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Attractiveness of black rhinoceros (*Diceros bicornis*) to tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) and other biting flies

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Abstract

During translocations of black rhinoceros (*Diceros bicornis* Linnaeus) in Kenya, we studied the relationships between the rhinoceros and biting flies. In trapping experiments, rhinoceros waste products (urine or dung) were substituted for known attractants such as cow urine, 1-octen-3-ol or acetone. Catches of *Glossina pallidipes* Austen, *Glossina longipennis* Corti, *Stomoxys* spp., and *Haematopota* spp. were not affected by these substitutions. NG2G and Vavoua traps sited near captive animals caught similar numbers and kinds of flies as traps set without animals. Any minor attractive properties of rhinoceros odours were probably due to the presence of known attractants such as 4-cresol and 3-n-propylphenol, which were confirmed to be present through gas chromatography–mass spectroscopy. In feeding trials with laboratory-reared tsetse, *Glossina brevipalpis* Newstead and *Glossina morsitans centralis* Machado fed well on immobilized animals, whereas *G. longipennis* fed reluctantly. Catches of *G. brevipalpis* were doubled in one trapping experiment when rhinoceros urine was used as odour bait. *Philoliche* spp., *Haematopota* spp. and other Tabanidae fed on captive rhinoceroses. Many species of Stomoxyinae were associated with rhinoceroses. Of these, the most frequent association was with *Rhinomusca dutoiti* Zumpt, a species found previously only in South Africa. *Rhinomusca dutoiti* was found in two highland rhinoceros sanctuaries, Nairobi National Park and Solio Ranch Game Reserve.

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Introduction

Although only 2550 black rhinoceroses (*Diceros bicornis*) now remain in Africa (Brooks, 1994), the species was once abundant in areas with large populations of tsetse (*Glossina* spp.), such as the Luangwa valley in Zambia (Leader-Williams, 1988), the Zambezi valley in Zimbabwe (Roth, 1967) and Tsavo Park in Kenya (Goddard, 1969). Recognition of the historical importance of the rhinoceros as a host of tsetse was reflected in the shooting of 476 animals for tsetse control in Zimbabwe prior to 1958 (Roth, 1967). More recently, the rhinoceros accounted for 4.8% of blood meals of *Glossina pallidipes* Austen and 10.5% of meals of *G. swynnertoni* Austen in the Maasai Mara, Kenya (Wilson *et al.*, 1972). These data were collected when the Mara contained only about one animal per 15 km^2 (Mukinya, 1973). Similarly in Zimbabwe, *G. pallidipes* took 10.6% of its meals from rhinoceroses when many animals still lived in the Zambezi valley (Phelps & Vale, 1978).

Historical surveys also documented an especially close relationship between the rhinoceros and a member of the *fusca* group of tsetse, *G. longipennis* Corti. When the black rhinoceros was abundant in East Africa in the 1950s, *G. longipennis* took 60% of its meals from this host (Moloo, 1993). *G. longipennis* is one of the few tsetse species that lives in hot, dry *Acacia–Commiphora* bushland, a favoured habitat of the rhinoceros in Kenya (Goddard, 1969). Despite this close association, dwindling numbers of free-ranging rhinoceroses in Kenya have not had any noticeable impact on *G. longipennis* numbers. High populations of this tsetse species have been encountered recently in Kenya at Nguruman (Kyorku *et al.*, 1990) and at Galana Ranch (Baylis & Nambiro, 1993).

The feeding preferences of tsetse reflect a complex endpoint in a series of linked behavioural responses to hosts. In the well-studied savannah species, olfactory cues appear to be particularly critical in host location (Colvin & Gibson, 1992). Compounds such as carbon dioxide, acetone and 1-octen-3-ol from breath (Vale & Hall, 1985), and phenols such as 4-cresol and 3-n-propylphenol (Vale *et al.*, 1988), or crude urine itself (Dransfield *et al.*, 1990), attract many species. Some of these compounds are now used extensively as odour baits for sampling and control of the *morsitans* group of tsetse. In Zimbabwe for example, insecticide-impregnated targets for the control of *G. pallidipes* and *G. morsitans* Westwood are baited with acetone, the two phenols and 1-octen-3-ol (Vale, 1993). Unfortunately, similar odour-based techniques have not been developed for the other major groups of tsetse. In the *palpalis* group, researchers have yet to find an attractant suitable for large-scale field use. In the *fusca* group, only small increases in the catch of *G. longipennis* have been achieved by baiting traps with acetone, urine or phenols (Baylis & Nambiro, 1993). Similarly, catches of *G. brevipalpis* Newstead have only been slightly enhanced through the use of acetone (Kyorku *et al.*, 1995). The successful exploitation of bovine odours for the control of some species of savannah tsetse (Vale, 1993) has yet to be repeated for other groups or other hosts.

