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EVOLUTIONARY ASPECTS OF THE ECOLOGY OF ARBOVIRUSES IN BRAZILIAN AMAZONIA, SOUTH AMERICA

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INTRODUCTION

The Amazonian region of Brazil is probably the world's richest reservoir of arboviruses. The 183 different types of arboviruses so far detected in this region (Table 1; Hervé *et al.*, 1986; Pinheiro *et al.*, 1986; Travassos da Rosa *et al.*, 1986, 1987, 1989 and unpublished data), account for near one third of the world's arboviruses: a total of 533 (Karabatsos, 1985). Of these, 136 (83.4 %) are endemic in this part of the neotropical zoogeographical region.

Of main interest in relation with the man-made modifications of the natural environment is the study of the ecological factors which allow the coexistence of such a great number and variety of viruses and their complex transmission cycles (Pinheiro *et al.*, 1977; Dixon *et al.*, 1981). The virological and serological data, obtained between 1954 and 1992 at the Evandro Chagas Institute / National Health Foundation (Belém), will be analysed from two complementary points of view: (i) the definition of the ecological niche of each arbovirus and (ii) the ecological factors which have possibly constrained their evolution.

The ecological niche concept has been used recently by Calisher (1994) in relation with the definition of the virus species: "*A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche*" (emphasis by us). Other definitions have been proposed by ecologists. One which best suits the arboviruses is as follows: "A niche is a multi-dimensional hypervolume of resource axes" (Colinvaux, 1986: 31). In the case of arboviruses, each host or alternatively, each component of the hosts' environment may represent one of these resource axes or variables, allowing for the quantification of the niche. Multifactorial data analysis methods, which have been used here for the first time with arboviruses (to our knowledge), seem particularly well suited to (i) the numerical definition of the niche of each virus species and (ii) the study of the ecological grouping of the viruses.

The question underlying our present work relates to the ecological factors which prevent arboviruses from multiplying randomly in all available hosts. There are probable constraints of various origins and located at various levels, from inside the cell to the ecosystem: genetical (or physiological) (Dubois, 1991), eco-ethological and historical (or biogeographical) (Barbault, 1991).

MATERIALS AND METHODS

SEROLOGICAL AND VIROLOGICAL METHODS

The crude data were represented by the isolated strains and the results of haemagglutination inhibition (HI) tests (the laboratory techniques are described in detail in Shope & Sather, 1979). The HI tests have been considered positive for a particular arbovirus if it showed a titer at least four-fold higher than any other tested antigen in the same serological (cross-reacting) group. This "four-fold titer criterion" has been established either from unpublished results of experiments conducted on mice, rats and fowls, or from follow-up serological studies in naturally infected people and sylvatic animals. This criterion may appear overrigorous but the main objective was to prevent the introduction of false positives in the data, even at cost of some false negatives. When sera were available, HI results which appeared doubtful have been checked by seroneutralization test (except in the case of turtle sera which do not contain neutralizing antibodies: APA Travassos da Rosa, unpublished data). A contingency table has been constructed, of which three subsets have been submitted to the treatments described below.

NUMERICAL AND STATISTICAL METHODS

The structure of the data was first explored qualitatively, using presence (1) vs. absence (0) of each virus in each host as characters (annexes 1 & 2). The hosts have been grouped at the order or genus/subgenus levels for vertebrates and arthropods, respectively. The subsets were as follows: (a) arboviruses known from arthropods only; (b) arboviruses known from vertebrates only and (c) arboviruses known from both types of hosts. These analysis were based on the phylogenetic method (Wiley, 1981; d'Udekem-Gevers, 1990; Hennig *in* Goujet *et al.*, 1988; Janvier *in* Goujet *et al.*, 1988) and have been conducted with the PAUP software (Swofford, 1993). For each subset, an unrooted consensus tree was computed, adopting the "50 %-majority rule" (Margush & McMorris *in* Swofford, 1993).

Quantitative treatments were done on the uncoded data matrix (contingency table) or subsets of it (Annexes 4-6). Two methods of multifactorial analysis, the factorial analysis of correspondances and the hierarchical

ascendent classification (AnaMul and ADDAD packages, respectively) have proved useful for this approach (Fénelon, 1981; Febvay & Bonnot, 1990). In order to study the ecological relationships existing between the arboviruses more specifically, the hosts have been grouped according to their terrestrial/ arboreal and diurnal/ nocturnal habits (variables number 32 to 37 in Annex 1). A more detailed study of the bird-associated arboviruses, based on data about habitat and level preferences of the hosts has been done in a separate paper by Dégallier *et al.* (1992b) and will only be summarized below.

RESULTS

The following sample sizes (number of specimens or pools) formed the basis of the present study: haematophagous Diptera, more than 515 000 pools; marsupials, 6,427; bats, 9,276; wild monkeys, 2,428; rodents, 18,741; edentates, 861; carnivorous, 361; ungulates, 3,374; birds, 12,423; reptiles, 6,052; amphibians, 1,509.

THE QUALITATIVE APPROACH

NUMBER OF HOSTS BY VIRUS

The number of different species of hosts for each virus group (table 2) gives a crude indication of their host spectrum, and consequently, of the intensity of their adaptive radiation. It is obvious from these data (compared with those in table 1) that the virus families with greater number of species are not necessarily those which were found in the greater variety of hosts. For example, the Flaviviridae and Togaviridae with only 8 viral species each in the Brazilian Amazon region, have been found associated with at least 57 and 56 different hosts, respectively. This compares with 54 hosts known for the Bunyaviridae which includes 45 species. On the other hand, the Reoviridae, accounting for 63 different viruses, are known from only 14 different species of hosts. Thus, the ecological diversification, in terms of number of hosts involved in transmission cycles, seems to be independant from the systematic diversity of the viruses (= number of species). However we will see in later discussion the importance of sampling bias on this pattern.

Table 1: Numbers of genera, serological groups and species for each family of arboviruses present in the amazonian region of Brazil; their order of enumeration follows decreasing number of species.

Family	genera	groups	species
Bunyaviridae	2	11	70
<i>Bunyavirus</i> ^(a)	-	10	45
<i>Phlebovirus</i>	-	1 ^(b)	25
Reoviridae	1	2 ^(c)	63
Rhabdoviridae	1	5 ^(d)	15
Togaviridae	1	1	8
Flaviviridae	1 ^(e)	1	8
Coronaviridae	1	1	1
Poxviridae	-	-	1
Unclassified	-	-	12
Arenaviridae ^(f)	1	1	3)
Herpesviridae ^(f)	-	-	1)
Paramyxoviridae ^(f)	-	-	1)

^(a) with 1 *Bunyavirus*-like virus included

^(b) with 4 ungrouped viruses included

^(c) with 3 ungrouped viruses included

^(d) with 3 ungrouped viruses included

^(e) with 1 ungrouped virus included

^(f) probably not arboviruses

NUMBER OF VIRUSES BY HOST

Table 3 shows the number of different species of viruses found in each ecological type and/or systematic group of hosts.

