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Transmission dynamics of lymphatic filariasis: vector-specific density dependence in the development of *Wuchereria bancrofti* infective larvae in mosquitoes

L. C. SNOW[1,2], M. J. BOCKARIE[3] and E. MICHAEL[1]

[1]Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College of Science, Technology and Medicine, London, [2]Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, New Haw, Addlestone, U.K. and [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

Abstract. The principles of meta-analysis developed in a previous study were extended to investigate the process of *Wuchereria bancrofti* (Cobbold) (Filarioidea: Onchocercidae) infection in mosquito (Diptera: Culicidae) hosts, focusing specifically on the functional forms and strength of density dependence in the development of ingested microfilariae (mf) to infective (third instar) larvae (L3). Mathematical models describing observed mf–L3 functional responses for each of the major three parasite-transmitting vector genera, *Aedes, Culex* and *Anopheles* mosquitoes, were fitted to paired mf–L3 data collated from all available studies in the published literature. Model parameters were estimated and compared by deriving and applying a data synthetic framework, based on applying a non-linear weighted regression model for fitting mathematical models to multistudy data. The results confirm previous findings of the existence of significant between-genera differences in the mf–L3 development relationship, particularly with regard to the occurrence of limitation in *Culex* mosquitoes and facilitation in *Aedes* and *Anopheles* mosquitoes. New and unexpected findings regarding L3 development from ingested mf were discovered as follows: (1) for *Culex*, overcompensation in L3 development at higher intensities of mf (or a peaked mf–L3 functional response) was detected; (2) for *Aedes* mosquitoes, facilitation (with an apparent asymptotic constraint on L3 development at high mf densities) was shown to be the major process governing L3 development, and (3) for *Anopheles,* a stronger facilitation type of response with no apparent saturation in L3 development appears to govern L3 output from ingested mf. These results yield major new insights regarding filarial vector infection dynamics and their potential impacts on parasite control, and demonstrate the efficacy of employing a data synthetic approach to reveal and estimate parasitic infection processes in host populations.

Key words. *Aedes*, *Anopheles*, *Culex*, *Wuchereria bancrofti*, density dependence, functional responses, infective stage larvae, lymphatic filariasis, meta-analysis, mosquitoes, non-linear mixed models, parasite transmission dynamics.

Introduction

The recent targeting of lymphatic filariasis for elimination as a global health problem has raised important questions concerning the role of low-density microfilaraemia in sustaining parasite transmission in communities during and following control programmes. A crucial observation is that mass drug treatment does not always clear microfilariae (mf) from the peripheral blood, leaving a small number of people with very low levels of infection (Mahoney & Kessel, 1971; Bryan & Southgate, 1976).

Correspondence: Edwin Michael, Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College of Science, Technology and Medicine, St Mary's Campus, Norfolk Place, London W2 1PG, U.K. Tel./fax: + 44 20 7594 3946; E-mail: e.michael@imperial.ac.uk

Resolving the role of such residual microfilaraemia in maintaining transmission has now become a pressing research need, particularly in the context of the global initiative to control the disease by mass chemotherapy. Epidemiological work has shown that the key to successfully resolving this issue is better quantitative understanding of infection processes in the mosquito vectors, and in particular how density-dependent processes in larval development might act synergistically or antagonistically with the currently recommended methods of control (Bryan & Southgate, 1976; Dye, 1992; Wada *et al.*, 1994; Subramanian *et al.*, 1998; Michael, 2002).

The development of transmission dynamics models for filarial diseases (Dietz, 1982; Chan *et al.*, 1998; Basanez & Boussinesq, 1999; Norman *et al.*, 2000; Michael, 2002; Michael *et al.*, 2004) means that quantitative tools are now available to study the complex relationships between human, parasite and vector populations, and to clarify how density-dependent factors in both the host and vector populations may contribute to observed population dynamics as well as the control of infection. One constraint of current models, however, is that parasite infection dynamics in the vector host are based on only one form of the functional relationship describing the mf to L3 development process (Plaisier *et al.*, 1998; Norman *et al.*, 2000). This is despite entomological evidence of genera-specific differences in the dynamics of larval infection in *Culex*, *Aedes* and *Anopheles* mosquitoes (Pichon *et al.*, 1974; Pichon, 1974; Southgate & Bryan, 1992). There is therefore a need for more explicit mechanistic models to inform and direct control measures in different endemic areas, which will depend upon a more precise quantification of the form and differences in the mf–L3 development relationship between these vectors.

Two types of functional relationships have been proposed for the relationship between the mean number of mf ingested and the mean number of L3 (or in some cases L1) that develop per mosquito. Limitation, thought to occur in *Culex* mosquitoes, describes an increase in the number of L3 developing with increasing mf intakes to either saturation or a decline (overcompensation) at high mf uptakes – a negative density-dependent process (Pichon, 1974; Southgate & Bryan, 1992; Subramanian *et al.*, 1998). By contrast, facilitation, thought to be important in *Anopheles* mosquitoes (Brengues & Bain, 1972; Pichon, 1974, 2002; Southgate & Bryan, 1992), can be thought of in ecological terms as an example of depensatory population dynamics (positive density dependence), arising when factors operating at low population densities cause a population to decline to extinction if depressed below some critical threshold (Myers *et al.*, 1995; Courchamp *et al.*, 1999; Chen *et al.*, 2001; Fowler & Ruxton, 2002; Regoes *et al.*, 2002). The significance for filariasis control is that this may lead to a non-zero threshold of infection in the host, below which the number of parasites recruited into the mosquito population will be too low to sustain transmission and infection will die out.

Although there is some evidence to support the operation of limitation, there are inconsistencies in the published results for all genera. The emphasis in the literature is on the examination of single datasets, and yet there is a large degree of variation between studies, which could reflect heterogeneities ranging from experimental to functional variations (Osenberg *et al.*, 1999). This means that it is still unclear if overall predictable patterns exist within and between vector genera in the mf–L3 relationship. For example, in previous investigations of the mf–L3 functional relationship in anopheline mosquitoes, which assessed the fits of linearized versions of potential mf–L3 development functions (Southgate & Bryan, 1992), only three of eight models fit observed mf–L3 datasets, highlighting the high degree of between-study variability in the available data. Recent advances in statistical ecology, however, have shown that the use of a synthetic approach to combining information from separate but similar studies may play a useful role in the detection and quantification of ecological processes by successfully accounting for the problems of single study heterogeneities (Arnqvist & Wooster, 1995; Myers & Mertz, 1998; Osenberg *et al.*, 1999; Gurevitch & Hedges, 2001; Myers, 2001). In particular, aggregated analysis may have great potential for the development of more powerful tests for differentiating between vector–host functional responses, as well as for facilitating more precise estimations of such processes (Myers & Mertz, 1998; Osenberg *et al.*, 1999; Myers, 2001; Snow & Michael, 2002).

Here we present the second part of our investigation into the population dynamics of filarial infection within vector mosquito populations based on a synthetic analysis of published and unpublished mosquito feeding experiments. This study has two specific aims. The first is to examine the existing data to determine and quantify the general form of the functional response between ingested mf and the development of L3 in each of the three major vector genera: *Culex* spp., *Aedes* spp. and *Anopheles* spp. The second is to test for significant differences in the general form and strength of density dependence between these vector genera. Following our modelling and statistical meta-analytical framework (Snow & Michael, 2002), we address both aims through a synthetic comparison of the statistical fits of simple, phenomenological, mathematical models, using relevant data from individual datasets for each mosquito genus.

Materials and methods

Sources of data used in the analysis

The investigation of the forms and degree of density dependence in the uptake and development of microfilariae by filarial vectors relied on information gathered from the published literature. The first stage in the analysis thus comprised a literature search for studies containing the required information. Electronic and manual literature searches yielded a total of 14 mosquito feeding experiments, consisting of studies on all three of the main filariasis transmitting genera (Table 1). Essential criteria for inclusion were that studies (1) used human volunteers infected with *Wuchereria bancrofti* (Cobbold), and (2) contained paired data on the human microfilarial density at the time of feeding and the corresponding mean number of L3 per mosquito at the end of the incubation period (approximately 10–14 days). One additional unpublished study using *Anopheles punctulatus* Dönitz was included due to the unusually high mf density of the human volunteers ($>$ 11 000 mf/mL [310 mf/20 μL]),

Table 1. Details of studies and data used in this analysis.

