The kinetics of maturation of trypanosome infections in tsetse

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SUMMARY

Estimates of the time delay between the infective bloodmeal and maturation (incubation or maturation time) for 4 trypanosome stocks (2 *Trypanozoon* and 2 *Trypanosoma congolense*) show that maturation time in tsetse is not a parasite species-specific constant. The mean incubation time of a *Trypanosoma brucei rhodesiense* stock (EATRO 2340 – 18 days) was not significantly different from one *T. congolense* stock (SIKUDA88 – 15·5 days) but was significantly greater than another (1/148 FLY9 – 12·5 days). There was no significant difference in incubation times between male and female *Glossina morsitans morsitans* for any of the stocks but in both of the *Trypanozoon* stocks the proportion of female flies producing mature infections was significantly less than in males. However, estimates of gene frequency, assuming a model in which maturation is controlled by an X-linked recessive allele, gave inconsistent results indicating that maturation cannot be controlled by a single sex-linked gene. Maturation was shown to be a tsetse sex-dependent phenomenon in *Trypanozoon* but not in *T. congolense* infections. Incubation time was quite variable even for a single trypanosome stock (e.g. standard deviation of 5 days for one *Trypanozoon* stock); we discuss how this variability can affect disease transmission, and the interpretation of age–prevalence data.

Key words: *Trypanosoma brucei, Trypanosoma congolense*, tsetse, maturation, age–prevalence.

INTRODUCTION

It is generally assumed that maturation of a trypanosome infection in tsetse flies is a constant, which depends only on temperature and the infecting species of trypanosome. The way age–prevalence data for tsetse are interpreted and vectorial capacity estimated usually depends on this assumption. The study of maturation of *Trypanozoon* infections in tsetse has received little attention, primarily because of the problems associated with obtaining sufficient salivary gland infections for analysis. Very wide-ranging estimates have previously been given for the time taken for infections to mature in tsetse e.g. 17–45 days for *Trypanozoon* and 19–53 days for *T. congolense* (Hoare, 1970). The present study was facilitated by the availability of a highly susceptible line of *G. m. morsitans* which provided consistently high infection rates so permitting a detailed analysis of the timing and incidence of maturation in flies infected with different trypanosome stocks.

A problem arises in studying maturation in *Trypanozoon* stocks as male tsetse show higher salivary gland infection rates than females when infected in the laboratory. Significant differences between the sexes in maturation have not, however,

been observed in flies infected with *Nannomonas* species. In the absence of breeding experiments we associated this phenomenon seen in tsetse infected with *Trypanozoon* with the action of a Mendelian gene by fitting a model for a recessive sex-linked gene to infection rate data (Maudlin, Welburn & Milligan, 1991). The aim of the present work was to examine the dynamics of maturation of different *Trypanozoon* species and *T. congolense* stocks and to test further the sex-linkage hypothesis with a larger body of data.

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 throughout.

Trypanosomes

The following trypanosome stocks were used. *T. b. rhodesiense* stock EATRO 2340 (a cloned tsetse transmissible line derived from EATRO 2340 (GUT at 7.13) was used throughout (see Cornellisen *et al.* (1985) for details)). *T. b. brucei* stock BUTEBA 135 isolated (by I.M.) from cow in Tororo district, Uganda 1990. *T. congolense* stock SIKUDA 88

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isolated (by I.M.) from cow in Tororo district, Uganda 1990. *T. congolense* a cloned stock 1/148 FLY9 (see Young & Godfrey (1983) for details).

Fly infection rates

Teneral flies were infected on the day following emergence from the puparium. Infective feeds were given *in vitro* using frozen stabilates of trypanosomes suspended in defibrinated pig blood (Welburn & Maudlin, 1987). Following infection all flies were maintained at 25 °C and 70 % relative humidity and fed on defibrinated pig blood through an artificial membrane. Flies were dissected at intervals following infection when midguts and mouthparts (*T. congolense*) or salivary glands (*Trypanozoon*) were examined by phase-contrast microscopy (× 400).

Data analysis

Distribution of incubation times. We assume that some flies are unable to produce mature infections, no matter how long they survive after infection; further we assume that the conditional distribution of incubation times is a gamma distribution with scale and shape parameters ρ and k with probability density:

$$g(t) = \rho^k (t-\delta)^{k-1} e^{-\rho(t-\delta)} / \Gamma(k) \quad (t \geqslant \delta),$$
$$g(t) = 0 \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad (t < \delta),$$

where g is the probability density of the distribution of incubation times.

