Trypanosome infections and survival in tsetse

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SUMMARY

The effect of trypanosome infection on vector survival was observed in a line of *Glossina morsitans morsitans* selected for susceptibility to trypanosome infection. The differential effects of midgut and salivary gland infections on survival were examined by exposing flies to infection with either *Trypanosoma congolense* which colonizes midgut and mouthparts or *Trypanosoma brucei rhodesiense* which colonizes midgut and salivary glands. A comparison of the survival distributions of uninfected flies with those exposed to infection showed that salivary gland infection significantly reduces tsetse survival; midgut infection had little or no effect on the survival of tsetse. The significance of these findings is discussed in relation to the vectorial capacity of wild flies.

Key words: *Trypanosoma brucei rhodesiense*, *Trypanosoma congolense*, tsetse, survival.

INTRODUCTION

The effects of trypanosome infections on fitness in tsetse are difficult to determine given the normally low rate of infection observed both experimentally and in the wild. Effects of infection on vector survival are of interest for the evolution of parasite/vector interactions since parasite transmission depends strongly on vector survival and the frequency of genetic factors controlling vector susceptibility depends on the fitness of infected vectors. Evidence for the effect of trypanosomes on survival in tsetse is equivocal with some studies showing no effect (Baker & Robertson, 1957; Moloo & Kutuza, 1985) but studies by Nitcheman (1988) showed effects of *T. congolense* on survival and studies by Golder *et al.* (1982, 1984) showed increased effects of insecticides on infected flies. In the present study the effects of trypanosome infection on tsetse longevity were examined using a line of *G. m. morsitans* selected for susceptibility to infection so that any effects of infection could be more readily quantified and data from earlier studies is re-interpreted.

MATERIALS AND METHODS

Flies

An iso-female line of *G. m. morsitans* (line 1.6 – for details see Welburn & Maudlin, 1991) selected for susceptibility to midgut infection was used.

Trypanosomes

Two trypanosome stocks were used: *T. b. rhodesiense* stock EATRO2340 (see Cornellissen *et al.* 1984 for details of stock history) and *T. congolense* stock 1/148FLY (for stock details see Young & Godfrey, 1983).

Frozen stabilates of infected mouse blood were used for the infective feeds as previously described (Welburn & Maudlin, 1987).

Fly infection and survival

Flies of both sexes in groups of 400 (50 flies per cage) were infected on the day following emergence from the puparium with either *T. b. rhodesiense* or *T. congolense*; a control group was left uninfected. Flies were kept at 25 °C, approximately 70% r.h. and fed daily on defibrinated pig blood through an artificial membrane. At daily intervals up to 13 weeks post infection the cages were examined and any dead flies were removed. Survival of flies exposed to infection and uninfected flies was compared using a Wilcoxon test (used in preference to a logrank test because the hazard ratio is not constant – Collett, 1994). Gompertz curves were fitted to the survival data by maximum likelihood (Garg, Rao & Redmond, 1970).

RESULTS AND DISCUSSION

Salivary gland infection, fly sex and survival

Fig. 1A–D shows survival and hazard curves for male and female *G. m. morsitans* exposed to infection with either *T. b. rhodesiense* or *T. congolense* compared with uninfected flies. The survival distributions are well described by the gompertz distribution; in this model, which has been widely used to fit insect survival data (e.g. Clements & Paterson, 1981), the hazard of dying increases exponentially with age being modelled as $\alpha.\exp(\beta a)$ where α and β are fitted parameters and a is the fly age. The fitted values and calculated expected mean survival time for the fitted model are shown in Table 1 and results

