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# TRYING TO PREDICT AND EXPLAIN THE PRESENCE OF AFRICAN TRYPANOSOMES IN TSETSE FLIES

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ABSTRACT: Trypanosome infections identified by polymerase chain reaction on field-caught tsetse flies from various locations were analyzed with respect to factors intrinsic and extrinsic to the trypanosome–tsetse association. These factors were then simultaneously analyzed using artificial neural networks (ANNs) and the important factors were identified to predict and explain the presence of trypanosomes in tsetse. Among 4 trypanosome subgroups (*Trypanosoma brucei* s.l., *T. congolense* of the 'savannah' and of the 'riverine-forest' types, and *T. simiae*), the presence of the 2 types of *T. congolense* was predictable in more than 80% of cases, suggesting that the model incorporated some of the key variables. These 2 types of *T. congolense* were significantly associated in tsetse. Among all the examined factors, it was the presence of *T. congolense* savannah type that best explained the presence of *T. congolense* riverine forest type. One possible biological mechanism would be 'hitchhiking,' as previously suspected for other parasites. The model could be improved by adding other important variables to the trypanosome tsetse associations.

Trypanosomes are widespread in Africa, where they cause diseases of medical and veterinary importance, e.g., human 'sleeping sickness' and 'nagana.' Although mechanical transmission by biting flies may take place, these protozoan parasites are mainly transmitted cyclically by tsetse flies (*Glossina*). In epidemiological studies on trypanosomosis, it is important to know the infection rates of tsetse flies and the kinds of trypanosomes they carry. These parameters are part of the vectorial capacity of tsetse, which is a main component of disease transmission risk and which depends on several factors (Molyneux, 1980; Welburn and Maudlin, 1999). These factors can be grouped into intrinsic risk factors (restricted to trypanosome–*Glossina* interactions, so called vectorial competence) and extrinsic risk factors (ecological, most of which remain to be identified) (Reisen, 1988; Reifenberg, Cuisance et al., 1997). Routine identification of trypanosomes in tsetse has largely relied on dissection and microscopic examination of tsetse organs (Lloyd and Johnson, 1924), but this method is only accurate to subgenus. Moreover, mixed infections in a single fly (for example of *Nannomonas* Hoare, 1964, and *Duttonella* Chalmers, 1918) cannot be detected by this method; neither can immature midgut infections be distinguished; nor can infection with a few parasites be detected by microscopy. Polymerase chain reaction (PCR), using repetitive DNA sequences specific for each species or subgroup of trypanosome (Moser et al., 1989; Masiga et al., 1992; Majiwa et al., 1994), overcomes problems of sensitivity and specificity associated with the traditional methods of identification. PCR has been used extensively for accurate identification of trypanosomes in naturally infected tsetse in several African countries (McNamara et al., 1995; Solano et al., 1995, 1996; Masiga et al., 1996; Woolhouse et al., 1996; Reifenberg, Solano et al., 1997; De La Rocque et al., 1998; Lefrançois et al., 1998, 1999; Morlais et al., 1998).

In the present study, the results of several studies were compiled in which trypanosomes were identified in the midgut of wild tsetse using PCR. The objective was to determine the most important factors explaining the occurrence of trypanosomes in tsetse flies, which may aid in predicting disease occurrence, reemergence, or resurgence.

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## MATERIAL AND METHODS

### Composition of data set

Our investigations focused on midgut infections only, because the establishment of a trypanosome infection in tsetse and its maturation may depend on distinct factors (e.g., Maudlin et al., 1991). Only the presence of the trypanosomes was taken into account, not their absence (since trypanosomes not recognized by the sets of primers used may exist).

