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MODELLING OF VIRUS INTERRELATIONSHIPS FOR COMPUTER ANALYSIS OF INHIBITION DATA

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Abstract—This paper proposes a mathematical model of the evolution of viruses. The model allows computer-assisted determination of ancestry among viruses. We establish a bi-directional correlation between the evolutionary tree of a group of related viruses and the inhibitive relatedness among those viruses. In particular, we show how to generate the evolution tree from data about the ability of some viruses to induce antibodies inhibiting other viruses.

1. INTRODUCTION

The evolution tree ("phylogenetic tree") of a group of related species is the hierarchy of ancestry relationships among the species. The determination of evolution trees among groups of related viruses would greatly broaden our knowledge about viruses and suggest possible ways of inhibition of some viruses.

A virus can induce antibodies which are capable of inhibiting that virus. In the case of a similar viruses, the antibodies induced by one virus may also be capable of inhibiting the other virus, though with lesser potency. In such a case, we say that there is an inhibitive ("antigenic") relationship between the two viruses.

In this paper, we establish a bi-directional correlation between the evolutionary tree of a group of related viruses and the inhibitive relatedness among those viruses. In particular, we show how to generate the evolution tree from data about inhibitive relatedness.

An obvious criterion of ancestry—precedence in time of the species emergence in nature—which can be determined from paleontological data, is often unusable due to the absence of such paleontological data. Since there are no viral fossils, the only time-marking datum is the registered data of the virus isolation. However, the date of virus isolation can be an incidental case not at all corresponding to the real chronology of the virus' "first appearances" ("origin") in nature. (An exception is the case of a few pandemic viruses isolated during the pandemic and post-pandemic period as a result of the worldwide surveillance.)

Another appropriate criterion—the degree of similarity between the related species—is more often available for investigation. However, the latter criterion, even in clear cases when the relatedness can be estimated quantitatively, does not determine the evolutionary direction. For example, even if we can quantify the similarity between two viruses v and w, we do not know whether v is the predecessor of w, or vice versa, or they are siblings. One "heuristic" in this respect, is the idea that the descendant virus should be "more complex" than the ancestor virus. This "heuristic" may be a valuable criterion for the determination of the evolutionary direction for most species. Although in some cases, especially in the case of parasitic species, we notice evolutionary reduction and simplification instead of complication, this "heuristic" seems to be reasonable since the evolution of the biosphere as a whole proceeds progressively from primitivity to complexity. However, according to some modern views, the "directionality in life's history" exhibits "vectorial" properties [1] not necessarily in parallel with the "progressive" complication principle. In the kingdom of viruses, the evolutionary process is expressed immediately at the molecular level since the genetic material presented by the viral nucleic acid and the viral proteins are polymeric macromolecules whose primary structures are strictly correlated with each other through the triplet

genetic code. Therefore, the difference between two related virus strains can be measured directly as the difference between the primary structures of their nucleic acids or proteins (which is, essentially, the same since they can be deduced from each other).

In the above comparison of two related viruses, the "from-simpler-to-more-complex heuristic" of the evolutionary direction (ancestry) loses any sense. We therefore avoid the use of this "heuristic" for the determination of ancestry among viruses.

The present paper proposes a mathematical model of virus evolution. The model allows computer-assisted determination of ancestry and construction of a phylogenetic tree.

2. EXPERIMENTAL BACKGROUND

The experimental data on the antigenic relationships described in Refs [2, 3] were employed in the present studies. The data resulted from experimentation with the following group of viruses (avian paramyxoviruses, PMV):

Virus Designation	
NDV	D199/HK
Yucaipa	Dove/Tn
Ty/Wisc	Goose/Del
Pk/Neth	Duck/NY
D3/HK	Pigeon/Ot

For each pair of the above viruses, v and w, the following data is available:

—the ratio between

the ability of the antibodies induced by v to inhibit virus w

and

those antibodies' ability to inhibit virus v itself;

—the ratio between

the ability of the antibodies induced by w to inhibit virus v

and

those antibodies' ability to inhibit virus w itself;

—each of the above ratios is available for each of two activities—HA inhibition (HI) and Nase inhibition (NI).

3. THE MODEL

3.1. Postulates of the general hypothesis

Postulates (1)–(3) are similar to those used in the combinatorial model of the antigenic kinship [4], namely:

(1) Each PMV virion contains a number C of identical HN molecules.
(2) Each HN molecule contains two distinct antigenic domains—HA and Nase—spatially arranged around HA and Nase functionally active sites.
(3) Each HA as well as Nase domain consists of numbers Dh and Dn, respectively, of antigenic determinants.

