

Application of DNA markers to identify the individual-specific hosts of tsetse feeding on cattle

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Abstract. Primer sets for five different ungulate loci were used to obtain individual microsatellite DNA profiles for 29 Mashona cattle from a herd in Zimbabwe. There were 3–13 alleles for each locus and, using the entire suite of five loci, each animal within the herd, including closely related individuals, could be unequivocally distinguished. Wild-caught *Glossina pallidipes* Austen (Diptera: Glossinidae) were fed on specific cattle and the bloodmeal was profiled 0.5–72 h after feeding. The individual specific sources of the bloodmeals, including mixed meals produced by allowing tsetse to feed on two different cattle, were reliably identified up to 24 h after feeding. The technique was used in field studies of host selection by *G. pallidipes* and *G. morsitans morsitans* Westwood (Diptera: Glossinidae) attracted to pairs of cattle. When the pair comprised an adult and a calf, 100% of meals were from the adult. For some pairs of adult cattle, tsetse were biased significantly towards feeding on one animal, whereas for other pairs there was no such bias. In general, feeding was greater on the animal known to have a lower rate of host defensive behaviour. Results suggest that relatively slight differences in the inherent defensive behaviour of cattle produce large differences in host-specific feeding rates when the hosts are adjacent. For flies attracted to pairs of cattle, <2% contained blood from both hosts. The DNA profiling technique will be useful in studying the epidemiology of vector-borne diseases of livestock.

Key words. *Glossina*, bloodmeal, cattle, feeding behaviour, microsatellite DNA, tsetse fly, Zimbabwe.

Introduction

Knowledge of the source of an insect's bloodmeal provides important information relating to the epidemiology of vector-borne diseases. In the past, interest has focused mainly on determining the species of host that provided the meal. In studies of malaria for instance, the percentage of meals from humans (anthropophilic index) is an important component of a mosquito's vectorial capacity (Dye, 1992). Knowledge of the host species of tsetse similarly formed the basis of a successful, albeit ecologically unacceptable, control technique based on the selective eradication of important host species (Vale & Cumming, 1976). Methods for determining the specific source

of a meal have been based on various immunological methods (Weitz, 1970; Clausen *et al.*, 1998).

There has also been much interest in identifying individual-specific sources of bloodmeals. One of the most important parameters controlling the epidemiology of insect-borne diseases such as malaria or trypanosomiasis is the vector's biting rate. Specifically, the vectorial capacity of an insect changes as the square of the biting rate (Dye, 1992). An insect's biting rate is the reciprocal of the feeding interval and this is taken to be the period between successive full bloodmeals, assuming that each feed is not interrupted. However, if a feeding vector is interrupted, and if this leads to repeated probing of different hosts, then the biting rate increases. Consequently attempts have been made to determine the frequency of multiple bloodmeals.

Serological (Boreham & Garrett-Jones, 1973; Guzman *et al.*, 1994) and histological (Wekesa *et al.*, 1995) techniques have

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been used in studies of various vectors but the limited number of sero- or histotypes identified by these procedures has limited their utility. Coulson *et al.* (1990) described a novel method for identifying the individual source of a bloodmeal from a mosquito that had fed on a human, based on the amplification and analysis of human DNA in the bloodmeal. More recently, researchers have started to use various molecular biology techniques to characterize the sources of meals from mosquitoes resting in houses and thought to have fed on humans from a particular hut (Gokool *et al.*, 1993) or village (Koella *et al.*, 1998).

The same type of technique could be particularly valuable in studies of the feeding behaviour of tsetse, for at least two reasons. First, there is much debate regarding the feeding interval of tsetse (Randolph *et al.*, 1992; Hargrove & Packer, 1993) which has important implications for the epidemiological role of tsetse (Rogers, 1988). Much of the work informing this debate is based on the measurement of haematin levels in tsetse, which declines exponentially with time since feeding (Brady, 1975; Loder *et al.*, 1998). The epidemiological implications from this work are premised on the assumption that bloodmeals are derived from a single meal taken from an individual animal. A technique that can determine whether bloodmeals are derived from different individuals is needed to examine this assumption.