A total of 256 PCR-identified infections was analyzed. This data set represents a total of 4,885 field-dissected flies (prevalence varied between 0 and 20%). The data were taken from 7 published sources that report PCR identification of trypanosomes found in the midgut of 5 tsetse species or subspecies of major medical or veterinary importance in West Africa (McNamara et al., 1995; Solano et al., 1995, 1996; Masiga et al., 1996; Reifenberg, Solano et al., 1997; Lefrancois et al., 1998, 1999). The 4 trypanosome taxonomic subgroups considered reflect the most widely used PCR primer sets in tsetse fly surveys and were all used in the above-cited references. They are *Trypanosoma congolense* (Broden, 1904) savannah type, *T. congolense* riverine-forest type, *T. simiae* (Bruce, 1912), and *T. brucei* s.l. Dutton, 1902. *Trypanosoma vivax* Zieman, 1905, could not be included because the development of this parasite is restricted to the mouthparts of the tsetse fly. Other studies that used this methodology in other parts of Africa could not be incorporated in this present investigation, owing to different sampling techniques which could have biased the analyses; for example, Woolhouse et al. (1996) did not look for *T. brucei* in the dissected flies and Morlais et al. (1998) did not look for *T. congolense* savannah type.

### Explanatory variables

Variables studied, both intrinsic and extrinsic to the vector parasite association, are listed in Table I. As intrinsic variables, the tsetse taxa included represent those of the *palpalis* and *morsitans* groups that are important vectors of both human and nonhuman trypanosomoses. For phylogenetic position of tsetse, 3 categories were defined that represent discrete levels of genetic distances between taxa. Category A separates tsetse of *Glossina* Zumpt, 1935 (*morsitans* group, *Gms* and *Gl*), from those of *Nemorhina* Robineau-Desvoidy, 1830 (*palpalis* group, *Gpp, Gpg,* and *Gt*) (see Table I for abbreviations). Then, category B within the *palpalis* group separates *Gt* from both *Gpp* and *Gpg*; category C, at the within-species level, separates *Gpp* from *Gpg*. Because various trypanosome taxa may occur together in a given tsetse midgut (e.g., Solano et al., 1995; Woolhouse et al., 1996), the presence of trypanosomes other than the one under analysis was entered as a variable.

As extrinsic variables, the tsetse habitat characteristics were those reported in the literature (see, e.g., Buxton, 1955; Laveissière, 1986). The tsetse distribution range was estimated as the number of countries where a given tsetse taxon occurs (Brunhes et al., 1999). For bloodmeal

TABLE I. Explanatory variables included in the study. A random variable was also included (see text).

| Variable | Composition | Type* |
|---|---|---|
| Tsetse taxa | *Glossina palpalis palpalis* (*Gpp*)<br>*G. palpalis gambiensis* (*Gpg*)<br>*G. tachinoides* (*Gt*)<br>*G. longipalpis* (*Gl*)<br>*G. morsitans submorsitans* (*Gms*) | Intrinsic |
| Phylogenetic position of tsetse taxa | Categories A, B and C | Intrinsic |
| Trypanosome taxa | The 3 other taxa than the one under analysis | Intrinsic |
| Tsetse habitat | Savannah, humid savannah, forest, forest gallery | Extrinsic |
| Tsetse distribution range | Number of countries where the taxa is found | Extrinsic |
| Geographic location of the survey | West African savannah, West African forest | Extrinsic |
| Bloodmeal preferences | Bovidae and suidae, bovidae mainly, human and other available hosts | Extrinsic |

* Intrinsic or extrinsic to the trypanosome–tsetse association.

preferences, the taxa included in the present study represent 3 of the 5 distinct feeding groups of tsetse (Weitz, 1963; Clausen et al., 1998).

A random number variable was inserted in the database to represent a factor that had initially no influence upon the extent of trypanosome occurrence. A low contribution of the random variable would indicate little effect of chance on the infection, whereas a high value would indicate a large effect of chance (Ball et al., 2000; de Garine-Wichatitsky et al., 1999).

