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Transmission dynamics of lymphatic filariasis: density-dependence in the uptake of *Wuchereria bancrofti* microfilariae by vector mosquitoes

L. C. SNOW and E. MICHAEL
Department of Infectious Disease Epidemiology, Imperial College of Science Technology and Medicine, University of London, U.K.

Abstract. Gaining a better understanding of parasite infection dynamics in the vector mosquito (Diptera: Culicidae) population is central to improving knowledge regarding the transmission, persistence and hence control of lymphatic filariasis. Here, we use data on mosquito feeding experiments collated from the published literature to examine the available evidence regarding the functional form of the first component of this parasite–vector relationship for *Wuchereria bancrofti* (Filarioidea: Onchocercidae) causing Bancroftian filariasis, i.e. the rate of microfilariae (mf) uptake from the blood of infected humans by the feeding mosquito vector. Using a simple logarithmic regression model for describing the observed relationships between the mean numbers of mf ingested per mosquito and parasite load in humans in each study, and a linear mixed-effects meta-analytical framework for synthesizing the observed regressions across studies, we show here for the first time clear evidence for the existence of density-dependence in this process for all the three major filariasis transmitting mosquito vectors. An important finding of this study is that this regulation of mf uptake also varies significantly between the vector genera, being weakest in *Culex*, comparatively stronger in *Aedes* and most severe and occurring at significantly lower human mf loads in *Anopheles* mosquitoes. The analysis of the corresponding mf uptake prevalence data has further highlighted how density-dependence in mf uptake may influence the observed distributions of mf in vector populations. These results show that whereas strong regulation of mf uptake, especially when it leads to saturation in uptake at low human parasite intensities, can lead to static distributions of mf per mosquito with host parasite intensity, a weaker regulation of mf ingestion can give rise to changes in both mean mf loads and in the frequency distribution of parasites/mosquito with increasing human parasite intensity. These findings highlight the importance of considering local vector infection dynamics when attempting to predict the impacts of community-based filariasis control. They also emphasize the value of developing and applying robust meta-analytic methods for estimating functional relationships regarding parasitic infection from population ecological data.

Key words. *Aedes*, *Anopheles*, *Culex*, *Wuchereria bancrofti*, density-dependence, filariasis control, linear mixed-effects models, logarithmic regression, lymphatic filariasis, meta-analysis, microfilariae, mosquitoes, negative binomial model, parasite transmission dynamics, prevalence.

Correspondence: Lucy Snow, 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. E-mail: lucy.snow@ic.ac.uk

Introduction

Gaining a quantitative understanding of parasite infection dynamics in the vector mosquito population is a key requirement to improving knowledge regarding the transmission, persistence and control of lymphatic filariasis (Bryan & Southgate, 1976; Dye, 1992; Wada *et al*., 1994; Subramanian *et al*., 1998; Michael, 2002). Despite this, and in spite of a significant body of work in this area (Samarawickrema *et al*., 1985; Bryan & Southgate, 1988a,b; Bryan *et al*., 1990; Southgate & Bryan, 1992; Subramanian *et al*., 1998), the forms of the various components of the macroparasite-vector infection process, including uptake of microfilaria (mf) from the human host, development of mf to the infective stage larvae (L3), and vector mortality rate, within (and between) the three major vector genera, i.e. *Culex*, *Aedes* and *Anopheles* spp., transmitting filariasis in endemic regions are still not fully worked out. A systematic and accurate quantification of these processes has now become urgent, given the current global initiative for controlling filariasis transmission by mass chemotherapy (Ottesen *et al*., 1997). For example, current models of filariasis transmission and control presume a single functional relationship for the parasite–vector infection process, whereby the number of infective larvae (L3) that develop in the vector population is inversely related to the number of mf ingested (Plaisier *et al*., 1998; Norman *et al*., 2000), which not only assumes the operation of a single infection process but also overlooks potential between-vector differences in the dynamics of larval infection (Pichon, 1974; Pichon *et al*., 1974; Southgate & Bryan, 1992).

A fundamental question in the analysis of any parasitic infection process also relates to the role and impact of density-dependent mechanisms controlling parasite population size or growth (Dietz, 1988). Such regulatory processes, whereby vital rates (e.g. larval uptake and development and vector mortality rates) are influenced by (parasite) population density, have the potential to stabilize and hence promote the long-term persistence of infection (Dietz, 1988). As they also make parasite population dynamics become nonlinear, the presence of these processes can additionally act either antagonistically or synergistically with the current recommended methods of control (Dietz, 1988; Dye, 1992; Wada *et al*., 1994). A further necessary requisite for the analysis of these processes in vector-borne infections is also to quantify precisely where and at what magnitude in such complex life cycles regulation operates, as overall population regulation may depend on the occurrence and relative strengths of stabilizing forces acting on different stages of such life cycles (Dietz, 1988; Hellriegel, 2000; Neubert & Caswell, 2000).

Several studies in the past have attempted to investigate the functional forms of the three major filarial parasite-vector infection processes, i.e. mf uptake, L3 development and mosquito mortality rate (see examples for uptake studies in Table 1). Although these studies have provided valuable information regarding filarial infection of mosquitoes, particularly with respect to possible vector differences in the survival and development of ingested mf to L3, i.e. a likely proportional development of L3 from ingested mf in the *Brugia malayi–Mansonia bonnae* parasite/vector complex, a positive density-dependent mf/L3 relationship in some *Wuchereria bancrofti–Anopheles* combinations, and a negative density-dependent or limiting relationship for this process for the *W. bancrofti–Culex quinquefasciatus* complex (Pichon, 1974; Southgate & Bryan, 1992), a major obstacle to drawing firm conclusions regarding these results has been the finding of significant between-study variation in observed relationships – in terms of both process and sampling heterogeneity (see below; Southgate & Bryan, 1992) – and the lack so far of a more robust synthetic analysis of the data from individual studies. The latter is a particularly glaring gap, as an aggregated analysis of data from single studies, when properly carried out, has the potential for allowing patterns to be determined that may not be detected otherwise (Michael *et al*., 1994; Gurevitch & Hedges, 2001; Myers, 2001). This is highlighted by the recent applications of new approaches based on the principles of meta-analysis to population ecology (Arnqvist & Wooster, 1995; Myers *et al*., 1997; Myers & Mertz, 1998; Gurevitch & Hedges, 2001; Myers, 2001), including helminth epidemiology (Michael *et al*., 1994; Poulin, 1996), which have demonstrated the value of such methods for resolving complex ecological questions via the efficient synthesis of information from separate but similar studies.

In this paper we present the first part of our investigations into the population dynamics of filarial infection within vector mosquito populations based on the meta-analyses of existing data in the published literature. We focus here on the first component of the parasite–vector relationship: the rate of uptake of mf from the blood of infected human hosts by the feeding mosquito vector. The aims of this study are primarily twofold: (i) to determine the overall structure, and particularly evidence for density-dependence, of this parasite-vector infection process, and (ii) to compare the forms of the uptake relationship across the three major mosquito vector genera: *Culex*, *Aedes* and *Anopheles*. We also illustrate the use of the multilevel modelling approach to the meta-analysis of ecological data as a research tool for the efficient summation and comparison of the uptake relationships across studies and vector genera (Goldstein, 1995; Smith *et al*., 1995; Stram, 1996).

Materials and methods

Our analysis in essence entailed: (1) compiling all the relevant published data on the uptake of mf from infected humans by feeding mosquitoes belonging to the three major vector genera; (2) developing and applying simple models to describe the mf uptake relationship within studies; and (3) using multilevel modelling methods based on linear mixed models to combine and compare results across the various studies. Specific methods employed for each of the above stages are as follows.

