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Improved models for the relationship between age and the probability of trypanosome infection in female tsetse, *Glossina pallidipes* Austen

J. W. Hargrove[1] and J. Van Sickle[2]

[1]SACEMA, University of Stellenbosch, Stellenbosch, South Africa and [2]Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon, USA

Research Paper

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Corresponding author:
J. W. Hargrove; Email: jhargrove@sun.ac.za

Abstract

Between 1990 and 1999, at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, 29,360 female *G. pallidipes* were dissected to determine their ovarian category and trypanosome infection status. Overall prevalences were 3.45 and 2.66% for *T. vivax* and *T. congolense*, respectively, declining during each year as temperatures increased from July – December. Fits to age-prevalence data using Susceptible-Exposed-Infective (SEI) and SI compartmental models were statistically better than those obtained using a published catalytic model, which made the unrealistic assumption that no female tsetse survived more than seven ovulations. The improved models require knowledge of fly mortality, estimated separately from ovarian category distributions. Infection rates were not significantly higher for *T. vivax* than for *T. congolense*. For *T. congolense* in field-sampled female *G. pallidipes*, we found no statistical support for a model where the force of infection was higher at the first feed than subsequently. The long survival of adult female tsetse, combined with feeding at intervals ≤3 days, ensures that post-teneral feeds, rather than the first feed, play the dominant role in the epidemiology of *T. congolense* infections in *G. pallidipes*. This is supported by estimates that only about 3% of wild hosts at Rekomitjie were harbouring sufficient *T. congolense* to ensure that tsetse feeding off them take an infected meal, so that the probability of ingesting an infected meal is low at every meal.

Introduction

Animal African Trypanosomiasis continues to be a serious veterinary problem, costing 37 African countries USD 4.5 billion annually (FAO, 2017). The trypanosome species of veterinary interest in eastern and southern Africa are *Trypanosoma vivax* and *T. congolense*. Woolhouse *et al.* (1993) used a catalytic function to model age-related changes in the prevalence of *T. vivax* infections in female *G. pallidipes*. For *T. congolense*, they suggested a slightly more complex model that allowed for a reduction in prevalence among older flies. Age was estimated using ovarian dissection, which can provide precise estimates of chronological age for flies in their first ovarian cycle – i.e., flies that have ovulated fewer than four times – but not, in general, for older flies (Challier, 1965). Woolhouse *et al.* (1993) assumed for their sample, however, that negligible numbers of flies survived to a third ovarian cycle so that uncorrected ovarian category is identical to ovarian age – i.e., the number of times the fly has ovulated – and is thus a valid index of chronological age. This assumption was carried over into other modelling of the same data (Woolhouse and Hargrove, 1998; Lord *et al.*, 1999). The larger numbers dissected in the current study makes clear that the above assumption cannot be sustained. Accordingly, we develop improved models that account for the longer survival of adult flies.

In so doing, we also investigated the suggestion that the probability of a tsetse acquiring a trypanosome infection is higher when the fly takes its first blood meal – i.e., as a teneral fly – than at all subsequent feeds. This so-called 'teneral effect' is well established for *T. brucei*, being first described by Van Hoof *et al.* (1937) for the acquisition of *T. b. gambiense* by *G. palpalis*. Haines (2013) provides an excellent review of the subject and notes that refractoriness to *T. brucei* infections in non-teneral tsetse can be reduced among starved flies (Kubi *et al.*, 2006). In his general model for the African trypanosomiases, Rogers (1988) assumed that there was no such teneral effect in the case of *T. congolense*, which causes disease in livestock, but not humans. Distelmans *et al.* (1982) had, however, found that teneral *G. p. palpalis* were more readily infected with *T. congolense* than older flies. Later, Welburn and Maudlin (1992) using laboratory *G. m.* morsitans, found the risk of acquiring a *T. congolense* infection was seven-times higher in tenerals than in all older flies. This led them to suggest that non-teneral flies do not play a significant part in the epidemiology of *T. congolense*. The motivation for our

paper is thus two-fold: (i) to provide improved models for the dynamics of trypanosome infection in the field; (ii) to assess the importance of the teneral effect in the dynamics of infection of *G. pallidipes* females with *T. congolense*.

Methods

Study area

The study was carried out between September 1990 and April 1999 at Rekomitjie Research Station (16°10′S, 29° 25′E, altitude 520 m), Zambezi Valley, Zimbabwe. Daily maximum and minimum temperatures were recorded using mercury thermometers housed in a Stevenson screen at the Station, and rainfall was recorded using a gauge placed 4 m from the Stevenson screen.

Fly sampling

Female *G. pallidipes* were collected at various sites within 2 km of the Research Station; >99% of flies were captured in epsilon traps (Hargrove & Langley, 1990) baited with artificial host-odour consisting of acetone, 1-octen-3-ol, 3-n-propyl phenol and 4-methyl phenol released at ∼200, 0.4, 0.01 and 0.8 mg h^{-1}, respectively. The cages in which flies were captured were wrapped in a heavy-duty black cotton cloth and kept in a polystyrene box; in about 90% of cases, traps were cleared at 30-minute intervals. Flies were dissected within 24 hours of capture.

Classification of flies by ovarian and wing fray category and trypanosome infection status

We followed the general dissection procedure described by Woolhouse *et al.* (1993). We dissected the ovaries and uterus of female tsetse and assigned each to an ovarian category. The general technique was originally developed for estimating the age of female mosquitoes (Detinova, 1949, 1959, 1962). It was then applied to tsetse by Saunders (1960, 1962) and improved by Challier (1965). Details of the age estimation procedure, as applied to female tsetse, are provided by the above authors, and the technique has been used and cited by many others since then (Rogers *et al.*, 1984; Dransfield *et al.*, 1989; Randolph *et al.*, 1990; Hargrove, 1993, 1995, 1999*a*, 1999*b*; 2012; Hargrove *et al.*, 2019). Nonetheless, for convenience – and particularly given the complexities of the technique – we provide the following details of the age estimation procedure, based largely on the description provided by Hargrove (2012).

Figure 1 illustrates the disposition of the four oocytes within the paired ovaries at different stages of the life of a female tsetse fly. In the normal fly, oocytes develop and are ovulated in the strict order: right inner (labelled A), left inner (C), right outer (B) and left outer (D). Ovulation of all four oocytes comprises a single ovarian *cycle*. The relative size of the oocytes allows distinction between flies that have ovulated zero, one, two or three times. The oocytes of a fly that has ovulated four times have, however, the same conformation as a fly that has not yet ovulated (fig. 1). Flies that have ovulated 0 and 4 times can, however, be distinguished because ovulation results in the ovariole bearing a follicular relic of the previous ovulation. A fly that has ovulated four times has a relic on each ovariole, whereas a fly that has not yet ovulated for the first time has no relic on any ovariole. Similarly, since flies in ovarian categories 1, 2 and 3 have 1, 2 or 3 relics, respectively, they can be distinguished from flies that have ovulated 5, 6 or 7 times – all of which have four relics.

Problems arise for flies that have ovulated more than seven times because, in tsetse – though not in mosquitoes (Detinova, 1962) – only one relic is ever seen on an ovariole. Further ovulations do not result in changes in the appearance of the relics. Since it is not therefore possible to distinguish a fly that has ovulated 4 times from one that has ovulated 8, 12, 16 … , etc., times, we define ovarian category $4 + 4n$, $n = 0, 1, 2, 3\ldots$ as a category that includes flies of all these ovarian age groups. Ovarian categories $5 + 4n$, $6 + 4n$ and $7 + 4n$ are similarly defined. In what follows we distinguish between a fly's ovarian *category* – as defined above – and its ovarian *age group*, being the number of times the fly has ovulated.