Second, there is much interest in using insecticide-treated cattle to control tsetse (Bauer *et al.*, 1995; Baylis & Stevenson, 1998; Vale *et al.*, 1999). Recent evidence indicates that there are up to 20-fold differences in the efficacy of different cattle as baits (Torr & Mangwiro 2000). In the traditional farming systems of Africa, livestock keepers typically own heterogeneous herds of cattle such that animals of different age, sex and size are kept together. Consequently, there may be potential opportunities for increasing the cost-effectiveness of the cattle-treatment technique by selectively treating those animals that are effective baits. It would therefore be useful if we could determine whether tsetse attracted to a herd of cattle contact only a few 'attractive' animals within the herd.

In this study we applied ungulate microsatellite primers to tsetse bloodmeals to determine if individual-specific DNA profiles could be generated, using variable microsatellite markers, to identify the individual source(s) of bloodmeals. Microsatellite loci are highly polymorphic regions composed of repeat units of between 2 and 5 base pairs (bp) that are arranged in tandem array and maintain allelic diversity based on differences in the array size (Bruford & Wayne, 1993). Ungulate microsatellite loci have been used in forensic identifications and paternity assignment in cattle (Beamonte *et al.*, 1995).

Materials and Methods

All field studies were carried out between January 1998 and April 1999 within 3 km of Rekomitjje Research Station in the Zambezi Valley of Zimbabwe where *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood occur. The research station is in the Mana Pools National Park where wild

ungulate hosts such as buffalo (*Syncerus caffer* Sparman), bushbuck (*Tragelaphus scriptus* Pallas), kudu (*Tragelaphus strepsiceros* Pallas) and warthog (*Phacochoerus aethiopicus* Pallas) are abundant. Apart from a herd of ~30 research cattle at Rekomitjje, no domestic livestock are found within 50 km of the station.

Specimens

Cattle. Mashona cattle, a breed of short-horned Sanga indigenous to Zimbabwe, were used in all studies. Blood samples were taken from cattle by collecting blood from the ear vein onto a filter paper. The sample was air dried and placed in an aluminium foil envelope that was heat-sealed and then stored in a -10°C freezer, pending despatch to Trent University, Peterborough, Canada for DNA extraction and profiling.

Tsetse. Unfed tsetse were caught from Epsilon traps (Muzari & Hargrove, 1996) baited with a blend of acetone (500 mg/h), 1-octen-3-ol (0.5 mg/h), 4-methylphenol (1 mg/h) and 3-*n*-propylphenol (0.1 mg/h) dispensed by the methods of Torr *et al.* (1997). The traps were operated between 1400 and 1800 h, > 3 km from Rekomitjje Research Station. The trapped flies were killed within 30 min of collection by placing them in a freezer for 10 min. Fed tsetse were obtained by three different methods.

First, flies were caught in the late afternoon after they had fed off a bait ox. They were retained singly in a glass tube (2.5 cm × 7.5 cm) with a cork stopper at one end and netting at the other. Caught tsetse were kept in a polystyrene cool box and later transferred to a laboratory where they were killed at various intervals after feeding.

Second, unfed flies were caught from traps in the late afternoon. The flies were transferred singly to glass tubes (2.5 cm × 7.5 cm) with a cork stopper at one end and netting at the other and held alive in an insectary (Temp 23–25°C, r.h. 60–70%) overnight. The following morning, the tubed flies were allowed to feed on an ox by placing the tube against the flank of the animal. After each fly had partially fed, it was moved to a second animal where it completed feeding. The flies were killed within 30 min of engorgement.

Third, tsetse that had fed naturally in the field on cattle were sampled following the method of Vale (1977). One or two cattle were placed at the centre of an incomplete ring (8 m diameter) of six electric nets (Vale, 1974). Flies that struck the net were killed or stunned and fell onto metal or plastic trays. Tsetse caught on the outside or the inside of the ring were presumed to be approaching or leaving the ox, respectively. Feeding efficiency was estimated as the number of fed tsetse on the inside of the ring of nets expressed as a percentage of the total catch from the inside of the ring. To enhance the numbers of tsetse attracted to the cattle, a blend of carbon dioxide (120 l/h), acetone (500 mg/h), 1-octen-3-ol (0.5 mg/h), 4-methylphenol (1 mg/h) and 3-*n*-propylphenol (0.1 mg/h) was dispensed ~2 m downwind of the animal.