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

Reference	Vector species	Blood sampling technique	No. mf densities studied	Mean human mf density (range)	No. mosquitoes dissected per mf density	Mean mf per mosquito (range)
Bryan & Southgate (1988a)	*An. arabiensis*	1 mL venous blood	18	285.9 mf/mL (0–1140)	12–50	1.022 (0–4.4)
	An. gambiae	1 mL venous blood	20	312.1 mf/mL (0–1140)	16–50	1.465 (0–5.44)
	An. melas	1 mL venous blood	11	434.6 mf/mL (0–1140)	4–20	1.066 (0–2.8)
Calheiros *et al.* (1998)	*Ae. aegypti*	1 mL venous blood	4	1280 mf/mL (906–1830)	Not given	5.75 (3.5–7.2)
	Cx. quinquefasciatus	1 mL venous blood	4	1280 mf/mL (906–1830)	Not given	18.3 (8.8–26.2)
Failloux *et al.* (1995)	*Ae. polynesiensis*	1 mL venous blood	24	3749 mf/mL (603–9231)	14–45	15.81 (4.2–38.4)
Gubler *et al.* (1973)	*Cx. quinquefasciatus*	20 μL fingerprick blood	14	330.9 mf/20 μL (3–669)	4–10	78.5 (7.6–184.7)
Jayasekera *et al.* (1991)*	*Cx. quinquefasciatus*	1 mL venous blood	4	97 mf/mL (2.5–323)	44–390	2.61 (0.5–5.55)
Jordan & Goatly (1962)	*Cx. quinquefasciatus*	20 μL fingerprick blood	17	178.1 mf/20 μL (25–471)	19–25	63.6 (5.4–206.2)
Lowrie *et al.* (1989)	*Cx. quinquefasciatus*	1 mL venous blood‡	21	9 mf/mL (1–28)	3–100	1.529 (0.2–4)
McGreevy *et al.* (1982)*	*Ae. aegypti*	200–600 μL fingerprick blood†	28	235.3 mf/mL (1–1395)	8–42	2.85 (0–15.94)
	An. gambiae	200–600 μL fingerprick blood†	16	376.6 mf/mL (1–2667)	9–90	1.298 (0–7.56)
	Cx. quinquefasciatus	200–600 μL fingerprick blood†	17	204.2 mf/mL (1.3–1395)	19–68	3.996 (0–19.53)
Samarawickrema *et al.* (1985)	*Ae. polynesiensis*	1 mL venous blood	8	449.2 mf/mL (5–1464)	24–70	1.145 (0.02–5.92)
	Ae. samoanus	1 mL venous blood	8	459.8 mf/mL (5–1542)	33–82	0.838 (0.01–2.73)
Sabry (1987)	*Cx. pipiens molestus*	1 mL venous blood	21	1283 mf/mL (4–7343)	50	5.346 (0–43.66)

*Mosquitoes used in the experiments were from laboratory colonies.
†Counting chamber; mf uptake data from these studies were in the range seen for 1 mL venous blood sampling and hence considered as belonging to this group in the analyses described in the text.
‡Knott technique.

Sources of data

The present analyses required paired information on human host mf density and the corresponding ingested parasite load and prevalence in samples of feeding mosquitoes. An extensive literature survey located a total of 10 studies containing such data suitable for analysis (Table 1). All studies are mosquito feeding experiments conducted on human hosts infected with *W. bancrofti*. It was decided not to include studies using *Brugia* spp., non-human hosts or those investigations using artificial feeding techniques, both due to the paucity of such studies and because of incomplete information on how the latter factors may influence uptake. All selected studies contained data on host mf density at the time of feeding and results of mosquito dissections. All studies also contained data on at least three host mf densities. The studies covered all three major mosquito vector genera.

It was important that enough information was given in the paper for the parasitological and entomological methods used to be understood and evaluated, as a key criteria for study selection was uniformity as far as possible in the experimental methods followed. Thus, in all cited studies, mosquitoes were fed on human volunteers with known mf densities as determined by either stained slide or nucleopore filtration at the time of feeding (using fingerprick and venous blood, respectively). Mosquito dissection was carried out immediately following feeding with the mosquitoes cooled on ice to retard penetration of mf through the midgut, or frozen at −10°C and dissected the following morning (Gubler *et al.*, 1973; Jayasekera *et al.*, 1980). In such instances, dissection was carried out by removing the mosquito midgut and dissecting out the bloodmeal. In some studies, however, the whole mosquito was dissected in order not to miss any larvae that might have already passed out of the midgut (Gubler *et al.*, 1973; Lowrie *et al.*, 1989).

Other relevant details of the studies selected and used are given in Table 1.

Standardization of human mf density in blood samples

Except for two studies involving *C. quinquefasciatus*, which used 20 μL fingerprick blood for making thick smear slides, the rest of the studies selected for analysis predominantly employed the method of membrane filtration of 1 mL of venous blood to estimate mf density in infected human volunteers (Table 1). Because mosquitoes ingest blood from the peripheral circulation, an essential scaling requirement was the conversion of all host mf densities estimated using the more sensitive filtration method to reflect numbers expected using the smaller peripheral blood volume of 20 μL of fingerprick blood. This necessitated estimating the likely relationship between parasite densities estimated using the 20 μL fingerprick and 1 mL venous blood samples (for the stained thick smear and nucleopore filtration diagnostic methods, respectively). A search of the literature revealed five studies that provided data on parallel mf counts in paired samples of 20 μL fingerprick and 1 mL venous blood taken from the same individual (see legend to Fig. 1), and we use these data here to estimate the conversion factor for performing the required data transformation. Specifically, this was done through the estimation of the regression relationship between the observed parallel parasite counts obtained in these studies using the two methods. Estimation of the global regression function