Relatively few researchers have studied the attraction of tsetse to hosts other than cattle, with studies to date only on *G. pallidipes*, *G. morsitans* and a selection of wild bovids and suids (Vale, 1977; Owaga, 1984; Vale *et al.*, 1986; Torr, 1994). The black rhinoceros is a potentially interesting host as it is a monogastric browser in the Perissodactyla (Clemens & Maloiy, 1982), in contrast to the bovine, which is a grazing ruminant in the Artiodactyla. It is a sedentary species, living a mostly solitary life in a well-defined home range. It sprays objects with urine and defecates at regular sites, probably for territorial marking (Goddard, 1967). These distinctive features of behaviour and metabolism suggest rhinoceros waste products might provide novel cues for host location. We therefore took advantage of access to rhinoceroses during translocations in Kenya (Mihok *et al.*, 1992a) to examine the attractiveness of animals and their waste products to tsetse and other biting flies.

Materials and methods

Study areas

Trapping experiments were conducted in three areas in Kenya: Nguruman, a hot, dry area west of Lake Magadi with extensive tsetse populations in dense thickets and woodlands following the Oloibortoto River (Dransfield *et al.*, 1990); Ngulia, a similarly hot and dry area with scattered tsetse populations in Tsavo West National Park (Mihok *et al.*, 1992a); and Shimba Hills National Reserve, a warm, humid area of coastal forest south of Mombasa (Kyorku *et al.*, 1995).

Additional trapping experiments and observations of flies around rhinoceroses were made in five areas during translocations when animals were being immobilized at first capture or after 2–8 weeks of confinement (Brett, 1990). The areas included the two largest highland populations of rhinoceros in Kenya (Nairobi National Park, Solio Ranch Game Reserve) and three smaller lowland populations (Ngulia in Tsavo West National Park, Lugard Falls in Tsavo East National Park, Lewa Downs Ranch near Isiolo).

Traps

Three kinds of tsetse trap, made from blue/black cotton cloth and white polyester mosquito netting, were used: Vavoua, NG2G and Siamese. The Vavoua trap (Laveissière & Grébaut, 1990) was designed for *palpalis* group tsetse; it is also an excellent trap for stable flies, *Stomoxys* spp. (Mihok *et al.*, 1995). The NG2G trap (Brightwell *et al.*, 1991) was designed for *G. pallidipes* and *G. longipennis*; it is also a good trap for tabanids (Amsler *et al.*, 1994). The Siamese trap was designed for mixed populations of *G. pallidipes* and *G. brevipalpis* (Kyorku *et al.*, 1995). All traps were made to original designs, except for the Vavoua, which was made with screens 10 cm longer to suit the dimensions of local cloth.

Odour baits

Urine was collected from two rhinoceroses held in bomas at Ngulia and from Zebu cattle owned by Maasai pastoralists at Nguruman. All hosts fed or were fed on natural forage. Dung for odour baits and for chemical analyses was collected from the two black rhinoceroses at Ngulia, from free-ranging elephants at Tsavo, and hippopotami and white rhinoceroses at the Maasai Mara. Urine from cattle was pooled from different animals and held at room temperature for three weeks in glass bottles. It was then frozen for storage and thawed for use in experiments as required. Rhinoceros urine was handled similarly, except it

Table 1. Outline of experiments testing waste products of the black rhinoceros (*Diceros bicornis*) as odour baits for *Glossina* spp. and other biting flies in Kenya.

Expt	Season	Habitat	Odours tested	ANOVA design	Trap	Standard odours	Purpose/contrast
Ngulia: Tests of fresh rhinoceros dung, targeting *G. longipennis* and *G. pallidipes*							
1/2	dry	thicket	A+RD A+CU	cross 16 rep each expt	NG2G	A+CU	rhino dung vs cow urine with acetone present in two separate experiments
3/4	dry	riverine thicket	A A+RD A+CU	3×3 LSQ 3 rep each expt	NG2G/ Vavoua	A	rhino dung vs cow urine with acetone present, vs acetone, two experiments with different traps
Nguruman: Tests of rhinoceros urine targeting *G. pallidipes*, *G. longipennis* and biting flies							
5	wet	thicket	A CU RU 1, 2, 3	5×5 LSQ 2 rep	Vavoua	A	rhino urine kept 1–3 weeks vs acetone and cow urine using trap efficient for *Stomoxys* during rainy season
6	dry	woodland	CU RU 2 wk RU 3 wk	3×3 LSQ 1 rep	NG2G	CU	rhino urine vs cow urine in a preliminary survey in an area with a high density of *G. longipennis*
7	dry	woodland	RU RU+A RU+O RU+A+O CU+A+O	5×5 LSQ 3 rep	Vavoua	CU+A+O	rhino urine (kept 4 weeks) combined with odour baits for tsetse such as acetone and octenol vs standard combination for tsetse
Shimba Hills: Tests of rhinoceros urine and elephant/rhinoceros dung targeting *G. pallidipes*, *G. brevipalpis*, *G. austeni* and biting flies during rainy season							
8	wet	forest	A CU 3 wk RU 1, 2, 3 wk	5×5 LSQ 3 rep	Vavoua	A	replicate of Expt 5 in rainy season using trap efficient for *Stomoxys*
9	wet	forest	A A+CU A+RU A+RD A+ED	5×5 LSQ 2 rep	Siamese	A	rhino urine (kept 5 weeks) and rhino and elephant dung with acetone present, using trap efficient for *G. brevipalpis*

