Inter-relation of Sylvatic and Domestic Transmission of *Trypanosoma cruzi* in Areas with and without Domestic Vectorial Transmission in Minas Gerais, Brazil

L Diotaiuti, AS Pereira*, CF Loiola**, AJ Fernandes**, JC Schofield***, JP Dujardin****, JCP Dias**, E Chiari

Departamento de Parasitologia *Departamento de Bioquímica, ICB, UFMG, Caixa Postal 2486, 31270-901 Belo Horizonte, MG, Brasil **Centro de Pesquisas René Rachou-FIOCRUZ, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil ***London School of Tropical Medicine and Hygiene, WC1 E7HT, London, UK ****ORSTOM, BP 5445, 34032 Montpellier, France

During the period 1980-1986, we captured triatomine bugs and mammalian reservoir hosts from sylvatic and domestic situations in different municipalities of the State of Minas Gerais. Trypanosoma cruzi *was isolated from captured bugs, mammals and patients. After cultivation in LIT medium, the electrophoretic enzyme profiles were determined. We obtained a total of 32 parasite isolates from regions with active domestic transmission, and 24 isolates from areas under control. For the first areas the results suggest introduction of* T. cruzi *from sylvatic habitats, through incursion of infected opossums and/or sylvatic* T. sordida, *which appears to have given rise to at least one acute human infection. Of particular interest is the finding of sylvatic opossums and a* T. sordida *nymph infected with ZB, that could indicate return of parasites from chronic human infections to sylvatic transmission cycles. For the areas under control we also interpret the results as interaction between sylvatic and domestic cycles of transmission, here through the invasion of houses by bugs carrying the Z1 zymodeme from the sylvatic environment. The Multivariate Correspondence Analysis gives a spatial description between the different parasite isolates and confirms the existence of a bridge in the opposite direction in the region with active vectorial transmission including the exporting of Z2 through the peridomestic environment into the sylvatic cycle. For the other areas this bridge corresponds especially to* Panstrongylus megistus, *importing Z1 into the domestic environment.*

Key words: *Trypanosoma cruzi* - isoenzyme - Chagas' disease

Enzymatic variation in *Trypanosoma cruzi* seems largely due to clonal expansion of the parasite under different conditions of selection (Tibayrenc & Ayala 1988). However, the electrophoretic isoenzyme patterns can be grouped into major zymodemes that are each associated with particular epidemiological situations (Miles 1983). In Brazil for example, zymodemes Z1 and Z3 are principally associated with sylvatic transmission (Z1 mainly with opossums, and Z3 mainly with armadillos) (Miles 1985), while Z2 has been mainly isolated from patients in the chronic phase of Chagas' disease, particularly in the Southern and Central regions (Romanha 1982, Miles 1983, Luquetti et al. 1986), which correspond to the principal *Triatoma infestans* distribution area in the country. The association *T. infestans*/Z2 zymodeme becomes weaker in the North and Northern regions of the country, where the Z2 zymodeme is episodic in the State of Ceará (Alencar 1987) and absent in Amazonia and Venezuela (Miles 1985). Mixture areas in the State of Bahia (Barrett et al. 1980) are probably explained by the close distribution of *T. infestans* with the strictly domestic *Panstrongylus megistus*.

Studying chagasic patients from Bambuí, Minas Gerais, Brazil, Romanha (1982) classified the parasite isolates in four zymodemes, ZA, ZB, ZC, and ZD. ZA is equivalent to Z2 of Miles et al. (1977), while ZB and ZC are distinct (Bogliolo et al. 1986) and ZD represented a mixture of parasites with patterns ZA and ZC. Romanha's study showed a predominance of ZA (or Z2) amongst the isolates from humans. Different papers confirm the great homogeneity of the parasites in the sylvatic environment, showing prevalently Z1 isozyme profiles (Miles et al. 1981, Póvoa et al. 1984, Fernandes et al. 1991, Steindel et al. 1993). The similarity of banding patterns between para-