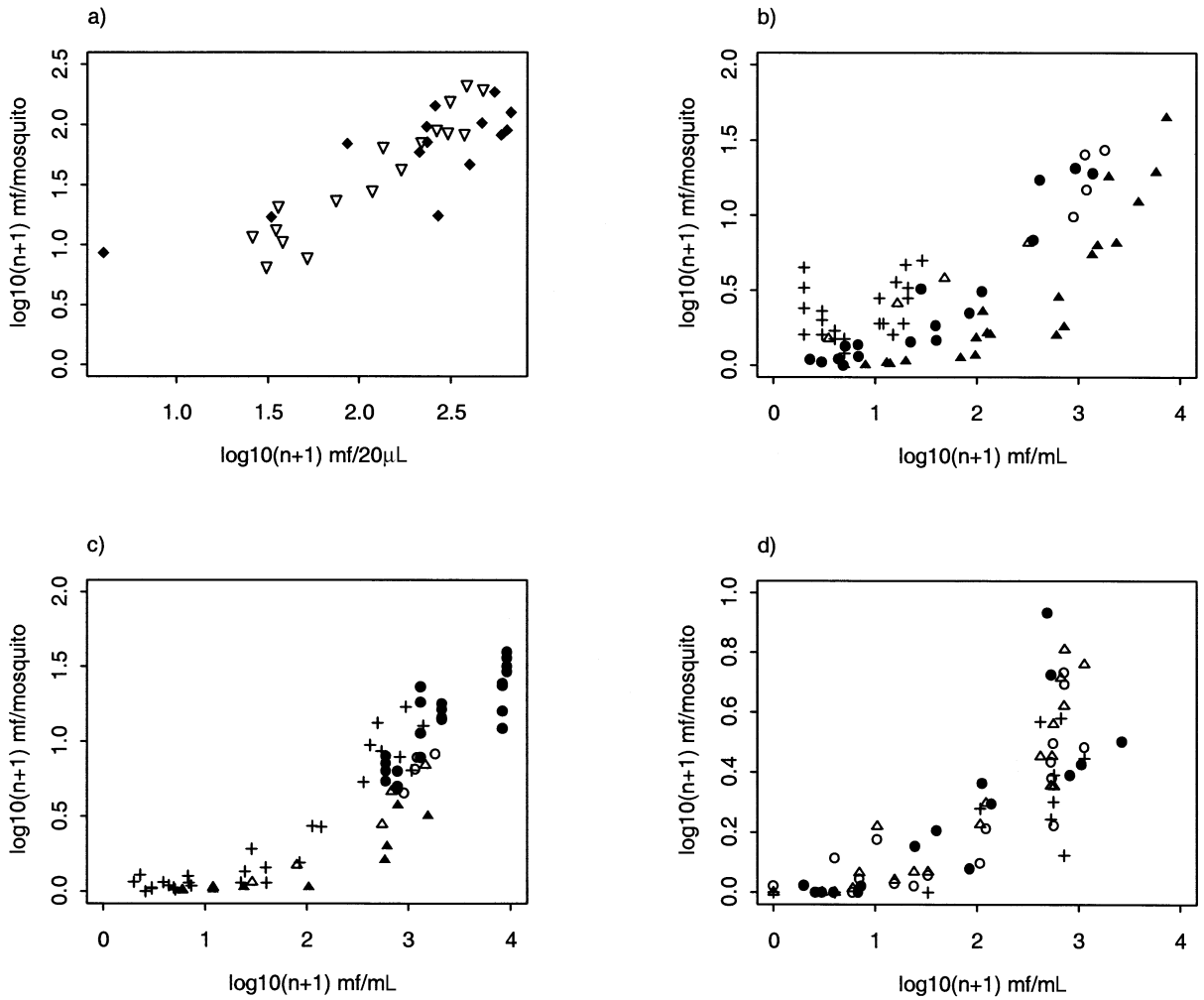

Fig. 1. Scatter plots of the logarithmic relationship between the numbers of mf ingested per mosquito (log $n+1$) and human host mf intensity (log $n+1$) by study (each denoted by an individual symbol) for (a) *Culex* mosquitoes based on human parasite counts estimated using 20 μL fingerprick blood samples, and (b) *Culex* mosquitoes, (c) *Aedes* mosquitoes and (d) *Anopheles* mosquitoes all based on human mf counts estimated using 1 mL venous blood. Data sources: (a) ◆ = Gubler *et al.* (1973); ▽ = Jordan & Goatly (1962); (b) ● = McGreevy *et al.* (1982); △ = Jayasekera *et al.* (1980); ○ = Calheiros *et al.* (1998); + = Lowrie *et al.* (1989); ▲ = Sabry (1987); (c) ● = Failloux *et al.* (1995), ○ = Calheiros *et al.* (1998); + = McGreevy *et al.* (1982); △ = Samarawickrema *et al.* (1985) (*Ae. polynesiensis*); ▲ = Samarawickrema *et al.* (1985) (*Ae. samoanus*); (d) ○ = Bryan & Southgate (1988a) (*An. arabiensis*); △ = Bryan & Southgate (1988a) (*An. gambiae*); + = Bryan & Southgate (1988a) (*An. melas*); ● = McGreevy *et al.* (1982).

was carried out using a mixed model framework as outlined below.

The relationship between the intensity of mf uptake by mosquitoes and host parasite density

Given that a specific aim of this study was to examine the mf uptake data for the presence of density-dependence in the process, we evaluated the relationship between mean mf uptake/ingested per mosquito and mean mf load per 20 μL blood sample per host using the logarithmic regression or the so-called 'log-log unit slope' method for detecting density-dependence (Morris, 1963a,b), whereby $\log_{10}$ mean mf $(n+1)$ uptake per mosquito is regressed against $\log_{10}$ mean mf $(n+1)$ per host giving:

$$\log y = \log \alpha + \beta(\log x), \tag{1}$$

which is a linearization of the power curve:

$$y = \alpha x^{\beta}, \tag{2}$$

where y is mean mf uptake per mosquito, x is the mean mf density per host, $\log \alpha$ is the intercept in the linearized function, and β, the slope of the logarithmic regression, is the parameter associated with the form of the curve. $\beta < 1$ would indicate negative density-dependence, $\beta > 1$ inverse or positive density-dependence and $\beta = 1$ linearity or proportionality between the original variables (Morris, 1963a,b; Maelzer, 1970; Basanez *et al.*, 1994). Arithmetic mean mf per dissected mosquito or host constituted the original measure of central tendency in all analyses. The logarithmic regressions were carried out separately for each study, and the results were statistically combined and summarized for each genus using the multilevel mixed model approach described below.

Prevalence of mf uptake

For likely aggregated processes such as the number of mf ingested per mosquito, the relationship between the proportion of mosquitoes infected (per sample) and the mean mf intake ($Y(x)$) can be approximated using the following function from the negative binomial distribution (Medley *et al.*, 1993):

$$p(y(x); \kappa) = 1 - (1 + y(x)/\kappa)^{\kappa} \tag{3}$$

where $p(y(x); (\kappa)$ is the prevalence expected from a sample of mosquitoes, $y(x)$ is the functional relationship between the mean intake (y) and the mean blood mf intensity in the host (x), and κ is the over-dispersion parameter. We explicitly assess if κ or aggregation changes with level of infection by defining κ as a function of $y(x)$ and comparing the corresponding fits of functions up to the quadratic:

$$\kappa(y(x)) = \kappa_0 \tag{4}$$

$$\kappa(y(x)) = \kappa_0 + \kappa_1\ y(x) \tag{5}$$

$$\kappa(y(x)) = \kappa_0 + \kappa_1\ y(x) + \kappa_2\ y(x)^2 \tag{6}$$

The equations were fit to data using maximum likelihood (Medley *et al.*, 1993) with the functional relationship $y(x)$ (Eq. 2) determined using Eq. (1) fixed for each genera. Unlike the analysis of the intensity of mf uptake, all data for a particular vector genus were pooled in order to carry out the analysis, i.e. separate analyses by study were not performed due to sparseness of data within the subset of individual studies containing these data (see study references in legend to Fig. 5 below).

Meta-analysis of individual study results using linear mixed models

The relationship between the number of mf ingested and host blood mf intensity was summarized for each mosquito genus and compared between genera in this study by a meta-analytic synthesis of information across the relevant single studies (Goldstein, 1995; Smith *et al.*, 1995; Stram, 1996; Goldstein & Leyland, 2001; Thompson *et al.*, 2001). The use of meta-analysis here is motivated by the likelihood that any single study, as in most ecological investigations, is unlikely to provide a complete description of the uptake relationship. Single studies are likely to be highly variable and sampling may not encompass the full range of the response studied. Multilevel meta-analytic approaches are particularly applicable for carrying out the present analysis, given that these methods also take account of the hierarchical structure of the data, where individual observations are nested or clustered within different studies, while combining these grouped and therefore statistically non-independent data across the study units. As we are interested in summarizing and comparing the vector genera-specific (log) regression relationships between mf uptake and parasite intensity in human host blood (Eq. 1), the linear mixed effects modelling approach provides a natural framework for analysis (Hokka, 1997; Pinheiro & Bates, 2000). The general random intercept linear mixed regression model for a two-level data structure is given by:

$$y_{ij} = \mu + \eta_j + \beta x_{ij} + \varepsilon_{ij}, \eta \sim N(0, \sigma^2{}_n) \tag{7}$$

where i and j are observation (paired human–mosquito mf data) and study indices y and x are expected $\log_{10}$ mean mf uptake $(n+1)$ by mosquitoes and $\log_{10}$ host mf density $(n+1)$, respectively, β is the slope of the relationship between mf uptake and host blood mf intensity, which is assumed to be same for all studies, η_j is the difference the overall intercept

(μ) and study is intercept and is estimated as a random effect distributed normally and independently of ε_{ij} the model error variance ($\sim N(0, \sigma^2)$). This model thus combines information regarding the mean mf uptake – mean host mf intensity logarithmic regression relationship from the disparate studies while considering that these studies may vary in mean uptake (i.e. have different regression intercepts). Likewise, we could also consider a random effect for the slope using :

$$y_{ij} = \mu + \eta_j + (\beta + \varsigma_j)x_{ij} + \varepsilon_{ij}, \varsigma \sim N(0, \sigma_\varsigma^2) \quad (8)$$

where there are now two random effects, a random intercept η_j and a random slope or coefficient ς_j.