Notes: A=acetone; CU=cow urine (kept 3 weeks); ED=elephant dung; O=octenol; RD=rhinoceros dung; RU=rhinoceros urine; Expt=experiment; cross=crossover design switching odours or traps at each site between days; LSQ=Latin square design rotating odours among sites and days; Rep=number of replicates.

was not pooled. As only a small quantity of rhinoceros urine was obtained, some batches were re-used in Experiments 7 and 9 (table 1). Dung was available in large quantities and was therefore never re-used. It was collected at sunrise from overnight defecation and used immediately in experiments at Ngulia, or frozen about 6 hours after collection for use at other sites. Dung (2 kg batches mixed with 500 ml water) was dispensed in plastic bags with an open top (20 cm diameter). Each bag was filled with new dung daily.

Acetone and urine samples were dispensed through 2 cm and 7 cm diameter apertures, respectively, in bottles placed at the base of traps; octenol (1-octen-3-ol) was dispensed from polythene sachets (Vale, 1993). Release rates were highest at Nguruman and Ngulia in the dry season when maximum daily temperatures often exceeded 35°C. Maximum rates were about 2500 mg/h (acetone), 1000 mg/h (urine) and 1.0 mg/h (octenol). During wet seasons and at Shimba Hills, release rates were about one third of the above. Dry ice in a leaky container was used once at an evaporation rate of about 2 litres CO_2 per minute.

Experimental trapping

Waste products from rhinoceros were compared with known attractants for tsetse such as acetone, cow urine and octenol, targeting tsetse and biting flies with traps appropriate for different species in areas where these species were present (table 1). Experiments were mostly Latin square designs where the number of sites and days were equal to the number of treatments (Perry *et al.*, 1980). In two experiments only, crossover designs were used, where two odour treatments were exchanged at each of many sites between days.

Statistical analyses were done with log(n+1)-transformed data. For Latin square designs we tested for the effects of odour treatments, days, squares and sites within squares. Interaction effects were never significant and were therefore excluded from the ANOVA model. Differences among means were tested with the Student-Newman-Keuls test (SNK at $P < 0.05$). For comparison, results are tabulated as detransformed means and as indices of increase (ratio of detransformed treatment mean to detransformed mean of a selected odour treatment).

Rhinoceros observations

On four occasions when 1–4 rhinoceroses were held in bomas at Ngulia, NG2G and Vavoua traps were set next to the animals for 1–2 months. Traps were set just outside the boma walls. Traps were emptied daily and were set both with and without odour baits (acetone, cow urine, octenol). Altogether 174 trap-days of data were produced. A similar trapping effort was also conducted 2 km away from the bomas. With the exception of one 28-day rainy-season experiment, trap data were collected mainly during dry periods when fly numbers were low. Flies were also trapped on a single occasion three weeks after eight animals were held in adjacent bomas near Lugard Falls. One NG2G trap and two Vavoua traps were set next to the bomas for one day; the traps were baited with acetone, cow urine and octenol (NG2G), or CO_2 and octenol (Vavoua). The boma area was saturated with naturally dispensed rhinoceros odours, with about 1000 kg of dung piled nearby.

Additional trapping was done in two areas of Kenya with high densities of black rhinoceros: Nairobi Park (0.5/km^2) and Solio Ranch Game Reserve (1.0/km^2, Brett, 1990). Extensive surveys in Nairobi Park have already been reported (Mihok *et al.*, 1995). At Solio Ranch, we conducted one survey of the riverine *Acacia xanthophloea* woodland in March 1994 (dry season) using Vavoua traps baited with octenol (24 trap-days).

When rhinoceroses were immobilized at first capture, or when they were held in bomas, we observed and attempted to identify the kinds of flies on them. This was done during 44 immobilizations, mostly at Tsavo National Park and at Solio Ranch Game Reserve. Hand-net catches of flies were also made occasionally. Finally, on 18 occasions for 12 animals, teneral laboratory-reared tsetse (*G. morsitans centralis* Machado, *G. longipennis* and *G. brevipalpis*) were fed directly on immobilized animals to monitor feeding success and to diagnose the presence of trypanosomes (Mihok *et al.*, 1992a, 1992b). Groups of 25–50 flies of each species in separate cages were used. These trials were done mainly at Ngulia, between 11.00 and 14.00 h, at temperatures of 25–30°C. Cages of flies were strapped together so that all tsetse species fed under identical conditions. About half of the time, we were able to feed flies by resting cages on the nape of the neck. This minimized effects of close-proximity odours from the presence of a human hand holding the cages (Hargrove, 1976). Unfortunately, general odour effects could not be prevented, as the rhinoceros was usually surrounded by people during immobilizations.

Rhinoceros dung was screened for the presence of dipterous larvae or pupae on at least one occasion at each study area, and on many occasions at Nairobi Park. Eight batches of dung of about 10 kg each were also collected from Ngulia and Nairobi Park in different seasons. Each batch was sealed in a plastic bag with an exit cage to monitor emergence. A few batches of dung were also mixed with water and screened for floating pupae.