For flies in ovarian categories 0 to 3, only, the ovarian category and ovarian age group are identical. In principle n has no upper bound but the relationship between wing fray and age in *G. pallidipes* females suggests that <1% of *G. pallidipes* females survived to ovulate >11 times in the field at Rekomitjie (Hargrove, 2020). Negligible numbers of females would thus survive long enough to ovulate more than 15 times, and the same conclusion can be reached from field estimates of the age-specific mortality of *G. m. morsitans* (Hargrove *et al.*, 2011). These results suggest n takes a maximum value of 3 for our study situation and we have assumed this is the case. Flies are thus assumed to survive a maximum of four ovarian cycles; ovarian category $4 + 4n$ includes flies in ovarian age groups 4, 8 and 12 – and analogous statements apply to flies in ovarian categories $5 + 4n$, $6 + 4n$ and $7 + 4n$.

We used the classification of Jackson (1946) to assign each fly to one of six wing fray categories. While ovarian dissection provides a more accurate estimate of a fly's chronological age for tsetse, this is only true for flies in ovarian age group <4. Wing fray, by contrast, provides a relative measure of age for all flies and allows us to gauge how trypanosome prevalences change over flies' entire lives.

The mouthparts of each fly were dissected, and the labrum and hypopharynx were examined under a light microscope at 240 × magnification. If no trypanosomes were found in the mouthparts the fly was diagnosed as negative for all trypanosome infections and no further dissection was carried out. If trypanosomes were detected, the midgut was dissected out and screened for infection. Where trypanosomes were detected in both the midgut and mouthparts, the fly was diagnosed as being infected with *T. congolense*; if trypanosomes were found only in the mouthparts the fly was diagnosed as being infected with *T. vivax*. It is also possible for a fly to be infected with both trypanosome species.

The delay between the time when a fly becomes infected and when it is possible to detect trypanosomes in the fly by dissection is such that there is effectively zero probability of finding trypanosomes in a fly before it has ovulated for the first time. Thus, among 1228 flies dissected that were adjudged to have recently ovulated for the first time and were either 8 or 9 days old – 0 and 2 were found to harbour a *T. vivax* or a *T. congolense* infection, respectively. The estimated prevalences, with 95% confidence interval, were thus extremely close to zero: 0 (0–0.003) and 0.0016 (0.0002–0.006), respectively. Flies in ovarian category 0, which are by assumption younger than 8 days, will have expected prevalences even lower than the above values. Accordingly, flies found to be in ovarian category 0 were not dissected further to look for trypanosomes, and the trypanosome prevalence of such flies was assumed to be identically zero.

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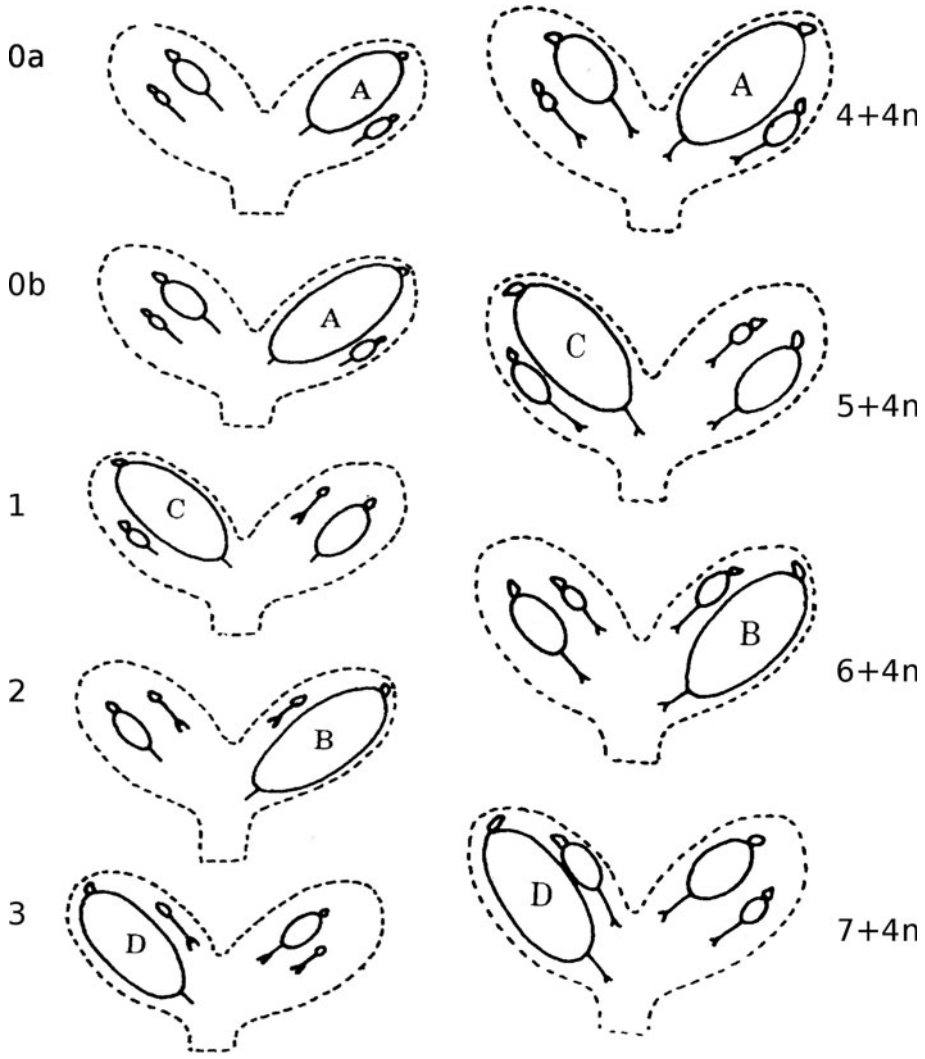

Figure 1. Diagrammatic representation of changes in the appearance of tsetse fly ovaries during successive ovulations. Symbols next to each diagram indicate the ovarian category: 0, 1, 2, 3, 4 + 4*n*, 5 + 4*n*, 6 + 4*n*, 7 + 4*n*. 0a and 0b indicate females that have not yet ovulated for the first time and where the largest oocyte is shorter, or longer, respectively, than about 0.6 mm.

Modelling patterns of acquisition of trypanosome infections

Catalytic model: assuming no flies ovulate more than seven times

Woolhouse *et al.* (1993) used a catalytic model to explain age-related changes in the prevalence of *T. vivax* infections in female *G. pallidipes*, fitting their data using the function:

$$p(k) = 1 - \exp\,(-\lambda(k-\tau)) \qquad (1)$$

In equation 1, $p(k)$ is the predicted trypanosome prevalence among flies of age k days. Equivalently $p(k)$ can be interpreted as the probability that any fly will become infected by the time it reaches the age of k days. The parameter λ is the rate of infection and τ is the number of days between the acquisition of an infection and the time when trypanosomes can first be detected in the fly. Equation 1 predicts prevalence directly for flies of any known daily age, without requiring knowledge of fly mortality, and without using explicit predicted values for the numbers of infected and uninfected flies. We follow Woolhouse *et al.* (1993) in applying our models to data pooled over time, so that model parameter estimates are viewed as long-term averages.

To help interpret λ, one can replace the exponential function in equation 1 with its power series expansion. For fractional values of $\lambda(k-\tau)$ close to zero, as in our estimates below, the squared and higher-power terms of the power series are negligible in magnitude. Thus, equation 1 can be closely approximated by $p(k) \approx \lambda(k-\tau)$. Since $p(k+1) - p(k) \approx \lambda$, the λ parameter of equation 1 can be interpreted as the increase in a fly's infection probability from any one day to the next. That is, λ is the approximate probability that any uninfected fly will become infected within the next 24 hours.