Results

Data features and preliminary analysis

The data sources available for use in this study varied considerably in the measurements of interest essential to carrying the present analysis (Table 1). Studies differed not only in sample sizes of mosquitoes used but more pertinently also in the number of human volunteer mf intensities as well as the range of densities studied. A significant feature of relevance to comparability of data is that these studies also used different human blood sampling volumes and techniques to enumerate mf intensity in infected volunteers (Table 1). These between-study variabilities and the restriction of human mf intensities in single studies clearly highlight the need for a meta-analytic combination of information across studies, which takes account of precision and correlation of results within each study while allowing for appreciable heterogeneity between studies.

Figure 1 plots the $\log_{10}$ transformed paired mean mf uptake – human mf density data by study and vector genus, and shows that despite the between-study variability, a consistently similar positive association occurs between the two variables within and across all the studies for each vector genus. These preliminary plots, however, also demonstrate the confounding role that differences in human blood sampling techniques can play in the interpretation of mf uptake results. This is highlighted by the results for the *Culex* mosquitoes portrayed in Figs 1(a) and (b), which appear to suggest that for similar values of human mf intensity, mean uptake by the vector is higher in the two studies where human parasite density was quantified using 20 μL fingerprick blood compared to that obtained with larger blood volumes using more sensitive methods. Although peripheral blood may contain proportionately more mf than parallel venous blood (Desowitz & Southgate, 1973; Nathan & Raccurt, 1979; Eberhard *et al.*, 1988; Sabry, 1991; Dreyer *et al.*, 1996), it is more likely that underestimation of the true blood mf density by the fingerprick method may underlie the apparently enhanced uptake observed for this method in the present case (Fig. 1a). This finding again emphasizes the crucial importance of applying uniform indicators of infection when undertaking comparative data analyses in lymphatic filariasis (Michael *et al.*, 1994, 2001; Michael & Bundy, 1998). Here, we attempt this standardization by transforming the human mf data from all the studies which used the sampling procedures based on larger blood volumes (predominantly 1 mL venous blood) to reflect enumeration based on the 20 μL fingerprick method (see below). As noted in the Methods, conversion to expected 20 μL peripheral fingerprick densities was also chosen, as estimates from this standardization are more likely to reflect the numbers of mf encountered by mosquitoes when feeding on host peripheral blood.

Standardizing human mf intensity data

As outlined in the Methods section, the standardization of human mf intensity essentially involved estimating the regression relationship between paired mf counts obtained from infected individuals using the two blood sampling techniques in question, and applying the regression equation so derived to convert the mf intensity data from the studies employing the 1 mL venous blood sampling method. Data for the regression analysis were derived from five separate published studies (see study references in legend to Fig. 2), the bivariate plot in Fig. 2 depicting the observed overall and study-specific relationships between the paired parasite counts on the $\log_{10}$ scale. Given the two-level data structure (observations nested or clustered within studies) and the meta-analytic rationale outlined previously, we

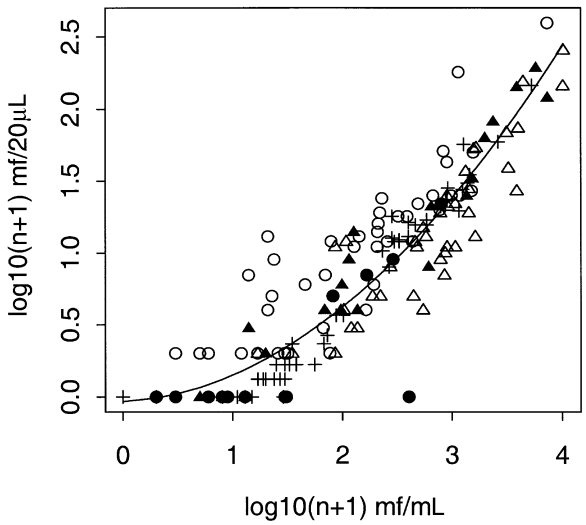

Fig. 2. The logarithmic relationship between mf counts ($\log n + 1$) obtained from paired fingerprick and venous samples of blood obtained from the same individual plotted by study (symbols). The line is the mean quadratic regression curve from the best-fit linear mixed-effects model detailed in Table 2. Data sources: ○ = Eberhard *et al.* (1988); △ = Moulai-Pelat *et al.* (1992); ▲ = Sabry (1991); + = Samarawickrema *et al.* (1985); ● = Schreiber *et al.* (1976).

Table 2a. Results of fitting linear mixed-effects models to parallel parasite count data (log scale) estimated using 20 μL fingerprick and 1 mL venous blood samples.

Model	d.f.	Loglik*	Test	L. Ratio	*P*-value
1. Linear, random intercept	4	−10.8239			
2. Quadratic, random intercept	5	13.4613	1 vs. 2	4.570	<0.0001
3. Quadratic, random slope and intercept	7	14.9022	2 vs. 3	2.882	0.2367

Table 2b. Parameters of the best-fit model used to standardize human mf densities.

Model parameters	Value	SE	d.f.	*t*-value	*P*-value
Model 2. Quadratic, random intercept					
A	0.1449	0.0193	160	7.5151	<0.0001
B	0.0370	0.0838	160	0.4414	0.6595
C	−0.0309	0.1007	160	−0.3068	0.7594

*Higher log-likelihood (loglik) values indicate better fitting models.

applied a linear mixed effects modelling framework to estimate the mean logarithmic regression relationship between the two counts from these data. Both random intercept and random coefficient models (adopting Eqs 7 and 8), as well as fits of linear and quadratic terms in the regression function, were tested in sequence (Pinheiro & Bates, 2000; Everitt & Rabe-Hesketh, 2001). Table 2 shows the log-likelihoods of each model fitted and the likelihood ratios of sequentially evaluating the quadratic and random coefficient models to the baseline random intercept linear model, which indicate that the present data are best described by the random intercept model including a positive quadratic term. The parameter values for this best-fit mean quadratic regression function (allowing for random between-study intercepts) are also given in Table 2: the curve in Fig. 2 represents the prediction of this model and demonstrates a good visual fit to observed data. The estimated positive quadratic function implies that as host mf intensities increase, progressively more mf are enumerated in 20 μL fingerprick blood samples compared to the estimates made using 1 mL venous blood from the same individual, thereby supporting and clarifying previous assertions for the greater sensitivity of the fingerprick method in relation to venous blood sampling for quantifying human mf density (Desowitz & Southgate, 1973; Nathan & Raccurt, 1979; Eberhard *et al.*, 1988; Sabry, 1991; Dreyer *et al.*, 1996). The results also indicate that while there was significant between-study heterogeneity in the intercept (mean mf levels), the estimated quadratic regression relationship between the two mf counts, at least on a logarithmic scale, is essentially similar (non-significant random effects for the slope; Table 2).