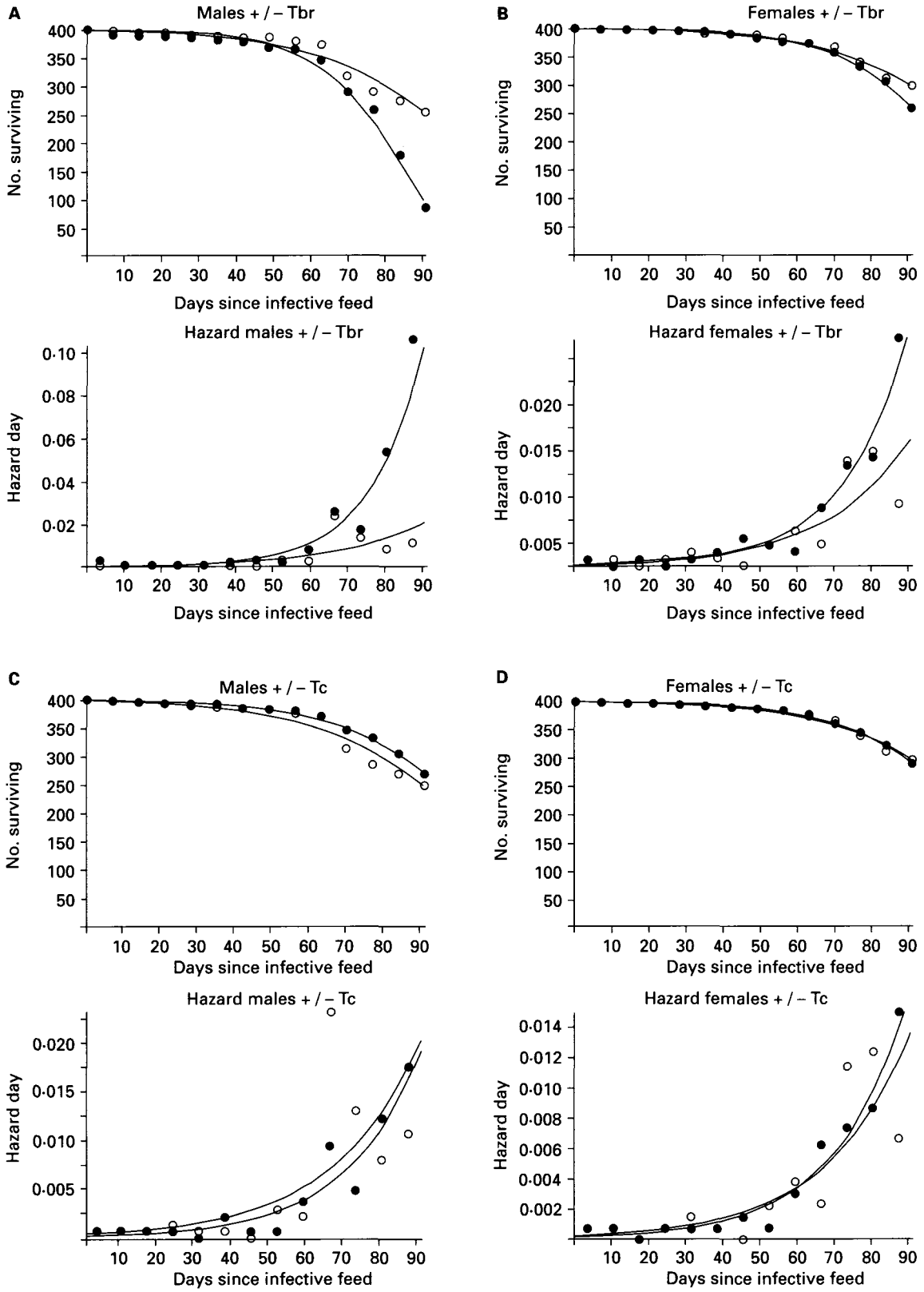

Fig. 1. Survival of male and female uninfected (open circles) *G. m. morsitans* compared with flies exposed to infection with *T. b. rhodesiense* (Tbr) (Fig. 1A and B) or *T. congolense* (Tc) (Fig. 1C and D) (solid circles). The fitted lines were obtained by fitting a gompertz distribution to the data by maximum likelihood.

of Wilcoxon tests comparing survival of infected and uninfected flies are given in Table 2. Survivorship of uninfected male and female cohorts differs in the estimate for the baseline hazard, α, but the estimates for β are similar, implying that the hazard increases at the same rate but begins at a higher level in males (the hazard of mortality for males is the same as that for females about 12 days older).