sites from a fatal acute case from the north of the State of Minas Gerais and two isolates from opossums led Romanha (1982) to suggest that *T. cruzi* from sylvatic transmission cycles had been recently introduced into the domestic situation. Use of isozyme profiles as epidemiological markers is a helpful tool for examining the relationship between sylvatic and domestic transmission cycles of *T. cruzi*. The technique was developed after *T. infestans* had become established as the principle domestic vector in Brazil (Schofield 1988), but before major control interventions against this vector had been carried out over the greater part of the country (Dias 1987). As a result, the initial interpretations of association between Z1 zymodeme with the sylvatic cycle, and Z2 zymodeme with human disease, although representing considerable simplification, for the southern and central Brazil probably does reflect a generalised distinction between domestic transmission involving *T. infestans* and sylvatic transmission involving other species of Triatominae. *T. infestans* is thought to have invaded Brazil since the turn of this century, mainly by passive dispersal in association with human migrations (Schofield 1988). Its origins seem to have been in the Cochabamba region of Bolivia (Dujardin et al. 1987), and it may have been responsible for the spread of the zymodeme Z2 of *T. cruzi* from that region, introducing it into Brazil where the predominant zymodeme had previously been Z1. The association between the zymodeme Z2 and *P. megistus* in the State of Bahia reinforces the correlation of this pattern with the domestic cycle of *T. cruzi* where this triatomine accomplishes the epidemiological behaviour of *T. infestans* in Recôncavo Bahiano (Barrett et al. 1980). In at least one case, introduction of *T. infestans* into northeastern Brazil was associated with an outbreak of *T. cruzi* transmission involving zymodeme Z2 (Barrett et al. 1980). In Brazil, there has been considerable success in interrupting domestic cycles of *T. cruzi* transmission by eliminating domestic species of the triatomine bug vectors (Dias 1987). However, there is an ever present risk of reinvasion of treated houses by sylvatic species of Triatominae, which may lead to important changes in the epidemiology of Chagas' disease in the treated regions. For example, after elimination of *T. infestans* as the major domestic vector in parts of the State of Minas Gerais, two other triatomine species - *T. sordida* and *P. megistus* - have become more frequently reported from the rural houses, representing reinvasion from sylvatic ecotopes (Diotaiuti et al. 1993). The aim of the present study is therefore to compare the epidemiological patterns of *T. cruzi* transmission in regions of Minas Gerais where the vectorial transmission of Chagas' disease is still occurring, and regions where this transmission was interrupted.

MATERIALS AND METHODS

During the period 1980-1986, we captured triatomine bugs and mammalian reservoir hosts from sylvatic and domestic situations in different municipalities of the State of Minas Gerais, East region of Brazil. This period covered the start and conclusion of a major national control campaign against *T. infestans*, the principal domestic vector of *T. cruzi* in the region (Dias 1987).

T. cruzi was isolated from captured bugs by inoculation of bugs faeces into mice. Xenodiagnosis using laboratory-bred *T. infestans* nymphs was carried out on the captured mammals, and faeces from these bugs were also inoculated into mice. Using the same procedure, we also obtained parasite isolates from two chronic-phase patients from a region where no Chagas' disease transmission had been reported for many years, and from one acute-phase patient from a region where vector transmission is still active.

Parasites from the inoculated mice were cultivated in LIT medium (Camargo 1964), and their electrophoretic enzyme profiles determined using the methods of Romanha (1982). Each enzyme test-plate included control samples representing Z1, Z2 and Z3 (Miles et al. 1977), and control samples of ZA, ZB, ZC and ZD (Romanha 1982). Six enzymes were developed: ALAT, (EC 2.6.1.2), ASAT (EC 2.6.1.1), GPI (EC 5.3.1.9), PGM (EC 2.7.5.1), G6PD (EC 1.1.1.49) and ME (EC 1.1.40).

Data were considered by area, with or without actual transmission (Tables I, II), and were analyzed by a Multiple Correspondence Analysis (Thioulouse 1990). The factor maps from this analysis are presented for the two first factor coordinates (Figs 1, 2), corresponding to Tables I and II, respectively.

RESULTS AND DISCUSSION

We obtained a total of 32 parasite isolates from regions with actual active domestic transmission, and 24 isolates from areas under control. The zymodeme profiles all corresponded to ZB and ZC of Romanha (1982), or Z1 and Z2 of Miles et al. (1977) (Tables I, II).