Author	Vector species	No. of mosquitoes dissected per mf density	Range of human mf densities studied	No. of mf densities studied*	Range of L3 per mosquito
Bockarie (unpublished)	*An. punctulatus*	72–152	2–11 150 mf/mL	3	0–5.13
Brito *et al.* (1997)	*Cx. quinquefasciatus*†	20–98	17–700 mf/mL	6	1–2.62
Bryan & Southgate (1988)‡	*An. arabiensis*	35–294	0–1140 mf/mL	14	0.01–0.81
	An. gambiae	80–276	0–1140 mf/mL	18	0–0.7
	An. melas	1–110	0–1140 mf/mL	8	0.06–1.37
Failloux *et al.* (1995)‡§	*Ae. polynesiensis*†	4–72	603–9231 mf/mL	6	2.7–18.81
Gasarasi (2000)	*Cx. quinquefasciatus*	20–35	120–1254 mf/mL	6	0.5–3.95
Gelfand (1955)	*An. gambiae*†	20–35	13–113 mf/20 μL	6	0.24–2.53
	An. melas†	24–51	20–116 mf/20 μL	4	0.34–3.92
Jordan & Goatly (1962)‡	*Cx. quinquefasciatus*†	Not given	10–471 mf/20 μL	21	1.02–7.21
Jayasekera *et al.* (1980)‡	*Cx. quinquefasciatus*†	7–50	26.5–222 mf/20 μL	12	1.3–6.4
	Cx. quinquefasciatus†	8–38	18–389 mf/20 μL	12	1.1–9.6
McGreevy *et al.* (1982)	*Cx. quinquefasciatus*†	21–140	1.3–2705 mf/20 μL	14	0–3
Rajagopalan *et al.* (1997)	*Cx. quinquefasciatus*	4–24	22–203 mf/20 μL	13	0–8.9
Rosen (1955)§	*Ae. polynesiensis*	13–138	0.4–555.1 mf/20 μL	23	0.05–21.7
Samarawickrema *et al.* (1985b)§*	*Ae. polynesiensis*†	15–271	1–5290 mf/mL	40	0–11.98
	Oc. samoanus¶	37–126	1–1449 mf/mL	18	0–4.28
	Ae. polynesiensis†	59–490	1–5290 mf/mL	29	0–10.8
Samarawickrema *et al.* (1985a)§	*Ae. samoanus*	93–296	1–1449 mf/mL	18	0–6.3
Sabry (1987)‡	*Cx. pipens molestus*	15–178	4–7343 mf/mL	21	0–17.01

*mf densities of human volunteers.
†Mosquitoes originated from laboratory colonies.
‡Studies containing actual observed ingested mf data.
§Subperiodic strains of *W. bancrofti* used.
¶Formerly *Ae. samoanus* and classed as such in this analysis.

enabling us to examine ranges of mf uptakes in *Anopheles* much higher than present in the published literature (Table 1). As before (Snow & Michael, 2002), a key criteria for study inclusion was also clarity regarding the parasitological and entomological procedures to ensure that the studies selected were uniform, as far as possible, in the experimental methods followed.

Standardization of human mf densities and estimation of mf numbers ingested by vectors

Unfortunately, most published studies contain only data on the mean numbers of L3 that develop per mosquito after feeding on volunteers with known mf densities, and fail to examine or record the number of mf ingested by mosquitoes immediately after feeding. Standardization was therefore required and performed in two stages. First, all human mf densities recorded in the studies were converted to mf per 20 μL, using a scaling factor for converting 1 mL of venous blood to 20 μL of fingerprick blood, as described in Snow & Michael (2002). This reduced variation caused by the use of different means of sampling human blood and also provided a better reflection of the likely numbers of mf ingested by mosquitoes as a result of ingesting blood from the peripheral circulation (Snow & Michael, 2002). Once the human mf densities had been standardized, we then used the previously estimated vector-specific functions for mf uptake by mosquitoes from feeding on human mf density per 20 μL (Snow & Michael, 2002) to calculate the mean number of mf that would be expected to be ingested per mosquito.

Analytical methods

Models for describing the functional relationship between mean L3 output and mean mf intake per mosquito. We compared and tested the fits of the following non-linear models to the observed relationships between the mean number of L3 and the predicted mean number of mf ingested per mosquito. Based on visual inspection of the raw data, we initially tested two hyperbolic models for describing a saturating or asymptotic limit to L3 development with increasing mf density. The first model, of the form:

$$y = \alpha x/(1 + (x/\beta)) \quad (1)$$

thus describes a simple saturating, limitation type of relationship, where x represents mean mf ingested per mosquito, y is mean L3 per mosquito and α and β are positive parameters describing the shape of the curve. Mechanistically, α may denote the density-independent number of L3 produced at low mf burdens, whereas β controls the degree of density dependence in the development of L3 as mf numbers increase. This model thus describes a larval development process, which allows for an increase in mean L3 numbers with increasing mean mf up to a saturation number of L3 produced at high mf intakes.

To test for facilitation in L3 development at low mf numbers, this model was modified to include a term for depensation to yield a sigmoid-type relationship, which allows for saturation in L3 development at high mf intakes as follows:

$$y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta)) \quad (2)$$

where parameters x, y, α and β are as described before and ς controls the rate of depensation in the development curve. If ς is ≤ 1 then there is no depensation and the curve is a simple saturation curve as described by equation 1. If ς is significantly > 1, then the development curve is sigmoid in shape, suggestive of positive density dependence or facilitation at low mf numbers (Myers *et al.*, 1995; Myers & Mertz, 1998; Myers, 2001).

We also fitted and tested a second set of models to determine if a loss in infective larvae occurred at high mf intakes due to either larval mortality or loss of infected mosquitoes. In particular, a Ricker-type model is used to describe the situation where there might be a decrease in L3 development at high mf intakes (Chen *et al.*, 2001; Myers, 2001):

$$y = \alpha x^{*}\exp(-\beta x) \tag{3}$$

where x and y are as before, and α is the slope at the origin. The rate of density-dependent mortality is given by the value of β. Where the data showed a limitation model to be the best fit (equation 1), we used this model to test for loss of larvae at high mf intakes. Where depensation was present (equation 2), equation 3 was modified to include a larval loss term as follows:

$$y = \alpha\beta x^{(1+\varsigma)}*\exp(-\beta x) \tag{4}$$

where α and ς are parameters defining the shape of the relationship as before and β defines the degree of density-dependent loss of larvae.

Model selection and parameterization. We employed the non-linear mixed effects model framework in order to fit and parameterize the models described in equations 1–4 above (Schall, 1991; Breslow & Clayton, 1993; Pinheiro & Bates, 2000; Venables & Ripley, 2002). This methodology was implemented using the 'nlme' function available within the software programme S-PLUS (Venables & Ripley, 2002), and the following general form of the non-linear mixed effects model:

$$y_{ij} = f(x_{ij}, \beta_{ij}) + \varepsilon_{ij} \tag{5}$$

where i is the index of each study used in the analysis, j represents the measurements (observed paired mean L3 [y_i] and mf [x_i] values) from each study, f is a general, real-valued differentiable (non-linear) function of the group or study specific parameter vector β_{ij} arising from the fit of each model (equations 1–4) to the covariate vector x_{ij} in each study, and ε_{ij} is a normally distributed within-group or study error term (Pinheiro & Bates, 2000; Venables & Ripley, 2002).

