Genetic Composition of a High-Yielding Influenza A Virus Recombinant: A Vaccine Strain Against "Swine" Influenza

Abstract. *Analysis of the RNA migration pattern of a high-yielding influenza virus recombinant, X-53, used in vaccine production, reveals that only the two genes coding for hemagglutinin and neuraminidase antigens were derived from the "swine" influenza virus parent, A/New Jersey/11/76, while six were acquired from the A/PR/8/34 (H0N1) parent, donor of the high yield characteristic.*

Artificial immunization against influenza presents unusual problems because of frequent antigenic mutations of the virus that dictate correspondingly frequent changes in the viral composition of vaccines. Because optimal yields of virus are rarely obtained following inoculation of the allantoic sac of the chick embryo with influenza virus strains newly isolated from man, tedious and time-consuming "adaptation" of the virus generally has been required to make vaccine production feasible. In recent years, high-yielding vaccine viruses have been produced by recombination through mixed infection of various H3N2 (Hong Kong) (*1*) variants with a high-yielding donor strain A/PR/8/34 (H0N1) (*2*). Selection of recombinants of desired antigenicity and rapid growth capacity is effected by suppression of virus of PR8 external antigen phenotype with antiserum and by subsequent passage of virus at high dilution. Thus, the selected virus bears the external (hemagglutinin and neuraminidase) antigens of the new isolate but has acquired enhanced capacity for replication in the allantoic sac—presumably related to the incorporation of PR8 genes. In this report, we present analysis of the genetic composition of a high-yielding recombinant, X-53, which was derived from a potential pandemic ("swine") influenza virus A/NJ/11/76 isolated early this year at Fort Dix (*3*) and the laboratory strain, influenza A/PR/8/34 virus (*2*). This recombinant is now being used in vaccine production.

Table 1. Comparative viral yields (HA titer) and antigenic characterization of recombinant X-53 and parental viruses.

Virus	HA titer*	Hemagglutination-inhibition antiserum		Neuraminidase-inhibition antiserum	
		PR8/HK†	A/NJ/11/76	HK/PR8‡	A/NJ/11/76
A/PR/8/34 (H0N1)	8192	320§	<10	5120	< 10
A/NJ/11/76 (Hsw1N1)	16	< 10	2560	640	640
X-53	512	< 10	1280	320	1280

*The yields (HA titer) of influenza A/NJ/11/76 and X-53 viruses were compared after equal number of passages (four) in embryonated eggs after recombination. †Antiserum to antigenic hybrid virus $H_{PR8}N_{HK}$ (H0N2). ‡Antiserum to antigenic hybrid virus $H_{HK}N_{PR8}$ (H3N1). §Reciprocal of serum dilution at end point (*6*).

