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New tools for trypanosomiasis field studies

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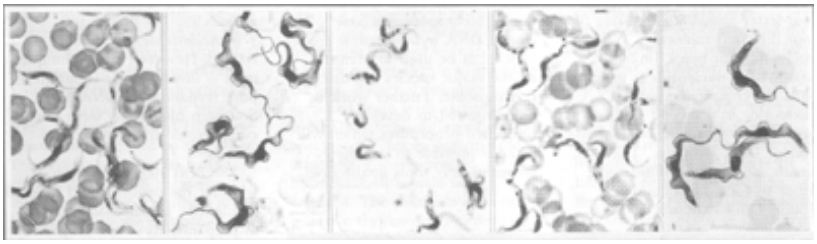
One goal of ILRAD's trypanosomiasis research program is to develop simple, sensitive and accurate methods to detect trypanosomes in infected tsetse flies and mammals, and to determine parasite species and antigenically distinct populations, called serodemes. This work is important for trypanosomiasis diagnosis and treatment and for epidemiological studies, for instance to predict the level of trypanosomiasis risk in an area before introducing susceptible livestock.

Different species of trypanosome infect nearly every type of domestic animal, many species of wild animal and man. The most important species in economic terms are those which infect cattle, sheep and goats in Africa: *Trypanosoma congolense, T vivax* and *T brucei brucei.* Closely related *T brucei* sub-species, *T b rhodesiense* and *T b gambiense,* cause human sleeping sickness. *T simiae,* which causes a severe disease in pigs, is related to *T congolense,* while *T evansi,* which infects camels, is related to the *T brucei* group.

Most African trypanosomes are transmitted cyclically by tsetse flies, but *T evansi* is transmitted mechanically by other types of biting flies.

Present methods for species identification

Field workers and laboratory staff identify trypanosome infections by microscopic examination of tsetse organs and mammalian blood. The bloodstream forms of the three major trypanosome species can be distinguished by their appearance, but the number of parasites present in the blood of an infected animal varies widely during the course of infection. The microscopy techniques which work best for detecting parasites in small numbers are not as well suited for distinguishing trypanosome species.



Can you name the parasites? These micrographs show bloodstream forms of five different species of African trypanosome —Trypanosoma brucei brucei, T congolense, T vivax, T evansi *and* T simiae *—though not in this order. The first three species were produced at ILRAD and the other two at the Kenya Government's Veterinary Research Laboratory. Can you tell which is which? The answers are on the last page.*

It is also difficult to distinguish trypanosome species in infected tsetse. An experienced technician can dissect tsetse organs and tentatively identify the infecting trypanosome species based on the parasites' location. This is possible because the three *T brucei* subspecies develop in the gut and salivary glands while *T congolense* and *T simiae* develop in the gut and proboscis and *T vivax* is found almost exclusively in the proboscis. It is not possible to identify particular species within the major groups by this method, for instance to distinguish *T simiae* from *T congolense* or *T b brucei* from *T b gambiense* and *T b rhodesiense.* This is done by subinoculation into experimental animals. For instance, if *T congolense*-type parasites are inoculated into a pig and a severe parasitaemia develops the trypanosomes are identified as *T simiae.* Clearly, this approach is expensive and time consuming and there are drawbacks in terms of animal welfare.

Another major problem is that many infections encountered in the field—particularly in mammals and at times also in tsetse—are mixed, consisting of more than one trypanosome species. In this situation, the process of species identification by microscopic examination and subinoculation into experimental animals can become long and complex.

Comparison of trypanosome DNA

Given the problems inherent in current methods, scientists in several laboratories are working to develop better diagnostic tests for the identification of trypanosome species. Such diagnostic tests must be sensitive enough to identify parasites present in small quantities of crude materials collected in the field, specific enough to distinguish the most important trypanosome species and simple enough to be performed on a routine basis in areas lacking sophisticated laboratory facilities. Each living organism contains its own characteristic deoxyribonucleic acid—DNA. DNA carries the blueprints for all the functional protein components of a plant or animal, as well as elements which control when and in what quantities these components are produced. This information is encoded in a four-letter alphabet consisting of four different molecules, called nucleotides or bases, linked in a chain. Each nucleotide is matched with the complementary nucleotide in a second chain to form a base pair, the fundamental unit of DNA structure. The two chains are wound around each other to form the famous double helix structure of DNA.