Analysis of vector mean mf uptake as a function of host mf density

Figures 3(a)–(c) portray the vector-specific logarithmic relationships observed between mean ingested mf load per

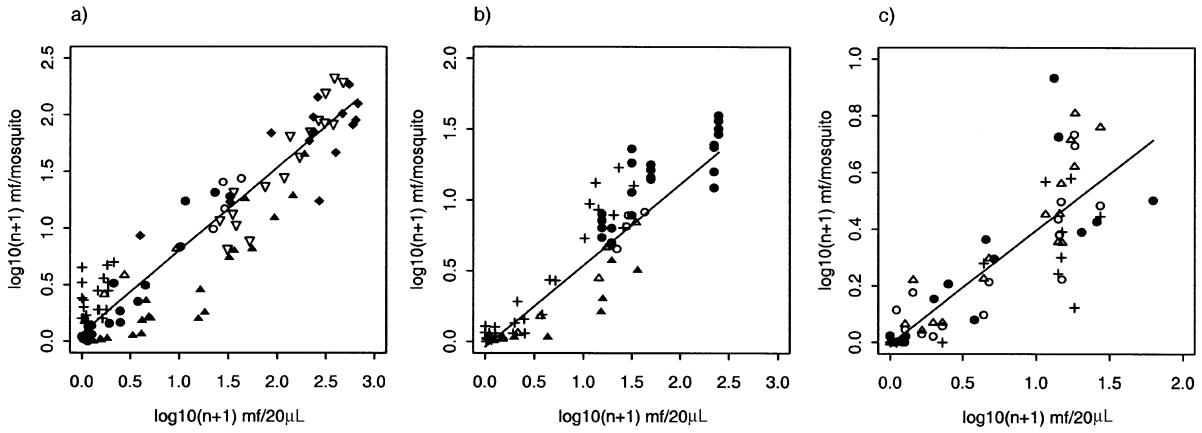

Fig. 3. The logarithmic relationships between the numbers of mf ingested per mosquito (log $n+1$) and human host mf intensity (log $n+1$) plotted by study (symbols) for (a) *Culex* mosquitoes, (b) *Aedes* mosquitoes and (c) *Anopheles* mosquitoes based on standardized human infection data. The lines in each graph denote the best-fit mean regression lines estimated by the linear mixed-effects models fitted to the data for each genus (Table 3). Data sources for the analysis are as described in the legend to Fig. 1 for each genus.

mosquito and the corresponding host parasite density following the standardization of host infection intensity to reflect worm numbers based on the 20 μL fingerprick technique. The fact that the standardization carried out appropriately re-scaled the data in this study is indicated by the fact that the converted data (and hence the mf uptake relationship) were not obviously different from the responses shown by the two *Culex* studies whose data were left unchanged (see Fig. 3a). One consequence of re-scaling the host mf densities to the equivalent density per 20 μL of fingerprick blood, however, is that by reducing observed mf densities (but not the mean mf uptake in mosquitoes) the

Table 3a. Results of linear mixed-effects model fits to mf uptake data (log scale) from different studies.

Model	d.f.	Loglik	Test	L. Ratio	*P*-value
1. Linear, random intercept	4	13.207			
2. Quadratic, random intercept	5	13.922	1 vs. 2	1.4294	0.2319
3. Linear, random slope and intercept	6	16.356	1 vs. 3	6.2970	0.0429

Table 3b. Parameter values of the best-fit mean regression model for *Culex* uptake.

Model parameters	Value	SE	d.f.	*t*-value	*P*-value
Model 3. Linear, random slope and intercept					
Intercept	0.0714	0.1284	90	0.5560	0.5796
Slope	0.7319	0.0734 (0.588–0.876)*	90	9.9745	<0.0001

*95% confidence intervals for the slope.

Table 3c. Results of linear mixed-effects model fits to mf uptake data (log scale) from different studies.

Model	d.f.	Loglik	Test	L. Ratio	*P*-value
1. Linear, random intercept	4	28.503			
2. Quadratic, random intercept	5	30.078	1 vs. 2	3.1501	0.0759
3. Linear, random slope and intercept	6	33.318	1 vs. 3	9.6303	0.0081

Table 3d. Parameter values of the best-fit mean regression model for *Aedes* uptake.

Model parameters	Value	SE	d.f.	*t*-value	*P*-value
Model 3. Linear, random slope and intercept					
Intercept	−0.0371	0.0332	66	−1.1178	0.2677
Slope	0.5741	0.0631 (0.450–0.698)*	66	9.0928	<0.0001

*95% confidence intervals for the slope.

Table 3e. Results of linear mixed-effects model fits to mf uptake data (log scale) from different studies.

Model	d.f.	Loglik	Test	L. Ratio	*P*-value
1. Linear, random intercept	4	37.089			
2. Quadratic, random intercept	5	37.288	1 vs. 2	0.3979	0.5282
3. Linear, random slope and intercept	6	37.756	1 vs. 3	1.3352	0.5129

Table 3f. Parameter values of the best-fit mean regression model for *Anopheles* uptake.

Model parameters	Value	SE	d.f.	*t*-value	*P*-value
Model 1. Linear, random intercept					
Intercept	−0.0061	0.0288	60	−0.2122	0.8327
Slope	0.4010	0.0321 (0.338–0.464)*	60	12.492	<0.0001

*95% confidence intervals for the slope.

standardization better linearized the observed relationships for all vector genera (compare Fig. 1 with Fig. 3).

Table 3 shows the results of fitting various linear mixed models to the $\log_{10}$ mf uptake vs. $\log_{10}$ host mf density data for each vector genus. As above, we again evaluated the sequential addition of shape (linear against quadratic) and between-study heterogeneity (random intercepts against random slopes) elements to the observed mf uptake–host density relationship for each of the transmitting vectors. The results clearly substantiate the high between-study variability in the data (hinted at in Fig. 3), with the best-fitting models for *Culex* and *Aedes* signifying significant between-study variation in both regression intercepts and slopes (*P*-value of adding random effects for the slope to the baseline random intercept model <0.05 in each case), and the best-fit model for *Anopheles* indicating the presence of significant between-study variation in the intercept (see Tables 3a–f). However, it is clear that after adjusting for such between-study heterogeneities, significant linear logarithmic relationships exist between vector mean mf uptake and host parasite density for all three vector genera. For all three genera, the intercepts of these mean relationships were also not statistically significant, indicating that mf uptake by vectors was simply proportional to host blood mf density in each case (see model parameters given in Tables 3a–f and fits of the corresponding models (portrayed by lines) to data for each vector genus in Figs 3(a)–(c). A major finding from this analysis is that although significantly different from zero, the values of the slopes of the linear logarithmic relationship for each vector genus were all significantly lower than 1 with 95% confidence intervals excluding 1 in each case, indicating the occurrence of significant negative density-dependence in *W. bancrofti* mf uptake by mosquitoes regardless of vector genus (Tables 3a–f).

This can be more clearly observed in Figs 4(a)–(c), which plot the data and the estimated uptake function for each genus on the original (non-logarithmic) scale. The solid curve in each graph represents the 1 : 1 replacement line or the expected uptake load in the absence of density-dependence, and the dashed curves denote the respective power models (Eq. 2) estimated via the logarithmic regressions for each genus (see Methods section). The area between the two curves in each graph represents the degree of density-dependence acting on mf uptake, and it is clear that although ingestion of mf by mosquitoes is density-dependent for all three genera, the degree of such regulation varied between the vector genera. Density-dependence in mf uptake was least in *Culex* mosquitoes (β-value at 0.7319 comparatively higher and closer to 1.0), whereas it is higher in *Aedes* (lower $\beta = 0.5741$) and apparently most severe in *Anopheles* ($\beta = 0.401$).