In areas with actual active domestic transmission we found Z1, Z2 and ZB zymodemes of *T. cruzi* (Table I). Most *T. infestans* had Z2 or ZB, which are considered characteristic of human infections in southern and central Brazil (Miles et al. 1977, Romanha 1982, Luquetti et al. 1986). However, some of the *T. infestans*, most *T. sordida* and most opossums showed Z1 zymodeme which is generally considered characteristic of sylvatic

TABLE I

Local of capture, origin and electrophoretic isoenzyme patterns of *Trypanosoma cruzi* isolates from human patients, triatomines and reservoirs in areas with active vectorial transmission of Chagas' disease, in the State of Minas Gerais, Brazil

Isolate number	Municipality	Local of capture	Origin	Zymodeme
1	Itacarambi	domestic	*T. infestans*	B
2	Itacarambi	domestic	*T. infestans*	Z1
3	Itacarambi	domestic	*T. infestans*	Z1
4	Itacarambi	domestic	*T. infestans*	Z1
5	Itacarambi	-	Human/acute	Z1
6	Itacarambi	domestic	*T. infestans*	Z1
7	Itacarambi	peridomestic	*T. sordida*	Z1
8	Manga	domestic	*T. infestans*	B
9	Manga	domestic	*T. infestans*	B
10	Manga	domestic	*T. infestans*	B
11	Montalvânia	peridomestic	*T. sordida*	B
12	Montalvânia	domestic	*T. sordida*	Z1
13	Mato Verde	peridomestic	*T. sordida*	Z1
14	Mato Verde	domestic	*T. infestans*	B
15	Mato Verde	sylvatic	*T. sordida*	Z1
16	Mato Verde	sylvatic	*D. albiventris*	B + Z1
17	Mato Verde	sylvatic	*D. albiventris*	B + Z1
18	Mato Verde	sylvatic	*T. sordida*	B
19	Mato Verde	sylvatic	*D. albiventris*	Z1
20	Mato Verde	sylvatic	*D. albiventris*	Z1
21	Mato Verde	domestic	*T. infestans*	Z2
22	Mato Verde	domestic	*T. infestans*	B
23	Mato Verde	sylvatic	*T. sordida*	Z1
24	Mato Verde	peridomestic	*D. albiventris*	Z1
25	Mato Verde	sylvatic	*D. albiventris*	Z1
26	Mato Verde	sylvatic	*D. albiventris*	Z1
27	Mato Verde	sylvatic	*D. albiventris*	Z1
28	Mato Verde	sylvatic	*D. albiventris*	Z1
29	Mato Verde	sylvatic	*D. albiventris*	Z1
30	Mato Verde	sylvatic	*D. albiventris*	Z1
31	Mato Verde	sylvatic	*D. albiventris*	Z1
32	Mato Verde	sylvatic	*D. albiventris*	Z1

T. cruzi. This strongly suggests introduction of *T. cruzi* from sylvatic habitats, through incursion of infected opossums and/or sylvatic *T. sordida*, and appears to have given rise to at least one acute (ie. recent) human infection (Table I). Of particular interest here, however, is the finding of sylvatic opossums and a *T. sordida* nymph infected with ZB zymodeme of *T. cruzi*. These were captured in a wood about 50m from the nearest house (where at least one inhabitant was seropositive for *T. cruzi*) and could possibly indicate return of parasites from chronic human infections to sylvatic transmission cycles.

The areas under control represent regions where vectorial transmission of *T. cruzi* was previously high, but now do not record the presence of triatomine colonization within the houses. Nev-

ertheless, some autochthonous species of Triatominae are found in sylvatic ecotopes and, occasionally, inside houses. In these regions (Table II) we verified the presence of Z1 in sylvatic Triatominae and opossums, and in most of the bugs (*P. megistus*) captured in and around houses. Two patients with chronic infections showed Z2 isoenzyme profiles, and four of the *P. megistus* showed ZB or Z2 profiles which are more usually associated with chronic human disease. Again therefore, we interpret these results to indicate interaction between sylvatic and domestic cycles of transmission. Here, it would appear that invasion of houses by otherwise sylvatic bugs can not only introduce Z1 into the domestic environment, but these bugs can also reconstitute domestic transmission of Z2. However, Tibayrenc et al. (1986, 1990) have pre-

TABLE II

Local of capture, origin and electrophoretic isoenzyme patterns of *Trypansoma cruzi* isolates from human patients, triatomines and reservoirs in areas without active vectorial transmission of Chagas' disease, in the state of Minas Gerais, Brazil