### Analyses

Artificial neural networks (ANNs) were used to model both intrinsic and extrinsic parameters of trypanosome occurrence in tsetse. These models may combine a set of source variables (e.g., intrinsic and extrinsic) to predict an effect (Craig et al., 1999)—in this case, the likelihood of the presence of a given trypanosome occurring in tsetse midguts. In addition, ANN methods do not require a linear relationship between variables and so may be better suited to model nonlinear phenomena. ANNs also differ from general linearized models, e.g., logistic regression, in the way that the relationships between independent parameters and the predictor variables are estimated by an iterative trial-and-error procedure (Lek and Guégan, 1999).

The back-propagation algorithm for training the database with a typical 3-layer feed-forward (n-3–4) network (Guégan et al., 2000) was used; that is, n input neurons corresponding to the n independent parameters introduced into the model, 3 hidden neurons determined as the optimal configuration to obtain a best compromise between bias and variance, and 4 output neurons for the presence of each trypanosome taxonomic group. To assess the performance of the model, the total data set was partitioned into a first subset to train the model and a second subset to test its predictive power. The test used was a 'leave-one-out' or jackknife procedure (Efron 1983). This procedure leaves out a test set (1 tsetse fly × n inputs) from the training set (256–1 × n inputs), and this is repeated for each infected tsetse. The model deduced from the training set is then used to predict the presence or absence of a given trypanosome category in the test set. This was repeated for a maximum of 5,000 iterations for each tsetse fly.

First, the predictive value of each model was tested for assessing simultaneously the presence of each trypanosome taxonomic group in the midgut of the tsetse. The predictions obtained from the neural network model, e.g., predicted infected or predicted noninfected, were compared to the observed outcomes (e.g., infected, which are the true positives, and noninfected, which are the true negatives), and a percentage of 'good classification' was obtained. Second, and only when the model was shown to be useful, the contribution of each variable to the total variance of the trypanosome occurrence response was calculated by repeating 10 simulations of the same test. This test is based on Goh's (1995) algorithm, which allows discrimination of the effect of each independent parameter on the presence or absence of an event. This provides a way to obtain mean contribution values and confidence values around the mean for each predictor entered in the model (Lek and Guégan, 1999).

An association test using a correlation coefficient for binary data (Janson and Vegelius, 1981) was calculated to see whether associations between trypanosome groups were observed more frequently than by chance. In this test, all cases where the 2 trypanosomes occurred together were taken into account. All statistical analyses were performed with MatLab 5.0 for Macintosh software.

TABLE II. Taxonomic composition of the different coinfections identified in the midgut of the tsetse. *Tcs, Trypanosoma congolense* savannah type; *Tcf, T. congolense* forest type; *Ts, T. simiae*; *Tb, T. brucei.*

| Type of multiple infection | No. of occurrences |
|---|---|
| *Tcs/Tcf* | 53 (65%) |
| *Tcs/Tcf/Tb* | 12 (15%) |
| *Tcs/Tcf/Ts* | 1 (1%) |
| *Tcs/Tb* | 5 (6%) |
| *Tcs/Ts* | 3 (4%) |
| *Tcf/Ts* | 3 (4%) |
| *Tcf/Tb* | 4 (5%) |
| Total | 81 |

## RESULTS

### Identity of the trypanosomes found in the midgut of tsetse

The most prevalent trypanosome subgroup detected by PCR was *T. congolense* savannah type (148 occurrences), followed by *T. congolense* riverine-forest type (116 occurrences), *T. simiae* (54 occurrences), and *T. brucei* (32 occurrences). The total number of identified trypanosome infections (350) was greater than the number of infected tsetse (256) because of multiple infections. Among the 256 infections, 175 were due to a single trypanosome group, 68 to coinfections by 2 trypanosome groups, and 13 to coinfections involving 3 trypanosome groups. Among the mixed infections, the trypanosome associations most frequently found together were savannah and riverine-forest types of *T. congolense* (65%), followed by a 3-way association involving these 2 groups with *T. brucei* (15%) (Table II).