We applied the catalytic model to our data. Each dissected fly in our data was identified only to its ovarian category; to apply equation 1, we specified the range of daily ages spanned by each category. Following Hargrove (1995, 2012) we allocate flies of age 0–7 days to category zero: thereafter we assume a 9-day pregnancy duration, so that successive ovarian age groups include successive 9-day intervals. For the catalytic model only, we follow Woolhouse *et al.* (1993) in assuming that no flies survived to ovulate more than seven times so that, for this model only, ovarian age group i and ovarian category i are identical for $i = 1,7$. Thus, ovarian category 1 includes days 8 to 16, category 2 includes days 17 to 25, and so forth. In general, category i includes the daily ages $(i*9-1)$ to $(i*9+7)$, with all lifespans terminating at $(7*9+7) = 70$ days.

We then assume that the predicted prevalence $P(i)$, for flies of ovarian category i, $i = 1,7$ is equal to the mean of the daily

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prevalences predicted for all 9 days included in that ovarian category. That is,

$$P(i) = \left(\sum_{(i*9-1)}^{(i*9+7)} p(k) \right) / 9 \tag{2}$$

As explained above, we assume $P(0) = 0$. In words, equation (2) says that we find the average prevalence for any given ovarian category by summing the prevalences for each day of pregnancy and dividing by 9.

Susceptible-infected SI model: assuming flies can survive to ovulate more than seven times

When fitting the catalytic model, no account was taken of fly survival – nor, explicitly, of the numbers of susceptible and infected flies found in each ovarian category. Instead, trypanosome observed and predicted prevalences were compared directly. As discussed earlier, however, it is clear that significant proportions of female tsetse do survive to ovulate more than seven times. Under these circumstances we need to take fly mortality into consideration and the catalytic model of Woolhouse *et al.* (1993) is not appropriate. Instead, we used an SI model to predict, separately, the numbers of infected (I), noninfected (susceptible, S), and total ($N = S + I$) flies, that would occur at each daily age, k. We assume that, as flies age, they progressively transition from the susceptible to the infected group, with equation 1 again predicting the probability that a surviving fly will become infected by the age of k. We pool the predicted daily values of S and I into their respective ovarian age groups and for, age groups >3, further pool these for ovarian categories $4 + 4n$ to $7 + 4n$.

To model fly mortality, we assume that flies of all ages, and infection status, die at the instantaneous daily rate μ, so that an initial, total number, $N(0)$ of newly-emerged flies will have decreased to $N(k) = N(0)\ \exp(-\mu k)$ by age k. The daily survival probability of any fly is $\varphi = \exp(-\mu)$. With this mortality model, the numbers of surviving flies that are infected is given by: $I(k) = N(0)\ \exp(-\mu k)(1 - \exp(-\lambda(k - \tau))$, where λ and τ are as defined for equation 1. The total numbers, and numbers infected, at each daily age are again pooled into 9-day ovarian age groups, now indexed by $i = 1,2, \ldots ,15$. As before, ovarian age group i includes the daily ages (i*9-1) to (i*9 + 7), but all flies are now assumed to have died by the age of (15*9 + 7) = 142 days. For ovarian age groups $i = 1$ to 3, and daily age indexed by k, we sum the numbers predicted for each daily age within each age group, to get group totals:

$$N(i) = \left(\sum_{i*9-1}^{i*9+7} N(k) \right)$$

$$I(i) = \left(\sum_{i*9-1}^{i*9+7} I(k) \right)$$

For the ovarian age groups 1 to 3, prevalence is then predicted as

$$P(i) = I(i)/N(i) \quad i = 1, 3 \tag{3}$$

For older flies, we need to further pool the ovarian age *groups* to calculate the prevalence for the corresponding ovarian *category*, as is shown for the (4 + 4n) category:

$$\begin{aligned} &N(4 + 4n) = N(4) + N(8) + N(12) \\ &I(4 + 4n) = I(4) + I(8) + I(12) \\ &\text{so that} \quad P(4 + 4n) = I(4 + 4n)/N(4 + 4n) \end{aligned} \tag{4}$$

and analogous equations are used for flies in ovarian categories $5 + 4n$, $6 + 4n$ and $7 + 4n$. Note that $N(0)$ can be an arbitrary value because it divides out when calculating $P(i)$.

Susceptible, exposed and infective (SEI) model

The fixed time delay (τ) in the SI model is biologically unrealistic in assuming a sharp boundary between the ages when tsetse are and are not infective. As an alternative, we consider a model where we partition flies into three states – Susceptible, Exposed and Infective (SEI). Instead of a fixed delay (τ) we model the proportions of flies transitioning from the S to E to I compartments during each age increment, again indexed by k. Susceptible flies are those that have never acquired a trypanosome infection. Exposed flies have been so infected, but the trypanosome infection has not developed to the point where the fly can pass on that infection – i.e., it is not infective. Infective flies can pass on the trypanosome infection; for both the SI and SEI models we assume that flies, once infected, stay infected for life. They thus only leave the infective state by dying. They are also the only flies in which trypanosome can be detected using dissection techniques; accordingly, only the infective flies are included in compartment I in the modelling. Our SEI model assumes the same extended set of 16 ovarian age groups (including category/group 0) as were used in the SI model, and also assumes the same mortality model.

We assume an arbitrary initial number S_0 of susceptible flies. Since none has yet been exposed or infected, $E_0 = 0$ and $I_0 = 0$. Then the following transition equations apply for the SEI model as the flies age:

$$\begin{aligned} S_{k+1} &= \varphi(1-\lambda)S_k \\ E_{k+1} &= \varphi(\lambda S_k + (1-\gamma)E_k) \\ I_{k+1} &= \varphi(\gamma E_k + I_k) \end{aligned} \tag{5}$$

where, for each unit of age φ is again the probability that a fly will survive to the next age, regardless of its infection status. The parameter λ is the probability that susceptibles (S_k) move to the exposed class (E_{k+1}). Since all flies surviving the Exposed class ultimately become infected, λ is again the probability of becoming infected in a single age increment. The γ parameter is the probability of moving from the exposed class (E_k) to the infective class (I_{k+1}), between ages k and $k + 1$. The total count is given by $N_k = S_k + E_k + I_k$. When implementing equations 5 we used the same, separately estimated, value of φ as used in the SI model.

When applying this model, we changed the age increment from 1 to 3 days, assuming that the latter increment approximated the interval between successive feeds, and that the flies take an average of three meals in a 9-day pregnancy (Randolph *et al.*, 1992). The change enabled us to investigate the suggestion that the force of infection is higher at the first blood meal than at subsequent meals (Welburn and Maudlin, 1992). This was achieved by allowing that λ in equation 5 could take a different (presumed higher) value when the fly took its first blood meal, compared with all later meals. Notice that the first meal is taken during ovarian category 0 when no flies can yet be in category I and when,

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therefore, the numbers play no role in the model fitting. If, however, λ were indeed very much higher at the first meal, we should expect a surge of infectives during ovarian category 1.

With a 3-day age increment, there are 3 increments per 9-day pregnancy. We now begin ovarian age group 1 at day 7, rather than day 8, so that age group 0 includes the 3-day age increments $k = 1$ and 2, while group 1 includes increments $k = 3$, 4, and 5. In general, ovarian age group i includes the age increments from $k = (3^*i)$ to $k = (3^*i + 2)$, for $i = 1, 2, \ldots 15$. Thus, predicted total values of S, E, I and N for each of the 15 ovarian age groups are given by:

$$S(i) = \sum_{3*i}^{3*i+2} S_k$$

$$E(i) = \sum_{3*i}^{3*i+2} E_k$$

$$I(i) = \sum_{3*i}^{3*i+2} I_k, \text{ and}$$

$$N(i) = S(i) + E(i) + I(i) \quad (6)$$

Finally, the values of $I(i)$ and $N(i)$ from equation 6 are inserted into equations 3 and 4, to calculate the SEI- predicted prevalences for the younger ovarian categories $i = 1$, 2, 3 and for the older, pooled ovarian categories $4 + 4n$ to $7 + 4n$.