Some types of hosts seem to be more favorable to the speciation of arboviruses than others. Among the arthropods, sandflies are almost the sole hosts for the majority of Reoviridae known from our region. As these viruses do not form agglutinins in vertebrates, it is not yet possible to know if they are diversified in this respect. Due to their minuteness and the lack of an identification key for fresh females, the phlebotomine sandflies were not identified and thus, may contain many species with various habits. On average, nocturnal mosquitoes harbor more different viruses than diurnal ones. This difference results mainly from the number of Bunyaviridae transmitted by nocturnal mosquitoes. Among the vertebrates, the same may be said, i. e., that the nocturnal ones harbor a larger variety of viruses, due especially to the predominance of the bunyaviruses. The Flaviviridae seem to be as "diurnal" than "nocturnal" but the Togaviridae may be more "diurnal" if we consider their vertebrate hosts.

Nevertheless, the data discussed in the two previous paragraphs are very crude and will be examined in more details in the following sections.

The phylogenetic assumptions

Any phylogenetic study may ideally need assumptions about (i) the transformation sequence of the states of the characters, and (ii) the states which may be considered as apomorphic (= derived or specialized) or plesiomorphic (= ancestral or primitive).

However, in the case of arboviruses, few authors have addressed this question. Mattingly (1960) and Calisher (1988) supposed that the *Culex* - bird cycle of many arboviruses may be primitive (plesiomorphic). In fact, if the arboviruses can be considered to have originated in arthropods before they become adapted to vertebrates (Goldbach & Wellink, 1988) have suggested the same for insect viruses which adapted to plant); it would be reasonable to associate ancestry of the vertebrate group with diversity of arboviruses. The phylogeny of mosquitoes is even worse known and cannot give any indication to solve this question although culicines were first recorded from the Oligocene. Thus, the present study has been done, without doing any *a priori* polarization of the characters.

Results of the phylogenetic analysis

Figures 1, 2 and 3 show hypothetical phylogenies of the arboviruses whose arthropod and vertebrate, only arthropod, or only vertebrate hosts are known, respectively.

Fifty, 34 and 29 viruses have been included in these respective subsets. Sixty different viruses are known only from phlebotomine sandflies (Travassos da Rosa *et al.*, 1983, 1984; see also annex 3) and, as such, have been grouped under the denomination: "sandfly borne".

The first consensus tree (Figure 1) was computed from a total of 500 trees, each of 141 steps. Its resolution is not perfect as it shows yet some polychotomies. Thus, the viruses Acara to Nepuyo, Benfica to Guajara, Benevides to Itaquí, Irituia to Tapara and Jurona need to be studied in more detail with respect to their hosts and/or the possible sampling bias. Some ecological groups seem better established if we consider the value of the majority-rule index: Moju to Una, Marituba to SLE, Turlock to Trinité, and Guaroa to Oropouche.

The second consensus tree (Figure 2), obtained from 500 most parsimonious trees, each 27 steps-long, and which shows the hypothetical relationships of the viruses isolated only from haematophagous arthropods, appears even less resolved than the previous one. With the exception of little groups like Buritirana/Itacaiunas and Taiassui/Wyeomyia, the groups are either polychotomous or with poor consensus rates. However, despite its polychotomy, the Arumateua to Tucuruí group was present in more than half of the trees.

Remarks of the same order than the preceding can be made about the tree presented in Figure 3, where viruses only known from vertebrate hosts have been considered (consensus tree obtained from 102 distinct trees, each 26-steps-long). The relationships between the viruses from Agua Preta to Parí and Belém to Jatobá are poorly resolved. On the other hand, some associations appeared at greater rates: Anhangá/Jari; Araguari/Itaituba/Pirí and Bocas to Timbó. One group seems to be fairly resolved: Urucuri to Utinga.

Table 2: Minimum numbers of different species of vertebrate and arthropod hosts for each family and/or genus of arboviruses present in the amazonian region of Brazil; their order of enumeration follows decreasing total number of species.

Family/Genus	Virus/Min. Nr. of different species		
	Vertebrates	Arthropods	Total
Flaviviridae	37	20	57
Togaviridae	39	17	56
Bunyaviridae			
<i>Bunyavirus</i>	15	39	54
<i>Phlebovirus</i>	9	3	12
Reoviridae	2	12	14
Rhabdoviridae	3	6	9
Coronaviridae	1	-	1
Poxviridae	1	-	1
Unclassified	-	2	2

THE QUANTITATIVE APPROACH

The quantitative analysis have been done with the viruses whose cycles are better known, i.e. which have been found in both vertebrate and arthropod hosts. A special analysis has been done previously with the viruses known from birds (Dégallier *et al.*, 1992b).

The multidimensional ecological niche

The first four factors which resulted from the correspondance analysis, account for 91.9 % of the total inertia of the dot cloud. The representation of the variables is best along the factors 1, 2, 3 and 4 (Figure 4). The former seems to be oriented from diurnal to nocturnal canopy vertebrates, from diurnal to nocturnal ground vertebrates and from diurnal to nocturnal mosquitoes. The third factor is less discriminant for these same ecological variables. The fourth factor separates well the mosquito-related variables (diurnal/nocturnal). However, in order to interpret graphically the relationships between these variables and the viruses, we have to consider the relative contribution of each of the latter to the same factors. Figures 5 and 6 show simultaneously the projections of the variables and viruses on the planes formed by the factors 2 and 3, and 2 and 4, respectively. Only the elements which contributed for at least 20 % of the total inertia of at least one of the two factors have

Table 3: The hosts of sylvatic arboviruses in Brazilian Amazonia, grouped according to their ecological characteristics. For each type of host is indicated the number of virus species associated with.

Arbovirus Families	Mosquitoes		Sandflies	Midges	Ticks	Vertebrates			
	Nocturnal	Diurnal				Diurnal		Nocturnal	
						terr.	canopy	terr.	canopy
Flaviviridae	4	4	0	0	0	6	6	6	6
Togaviridae	6	6	0	0	1	6	7	5	5
Bunyaviridae	37	19	2	2	2	13	17	21	17
Reoviridae	8	5	51	0	0	0	1	2	0
Rhabdoviridae	3	3	3	0	0	4	2	1	1
Coronaviridae	0	0	0	0	0	1	0	0	0
Poxviridae	0	0	0	0	0	0	0	1	0
Unclassified	4	2	2	2	0	0	0	0	3
Total	63	40	69	4	3	36	40	45	38

been retained. For clarity, the labels of the elements which contributed significantly to the two factors are in boldface and those which contributed only to the ordinate factor are in italics.

The following associations may be deduced from the Figures 5 and 6:

- BEN, ICO, BSB, MOJU, ACA, BSQ, CAR, CAR-like, CATU, MUC, NEP, CAP, GMA, BVS, ITQ, MUR, ORI, BIM, GJA, GAM, AURA, Trombetas: nocturnal terrestrial vertebrates/nocturnal mosquitoes;

- UNA, MCA, ILH, TNT, KRI, MAG (+ KWA-like, ANU ?): diurnal terrestrial vertebrates/diurnal mosquitoes;

- YF, GRO, MAY, SLE, TCM, TUR, WEE, ORO, TCM, JUR: diurnal canopy vertebrates/diurnal mosquitoes;

The viruses EEE and PAC-like seem to localize at intermediate positions, between diurnal and nocturnal and between canopy and ground-dwelling hosts. In the following section, we will "go back" to the uncoded data to examine the exact ecological components of each of these groups.