Flies were collected from the trays at 30-min intervals and placed individually into single tubes that were then stored in a

Table 1. Ungulate primer sequences for five microsatellite loci.

| Primer | Primer sequences | Reference |
|-------------|-----------------------------------------|----------------------------------|
| BM4513-F | 5'-GCG CAA GTT TCC TCA TGC-3' | Bishop <i>et al.</i> , 1994 |
| BM4513-R | 5'-TCA GCA ATT CAG TAC ATC ACC C-3' | |
| BM1225-F | 5'-TTT CTC AAC AGA GGT GTC CAC-3' | Bishop <i>et al.</i> , 1994 |
| BM1225-R | 5'-ACC CCT ATC ACC ATG CTC TG-3' | |
| MAP2C-F | 5'-TTT ACC AGA CAG TTT AGT TTT GAG C-3' | Moore <i>et al.</i> , 1994 |
| MAP2C-R | 5'-AAG GAT TCT GTC TGA TAC CAC TTA G-3' | |
| IGF-1-F | 5'-GCT TGG ATG GAC CAT GTT G-3' | Kirkpatrick <i>et al.</i> , 1992 |
| IGF-1-R | 5'-CAC TTG AGG GGC AAA TGA TT-3' | |
| OLADRB-int | 5'-CGT ACC CAG AKT GAG TGA AGT ATC-3' | Paterson <i>et al.</i> , 1998 |
| ODADRB-schw | 5'-TGK GCA GCG GCG AGG TGA G-3' | |

fridge at 4°C. Tsetse were classified according to the side of the net where they were caught and as fed or unfed according to the presence or absence of fresh red blood visible through the abdominal wall. On days when a calf + ox were in the ring of nets, observers viewed the cattle from a tower (Torr, 1994) and counted the number of tsetse seen on each animal.

Field processing of tsetse

In Zimbabwe, the abdomens of both fed and unfed flies were squashed singly onto filter papers within 4 h of collection. To prevent cross-contamination, all materials were handled with disposable gloves and instruments. The papers were air dried, sealed in aluminium envelopes and then stored at -10°C.

DNA extraction and microsatellite analysis

DNA from samples of tsetse bloodmeals and cattle blood was extracted following a modified Qiagen (Qiagen Inc, Mississauga, Canada) extraction protocol using lysis buffer (Guglich *et al.*, 1994). The extracted DNA was then amplified with four bovine-specific microsatellite primer sets (Research Genetics, Huntsville, Alabama, U.S.A.) and one ovine microsatellite set (Paterson *et al.*, 1998) (Table 1) as follows.

Samples were amplified using 4.6 pmol $\lambda^{33}\text{P}$ T4 polynucleotide kinase end labelled primer ATP in a total reaction volume of 10 μl per tube using 25 ng of genomic DNA, 200 μM dNTPs, 1x amplification buffer, 2.0 mM MgCl_2 , unlabelled primer (0.2 mM), labelled primer, and 0.5 units of *Taq* polymerase (Life Technologies, Burlington, Canada). Products were amplified under the following conditions: 94°C for 5 min, 55–65°C for 30 s, 72°C for 15 s 1 cycle; 94°C for 15 s, 55°C for 30 s, 72°C for 15 s 30 cycles; 94°C for 15 s, 55°C for 30 s, 72°C for 2 min 1 cycle. Products were then mixed with 0.4 volume of formamide loading buffer and were heated at 95°C for 5 min before loading onto a 6% sequencing gel containing 50% (w/v) urea. A control sequencing reaction of phage M13 DNA was run adjacent to the samples to produce size markers for the microsatellite alleles.

Cytochrome b gene sequencing and sequence analysis

For a few bloodmeal samples where the blood was found to be not from cattle, the host species was identified using the following primers to amplify a 305-bp region of the mitochondrial DNA cytochrome b gene (Kocher *et al.*, 1989).