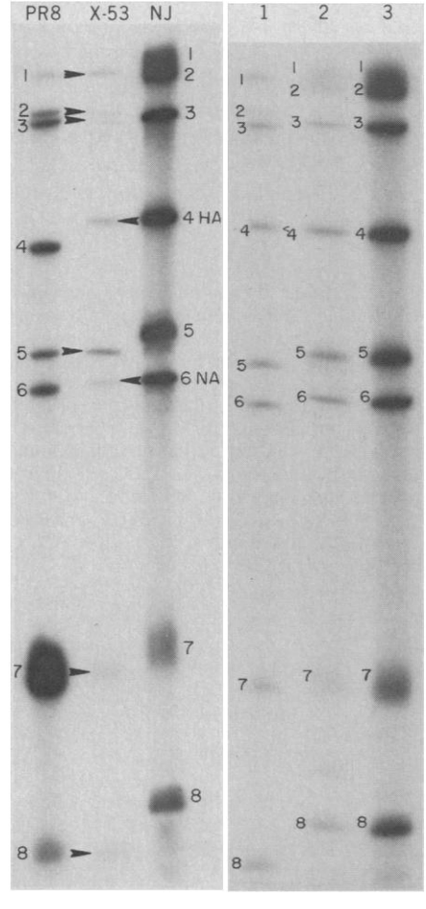

Fig. 1 (left). Analysis of ^{32}P-labeled RNA's of influenza virus recombinant X-53 and the two parent viruses, influenza A/PR/8/34 and A/NJ/11/76 virus, on urea-polyacrylamide gels. Lane 1, influenza A/PR/8/34 virus; lane 2, recombinant virus X-53; and lane 3, influenza A/NJ/11/76. In order to label the viral RNA's, all viruses were grown in MDCK (canine kidney) cells after infection with one to ten plaque-forming units per cell (*4*, *5*). Isolation and separation of the ^{32}P-labeled RNA's on 2.6 percent urea-polyacrylamide gels and autoradiography was done as described (*4*, *5*). Migration is from top to bottom. The RNA's of the parent viruses (lanes 1 and 3) are numbered 1 through 8. The arrows next to the RNA segments of the recombinant X-53 indicate the derivation of the RNA from influenza A/PR/8/34 virus or the "swine" virus A/NJ/11/76. It should be noted that under these conditions of virus growth and RNA labeling equimolar distribution of labeled RNA's is not always observed. Fig. 2 (right). Analysis of ^{32}P-labeled RNA's of a recombinant virus and its parent influenza virus A/NJ/11/76. Lane 1, influenza virus recombinant (A/NJ/11/76[Hsw1]-A/PR/8/34[N1]) derived from influenza virus A/NJ/11/76 and influenza virus A/PR/8/34; lane 2, influenza virus A/NJ/11/76; and lane 3, influenza virus A/NJ/11/76 (same isolate as in lane 2 but different ^{32}P-labeled preparation). Conditions of experiment are as in Fig. 1. The RNA segments of all three samples are numbered 1 through 8. The arrow next to RNA 4 of the recombinant (lane 1) indicates its derivation from the parent virus A/NJ/11/76 (lanes 2 and 3). Bands 7 are not clearly resolved on this gel.

The antigenic characteristics and comparative titers of X-53 and its parental viruses are presented in Table 1. As defined by hemagglutination-inhibition and neuraminidase-inhibition tests, X-53 is identical antigenically with the parental "swine" influenza virus A/NJ/11/76. The hemagglutination (HA) titer of X-53 in allantoic fluid 40 hours after infection of embryonated eggs is 32-fold that of the New Jersey isolate. Polyacrylamide gel analysis of the RNA's (*4*, *5*) of the two parent viruses, influenza A/NJ/11/76 and influenza A/PR/8/34, and of the high-yielding recombinant X-53 is shown in Fig. 1. All three viruses contain eight RNA segments, although the two slowest moving segments of the New Jersey strain are not separated in Fig. 1. The PR8 virus studied previously (*4*, *5*) contains a cluster of three slowly moving RNA's, a cluster of three RNA's in the center, and two fast moving RNA's (lane 1). The corresponding RNA segments of the swine virus (lane 3) are clearly distinguishable from those of PR8 virus. A comparison with the RNA's obtained from the recombinant X-53 (lane 2) shows that only band 4 and band 6 of the recombinant correspond to those of the swine virus. RNA segments 4 and 6 of PR8 virus have been previously shown to code for the hemagglutinin and neuraminidase, respectively (*5*). Consequently, of the eight RNA segments contained in the X-53 recombinant virus only those coding for the hemagglutinin and the neuraminidase are derived from swine virus. The remaining RNA seg-

ments coding for the P proteins, nucleoprotein, membrane protein, and nonstructural protein are derived from PR8 virus.

In order to identify which RNA of X-53 codes for hemagglutinin and which for neuraminidase, the RNA pattern of another recombinant derived from influenza A/PR/8/34 and A/NJ/11/76 virus was analyzed. Serologic analysis of this recombinant demonstrated that like X-53 it derived its hemagglutinin from A/NJ/11/76 virus but its neuraminidase was shown to be of PR8 virus origin. RNA analysis of this recombinant (Fig. 2, lane 1) reveals that it has derived only the fourth RNA segment from the "swine" virus (lanes 2 and 3). This demonstrates that, as is the case with PR8 virus, the fourth RNA of the swine virus codes for the hemagglutinin and the sixth RNA for neuraminidase.