Association tests between trypanosome groups yielded significant positive results for the savannah and riverine-forest types of *T. congolense* (Table IIIA; $P < 0.05$). None of the other association tests was significant (see Table IIIB for example). Applying the same test, but taking into account all the dissected tsetse (even the uninfected ones), the test became highly significant ($P < 0.0001$).

### Prediction of presence of trypanosomes

Based on information entered into the model, the percentage of good classification scores were as follows: 60 and 88% for *T. congolense* savannah and riverine-forest types, respectively,

TABLE III. Association tests (correlation coefficient and corresponding chi-square statistics for binary data) illustrating the more represented trypanosome pairwise associations in the data set.

A. *Trypanosoma congolense* savannah type versus *T. congolense* riverine-forest type.

| | | *T. congolense* savannah type | | |
|---|---|---|---|---|
| | | Present | Absent | Total |
| *T. congolense* riverine-forest type | Present | 66 | 50 | 116 |
| | Absent | 82 | 58 | 140 |
| | Total | 148 | 108 | 256 |
| Chi square | 6.223 | $P < 0.05$ | | |

B. *Trypanosoma congolense* savannah versus *T. brucei.*

| | | *T. congolense* savannah type | | |
|---|---|---|---|---|
| | | Present | Absent | Total |
| *T. brucei* | Present | 17 | 15 | 32 |
| | Absent | 131 | 93 | 226 |
| | Total | 148 | 108 | 256 |
| Chi square | 1.505 | $P > 0.05$ | | |

87% for *T. brucei,* and 79% for *T. simiae.* However, negative scores, e.g., predicted uninfected/observed uninfected, varied greatly between 13 and 100% (Table IV). Positive scores (predicted infected/observed infected) also varied between 0 and 95%.

The best scores were obtained for the *T. congolense* riverine-forest type, with good classification scores for positive (90%) and true (87%) cases. For *T. congolense* savannah type, the classification was better able to detect its presence (95%) than its absence (13%). This could indicate that there was a bias in modeling capacity to detect the absence of *T. congolense* savannah type. Although good classification scores were obtained for *T. simiae* and *T. brucei,* their presence could not be predicted at all because of the low number of tsetse infected by these trypanosomes.

### Contribution of explanatory variables to prediction

The contribution of each variable to the model for the 2 groups of *T. congolense* was calculated based on the best value obtained to model their presence. It should be stated here that, after a first look at the whole database, it appeared that some variables showed complete collinearity. For example, *G. p. palpalis* was the only tsetse taxon in the forest habitat, so these 2 variables were merged. The same was done for *G. morsitans submorsitans* and tsetse feeding on bovidae, as well as *G. longipalpis* and tsetse feeding on bovidae and suidae.

For savannah-type *T. congolense,* the results suggest that rather than only 1 or 2 variables, it is the simultaneous confounding influence of several independent variables that accounted for the presence of this trypanosome in the midgut of the tsetse analyzed. No variable contributed more than 7% to the total classification score, and the "random" variable introduced as an independent variable explained the presence of the savannah-type *T. congolense* as much as other independent parameters.

Alternatively, the presence of *T. congolense* riverine-forest type was best explained by the variable *Tcs* (i.e., the presence of *T. congolense* savannah type in the midguts of the tsetse), although its role was not significantly different from other independent variables after 10 runs (Fig. 1).

TABLE IV. Percentage of good total classification scores for each trypanosome taxonomic group, including positive (predicted infected/observed infected) and negative (predicted uninfected/observed uninfected) scores after 5,000 iterations.

| | Good classification scores (%) | % Positive | % Negative |
|---|---|---|---|
| *Trypanosoma congolense* savannah type | 60.7 | 95 | 13 |
| *T. congolense* riverine-forest type | 88.7 | 87 | 90 |
| *T. simiae* | 78.9 | 0 | 100 |
| *T. brucei* | 87.5 | 0 | 100 |

## DISCUSSION

In the present study, the factors affecting the presence of trypanosomes in the midgut of tsetse were analyzed simultaneously in order to find the most significant. This approach has already been used in other host–parasite systems, e.g., to explain the prevalence of avian hemoparasites (Tella et al., 1999). To our knowledge, this is the first time it has been used in tsetse–trypanosome associations.