Primer 1 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'
Primer 2 5'-CCC TCA GAA TGA TAT TTG TCC TCA-3'

The cytochrome b gene was amplified in a total reaction volume of 20 μl per tube using 25 ng of genomic DNA, 200 μM dNTPs, 1x amplification buffer, 2.0 mM MgCl_2 , primers 1 and 2 (0.2 mM) and 1.0 units of *Taq* polymerase. Products were amplified under the following conditions: 94°C for 5 min, 55°C for 30 s, 72°C for 30 s 1 cycle; 94°C for 30 s, 55°C for 30 s, 72°C for 30 s 35 cycles; 94°C for 30 s, 55°C for 30 s, 72°C for 2 min 1 cycle. Products were re-amplified and cleaned through QIAquick (Qiagen, Canada) for DNA sequencing using dye-terminator cycle sequencing using a ABI Prism 373 DNA Sequencer (MOBIX, McMaster University, Hamilton, Ontario, Canada)

Genetic analysis of microsatellite and mitochondrial DNA

Individual genotypes were scored from the samples. Multiple bloodmeals were detected based on the presence of more than two alleles per locus. The confidence in assigning a specific genotype to an individual was assessed using a probability of identity (POI) (Paetkau & Strobeck, 1994; Waser & Strobeck, 1998). This measure is used in forensic identification when calculating the probability of a match or the probability that two random and unrelated animals will share the same DNA profile by chance. The POI values were calculated for the herd of cattle ($n = 29$).

DNA sequences obtained for the mtDNA cytochrome b region were aligned using the programs Sequp and ClustalW. Individual sequences were compared to GENBANK/EMBL submitted sequences using the BLAST program (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov/) to identify sequences with the highest similarity.

Table 2. Genetic variation, allele frequencies and expected heterozygosity (H_E) of five microsatellite loci in Mashona cattle ($n=29$).

| Allele | Frequency | Allele | Frequency |
|----------------------|-----------|----------------------|-----------|
| BM4513 | | Map2C | |
| 138 | 0.0172 | 97 | 0.0172 |
| 140 | 0.0690 | 99 | 0.2414 |
| 142 | 0.0172 | 101 | 0.5690 |
| 146 | 0.3621 | 107 | 0.0690 |
| 148 | 0.2931 | 111 | 0.0862 |
| 150 | 0.0172 | 113 | 0.0172 |
| 152 | 0.1552 | H_E | 0.616 |
| 158 | 0.0172 | | |
| 162 | 0.0172 | | |
| 170 | 0.0345 | | |
| H_E | 0.765 | | |
| OLADRB | | BM1225 | |
| 150 | 0.0172 | 227 | 0.0172 |
| 152 | 0.0172 | 229 | 0.0172 |
| 158 | 0.0517 | 237 | 0.0172 |
| 160 | 0.0690 | 241 | 0.7586 |
| 164 | 0.1207 | 243 | 0.0862 |
| 168 | 0.0690 | 247 | 0.0517 |
| 170 | 0.1034 | 251 | 0.0517 |
| 172 | 0.0862 | 166 | 0.0172 |
| 174 | 0.0517 | H_E | 0.418 |
| 180 | 0.1724 | | |
| 192 | 0.0517 | | |
| 194 | 0.1724 | | |
| H_E | 0.905 | | |
| IGF-1 | | | |
| 227 | 0.5345 | | |
| 229 | 0.0172 | | |
| 231 | 0.4483 | | |
| H_E | 0.522 | | |

Results

Analyses of cattle blood

The results of the microsatellite profiles for 29 cattle show that there were 3–13 alleles for each locus with expected heterozygosities ranging between 0.418 and 0.905. The OLADRB locus demonstrated the most genetic variability within the herd and IGF-1 was the least variable; the latter primer was consequently the least useful for distinguishing cattle (Table 2). Each of the cattle at Rekomitjie had unique microsatellite profiles despite the herd being isolated and consequently inbred with many closely related individuals as illustrated by the profiles of a cow and two of her calves (Fig. 1). The Probability of Identity (POI) values ranged between 6.35×10^{-8} and 9.55×10^{-4} ; the latter probability indicates that in a sample of 1000 cattle, two unrelated animals might be expected to share this DNA profile by chance.