Our preliminary application of this mixed effects modelling approach to estimation, however, led to successful convergence only in the case of the data for *Culex*. The failures in convergence for the observed data for *Aedes* and *Anopheles* persisted despite using a range of starting values; this was most probably due to the paucity of the data within studies and the restricted ranges of x-values associated with them. As a consequence, we carried out the fitting of equations 1–4 using weighted non-linear least squares regression methods (Chambers & Hastie, 1992; Venables & Ripley, 2002). Model fits were carried out using the 'nls' function available within S-PLUS, and the following form of the non-linear regression model:

$$y_i = \eta(x_i, \beta) + \varepsilon_i \tag{6}$$

where y_i is mean L3 and x_i is a vector of covariates (paired mf here) for the i'th datum, β is a ρ-component vector of unknown parameters, and η is the known non-linear function (equations 1–4) of x and β, and ε_i is an $N(0,\sigma^2)$ error term (Bates & Watts, 1988; Venables & Ripley, 2002). The models were fit using pooled data for each genera. Between-study variation in the response function was incorporated in this formulation by simply weighting the data from each individual study, based on the product of the range of host mf densities observed and the number of densities studied, thus assigning greater weights to larger studies covering a broader range of mf intakes. Models were fit using the default Gauss–Newton algorithm in each case. Determination of the best-fit model (with or without density-dependent loss of larvae) was based on Akaike's information criterion values (AIC), with the best-fit models giving the lowest AIC values. Weighted AIC values and evidence ratios to guide model selection were calculated as in Burnham & Anderson (2002).

Addressing uncertainties. To deal with the non-normal distribution of our data due to aggregation of parasite counts within mosquitoes, bootstrap re-sampling techniques were used to estimate the 95% confidence intervals around the model parameters from the fitted weighted non-linear regression models (Venables & Ripley, 2002). A total of 1000 bootstrap samples were drawn from each relevant dataset to estimate the 95% bias corrected confidence intervals for each parameter of a given fitted model. In addition, the effect of measurement errors in predictor variables, which can lead to underestimation of the regression coefficients (Carroll *et al.*, 1995), was addressed in the analyses by simulating a 10% random error in the x variable values (using a rejection sampling method to restrict the simulated data to zero and positive x-values only), and comparing model fits and parameter estimates with and without error in the predictor variable. Average behaviour of the effects of measurement error in the predictor variable was determined by repeating the error simulations 1000 times for each model and obtaining the mean values and standard errors for each model parameter from the resultant parameter distributions arising from these simulations.

Results

Estimation of mf uptake

As described above, few of the published studies provided the required paired data on both mean mf ingested and mean L3 per mosquito for each individual batch of mosquitoes used in the reported feeding experiments (Table 1). This meant that it was necessary to estimate the mf intake for each batch of mosquitoes based on the host mf–vector infection uptake functions we had previously quantified for each vector genus (Snow & Michael, 2002). Figure 1 shows the vector-specific relationships between mean L3 per mosquito and both the actually observed and expected or estimated mean mf per mosquito for those five studies that provided raw data on mf intake (details of these studies are shown in Table 1). Although there is much variability, using expected mf intakes may not significantly distort the true mf–L3 development relationship observed in these studies for each

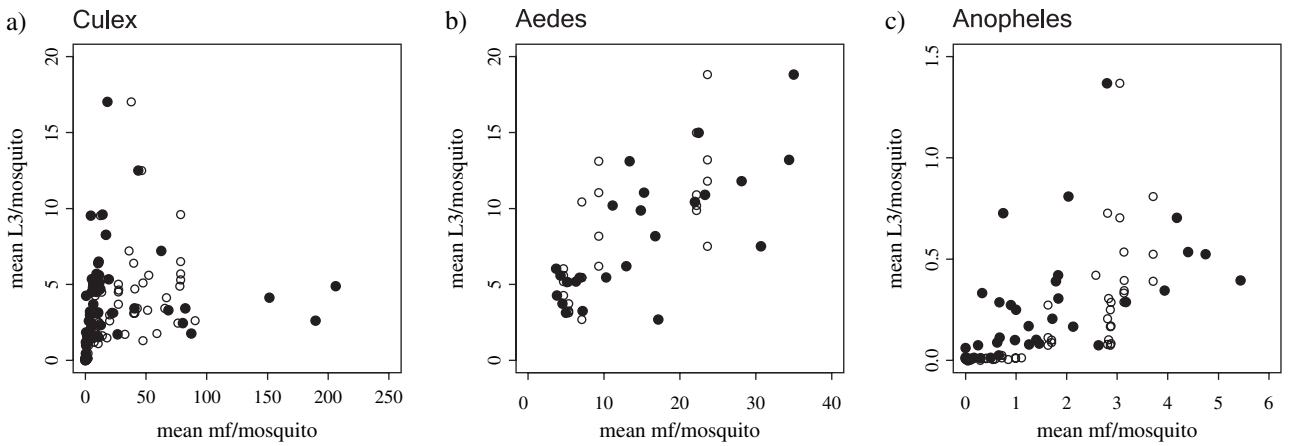

Fig. 1. Scatterplots of the relationships between mean mf ingested per mosquito and mean infective L3 larvae per mosquito based on observed (●) and predicted (○) mean numbers of mf ingested per mosquito for (a) *Culex*, (b) *Aedes* and (c) *Anopheles* mosquitoes. Details of studies providing observed data on the density of ingested mf from feeding on infected human volunteers are given in Table 1.

vector genus (Fig. 1). This was further supported by the results of a two-sample permutation-based *t*-test applied to these data that revealed no significant difference in the predicted and actual mf intake values for *Culex* and *Aedes* vectors ($P > 0.25$), and only a marginal statistical difference between the two mf datasets for *Anopheles* mosquitoes ($P = 0.035$). These high *P*-values and the large variability visible in actual values of mf uptake (Fig. 1) mean that using the predicted mf values is unlikely to overtly bias our proposed analysis in this study and indeed would more likely minimize the effects of data variability. All subsequent analyses of the mf–L3 relationship were therefore carried out using the predicted values of mf intake.

Fits of phenomenological mathematical models to observed mf–L3 functional relationships

To determine the best-fitting model for the mf–L3 functional response, we used an investigative framework, beginning with the fit of the simplest model, the base hyperbolic model describing a saturating limit to L3 development with increasing mf density (equation 1), followed by sequential assessments of the fits of the hyperbolic model with a depensatory term included (equation 2) and the corresponding more complex models describing a density-dependent decrease of L3 at high mf densities (equations 3 and 4). This framework of analysis allowed the