Prevalence of mf uptake

In contrast to the mean mf uptake–host infection density relationship, the association between the prevalence of mosquitoes with ingested mf per vector feeding sample and host blood mf density was highly non-linear for all three genera (Fig. 5). For *Culex* and *Aedes* mosquitoes, prevalences rise rapidly with host mf densities before levelling off at around 100% (slightly lower in the case of *Aedes*) at around 50mf/20 μL, whereas in the case of *Anopheles* the levelling of infection occurred at an apparently lower prevalence (Figs 5a–c), although this may be a function of the smaller range of host mf densities studied by the available investigations for this vector.

The fitted functions for κ using Eqs (4)–(6) are given in Table 4, and indicate this parameter to be a linear function of mean ingested mf density for *Culex* and *Aedes* mosquitoes but independent of mean uptake for *Anopheles* mosquitoes. The corresponding negative binomial model (Eq. 3) predictions are plotted in Figs 5(a)–(c) and exhibit good visual fits to the observed data in each case. The small estimated values of κ in this study suggest that ingested mf per mosquito is likely to be highly over-dispersed for all the three genera.

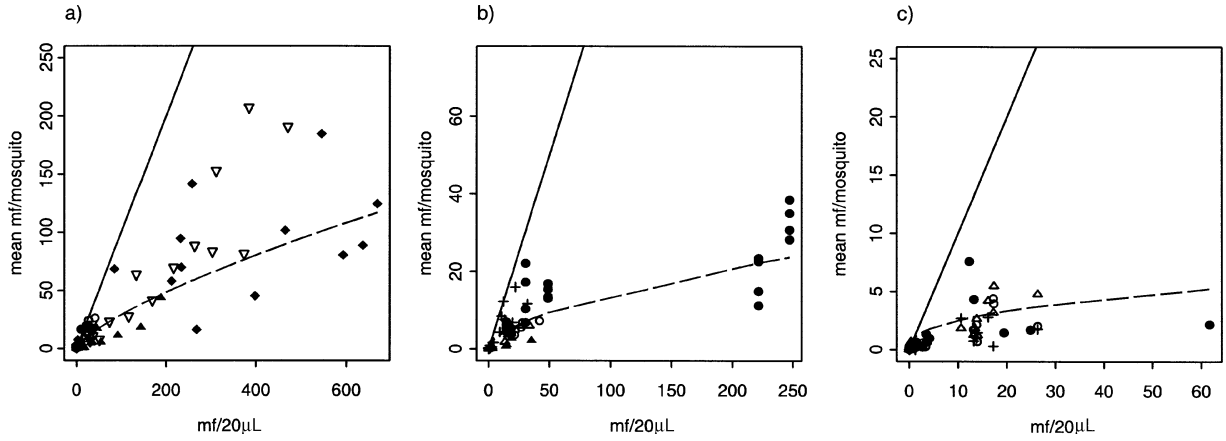

Fig. 4. Observed and predicted uptake of mf for (a) *Culex*, (b) *Aedes* and (c) *Anopheles* mosquitoes plotted on the original (arithmetic) scale. As before, symbols portray observed data from individual studies, and the dashed lines represent the best-fit uptake function (Eq. 2) for each genus. Solid curves denote the 1 : 1 replacement line expected in the absence of density-dependence. Note the marked variation in *y*-axis values between the three genera, in particular the low numbers of mf observed for *Anopheles* vectors. Symbols as described in Fig. 1.

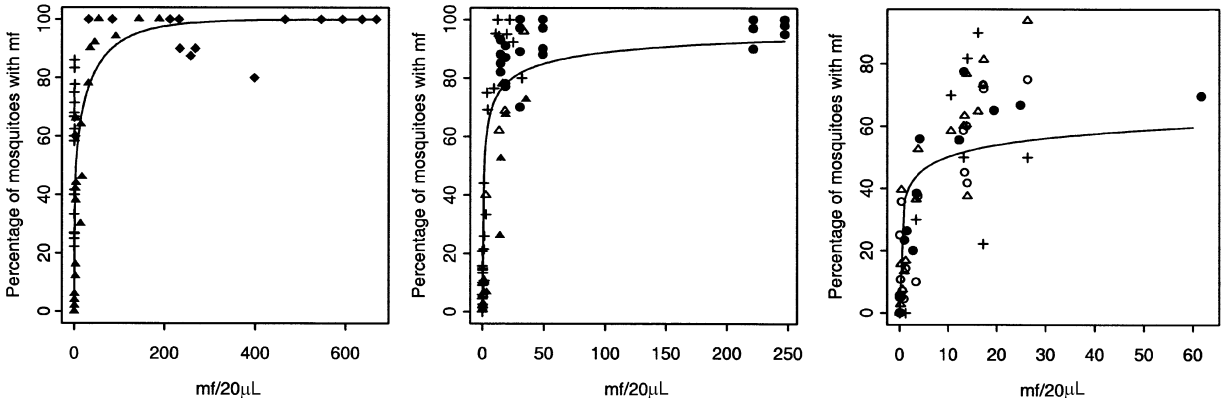

Fig. 5. The relationships between the percentage of mosquitoes with ingested mf and mean human parasite load for (a) *Culex*, (b) *Aedes* and (c) *Anopheles* mosquitoes. Symbols denote observed data from single studies (study references as described in the legend to Fig. 1).The lines show the corresponding best-fit negative binomial models (Eq. 3 and Table 4) in each case. Note the wide variation in *x*-values (human parasite loads) studied between the three genera.

The expected mf frequency distributions in individual mosquitoes in each of three theoretical vector populations using the estimated κ-values in Table 4 for *Culex* and *Anopheles* mosquitoes, respectively, are illustrated in Figs 6(a) and (b). The mf distributions expected in mosquito populations feeding on mean host intensities of 2.5, 25 and 100 mf per 20 μL blood sample are shown for each vector genus. The results show that the degree of (ingested) mf aggregation may decrease rapidly in the theoretical *Culex* populations as host mf density increases, whereas parasite distribution is likely to change little, at least up to the highest host density analysed (100 mf/20 μL fingerprick blood) for the simulated *Anopheles* populations. As discussed below, this differential theoretical results arise jointly from the genus-specific variations estimated in this study in both the degree of density-dependence in parasite uptake and the dependence of κ on mean ingested parasite loads for each vector.

Discussion

We have used a meta-analysis methodology based on linear mixed models and a simple logarithmic regression method for detecting density-dependence (Morris, 1963a,b) to examine the available evidence from published studies regarding the functional relationships observed between mean mf uptake by feeding vector mosquitoes and human parasite load in lymphatic filariasis. This framework has allowed us to reveal for the first time evidence not only for the presence of density-dependence in this filarial transmission process in each of the three major vector mosquito genera, but also for the existence of significant variation in the degree of such regulation between these vectors. These results represent significant new findings regarding filariasis transmission, and are thus of great value to our overall aim of gaining a better quantitative understanding of the population dynamics and control of this parasite (Chan *et al*., 1998; Michael *et al*., 1998; Norman *et al*., 2000; Michael, 2002).