Isolate number	Municipality	Local of capture	Origin	Zymodeme
1	Caeté	sylvatic	*D. albiventris*	Z1
2	Patos de Minas	domestic	*P. megistus*	Z2
3	Patos de Minas	-	Human/cronic	Z2
4	Patos de Minas	domestic	*P. megistus*	Z1
5	Patos de Minas	domestic	*P. megistus*	Z1
6	Patos de Minas	peridomestic	*P. megistus*	Z1
7	Virgem da Lapa	domestic	*P. megistus*	Z2
8	Virgem da Lapa	domestic	*T. vitticeps*	C
9	Jaboticatubas	-	Human/cronic	Z2
10	Contagem	domestic	*P. megistus*	Z1
11	Betim	peridomestic	*P. megistus*	Z1
12	Betim	peridomestic	*P. megistus*	Z1
13	Ibirité	domestic	*P. megistus*	B
14	Minas Novas	domestic	*P. megistus*	B
15	Guaranésia	domestic	*P. megistus*	B
16	Guaranésia	peridomestic	*P. megistus*	Z1
17	Monte Belo	peridomestic	*P. megistus*	Z1
18	Brumadinho	peridomestic	*P. megistus*	Z1
19	C. Mato Dentro	domestic	*P. megistus*	Z2
20	Itapagipe	domestic	*P. megistus*	Z2
21	Itapagipe	sylvatic	*R. neglectus*	Z1
22	Itapagipe	sylvatic	*P. megistus*	Z1
23	Itapagipe	sylvatic	*D. albiventris*	Z1
24	Itapagipe	sylvatic	*D. albiventris*	Z1

viously noted the presence of mixed zymodemes in infections of individual humans and triatomines, while Finlay and Dvorak (1987) have modelled the rapid overgrowth of fast-growing *T. cruzi* clones during the passage of new isolates. We cannot therefore exclude the possibility of loss of some biological clones during our passage of isolates through cultures and mice, such that Z2 zymodeme for example, may have been present among some of the primary isolates subsequently identified as Z1 zymodeme.

The Multivariate Correspondence Analysis helps to illustrate the apparent interactions between sylvatic and domestic transmission in the two areas, by giving a spatial description of correspondence between the different parasite isolates. For the areas with actual active transmission, the analysis shows eight grouping of parasite isolates (Fig. 1). Groups *a* and *b* correspond to all the domestic isolates from *T. infestans* and from a human acute case, while groups *c* and *d* correspond to all the sylvatic isolates from *Didelphis*. These groups are clearly distinguished from each other by the *y* factor axis, and by the *x* factor axis from

the remaining groups which correspond to isolates from *T. sordida* from sylvatic, peridomestic and domestic habitats, together with the single peridomestic isolate from *Didelphis*. The implication of this correspondence is that *T. sordida* is acting as an effective brigde between sylvatic and domestic habitats, importing parasites of zymodeme Z1 from the sylvatic cycle to the domestic environment. However, the position of groups *d* and *h* could imply also a bridge in the opposite direction, exporting Z2 zymodeme of *T. cruzi* through the peridomestic environment into the sylvatic cycle.