The next two postulates are the core of the present hypothesis:

(4) The antigenic determinants are grouped into classes, each class including both identical and similar ("related") determinants.
(5) Each determinant of a certain class induces a sort of antibodies which, besides the identical (homological) determinants, can also inhibit the related determinants of the same class with the effectiveness determined by the degree of the relatedness.

3.2. Definitions of antigenic kinship and antigenic distance

This paper models two kinds of inhibition tests: HI and NI. The following definitions are proposed for the HI tests. The NI definitions are analogous.

Let us consider the following hypothetical situation:

> A virus x has evolved into a virus y. This evolution was a result of mutational changes in the determinant(s) belonging to a class c. The changed determinants are still "similar" or "related" to initial determinant before mutation, i.e. they still belong to the same class c.

Then, the antigenic kinship between the viruses x and y *with respect to the determinants of the class* c determined by hypothetical inhibition tests is defined as the ratio between

(a) The inhibition of the class-c determinants of x by the antibodies against the class-c determinants of y

and

(b) the inhibition of the class-c determinants of virus x by its own antibodies.

(The "inhibition" is expressed quantitatively as the number of determinants inhibited by a fixed amount of antibodies.) We denote this type of kinship as $k(x, y, c)$. Note that $k(x, x, c) = 1$. It is not at all necessary that $k(x, y, c) = k(y, x, c)$ (because of the possibility of asymmetric cross-reactivity [2–4]).

The general antigenic kinship between the viruses x and y *with respect to all the classes of antigenic determinants* detected by the functional inhibition tests (HI or NI) is defined as the ratio between

(a) the inhibition of the determinants of the virus x by the antibodies against the determinants of the virus y

and

(b) the inhibition of the determinants of x by its own antibodies.

This general kinship is denoted as $K(x, y)$. Note that $K(x, x) = 1$. It is not necessary that $K(x, y) = K(y, x)$.

It follows from the experimental data [2] that $K(x, y)$ is normally several orders of magnitude smaller than 1, while $K(x, x) = 1$. In some cases, $K(x, y) = 0$ (no kinship).

On the basis of the above postulates and definitions, the primary hypothesis of the antigenic kinship in the first approximation is expressed by the following equation:

$$K(x, y) = k(x, y, c_1) * k(x, y, c_2) * \cdots * k(x, y, c_n).$$

(The "$*$" is multiplication.)

A notion of "antigenic distance", which is the inverse of the notion of antigenic kinship and which will be used in the further computations in order to simplify formulas, is expressed as

$$D(x, y) = -\log_2[K(x, y)].$$

3.3. An auxiliary theorem

Let us assume that:

(a) A virus y is a descendant of a virus x.
(b) A virus z is a descendant of the virus y.
(c) The determinant pattern of the virus z differs from the determinant pattern of the virus x in what the virus z differs from the virus y, plus in what y differs from x. This means that if the drift from x to y caused changes in some classes of the

determinants, the drift from y to z caused changes in OTHER classes of determinants, rather than changes in the same classes again.

Then:

(i) The antigenic distance from x to z is equal to the antigenic distance from x to y plus the antigenic distance from y to z.
(ii) The antigenic distance from z to x is equal to the antigenic distance from z to y plus the antigenic distance from y to x.

3.3.1. Proof of the theorem. Let us assume that the change from x to y was in the classes c_1 and c_2, and the change from y to z was in the class c_3. Then

$$\begin{aligned} K(x, y) &= k(x, y, c_1) * k(x, y, c_2) * \cdots * k(x, y, c_n) \\ &= k(x, y, c_1) * k(x, y, c_2) * 1 * \cdots * 1 \\ &= k(x, y, c_1) * k(x, y, c_2). \end{aligned}$$

Similarly,

$$K(y, z) = k(y, z, c_3)$$

and, similarly, since x and z differ in three classes:

$$K(x, z) = k(x, z, c_1) * k(x, z, c_2) * k(x, z, c_3).$$

But c_1 and c_2 have not been changed during the drift from y to z, and c_3 has not been changed during the drift from x to y. Thus, for example, $k(x, z, c_1) = k(x, y, c_1)$ and, hence,

$$K(x, z) = k(x, y, c_1) * k(x, y, c_2) * k(y, z, c_3).$$

Thus,

$$K(x, z) = K(x, y) * K(y, z)$$

and, hence,

$$\begin{aligned} D(x, z) &= -\log[K(x, z)] = -\log[K(x, y) * K(y, z)] \\ &= -\log[K(x, y)] + -\log[K(y, z)] = D(x, y) + D(y, z). \end{aligned}$$

Similarly,

$$D(z, x) = D(z, y) + D(y, x)$$

This completes the proof.