Among other approaches, molecular biologists at ILRAD and elsewhere are developing diagnostic tests based on differences in parasite DNA. A high proportion of the trypanosome genome is composed of repetitive DNA sequences, most of which may be unique to each trypanosome species or subspecies. Other biochemical methods for species identification, such as isoenzyme analyses, require large numbers of parasites that must be grown either *in vitro* or in experimental animals. By contrast, the identification and use of species-specific repetitive DNA sequences offers the possibility of not having to grow the parasites at all: trypanosomes can be identified directly from blood samples taken from infected animals or preparations from infected tsetse.

In the past few years, ILRAD scientists have developed DNA typing techniques and have cloned DNA hybridization probes which can be used to identify all the major species of African trypanosome. Further work is now required to develop simplified procedures which

will make it possible to use these powerful new tools in the field.

Species identification based on VSG genes

The first DNA hybridization experiments at ILRAD originated from studies on the genes coding for trypanosome variable surface glycoproteins (VSGs). Three VSG genes and a DNA fragment located in the flanking region next to one of the genes had been cloned from different stocks of *T b brucei.*

Trypanosomes were isolated from the blood of rats, each infected with a stock or clone of *T b brucei, T b gambiense, T b rhodesiense, T congolense, T evansi* or *T vivax*. Parasite DNA was purified, split into fragments by restriction enzymes and the fragments separated by electrophoresis on agarose gels. The DNA was then transferred from the gels onto nitrocellulose filters by a technique called Southern blotting. The three genes and the flanking region were labeled with radio-isotopes and applied to these filters which were already bearing single-stranded DNA fragments. The radio-labeled DNA hybridized to complementary strands of DNA on the filters. When the filters were exposed to X-ray film, the spots where DNA hybridization had occurred appeared on the film as dark bands. The patterns of banding revealed that all four probes hybridized with DNA from all the stocks and clones of *T b brucei* and *T b rhodesiense* tested but with no stocks or clones of any of the other species.

These experiments showed that DNA hybridization techniques could be used to identify trypanosome species. They also confirmed previous observations that the two trypanosome subspecies, *T b brucei* and *T b rhodesiense,* are closely related. However, the techniques used—involving the isolation of pure trypanosome DNA and Southern blotting—were too cumbersome for general field use.

The next step was to see whether the probes could be used to identify trypanosome species in preparations of whole parasites or blood from infected animals. This work showed that these probes were not sensitive enough to be useful in the field. Using whole trypanosomes, a minimum of 10,000 parasites was required to obtain a positive hybridization signal after a 16-hour exposure; using blood from infected animals, the probes could not detect fewer than 100,000 parasites.

Search for more sensitive probes

Since specificity and sensitivity are important features of a diagnostic test, scientists began searching for DNA sequences in the genomes of the most important trypanosome species which would hybridize exclusively with DNA from the same species and which would recognize DNA from a small number of parasites. The original *T brucei* DNA probes met the first criterion, but not the second.

The VSG genes and genes encoding other proteins are only present in one or a small number of copies in the parasite genome. The remainder of the genome consists of sequences of DNA which may be present in hundreds or thousands of copies. The function of most of these repeated sequences is unknown but they tend to be specific to particular trypanosome species. Because they are present in such large numbers, these sequences are much more easily detected in small numbers of parasites than single-copy genes.

To identify sensitive, species-specific DNA probes, total DNA was purified from cloned parasites of various species, the DNA was split into fragments with restriction enzymes and the fragments were separated on agarose gels by electrophoresis. This involves passing electrical charges through the gels which allow the fragments of DNA to move from one end to the other. The fragments move at various rates depending on their size so that the smallest fragments move all the way to the bottom of the gel while the largest remain near the top. Identical fragments, representing DNA sequences which occur in multiple copies, move at the same rate and thus end up clustered at the same position in the gel.

The fragments of trypanosome DNA were transferred onto nitrocellulose filters and

incubated with radio-labeled whole DNA from the same and different parasite species. In this case, the whole DNA was used as a probe. Conditions for hybridization were selective —only a small amount of radio-labeled whole DNA was used and the incubation period was short (12 to 15 hours). Under these conditions, hybridization would occur preferentially at places where multiple copies of identical DNA fragments were clustered. Again, the points where hybridization occurred could be observed as dark bands on X-ray film. Nearly all hybridization signals detected in this way appeared to be species specific: they were only seen when whole radio-labeled DNA was applied to filters containing DNA from the same species.