For the areas under transmission control, the same analysis drew fewer groups, but again illustrated a correspondence between the parasite isolates in accordance with their domestic, peridomestic or sylvatic origins (Fig. 2). Groups *a* and *b* correspond to domestic isolates showing ZB, Z1 and Z2 zymodemes of *T. cruzi*, group *c* corresponds to peridomestic Z1 zymodeme isolates, and group *d* corresponds to sylvatic Z1 zymodeme isolates. Again the distribution of parasite isolates within these groups implies a bridge, this time by *P.*
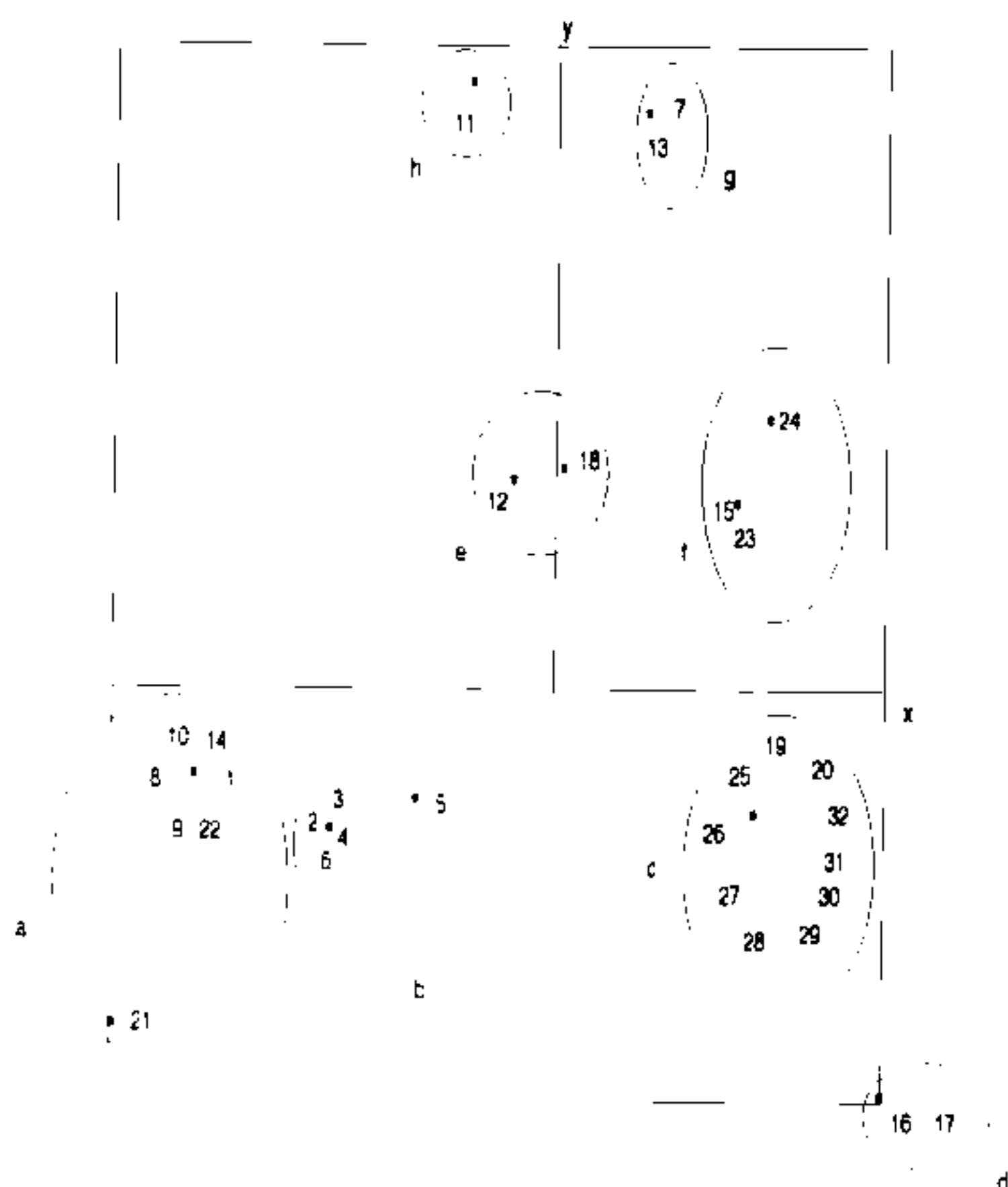
Fig. 1: factor map of the multiple correspondence analysis performed on Table I. The two first (out of 9) factor coordinates are the x and y axis, respectively, and represent 43% of the total variability. Domestic, peridomestic and sylvatic transmissions are separated mainly by the first axis (r = 0.81), while the vectors and zymodemes were more correlated to the second axis (r = 0.97 and 0.88, respectively). r is the correlation between the variable and the factor.
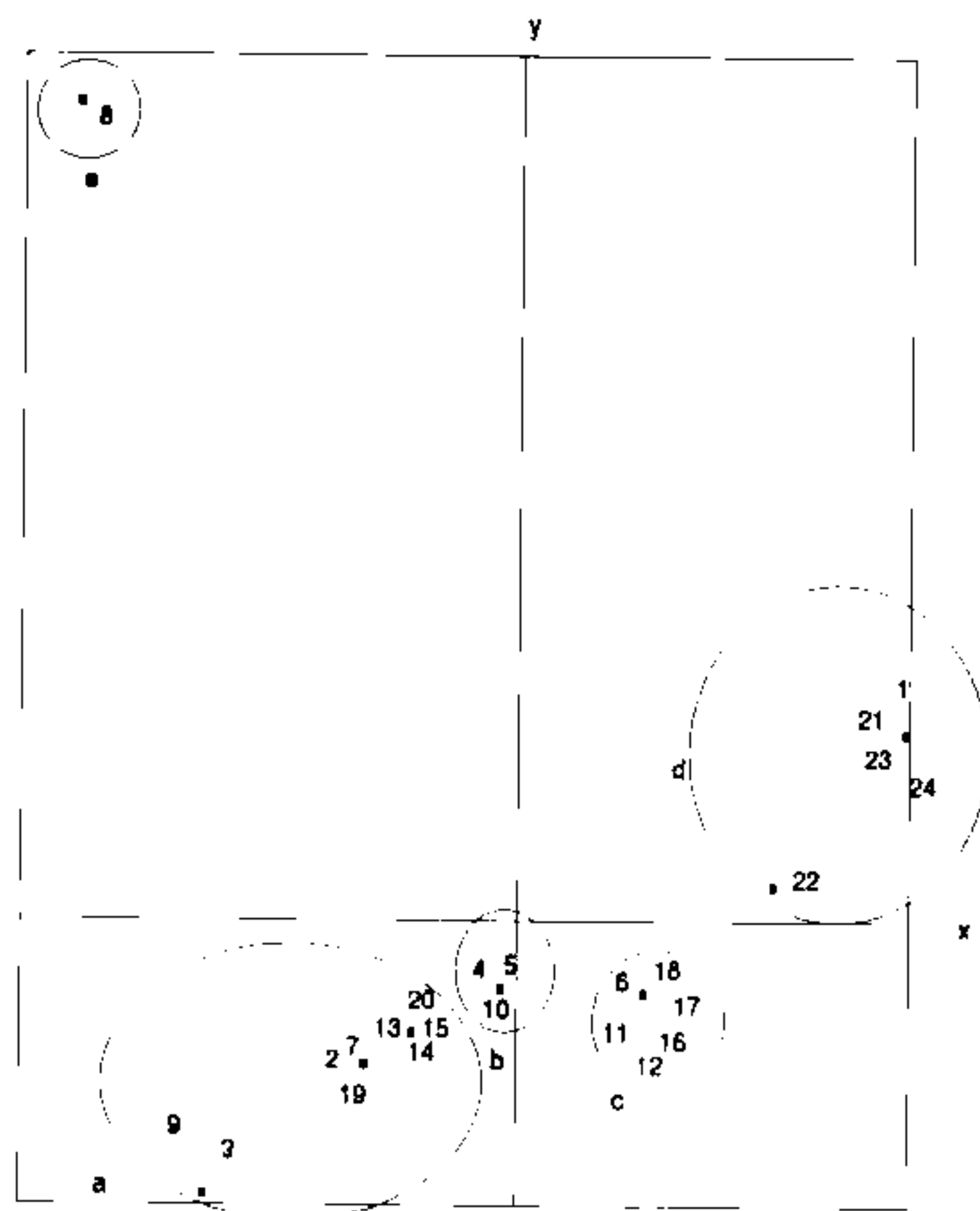