THE ECOLOGICAL (NUMERICAL) CLASSIFICATION

The ecological groups obtained by the ascendent hierarchical classification algorithm are shown in Figures 7 and 8 for the viruses and their serological groups, respectively. The proportions representing the different types of hosts have been put on the ordinate scale. Serological group A and bunyaviruses may contain some viruses which do not cross react inside these groups, thus they have been indicated by an asterisk.

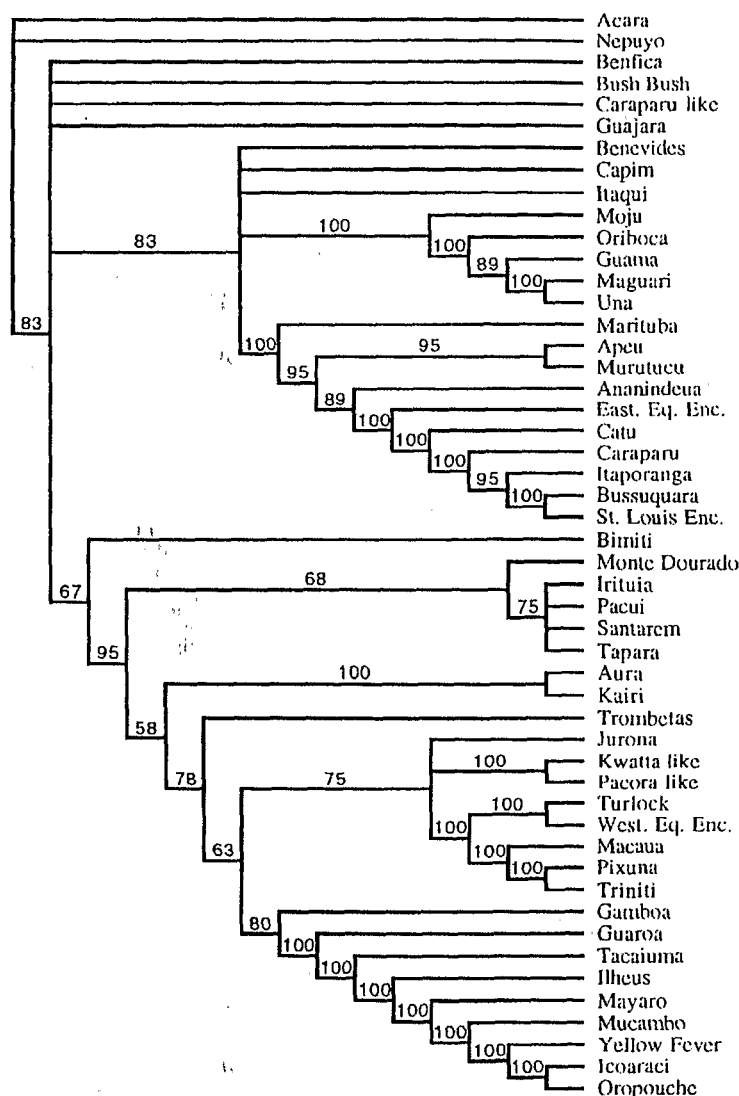


Figure 1. Unrooted, 50 % - majority rule consensus tree of the arboviruses known from both vertebrate and arthropod hosts in Brazilian Amazonia, 1954-1992. The value at base of each group is the proportion (%) of the 500 most parsimonious trees which show the group.

Some remarks which remain of preliminary nature can be drawn from this analysis: (i) there is a continuum (or gradient) from “diurnal” to “nocturnal” viruses and from “arboreal” to “terrestrial”; (ii) some groups like the GAM to WEE, TUR to ACA and Trombetas to GRO were mostly found in “diurnal canopy vertebrates” and “nocturnal mosquitoes”; (iii) the PIX/ TNT/ MAG group is predominantly “diurnal” and “terrestrial”, opposing to the almost strictly “canopy-liking” viruses MAY, ILH, YF and UNA; (iv) the ecology of some groups of viruses like ORO/ ANU, Tapara to KRI and ICO needs more information to be gathered, especially about their vectors; (v) in fig. 8, we see that with some exceptions, each serological group has but one virus in one ecological group; we need finer definitions of the niches of 21 viruses pertaining to A, B, BUN, C, CAP, CGL and GMA serological groups.

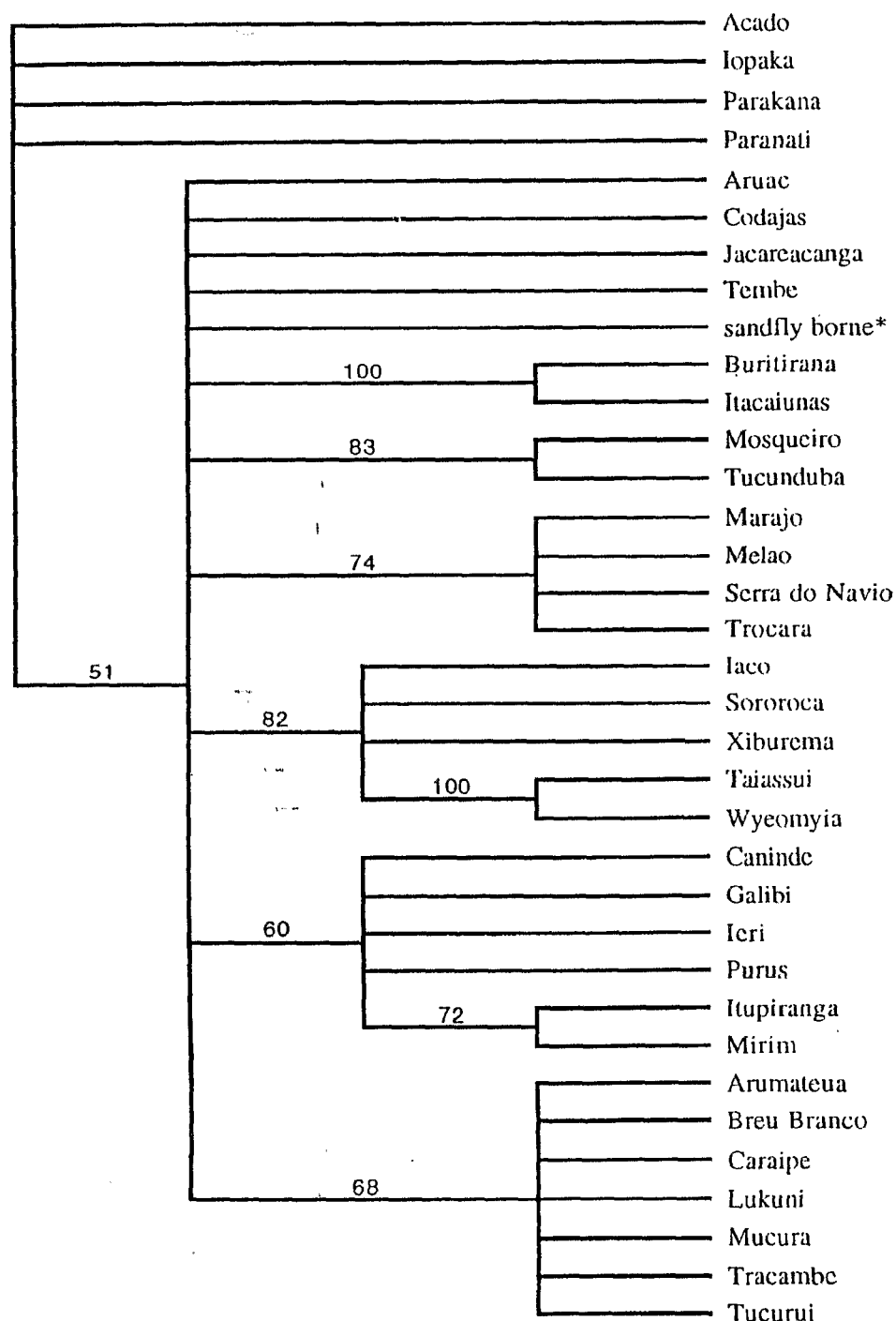


Figure 2. Unrooted, 50 % - majority rule consensus tree of the arboviruses known only from arthropod hosts in Brazilian Amazonia, 1954-1992. The value at base of each group is the proportion (%) of the 500 most parsimonious trees which show the group. Under the “sandfly borne*” label are 60 different viruses which are known exclusively from phlebotomine sandflies.