Chemical analyses

The phenolic composition of a pooled sample of urine kept for 3 weeks from Zebu cattle was compared with four samples of rhinoceros urine from two animals originating from Nairobi Park and held in bomas at Ngulia: one sample kept 1 day from an adult male, one sample kept 2 days from a subadult female, one sample kept 1, 2, or 3 weeks from the adult male, and one sample kept 3 weeks from the same male but collected at a different time. Samples were collected a few weeks after the animals had been confined in the bomas; during this time they were fed on natural forage. A pooled batch of rhinoceros dung from the male and the female was also analysed as well as pooled samples of dung from elephant, hippopotamus and white rhinoceros. Phenols present in these waste products were identified following the methods of Hassanali *et al.* (1986).

Volatiles from dung samples were isolated by steam distillation for 2 h. Distillates and urine samples (120 ml) were extracted with HPLC grade dichloromethane (30 ml×3, Aldrich Chemical Ltd, UK). The combined extract was dried over anhydrous sodium sulphate, filtered and evaporated to dryness (50°C). The extracts were then dissolved in dichloromethane (2 ml) and stored at −20°C. Portions of these samples were diluted (×3) and analysed

Table 2. Captures of *Glossina pallidipes* using rhinoceros waste products relative to known attractants for this tsetse species.

Experiment/Details	Odours	Mean	Maximum	Index
1. Ngulia (NG2G traps, $n=32$, $CV=47$, fresh dung)				
$F=1.30$, $df=1,20$	A+RD	4.4[a]	25	0.73
$P=0.27$	**A+CU**	**6.0[a]**	**22**	**1.00**
2. Ngulia (NG2G traps, $n=32$, $CV=20$, fresh dung)				
$F=0.32$, $df=1,20$	A+RD	37.7[a]	152	1.15
$P=0.58$	**A+CU**	**32.7[a]**	**134**	**1.00**
5. Nguruman (Vavoua traps, $n=50$, $CV=34$)				
$F=4.61$, $df=4,32$	**A**	**37.0[a]**	**59**	**1.00**
$P=0.005$	RU 3 wk	17.6[ab]	45	0.48
	RU 2 wk	14.7[ab]	52	0.40
	RU 1 wk	10.6[b]	47	0.27
	CU	7.3[b]	39	0.20
6. Nguruman (NG2G traps, $n=9$, CV=11)				
$F=0.01$, $df=2,2$	RU 2 wk	37.5[a]	50	1.06
$P=0.99$	RU 3 wk	37.4[a]	52	1.05
	CU	**35.5[a]**	**57**	**1.00**
7. Nguruman (Vavoua traps, $n=75$, $CV=59$, RU at 4 wk)				
$F=4.82$, $df=4,52$	**CU+A+O**	**5.6[a]**	**108**	**1.00**
$P=0.002$	RU+O	2.7[b]	22	0.48
	RU+A+O	2.7[b]	17	0.48
	RU+A	2.7[b]	42	0.48
	RU	2.2[b]	11	0.39
8. Shimba Hills (Vavoua traps, $n=75$, $CV=41$)				
$F=0.65$, $df=4,52$	**A**	**21.5[a]**	**579**	**1.00**
$P=0.63$	CU	20.1[a]	251	0.94
	RU 1 wk	15.9[a]	409	0.74
	RU 2 wk	14.3[a]	309	0.67
	RU 3 wk	11.9[a]	325	0.56
9. Shimba Hills (Siamese traps, $n=50$, $CV=15$, RU at 5 wk)				
$F=3.73$, $df=4,32$	A+CU	151.0[a]	382	2.88
$P=0.01$	A+RD	75.2[a]	296	1.43
	A+RU	67.6[b]	266	1.29
	A+ED	65.6[b]	199	1.25
	A	**52.5[b]**	**153**	**1.00**

*Mean, detransformed mean in flies/trap/day; Index, ratio of detransformed mean to detransformed mean of standard; means within experiments followed by the same letter were not significantly different (SNK test, $P < 0.05$); *n*, sample size for whole experiment, *CV*, coefficient of variation for transformed data, other abbreviations as in table 1. The standard odour treatment is set in boldface type.

on a Hewlett Packard gas chromatograph 5890A, fitted with a 50 m carbowax column (0.32 mm ID and 0.3 μm film thickness) equipped with a FID. Nitrogen was used as the carrier gas and analysis was carried out with the following temperature programme: 100°C to 180°C at 3°/min, 25 min hold at final temperature. The peaks were integrated on a 3396 Hewlett Packard integrator. To confirm the identity of selected peaks, samples were analysed on a VG 12–250 quadrupole mass spectrometer (EL, 70 eV) coupled to a Hewlett Packard 5790A gas chromatograph. Identity was confirmed by co-injection with authentic samples.

