 times more frequently detected than Ad3 (33). Ad7, but not Ad3, has been responsible for outbreaks of pneumonia among military conscripts.

TABLE 3. Test of significance of the means of PCRFs within and between genomic clusters[a]

Comparison of groups[b]:		χ^2
Within one cluster	Between two clusters	
3GC1 (156.7/7.3)	3GC1-3GC2 (114/47.1)	34.37
3GC1 (156.7/7.3)	3GC1-3GC3 (121.1/41.7)	27.38
3GC3 (151.6/11.1)	3GC3-3GC1 (121.1/41.7)	21.15
3GC3 (15.6/11.1)	3GC3-3GC2 (103.3/58.1)	41.07
7GC1 (158/11)	7GC1-7GC2 (120.0/44.5)	25.36
7GC1 (158/11)	7GC1-7GC3 (122.4/45.8)	25.84
7GC3 (153.1/14.4)	7GC3-7GC1 (122.4/45.8)	19.80
7GC3 (153.1/14.4)	7GC3-7GC2 (126.8/38.5)	13.44

[a] All values are significant at $P < 0.001$.

[b] 3GC1 is the abbreviation for the Ad3 genomic cluster 1; 7GC1 is the abbreviation for the Ad7 genomic cluster 1, and so on. Values in parentheses indicate mean PCRFs/mean of different fragments.

Differences in pathogenicity among strains influence the representativity of any selection of virus strains, with the exception of studies designed as part of the Virus Watch Program (35). An inherent limitation in the studies of the evolution of viruses is their restriction to isolates that cause disease of a sufficient severity to merit isolation.

The prototype Ad3 and Ad7 strains originally isolated in 1953 and 1954 can no longer be identified. However, the Ad3p1 and Ad7p1 members of these two clusters have recently been isolated. This can be taken as a sign of a divergent evolution. During epidemic outbreaks several genome types of the same serotype frequently cocirculate, e.g., in Beijing from 1958 to 1959, Ad7a1, Ad7a4, Ad7b, and Ad7g; in Japan from 1979 to 1980, Ad3a and Ad3a3; and in

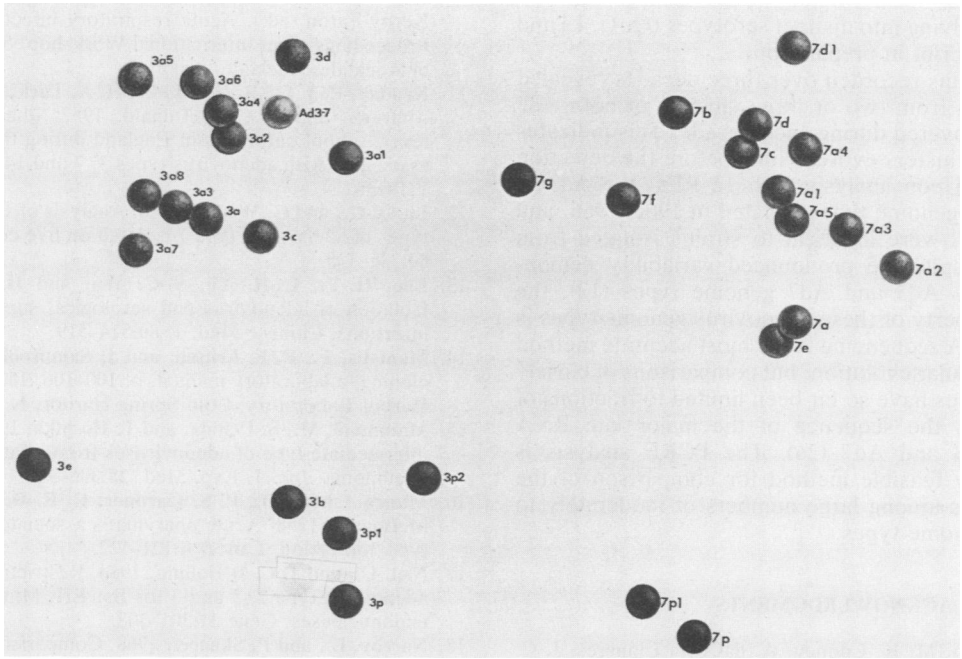
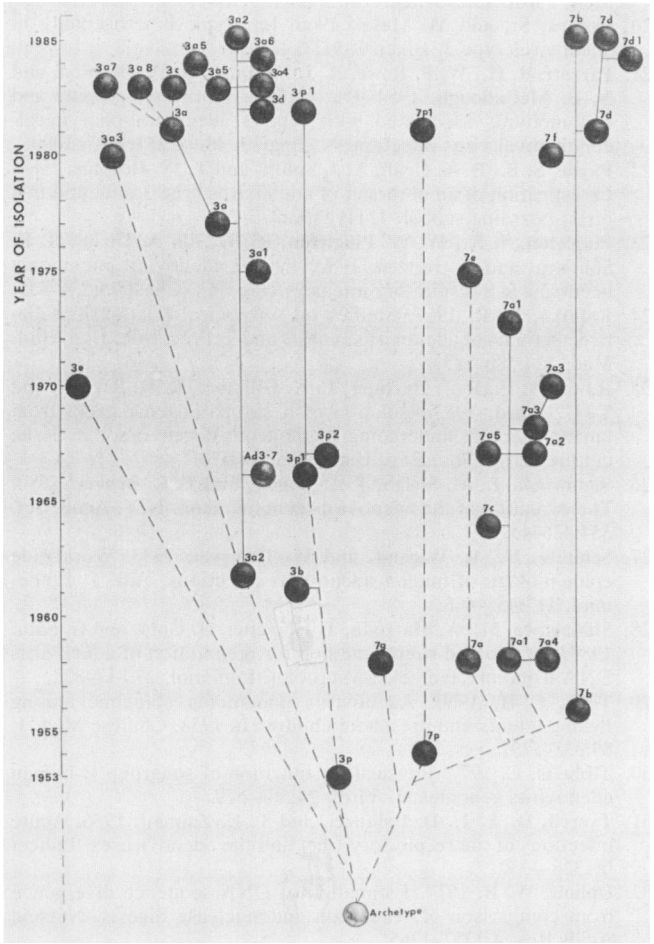