Statistical methods

Prevalence models: parameter estimation and model assessment

We estimated the parameters of all models using maximum likelihood (ML), as implemented in the R package *maxlik* (Henningsen and Toomet, 2011). For the catalytic, SI and SEI models, the predicted prevalence can also be interpreted as the predicted probability that a randomly sampled fly is infected. Thus, the log likelihood for each of the three models has the binomial form:

$$Loglik = \sum_i [n_I(i)log(P(i)) + n_U(i)log(1 - P(i))] \quad (7)$$

where $n_I(i)$ and $n_U(i) = n(i) - n_I(i)$ are the observed counts of infected and uninfected flies, respectively, in the 7 ovarian categories $i = 1$ to $7 + 4n$. The observed total count in each category is $n(i)$.

The three prevalence models differ only in how they predict the $P(i)$ values of equation 7. For the catalytic model, $P(i)$ is given by equation 2 and, for the SI model, the predicted $P(i)$ values are given by equations 3 and 4. For the SEI model, predicted values of $P(i)$ are again given by equations 3 and 4, but with the components of $P(i)$ (that is, $I(i)$ and $N(i)$) now calculated from equations 6.

We report 95% confidence intervals for all estimated parameters. In addition, we used the Akaike information criterion (AIC), as well as graphical displays, to assess and compare how well the prevalence models fit the data (Burnham and Anderson, 2002). AIC measures a model's quality of fit, with a penalty for the number of estimated parameters needed to achieve that quality. In calculating AIC for the SI and SEI models, we also included φ in the count of estimated parameters. As a rule of thumb, models having AIC values within 2 units of each other do not differ in their quality of fit (Burnham and Anderson, 2002).

Estimation of survival probability

We also used ML to estimate the survival probability φ separately from the prevalence models and based only on the total counts in the ovarian categories. This estimate was then used as a fixed parameter value when estimating the other parameters of the SI and SEI models. To estimate φ, we assumed that the total counts, $N(i)$, represent a sample from a stationary age distribution of live flies. Then, the number surviving to ovarian age group i is given by $N(i) = N(0)\varphi^i$, where $N(0)$ is an arbitrary initial count of age 0 flies, and φ is now the 9-day survivorship probability. Assuming that no flies survive beyond 15 ovulations, the grand total count of all flies is $N_T = N(0)\sum_i \varphi^i$, where the sum ranges over $i = 1,2 \ldots 15$. Then, the ratio $N(i)/N_T$ expresses the age distribution as a multinomial distribution of the probabilities $p(i)$ that a randomly-selected fly is of ovarian age group i.

For ovarian categories $i = 1$, 2, 3, the probabilities are:

$$p(i) = N(i)/N_T = \varphi^i / \sum_i \varphi^i \quad (8)$$

The number of survivors in ovarian category $4 + 4n$ includes all flies that survived through ovarian age groups 4, 8 or 12, and similarly for the other pooled categories. Thus the survival probabilities for the grouped ovarian categories $4 + 4n$ and $7 + 4n$ are:

$$p(4 + 4n) = [\varphi^4 + \varphi^8 + \varphi^{12}] / \sum_i \varphi^i$$

$$p(7 + 4n) = [\varphi^7 + \varphi^{11} + \varphi^{15}] / \sum_i \varphi^i \quad (9)$$

with analagous expressions for 5+6n and 6+6n. Finally, the log likelihood is given by

$$loglik = \sum_i n(i)log(p(i)) \quad (10)$$

where $n(i)$ are the observed total counts in ovarian categories 1, 2, … $7 + 4n$, and the probabilities $p(i)$ are given by equations 8 and 9. We maximized equation 10 directly. Equivalently, Hargrove (1993) set the derivative of Equation 10 to zero, thus yielding a closed form for the maximum, which was then solved numerically.

As defined above, φ is the probability of survival for 9 days. We converted the estimate of φ into 3-day and 1-day survival probabilities, for use in the SEI and SI models, by calculating its cube root and ninth root, respectively.

Results

Temporal variations in temperature and rainfall, and in trypanosome prevalence

Rekomitjie Research Station has a hot tropical climate with temperatures increasing between July and November, leading up to

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the start of the single rainy season, largely restricted to November-February (fig. 2A). A total of 29,360 female *G. pallidipes* were dissected for determination of ovarian category and trypanosome infection status. Of these, the wing fray category was gauged in 27,951 flies. *T. vivax* and *T. congolense* infection prevalences – for all flies pooled on age – were 3.45 and 2.66%, respectively and declined during each year as temperatures increased between about July and December. Thereafter they increased sharply for 1–2 months, then more slowly to peak again in July (fig. 2B).

Age distribution of all flies dissected, and of those found infected with trypanosomes

Among all flies dissected, there were similar numbers in ovarian categories 1 and 2, smaller numbers in ovarian category 3, and then a marked jump in numbers when moving to ovarian category $4 + 4n$ (fig. 3A) – in accord with the fact that ovarian category $4 + 4n$ includes flies that have ovulated 4, 8 or 12 times (see above). For infected flies, numbers increase between ovarian categories 1 and 3 and then take an even larger jump for those in ovarian category $4 + 4n$ – reflecting the fact that flies can accrue infections throughout life (fig. 3B). The numbers of infected flies only begin to diminish for flies of ovarian category $4 + 4n$, reflecting the changing balance between reduced numbers of susceptible flies, and thus new infections, and deaths of those that are already infected. When wing fray was used as a cruder measure of age, the numbers dissected increased until wing fray category 4, and the numbers of infected flies until category 4 or 5 (fig. 3C, D). The difference reflects the fact that wing fray cannot decrease with age – and will naturally increase at different rates in different flies.

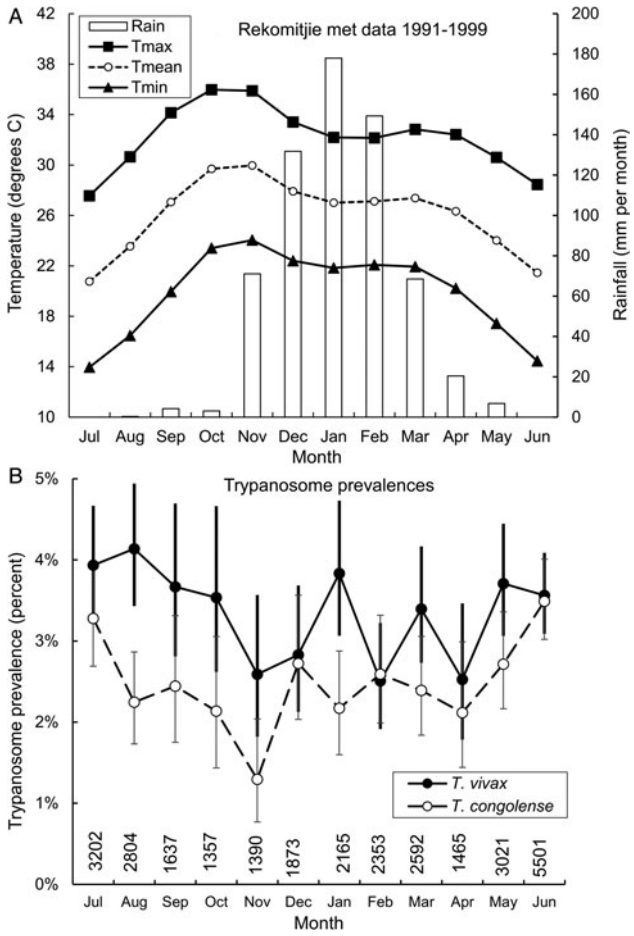

Figure 2. (A) Minimum, mean and maximum daily temperatures and mean monthly rainfall 1991–1999, Rekomitjie Research Station, Zambezi Valley, Zimbabwe. (B) Monthly prevalence of infections of *T. vivax* and *T. congolense* in female *G. pallidipes* captured and dissected between 1990 and 1999. Error bars indicate the 95% confidence intervals. Samples sizes are for each month, pooled on year.