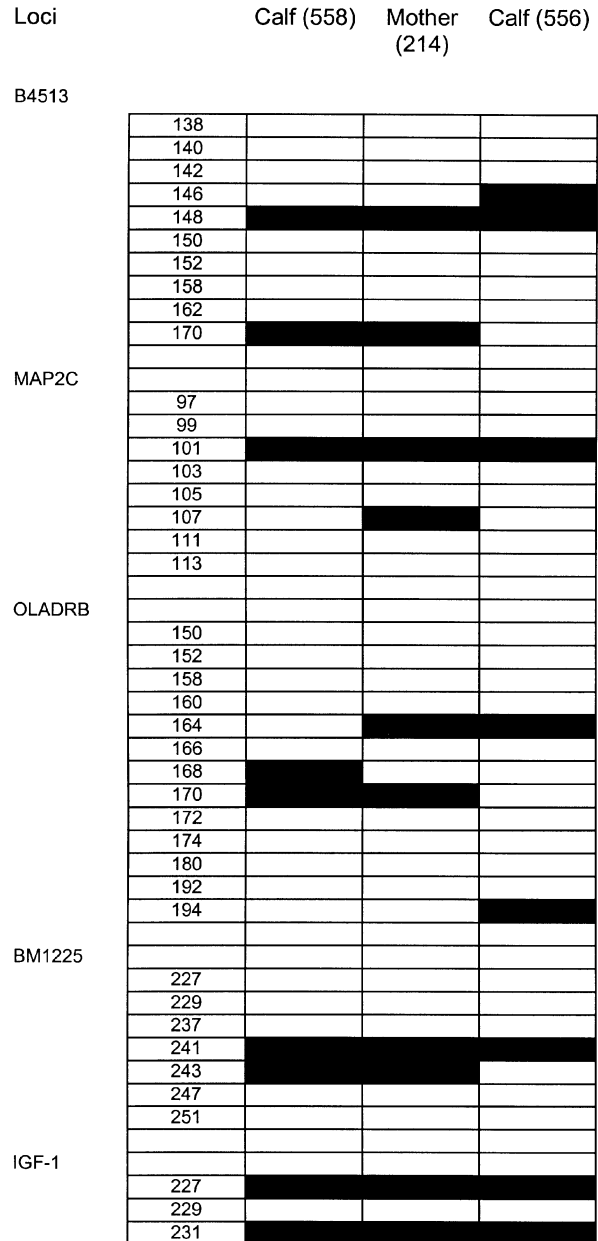


Fig. 1. Microsatellite profiles of a cow (no. 214) and her calves born in 1995 (556) and 1997 (558). The father(s) of the calves is unknown. While each calf shares at least one allele at each locus with its mother, and some loci are identical for different animals (IGF-1), the overall profile from the five loci is unique for each animal.

Analysis of tsetse with known feeding histories

Unfed flies. The gut contents of six unfed flies were analysed. No cattle DNA was detected in five of the samples but one sample amplified a weak product at locus BM4513. This could represent the remnants of a bloodmeal from cattle or a wild ungulate.

Table 3. Percentages of bloodmeals from tsetse containing DNA from either the test animal(s), other unidentified ungulate or with no detectable bovine DNA. Flies were classed as being fed or unfed and were caught from the inside or the outside of the ring of nets.

| Feed status | Catch position | <i>n</i> | Test animal (%) | Other ungulate (%) | No amplification (%) |
|-------------|----------------|----------|-----------------|--------------------|----------------------|
| Fed | Inside | 219 | 86.3 | 5.0 | 8.7 |
| | Outside | 18 | 72.2 | 11.1 | 16.7 |
| Unfed | Inside | 70 | 4.3 | 28.6 | 67.1 |
| | Outside | 106 | 1.9 | 12.3 | 85.8 |

Fed flies. Male *G.pallidipes* were caught after feeding on one of three cattle and samples of these flies were killed within 30 min of feeding or at 24 h, 48 h or 72 h thereafter. Cattle DNA was detected in the gut contents of all flies killed 30 min ($n=15$) or 24 h ($n=5$) after feeding, and the microsatellite profiles from the bloodmeals matched that of the known host. For three of 15 samples taken at 30 min after feeding, amplified products from other, unknown animals were also apparent. These products could be from other cattle or wild ungulates. Complete microsatellite profiles were achieved for five ($n=6$) flies killed 48 h after feeding and two ($n=3$) of those killed 72 h after feeding. Although the samples sizes were small, the data suggest that the technique is less reliable >48 h after feeding.

Multiple meals. Analysis of the gut contents of six tsetse known to have fed from two different cattle gave allelic patterns that were consistent with the microsatellite profiles of blood from the two known hosts.