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Table 1. Parameters estimates for the fitted gompertz model for survival of uninfected *G. m. morsitans* and flies infected with *T. b. rhodesiense* (Tbr) or *T. congolense* (Tc)

(L is the expectation of life for the fitted model in days (standard errors in brackets). α is the baseline hazard in young flies; β is the rate at which the hazard increases with age.)

	Uninfected	Tc	Tbr
Males			
L	95·7 (2·02)	98·7 (1·97)	77·8 (0·86)
β	0·043 (0·0041)	0·051 (0·0049)	0·072 (0·0037)
α	0·0004 (0·00011)	0·0002 (0·00007)	0·0002 (0·00004)
Females			
L	105·1 (2·84)	102·2 (2·31)	95·2 (1·52)
β	0·045 (0·0051)	0·051 (0·0053)	0·059 (0·0050)
α	0·0002 (0·00009)	0·0002 (0·00006)	0·0001 (0·00005)

Table 2. Comparison of survival time of uninfected *G. m. morsitans* (Gmm) of each sex with flies infected with *T. b. rhodesiense* (Tbr) or *T. congolense* (Tc)

	Standardized Wilcoxon rank sum statistics	2-sided *P* value
Effect of Tbr infection		
Female Gmm	−2·53	0·012
Male Gmm	−9·46	< 0·0001
Effect of Tc infection		
Female Gmm	−0·31	0·76
Male Gmm	+1·96	0·05

The results show that the hazard of dying increases with age more rapidly in flies exposed to infection than in uninfected cohorts of flies and this effect is more marked in males than in females. The difference in survival of uninfected flies and flies exposed to infection with *T. b. rhodesiense* is statistically significant; the difference between uninfected flies and flies exposed to infection with *T. congolense* is slight and not statistically significant for females and of borderline significance for males.

There have been few studies examining the effects of trypanosome infection on fitness in tsetse flies as such work is limited by the flies inherent refractoriness to infection which presents a problem in looking for significant effects, especially in examining the effects of salivary gland infection which are normally rare (for review of experimental data see Maudlin, Welburn & Milligan, 1991). The present study used a line of *G. m. morsitans* selected for susceptibility to trypanosome infection in which very high levels of midgut and mature infection rates are routinely achieved. Using the same line of *G. m. morsitans* (1.6) and the same trypanosome stocks as in the present work we obtained the following infection rates (figures in parentheses are numbers of flies dissected): *T. congolense* FLY1/148 midgut % males 76 (900), females 71 (800); hypopharynx % males 44, females 49; *T. b. rhodesiense* EATRO2340 midgut %: males 72 (945), females 81 (773); salivary glands %: males 27, females 11 (data from Dale *et al.* 1995). In the present study *G. m. morsitans* (line 1.6) exposed to infection with *T. b. rhodesiense* had significantly different survival distributions from uninfected control flies whereas the survival distribution of *T. congolense* exposed flies did not differ from control flies. Since only about 70–80 % of flies exposed to infection would have acquired midgut infection, and of these only 20–40 % would have developed salivary gland infection, the effect of infection on survival is underestimated by our experiments, perhaps considerably. In parallel experiments, reported elsewhere (Dale *et al.* 1995), we estimated the proportion of male and female flies that developed mature infections and the distribution of maturation time for these parasite stocks (time elapsed between infective feed and detection of parasites in the salivary glands (*T. b. rhodesiense*) or proboscis (*T. congolense*)); 37 % of males and only 14 % females with midgut infections developed mature salivary gland infections with *T. b. rhodesiense* stock EATRO2340. This is consistent with the greater effect of parasite induced mortality of male flies observed in the present work. However, the mean maturation time for stock EATRO2340 was 18 days (Dale *et al.* 1985), whereas the additional mortality in the infected cohorts of flies observed here occurred after 50 days. Therefore the additional mortality would seem to be not directly related to the time of initial invasion of the salivary glands. However in Baker and Robertson's (1957) experiments on the effects of infections on mortality in tsetse, divergence between control and infected cohorts of flies began around day 20 after the infective feed.