The analysis was made possible by using the PCR technique, which allows accurate identification of trypanosome taxa at the specific and intraspecific levels. PCR reactions were applied only on infected midguts (diagnosed using microscopy). Subsequently, only PCR positive results on these infected midguts were incorporated into the data set. Together with the assumption that DNA is rapidly degraded in the midgut (McNamara et al., 1995), this allows us to hypothesize that the trypanosomes identified represented established infections.

As a first attempt to predict the occurrence of trypanosomes in tsetse midguts, it was found more interesting to predict the presence of the trypanosomes than their absence, because the latter could be due to confounding factors (e.g., too few cases), the presence of undetected trypanosomes, or the lack of adequate variables.

The fact that the presence of *T. congolense* savannah and riverine-forest types could be predicted by the model suggests that the model incorporated some of the important variables influencing the establishment of a trypanosome infection in the vector. The model could be improved by including other factors that have not yet been incorporated in field studies (e.g., precise local climatic conditions, local habitat of the tsetse, availability of hosts, and intrinsic factors such as the presence of endosymbionts). The model could also benefit from replacing the discrete categorical variables by continuous ones, which would allow more powerful analyses. For example, values of normalized difference vegetation indices (NDVIs) provided by meteorological satellites could be used as climatic factors because they have been shown to be good predictors of the presence of tsetse in West Africa (Rogers and Randolph, 1991; Rogers et