Fig. 2: factor map of the multiple correspondence analysis performed on Table II. The two first (out of 8) factor coordinates are the x and y axis, respectively, and represent 49% of the total variability. Domestic, peridomestic and sylvatic transmissions, as well as the different vectors, are separated mainly by the first axis (r = 0.87 and 0.93, respectively), while the zymodemes were more correlated to the second axis (r = 0.60). r is the correlation between the variable and the factor.

megistus, importing Z1 zymodeme into the domestic environment.

In a few areas of Brazil - especially in the municipality of Bambuí, Minas Gerais - control of *T. infestans* has been effected for many years. In much of the country, however, control of this vector has only been effective since 1983 (Dias 1987). It would appear that control of this domestic vector can significantly reduce the transmission of *T. cruzi* Z2 zymodeme. However, the reinvasion of houses by other species of Triatominae from sylvatic environments can initiate domestic transmission of Z1 zymodeme and may reconstitute domestic transmission of Z2 zymodeme. Opossums, which are often infected with *T. cruzi*, appear to play an important role in introducing Z1 pattern into domestic and peridomestic environments.This fact emphasises the need for continued vigilance of controlled areas in order to avoid colonization of houses by the bugs and opossums which are the main components of the sylvatic (Z1 zymodeme) transmission cycle of *T. cruzi*.

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