The present results have highlighted the important role that meta-analytic approaches can play in facilitating a better understanding of the population ecology of infectious disease (Michael *et al*., 1994; Poulin, 1996). Previous single studies of the mf uptake relationship for different vector–*W. bancrofti* combinations have yielded variable results regarding the functional form of this parasite–vector interaction, ranging from an apparently non-linear saturating relationship (McGreevy *et al*., 1982; Bryan & Southgate,

Table 4. Maximum likelihood estimates of the fits of the negative binomial model to mf uptake prevalence – human mf intensity data for each vector genus describing the relationship between mf uptake ($y(x)$) and the clumping factor of the negative binomial distribution κ. Results for a model where κ is independent of mean uptake and a model where κ is a linear function of mean parasite uptake are shown (Eqs 4 and 5).

	Model	Negative Log Likelihood*	κ_0	κ_1
Culex	Constant	875.84	0.44214	–
	Linear	849.00	0.24453	0.01440
Aedes	Constant	1035.75	0.61655	–
	Linear	1029.23	0.58565	0.00303
Anopheles	Constant	914.62	0.31606	–
	Linear	914.56	0.31577	0.00010

*Lower values indicate better fitting models in each case.

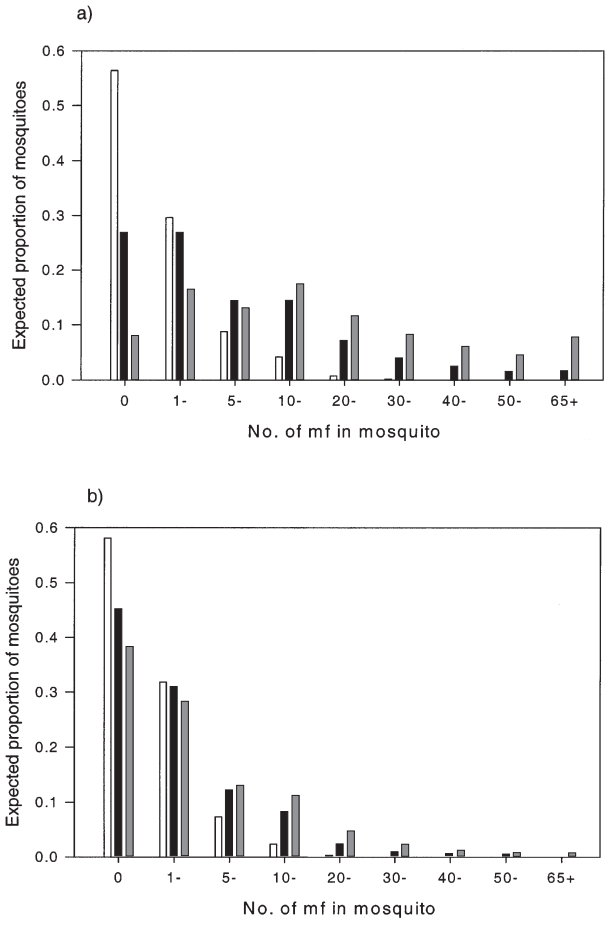

Fig. 6. The expected frequency distribution of mf counts/mosquito for three theoretical populations of (a) *Culex* and (b) *Anopheles* given the parameters of best-fit negative binomial models for each of these vectors (Table 4) in relation to host mf densities of 2.5 mf/20 μL (open bars), 25 mf/20 μL (black bars) and 100 mf/20 μL (shaded bars).

1988a,b; Subramanian *et al.*, 1998) to a linear association (Jordan & Goatly, 1962; Lowrie *et al.*, 1989; Jayasekera *et al.*, 1991). We show that one major factor that may underlie such variability is the considerable heterogeneity in the design of the single investigations carried out so far, which include variations in study sample sizes used, methods deployed for enumerating host and mosquito mf counts, and host mf densities studied (Table 1 and Fig. 1). Note that the effect of the latter variable by itself would lead to different studies measuring different parts of the mf uptake dose–response relationship, thereby contributing to the apparent observed heterogeneity in this parasite–vector infection process (see Fig. 1). These study design differences clearly suggest that further progress in understanding the filarial mf uptake relationship using available data requires the application of comparative analysis methods that allow statistically robust combination of results from separate studies. Here, we have illustrated how the linear mixed model framework may offer a particularly appropriate technique for combining evidence regarding the mf uptake relationship (as indeed for the meta-analysis of any other functional relationship (Myers *et al.*, 1997; Myers & Mertz, 1998; Myers, 2001) from disparate experimental studies. An important feature of this method is that it takes explicit account of the hierarchical nature of the data (i.e. observations are correlated within study units), when combining different study findings to form the overall global estimate (Pinheiro & Bates, 2000; Everitt & Rabe-Hesketh, 2001). In addition, a random effects formulation allows for a simple adjustment of between-study variability by considering that the effects determined in individual studies, rather than measuring a single fixed effect, derive instead from some population distribution (often specified to be normal), the dispersion of which may be taken to represent study heterogeneity (Goldstein, 1995; Stram, 1996; Goldstein & Leyland, 2001; Thompson *et al.*, 2001). In essence, these methods therefore quantify the average effect or response from single studies by focusing on the mean of such estimated distributions. This incorporation of the need to account for both within- and between-study variance when estimating an average response contrasts with traditional methods for estimating mean responses from individual studies, which have been based on either a simple pooled analysis of all the available data or a counting of the number of significant single studies (Southgate & Bryan, 1992; Basanez *et al.*, 1994). The former method may overestimate the significance of any finding (by deflating the variance), whereas the latter practice, also known as 'vote-counting', has been shown to favour a bias towards the null hypothesis of no effect (Hedges & Olkin, 1985). The meta-analytic procedures followed in this study for quantifying the mean mf uptake relationship from published data therefore represent a key methodological improvement to previous work investigating this process.

Although our primary aim in assessing between-study heterogeneity was to account for this variability when quantifying the overall average mf uptake response for each vector genus, a further advantage of employing the mixed effects meta-analytic framework is that these models can also be readily extended to investigate the factors that may underlie the observed study variance (Stram, 1996; Everitt & Rabe-Hesketh, 2001; Goldstein & Leyland, 2001; Thompson *et al.*, 2001). The relevant covariates for undertaking such an analysis, however, were not recorded in any study, but it is worth noting that several factors may underlie the significant between-study heterogeneity estimated here for the uptake relationship for each vector (Tables 3). These are likely to range from the effects of differences in host mf densities investigated by individual studies to factors relating to differences in the parasite and mosquito strains used (Jayasekera *et al.*, 1980; Curtis *et al.*, 1981; Zielke, 1992). Except for the host mf densities and perhaps the experimental conditions themselves, the other more subtle differences are rarely recorded by individual studies. This clearly further enhances the value of considering between-study heterogeneity as an inevitable component of any meta-analysis, and the use of random effects meta-analysis methods, as employed in this study, for treating such

heterogeneity as random factors when estimating overall effects (Thompson *et al*., 2001).