The bird-associated viruses

We will summarize below the main results of this specific study, already published by Dégallier *et al.* (1992a). The subset including the bird-associated arboviruses accounted for 30 different arboviruses. An ascendent hierarchical classification has been obtained, using as ecological variables five types of vegetation, two of which (igapo or inundated forest and "terra firme" forest) has been subdivided in two and five strata,

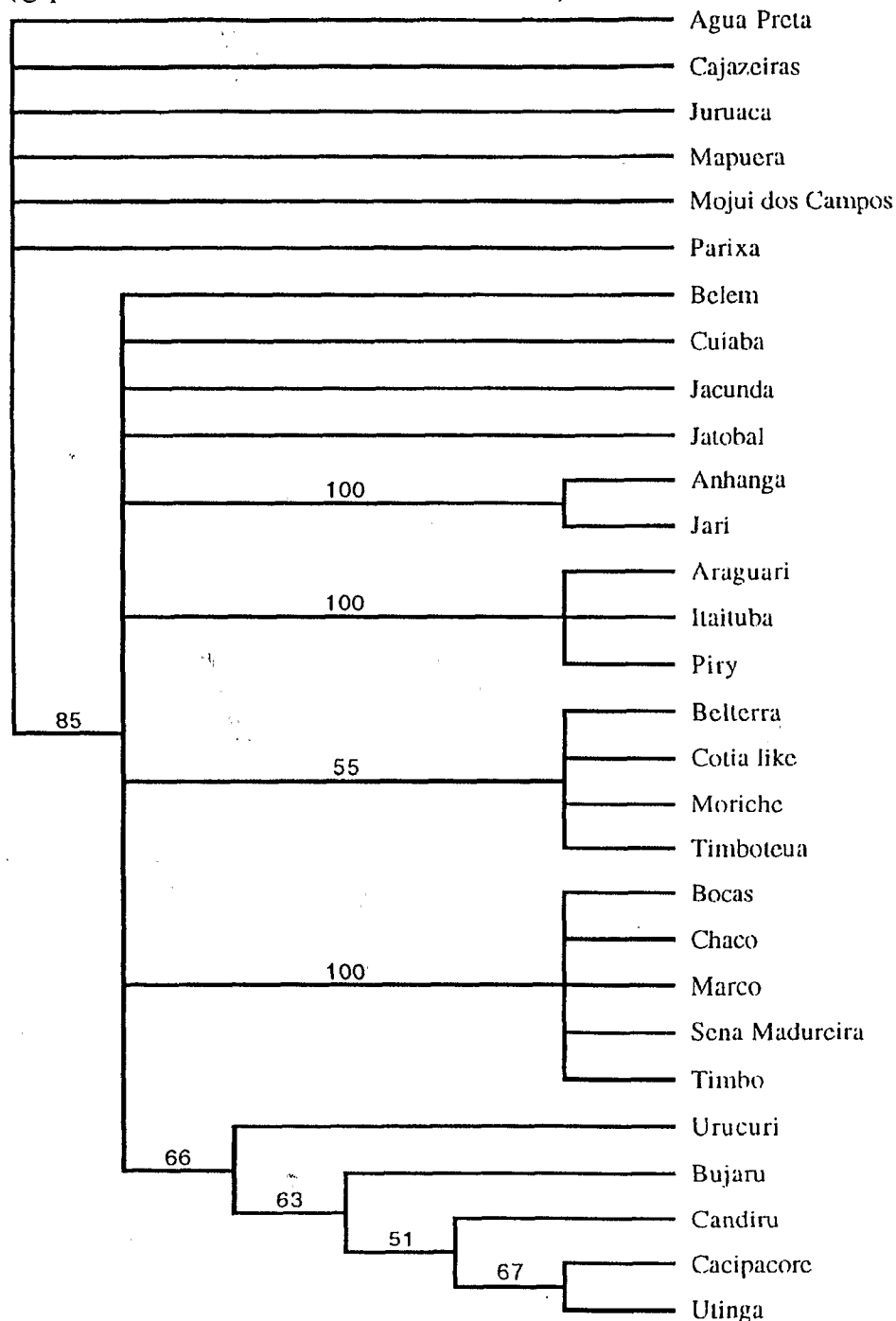


Figure 3. Unrooted, 50 % - majority rule consensus tree of the arboviruses known only from vertebrate hosts in Brazilian Amazonia, 1954-1992. The value at base of each group is the proportion (%) of the 102 most parsimonious trees which show the group.

respectively. A gradient (continuum) has been observed between bird species which prefer secondary vegetation or forest (= "capoeira") and, those which are found mainly in primary forest.

CDU virus has been found mainly in canopy birds which are dwelling mainly (50 %) above 15 and 30 m. This virus, as for some others (CPC, MAY, ILH, TCM), has an important secondary forest component (25 %). The birds which are hosts of UTI, KWA, GAM and ICO viruses are species living exclusively in the "terra-firme" forest.

EEE virus has been "found" in all but one rare type of vegetation or strata (forest on sandy ground) and is considered as ecologically versatile. This may be linked to a great potential of this virus to colonize new niches, including in urban environment (strains isolated from mosquitoes in Fortaleza, Ceará, Brazil).