3.4. Our approach to solution of the problem of ancestry

On the basis of the above theorem, let us designate the viruses x, y and z as a parent, a child and a grandchild, respectively. According to assumption (c) of the theorem, not every triple of x–y–z has to satisfy conclusions (i) and (ii) of the theorem. When a triple of viruses x–y–z does satisfy both conclusions (i) and (ii), then there is a good chance that they are related according to either of the schemes: parent–child–grandchild or grandchild–child–parent. The fact that the antigenic distances (D) between different pairs of related viruses (i.e. those responding to the parent–child scheme) are quantitatively different [2, 4] is compatible with the view that the evolutionary changes between the related viruses are not due to one drifting step but rather are the results of several such steps.

Now let us assume that several triples of viruses, e.g. x–y–z_1, x–y–z_2 and x–y–z_3, satisfy both conditions (i) and (ii) of the theorem. From each triple taken separately one cannot affirm whether

$$x \text{ is the parent of } y \text{ and } y \text{ is the parent of } z$$

or vice versa:

$$z \text{ is the parent of } y \text{ and } y \text{ is the parent of } x.$$

However, the several triples considered together provide strong evidence that the virus x is the parent of y, while z_1, z_2 and z_3 are the children of y. If this were not true, then z_1, z_2 and z_3 each would have a strong probability of being the parent of y. The latter is quite unlikely, since y may have only one parent.

Further support for the decision about the parent–child relation between x and y can be given by another triple w–x–y when such a triple also satisfies the conditions (i) and (ii) of the theorem but involves another virus w on the left-hand side, instead of the virus z on the right-hand side in the triple x–y–z. The information about the triple w–x–y itself does not determine absolutely who is a parent of whom, but strongly supports a conclusion that either x is the parent of y, or y is the parent of x. Thus, data about triples like w–x–y give additional support to conclusions from the triples x–y–z.

The data about the antigenic distances between every pair of viruses have been obtained experimentally. Our computer program has analyzed all triples of viruses and extracted those triples where $D(x, z) = D(x, y) + D(y, z)$ approximately (up to the precision of the experimental method) and, at the same time, $D(z, x) = D(z, y) + D(y, x)$. These triples are likely to have a parent–child–grandchild relationship in one of the directions. Although an individual triple x–y–z alone does not tell whether it displays a parent–child–grandchild direction or an opposite grandchild–child–parent direction, the totality of all the data treated by a global analysis together with combinatorial consideration (based on above principles) have provided for only one probable solution.

3.5. Ancestry relationships between the viruses according to the data of HI tests

The treatment of the experimental data related to the HI test [2] has revealed 12 triple combinations of viruses (from the whole total of 720 possible triples) which could be arranged by the above expression of x–y–z triples satisfying (with a minor deviation) both conditions (i) and (ii) of the theorem. That means that the 12 triples were 6 pairs of triples, each pair of triples corresponding to the direct and reverse directions of the ancestry [conditions (i) and (ii)]. The results obtained (Tables 1 and 2) appeared to be a basis for construction of the ancestry tree. The example below demonstrates the approach.

Table 1. Six triples satisfying condition (i) of the auxilliary theorem

Triples $x;y;z$	$D(x, y)$	$D(y, z)$	$D(x, y) + D(y, z)$	$D(x, z)$
D/3HK–Ty/Wisc–Pk/Neth	2.18 ± 0.47	1.75 ± 0.64	3.93 ± 0.86	4.07 ± 1.13
NDV–Pigeon/Ot–Dove/Tn	3.61 ± 0.43	0.05 ± 0.36	3.66 ± 0.56	3.29 ± 0.22
Pk/Neth–Pigeon/Ot–Dove/Tn	3.00 ± 0.34	0.05 ± 0.36	3.05 ± 0.50	3.35 ± 0.20
Ty/Wisc–Pk/Neth–Pigeon/OT	1.75 ± 0.64	3.00 ± 0.34	4.75 ± 0.72	4.19 ± 0.69
D3/HK–Dove/Tn–Pigeon/Ot	3.14 ± 0.70	2.40 ± 0.22	5.54 ± 0.73	5.00 ± 0.93
D199/HK–Dove/Tn–Pigeon/Ot	3.50 ± 0.50	2.40 ± 0.22	5.90 ± 0.55	6.01 ± 0.22

The boxed pairs demonstrate $D(x, y) + D(y, z)$ is approximately equal to $D(x, z)$.