Results

Experimental trapping

Eight experiments yielded sufficient data for analysis of *G. pallidipes* catches (table 2). At Ngulia in Experiments 1 and 2, traps baited with acetone and fresh rhinoceros dung caught as many tsetse as traps baited with acetone and cow urine. At Nguruman in Experiments 5 and 6 and at Shimba Hills in Experiment 8, traps baited with rhinoceros urine kept for 1–3 weeks caught as many tsetse as traps baited with cow urine. Traps baited with acetone caught more tsetse than traps baited with cow or rhinoceros urine in Experiments 5 and 7. At Nguruman in Experiment 7, a combination of baits used for control and sampling of *G. pallidipes* (acetone, octenol, cow urine) caught more tsetse than any combination of rhinoceros urine with octenol and/or acetone. At Shimba Hills in Experiment 9, acetone and cow urine caught significantly more tsetse than any combination of acetone with rhinoceros dung or urine, or elephant dung.

Table 3. Captures of *Glossina longipennis* and *G. brevipalpis* using black rhinoceros waste products relative to known attractants for these tsetse species.

Experiment/Details	Odours	Mean	Max	Index
3. Ngulia (Vavoua traps, $n=27$, $CV=53$, fresh dung)				
G. longipennis	A+CU	3.8[a]	8	1.85
$F=4.01$, $df=2,14$	A+RD	2.2[a]	7	1.09
$P=0.04$	**A**	**2.0[a]**	**4**	**1.00**
4. Ngulia (NG2G traps, $n=27$, $CV=33$, fresh dung)				
G. longipennis	A+CU	4.3[a]	15	1.86
$F=3.11$, $df=2,14$	A+RD	2.6[a]	8	1.11
$P=0.08$	**A**	**2.3[a]**	**8**	**1.00**
6. Nguruman (NG2G traps, $n=9$, $CV=33$)				
G. longipennis	**CU**	**27.7[a]**	**33**	**1.00**
$F=0.62$, $df=2,2$	RU 2 wk	24.9[a]	43	0.90
$P=0.62$	RU 3 wk	12.0[a]	47	0.43
7. Nguruman (Vavoua traps, $n=75$, $CV=71$, RU at 4 wk)				
G. longipennis	**CU+A+O**	**3.3[a]**	**14**	**1.00**
$F=4.20$, $df=4,52$	RU+A+O	2.6[ab]	14	0.78
$P=0.005$	RU+O	2.2[ab]	8	0.67
	RU	1.9[b]	4	0.57
	RU+A	1.5[b]	5	0.45
9. Shimba Hills (Siamese traps, $n=50$, $CV=53$, RU at 5 wk)				
G. brevipalpis	A+RU	4.7[a]	30	1.95
$F=3.22$, $df=4,32$	A+CU	4.0[a]	26	1.65
$P=0.03$	**A**	**2.4[a]**	**8**	**1.00**
	A+RD	2.4[a]	13	0.99
	A+ED	2.3[a]	14	0.96

Abbreviations as in tables 1 and 2.

Table 4. Results of experiments on the attractiveness of black rhinoceros waste products to biting flies other than tsetse.

Experiment/Details	Odours	Mean	Max	Index
5. Nguruman (Vavoua traps, $n=50$, $CV=30$)				
Stomoxys spp.	**A**	**43.5[a]**	**2075**	**1.00**
$F=0.10$, $df=4,32$	RU 3 wk	41.4[a]	2508	0.95
$P=0.98$	RU 1 wk	41.1[a]	728	0.94
	CU	36.4[a]	832	0.84
	RU 2 wk	35.6[a]	1778	0.82
7. Nguruman (Vavoua traps, $n=75$, $CV=59$, RU at 4 wk)				
Stomoxys spp.	**CU+A+O**	**3.5[a]**	**375**	**1.00**
$F=0.92$, $df=4,52$	RU+A+O	3.0[a]	252	0.85
$P=0.46$	RU	2.7[a]	266	0.76
	RU+A	2.6[a]	579	0.75
	RU+O	2.4[a]	252	0.67
8. Shimba Hills (Vavoua traps, $n=75$, $CV=78$)				
Stomoxys spp.	RU 1 wk	9.3[a]	3000	1.97
$F=0.48$, $df=4,52$	RU 2 wk	8.2[a]	2009	1.72
$P=0.75$	RU 3 wk	7.5[a]	2553	1.58
	CU	6.1[a]	2061	1.29
	A	**4.7[a]**	**2000**	**1.00**
9. Shimba Hills (Siamese traps, $n=75$, $CV=40$, RU at 5 wk)				
Stomoxys spp.	A+CU	49.4[a]	451	1.95
$F=0.38$, $df=4,52$	A+RD	29.4[a]	83	1.03
$P=0.83$	**A**	**28.6[a]**	**86**	**1.00**
	A+ED	28.4[a]	347	0.99
	A+RU	25.3[a]	401	0.89
8. Shimba Hills (Vavoua traps, $n=75$, $CV=34$)				
Tabanidae	CU	16.3[a]	502	1.43
$F=0.66$, $df=4,52$	RU 2 wk	13.1[a]	1202	1.14
$P=0.62$	RU 3 wk	12.5[a]	191	1.09
	A	**11.5[a]**	**403**	**1.00**
	Ru 1 wk	10.0[a]	101	0.87

Abbreviations as in tables 1 and 2.