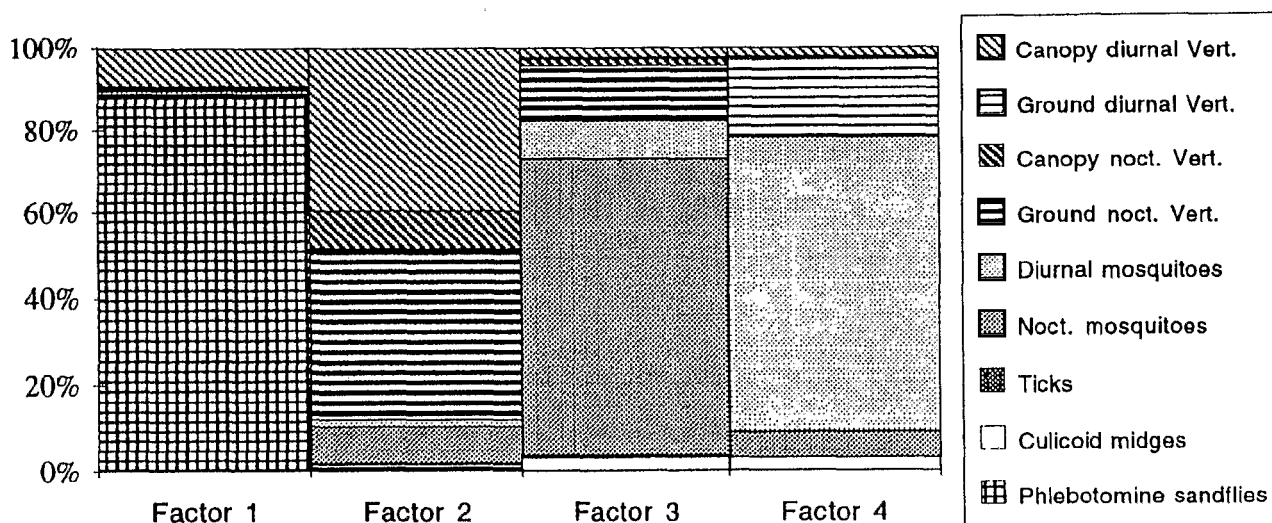


Figure 4. Contribution (% of explained inertia) of the nine ecological variables to the factors 1-4 of the correspondance analysis of the arboviruses known from both arthropod and vertebrate hosts in Brazilian Amazonia, 1954-1992.

The viruses which may be considered the more prone to infect human people in rural places are BSQ, GMA, MUC, MUR and APEU because they have been found in birds which are living in the secondary (or degraded) environments mixed with cultivated areas as is often the case in Amazonia. They are actually fairly prevalent in human sera.

DISCUSSION

The ecological niche concept for arboviruses

Previous works have already described the probable sylvatic cycles of many amazonian arboviruses (Woodall, 1967; Dégallier, 1982; Hervé *et al.*, 1986). However, the grouping of ecologically similar viruses was made mainly after the number and nature of the hosts, i. e. the relative "complexity" of the cycles. We have reexamined the same data in a phylogenetic perspective. In a quantitative ecological study of the viruses

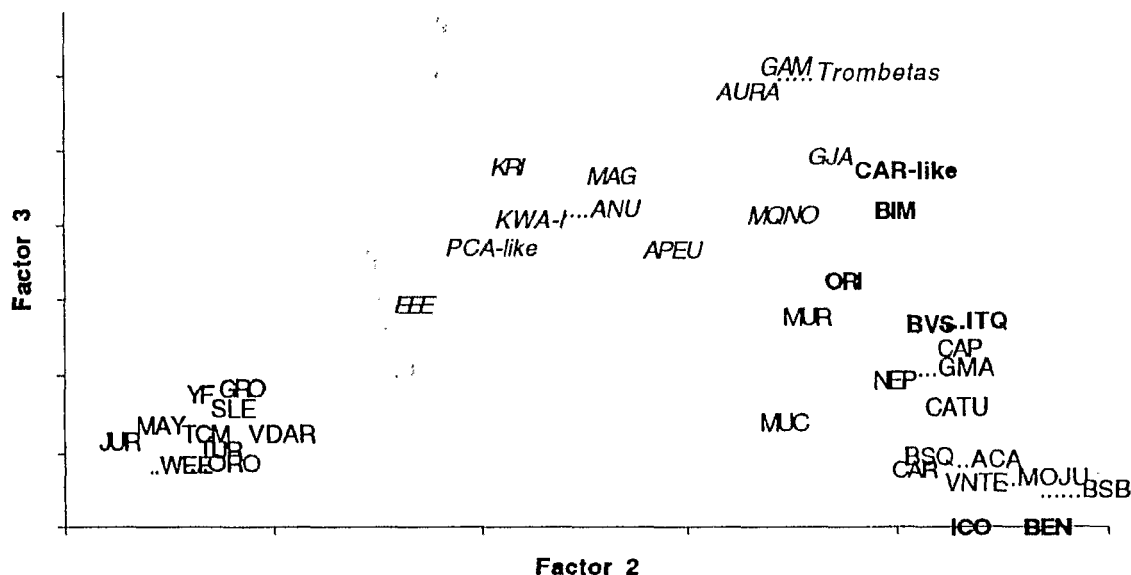


Figure 5. Graphical output of the correspondance analysis of the ecological data available for the arboviruses known from both arthropod and vertebrate hosts in Brazilian Amazonia, 1954-1992. Projections of viruses and ecological variables with an at least 20% contribution on one (italics: fact. 3; standard: fact. 2) or both (boldface) of the factors.

pertaining to the group C of *Bunyavirus*, Woodall (1979) has shown a marked niche separation between canopy- and ground level-transmitted viruses and, when two viruses appeared to share the same niche, that the vectors were distinct species. Thus, for establishing themselves in a locally stable equilibrium, related viruses cannot share the same arthropod and/or vertebrate hosts. It may even be said that the less they are serologically related, the more they can share the same ecological niche.

The complexity of the amazonian environment, exemplified by an extreme diversity of vertebrate and arthropod species, and consequently of niches, have favoured the diversification and sympatry of many

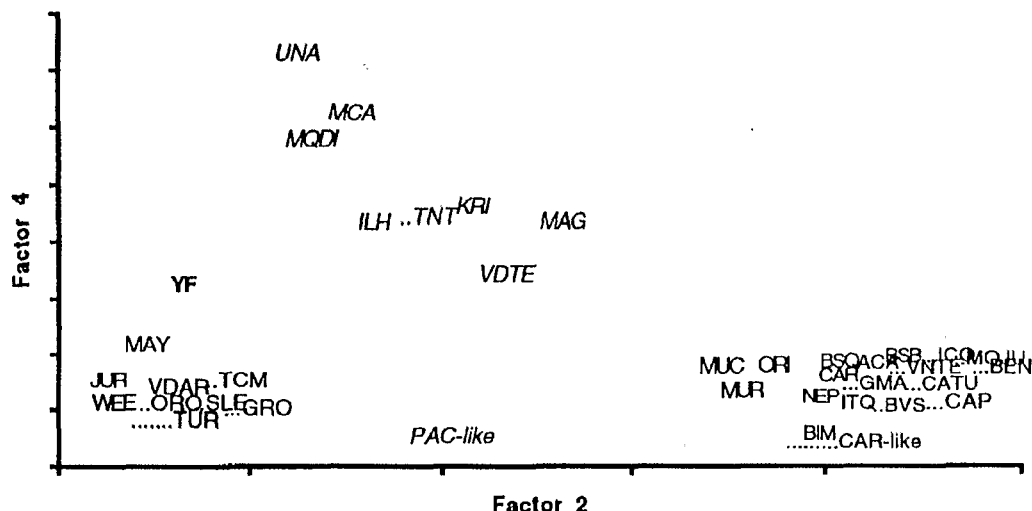


Figure 6. Graphical output of the correspondance analysis of the ecological data available for the arboviruses known from both arthropod and vertebrate hosts in Brazilian Amazonia, 1954-1992. Projections of viruses and ecological variables with an at least 20% contribution on one (italics: fact. 4; standard: fact. 2) or both (boldface) of the factors.

arboviruses of the same group. As the present study has shown, the presence of two or more different viruses of the same serological group in what has been characterized as one niche may be due to the coarseness of the ecological variables which have been considered. As many ecologists have shown, temporal and spatial variables may also be included in the multidimensional definition of the niches. The isolations of strains and/or serological conversions in sentinel animals should be interpreted in the future for the "temporal" characterization of the niches.