Table 2. The 6 triples satisfying condition (ii) of the auxilliary theorem (combined in reverse order compared with the triples displayed in Table 1)

Triples z–y–x	$D(z, y)$	$D(y, x)$	$D(z, y) + D(y, x)$	$D(z, x)$
Pk/Neth–Ty/Wisc–D3/HK	2.95 ± 0.66	1.37 ± 0.21	4.32 ± 0.69	5.52 ± 0.60
Dove/Tn–Pigeon/Ot–NDV	2.40 ± 0.22	3.21 ± 0.43	5.61 ± 0.48	5.37 ± 0.43
Dove/Tn–Pigeon/Ot–Pk/Neth	2.40 ± 0.22	2.05 ± 0.52	4.45 ± 0.56	3.81 ± 0.60
Pigeon/Ot–Pk/Neth–Ty/Wisc[a]	2.05 ± 0.52	1.37 ± 0.21	3.42 ± 0.56	1.75 ± 0.31
Pigeon/Ot–Dove/Tn–D3/HK	0.05 ± 0.36	5.58 ± 0.72	5.63 ± 0.85	4.06 ± 0.78
Pigeon/Ot–Dove/Tn–D199/HK[b]	0.05 ± 0.36	∞	∞	5.04 ± 0.96

The boxed pair demonstrate $D(z, y) + D(y, x)$ is approximately equal to $D(z, x)$.
[a]The difference between the boxed pairs is on the verge of statistical significance.
[b]The only case not satisfying condition (ii) of the auxilliary theorem.

3.5.1. Example. The following two triples were found to satisfy conditions (i) and (ii):

1. Dove/Tn–Pigeon/Ot–Pk/Neth.
2. Dove/Tn–Pigeon/Ot–NDV.

In each of these cases taken separately, there is a good reason to suspect ancestor–descendant relatedness, but the ancestry could exist with equal chance in two oposite directions, namely:

Dove/Tn→Pigeon/Ot→Pk/Neth

or

Pk/Neth→Pigeon/Ot→Dove/Tn

and

Dove/Tn→Pigeon/Ot→NDV

or

NDV→Pigeon/Ot→Dove/Tn.

However, if we consider both triples together, the chance for the ancestry in the direction FROM the Dove/Tn significantly increases, namely:

Dove/Tn→Pigeon/Ot→Pk/Neth

and

Dove/Tn→Pigeon/Ot→NDV.

The chance for such direction of the ancestry further increases while considering the other triples satisfying conditions (i) and (ii) and containing other combinations of the members of the above two initial triples, e.g.

Pigeon/Ot–Pk/Neth–Ty/Wisc

and

Pk/Neth–Ty/Wisc–D3/HK.

Such analysis, involving all the triples (Tables 1 and 2), led to the following conclusions.