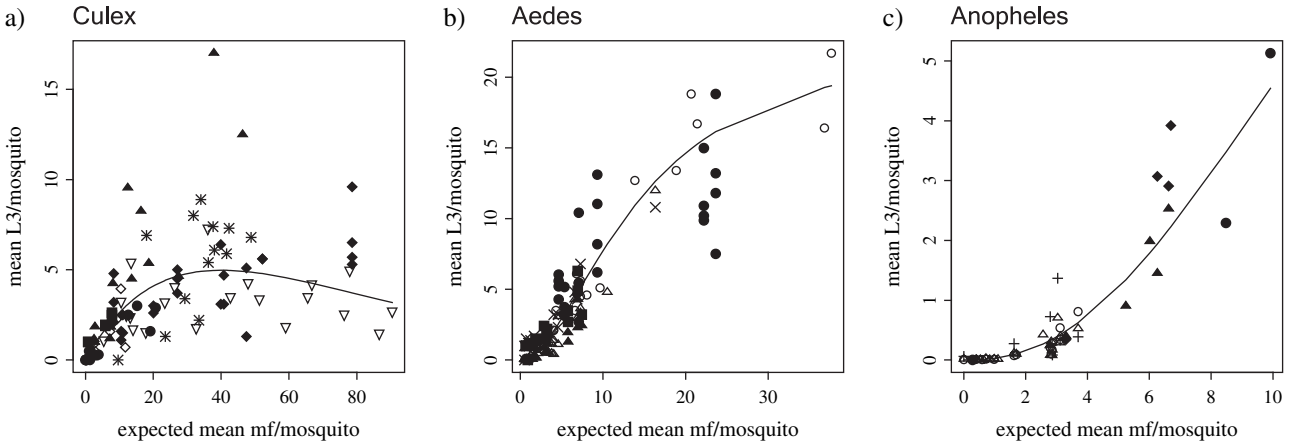

Fig. 2. The relationship between the mean numbers of L3 per mosquito and mean numbers of mf ingested per mosquito by study for (a) *Culex*, (b) *Aedes* and (c) *Anopheles* mosquitoes. The solid line in each graph represents the predictions of the best-fit non-linear model (Table 2) describing the functional form of the mf–L3 relationship for each genus, while the symbols portray the corresponding data in each case. Data sources: (a) ♦ = Jayasekera *et al.*, 1980, 1991; ∇ = Jordan & Goatly 1962; ◇ = Gasarasi (2000); ● = McGreevy *et al.*, 1982; ▲ = Sabry, 1987; ■ = Brito *et al.*, 1997; * = Rajagopalan *et al.*, (1977); (b) ● = Failloux *et al.*, 1995; Δ = Samarawickrema *et al.*, 1985a (*Ae. polynesiensis*); ■ = Samarawickrema *et al.*, 1985a (*Ae. samoanus*); + = Samarawickrema *et al.*, 1985b (*Ae. polynesiensis*); ▲ = Samarawickrema *et al.*, 1985b (*Ae. Samoanus*); ○ = Rosen (1955); (c) ○ = Bryan & Southgate, 1988 (*An. arabiensis*); Δ = Bryan & Southgate, 1988 (*An. gambiae*); + = Bryan & Southgate, 1988 (*An. melas*); ● = Bockarie (unpublished) (*An. punctulatus s.l*); ▲ = Gelfand, 1955 (*An. gambiae*); ♦ = Gelfand, 1955 (*An. melas*).

implementation of a 'model selection approach' to identifying the best model from the set of plausible candidate models for describing the mf–L3 functional response within each vector genus (Hilborn & Mangel, 1977; Burnham & Anderson, 2002; Johnson & Omland, 2004).

Figure 2(a–c) illustrates the patterns observed between mean L3 per mosquito and the corresponding predicted mean mf intake per mosquito for each of the three vector genera based on the collated data from each individual study (symbols in the graphs identify data from particular studies). The scatterplots highlight the differences in the ranges and levels of mean L3 and mf intakes covered by the data for each vector genus, but show clear graphical evidence of a systematic difference in the patterns of the mf–L3 functional relationship occurring between *Culex* mosquitoes on the one hand and *Aedes* and *Anopheles* mosquitoes on the other. Thus, while the data depicted in

Table 2. Results of fitting non-linear phenomenological models (equations 1–4) to paired mf–L3 data from each of the three vector genera. Parameter values from the best-fit model are shown along with corresponding bias corrected bootstrap confidence intervals. In addition, the non-linear mixed effects model fit to the *Culex* data is shown in brackets.

(a) *Culex*

Model	AIC	w(AIC)*	Test	Evidence ratio*
Equation 1 $y = \alpha x/(1 + (x/\beta))$	1352.619	0.023		
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$	1352.283	0.027	2v1	1.183†
Equation 3 $y = \alpha x * \exp(-\beta x)$	1345.163	0.950	3v1	41.596
Parameter	**Value**	**SE**	**Bca 95% CI**	
Equation 3 $y = \alpha x * \exp(-\beta x)$				
α	0.338 (0.278‡)	0.043 (0.034‡)	0.267–0.426 (0.211–0.345‡)	
β	0.025 (0.016‡)	0.003 (0.004‡)	0.020–0.030 (0.019–0.031‡)	

(b) *Aedes*

Model	AIC	w(AIC)*	Test	Evidence ratio*
Equation 1 $y = \alpha x/(1 + (x/\beta))$	1757.625	8.31×10^{-10}		
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$	1717.673	0.394	2v1	4.74×10^{8}*
Equation 4 $y = \alpha\beta x^{(1+\varsigma)} * \exp(-\beta x)$	1716.810	0.606	4v2	1.539§
Parameter	**Value**	**SE**	**Bca 95% CI**	
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$				
α	0.183	0.051	0.059–0.339	
β	125.109	28.820	77.68–262.586	
ς	1.803	0.138	1.409–2.371	

(c) *Anopheles*

Model	AIC	w(AIC)*	Test	Evidence ratio*
Equation 1 $y = \alpha x/(1 + (x/\beta))$	505.772	5.430×10^{-26}		
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$	390.94	0.468	2v1	8.619×10^{24}¶
Equation 4 $y = \alpha\beta x^{(1+\varsigma)} * \exp(-\beta x)$	390.684	0.531	4v2	1.137
Parameter	**Value**	**SE**	**95% CI****	
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$				
α	0.030	0.022	– 0.013–0.073	
β	482.568	262.536	– 29.377–944.513	
ς	2.352	0.476	1.394–3.31	

*Evidence ratio = Model 1 w(AIC)/Model 2 w(AIC), where akaike weights (w(AIC) are estimates of relative likelihoods of each model in question {exp(– ½Δ(AIC)} divided by the sum of the likelihoods of all competing models (Burnham & Anderson, 2002), is an information theoretic measure that quantifies how many times more likely a particular model is better than the one tested against. For example, equation 3, being approximately 42 times more likely than equation 1 in describing the present data, is the preferred model compared with equation 2 (which is only 1.2 times better).

†ς not significantly different from 1 ($\varsigma = 2.1549700$, t = 1.731800) (see text).

‡Non-linear mixed effects model parameter values (see text).

§ς significantly different from 1 ($\varsigma = 1.805690$, t = 13.01930).

¶ς significantly different from 1 ($\varsigma = 2.0326200$, t = 4.93892).

**Least squares confidence intervals given for this genus due to convergence problems in fitting the models to bootstrap samples.

Fig. 2(a) show that the larval infection pattern for *Culex* vectors is likely to represent a hyperbolic or saturating type of relationship between mf intake and L3 development, the corresponding data for *Aedes* and *Anopheles* provide evidence of a positive density-dependent sigmoidal type of relationship in this larval infection process (Fig. 2b, c).

Table 2 shows the results of fitting equations 1 and 2 first (i.e. the basic hyperbolic saturating and facilitation-type models, respectively) to the data for each vector genus, and then, based on these results, additional models that allow for density-dependent loss of larvae at high densities (equations 3 and 4). The AIC values for each fitted model are shown along with parameter values and the corresponding bias-corrected bootstrap confidence intervals of these values for the best-fit model for each of the three vector genera. Least squares confidence intervals are given for *Anopheles* due to convergence problems in fitting the models to the bootstrap samples, probably due to the small size and overly dispersed nature of the available dataset. The results in Table 2 clearly support the visual impressions portrayed in Fig. 2 of significant between-genera differences in the form of the relationship between mean L3 per mosquito and the corresponding mean mf per mosquito. The depensation term ς was highly significant for *Aedes* and *Anopheles* mosquitoes ($P < 0.05$), supporting the presence of positive density dependence (facilitation) in the mf–L3 relationship for these vectors (see predictions of the best-fitting facilitation-type model, (equation 2) as portrayed by the lines in Fig. 2(b, c)), whereas the same term was not significant for *Culex* mosquitoes (Table 2). For this vector, the results indicate that the best-fit model describes a negative density-dependent relationship, but also with a significant decrease in mean L3 at high mf intakes (Table 2, Fig. 2a). The dome-shaped curve embodied by equation 3 and depicted in Fig. 2(a) provides a significantly better fit than the predictions of equation 1 as determined by AIC values ($P < 0.05$). Visual inspection of the data for *Aedes* and *Anopheles* gave no indication of a similar decrease in mean L3 at the highest intakes (Fig. 2b, c), a result confirmed by the failure of model 4 (equation 4) to significantly improve the fit of the basic hyperbolic facilitation-type model (equation 2) to either of the datasets for these vectors (Table 2).