Excepted for some viruses which are ecologically very distinct (PAC-like, CDU), there is no clear-cut separation between one group and the next along the "ecological transects" defined either by the preferred vegetation types and strata, or by the habits of the hosts. This may mean that, with similar historical (= biogeographical + genetical) constraints, the arbovirus population in a defined community forms a dynamic equilibrium. Subsets of this population may share the same niche, at least at the two levels studied here, and each niche is separated from the others by ecological constraints acting on the host populations. However, two very different types of perturbations can modify this equilibrium. When some fluctuations are going on in host populations, as occurs seasonally for non-immune hosts, only the arbovirus transmission levels are affected. On the contrary, when the host populations are permanently modified, the equilibrium of arbovirus populations need to shift to a new state. Eventually, new niches may appear which would be filled after a short time. These may be colonized by new genotypes, obtained by recombination, reassortment or introduction from adjacent communities (Calisher, 1988). These relatively rapid adjustments of equilibrium have been noted in the case of the important perturbations induced by the filling of a dam reservoir, where "new" arboviruses appeared in the area, either as exogenous material or as autochthonous speciation.

The phylogenetic study of arboviruses, based on the viruses-hosts associations is yet very tentative because of the lack of phylogenetic classifications of either group (Eldridge, 1990). This author has looked for some evidence of a host-parasite coevolution in the case of the California serogroup viruses. What we have defined as historical constraints are doubtlessly related with some effects of coevolution but it remains difficult to distinguish these from the constraints arising from virus competition after horizontal transfers (Tabachnick, 1991). These hypothesis need to be evaluated by molecular biologists.

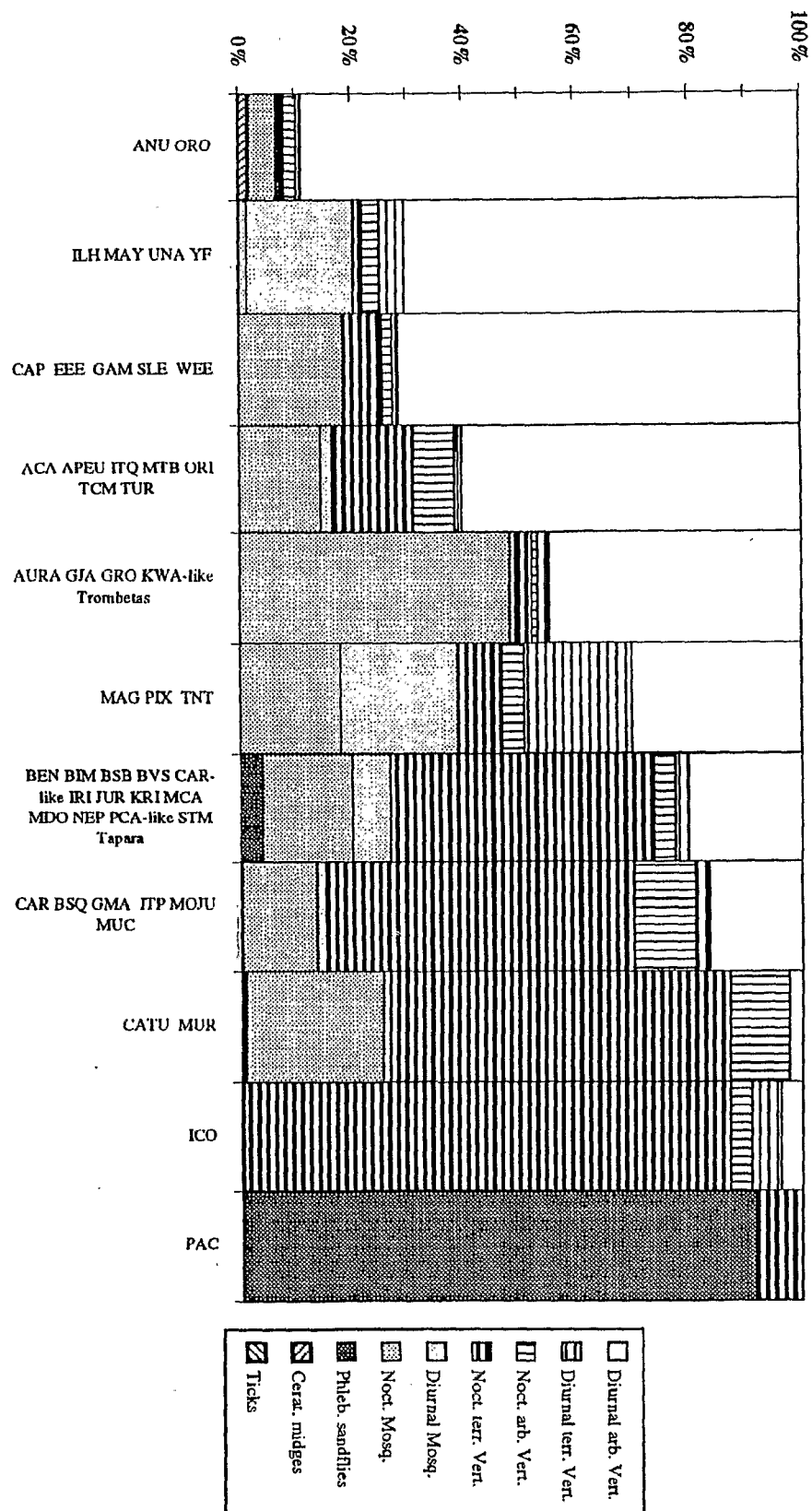


Figure 7. Ecological composition of the groups of viruses, obtained by the hierarchical ascendent classification analysis of the ecological data, available for the agents known from both arthropod and vertebrate hosts in Brazilian Amazonia, 1954-1992.

THE EVOLUTIONARY ASPECTS OF THE ECOLOGY OF ARBOVIRUSES

What happened with the viruses for which some evolutionary hypothesis are available, based on protein sequencing? Levinson *et al.* (1990) furnished some interesting hypothesis which will be discussed from an

ecological point of view. MAY (with UNA, not studied by these authors), EEE/WEE, AURA and MUC (member of VEE complex) seem to have diverged in four different directions (fig. 7). As Hahn *et al.* (1988) have shown, WEE virus arose probably as a recombinant between EEE and another *Alphavirus* which may be AURA. EEE seems to be a very versatile virus, especially in birds (fig. 9) whereas AURA has been found only in monkeys. The ecological link between the two may be the *Melanoconion* and *Ochlerotatus* subgenera of *Culex* and *Aedes* mosquitoes, respectively.