These preliminary investigations suggested that for tsetse feeding on cattle at Rekomitjie, the individual source(s) could be uniquely identified using a suite of five microsatellite loci. Accordingly, studies were undertaken to analyse the feeding responses of wild tsetse feeding naturally on cattle.

Analysis of wild tsetse

Studies were made of the feeding responses of tsetse to either single or paired animals. The pairs comprised either two oxen or a calf and an ox. In 1998, a total of 964 tsetse were caught, of which 97% were *G.pallidipes* compared to 72% ($n=516$) in 1999.

All studies concerned with the responses of tsetse to either single animals or a calf + ox were carried out in 1998. Pooling the catch data for all tsetse, the mean feeding rate on a single calf was 16% ($n=109$) compared to 35% (229) on an ox and 39% (170) for an ox + calf. For four days when an ox + calf were in the ring, 96 tsetse were observed on the ox compared to 19 on the calves. The differences in the mean feeding rates for calves and oxen, and the numbers seen landing on oxen and calves, are consistent with more extensive data (Torr & Mangwiro 2000) showing that tsetse feed more successfully on oxen than calves. In studies of the responses of tsetse to

pairs of oxen, the mean feeding rates varied between 27% and 54%.

Microsatellite profiles

Results from the analyses of 413 tsetse attracted to cattle (Table 3) were in general accord with expectations. First, 85% ($n=237$) of the fed flies were identified as containing blood from the test animal(s) within the ring of nets on that day, compared to 2.8% (176) of the unfed flies; Fig. 2 shows examples of profiles obtained from fed wild flies. Of the fed flies, 5.5% contained blood from another host and in 9.8% of samples no host DNA was detected. For the unfed flies, 18.8% contained blood from nontest host(s) and 78.4% of samples had no detectable host DNA.

A few flies ($5/413=1.2%$) contained bovine DNA from nontest animals, which were identified, using the profile data for the entire herd, as being from known cattle which were not in the ring of nets on that particular day. A few flies ($8/413=1.9%$) with a clear host identity, were also found to contain DNA from a second unknown ungulate host. The DNA profiles classed as belonging to another ungulate comprised some with alleles found within the cattle as well as some alleles not found in the cattle. Three specimens containing the latter were amplified at the cytochrome b region and identified as being from African buffalo (*Syncerus caffer*).

Tsetse found to have unidentified bovine DNA presumably represent flies that contained remnants of DNA from bloodmeals derived from other wild hosts or possibly small traces of blood from a cattle meal that were not sufficient to obtain a clear identity.

Paired cattle

For fed tsetse caught on days where there was an ox + calf in the ring of nets, 100% of identified meals were from the ox (Table 4). When two oxen were in the ring, the percentages varied between one pairing where all meals were from one animal to three pairings where roughly equal numbers of meals were from each animal. The frequency of mixed meals was very low; overall for days where two hosts were in the ring of nets, only 1.5% (3/202) contained blood from both hosts.