We have used the simple 'log-log unit slope' method here for testing for density-dependence in the ingestion of *W. bancrofti* mf by vector mosquitoes (Morris, 1963a,b; Lebreton, 1989). Within this framework, a regression slope smaller than unity is taken as evidence for the occurrence of density-dependence. Based on this criterion, we have demonstrated for the first time that mean *W. bancrofti* mf uptake by feeding mosquitoes is not only density-dependent in all the three major vector genera transmitting this filarial parasite, but also that the degree of observed density-dependence may vary significantly between these genera. Density-dependence in mf uptake appears to be strongest (despite the fact the available studies here covered a significantly smaller range of host mf densities; Fig. 4) for *Anopheles* mosquitoes (slope, $\alpha = 0.401$ with confidence intervals (CL) of 0.338–0.464), followed by *Aedes* ($\alpha = 0.5741$, CL 0.450–0.698), and is weakest in *Culex* ($\alpha = 0.7319$, CL 0.588–0.876). These results clearly present major new insights regarding this initial component of the transmission dynamics of lymphatic filariasis in the vector population; however, a caveat which needs to be borne in mind when interpreting these results is that a slope tending towards zero may also result if mf densities in both vectors and humans are measured with error (Bulmer, 1975; Lebreton, 1989; Basanez *et al*., 1994). Thus, slopes of the regressions may be less than one, even if density-dependence is lacking simply as statistical artefacts. A realistic treatment of the impact of measurement error in this study was hampered by both the paucity of knowledge regarding the expected magnitude of error surrounding estimates of human mf density and the lack of readily applicable methods for accounting for this error especially within a multilevel modelling framework (but see Woodhouse *et al*., 1996). However, a preliminary analysis carried out using a standard error-in-variables regression method applied to the overall pooled data for each vector genus (Stata, 2001) indicated that errors in measured human mf density needed to be more than 15% to affect the results reported here (data not shown). Note also that the effect of measurement error is likely to a problem primarily only for the result estimated for *Culex* in this study, as the regression slopes estimated for *Aedes* and *Anopheles* vectors are appreciably smaller than one (Tables 3c–f). Nonetheless, we indicate a need for future work in filariasis epidemiology (for example, through repeated sampling) to quantify the scales of error to be typically expected when measuring human blood mf levels.

As noted above, a major result of this study is that the strength of density-dependent mf uptake is found to vary markedly between vector genera. All three genera show a non-linear saturating relationship for mf ingestion, but the level and point at which this saturation is reached differed, being significantly higher in *Culex* compared to either of the other two genera (Fig. 4). Several factors may underlie both the observed density-dependence in general and the observed between-genera variation. First, it has been shown that mosquitoes ingest more mf than would be expected based on bloodmeal volume, and that this concentration may be density-dependent (Samarawickrema *et al*., 1985; Bryan & Southgate, 1988a; Failloux *et al*., 1995). This could clearly account for the observed density-dependence in mf uptake portrayed in Fig. 4, and suggests that between-genera differences in uptake may be a result of genus-specific variation in mf concentration capacity. Such a differential capacity has been proposed to be the specific cause of higher uptakes of mf by *Cx. quinquefasciatus* mosquitoes, in particular when compared with *An. gambiae* (Crans, 1973). A second factor that may underlie genera differences in the rate of mf uptake is thought to be variation in mosquito size and hence ingested bloodmeal volume, with *Cx. quinquefasciatus* shown to ingest larger blood volumes (and hence mf) compared to *Ae. aegypti* and *An. gambiae* in that order (McGreevy *et al*., 1982; Albuquerque *et al*., 1999). A further explanation that has been proposed involves differences in the ways in which mosquitoes feed. It has been suggested that some mosquitoes will take blood directly from the vessel, whereas others lacerate vessels and feed on the subcutaneous haemorrhage that forms. The latter is thought to lead to a higher uptake of mf but there is little evidence to support both this and how vector feeding varies between genera. The possibility of a genetic mechanism for refractoriness to filarial larvae (Beerntsen *et al*., 1995) may also contribute to the observed variation but requires further investigation, especially regarding how resistant loci are distributed in mosquito populations. By contrast, however, genus-specific differences in pharyngeal and cibarial armatures, rows of small teeth or spines that protrude into the lumen of the foregut, are unlikely to affect the numbers ingested unless the armature prevents mf from reaching the midgut (Bryan *et al*., 1976; McGreevy *et al*., 1978; Bryan & Southgate, 1988b). These observations suggest that it is more than likely that more than one mechanism may act to regulate mosquito mf uptake and the observed between-vector heterogeneity in this relationship.

The analysis carried out in this study of the association between the prevalence of mf uptake by the vector and blood worm intensity in the human host has provided further insights into the relations between filarial mf uptake, prevalence of infection and parasite aggregation in vector populations (Basanez *et al*., 1994; Subramanian *et al*., 1998). Our analysis has shown that the markedly non-linear relationship observed between the prevalence of ingested mf in vectors and parasite load in humans for all three vector genera is likely to be a joint function of both vector-specific rates of mean mf uptake and degrees of aggregation in parasite ingestion. In particular, the fits of the negative binomial model to the vector mf prevalence–human parasite density data indicated that the observed non-linearity in this relationship for all three vector genera may be due to the occurrence of high levels of aggregation (as evidenced by the low values obtained for the clumping factor, κ) of the numbers of mf ingested per mosquito (Figs 5 and 6; Table 4). The results also show that both the mean and parameter κ of this distribution may vary with human mf

density for the *Culex* and *Aedes* vectors but not in the case of the data available for *Anopheles* mosquitoes. This paradoxical result (decreasing κ with increasing mean mf uptake implies the operation of density-dependence; Anderson & Gordon, 1982; Pacala & Dobson, 1988; Hanski, 1990; Medley *et al*., 1993; Subramanian *et al*., 1998) may be a function of the restricted human mf densities fitted for the *Anopheles* vectors (Fig. 5), but it could also reflect the significantly higher mean mf uptakes (as a result of weaker regulation of mf ingestion) observed for *Culex* and *Aedes* mosquitoes compared to that observed for *Anopheles*. This implies that the static κ and the corresponding invariant vector mf over-dispersion likely to be observed for *Anopheles* with changing human mf density (Fig. 6) may be a direct function of the strong density-dependence in mf uptake observed for this vector. As shown in Fig. 4(c), such regulation may saturate the uptake of mf at significantly lower human mf loads, thereby limiting any further build-up of or change in mean mf load (and hence in κ) in these mosquitoes as host densities increase. These observations further support this study's conclusion regarding the existence of strong between-genera differences in the dynamics of mf uptake from infected humans in filariasis. Individual mosquito data to evaluate the predictions of these analyses are not available to us currently, but it is notable that essentially similar conclusions regarding density-dependence and parasite aggregation were reached when such data were analysed with respect to *W. bancrofti* mf uptake by *Cx. quinquefasciatus* from infected volunteers living in an endemic region in Southern India (Subramanian *et al*., 1998).

In conclusion, our meta-analysis of the published data regarding *W. bancrofti* mf uptake by mosquitoes has provided evidence for the first time regarding the existence of density-dependence in this transmission process in all the three major filariasis-transmitting vector genera. We have also shown that the strength of this regulation is strongly genera dependent, with density-dependence being weakest in *Culex*, comparatively stronger in *Aedes*, and most severe in *Anopheles*. The results also show how these vector genera-specific differences in the regulation of mf uptake could play an important role in influencing observed parasite distributions in mosquito populations, with mf frequency distribution per vector unlikely to change with host parasite load when there is strong regulation of this process (and which becomes operational at very low host mf densities). These findings have important implications for predicting the effects of control on parasite transmission in different endemic areas as they argue for the need to consider local vector genus-specific infection dynamics in making such estimations. By contrast, available transmission models for filariasis currently only reflect the dynamics of infection within *Culex* mosquitoes (Plaisier *et al*., 1998; Norman *et al*., 2000). However, parasite ingestion is not the only area where density-dependence is likely to play a role in filariasis transmission dynamics, as population regulation can also occur in other components of the infection process in vectors, including development of mf to the infective L3 stage and vector survival (Pichon, 1974; Dye, 1992; Southgate & Bryan, 1992; Basanez *et al*., 1994; Subramanian *et al*., 1998). The results of this study indicate that any realistic attempt to undertake an analysis of their relative importance to parasite transmission and control requires proper quantification of these processes. Evidently meta-analytical approaches, similar to those used here, will play a vital role in estimating the functional forms of these processes. These analyses, by extending the multilevel modelling approach described in this study, will be reported in subsequent publications.

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