Moloo & Kutuza (1985) found no effect of infection on reproductive performance and survival

of female *G. m. morsitans* infected with either *T. vivax*, *T. congolense* or *T. brucei* but significantly the flies in that study were maintained for only 63 days and, as we have seen from the present work, differences in survival only become apparent after day 50. Moreover, Moloo & Kutuza (1985) were unwittingly choosing flies (females only were used) with the lowest expectation of maturation and were unlikely to have detected any significant differences even in *T. brucei*-infected flies. In a later study in which flies were kept until death, Makumi & Moloo (1991) found that *T. vivax* infections reduced (but not significantly) survival of female but not male *G. palpalis gambiensis*. As *T. vivax* infections are restricted to the mouthparts in tsetse we may conclude that such infections have little or no effect on survival. Nitcheman (1988, 1990) noted reduced longevity in female *G. m. morsitans* infected with *T. congolense*; these differences were noted between 30 and 60 days post infection. The data of Nitcheman (1988 and personal communication P. J. M. Milligan) show significant difference in survival of infected and uninfected flies (mean survival time 94 days for infected flies, 284 days for uninfected flies, $p = 0{\cdot}006$).

Baker & Robertson (1957) reported no increase in mortality of infected flies but rather a slight decrease. However this interpretation of their data is based on survivorship curves in which males and females have been pooled. Re-analysis of these data (Table 3*a*) by sex shows that in three of the four experiments the average lifetime of infected tsetse is less than controls. In one experiment (*T. b. rhodesiense* in female *G. m. morsitans*) the average lifetime of infected flies is greater than the controls but in this experiment the survival of uninfected flies is reduced, rather than the survival of infected flies being enhanced. When the data from this experiment were pooled with the rest it appeared there was no effect of infection on survival. Mantel–Haentzel tests (Table 3*b*) show that the distribution of survival times of infected flies is the same in the two experiments with males and females but the uninfected controls differ in comparable experiments, very markedly in the case of females ($\chi^2 = 58{\cdot}2$, $P < 0{\cdot}001$). The data of Baker & Robertson (1957) also show mean lifetime of males to be less than females and with infected males less than uninfected males.

Survival under field conditions

It has been argued (Dye & Williams, 1995) that, since vector survival is so important for transmission, there will be strong selection for parasites that do not impair survival. However, there is a trade-off since there will also be selection for increased infectivity of parasites. Several studies have shown the effects of parasites on survival in the laboratory but this has not been convincingly demonstrated in natural populations. If parasite infection reduces survival we would expect to see prevalence of infection increase with age in young flies and level off in older flies rather than continue to steadily rise with age as reported from field studies (Harley, 1967; Rogers & Boreham, 1973; Ryan *et al.* 1982; Woolhouse *et al.* 1993, 1994). In our data parasite effects on survival become noticeable after about 50 days from the start of the experiment; it is possible that wild flies do not live long enough for parasite-induced mortality to play a role. Expectation of life of flies is much less in the field than in laboratory conditions (e.g. 77–105 days in this study, compared with 23 days estimated mean life span for male tsetse (Jackson, 1940)). However, since stresses of field conditions reduce life expectancy, effects of trypanosome infection on survival may be correspondingly greater in wild flies. This may partly explain the very low levels of salivary gland infection observed in wild flies ($< 1\%$) even when there is an abundance of infected hosts, as in the recent sleeping sickness epidemic in Busoga, Uganda (Okoth & Kapaata, 1986; Maudlin *et al.*, 1990). We cannot rule out that in the field *T. congolense* as well as *Trypanozoon* infections are deleterious given the metabolic load applied to the midgut by trypanosome infection (Bursell, 1981).