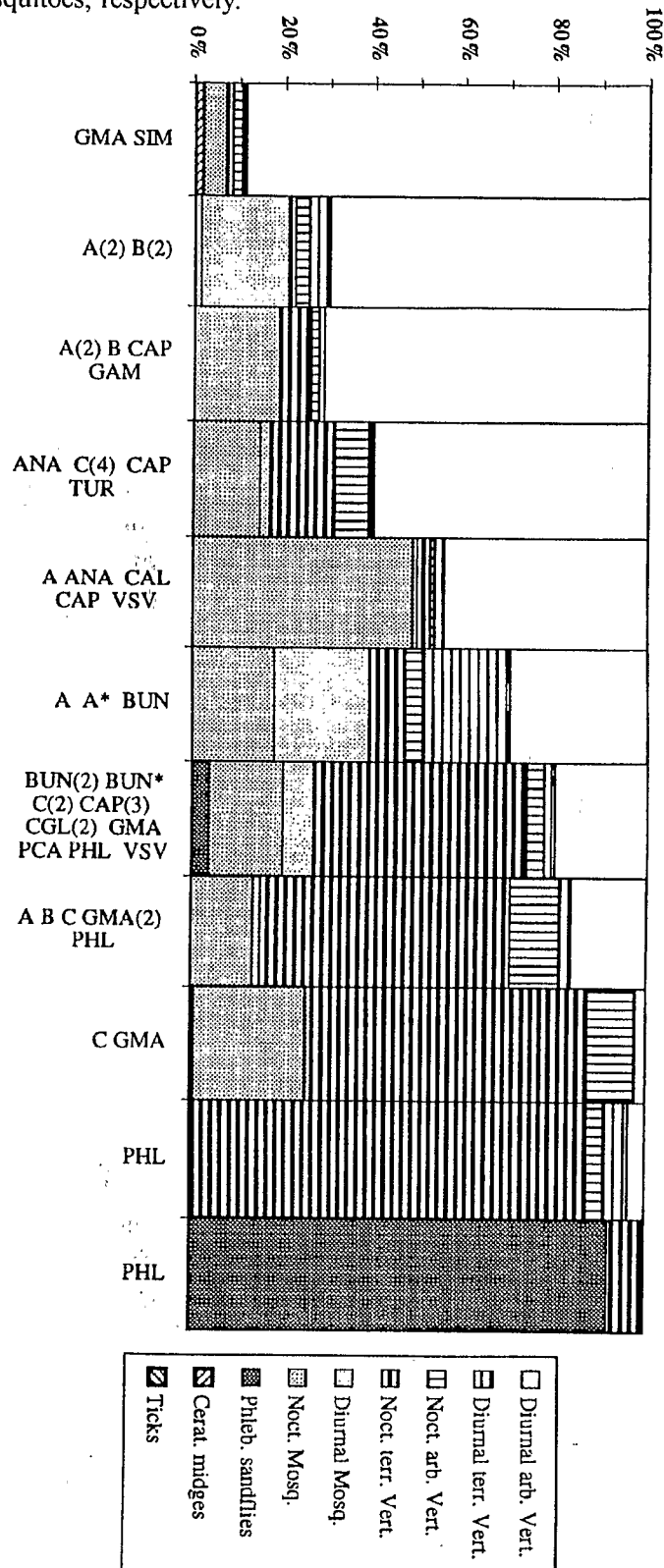


Figure 8. Graphic representation of the same data as in fig. 7, but with the serological groups of viruses in place of their actual names; when more than one virus is included in a group, the number of virus species is shown in parenthesis; asterisks refer to ungrouped viruses, i. e. viruses which do not cross react serologically inside their group.

CONCLUSIONS

The present study was not detailed enough to explain why some viruses of the same serological group coexist and other do not, but it allowed us to define the main ecological groups. Each of these groups further needs to be studied separately as it has been done with the bird-borne arboviruses.

Despite the great number of tested pools between 1954 and 1992, serological and virological data are lacking either for viruses which do not form agglutinins, or about potential hosts which are difficult to collect. It has been seen that less than one third (28.9 % or 50/173) of the viruses known from sylvatic hosts have been found in both vertebrate and arthropod hosts, 17.7 % (29/173) are known only from vertebrate hosts and 54.3 % (94/173) only from arthropods. Among the latter, 63.8 % (60/94) are known only from phlebotomine sandflies and represent probably a very complex ecological system, even if their vertebrate hosts are very diverse.

Sampling bias may account for some distortions in the quantitative delineations of the niches. For example, ground dwelling rodents, marsupials and birds are much easier to trap than canopy frequenting hosts and among them those which cannot be attracted by any type of baited trap. Thus, many species are poorly known, not only for the viruses they may harbour but also for their bioecology. An important ecological "axis" which has been yet neglected is the time or seasonal one. It is quite conceivable that some hosts may harbour different viruses of the same serological group at different times of the year. This may be especially the case with bunyaviruses whose antibodies are not life-long lasting (Shope *et al.*, 1967). In future studies, more precise serological tests may allow us to define each virus-host association better.

Nevertheless, the methodology followed in the above quantitative analysis revealed itself adequate to the study of the multidimensional niche concept of arboviruses, and it will also be a useful tool to make predictions about the evolution of the arboviruses in response to modifications of the environment.

Acknowledgments

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Annex 1. Types of hosts used as variables in qualitative and quantitative ecological analysis of the sylvatic arboviruses found in Brazilian Amazonia, 1954-1992.

Anopheles (Nyssorhynchus) spp.
Anopheles (Anopheles) spp.
Anopheles (Stethomyia) spp.
Culex spp. (not *Culex* or *Melanoconion*)

Annex 2. Data matrix used in the qualitative analysis of the sylvatic arboviruses found in Brazilian Amazonia, 1954 - 1992; The three successive subsets correspond to viruses known from: *arthropod and vertebrate* hosts; *only arthropods* and *only vertebrates*; the columns represent the variables 1-31 listed in annex 1; the "Phlc" (= sandfly-borne) viruses are listed in the annex 3.

ACA	0001000000000000001100011000000
ANU	0001010001000100001000000010010
APEU	00010101000000000001010000010010
AURA	0000010100000000000000000000010
BEN	000100000000000000000000010000000
BIM	000001000000000000000000010000000
BSB	0001000000000000010000010000000
BSQ	0001110001010000111101011010110
BVS	000101000000000000010000100000000
CAP	000111000000000000010000100000000
CAR	0001110000100001101011010011110
CARI	000101000000000000000000010000000
CATU	0011110001000010001010010000110
EEE	0001110101000000011000010010011
GAM	10000010000000000000000000010000
GJA	0001000000010000000000010000000
GMA	0001010101111000001000010010101
GRO	100000000000000001000000011110
ICO	000000000000100010111111010111
ILH	0000000110100001100000011011111
IRI	0000000000001000000000010000000
ITP	0001010001000000101010011011111
ITQ	0001010000000000001000110000000
JUR	00000000100000000000000000010000
KRI	0000000100110000000000000000010
KWAI	00001000000000000000000000010000
MAG	10100101101100000010000100000001
MAY	0000000010010010111110011011110
MCA	0000000000010000000000011010000
MDO	0000000000001000000000100000000
MOJU	0001010001011000001010010000000
MTB	0001010000000000001000000010000
MUC	0001010111010000001011011011110
MUR	0001010000010010001010010010010
NEP	0001000000000000001000010000010
ORI	0001010101110000001000010000000