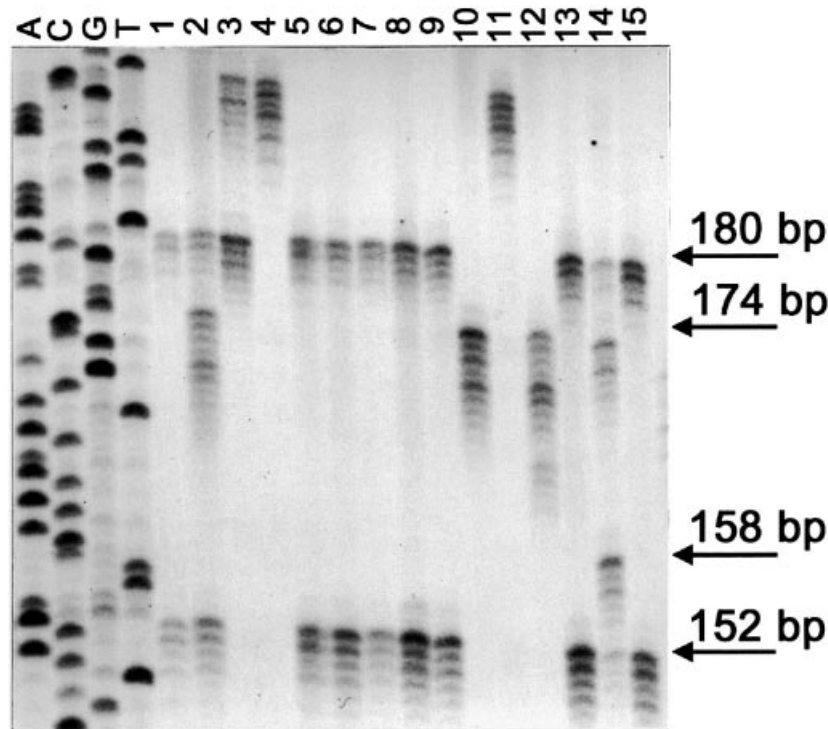


Fig. 2. Examples of DNA profiles from bloodmeals of *G. pallidipes* caught after feeding naturally on cattle in the field. This example shows the results from PCR amplification with the OLADRB primer pair. Arrows show the position of 152 bp, 158 bp, 174 bp and 180 bp alleles. Lanes 5–9 show the same profiles and are meals from a known ox and these profiles differ from lanes 3 and 4. Lanes 2, 12 and 14 have more than two bands, providing evidence of meals from at least two different hosts. Lanes ACGT are the DNA sequencing ladder.

Table 4. Mean feeding rates and bloodmeal identities for tsetse attracted to pairs of cattle. Bloodmeal identities indicate the percentages of bloodmeals identified as being a single feed from either animals no. 1 or no. 2, or a mixed feed comprising blood from no. 1 and no. 2. Sample sizes for feeding rates and bloodmeal identities are n_f and n_b , respectively, and P indicates the probability that the numbers of meals from the pair of animals are significantly different at the $P < 0.05$ (*) or $P < 0.001$ (***) level of probability. Only data for those bloodmeals that contained blood identified as being from at least one of the animals within the ring of nets are shown.

| Treatment | Animal | | Feeding rate | | Bloodmeal identities (%) | | | n_b | P |
|---------------|--------|-------|--------------|-------|--------------------------|-------|---------------|-------|-----|
| | no. 1 | no. 2 | % | n_f | no. 1 | no. 2 | no. 1 + no. 2 | | |
| Ox vs. calf † | Ox | Calf | 39 | 170 | 100 | 0 | 0 | 38 | *** |
| Ox vs. Ox | 167 | 225 | 54 | 13 | 100 | 0 | 0 | 6 | * |
| | 167 | 537 | 52 | 81 | 55 | 40 | 5 | 38 | NS |
| | 556 | 219 | 27 | 73 | 58 | 42 | 0 | 12 | NS |
| | 167 | 556 | 36 | 83 | 68 | 38 | 4 | 28 | NS |
| | 537 | 219 | 52 | 96 | 79 | 21 | 0 | 42 | *** |

†Results for ox vs. calf pairings are the pooled results from four separate ox + calf pairs.

Discussion

Practical implications

Present results show that the individual source(s) of a tsetse bloodmeal from cattle can be identified using a suite of five microsatellite loci. For flies that took a full meal, the technique provided clear and unequivocal identification of the source for

at least 24 h post feeding and could also be used to identify the hosts in mixed meals that were derived from two hosts. Closely related animals such as a cow and her calf could be readily distinguished. This is particularly important for studies of veterinary pests where there is frequently much in-breeding within a herd of livestock.

Tsetse are thought to digest ~60% of a bloodmeal within 24 h of feeding and ~95% within 48 h (Loder *et al.*, 1998). The

fact that we could identify all meals at 24 h postfeeding and >80% at 48 h, provides some evidence that we could have detected DNA from small incomplete meals. Although the sample sizes were small, the present results do suggest that the technique is less reliable for identifying meals >48 h after feeding. This accords with extensive data showing that tsetse completely digest their meals within ~72 h of feeding; during this period host DNA will be degraded so reducing the efficacy of the technique.

When the technique was used to study the responses of flies feeding naturally, the success rate of the technique was slightly less than that observed with the initial laboratory studies (85% vs. 100%). The difference is partly because a proportion (5.5%) of those flies classed as fed had not taken a meal from the host(s) within the ring but from another unidentified animal. The technique described here should be of general use in studies of other vector-borne diseases of cattle. Indeed, the same technique has been successfully used in studies of the feeding behaviour of *Stomoxys* spp. (A. Prior, unpublished data).