The DNA fragments showing the strongest hybridization signals, as visualized on the X-ray film, were cut out of identical agarose gels and the DNA inserted into a plasmid vector which was used to infect the bacterium *Escherichia coli.* The bacterium was then multiplied in culture. Bacterial colonies were screened again with radio-labeled total DNA from different parasite species and clones showing the strongest species-specific hybridization signals were selected. The plasmids containing putative trypanosome repetitive DNA sequences were purified from these bacteria and radiolabeled: they were then ready for use as hybridization probes.

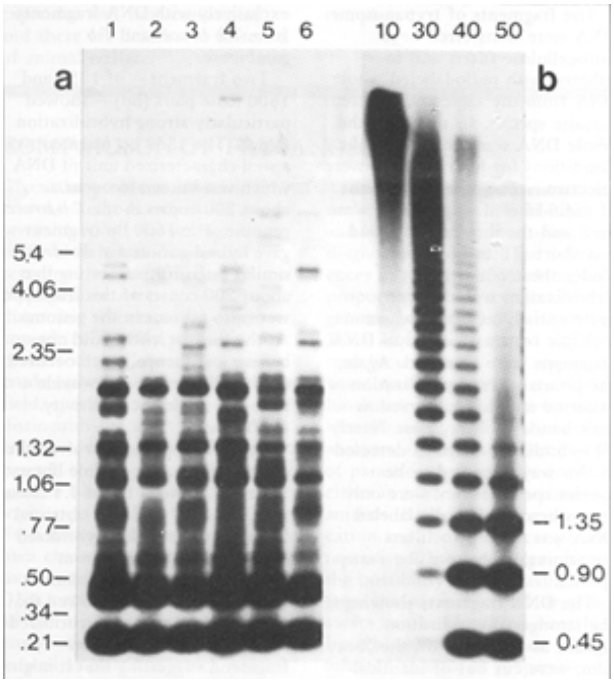
A probe for *T brucei*

Using these techniques, radio-labeled whole DNA from one clone of *T b brucei* (ILTat 1.4) was allowed to hybridize with DNA fragments derived from clones of the major trypanosome species. Under selective conditions the radio-labeled DNA hybridized exclusively with DNA fragments from *T b brucei* and *T b gambiense.*

Two fragment—of 1350 and 1600. base pairs (bp)—showed particularly strong hybridization signals. The 1350 bp fragment was a well-characterized unit of DNA which was known to occur as about 200 copies in the *T b brucei* genome. The 1600 bp fragment gave hybridization signals of similar intensity, suggesting that about 200 copies of this fragment were also present in the genome. At the time, scientists did not believe a sequence which occured only 200 times would provide a probe of sufficient sensitivity for field use.

Another approach involved the construction of a genomic library from the *T brucei* ILTat 1.4 clone. A sequence of 9500 bp obtained from this library also hybridized exclusively with DNA from *T b brucei* and *T b gambiense.* Further experiments showed that the 1600 bp fragment hybridized strongly with the 9500 bp fragment, suggesting that it might be contained within the larger DNA sequence. This was subsequently confirmed and the repeated element within the larger fragment was shown to be 5280 bp long. Two different copies of the whole 5280 bp fragment have now been sequenced, one by scientists at ILRAD and the other by colleagues at the Université Libre de Brussels in Belgium. Between 150 and 250 copies of this sequence occur in the *T brucei* genome, which— scientists now realize—probably made it adequate for use as a hybridization probe. Since the 5280 sequence was isolated from an African trypanosome, ILRAD scientists have named it *ingi*, the Kiswahili root adjective meaning many. A plasmid vector, called pgDR-1, has been purified from DNA containing the 1600 by portion of this sequence. Under stringent conditions, this probe hybridizes exclusively with trypanosomes of the *T brucei* group, including *T b brucei, T b gambiense, T b rhodesiense* and *T evansi.* It can be used to identify as few as 100 parasites in the blood of infected animals and has also been used to detect trypanosomes in tsetse.