Our finding that the high-yielding recombinant X-53 has derived six of its genes from the PR8 virus does not necessarily indicate that the high yield characteristic requires all six PR8-derived genes. In the course of genetic reassortment PR8 genes not related to enhanced replication may have been incorporated by chance into X-53. Alternatively, because the PR8 virus replicates much faster than the "swine" virus in the allantoic cavity of embryonated eggs, a greater number of PR8 genes is thus available for recombination and might have been incorporated into the genome of the recombinant including some not required for transfer of the high yield characteristic. A definitive answer to the question of how many and which genes are necessary for the transfer of the property of "good growth" to an influenza virus recombinant awaits further genetic analysis.

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References and Notes

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Synchronization of Periodical Cicada Emergences

Abstract. *Synchronized insect emergences are shown to be a possible consequence of predation in the presence of a limited environmental carrying capacity through a mathematical model for cicada populations that includes these two features. Synchronized emergences, like those observed in 13- and 17-year cicadas, are predicted for insects with sufficiently long life-spans. Balanced solutions, in which comparable emergences occur each year, are found for insects having sufficiently short life-spans, such as 3-, 4-, and 7-year cicadas. For the values used here, synchronized emergences occur for insects with life-spans of 10 years or more, and balanced emergences occur for life-spans of fewer than 10 years.*

Thirteen-year cicadas, *Magicicada* spp., appear in large numbers every 13 years, but practically none appear in intervening years (*1*). Seventeen-year cicadas emerge according to a similar pattern but they can bear both 13-year and 17-year progeny (*2*). The synchronization yields a periodic birth rate that is analogous to population waves studied in demography (*3*). However, the extreme form of the cicada periodicity cannot be maintained without mechanisms other than the usual birth and death schedules.

It has been suggested that predation and environmental carrying capacity can act in combination to bring about this synchronization (*1*, *4*). We have shown that this is indeed possible by constructing and analyzing a model of a cicada population that incorporates a predation threshold (*5*) (or predator satiation) and a limited carrying capacity. This model produces many features of cicada populations, such as synchronized emergences for cicadas with sufficiently long life-spans. The synchronized emergence is a consequence of the two conflicting requirements imposed by the predation threshold and the limited carrying capacity of the environment. On the one hand, the number of progeny produced in any year must exceed the predation threshold or they will be eliminated. On the other hand, their number cannot exceed the carrying capacity minus the living progeny produced in earlier years (residual carrying capacity). These requirements can best be met by a synchronized population when the life-span is long enough. However, they can also be met by a population with a short life-span having the same rate of emergence each year.

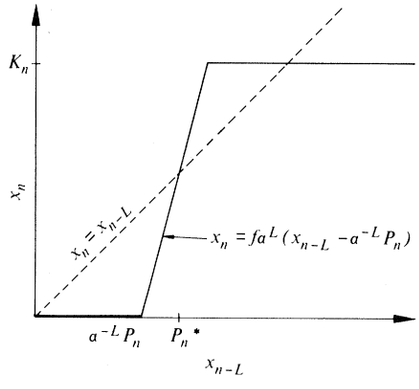

Fig. 1. The piecewise linear reproduction curve (Eq. 4), which determines the number (x_n) of nymphs becoming established underground in year n in terms of the number (x_{n-L}) in year $n - L$. This curve changes each year with the predation threshold (P_n) and the residual carrying capacity (K_n). The straight line $x_n = x_{n-L}$ is shown for reference.

Consider a species having a life-span of L years, with reproduction occurring in year L, followed by the death of the parents. Let x_{n-L} be the number of nymphs becoming established underground in year $n - L$. If α is their survival rate per year, then $x_{n-L}\alpha^L$ of them will survive L years and emerge as adults in year n. We assume that, when they emerge, predators will eliminate as many as P_n of them. There will be none left for mating if $x_{n-L}\alpha^L \leq P_n$; otherwise, there will be $x_{n-L}\alpha^L - P_n$ adults left. We denote this number by $(x_{n-L}\alpha^L - P_n)_+$ (*6*). If f is the number of hatched nymphs becoming established underground that each adult produces in a breeding period, then the total number of nymphs produced in year n is

$$H_n = f(x_{n-L}\alpha^L - P_n)_+ \quad (1)$$