This distribution is conditional on the flies being able to produce mature infection and applies only to flies which can produce midgut infection and which received an infective feed when teneral. The parameter δ represents a translation to allow for the fact that there must be some delay between the infective feed and maturation (a maturation time of zero is not possible). The mean incubation time is $(\delta + k/\rho)$ and the variance is k/ρ^2.

Defining P as the proportion of flies able to produce mature infections, the expected fraction of flies with mature infections dissected on the ith day of the experiment, t days after the infective feed is:

$$d_i = P \int_0^{t_i} g(s)\, ds.$$

In fitting this model we allowed that the parameters may be sex dependent; estimates of parameter values are obtained by maximizing the log likelihood:

$$\log L = \text{constant} + \sum_i M_i \log d_i + (G_i - M_i) \log (1 - d_i),$$

where d_i is a function of the parameters ρ_m, ρ_f, k_m, k_f, P_m, P_f (we have introduced an obvious subscript notation for fly sex). G_i is the number of flies with midgut infections, out of those flies dissected on day i of the experiment; of these M_i had mature infections. The parameter δ was fixed in advance as 5 days for *T. congolense* and 10 days for *T. brucei* sspp., independent of fly sex. The value of δ was not allowed to vary during the fitting process. It was assumed that maturation could not take place in shorter times than δ days. The model was simplified by replacing ρ_m and ρ_f with a common parameter ρ and similarly k_m and k_f with k, if doing so did not cause a significantly poorer fit. Numerical estimates of the standard error for each parameter estimate were obtained; these can be used to compare parameter estimates between stocks.

Sex-linkage model. In experiments with different fly stocks, proportions of males and females developing salivary gland infections with *T. brucei* sspp. appeared to follow the pattern of a sex-linked recessive trait (Maudlin *et al.* 1991). If the frequency of such an allele is q, a proportion q of males and q^2 of females will be expected to possess the trait. This hypothesis can be tested with the present data by examining the change in the log likelihood when parameters P_m and P_f in the null model are replaced with q and q^2 respectively (except when $P_m = P_f = 1$, when the two hypotheses are indistinguishable).

This analysis does not allow for possible effect of mortality due to mature infection; to separate the potentially confounding effects of parasite-induced mortality and variability in incubation time, linked data on survivorship are required (manuscript in preparation).

RESULTS

Curves were fitted to the infection rate data (Fig. 1 A–D) and parameter estimates and their standard errors are given in Table 1. There was no significant difference in mean or S.D. of incubation times between males and females for any of the stocks. In the case of the *T. congolense* stocks there was no significant difference between P_m and P_f but in both of the *T. b. brucei* stocks the proportion of flies able to produce mature infections in females, P_f, was significantly less than the equivalent value for males.