Furthermore it may be difficult to find evidence of parasite-induced vector mortality in natural populations as many factors may affect the shape of tsetse age-prevalence curves. If the rate of infection of flies increases with age (this could happen if for example flies, as they age, become more likely to feed on bovids rather than suids (see Harley, 1966; Ryan *et al.* 1986)), or if the ageing technique is affected by parasite infection (e.g. wing fray, used as a measure of age, is increased by trypanosome infection (Ryan, 1984)), then age prevalence curves may continue to rise with increasing age even when there is parasite-induced mortality. If susceptibility to infection is controlled by genetic factors, these should be less frequent in older than in younger flies. It would be easier to establish a reduction in frequency with age, than a levelling off, so this would provide a simpler test for parasite induced mortality in populations where the prevalence of infection in tsetse is high enough to produce a detectable effect.

Mechanisms of parasite-induced mortality

The question arises as to the mechanism behind the increased mortality observed here in infected flies. Possession of the putative gene/s which block maturation (Milligan, Maudlin & Welburn, 1995) does not in itself affect longevity as only salivary gland infected flies were compromised in the present study. Jenni *et al.* (1980) found that flies with *Trypanozoon* infections probed more frequently than uninfected flies which they suggested may be due to

Table 3. (*a*) Mantel–Haentzel χ^2 tests for data of Baker & Robertson (1957) on the survival of infected male and female *G. morsitans* (G.m.). χ^2 values (significance) based on comparison between infected and uninfected flies

	No. flies	Mean lifetime (days)	χ^2
Female G.m.			
T. b. brucei			
Infected	24	26·54	5·117 (**)
Uninfected	25	27·00	
T. b. rhodesiense			
Infected	42	26·07	46·755 (***)
Uninfected	21	20·62	
Male G.m.			
T. b. brucei			
Infected	18	20·61	18·594 (***)
Uninfected	21	23·57	
T. b. rhodesiense			
Infected	20	21·70	34·133 (***)
Uninfected	11	26·18	

Table 3. (*b*) Mantel–Haenzel tests χ^2 (significance) between comparable cohorts from data of Baker & Robertson (1957)

	T. b. rhodesiense	
	Infected	Uninfected
Males		
T. b. brucei		
Infected	3·458 (N.S.)	—
Uninfected	—	9·293 (0·002)
Females		
T. b. brucei		
Infected	1·316 (N.S.)	—
Uninfected	—	58·200 (< 0·001)

impairment of their labral mechano-receptors. Moloo (1983) however found no such effect on the feeding behaviour of flies whether infected with *Trypanozoon*, *T. congolense* or *T. vivax*. Simple impairment of feeding behaviour may not be responsible for the present effect.

The salivary glands of tsetse produce an anticoagulant (Lester & Lloyd, 1928) which trypanosome infection of the glands could impair making feeding more difficult as the flies get older. Patel, Otieno & Golder (1982) found that saliva from infected glands was depleted in protein compared with that from uninfected glands. Damage to infected salivary glands is obvious on dissection and has been noted by Golder *et al.* (1987). Flies with *Trypanozoon* (Golder, Patel & Darji, 1982, 1984) and *T. congolense* (Nitcheman, 1990) infections have been shown to be more susceptible to insecticides indicating a general lack of 'fitness' due to infection.

Vectorial capacity of tsetse

The vectorial capacity of a blood-sucking insect (the rate at which future inoculations arise from a currently infective case) is sensitive to changes in vector survival (Dye, 1990). Theoretically, female tsetse will contribute less to vectorial capacity as fewer of them are able to mature salivary gland infections (Milligan *et al.* 1995) but females live longer than males in the wild (Jackson, 1940; Phelps & Vale, 1978) which would tend to negate this effect. The prevalence of salivary gland infection in natural populations is roughly the same in the two sexes (Harley, 1967). Fairburn & Culwick (1950) suggested that male tsetse, because they were more efficient transmitters, offered a greater threat to human populations at risk of contracting sleeping sickness than female flies. The present work has shown that the greater risk offered to the human population by males, due to their increased susceptibility to salivary gland infection compared with females, is probably balanced by the fact that their lives are correspondingly shortened by the infection so reducing their vectorial capacity.

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