The sequencing of the 5280 bp *T brucei* DNA fragment has evoked considerable scientific interest apart from its potential use as a diagnostic probe. *Ingi* appears to be a type of randomly repeated DNA sequence called a retroposon, a mobile element dispersed in the genome by retrotransposition to new sites. This is the first time a retroposon of this kind has been described in a protozoan. Its function is unknown.



Two types of repetitive DNA sequence, illustrated here, were used as probes to identify trypanosome species. The first radiograph (a) illustrates hybridization with a fragment of ingi, a randomly repeated DNA sequence in the genome of T b brucei. *This sequence was radio-labeled and incubated with DNA from four clones of* T b brucei *(lanes 1–4), one clone of* T evansi *(lane 5) and one clone of* T b gambiense *(lane 6). Purified DNA from these clones had been cut into fragments with a restriction enzyme and the fragments separated by electrophoresis on an agarose gel and transferred onto nitrocellulose filters. Hybridization occurred with fragments of many different lengths, suggesting that ingi elements are embedded within diverse sequence environments and widely dispersed in the parasite genome. The second radiograph (b) shows that the Kilifi-type* T congolense *DNA probe, cloned in the plasmid pgNIK-450, is made up of a series of tandemly repeated nucleotides in an arrangement characteristic of satellite DNA. Purified DNA from a clone of Kilifi-type T congolense was split with a restriction enzyme for different periods of time, as indicated in minutes at the top of each column, separated on an agarose gel and transferred onto a nitrocellulose filter. After a 10-minute exposure to the enzyme, most of the DNA remained in a large fragment which stayed at the top of the gel; as the exposure time was lengthened, the DNA was split into smaller fragments which moved further down the gel. The probe, which is 450 bp long, hybridized to a ladder of DNA fragments whose unit lengths were multiples of 450 bp.*

The *ingi* sequence resembles a type of retroposon called a long interspersed nuclear element (LINE) which is present in very large numbers in the genomes of mammals. Similarities in *ingi's* nucleotide sequence and mammalian LINE sequences suggest a significant evolutionary relationship. *Ingi* is even more similar to a retroposon found in the fruit fly *Drosophila melanogaster.* Could early trypanosomes have usurped the original *ingi* sequence from their mammalian or tsetse hosts? Or could this type of retroposon be more widely distributed than was previously apparent, suggesting a very early evolutionary origin?

A second DNA probe for *T brucei* has been identified by scientists at the National Cancer Institute in the Netherlands. This probe is based on a small sequence of 177 by which appears to be present in very large numbers in the *T brucei* genome, arranged as a series of tandemly repeated nucleotides. This type of repeated sequence is known as satellite DNA.

DNA studies distinguish two populations of *T congolense*

The classification of *T (Nannomonas) congolense* trypanosomes has attracted considerable attention since the first trypanosomes of this type were described early in this century.

These parasites occur over vast areas of tropical Africa, causing serious disease and death in livestock and substantial economic losses for farmers. Populations of *T congolense*-type parasites cannot be distinguished on the basis of appearance, but by other criteria they are highly diverse.

ILRAD scientists recently identified two distinct groups among East African isolates of *T congolense* which differ from each other in terms of the size distribution of chromosomes, visualized by a technique known as molecular karyotyping. Chromosome-sized DNA molecules from different *T congolense* clones were freed of protein and separated according to size by electrophoresis on agarose gels. The gels were stained with ethidium bromide and photographed under ultraviolet light, revealing chromosomes of different sizes of different positions along the gels.

This technique, known as orthogonal field alternation gel electrophoresis (OFAGE), revealed two clearly distinct chromosome-profile types. One group consisted of parasites isolated during an epidemiological survey at Kilifi on the Kenya coast, while the other consisted of populations of *T congolense* type trypanosomes isolated in savannah areas of East, West and Central Africa. To evaluate the significance of these differences, the chromosome profiles of these two *T congolense* groups were compared with those of *T b brucei* and *T evansi,* which are considered to be distinct species. The chromosome profiles of the two *T congolense* groups—derived from parasites which cannot be distinguished by appearance and which are considered to belong to the same species—were more different from each other than were the chromosome profiles of *T b brucei* and *T evansi.*