Trypanosome prevalence as a function of fly age and ambient mean temperature

Stepwise logistic regressions were carried out, using the prevalences of either *T. vivax* or *T. congolense* infections as the dependent variable. Ovarian, and wing fray, categories and mean ambient temperatures all accounted for significant fractions of the variance for both dependent variables. For both species of trypanosome, ovarian category removed the largest proportion of the variance, and the odds ratios increased steadily with increasing ovarian category (table 1). Once this variation was removed, the odds ratios for increases with increasing wing fray category were more modest.

Trypanosome prevalences, adjusted for ovarian and wing fray categories, declined significantly with increasing temperature (table 1) measured as the mean temperature averaged over the nine days prior to the day on which the fly was captured – this period approximating the duration of pregnancy. The result is in accord with the finding that trypanosome prevalences, pooled on age, generally decrease in the hottest months of the year (fig. 2B).

Fitting data using the catalytic function

The model defined in equation 1, based on the assumption that no flies ovulate more than seven times, provides a reasonable fit to the data for both species of trypanosome (fig. 4) – although there are clear trends in the residuals. For *T. vivax*, the best fit was obtained with $\lambda = 0.00141$ per day and $\tau = 9.7$ days (table 2), very close to the values of $\lambda = 0.00149$ per day and $\tau = 14.2$ days of Woolhouse *et al.* (1993), using only data for flies dissected in 1990 and 1991. For *T. congolense*, using the same model, the values were $\lambda = 0.00107$ per day and $\tau = 9.8$ days (table 2). There is considerable overlap of the 95% confidence intervals between species, for λ and for τ. Results obtained using our larger data set also suggest that there is no need to postulate a decline in *T. congolense* prevalence among older flies (fig. 4B, *cf.* Woolhouse *et al.*, 1993).

Examination of the raw data on which the prevalences were calculated shows, however, that the Woolhouse *et al.* (1993) assumption that a negligible fraction of flies survives to a third ovarian cycle cannot be sustained (fig. 3). The increase, between ovarian categories 3 and $4 + 4n$, in the total numbers of flies caught and dissected – and the large jump in the numbers of infected flies – make it obvious that significant proportions of flies do survive to a third ovarian cycle: and we need to account for this fact. Note that a jump in observed trypanosome prevalence is not predicted by the fitted catalytic model (fig. 4).

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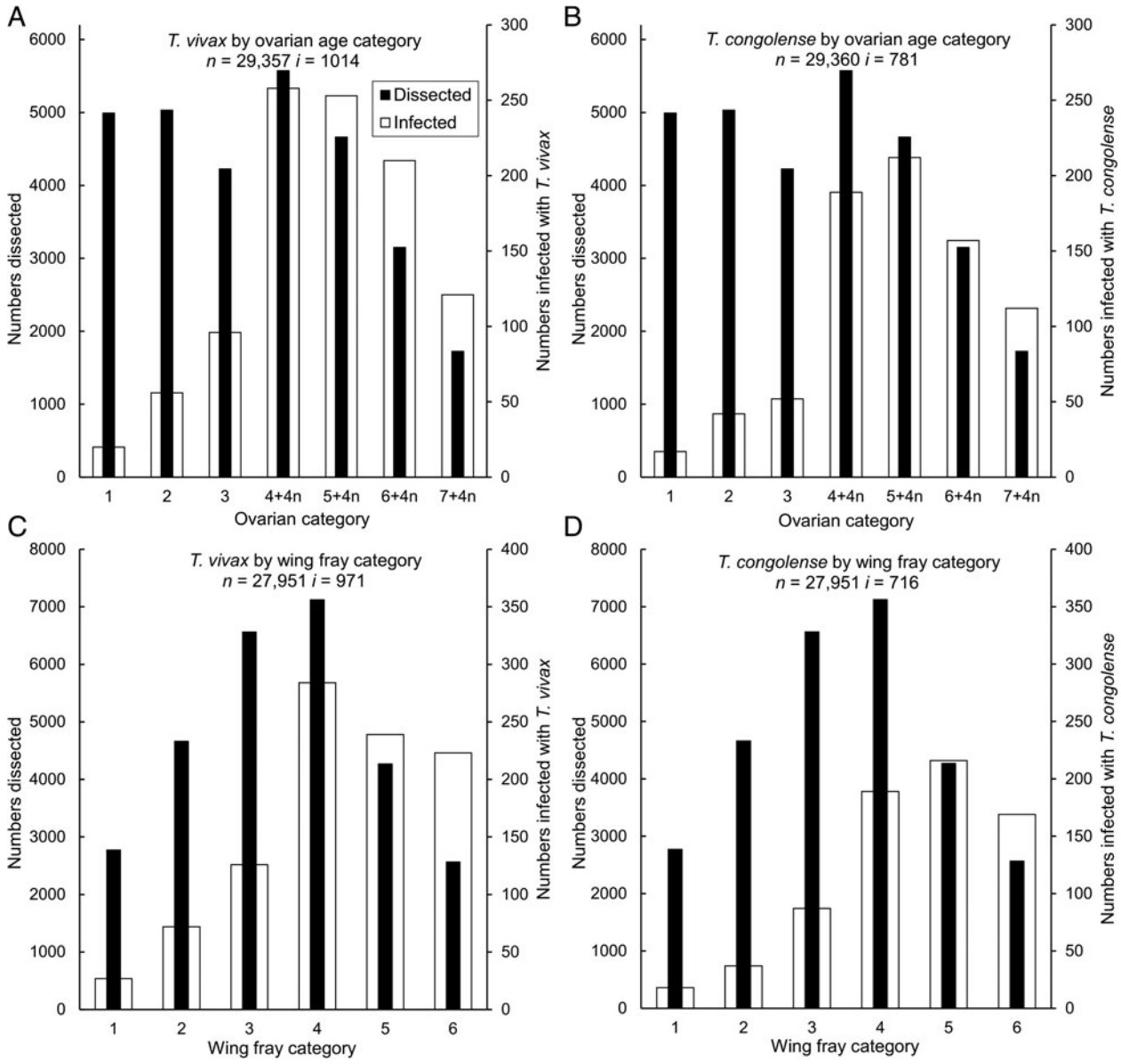

Figure 3. Distribution by ovarian or wing fray category of female *G. pallidipes* captured at Rekomitjie Research Station 1990-1999. Figures show numbers (*n*) dissected and numbers (*i*) infected. **A., C.** *T. vivax*; **B., D.** *T. congolense* by ovarian and wing fray categories, respectively. Notice the difference in scales on the left and right vertical axes on all graphs.

Fitting data using a susceptible-infected (SI) model

The SI model defined by equations (3) and (4) requires knowledge of the survival probability. From the ovarian category distribution of the total number of adult female *G. pallidipes*, dissected to detect infections of *T. vivax* (fig. 3A), we estimated that $\varphi = 0.976$ and the daily mortality rate $\mu = -\ln(\varphi) = 0.0242$ (95% CI: 0.0238–0.0246). Using this estimate, and the procedures leading to equations 3 and 4, resulted in improved fits to the data for *T. vivax*, relative to the catalytic model (*cf* figs 4A and 5A; AIC values (table 2) are lower by about 20).