While the technique is not perfect, the success rate is superior to that obtained in comparable studies of mosquitoes. Gokool *et al.* (1993) found for instance, that only ~35% of microsatellite profiles from fed *Anopheles gambiae* caught in the wild were readable, while Koella *et al.* (1998) achieved a success rate of ~65%. Assuming that there are no inherent differences in the ability of the appropriate primers to analyse human or bovid blood samples, then the difference must be related to the flies used in the different studies. In particular, the size of bloodmeals in tsetse (30–90 mg; Taylor, 1976; Torr & Hargrove, 1998) are greater than those of mosquitoes (~3 mg; Knaus *et al.*, 1993; Takken *et al.*, 1998). Moreover, in the present study, tsetse were caught and killed within seconds of feeding, whereas there was a delay of some hours in the study of mosquitoes. Thus the mosquitoes contained less host DNA in the first place and a longer period of delay allowed the DNA to be degraded.

Biological implications

Although the present study was primarily intended to establish a technique for identifying the sources of bloodmeals, the first application of the method has provided a number of new insights.

First, with regard to the use of Vale's (1977) method of using an incomplete ring of electric nets to study the feeding behaviour of tsetse, the present study shows that flies classed as being fed or unfed on the presence or absence of blood visible through the abdominal wall is providing a fair indication of feeding success. Most of the flies with visible blood did indeed have blood from the host, and flies classed as unfed did not have any bovine DNA products. Thus tsetse seem not to take small meals which are missed by field workers.

Second, the method showed that in situations where tsetse were presented with the choice of feeding on an adult or a calf, all the meals were from the adult. Previous studies, using rings of nets, indicate that tsetse are more successful in feeding on older animals and it is thought that this difference is related to host size and higher rates of defensive behaviour by young animals (Torr, 1994; Torr & Mangwiro 2000). The present study confirmed these findings by demonstrating that feeding success on the calves and oxen placed individually in the ring were different (16% vs. 35%). Moreover, when a calf and ox were placed together, more tsetse were seen to land on the adult, all the meals were from the adult, and the feeding rate for tsetse attracted to an ox + calf (39%) was closer to that of an ox alone (35%) rather than that of a calf (16%). This suggests either that tsetse were biased towards landing on the adult and/or that those flies which landed on the calf were interrupted before they began to feed and subsequently moved and fed on the neighbouring adult. The paucity of mixed meals suggests that tsetse that moved from the calf to the adult did so before they began to feed on the calf. This has interesting epidemiological implication since it suggests that suckling calves, which will be close to their mothers, will be bitten even less than suggested by the results of Torr & Mangwiro (2000). This phenomenon may partly explain why very young calves can survive long periods in tsetse-affected areas without contracting trypanosomiasis (Torr & Mangwiro (2000).

Third, when two oxen were placed in the ring we had variable results. In some cases, roughly equal numbers of tsetse fed from each ox, while in others significantly more meals were taken from one of the oxen. Torr & Mangwiro (2000) provide data on the feeding responses of tsetse to four of the oxen (numbers 225, 219, 167 and 537) used in this study. Their data show that ox 225 is an unusually poor host with only 39% of tsetse successfully feeding on it, compared to 63% for 167. When these two animals were paired in the present study, all the bloodmeals were from 167. Similarly the only other treatment, for which there was a significant difference in feeding success, was the pairing of ox 219 with 537 and the mean feeding rates for these two were, respectively, 51% and 61% (Torr & Mangwiro 2000). Conversely when oxen 167 and 537 with very similar feeding rates (63% vs. 61%) were paired, there were no significant differences in the numbers of meals from each ox. These data provide circumstantial evidence that relatively slight differences in feeding success between hosts translate into larger differences in biting rates and possibly in disease risks.

Lastly, the present data show that mixed meals were relatively rare when tsetse were attracted to pairs of oxen. This paucity of mixed meals suggests that tsetse, which were interrupted as they fed on one host, did not attempt to complete the meal on a neighbouring one, but returned to the original host or left the vicinity entirely. However, the present indications regarding host choice and the frequency of mixed meals were gained from studying the simplest 'herd' of two animals. Currently, studies are being made of the feeding responses of tsetse to larger and more natural herds of hosts.

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