These results were extended to include a comparison of repetitive DNA sequences within the genomes of these two *T congolense* groups by hybridization experiments using total DNA from different parasite clones. DNA sequences from *T congolense*-type clones isolated in Kilifi hybridized only with DNA from other Kilifi clones, whereas DNA sequences from savannah-type *T congolense* parasites isolated elsewhere in Africa hybridized exclusively with DNA from other non-Kilifi clones. The total absence of cross-hybridization between the DNA of these two groups contrasted with results for the *T brucei* group, showing considerable hybridization of DNA from *T b brucei* with DNA from *T b gambiense* and *T evansi* This suggests that some populations of *T congolense* are phylogenetically more distant from each other than are the two separate species, *T evansi* and *T brucei.*

What is the relationship between these two groups of *T congolense? T evansi* may have evolved from *T brucei* but it is unlikely that either one of the two *T congolense* groups evolved from the other—at least not recently. The simplest interpretation is that they are genetically distinct trypanosome species or subspecies; it is possible that they evolved independently from an ancestral trypanosome.

DNA probes for the two *T congolense groups*

Purified DNA from several trypanosome species was split into fragments and after electrophoresis on agarose gels was blotted onto nitrocellulose filters. The DNA fragments were hybridized with whole radiolabeled DNA from two *T congolense* clone*s,* one (ILNat 5.1) originating from Kilifi and the other (ILNat 2.1) originating from savannah-type *T congolense* parasites isolated in the Transmara region on the Kenya/Tanzania border. DNA fragments were identified which were characteristic of each clone and which produced strong hybridization signals. Neither sequence hybridized with DNA from the other *T congolense* group or from any other trypanosome species.

These two DNA fragments are made up of a series of tandemly repeated nucleotides in an arrangement characteristic of satellite DNA. They are smaller than the dispersed-repeat sequence identified in the *T brucei* genome. The fragment originating from the Kilifi clone consists of 450 bp, whereas the fragment from the other *T congolense* clone consists of 372 bp. Both sequences are present in large numbers: there are approximately 4000 copies of the 450 by fragment in the genome of the Kilifi parasites and 3000 copies of the 372 bp fragment in the genome of the savannah-type *T congolense.*

The sequences were cloned in *E coli* and recombinant plasmids were purified from the bacterial colonies which showed the strongest hybridization signals. The recombinant plasmid vector which hybridizes with *T congolense* parasites from Kilifi is called pgNIK-450; the vector which hybridizes with savannah-type *T congolense* parasites isolated from other parts of Africa is called pgNRE-372. Both can potentially identify DNA from as few as five trypanosomes.

A DNA probe which appears to recognize a third group of *T congolense* parasites, originating from a forested area of West Africa, has recently been identified by scientists at the University of Bristol (UK). This group has exchanged material with ILRAD and tests have been conducted with all three probes which showed that they each recognize a distinct group of parasites.

DNA probe for *T simiae*

There is a particularly urgent need for a method to identify *T simiae* and distinguish this species from *T congolense* because *T congolense* causes a severe disease in cattle while *T simiae* does not affect cattle but causes fatal infections in pigs. The two species cannot be distinguished reliably in terms of appearance. The methods currently used to distinguish them are laborious and can be extremely expensive.

Using the techniques already described, ILRAD scientists have identified a repetitive DNA sequence in the genome of *T simiae* which is specific for this species and is present in sufficiently high copy number to indicate its potential usefulness as a sensitive hybridization probe. Like the DNA probes for the two groups of *T congolense*, the *T simiae* probe consists of a tandem array of nucleotides; for *T simiae* the sequence is 550 bp long. The *T simiae* genome contains about 1000 copies of this sequence, located predominantly in the minichromosomes. The recombinant plasmid vector which contains this sequence, pgNS-600, can potentially be used to identify as few as 100 trypanosomes in an infected tsetse.

The specificity of ILRAD's three DNA probes—for *T simiae* and for the two groups of *T congolense*—has been tested using 7 clones and 1 stock of *T simiae* from one location in Kenya, 10 clones of *T congolense* from Kilifi, 7 clones of *T congolense* from different locations in Kenya, Tanzania, Uganda, Zambia and Burkina Faso and clones of *T brucei* and *T vivax*. So far, each probe has hybridized exclusively with the group of parasites from which it was derived.