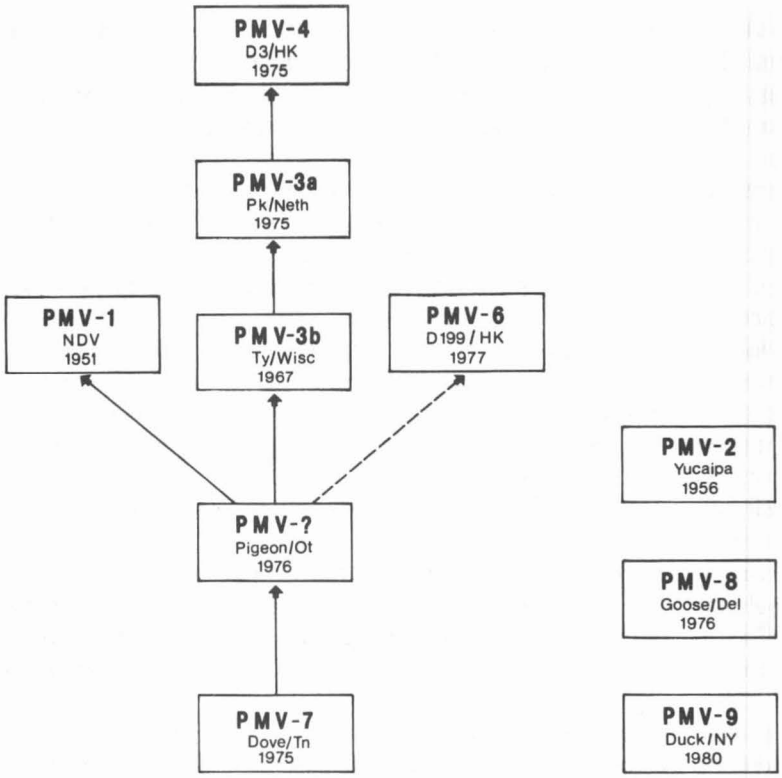

Fig. 1. Ancestry relationships between 7 viruses (7 serotypes of avian PMVs). ⟶ interconnections satisfying conditions (i) and (ii) of the theorem; ⟶ interconnections satisfying only condition (i) of the theorem.

All the triples, with the only exception of Pigeon/Ot–Dove/Tn–D199/HK, satisfy condition (ii) whenever they satisfy condition (i) and vice versa. The triples satisfying conditions (i) and (ii) span a clear ancestry tree (Fig. 1) with multiple evidence (from several triples) for each segment of the tree. As to the triple Pigeon/Ot–Dove/Tn–D199/HK, it satisfies condition (i) but does not satisfy condition (ii). The failure to satisfy condition (ii) is not in itself a contraindication to the existence of an ancestry relationship. Therefore, in the case of the D199/HK, the conclusion about its location upon the phylogenetic tree is supported only by one piece of evidence (the corresponding branch of the tree is indicated by the dashed arrow in Fig. 1), while the rest of the tree segments are supported independently by several pieces of evidence.

3.6. The results of the treatment of the NI test data

Among the 720 triples analyzed by the program, only very few triples satisfying conditions (i) and (ii) were found, but there was no mutual support between them as there was in the case of the HI test. There were some triples which provided a weak support for the results of the HI test. In addition, there were some triples with the viruses Goose/Del and Duck/NY (no ancestry relationship was found in the case of HI test data), but those triples were not compatible with the tree constructed from the HI test data (Fig. 1).

4. DISCUSSION

The suggested mathematical model is an alternative to the previously reported combinatorial model [4]. The new model is based on a different definition of the antigenic determinant which is postulated to be changed not according to the "all-or-none" law (by qualitative leaps) but gradually. This leads to the appearance of classes of related ("cross-reacting") determinants with different degrees of relatedness to each other.

In addition to another idea about the mechanism of antigenic kinship, the new model proposes an approach permitting the elucidation of the phylogenetic relationships, i.e. revealing the direction of the evolutionary changes which is reduced, essentially, to the recognition of ancestry among the members of a considered group of related viruses. The importance of the approach is due to the following reasons.

Even in the cases when the relatedness (antigenic or genetic) between the compared viruses is established and measured quantitatively, it remains unclear what strain must be considered as an "ancestor" to which all the other somehow differing members of the family can be arranged in a "phylogenetic tree" according to the quantitatively determined differences. However, the absolute criterion of the ancestry—a precedence in time—is not suitable in the case of viruses because of the absence of viral paleontology. Another "temporal" criterion—chronological time (the date) of the viruses' isolation—is not suitable either since the virus isolated can often be just an occasional incident not necessarily connected with the time of "creation" (emergence) of the isolated strain in nature. Although there are particular cases when the viruses seemed to appear "for the first time", this criterion is doubtful.

Probably, the situation with the AIDS virus(es) is also associated with the same problem which can be reduced to the following main question: whether the "novel" virus is really a newly emerged one, or it existed in nature for a long time and its "present appearance" is a matter of either incidence of its isolation, or it is a consequence of a puzzling phenomenon of sudden, "awakening" of a "slumbering" virus. The latter phenomenon is recognizable in the general theory of the living evolution when the period of slow and stately changes gave place to rapid and cataclysmic explosions [5].

As far as animal viruses are concerned, in particular, avian influenza and paramyxoviruses (PMV), such a temporal criterion seems to be completely unsuitable since in this case the virus isolation chronology is far less connected with the virus emergence in nature, as compared to human influenza pandemic viruses. Some data on the monoclonal antibody-mediated analysis of the avian influenza viruses may be an illustration of this [6, 7]. For example, some of the H7-containing strains (FPV-like influenza viruses) isolated recently in Israel turned out to have the same antigenicity as the homologous prototype FPV/Rostock/34 virus (the time interval between the isolations is 45–46 years) while some other avian influenza virus strains isolated during the same season differed significantly [7].