ORO	0000100100000100011111011010110
PAC	0000000000001000000000010000000
PCAI	0000100000000000000000000010000
PIX	0010000000010000001010011010100
SLE	0001110101010000101010111010110
STM	0000000000001000000000010000000
Tapa	0000000000001000001000010000000
TCM	1000000010000000000000011011110
TNT	1010000000010000000000011010000
Trom	10000000000000000000000000000001
TUR	0000110000000000000000001010010
UNA	0011000111110000000010000010000
WEE	0000010100000000100000001010101
YF	0000000110010000101011010000110
ANH	0000000000000000000010000000000
AP	000000000000000000000000000000100
ARA	0000000000000000001000000000000
Belt	000000000000000000000000010000000
BLM	0000000000000000000000000000010000
BOC	0000000000000000001000000000000
BUJ	000000000000000000001010011000011
Caja	000000000000000000000000000000100
CDU	0000000000000000000000000111010110
CHO	0000000000000000101000000000000
COTI	000000000000000000000000010000000
CPC	00000000000000001101010011010110
CUI	000000000000000000000000000100000
ITA	0000000000000000001000000000000
Jacu	000000000000000000000000010000000
JARI	0000000000000000000010000000000
Jato	000000000000000000000000000001000
Juru	000000000000000000000000000000100
MCO	0000000000000000001000000000000
MDC	000000000000000000000000000000100
MOR	000000000000000000000000010000001
MPR	000000000000000000000000000000100
Pari	000000000000000000000000000000100
PIRY	000000000000000000001000000000000
SM	0000000000000000001000000000000
TBT	000000000000000000000000010000000
TIM	0000000000000000001000000000000
URU	000000000000000000000000011000100
UTI	000000000000000000100010010010010
ACD	0000100000000000000000000000000
ARU	0000110000000000000000000000000
Arum	1000000000000000000000000000000
Breu	1000000000000000000000000000000
Buri	0000000000000100000000000000000
CAN	0000000000101000000000000000000

Cara	10000000000000000000000000000000
Coda	00000000100000000000000000000000
Gali	00000000001000000000000000000000
IACO	00000000000100000000000000000000
IERI	00000000000100000000000000000000
Iopa	00001000000000000000000000000000
Itac	00000000000001000000000000000000
ITU	00000001001000000000000000000000
JAC	00000100000000000000000000000000
LUK	10100001000000000000000000000000
Majo	00000001000000000000000000000000
MEL	00000001000000000000000000000000
MIR	00000101001000000000000000000000
MQO	00000100010100000000000000000000
Mucu	10000000000000000000000000000000
Prka	00001000000000000000000000000000
Prni	00001000000000000000000000000000
PUR	00000000001000000000000000000000
SDN	00000001000000000000000000000000
SOR	00000000000100000000000000000000
Taia	01110000000100000000000000000000
TME	00100000000000000000000000000000
Trac	10000000000000000000000000000000
Troc	00000001000000000000000000000000
Tuci	10000000000000000000000000000000
Tucu	10101101111100000000000000000000
WYO	01000000000100000000000000000000
XIB	00000000000100000000000000000000
Phle	00000000000010000000000000000000

Annex 3. Probable new arbovirus types, isolated only from phlebotomine sandflies in the Brazilian Amazonia, 1954 - 1992; NC = not classified; NG = not grouped.

Virus name	Prototype	Genus	Antigenic group
Acatinga	AR 482250	<i>Orbivirus</i>	Changuinola
Acurene	AR 446985	<i>Orbivirus</i>	Changuinola
Almeirim	AR 389709	<i>Orbivirus</i>	Changuinola
Altamira	AR 264277	<i>Orbivirus</i>	Changuinola
Ambe	AR 407981	<i>Phlebovirus</i>	Phlebotomus
Anapu	AR 496014	<i>Orbivirus</i>	Changuinola
Aracai	AR 425269	<i>Orbivirus</i>	Changuinola
Aratau	AR 428812	<i>Orbivirus</i>	Changuinola
Arawete	AR 505172	<i>Orbivirus</i>	Changuinola
Ariquemes	AR 485678	<i>Phlebovirus</i>	Phlebotomus
Aruana	AR 428815	<i>Orbivirus</i>	Changuinola
Assurinis	AR 482249	<i>Orbivirus</i>	Changuinola
Bacajai	AR 482267	<i>Orbivirus</i>	Changuinola
Bacuri	AR 496008	<i>Orbivirus</i>	Changuinola
Balbina	AR 478620	<i>Orbivirus</i>	Changuinola
Canoal	AR 433317	<i>Orbivirus</i>	Changuinola
Carajas	AR 411391	<i>Vesiculovirus</i>	V. S. V.
Catete	AR 495605	<i>Orbivirus</i>	Changuinola
Coari	AR 433343	<i>Orbivirus</i>	Changuinola
Cupixi	AR 502545	<i>Orbivirus</i>	Changuinola
Gorotire	AR 482251	<i>Orbivirus</i>	Changuinola
Gurupi	AR 35646	<i>Orbivirus</i>	Changuinola
Inhangapi	AR 177325	<i>Vesiculovirus</i>	NG
Ipixaia	AR 490496	<i>Orbivirus</i>	Changuinola
Iriri	AR 408005	NC	NG
Iruana	AR 496021	<i>Orbivirus</i>	Changuinola
Itaboca	AR 496034	<i>Orbivirus</i>	Changuinola
Jamanxi	AR 243090	<i>Orbivirus</i>	Changuinola
Jandia	AR 440489	<i>Orbivirus</i>	Changuinola
Jatuarana	AR 440497	<i>Orbivirus</i>	Changuinola
Joa	AR 371637	<i>Phlebovirus</i>	Phlebotomus
Jutai	AR 397374	<i>Orbivirus</i>	Changuinola
Kararao	AR 447024	<i>Orbivirus</i>	Changuinola
Maraba	AR 411459	<i>Vesiculovirus</i>	V. S. V.
Munguba	AR 389707	<i>Phlebovirus</i>	Phlebotomus
Oriximina	AR 385309	<i>Phlebovirus</i>	Phlebotomus
Ourem	AR 41067	<i>Orbivirus</i>	Changuinola
Pacaja	AR 440503	<i>Orbivirus</i>	Changuinola
Papura	AR 450572	NC	NG
Parauapebas	AR 415962	<i>Orbivirus</i>	Changuinola
Paru	AR 397370	<i>Orbivirus</i>	Changuinola
Pependana	AR 440504	<i>Orbivirus</i>	Changuinola
Pindobai	AR 482675	<i>Orbivirus</i>	Changuinola
Piratuba	AR 478781	<i>Orbivirus</i>	Changuinola
Saraca	AR 385278	<i>Orbivirus</i>	Changuinola
Serra Norte	AR 498935	<i>Orbivirus</i>	Changuinola
Surubim	AR 440507	<i>Orbivirus</i>	Changuinola
Tapirope	AR 434080	<i>Orbivirus</i>	Changuinola
Tekupeu	AR 505169	<i>Orbivirus</i>	Changuinola
Timbozal	AR 440541	<i>Orbivirus</i>	Changuinola
Tocantins	AR 486776	<i>Orbivirus</i>	Changuinola
Tocaxa	AR 505170	<i>Orbivirus</i>	Changuinola
Tuere	AR 484704	<i>Orbivirus</i>	Changuinola
Tumucumaque	AR 397956	<i>Orbivirus</i>	Changuinola
Turuna	AR 352492	<i>Phlebovirus</i>	Phlebotomus
Uatuma	AR 478626	<i>Orbivirus</i>	Changuinola
Uriurana	AR 479776	NC	NG
Uxituba	AR 452652	<i>Orbivirus</i>	Changuinola
Xaraira	AR 490492	<i>Orbivirus</i>	Changuinola
Xiwanga	AR 505171	<i>Orbivirus</i>	Changuinola

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