Additionally, the *T simiae* probe was used to test parasite material derived from infected tsetse captured at different locations in The Gambia. The probe hybridized specifically with *T simiae* whether the parasites were present in single or mixed infections. Material for these tests was provided by colleagues from the International Trypanotolerance Centre in The Gambia and from the University of Bristol.

DNA probe for *T* vivax

ILRAD's first *T vivax* DNA probe was derived from the genome of a West African clone, ILDat 1.2. A sequence of 10,000 bp was identified which gives strong, species-specific hybridization signals. DNA from a recombinant bacteriophage containing this sequence was purified and radiolabeled and the probe was named lambda gDIL-10. It is capable of detecting as few as 240 *T vivax* parasites in the blood of infected animals.

When this probe was tested against *T vivax* populations from different parts of Africa, it hybridized with parasites from West Africa, but not as well with *T vivax* originating from East Africa. A DNA probe has recently been developed from an East African *T vivax* clone which also appears to hybridize predominantly with parasites from its area of origin. *T vivax* parasites from East and West Africa cannot be distinguished by appearance: the only distinction scientists have observed in the past is a tendency for infections with West African parasites to be more acute. Up to now, ILRAD's two *T vivax* DNA probes have only been tested with a small number of parasite clones: further testing is required before conclusions can be drawn regarding differences between *T vivax* populations from the two regions and

from areas in between.

Simplified procedures for field use

The first step in simplifying procedures for trypanosome identification using DNA probes has been the development of methods for collecting and storing parasite material which can then be sent to a well equipped laboratory for analysis. Ultimately, the goal is to develop procedures which will allow the use of DNA probes for trypanosome characterization under field conditions with a minimum of equipment and material.

A serious limitation on the field application of experimental procedures was the requirement for purified DNA, which is obtained by long and complex procedures. ILRAD's DNA probes are sensitive enough to detect small numbers of trypanosomes in preparations of tsetse organs or blood from infected animals, but better procedures were needed for collecting, storing and preparing these samples for analysis. For detection of trypanosome infections in tsetse, various means of sample preparation from tsetse organs have been tested. Material from tsetse midguts or salivary glands was blotted onto nitrocellulose filters and incubated over night with ILRAD's pgDRI probe for *T brucei*. The probe gave strong hybridization signals for all material from *T brucei*-infected tsetse. The probe also hybridized, although weakly, with material derived from the proboscides of infected flies, but there was no hybridization with any material from uninfected tsetse.

Scientists also tested a simpler procedure involving cutting off the distal quarter of the tsetse abdomen to expose the guts. These were then squeezed and the contents sequentially touch-blotted, at up to eight different spots, on dry nylon filters. Caustic chemicals were added to the filters to kill the parasites and break up and fix their DNA, followed by addition of the pgNRE-372 *T congolense* probe. Although there was variation in the intensity of hybridization, positive signals could be observed in all eight touch blots for each of eight flies infected with *T congolense.* However, a filter touch-blotted with material from the same flies and stored for 10 days without further treatment showed no hybridization to the labeled probe. It thus appears necessary to fix the parasite DNA before filters are stored.

If DNA probes are ever to be used for identifying trypanosome species in the field, another method of labeling is required which does not rely on radioactive isotopes. Scientists have investigated the use of biotin labeling but it has not yet been possible to develop a procedure of sufficient sensitivity because mammalian blood or crude material from tsetse also tends to be labeled.

Recently, several procedures have been developed in different laboratories for chemically modifying DNA so that it can be detected by standard immunological techniques. ILRAD scientists are currently investigating the use of chemically derivatized DNA probes which might be suitable for the identification of trypanosome species under field conditions.