For *T. congolense*, similarly, the fit was markedly improved (*cf* figs 4B and 5B), with the AIC values again lower by about 20, relative to the catalytic model. As is obvious from the inspection of fig. 5, however, the fit to the *T. vivax* data is markedly better than for *T. congolense* when the SI model is applied to the data for both species.

Fitting data using a susceptible-exposed-infected (SEI) model

The relatively poor fit of the SI model to *T. congolense* data results from the use of the value (τ) to allow for the time taken to mature an infection. Using the SEI model, described by equation 5, results in an improved fit to the data for *T. congolense* (*cf* figs 6A and 5B). The AIC for the SEI(1) model was 6.0 lower than that for the SI model (table 2, rows 6 and 7). For *T. vivax* the AIC criteria achieved using the SI and SEI(1) models differed by only $1.0 < 2.0$ so that there was no 'quality of fit' difference between these two model forms (table 2, rows 2 and 3). Visually, the fit obtained using the SEI model (not shown) is almost indistinguishable from that for the SI model (fig. 5A).

In the SEI(2) model, our estimates of $\lambda 1$ were not significantly different from zero, for both *T. vivax* and *T. congolense* (table 2). Thus, our data provided no support for a teneral effect, in which

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Table 1. Results of logistic regression of the prevalence of *T. vivax* (A) and *T. congolense* (B) in female *G. pallidipes*, as a function of the fly's ovarian, and wing fray, category and the mean daily temperature (tbar91) over the nine days prior to its capture. Sample size 27,951

A. T. vivax		
	Odds ratio	95% Conf. interval
tbar91	0.956***	0.937–0.976
Ovarian category		
1	1.00	
2	2.78***	1.63–4.73
3	5.43***	3.26–9.04
4 + 4*n*	9.67***	5.90–15.87
5 + 4*n*	10.83***	6.56–17.86
6 + 4*n*	12.51***	7.53–20.77
7 + 4*n*	12.64***	7.48–21.37
Wing fray category		
1	1.00	
2	1.17	0.75–1.84
3	1.01	0.66–1.56
4	1.32	0.87–2.02
5	1.60*	1.04–2.46
6	2.42***	1.57–3.75
Constant	0.011	0.005–0.022
B. T. congolense		
	Odds ratio	95% Conf. interval
tbar91	0.949***	0.927–0.972
Ovarian category		
1	1.00	
2	2.69**	1.45–4.99
3	3.49***	1.88–6.46
4 + 4*n*	7.58***	4.23–13.58
5 + 4*n*	9.68***	5.39–17.40
6 + 4*n*	9.88***	5.44–17.92
7 + 4*n*	11.76***	6.39–21.63
Wing fray category		
1	1.00	
2	0.93	0.52–1.64
3	1.11	0.66–1.89
4	1.37	0.81–2.31
5	2.22**	1.32–3.76
6	2.76***	1.62–4.71
Constant	0.010	0.004–0.023

infection rates are higher for the first bloodmeal than for all subsequent bloodmeals. Our estimates of γ for both species, and of λ for *T. congolense,* were also not significant for the SEI(2) model (table 2). This suggests that our counts of infected and uninfected flies from only 8 ovarian categories cannot support reliable estimates of the 3 parameters of the SEI(2) model.

Note also that, in the single-lambda model, SEI(1), there was considerable overlap in CIs, between *T. vivax* and *T. congolense*, for the estimates of λ and γ (table 2, rows 3 and 7). Thus, while the point estimates of the two parameters indicate a higher probability of infection for *T. vivax* – and a shorter maturation time, resulting in higher predicted prevalences for this species (fig. 5B), the difference is not statistically significant.

Discussion

Recognition of the fact that significant proportions of female tsetse survive long enough in the field that they ovulate more than seven times means that it is not appropriate to use a catalytic model, which ignores fly mortality, to fit age-prevalence data for trypanosome infections. The more complex SI and SEI(1) models provide much improved fits to the data. Differences in the estimated infection rates between the models may appear small in absolute terms. However, the SI and SEI(1) values are 25 and 26% lower, respectively, than the values for the catalytic model in the case of *T. vivax* – and 29 and 16% lower in the case of *T. congolense*. This is as expected – because the catalytic model predicts the observed lifetime increase in prevalence over a lifespan of 70 days, in contrast to that same increase predicted over a lifespan of 142 days, for the SI and SEI(1) models.

The difficulty with the SI and SEI models is that they require knowledge of the mortality rate among adult female tsetse. This can be a problem because estimating this mortality from age distributions has proved to be extremely difficult (Hargrove and Ackley, 2015), particularly using samples collected over a short period of time. More believable estimates of mortality can be obtained, however, if samples are pooled over time periods of a year or more – such that problems associated with the seasonal variability of the population age structure are avoided (Van Sickle and Phelps, 1988; Ackley and Hargrove, 2017). Accordingly, we have only applied our analysis to data pooled over the whole period of the study and have not attempted to estimate the parameters λ, $\lambda 1$ and γ over shorter periods of time.

Coinfection rates

We assume in our analyses that the processes whereby female *G. pallidipes* acquire infections of the two trypanosome species, *T. vivax* and *T. congolense*, are completely independent of each other. In particular, being infected with one of the species should not alter the probability that a fly becomes infected with the other species. Coinfections, with more than one trypanosome species in a single fly, can be detected using techniques such as the polymerase chain reactions (PCR) that detect and identify, to species level, the DNA of different trypanosomes. The dissection process we used does not allow of such exact identification. Notice, however, that any fly that only has a mouthpart infection, can have only a *T. vivax*-type infection – because, if it also had a *T. congolense* infection, it would have trypanosomes in the midgut. The possibility of a *vivax-congolense* coinfection arises, therefore, only in flies where there are trypanosomes detected both in the mouthparts and the midgut. The following calculation suggests that the chances of this coinfection occurring for the female *G. pallidipes* in our study are negligible. Thus only 2.66 and 3.45% of all flies dissected were diagnosed to have a *T. congolense*-type or *T.*

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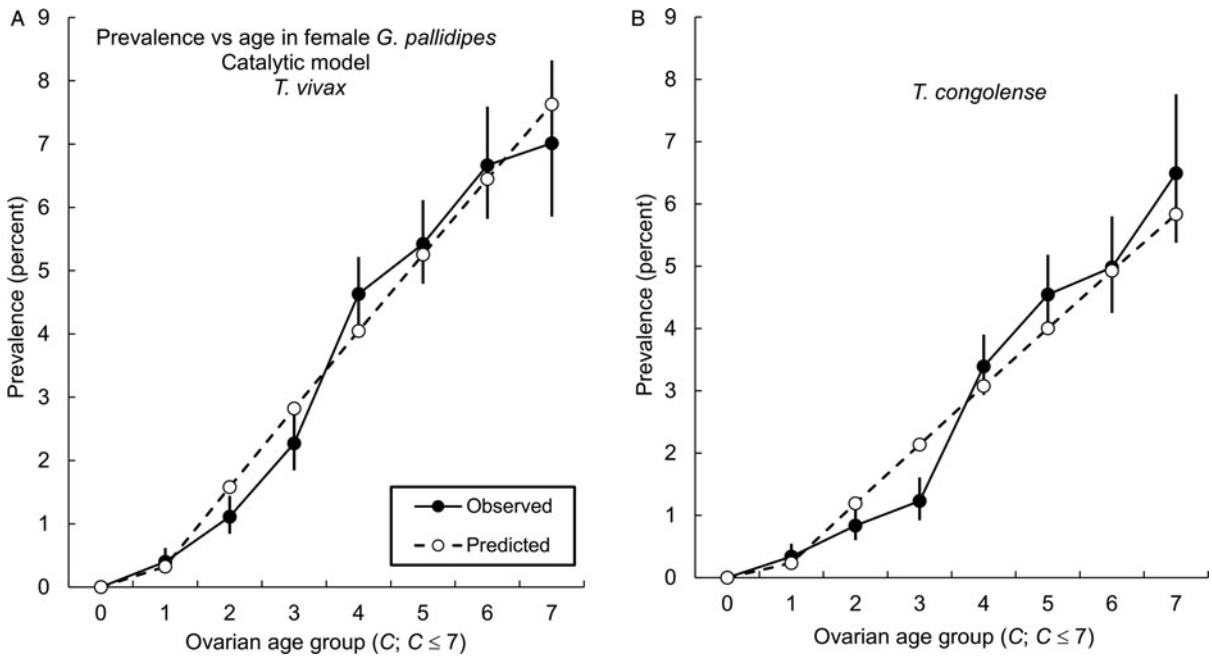

