Density-dependent processes in the transmission of human onchocerciasis: relationship between the numbers of microfilariae ingested and successful larval development in the simuliid vector

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SUMMARY

A previous paper reported that the intake of *Onchocerca volvulus* microfilariae (mff) by different species of *Simulium* is essentially proportional to the parasite load in the skin of infected carriers. This paper examines the fate of the ingested mff in susceptible vectors to assess the relationship between parasite intake and infective larval output in blackfly species with and without well-developed cibarial armatures. Analysis is based on data from 3 onchocerciasis endemic areas: Guatemala (*S. ochraceum s.l.*), West Africa (*S. damnosum s.l./S. sirbanum*) and the Amazonian focus between South Venezuela and Northern Brazil (*S. guianense* and *S. oyapockense s.l.*). The data, which include published and unedited information collected in the field, record experimental studies of parasite uptake by wild flies maintained in captivity until the completion of the extrinsic incubation period. The relationship between L3 output (measured as the mean number of successful larvae/fly or, as the proportion of flies with infective larvae) and average microfilarial intake, was strongly non-linear. This non-linearity was best represented by a sigmoid function in case of armed simuliids (*S. ochraceum s.l.*, *S. oyapockense s.l.*), or by a hyperbolic expression in that of unarmed flies (*S. damnosum s.l.*, *S. guianense*). These results are compatible, respectively, with the patterns of 'initial facilitation' and 'limitation' described in culicid vectors of lymphatic filariases. A maximum mean number of 1–3 L3/fly was observed in all 4 vectors. It is concluded that *O. volvulus* larval development to the infective stage is regulated by density-dependent mechanisms acting at the early phase of microfilarial migration out of the blackfly's bloodmeal. Damage by the bucco-pharyngeal armature may also be density dependent. A hypothesis, based on this density dependence is forwarded to explain initial facilitation, so far only recorded in vectors with well-developed cibarial teeth. Our results provide quantitative support for the conjecture that chemotherapy alone is likely to have a greater impact on reducing onchocerciasis transmission in endemic areas where the main vector has a toothed fore-gut than in foci where the vectors have unarmed cibaria.

Key words: *Onchocerca volvulus*, simuliid vectors, larval development, density dependence, limitation, facilitation.

INTRODUCTION

The life-cycle of filarial nematodes consists of populations of parasites within the vertebrate host and the insect host, linked by parasite stages transmitted in both directions. The regulation of these parasite populations is mediated by density-dependent mechanisms acting at one or several points of the two-host life cycle (Dietz, 1982; Plaisier *et al.* 1990, 1991).

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The demographic dynamics of macroparasites such as the filarial worms depends on the magnitudes of the birth, death, immigration and emigration rates within either or both hosts (Anderson & Gordon, 1982). Reproduction takes place only in the definitive host and there is no direct multiplication of parasites within either vertebrate or vector host. Density dependence translates into one or more of these rates being a function of the population density of the appropriate parasite stages (Dietz, 1988). This function may result in decreasing (negative feed-back) or increasing (positive feed-back) rates of population growth as density rises. Although only negative feed-back processes are instrumental in

Table 1. Sources of data used in this work

Vector species	Locality	Successful larvae	Type of mean used	References
Simulium ochraceum s.l.	Guatemala	L3	WM	De León & Duke (1966)
		Hmcl.* or	SQRTM§	Bain, Durette-Desset & De León (1974)
		Thor.† mff	SQRTM	Omar & Garms (1975)
		Thor. mff and L3	SQRTM	Collins *et al.* (1977)
Simulium damnosum s.l. (savanna spp.):	West Africa:			
S. damnosum s.s. and *S. sirbanum*	Upper Volta	L3	WM	De León & Duke (1966)
	Côte d'Ivoire	Hmcl. or	AM§	Bain (1971)
		Thor. mff	AM§	Philippon & Bain (1972)
			AM	Philippon (1977)
	Southern Ghana	Hmcl. mff	AM	OCP (1989)
				Alley *et al.* (1994)
Simulium guianense	Southern Venezuela	Thor. mff and L3	SQRTM§	Takaoka *et al.* (1984*b*)
			SQRTM§	This work
Simulium oyapockense s.l.	Southern Venezuela	Thor. mff and L3	SQRTM§	This work
	Northern Brazil	Thor. larvae‡ and L3	SQRTM§	Shelley *et al.* (1987)

* Haemocoelic microfilariae. Calculated from flies dissected between 0·5 and 12 h p.e. (see text).
† Thoracic microfilariae. Calculated from flies dissected between 0·5 and 12 h p.e. (see text).
‡ Thoracic larvae, computed from flies dissected 24 h p.e. (see text).
§ Means calculated from raw data.

constraining population growth, positive feed-back may also play a role in population dynamics by enhancing the chances of otherwise inefficient transmission mechanisms (Hairston & De Meillon, 1968).