The sex linkage model is a significant improvement on the null model in the case of EATRO 2340 and SIKUDA 88 but not for the other two stocks. The estimated values of q (the gene frequency assuming a model in which maturation is controlled by an X-linked gene) for EATRO 2340 and SIKUDA 88 were significantly different. The mean incubation times for the 2 congolense stocks were significantly different ($P < 0.05$) but there was no significant difference between the 2 *Trypanozoon* stocks. The mean incubation time for EATRO 2340 was, however, significantly greater than *T. congolense* 1/148 FLY ($P < 0.05$); BUTEBA 135 was not
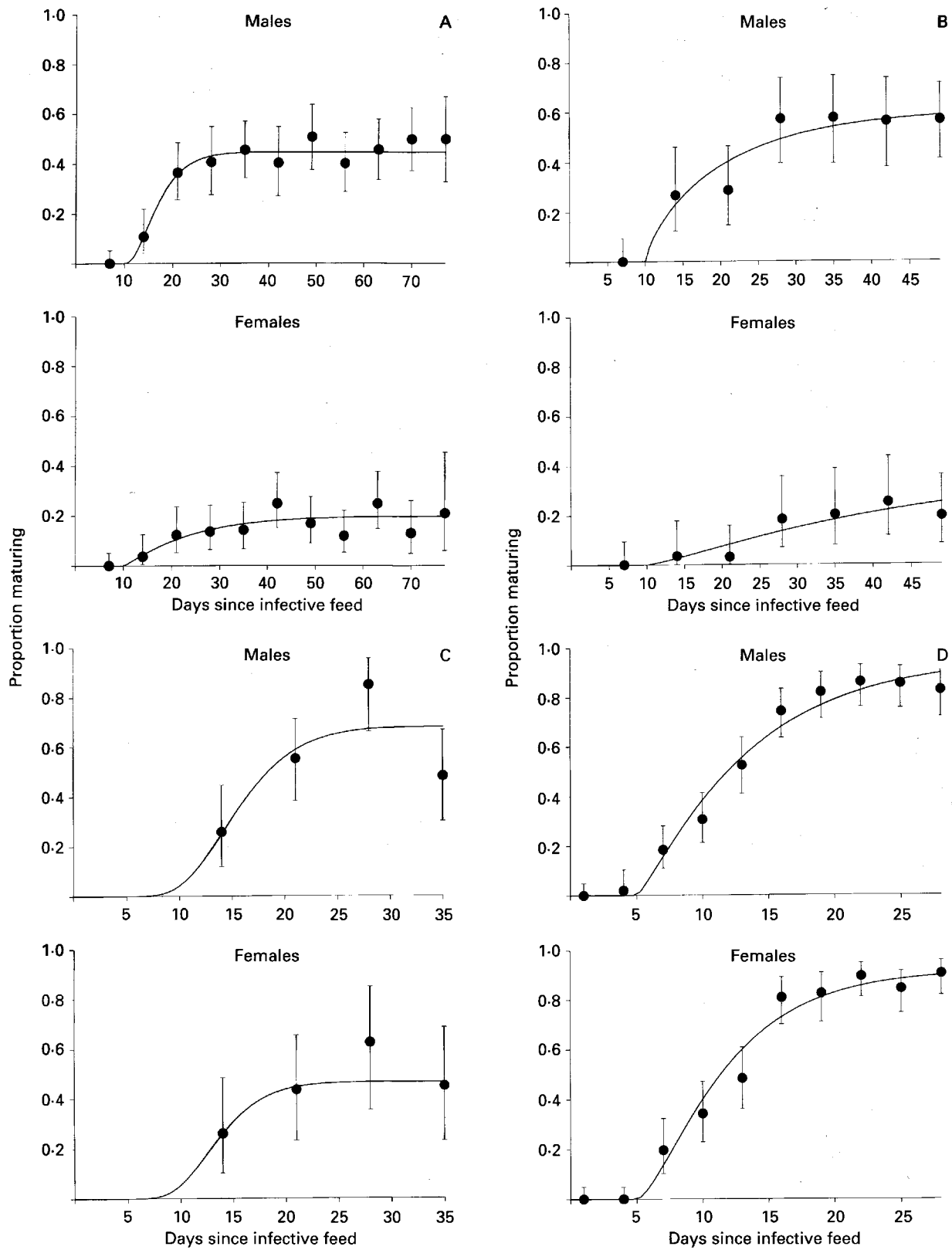
Fig. 1. (A–D) Proportion of midgut trypanosome infections maturing in male and female *Glossina morsitans morsitans* following infective feed with: (A) *Trypanosoma brucei rhodesiense* stock EATRO 2340; (B) *T. b. brucei* stock BUTEBA 135; (C) *T. congolense* stock SIKUDA 88; (D) *T. congolense* stock 1/148 FLY9. Numbers of flies dissected at each point (total number dissected): (A) 25–105 (1718), (B) 38–46 (422), (C) 40–48 (363), (D) 100 (1800). Error bars indicate 95 % confidence intervals.

Table 1. *Estimated mean and standard deviation* (S.D.) *of the distribution of maturation times (time from infective feed to mature infection) for 4 trypanosome stocks in* Glossina morsitans morsitans

(P_m *and* P_f *are estimates of the proportions of each sex able to produce mature infections. Standard errors* (S.E.) *are given for some parameter estimates to allow comparison between stocks. If the sex difference is not significant (χ^2 has P value < 0.05), the common estimate P_c is given. The fit of the sex-linkage model is indicated by the second χ^2, a P value > 0.05 indicates that the sex-linkage model is a significant improvement on the null hypothesis and the estimate of gene frequency q is then given.)*

Trypanosome stock	Incubation time (days) Mean (S.E.)	S.D.	P_m (S.E.)	P_f (S.E.)	χ^2 for P_m and P_f P value	P_c (S.E.)	χ^2 for sex-linkage model P value	q (S.E.)
EATRO 2340	18·00 (2·09)	5·22	0·461 (0·025)	0·174 (0·018)	100·3 $P < 0.001$	—	2·1 $P = 0.147$	0·436 (0·018)
BUTEBA 135	23·19 (8·18)	14·39	0·646 (0·127)	0·208 (0·054)	51·8 $P < 0.001$	—	8·46 $P = 0.004$	—
SIKUDA 88	15·54 (1·25)	4·60	0·638 (0·058)	0·522 (0·069)	2·04 $P = 0.153$	0·594 (0·050)	1·52 $P = 0.217$	0·690 (0·042)
1/148 FLY9	12·47 (0·65)	6·11	0·898 (0·032)	0·933 (0·031)	1·58 $P = 0.209$	0·901 (0·022)	6·42 $P = 0.01$	—

significantly different from other stocks in mean incubation time, however, the fit for this stock was poor and the standard error correspondingly large.