Three replicated trapping experiments at low tsetse density and one unreplicated experiment at high density provided data for analysis of catches of *G. longipennis* (table 3). In each experiment, there was no evidence for any extraordinary attractive properties of rhinoceros waste products. In fact, the highest catch was always obtained with known attractants such as acetone plus cow urine (Experiments 3 and 4), cow urine alone (Experiment 6), or acetone, cow urine, octenol (Experiment 7). However, catch differences among treatments were mostly not significant at $P < 0.05$.

Glossina brevipalpis was caught in sufficient numbers for analysis only in Experiment 9 when Siamese traps were used at Shimba Hills (table 3). Although the effect of odour treatments was marginally significant ($P=0.03$), the SNK test placed all odour treatments in a statistically homogeneous group. Traps baited with rhinoceros urine and acetone did, however, catch the highest number of *G. brevipalpis*.

Biting flies were caught in reasonable numbers only in experiments at Nguruman and at Shimba Hills in the rainy season (table 4). Sufficient data for analysis were obtained in four experiments for *Stomoxys* spp. and in one experiment for Tabanidae (mostly *Haematopota* spp.). Despite some very high maximum catches (e.g. 3000 *Stomoxys* spp., 1202 Tabanidae) as well as good mean catches in three of five analyses, no statistical heterogeneity was detected in any of

the odour combinations tested. The proportion of variance explained by the ANOVA models in these analyses for biting flies was similar to that found for tsetse, generally falling between 60–80%.

Rhinoceros observations

The only rainy season confinement of a rhinoceros at Ngulia (Mihok *et al.*, 1992b) took place before on-site staff were trained in the identification of biting flies. Tabanids (especially *Philoliche* spp.) were seen feeding on the animal during immobilizations and were also captured in traps next to the boma. More inconspicuous flies may also have been present, but were not monitored properly. All further translocations were done during dry periods, when flies of any kind were scarce. Only non-biting muscids were found in hand-net catches and by observations of insects on animals. Extended periods of trapping on various occasions yielded many non-biting muscids, but only very small numbers of *G. pallidipes*, *G. longipennis* and *Stomoxys calcitrans* (Linnaeus). Catches did not differ between traps set next to the rhinoceroses and traps set in similar habitat a few kilometres away. *Rhinomusca brucei* Malloch (Parsons & Sheldrick, 1964), a biting fly associated with skin lesions caused by *Stephanofilaria dinniki* Round (Round, 1964) was never encountered. Lesions were present in animals translocated from Nairobi Park, but healed a few weeks after animals arrived at Ngulia.

Traps set near Lugard Falls during the dry season next to eight animals in similar habitat to Ngulia caught very large numbers of non-biting muscids, and small numbers of *S. calcitrans*, *Haematobosca latifrons* (Malloch), *Haematobia* spp., *G. longipennis*, *Haematopota* spp. and *Tabanus gratus* Loew. *Haematopota* spp. and *Haematobia* spp. were observed feeding on rhinoceros. Like Ngulia, skin lesions in animals originating from Solio Ranch healed quickly after confinement near Lugard Falls.

Attempts to catch flies on free-ranging rhinoceroses were unsuccessful initially, as flies left by the time the animals were recumbent. We therefore attempted hand-net catches during helicopter-darting of animals at Solio Ranch, where it was possible to arrive at the animals's side shortly after darting. The ranch is the only place in Kenya where rhinoceroses are accompanied by large clouds of flies similar to those seen in Tsavo National Park in the 1960s (Parsons & Sheldrick, 1964). Of 242 flies netted from two animals in mid-morning, 77% were non-biting muscids. The remainder were 31 *Rhinomusca dutoiti* Zumpt, nine *Haematobia* spp., four *Stomoxys boueti* Roubaud and one *Haematobosca latifrons*. These flies were netted from animals with active skin lesions which were oozing blood. In the reserve, *Stomoxys niger niger* Macquart, *Stomoxys niger bilineatus* Grünberg, *Stomoxys varipes* Bezzi, *S. calcitrans*, *Haematobosca squalida* (Grünberg) and *Prostomoxys saegerae* (Zumpt) were captured in traps (in decreasing order of abundance). *Rhinomusca dutoiti* was netted from flies following vehicles, a phenomenon not observed elsewhere in Kenya.

Tsetse feeding experiments

For males, a similar but significantly larger percentage of *G. brevipalpis* than *G. m. centralis* fed on immobilized rhinoceroses (71%, N=209 vs 60%, N=768; $\chi^2=8.1$, $P < 0.05$). Female *G. brevipalpis* and female *G. m. centralis* fed equally well ($P=0.4$), but fewer females fed than males (*G. brevipalpis*: 58%, N=241, $\chi^2=7.3$, $P < 0.01$; *G. m. centralis*: 54%, N=678, $\chi^2=4.0$, $P < 0.05$). Feeding success was extremely poor in both male (29%, N=215) and female (29%, N=233) *G. longipennis* ($P \ll 0.001$ relative to the others).