Figure 4. Fitting the function $p(k) = 1 - \exp[-\lambda(k - \tau)]$ to ovarian age-specific prevalences for (A) *T. vivax* and (B) *T. congolense* in female *G. pallidipes* – where k is the daily age of the fly, λ the rate of infection and τ the delay between the time that a fly is infected and when trypanosomes can first be detected in the fly. Predicted daily prevalences are averaged within each ovarian age group to estimate the prevalence of each ovarian age. It is assumed that no flies survive >7 ovulations. Error bars on observed prevalences are exact 95% confidence intervals for a binomial proportion.

Table 2. Maximum likelihood estimates for catalytic, SI, and SEI infection models, for *T. vivax* and *T. congolense* infections of female *G. pallidipes*

		λ	λ1	τ (days)	γ	AIC*
		T. vivax				
1	**Catalytic**	0.00141 [0.00130, 0.00151]	–	9.7 [8.7, 10.7]		8426.2
2	**SI**	0.00101 [0.00093, 0.00108]		8.1 [6.7, 9.6]		8406.3
3	**SEI (1)**	0.00106 [0.00093, 0.00119]			0.091 [0.053,0.129]	8405.3
4	**SEI (2)**	0.00104 [0.00090, 0.00117]	0.00033 [−0.00185, 0.00251]		0.131 [−0.049,0.310]	8407.6
		T. congolense				
5	**Catalytic**	0.00107 [0.00098, 0.00115]		9.8 [8.7, 10.8]		6801.9
6	**SI**	0.00076 [0.00070, 0.00083]	–	8.4 [6.6, 10.2]		6778.7
7	**SEI (1)**	0.000903 [0.00073, 0.00108]			0.06133 [0.030, 0.093]	6772.7
8	**SEI (2)**	0.00367 [−0.00350, 0.10837]	0.02697 [−0.02777, 0.08170]		0.000433 [−0.004,0.0123]	6772.3

For the SEI(2) model the rate of infection ($\lambda 1$) at the first meal can be different from that at all subsequent meals. For all other models λ is identical for all ages. Units are daily for all models. Figures in parentheses adjacent to the estimate provide the 95% confidence interval (CI). We assume a fixed survival probability of 0.976/day, previously estimated via ML, from total count data only. If a CI spans 0, then that parameter is not significantly different from 0, when that parameter is added to a model already containing the other parameters. Lower AIC values within each species denote higher quality of model fit.
*Akaike information criterion.

vivax-type infection, respectively. If a fly's chances of acquiring an infection of one of the trypanosome species is independent of whether it is already infected with the other species, then the probability of finding a fly that is positive for both species is thus $0.0266 \times 0.0345 = 0.00092$ or about 1 in 1000 flies. Coinfections would be even rarer if infection with one species of trypanosome reduced the probability of acquiring an infection with another trypanosome species. Coinfections could be more prevalent than indicated above only if an infection with one species of trypanosome *increased* the probability of acquiring an infection with the other species. We are not aware of any suggestion in the literature that this might happen.

T. congolense *infections: the teneral effect*

Welburn and Maudlin (1992) found that laboratory *G. m. morsitans* were about 7-times more likely to acquire a *T. congolense* infection at their first meal than at any subsequent meal. By contrast, in *G. pallidipes* sampled in the field, we could not reject the null hypothesis of equality between the rate

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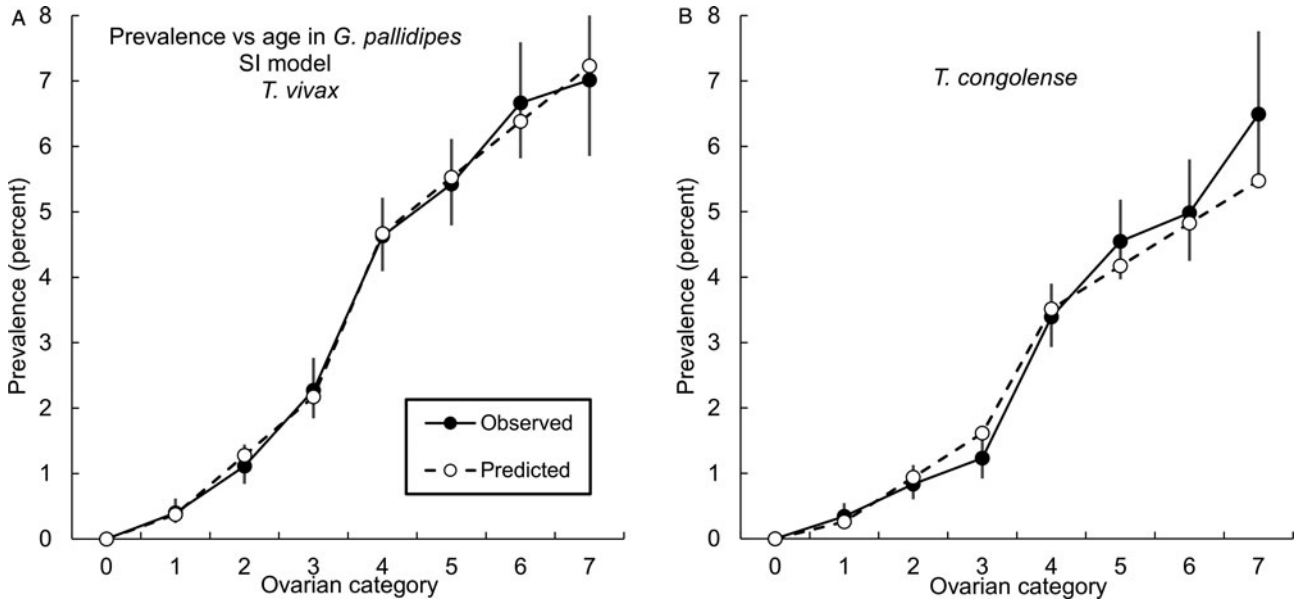

Figure 5. (A) Fitting age-specific percentage prevalences of **A.** *T. vivax* and **B.** *T. congolense* in female *G. pallidipes*, using a Susceptible-Infected (SI) model. Prevalences were calculated from the sums of the predicted numbers of infected and uninfected flies over successive 9-day pregnancy periods. It is assumed that negligible numbers of flies survive >15 ovulations. Error bars on observed prevalences are exact 95% confidence intervals for a binomial proportion.