DISCUSSION

Differences in maturation time have historically been attributed to the varying complexities of the life-cycles of different trypanosome species within tsetse so that *Trypanozoon* infections take longer to mature than a *T. vivax* infection (Buxton, 1955). However, estimates of mean incubation times for the 4 trypanosome stocks examined here reveal that maturation time is not strictly a species-specific parameter. The mean incubation time calculated for the maturation of a *T. congolense* stock (SIKUDA88 – 15·5 days) is similar to that for a *T. b. rhodesiense* stock (EATRO 2340 – 18 days). It would appear that the timing of maturation is more closely correlated with maturation success and preliminary analysis of further *T. b. brucei* stocks in the laboratory (data not shown) supports this hypothesis. In general terms, the longer the time taken for maturation of midgut infections, the poorer the transmission index. This may well account for the difficulties experienced by most laboratories trying to produce mature infections of *T. b. gambiense* (Dukes *et al.* 1989). We would suggest that *T. b. gambiense*, with its poor transmissibility, may take much longer to mature than generally anticipated; indeed it may be possible by extrapolation to predict the likely maturation time for stocks of this species.

The hypothesis assumed for the present analysis, based on a previous study (Maudlin *et al.* 1991), was that maturation is controlled by a single sex-linked gene in *Glossina*. Of the 4 further stocks examined

here only 2 fitted the sex-linkage hypothesis. However, estimates of q varied indicating that maturation cannot be controlled by a single sex-linked gene as the gene frequency q, within any given population, must remain fixed. Clearly maturation is a sex-dependent phenomenon in *Trypanozoon* but not in *T. congolense*. The underlying biochemical basis for sex differences in maturation of *Trypanozoon* is thought to involve production and/or uptake of lectin inhibitors within the fly midgut (Welburn & Maudlin, 1994). Breeding experiments involving parents with salivary gland infections have shown that it is not possible to select a line of flies with increased levels of maturation (unpublished observations) further suggesting that maturation is not controlled by a Mendelian character.

Vectorial capacity is usually calculated assuming that the incubation time is a constant. The component of vectorial capacity relevant here is the average times flies survive after they have developed mature, transmissible infections (the longevity factor). If the distribution of survival times is exponential with parameter b (mortality rate b independent of age) and still working with a gamma distribution of infection times, the longevity factor is:

$$\frac{1}{b}\left[\frac{\rho}{b+\rho}\right]^k e^{-\delta b}.$$

Since b/ρ is small, this is, to a good approximation, equal to $\exp(-b\tau)/b$, where $\tau = k/\rho + \delta$ is the mean incubation time, the approximation becoming exact as the variance decreases to zero. This latter expression is the one usually used for the longevity factor. However, the assumption of constant incubation time may make a difference with other

types of survivorship; to see this, suppose, as an extreme case, all individuals survive to age L and then die; the longevity factor is:

$$\frac{1}{2L} \int_0^L g(s)(s-L)^2 ds \quad \text{where } g \text{ is the distribution of}$$

incubation times.

But if incubation time were assumed a constant τ, the estimated longevity factor would be zero if $\tau > L$, and equal to $(L-\tau)^2/2L$ if $\tau < L$. More generally, when mortality rates increase with age, vectorial capacity estimates may be misleading if they do not take account of variation in incubation time. There is some evidence that mortality of adult tsetse does increase with age (Hargrove, 1991).

Variation in incubation time is an important factor in interpreting age prevalence curves. A steadily rising prevalence of *Trypanozoon* or *T. congolense* mature infection with fly age in the field (Harley, 1966; Tarimo *et al.* 1985; Woolhouse, Hargrove & McNamara, 1993) may reflect susceptibility at all ages or susceptibility only in young flies (Welburn & Maudlin, 1992) coupled with variation in incubation times. As Buxton (1955) has pointed out 'one cannot state that gland infections will develop by a certain day even at a particular temperature. One can only say that the earliest infections would be expected from a certain day but that other flies may develop a cyclical infection later, perhaps much later. This is a point of great importance...to the epidemiologist who would wish to relate infection to the age distribution of the fly'.

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