Whereas the relationship between mean L3 and mean mf ingested per mosquito was sigmoid in shape for both *Aedes* and *Anopheles* mosquitoes, the results also show that there is considerable difference in the parameter values of the best-fit depensation model (Table 2) between the two genera. Although the range of mf intakes estimated is far more restricted for *Anopheles* than *Aedes* or *Culex* mosquitoes, as a result of the strong density dependence in the uptake of mf from the human host in this vector (Snow & Michael, 2002), the parameter values for the depensation models indicate a much stronger regulation of L3 development at low mf intakes for *Anopheles* compared with *Aedes* vectors ($\varsigma_{\text{Aedes}} = 1.80$, $\varsigma_{\text{Anopheles}} = 2.35$). This can be observed in Fig. 3(a), which plots the data and the best-fit depensation model (equation 2) predictions for each of these two vector genera at low mf intakes (i.e. the region where facilitation or positive density dependence are thought to occur). The results clearly show that not only are L3 levels lower for *Anopheles* in this region, but the threshold mf density at which L3 output begins to occur is also higher for this vector compared with *Aedes* mosquitoes. Figure 3(b), which plots the paired mf and L3 data obtained for *Anopheles* at a similar mf intake range from three studies that gave observed data for these parasite stages, indicates that the sigmoid-type relationships observed in Figs 2(c) and 3(a) are unlikely to be artefacts of using predicted mf uptakes in our analysis, given that the observed data also portray a sigmoid-type relationship between mf density and L3 development at low mf uptakes. At the other end of the relationship (Fig. 2), whereas the maximum number of infective larvae that develops appears likely to saturate at around 20 L3 per mosquito in *Aedes* vectors, it is possible that saturation is never reached in *Anopheles* mosquitoes, as the maximum mf intake shown here (10 mf/mosquito) represents a human mf

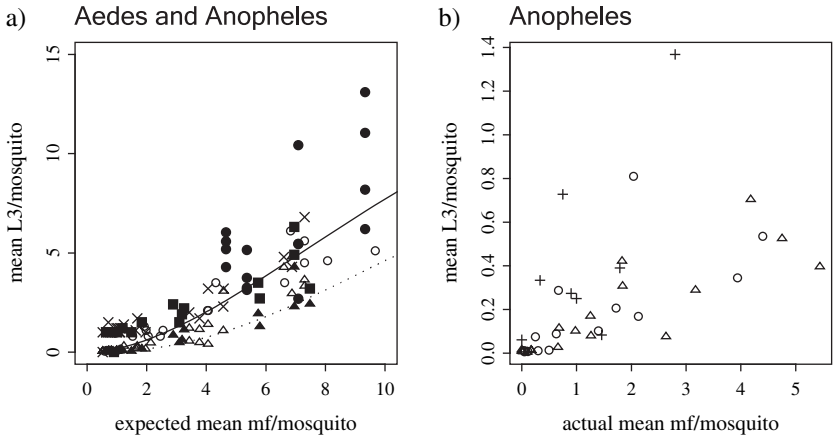

Fig. 3. Restricted axes plots showing (a) the observed and predicted differences in the initial facilitation stage of the mf–L3 relationship between *Aedes* and *Anopheles* mosquitoes. Symbols denote observed data while the lines represent the best-fit depensation model fits for both *Aedes (*solid line) and *Anopheles* (dashed line) (see Table 2). (b) The relationship between the observed mean numbers of mf ingested per mosquito and the mean number of L3 that develop for *Anopheles,* which illustrates that the estimated sigmoid-type relationship for this vector is unlikely to be an artefact of using predicted mean uptakes (see text). Symbols as in Fig. 2.

Table 3. Parameter values and 95% confidence intervals for the best-fit non-linear models described in Table 2 (equations 1–4) when fitted to data including 10% error in the mean uptake values.

(a) *Culex* Parameter	Value	Bca 95% CI
Equation 3 $y = \alpha x^{*}\exp(-\beta x)$		
α	0.303	0.242–0.371
β	0.023	0.019–0.028
(b) *Aedes*		
Parameter	**Value**	**Bca 95% CI**
Equation 2 $y = \alpha^{\varsigma}/(1 + (x^{\varsigma}/\beta))$		
α	0.123	0.037–0.242
β	181.051	109.059–429.240
ς	1.940	1.537–2.543
(c) *Anopheles*		
Parameter	**Value**	**Least squares 95% CI***
Equation 2 $y = \alpha x^{\varsigma}/(1 + (x^{\varsigma}/\beta))$		
α	0.257	0.218–0.296
β	610.641	– 164.106–1385.388
ς	2.377	1.377–3.377

*Least squares confidence intervals given for this genus due to convergence problems in fitting the models to bootstrap samples.

density of 11 150 mf/mL (310 mf/20 μL), an extremely high value for human infection in the field (compare Fig. 2b, c). By contrast, the maximum number of infective larvae predicted to develop in *Culex* is low (approximately five), a figure considerably lower than that predicted for *Aedes* (Fig. 2a).

The addition of 10% error in the *x* variable or to mf uptake values had no significant effect on the choice of best-fit model for any of the three vector genera. As shown in Table 3, the confidence intervals obtained for the depensation term when fitting equation 2 to data including 10% error in the mean mf uptake values did not include 1 for either *Aedes* or *Anopheles* mosquitoes, supporting the occurrence of a sigmoid-type functional relationship between mf uptake and L3 development for these vectors. Similarly, model 3 (equation 3) still proved the best fit to the *Culex* data (the β term significantly differed from 0), confirming a limitation-type relationship with overcompensation or loss of L3 at high mf uptakes for this mosquito vector (Table 3).

Discussion

The present study represents the second phase of our investigation into parasite infection processes among the mosquito vectors of lymphatic filariasis through the application of the principles of meta-analysis. The focus here has been on evidence for the presence and form of density dependence in the development of ingested mf to infective L3 stages within the mosquito host, extending our previous work on the uptake of mf from the human host (Snow & Michael, 2002). The results provide further evidence of the benefits of using this approach to estimate parasitic infection processes in host populations (Michael *et al.*, 1994; Poulin, 1996; Moore & Wilson, 2002; Snow & Michael, 2002). In particular, these results show that the major advantage of using such an approach is that by appropriately combining evidence across a range of similar studies, it may be possible to reduce or overcome the uncertainty or apparent variability surrounding a measured observation or biological relationship normally recorded in single studies. The problems encountered in our attempts to use the nlme function in S-PLUS software to fit the current mathematical models to grouped or multilevel data from separate individual studies, however, may reflect the need for further development of fitting algorithms for implementing non-linear multilevel random effects models, possibly via the use of empirical Bayes methods (Punt & Hilborn, 1997). The SAS procedure 'proc nlmixed' (Wolfinger, 1997) also failed to fit the present models. It is also clear from the between-study heterogeneity in the data available for the *Aedes* and *Anopheles* vectors (most probably arising from a mix of both paucity of data within studies and range restrictions in *x*-values between studies; Fig. 2b, c) that caution needs to be exercised when applying these meta-analytic techniques, as meta-analysis methods clearly do not provide a panacea for addressing excessive inherent biases or wide between-study heterogeneities in the available data. Indeed, a limitation of our present analysis has been the lack of data regarding infection, particularly in *Anopheles*, which almost certainly is the reason why estimation methods failed for this vector, both in relation to fitting the non-linear mixed model and bootstrap estimation of the confidence intervals for model parameters. This clearly suggests that more vector feeding studies need to be carried out over a wider range of human mf densities to gain a fuller understanding of vector–parasite infection dynamics as far as this genera is concerned.

We have attempted to partially overcome these problems of undertaking a synthetic analysis of model fits to the available mf–L3 data from different studies by deriving and implementing a simple, weighted, non-linear, least squares regression method. Although this formulation provides a simple mechanism for accounting for between-study variation, essentially by giving greater credence to larger studies covering a broader range of mf intakes, it basically fits a single mean response model to pooled data. This is in contrast to mixed effects modelling frameworks, which can not only account for random between-study effects in both the intercept and shape of functional responses while estimating mean responses from individual studies, but can also take explicit account of the hierarchical nature of such data (i.e. observations are correlated within study units) (Pinheiro & Bates, 2000; Everitt & Rabe-Hesketh, 2001). However, a comparison of results obtained by successfully fitting both the weighted non-linear regression and the non-linear mixed effects (as implemented by the nlme function) models to data for *Culex* mosquitoes (Table 2a) shows that the approximate weighted regression framework we employed in the present study is unlikely to drastically influence the overall findings of this investigation. As shown in Table 2, although parameter values were lower for the nlme formulation in comparison with those obtained from the weighted regression fits, the

observed differences were small (with overlapping confidence intervals) and did not affect the choice of the best-fit model for the data for this vector (the β parameter significantly differed from zero in both cases).