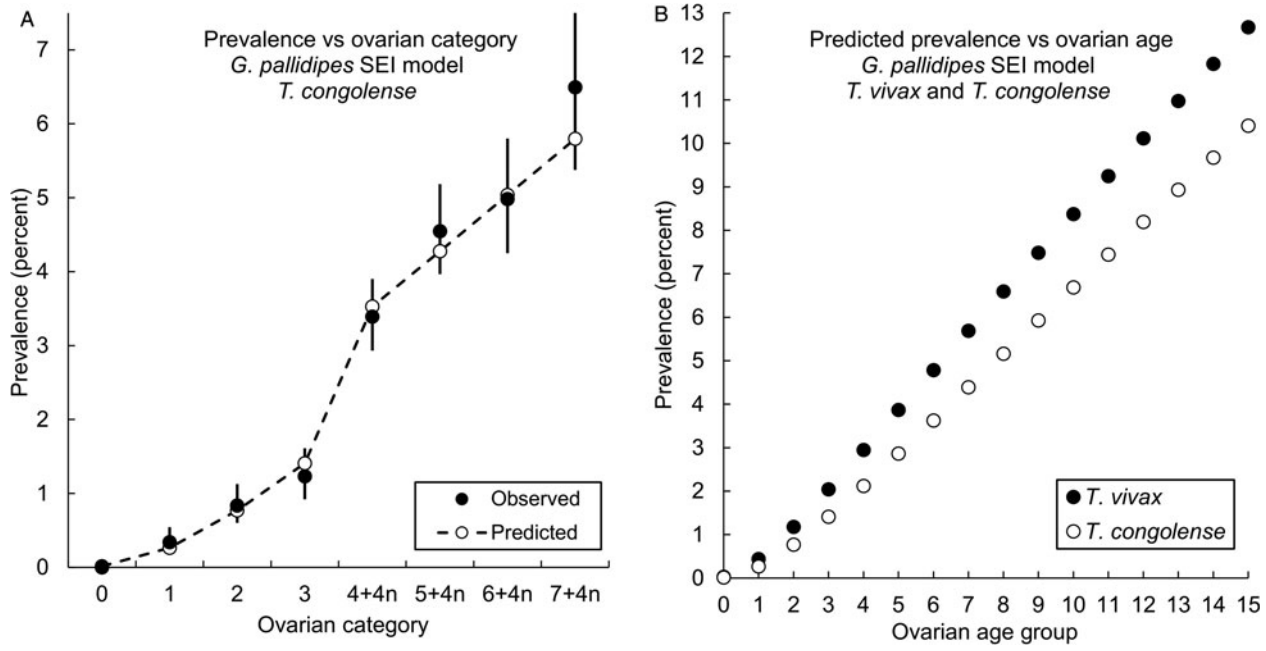

Figure 6. (A) Fitting age-specific percentage prevalences of *T. congolense* in female *G. pallidipes*, using a Susceptible-Exposed-Infected (SEI(1)) model. (B) Predicted prevalence for ovarian age groups 1–15, calculated from the sums of the predicted numbers of infected and uninfected flies over successive 9-day pregnancy periods. It is assumed that negligible numbers of flies survive >15 ovulations. Error bars on observed prevalences are exact 95% confidence intervals for a binomial proportion.

of infection for *T. congolense* at the first vs all later meals. Given the very large sample size available to us, and the excellent fit of the SEI(1) model to the data, we suggest that it might not even be feasible to separate $\lambda 1$ and λ using field data.

The following calculations suggest that, in any case, a teneral effect of the order estimated by Welburn and Maudlin (1992) would be of little importance even if it did occur – basically because of the longevity of tsetse, particularly females, and their need to feed every 2.5–3 days (Randolph *et al.*, 1992; Hargrove, 1999*a*, 1999*b*). Thus, suppose first that there were no teneral effect and that $\lambda = 0.00271$ at every meal, including the first (table 2, row 7). If, as we assume, a fly could live for 142 days (Hargrove *et al.*, 2011), we expect it to take about 47 blood meals. Its chances of remaining *uninfected* with *T. congolense*, throughout its life, is then estimated as $(1–0.00271)^{47} = 0.88$, and the probability that it is infected is $1–0.88 = 12\%$, in reasonable accord with the

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observed data and the predictions of our model (fig. 6B). Suppose, alternatively, that there is a teneral effect of the order suggested by Welburn and Maudlin (1992), such that $\lambda 1 = 7\lambda = 0.0190$. Then the estimated probability of remaining uninfected for 142 days is $(1–0.0190) \times (1–0.00271)^{46} = 0.87$ and the probability that the fly has become infected is 1–0.87 = 13%, only slightly higher than when there is no teneral effect. We thus find no support, at least in *G. pallidipes*, for Welburn and Maudlin's (1992) suggestion that bloodmeals other than the first are of no importance epidemiologically and conclude, conversely, that the so-called teneral effect is of minor importance in the epidemiology of *T. congolense* as transmitted by female *G. pallidipes*.

Trypanosome prevalence in vertebrate hosts

The probability (λ) that tsetse will mature a trypanosome infection after taking a bloodmeal is the product $\lambda = p1 \times p2$, where $p1$ is the probability that the bloodmeal they take is indeed infected with trypanosomes, and $p2$ is the conditional probability that a fly matures an infection given that it takes an infected meal. Results for the SEI(1) model (table 2, row 7) suggest $\lambda \approx 0.003$ per 3-day period (i.e., per bloodmeal) for *T. congolense* infections of female *G. pallidipes*. From Welburn and Maudlin (1992, their fig. 1) $p1$ for the first bloodmeal is about 0.7 and $p2$ for all later meals is about 1/7 of this value, i.e., $p2 \approx 0.1$. If $p2$ takes a similarly low value for non-teneral female *G. pallidipes* at Rekomitjie, this suggests that the proportion $p1$ that take a meal from a host infected with *T. congolense* is $p1 = \lambda/p2 = 0.003/0.1 = 0.03$. That is to say, of the hosts fed on by *G. pallidipes* at Rekomitjie, only about 3% are harbouring sufficient *T. congolense* to ensure that tsetse feeding off them take an infected meal. If that is the case then the proportion of flies developing a mature infection after their first meal is $\lambda = 0.03 \times 0.7 = 0.021$, or about 2.1% of the population. In fact, however, the 2% infection rate is only reached at the age of about 35 days (fig. 6A) – suggesting that $p1$ or $p2$, or both, are even lower than estimated.

For *T. vivax* our estimate for λ is also about 0.003 (table 2, line 3) but we do not have any estimate for $p2$. If it is markedly higher than for *T. congolense* then the proportion of animals with *T. vivax* infections is even lower than for *T. congolense*. For example, if $p2 = 0.5$ then $p1 = \lambda 2/p2 = 0.003/0.5 = 0.006$ and we predict that only 0.6% of vertebrate hosts would be harbouring sufficient *T. vivax* to provide an infective meal. Such low levels of infection in wild hosts support our suggestion that the teneral effect is unlikely to be important in the epidemiology of either *T. vivax* of *T. congolense*; the chances of becoming infected by a single meal is simply too small to explain the observed age-related changes in trypanosome prevalence.

Sex and species differences

In the current study we have considered only females of *G. pallidipes*, because there is no method available for gauging the chronological age of males. Nonetheless, the degree of wing fray in males does provide estimates of relative age, albeit with less accuracy than is associated with the use of ovarian dissection in females. We also have available less extensive data for age-specific trypanosome prevalences in female *G. m. morsitans* sampled at Rekomitjie. Future work will investigate whether similar models, to those developed here for female *G. pallidipes*, are also appropriate for the pattern of acquisition of trypanosome infection for male *G. pallidipes*, and for *G. m. morsitans*. Given that the teneral effect was suggested based on trypanosome infections acquired by *G. m. morsitans* in the laboratory, we need to decide, particularly, whether the effect is important in field populations of this tsetse species.

Data

All data used, and materials generated, in this publication are available from the authors on reasonable request.

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Author contributions. JWH designed the study, collated the data and carried out preliminary analyses. JVS carried out detailed statistical analysis. Both authors contributed equally to the preparation of the manuscript and the preparation of all figures and tables.

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Conflict of interest. The authors declare that they have no competing interests.

Ethical standards. Not applicable; the study does not involve mammalian subjects.

Consent for publication. Both authors gave consent for this publication. No other consent required.

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