The present data has shown clearly (Fig. 1) that there is no correspondence between the revealed phylogenetic relationships and the dates of the viruses' isolation. Thus, the difficulties in using temporal criteria for revealing ancestry in virus evolution enhance the importance of the suggested approach which permits the revealing of ancestry.

The presented evidence that the viruses Goose/Del and Duck/NY showed no ancestry relationship to the other avian PMVs (Fig. 1), in spite of the multiple antigenic cross-reactivity between them [2, 3] can be analyzed on the basis of the above considerations. Two possible explanations are as follows:

1. According to condition (c) of the theorem, the ancestry by the parent–child–grandchild (x–y–z) triple analysis can be established only if the changes from x to y and from y to z affect *different* classes of antigenic determinants (sequential mutation). If, however, the drift is due only to x–y changes, the phylogenetic relationship estimated by the criteria accepted here (several independent pieces of evidence obtained from the analysis of different triples) will be absent, while antigenic (inhibitional) relatedness would be expressed quite well.
2. The phylogenetic connections between the Goose/Del and Duck/NY and the other studied viruses are realized through "hypothetical" segments of the phylogenetic tree represented by the viruses which either "died out" (do not exist) or do exist (i.e. are circulating in the nature) but have not yet been isolated. For example, it can be easily imagined that the shape of the phylogenetic tree presented here would be quite different if the present analysis had been performed before 1976 when the Pigeon/Ot, occupying the crucial node in the tree (Fig. 1), had not yet been isolated.

The absence of the phylogenetic relationships found in the case of the NI test can be explained in the same way. However, the discrepancy between the data related to HI and NI tests remains a mystifying phenomenon. On the one hand, it does not compromise the approach as it is; yet, on the contrary, the failure to find phylogenetic relationships in the case of the NI test is in such contrast with the amazing consistency of the HI test data revealing the phylogenetic relationships, that it serves as a demonstration of a certain real regularity rather then just a game of figures. On the other hand, such discrepancy is astonishing if we take into account that both HA and Nase activities belong to the same HN glycoprotein molecule [8–10]. Such contrasting results on the phylogenetic relationships, together with the difference in the cross-reactivity [2, 3] between avial PMVs revealed by the HI and NI tests, seem to demonstrate the polar location of the HA and Nase functionally active sites on the three-dimensional body of the HN molecule, as well as the independent antigenic drift of the HA and Nase functionally active sites.

According to the general dogma, the genetic relatedness is considered as an intrinsic indication of evolution to which any other (phenotypic) indications, including antigenicity (inhibitional relationship), are related as secondary. In this respect, the phylogenetic tree describing and enterovirus–70 evolution [11] and constructed on the genetic basis, seems to be more genuine than that based on functional tests which is presented here. However, the main postulate underlying the genetic model of the phylogenetic tree is the assumption that the nucleotide base substitution occurs at a constant rate [12], although some oligonucleotide spots were found to be surprisingly conserved [11], which is not compatible with the random character of the assumption. In addition, this assumption, though possibly suitable for the case of enterovirus–70, does not take into account such phenomena as persistent and latent viral infections which accelerate mutational changes [13], and a combination of the "freezing" or conservation effect [6, 7, 14–16] with the phenomenon of microheterogenicity [17] of the influenza virus enigma. Therefore, the molecular evolution proceeding at the genomic level and expressed by the base substitution, and the antigenic drift proceeding at the protein tertiary structure level and expressed by serological cross-reactivity, may not proceed in parallel.

The mathematical model proposed here and that suggested previously are two alternatives based on essentially different hypotheses. Hence, a natural question is which of them is "correct". At present, however, the choice between them is based on the respective estimation of their "explaining capacity". Each of the models "explains" the described phenomena of the complicated labyrinth-like network of the cross-reaction relationships between avian PMV serotypes [2]. However, the new model presents an approach for the establishment of the phylogenetic (ancestry) relationships between the antigenically (inhibitionally) related viruses.

Selection of the better model from among the above is to be attempted via examination of the present model by finding such data which could provide independent proof of the model-predicted ancestry chain. This consists of using the results of appropriate comprehensive epizootiological surveillances of local PMV-caused outbreaks with massive virus isolations [18–20].

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