The application of these methods has clearly extended our understanding of the filarial mf–L3 development relationship. Thus, we have shown here for the first time the existence of a positive, non-linear peaked relationship between L3 output and mf uptake in *Culex* mosquitoes, in which strong overcompensation in L3 development begins to occur at higher intensities of ingested mf (above ∼ 40 mean mf/mosquito; Fig. 2a). Similarly, we have also presented clear evidence for the occurrence of facilitation-type relationships for this infection process for the first time in both *Aedes (*with an apparent asymptotic constraint on L3 development at high mf densities) and *Anopheles* (but with no apparent saturation in L3 development) vectors (Fig. 2b, c). While the finding for *Culex* mosquitoes augments previous demonstrations of the occurrence of a saturation or limitation type of function for the mf–L3 relationship in this vector (Prod'hon *et al.*, 1980; Sabry, 1987; Southgate & Bryan, 1992; Subramanian *et al.*, 1998), the evidence presented here for the occurrence of facilitation is less well supported in the literature, especially in the case of *Aedes*. These new findings clearly underscore the methodological advances made by using comprehensive data synthetic approaches that aim to determine and estimate ecological processes through combining data from multiple studies, in comparison with attempts to review evidence from different studies based on examining how often an outcome is statistically significant (Southgate & Bryan, 1992). As pointed out by Hedges & Olkin (1985), the results of the later exercise should be viewed with caution, as, rather than evaluating the importance of an ecological process, such analyses may simply examine the power of the tests used to detect it, which might thus encourage misleading conclusions.

Although we have presented evidence for the occurrence of significant facilitation-type relationships in the mf–L3 relationship for both *Aedes* and *Anopheles*, it is clear that the strength of this form of density dependence varies considerably between the two genera (Figs 2b, c and 3a). Not only are there much more severe constraints on parasite development at low mf values with a corresponding higher mf threshold at which L3 output occurs in *Anopheles* compared with *Aedes*, but there is another major difference in the lack of evidence for the occurrence of a saturation in L3 output as mf intake increases in *Anopheles*. Although this may reflect the lower range of mf densities studied for this mosquito genus (Fig. 2c), it is significant that, given the extremely strong density-dependent regulation of mf uptake in this vector (Snow & Michael, 2002), a saturation to L3 output is unlikely to be reached in *Anopheles* as the maximum mf intake shown in Fig. 2(c) (10 mf/mosquito) represents an extremely (and improbably) high value for human infection in the field (approximately 11 000 mf/mL). This means that despite the occurrence of a similar mf–L3 functional relationship, the development to L3 stage is much more efficient in *Aedes* than in *Anopheles*. Compared with the humped or peaked mf–L3 relationship estimated in this study for *Culex* mosquitoes, a corresponding density-dependent larval loss at higher mf intakes was not observed for either *Aedes* or *Anopheles*. The severe non-linear negative density dependence in L3 larval output, the relatively low maximum for L3 larval development (predicted to be about five; Fig. 2a) and the overcompensatory loss of larvae at high mf densities observed in *Culex* mosquitoes mean that *Aedes* mosquitoes are also likely to be more efficient than *Culex* mosquitoes at transmitting filariasis over comparable ranges of mf densities except at the very lowest (compare Figs 2a, b and 3a). Indeed, these results indicate that, except at very low mf uptakes or densities, *Aedes* mosquitoes may well be the most proficient of the major mosquito vectors implicated in transmitting lymphatic filariasis in the field. This is supported by the finding shown in Fig. 2c, which indicates that the maximum number of infective larvae that may develop in *Aedes* mosquitoes may reach up to 20 per mosquito, a number clearly higher than those predicted for *Culex* and most possibly *Anopheles* vectors (both likely to be around five per mosquito).

The infection of mosquitoes by filarial worms is likely to be mediated by many independent factors and the biological mechanisms that underlie the density-dependent processes operating in the mosquito vectors are thus likely to be complex (Basanez *et al.*, 1995). For example, negative density dependence such as that seen in *Culex* mosquitoes may arise as a result of larval competition for resources within the mosquito at high mf intakes or due to density-dependent mortality of the vector. While feeding experiments that measure mosquito survival at different mf uptake densities or compare larval densities in both dead and live mosquitoes at various times in the extrinsic incubation period in the vector (12–14 days) will ideally be required to differentiate the relative effects of these two factors, it is noteworthy that several researchers have highlighted the role that parasite-induced mortality might play in this process. Thus, Crans (1973) observed that mortality may be twice as high in *Culex quinquefasciatus* females infected with *W. bancrofti* compared with mosquitoes without infection, while Subramanian *et al.* (1998) reported a significantly higher percentage infected and intensity of infection among *Cx. quinquefasciatus* that died in their feeding experiments before 12 days compared with those recorded in the survivors. By contrast, factors that may underlie facilitation or the positive density dependence in L3 development observed for *Aedes* and *Anopheles* mosquitoes are thought to be many and include the interactions of ingested mf with cibarial teeth and peritrophic membrane, clotting agents and digestive enzymes acting in the bloodmeal and, intriguingly, factors that appear to operate at low mf intakes where parasites are more susceptible to insect host defences (Bain & Brengues, 1972; Brengues & Bain, 1972; Denham & & McGreevy, 1977; Townson & Chaithong, 1991; Southgate & Bryan, 1992).

The idea of an association between the presence of a cibarial armature and the operation of facilitation arises from evidence in which damage to ingested mf was found to be proportional to the degree of armature development in the respective vector (McGreevy *et al.*, 1978; Bryan & Southgate, 1988), which has traditionally implicated the operation of this phenomenon in vectors possessing well developed cibarial teeth, such as anophelines (Southgate & Bryan, 1992; Basanez *et al.*, 1995). The explanation of how the presence of cibarial teeth may produce positive feedback between mf intake and the number of infective larvae that develop revolves around the observation

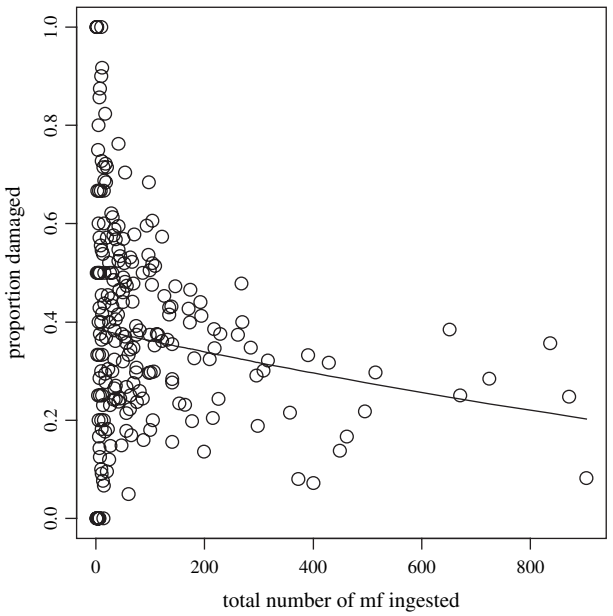

Fig. 4. The number of damaged mf found in the bloodmeal of *Anopheles punctulatus* as a proportion of the total number of mf ingested for each of 366 individual mosquitoes fed on infected human volunteers. The solid line represents the best-fit generalized linear logistic regression model fit to the data (regression slope $= -0.0010$, $P \leq 0.01$), indicating a significant, if weak, negative association between the two variables.

that, while it is possible for most mf to be ruptured by the teeth during low mf intakes, as more larvae are ingested the ones that become entangled may protect the remainder, which may then pass through undamaged (Bryan & Southgate, 1988). Figure 4 summarizes the results of a recent experiment (M. Bockarie, unpublished data) investigating the relationship between damage to mf and the total number of mf ingested obtained from bloodmeals of some 366 *An. punctulatus* mosquitoes fed on volunteers from Papua New Guinea. The results in the figure indicate that, while the proportion of mf damaged by the armature in this anopheline vector may overall be negatively dependent on host mf density, there may also be considerable variation surrounding the relationship, especially at the lower range of mf densities where the facilitation effect is likely to play a major role. This high variance suggests that although density-dependent damage of ingested mf by vectorial cibarial armature may play a role, this mechanism may not act to the exclusion of other factors, such as immunological defences against larvae, in underlying the observed phenomenon of facilitation in filarial vectors. Clearly the causal mechanisms that underlie facilitation and limitation in filarial vector infection dynamics need to be investigated further in an attempt to clarify and explain how and at what stage in the infection process larval mortality occurs in filarial vector populations.