Chemical analysis of waste products

Dichloromethane extracts of rhinoceros urine kept for one day contained only trace amounts of phenols. The potent tsetse odour attractant, 4-cresol, was present in small amounts in the sample kept for two days (8.7%). A variety of phenols were present in all urine samples kept for one week or more. The critical tsetse odour attractants, 4-cresol and 3-n-propylphenol, were present in ratios varying between 28:1 and 54:1 (4-cresol at 47.8% to 51.8% and 3-n-propylphenol at 0.96% to 1.77%) in the four rhinoceros urine samples kept for 1, 2 or 3 weeks. The overall phenolic composition of these samples was similar to that of cow urine kept for 3 weeks at room temperature (57.8% 4-cresol and 1.60% 3-n-propylphenol, ratio of 36:1). Two representative gas chromatograms are shown in fig. 1 with peaks for phenol, 3-cresol, 4-cresol, 3-n-propylphenol and 4-n-propylphenol identified. The presence of all of these compounds in rhinoceros urine was confirmed by GC-MS.

Chromatograms from distillates of wildlife dungs had an extremely large number of peaks, and hence, we did not pursue identification of the compounds present. Peaks potentially corresponding to important attractants such as 4-cresol and 3-n-propylphenol were present only as minor components.

Discussion

Overall, we did not find any special attractive properties of rhinoceros urine or dung that would justify more refined experiments for the development of odour baits from rhinoceros waste products. Catches of tsetse and other biting flies were minimally affected by the use of rhinoceros odours in place of, or in addition to, attractants such as cow urine, acetone or octenol. Analyses of rhinoceros urine revealed the presence of known phenolic attractants such as phenol itself, 3- and 4-cresol, and 3- and 4-n-propylphenol (Hassanali *et al.*, 1986; Bursell *et al.*, 1988). Small positive or negative effects on fly catches were presumably related to varying ratios of these phenolic volatiles in fresh and aged samples of urine and dung. Trapping was carried out in a variety of habitats and seasons with traps of proven efficacy for tsetse and other biting flies. It therefore seems unlikely that further investigations on rhinoceros waste products would be of value.

The relatively low catches of *G. longipennis* in traps baited with rhinoceros odours was unexpected, given historical data on the feeding preference of this tsetse for rhinoceroses (Moloo, 1993). *Glossina longipennis* was present in reasonable numbers at Ngulia and at Nguruman, and yet no large trap catches were obtained. In trials with immobilized animals, *G. longipennis* was also reluctant to feed on rhinoceroses. Although these feeding trials were done at a suboptimal time of the day (Kyorku & Brady, 1994), other tsetse such as *G. brevipalpis* and *G. m. centralis* fed well under similar constraints. These results suggest the relationship between *G. longipennis* and the rhinoceros may be a simple ecological

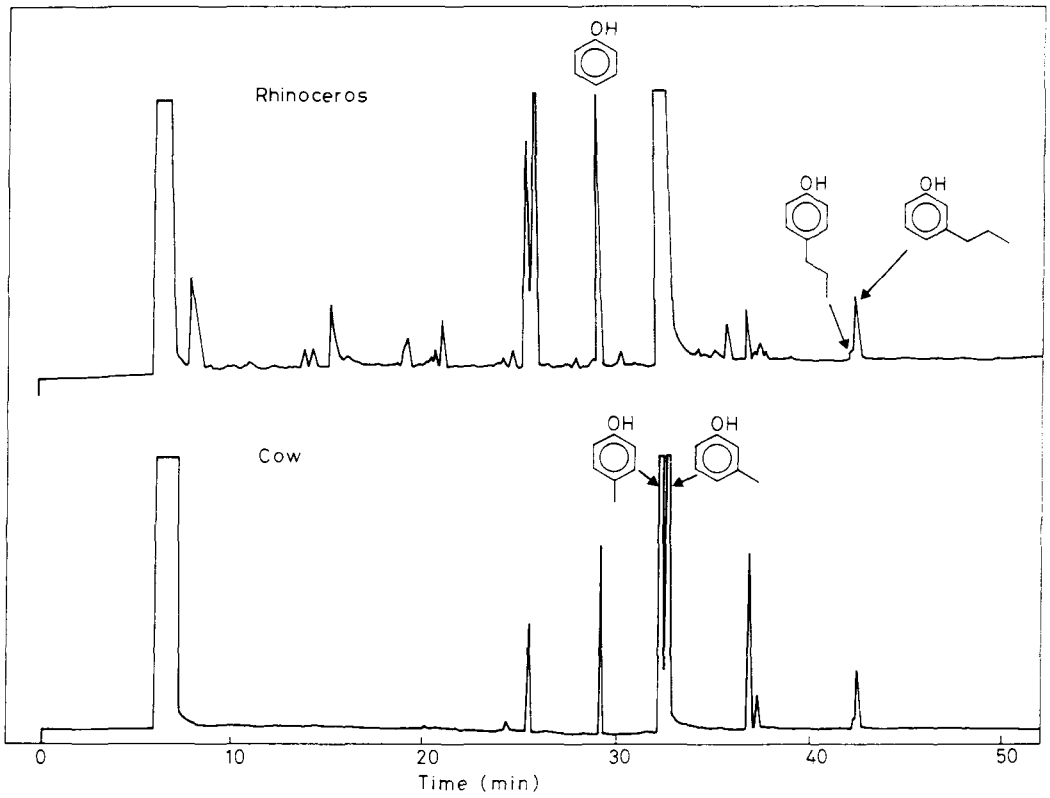

Fig. 1. Gas chromatograms for dichloromethane extracts of urine, kept for 3 weeks at room temperature in a stoppered bottle, from Zebu cattle and from an adult male black rhinoceros showing the major phenols present.

association. Future insights into the role of odour cues will clearly require a more refined experimental approach. Unfortunately, constraints on working with an endangered species prevented us from attempting more elaborate experiments.