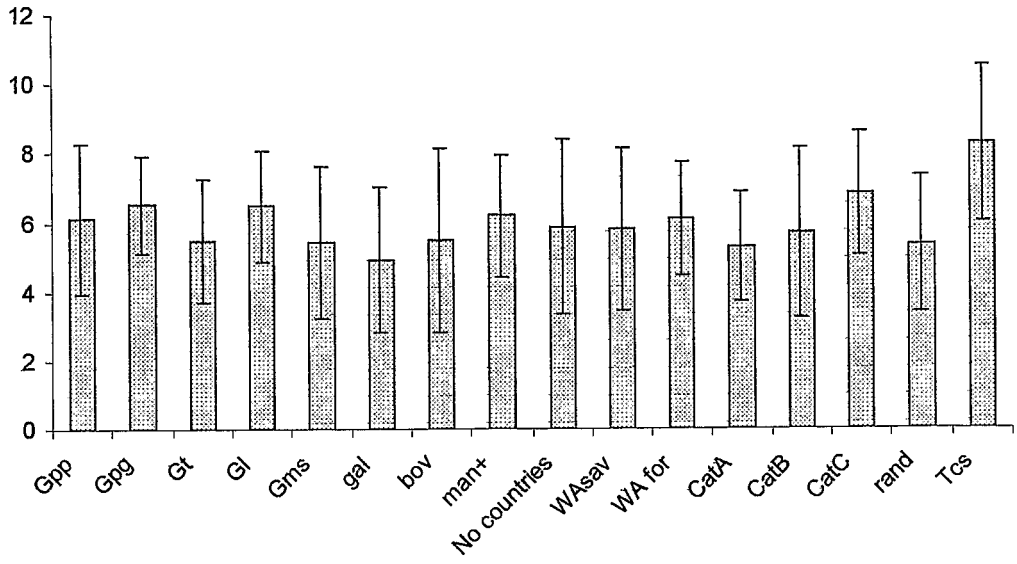


FIGURE 1. Histogram illustrating the contribution of different explanatory variables for the presence of *Trypanosoma congolense* riverine-forest type. Each bar gives the mean percentage of contribution for each explanatory variable with its corresponding 95% confidence interval after 10 runs. Legend: *Gpp, Gpg, Gt, Gl, Gms* are the 5 tsetse taxa investigated (see Table I); *gal,* forest gallery habitat; *bov,* tsetse preferentially feeding on bovids; *man+,* tsetse feeding preferentially on human and other available hosts; *no countries,* distribution range of tsetse taxa; *WAsav,* survey conducted in West African savannah zone; *WAfor,* survey conducted in West African forest zone; *cat A, B, C,* phylogenetic position of the tsetse (see text Materials and Methods: Explanatory variables); *rand,* random variable; *Tcs, Trypanosoma congolense* savannah type. Not all the initially chosen variables are represented because some were removed to avoid multicolinearity (see Results).

al., 1996). It will be also of great interest to extend this analysis to the trypanosomes found in the proboscis of the tsetse because these will represent mature infections, whereas an infection in the midgut will not necessarily lead to a mature infection (Maudlin et al., 1991; Reifenberg, Cuisance et al., 1997; Welburn and Maudlin, 1999).

A significant association between the savannah and riverine-forest taxonomic groups of *T. congolense* was demonstrated. Furthermore, the results suggested that the occurrence of *T. congolense* savannah type ranked first among all the potential influential factors when attempting to explain the presence of *T. congolense* riverine-forest type, although the reverse did not apply. These 2 taxonomic groups of *T. congolense* were defined first by Young and Godfrey (1983) on the basis of genetic differences revealed by enzyme electrophoresis. Thus, the riverine-forest group constituted stocks originating from the humid coastal zones of West Africa, whereas the savannah group contained stocks isolated from drier areas. Since then, the extensive use of PCR and DNA probes on naturally infected tsetse has shown that 'savannah' trypanosomes may be found in 'forest' tsetse (McNamara et al., 1995; Masiga et al., 1996; Morlais et al., 1998), as well as the reverse, i.e., 'forest' trypanosomes in 'savannah' tsetse (Lefrançois et al., 1999). A hypothetical representation of the interactions between these tsetse and the 2 types of *T. congolense* is presented in Figure 2. In several studies dealing with PCR identification of trypanosomes in tsetse, the riverine-forest type was rarely found alone (Solano et al., 1995). One exception was in Cameroon, in central humid Africa, where this group was the most prevalent in tsetse (Morlais et al., 1998). However, in Zimbabwe in a sample of more than 3,000 *G. pallidipes,* the riverine-forest type was never found without the savannah type of *T. congolense* in the same tsetse (Woolhouse et al., 1996). This latter result strengthens the observation that the 2 types of *T. congolense* were significantly associated in tsetse and that *T. congolense* savannah type appeared the most important variable accounting for the presence of the riverine-forest type.

Within the species *T. congolense,* the savannah type is the most prevalent in cattle in West Africa, whereas the riverine-forest type is very rare in cattle but more often present in domestic suids and small ruminants (Reifenberg, Solano et al., 1997; Lefrançois et al., 1998; Solano et al., 1999).

The riverine-forest type of *T. congolense* appears to originate from a forest biotope, seems to be poorly transmitted by various tsetse species (Reifenberg, Cuisance et al., 1997), and is rarely found alone in tsetse outside of its principal habitat. One has to reconcile these statements with the fact that it is frequently found in various tsetse taxa in immature and mature infections, together with its closely related savannah group of *T. congolense* in various ecological conditions (McNamara et al., 1995; Solano et al., 1995; Woolhouse et al., 1996).

A possible mechanism explaining its presence would be 'hitchhiking,' as proposed for other parasites (Thomas et al., 1998), e.g., the riverine-forest type of *T. congolense* would have a better chance to develop an infection and to be transmitted by tsetse already infected with the savannah type. To verify this hypothesis, further research must be conducted, focusing on experimental infections on tsetse sequentially infected with these 2 trypanosome types.