The existence of extinction thresholds in filariasis epidemiology has been much discussed lately with regard to the presence of differences in the form and strength of density dependence between vectors and their implications for parasite transmission (Webber, 1991; Dye, 1992; Southgate & Bryan, 1992; Wada *et al.*, 1994; Pichon, 2002). The main conclusion is that the occurrence of positive density dependence or facilitation in the mf–L3 relationship (as found here in both the *Anopheles* and *Aedes* mosquitoes) would make elimination of filariasis by reducing infection in human hosts easier to achieve in areas where these vectors prevail than in areas affected by *Culex*-mediated parasite transmission (Pichon *et al.*, 1974; Webber, 1991; Zhang *et al.*, 1991; Southgate & Bryan, 1992; Pichon, 2002). In reality, however, to fully understand the control impact of these transmission functions, we must also consider the processes affecting the parasite in the human host (Anderson & May, 1985; Basanez *et al.*, 1995). These considerations imply that incorporation of the vector–parasite infection processes derived here for the mf–L3 stage (including addressing the data gaps that still surround the estimation of anopheline infection dynamics) and previously for the mf uptake stage (Snow & Michael, 2002) into existing transmission models for lymphatic filariasis (Norman *et al.*, 2000; Michael *et al.*, 2004), the mathematical analysis of these models, and, crucially, their application to observed field filariasis control data from endemic areas differing in each of the major transmitting vectors, constitute the necessary next steps to understanding how vector–parasite infection processes may govern parasite transmission in the field. The current worldwide initiative to control lymphatic filariasis means that carrying out these studies has now become urgent if we are to provide more realistic predictions of how parasite transmission processes may interact with the efforts presently being proposed to achieve global control of this disease, thereby contributing to the knowledge necessary for their ultimate success.

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References

Anderson, R.M. & May, R.M. (1985) Helminth infections of humans – mathematical models, population-dynamics and control. *Advances in Parasitology*, **24**, 1–101.

Arnqvist, G. & Wooster, D. (1995) Meta-analysis – synthesizing research findings in ecology and evolution. *Trends in Ecology and Evolution*, **10**, 236–240.

Bain, O. & Brengues, J. (1972) Transmission of wuchereriasis and of bovine setariasis: histological study of the passage of microfilariae through the stomach wall of *Anopheles gambiae* A and *Aedes aegypti*. [In French]. *Annales de Parasitologie Humaine et Compare*, **47**, 399–412.

Basanez, M.G. & Boussinesq, M. (1999) Population biology of human onchocerciasis. *Philosophical Transactions of the Royal Society of London B Series*, **354**, 809–826.

Basanez, M.G., Remme, J.H., Alley, E.S., Bain, O., Shelley, A.J., Medley, G.F. & Anderson, R.M. (1995) Density-dependent processes in the transmission of human onchocerciasis: relationship between the numbers of microfilariae ingested and successful larval development in the simuliid vector. *Parasitology*, **110**, 409–427.

Bates, D.M. & Watts, D.G. (1988) *Non-linear Regression Analysis and its Applications*. Wiley, New York.

Brengues, J. & Bain, O. (1972) Passage des microfilaires de l'estomac vers l'hemocele du vecteur, dans les couples *Wuchereria bancrofti–Anopheles gambiae* A, *Wuchereria bancrofti–Aedes aegypti* et *Setaria labiatopapillosa–Aedes aegypti*. *Cah. O.R.S.T.O.M. Serie Entomologie Medicale et Parasitologie*, **10**, 235–249.

Breslow, N.E. & Clayton, D.G. (1993) Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association*, **88**, 9–25.

Brito, A.C., Williams, P., Fontes, G. & Rocha, E.M.M. (1997) A comparison of two Brazilian populations of *Culex quinquefasciatus* (Say, 1823) from endemic and non-endemic areas to infection with *Wuchereria bancrofti* (Cobbold 1877). *Memorias do Instituto Oswaldo Cruz*, **92**, 33–36.

Bryan, J.H. & Southgate, B.A. (1976) Some observations on filariasis in Western Samoa after mass administration of diethylcarbamazine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 39–48.

Bryan, J.H. & Southgate, B.A. (1988) Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 2. Damage to ingested microfilariae by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 138–145.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multi-Model Inference: a Practical Information-Theoretic Approach*. Springer, New York.

Carroll, R.J., Ruppert, D. & Stefanski, L.A. (1995) *Measurement Error in Non-linear Models.* Chapman & Hall, London.

Chambers, J.M. & Hastie, T.J. (1992) *Statistical Models in S*. Chapman & Hall, London.

Chan, M.S., Srividya, A., Norman, R.A. et al. (1998) Epifil: a dynamic model of infection and disease in lymphatic filariasis. *American Journal of Tropical Medicine and Hygiene*, **59**, 606–614.

Chen, D.G., Irvine, J.R. & Cass, A.J. (2001) Incorporating Allee effects in fish stock-recruitment models and applications for determining reference points. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 242–249.

Courchamp, F., Clutton-Brock, T. & Grenfell, B. (1999) Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution*, **14**, 405–410.

Crans, J.W. (1973) Experimental infection of *Anopheles gambiae* and *Culex pipiens fatigans* with *Wuchereria bancrofti* in coastal east Africa. *Journal of Medical Entomology*, **10**, 189–193.

Denham, D.A. & McGreevy, P.B. (1977) Brugian filariasis: epidemiological and experimental studies. *Advances in Parasitology*, **15**, 244–296.

Dietz, K. (1982) The population dynamics of onchocerciasis. *Population Dynamics of Infectious Diseases* (ed. by R.M. Anderson), pp. 209–241. Chapman & Hall, London.

Dye, C. (1992) Does facilitation imply a threshold for the eradication of lymphatic filariasis? *Parasitology Today*, **8**, 109–110.

Everitt, B. & Rabe-Hesketh, S. (2001) *Analysing Medical Data using S-PLUS*. Springer, New York.

Failloux, A.B., Raymond, M., Ung, A., Glaziou, P., Martin, P.M.V. & Pasteur, N. (1995) Variation in the vector competence of *Aedes polynesiensis* for *Wuchereria bancrofti*. *Parasitology*, **111**, 19–29.

Fowler, M.S. & Ruxton, G.D. (2002) Population dynamic consequences of allee effects. *Journal of Theoretical Biology*, **215**, 39–46.

Gasarasi, D.B. (2000) The transmission dynamics of bancroftian filariasis: the distribution of the infective larvae of *Wuchereria bancrofti* in *Culex quinquefasciatus* and *Anopheles gambiae* and its effect on parasite escape from the vector. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **94**, 341–347.

Gelfand, H.M. (1955) Studies on the vectors of *Wuchereria bancrofti* in Liberia. *American Journal of Tropical Medicine and Hygiene*, **4**, 52–60.

Gurevitch, J. & Hedges, L.V. (2001) Meta-analysis. Combining results of independent experiments. *Design and Analysis of Ecological Experiments* (ed. by S.M. Scheiner & J.G. Urevitch), pp. 347–369. Oxford University Press, Oxford.

Hedges, L.V. & Olkin, I. (1985) *Statistical Methods for Meta-analysis*. Academic Press, London.

Hilborn, R. & Mangel, M. (1977) *The Ecological Detective: Confronting Models with Data*, Princeton University Press, Princeton.