The presumed importance of ecological factors, as opposed to odour cues, is supported by research prompted by the feeding habits of *morsitans* group tsetse such as *G. swynnertoni, G. austeni* and *G. morsitans*. These tsetse have a strong preference for feeding on suids (Moloo, 1993). A few researchers have therefore investigated the potential of developing odour baits from warthog (Owaga, 1984) and bushpig waste products (Vale *et al.*, 1986). Experiments with living and stuffed warthogs baited with natural or synthetic odours, surrounded by electrocuting nets, have also been conducted (Torr, 1994). Torr's results have implied that warthog odours do not contain substantive new attractants for *G. morsitans* or *G. pallidipes*, leaving the question of how certain tsetse locate strongly-preferred hosts open to debate. Ecological factors such as an overlap of habitat and activity, defensive behaviours of the host, visual cues such as size, shape, colour, motion, etc. may be more important than odour cues in determining feeding habits for many tsetse. This may explain why the discovery of extremely efficient odour baits for the control of *G. pallidipes* and *G. morsitans* (Vale, 1993) has yet to be replicated for other species of tsetse (Küpper *et al.*, 1991).

A lone caveat on this observation is the possibility of developing a novel odour bait from rhinoceros for *G. brevipalpis*. In one experiment, traps baited with rhinoceros urine plus acetone caught twice as many flies as traps baited with acetone alone. *G. brevipalpis* also fed rapidly and in good numbers on immobilized animals during trials with laboratory-reared tsetse. In previous work, urine phenols or crude urine from cows, pigs and humans did not improve trap catches of *G. brevipalpis* (Kyorku *et al.*, 1995). *Glossina brevipalpis*, when present near rivers or lakes, tends to feed on hippopotamus, but it can easily adapt to other available hosts (Moloo, 1993). At Shimba Hills, it takes most of its meals from elephant (Mihok & Kang'ehte, unpublished data). Outside of Shimba Hills, *Glossina brevipalpis* is found in modest numbers in Kenya mainly in (former) rhinoceros habitat in the Kibwezi Forest. These observations suggest some potential in investigating the attractiveness to *G. brevipalpis* of both elephant and rhinoceros odours. Given the practical difficulties of field trials with such large animals, laboratory tests of volatiles would clearly be the most practical way to proceed (e.g. wind tunnel trials combined with GC–EAG: gas chromatography–electroantennographic detection).

In addition to our field experiments with waste products, regular access to translocated rhinoceros gave us the opportunity to make many unique observations on the ralationships between rhinoceros and biting flies. In 1993, roughly 417 black rhinoceros remained in Kenya (Brooks, 1994), with the two largest populations at Nairobi and Solio (Brett, 1990). Both areas harbour diverse communities of Stomoxyinae (Mihok *et al.*, 1995, this paper). In extensive surveys in other areas of Kenya, and Tanzania, we have yet to encounter a similarly high level of diversity of *Stomoxys*. Unfortunately,

priorities of translocation dictated access to rhinoceroses mostly during dry seasons and during mornings, when biting flies were neither numerous, nor active. We were also unable to do more than simply observe and catch flies in the vicinity of animals using traps or hand-nets. Hence, our short list of biting flies associated with rhinoceroses is very probably an underestimate.

The discovery of *Rhinomusca dutoiti* associated with the rhinoceros in a few highland areas in Kenya was unexpected. Previously, this species had been found only in South Africa. In the 1960s, Parsons & Sheldrick (1964) collected large numbers of the other member of the genus, *Rhinomusca brucei* Malloch, in Tsavo. We collected *R. dutoiti* only, and only in Nairobi National Park and Solio Ranch Game Reserve. We checked the identity of our specimens with a series of specimens of *R. dutoiti* collected by Zumpt in the 1960s held at The Natural History Museum, London. The museum also has a few specimens of *R. brucei* collected in the early 1960s from Tsavo. Our recent specimens of *R. dutoiti* from Nairobi and Solio clearly matched specimens of *R. dutoiti* from Natal, South Africa. We suspect that *R. brucei* may have become extinct in Kenya during the years of massive rhinoceros poaching, particularly in lowland areas such as Tsavo. In contrast, *R. dutoiti* appears to have survived in a few areas as a result of the timely creation of highland rhinoceros sanctuaries. *Rhinomusca dutoiti* may always have been present in the Kenyan highlands in association with rhinoceroses, but it has probably never been noticed by entomologists.

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