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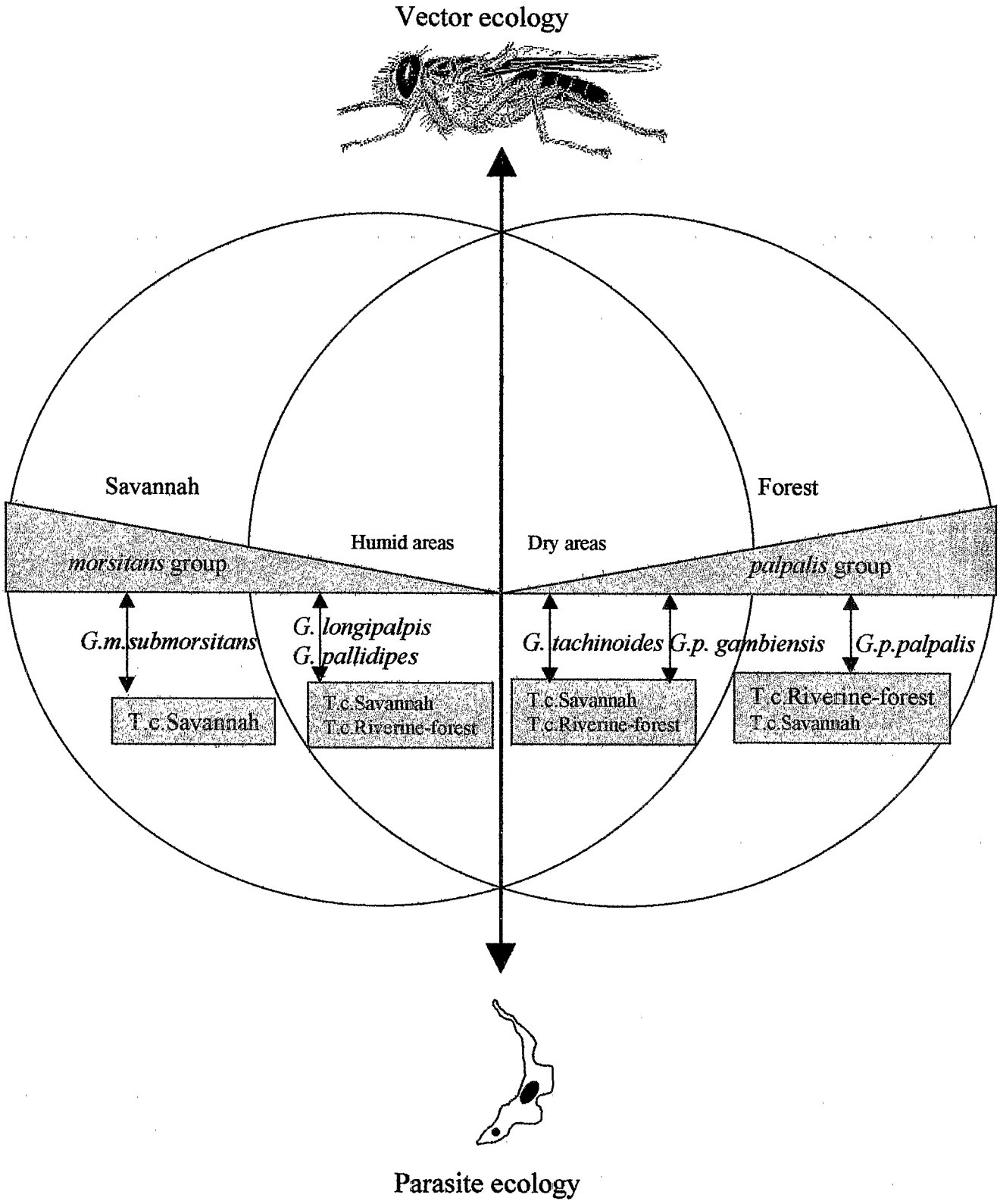


FIGURE 2. Hypothetical relationships between the 2 types of *Trypanosoma congolense* and their vectors, deduced from the results of field studies. Adapted from Reifenberg et al. (1996).

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