Jayasekera, N., Curtis, C.F., Zielke, E., Kuhlow, F., Jansen, C.G. & Chelliah, R.V. (1980) The susceptibility of Liberian *Culex quinquefasciatus* to *Wuchereria bancrofti* in Sri Lanka. *Tropenmedizin und Parastiologie*, **31**, 507–511.

Jayasekera, N., Kalpage, K.S. & De Silva, C.S. (1991) The significance of low density microfilaraemia in the transmission of *Wuchereria bancrofti* by *Culex (Culex) quinquefasciatus* Say in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 250–4.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in Ecology and Evolution*, **19**, 101–108.

Jordan, P. & Goatly, K.D. (1962) Bancroftian filariasis in Tanganyika: a quantitative study of the uptake, fate and development of microfilariae of *Wuchereria bancrofti* in *Culex Fatigans*. *Annals of Tropical Medicine and Parasitology*, **56**, 173–187.

Mahoney, L.E. & Kessel, J.F. (1971) Treatment failure in filariasis mass treatment programmes. *Bulletin of the World Health Organization*, **45**, 35–42.

McGreevy, P.B., Bryan, J.H., Oothuman, P. & Kolstrup, N. (1978) The lethal effects of the cibarial and pharyngeal armatures of mosquitoes on microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 361–368.

McGreevy, P.B., Kolstrup, N., Tao, J., McGreevy, M.M. & Marshall, T.F. (1982) Ingestion and development of *Wuchereria bancrofti*. *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 288–296.

Michael, E. (2002) The epidemiology of lymphatic filariasis control. *World Class Parasites: the Filariae* (ed. by T.V. Rajan & T. Klei), pp. 41–48. Kluwer Academic Publishers, Amsterdam.

Michael, E. Grenfell, B.T. & Bundy, D.A. (1994) The association between microfilaraemia and disease in lymphatic filariasis. *Proceedings of the Royal Society of London Series B*, **256**, 33–40.

Michael, E., Malecela-Lazaro, M.N., Simonsen, P.E., Pedersen, E.M., Barker, G., Kumar, A. & Kazura, J.W. (2004) Mathematical modelling and the control of lymphatic filariasis. *Lancet Infectious Diseases*, **4**, 223–234.

Moore, S.L. & Wilson, K. (2002) Parasites as a viability cost of sexual selection in natural populations. *Science*, **297**, 2015–18.

Myers, R.A. (2001) Stock and recruitment: generalizations about maximum reproductive rate, density dependence, and variability using meta-analytic approaches. *ICES Journal of Marine Science*, **58**, 937–951.

Myers, R.A., Barrowman, N.J., Hutchings, J.A. & Rosenberg, A.A. (1995) Population dynamics of exploited fish stocks at low population levels. *Science*, **269**, 1106–1108.

Myers, R.A. & Mertz, G. (1998) Reducing uncertainty in the biological basis of fisheries management by meta-analysis of data from many populations: a synthesis. *Fisheries Research*, **37**, 51–60.

Norman, R.A., Chan, M.S., Srividya, A. *et al*. (2000) EPIFIL: the development of an age-structured model for describing the transmission

dynamics and control of lymphatic filariasis. *Epidemiology and Infection*, **124**, 529–541.

Osenberg, C.W., Sarnelle, O., Cooper, S.D. & Holt, R.D. (1999) Resolving ecological questions through meta-analysis: goals, metrics and models. *Ecology*, **80**, 1105–1117.

Pichon, G. (1974) Relations mathématiques entre le nombre des microfilares ingérées et le nombre des parasites chez différents vecteurs naturels ou expérimentaux de filarioses. *Cah. O.R.S.T.O.M., Serie Entomologie Medicale et Parasitologie*, **4**, 199–216.

Pichon, G. (2002) Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against *Anopheles*-transmitted filariasis. *Annals of Tropical Medicine and Parasitology*, **96**(Suppl 2), 143–152.

Pichon, G., Perrault, G. & Laigret, J. (1974) Rendement parasitaire chez les vecteurs de filarioses. *Bulletin of the World Health Organization*, **51**, 517–524.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed Effects Models in S and S-PLUS*. Springer, New York.

Plaisier, A.P., Subramanian, S., Das, P.K. *et al*. (1998) The LYMFASIM simulation program for modelling lymphatic filariasis and its control. *Methods on Informatics in Medicine*, **37**, 97–108.

Poulin, R. (1996) Sexual inequalities in Helminth infections: a cost of being a male? *American Naturalist*, **147**, 287–295.

Prod'hon, J., Pichon, G., Rivière, F., DeJardin, J., Géry, M., Doué, F., Faugère, C. & Verneuil, M. (1980) Étude quantitative de la reduction parasitaire stomacale chez les vecteurs de filaroises. *Cah. O.R.S.T.O.M., Serie Entomologie Medicale et Parasitologie*, **18**, 13–25.

Punt, A.E. & Hilborn, R. (1997) Fisheries stock assessment and decision analysis: the Bayesian approach. *Reviews in Fish Biology and Fisheries*, **7**, 35–63.

Rajagopalan, P.K., Kazmi, S.J. & Mani, T.R. (1997) Some aspects of transmission of *Wuchereria bancrofti* and ecology of the vector *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research,* **66**, 200–215.

Regoes, R.R. Ebert, D. & Bonhoeffer, S. (2002) Dose-dependent infection rates of parasites produce the Allee effect in epidemiology. *Proceedings of the Royal Society of London Series B*, **269**, 271–279.

Rosen, L. (1955) Observations on the epidemiology of human filariasis in French Oceania. *American Journal of Hygiene*, **61**, 219–248.

Sabry, M. (1987) A study of the quantitative relationship between the blood density of *Wuchereria bancrofti* microfilariae and development of infective larvae in *Culex pipiens molestus* in Egypt. PhD Thesis, Faculty of Medicine, University of London.

Samarawickrema, W.A., Spears, G.F.S., Sone, F., Ichinori, K. & Cummings, R.F. (1985a) Filariasis transmission in Samoa. II. Some factors related to the development of microfilariae in the intermediate host. *Annals of Tropical Medicine and Parasitology*, **79**, 101–107.

Samarawickrema, W.A., Spears, G.F.S., Sone, F., Ichinori, K. & Cummings, R.F. (1985b) Filariasis transmission in Samoa. I. Relation between density of microfilariae and larval density in laboratory-bred and wild-caught *Aedes (Stegomyia) polynesiensis* (Marks) and wild-caught Aedes (Finlaya) samoanus (Gruenberg). *Annals of Tropical Medicine and Parasitology*, **79**, 89–100.

Schall, R. (1991) Estimation in generalized linear models with random effects. *Biometrika*, **78**, 719–727.

Snow, L.C. & Michael, E. (2002) Transmission dynamics of lymphatic filariasis: density-dependence in the uptake of *Wuchereria bancrofti* microfilariae by vector mosquitoes. *Medical and Veterinary Entomology*, **16**, 409–423.

Southgate, B.A. & Bryan, J.H. (1992) Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 523–530.

Subramanian, S., Krishnamoorthy, K., Ramaiah, K.D., Habbema, J.D.F., Das, P.K. & Plasier, A.P. (1998) The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology*, **116**, 243–255.

Townson, H. & Chaithong, U. (1991) Mosquito host influences on development of filariae. *Annals of Tropical Medicine and Parasitology*, **85**, 149–163.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer, New York.

Wada, Y., Kimura, E., Takagi, M. & Tsuda, Y. (1994) Facilitation in *Anopheles* and spontaneous disappearance of filariasis: has the concept been verified with sufficient evidence? *Tropical Medicine and Parasitology*, **46**, 27–30.

Webber, R.H. (1991) Can Anopheline-transmitted filariasis be eradicated? *Journal of Tropical Medicine and Hygiene*, **94**, 241–244.

Wolfinger, R.D. (1997) Comment: Experiences with the SAS macro NLINMIX. *Statistical Methods in Medical Research*, **16**, 1258–1259.

Zhang, S., Zhang, Q., Cheng, F., Wang, L. & Pen, G. (1991) Threshold of transmission of *Brugia malayi* by *Anopheles sinensis*. *Journal of Tropical Medicine and Hygiene*, **94**, 245–250.

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