FIG. 3. Projection of the three-dimensional model of the relationship among 33 genome types of Ad3 and Ad7.



Beijing from 1983 to 1984, Ad3a2, Ad3a4, Ad3a5, and Ad3a6.

The relations revealed by PCRFs presented in the closed-loop form enabled an evaluation of the genetic relatedness among the 33 genome types of Ad3 and Ad7. All genome types together formed six genomic clusters that were genetically related. The degree of PCRFs between distantly related genome types was of the same order within the two serotypes. The genetic relationship could, rather, be more distant between two clusters within one serotype than between clusters of two different serotypes.

The so-called intermediate strains Ad3-7 (15) and Ad3-16 (6) share epitopes defined by neutralization or hemagglutination inhibition assays (18). They have been considered as progeny of recombinants between the parental strains, bringing some disorder to the adenovirus taxonomy based on the serotype concept. Here it was demonstrated that Ad3-16 is a member of the Ad3 genomic cluster 1, whereas Ad3-7 belongs to the Ad3 genomic cluster 3.

The genomic clusters are considered as products of a divergent evolution that ultimately can be defined as separate genetic entities. However, differentiation of the epitope constellation on the exterior of the hexon is also required to justify distinction as separate serotypes. Structural constraints in the hexon polypeptide can be expected to conserve the epitopes that induce neutralizing antibodies. There are, in this respect, distinct differences between subgenus D, containing a multitude of closely related serotypes (1), and subgenus E, containing Ad4 only. This unique serotype hosts markedly different genomic clusters which nature has

FIG. 4. Branching diagram of genome types of Ad3 and Ad7 showing the time of identified appearance and the prevalence of genome types and the relation between the most closely related genome types. Symbols: ——, period of prevalence; ----, tentative relation between genome types.

refrained from evolving into distinct serotypes (Q.-G. Li and G. Wadell, manuscript in preparation).

Analyses of strains recorded over three decades revealed that genome types from two or three clusters of both Ad3 and Ad7 were recovered during each decade. This indicates that the genomic clusters evolved long before the detection of adenoviruses. Upon analysis with 12 REs, the Ad3a2, Ad3p1, and Ad7b genome types isolated in 1962, 1966, and 1956, respectively, were identical to strains isolated from 1983 to 1985. Despite the pronounced variability demonstrated among the Ad3 and Ad7 genome types (12), the characteristic property of these adenovirus genome types is conservation. DNA sequencing is the most accurate method for studying molecular evolution; but comparisons of closely related adenoviruses have so far been limited to fractions of the genome, e.g., the sequence of the major late RNA promoters of Ad3 and Ad7 (26). The PCRF analysis is presently the only feasible method for comparison of the genetic relatedness among large numbers of moderately to closely related genome types.

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