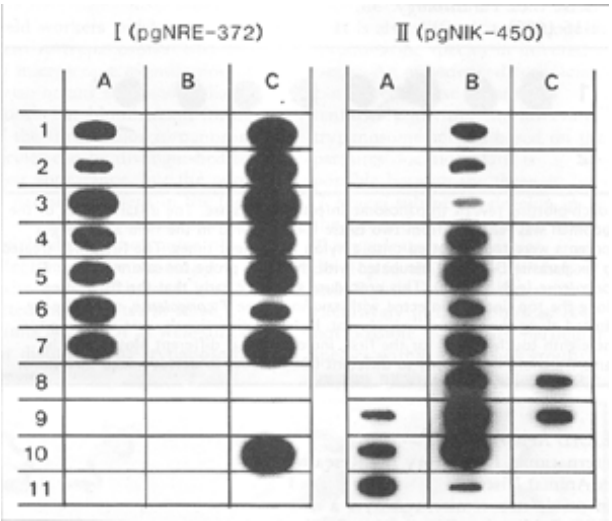
The first field applications

ILRAD's DNA probes have been used to identify trypanosome species in infected tsetse flies from East Africa and in blood samples collected from cattle in West Africa. The tsetse flies were collected from different sites in Kenya by cooperating scientists participating in the Trypanotolerant Livestock Network. The collection exercise was part of a Network training course which is conducted at ILRAD every year. Samples from infected cattle were collected in connection with ILRAD's collaborative project with the International Trypanotolerance Centre in The Gambia. In both cases, analysis with ILRAD's DNA probes revealed mixed infections with two or more trypanosome species which would have been difficult to detect by standard procedures.

Future directions

The highly repeated nucleotide sequences used as the basis of ILRAD's DNA probes tend to be specific for particular species of trypanosome. Other elements in the trypanosome

genome may be specific for larger or smaller categories of parasites. For instance, certain VSG genes which are important in the process of antigenic variation appear to be characteristic of particular serodemes. Potentially, DNA probes may be developed for serodeme analysis based on these genes.



DNA hybridization shows a clear distinction among T congolense *clones originating from different locations in Africa. Whole, ground-up trypanosomes were blotted at identical spots on duplicate nylon filters (I and II). Parasites in slots AI to A7 are clones of savannah-type* T congolense *isolated from different places in East and West Africa. Slots A9 to All and B1 to B11 contain* T congolense *clones derived from isolates from Kilifi on the Kenya coast. Slots C1 to C11 contain material from* T congolense *parasites isolated at Muhaka, a village near the Kenya coast about 90 km from Kilifi. Filter I was incubated with the DNA probe for savannah-type* T congolense *(pgNRE-372) and filter II was incubated with the Kilifi-type T congolense DNA probe (pgNIK-450). Each probe hybridized only with DNA from the parasite group from which it was derived. Interestingly, all the parasites isolated from Muhaka hybridized exclusively with one or the other probe—there was no cross-hybridization. This provides further evidence for the genomic distinction between Kilifi-type and savannah-type* T congolense.

Other elements in the trypanosome genome are characteristic of broader parasite categories. DNA probes may be developed which are based on sequences found in common in all members of a major trypanosome subgeneric group. A probe which could hybridize with all three groups of *T congolense* would be particularly useful. Such common sequences have been identified in the ribosomal DNA of filaria parasites, suggesting that ribosomal DNA might be a good place to look for DNA sequences with broader specificity in trypanosomes.

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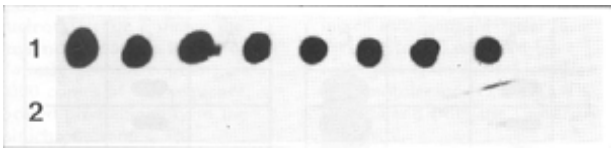

Touch-blotting reveals trypanosome infections in tsetse. The distal quarter of the abdomen was removed from two tsetse flies captured in the field and the gut contents were touch-blotted onto a nylon filter eight times. The filter was treated to fix parasite DNA and incubated with the DNA probe for savannah-type T congolense *(pgNRE-372). This procedure showed clearly that the fly blotted along the top line was infected with savannah-type* T.congolense*, while the fly blotted along the bottom line was not. Hybridization signals were as strong for the eighth touch-blot as for the first, indicating that different blots from the same fly could be exposed to different DNA probes to detect mixed infections.*

The trypanosomes pictured on page 1 are, from left to right, T congolense, T b brucei, T simiae, T vivax *and* T evansi. T congolense *and* T simiae *are similar to each other but they can be distinguished from the other species because they lack a free flagellum (the whip-like appendage extending from the anterior end).* T b brucei *and* T evansi *resemble each other.* T vivax *can be distinguished from* T brucei *and* T evansi *by the larger kinetoplast (which appears as a dark spot near the posterior) and often by a more rounded posterior end.*

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