In the part of the life-cycle taking place in the vector host, density dependence may act on the elements of vector competence (Spielman & Rossignol, 1985; Dye 1992*a*). More specifically it may influence the input of microfilariae (mff) into the insects, the survival and development of the larvae throughout the extrinsic incubation period, and the output of infective (L3) stages. More indirectly, it may act by affecting the survival, behaviour, etc. of the vector. In the particular case of human onchocerciasis, a previous paper (Basáñez *et al.* 1994) explored the first component of the transmission of *Onchocerca volvulus* from the human host to the simuliid vector, i.e. the microfilarial intake by the flies from the skin of infected carriers. Data from three different endemic areas in Africa and Latin America showed an almost linear relationship between dermal and ingested parasites in the ranges of microfilarial loads explored. However, it has been observed that the numbers of larvae successfully developing to the infective stage in filariasis vectors generally do not increase in the same proportional fashion (Jordan & Goatly, 1962; Obiamiwe, 1977; Rosen, 1955). On the contrary, in most cases, there is a significant loss of parasites throughout the period required for larval maturation.

Among the factors that can affect the survival and development of the parasites within the vector are (1) genetically determined host susceptibility or refractoriness to infection (Curtis & Graves, 1983), (2) the damage caused to ingested parasites by the cibarial/buccopharyngeal armature of the flies when it is present (Coluzzi & Trabucchi, 1968; Omar & Garms, 1975; McGreevy *et al.* 1978; Reid, 1978, 1994), (3) the dynamics of formation and structure of the peritrophic membrane, secreted around the bloodmeal (Lewis, 1950, 1953; Duke & Lewis, 1964; Reid & Lehane, 1984; Miller & Lehane, 1993) and (4) the mounting of specific and non-specific insect defences to parasite invasion (Townson & Chaithong, 1991; Ham, 1992). Some or all of these factors may be influenced by the density of the corresponding parasite stages.

The study of the events involved in the passage of ingested mff towards the site of final larval development in several vectors has led to the recognition of 3 main patterns, for which the terms 'limitation', 'facilitation' and 'proportionality' have been coined. They refer, respectively, to the fraction of successful larvae relative to the total parasite intake being a decreasing, an increasing or a constant function of the number of mff ingested (Bain, 1971, 1976; Pichon, 1974; Pichon, Perrault & Laigret, 1974; Prod'hon *et al.* 1980; Southgate & Bryan, 1992). They correspond to negative feed-back (the rate of successful incorporation of larvae within the insects decreases with parasite intake), positive feed-back (success increases along with density), and density independence respectively. In these studies, 'successful' mff have been defined as those larvae

managing to get out of the bloodmeal into the haemocoele or into the fly's location where maturation occurs, or actually completing their development to the infective stage.

It has been suggested that the non-linearities inherent in the concept of 'facilitation' and 'limitation' may have different and important implications for the control of these infections by, respectively, creating or not creating a transmission threshold below which the parasite will not persist in the host population (Brengues & Bain, 1972; Pichon *et al.* 1974; Bain, 1976; Webber, 1991; Southgate & Bryan, 1992). Such ideas are somewhat speculative at present due to the lack of a quantitative model for the population dynamics of the lymphatic filarial worms in both the definitive and vector hosts (Dye, 1992*b*, 1994; Dye & Williams, 1994). However, if these patterns are found to be equally applicable to the relationship *O. volvulus–Simulium*, a faster progress in testing these hypotheses could be made due to the more advanced stage of development of such models in human onchocerciasis (Dietz, 1982; Davies, Weidhaas & Haile, 1987; Plaisier *et al.* 1990; Anderson & May, 1991; Davies, 1993).

This paper investigates the evidence for and nature of density dependence in the second step of the incorporation of *O. volvulus* parasites by the simuliid vectors, namely, the rate at which mff from an infected bloodmeal succeed in reaching the fly's thoracic muscles, where they undergo further development. We examine several parasite–vector combinations present in endemic areas of human onchocerciasis. These combinations include vectors with well-developed cibarial teeth (*S. ochraceum s.l.* from Guatemala and *S. oyapockense s.l.* from the Venezuelan–Brazilian border) as well as species without such an armature (*S. damnosum s.l.* from West Africa and *S. guianense* from South Venezuela). Analyses are based upon published and unpublished data sets, and on new field studies conducted in Latin America.

We present the analyses of three different types of data sets. First, the relationship is explored between the mean numbers of mff ingested and the resulting mean numbers of successful larvae in blackfly species with and without a cibarial armature. A functional relationship between microfilarial input and larval output is thus obtained. Second, the fraction of flies harbouring successful larvae for different mean successful larval loads is studied in order to obtain an estimate of the degree of larval aggregation among these flies. Third, these estimates of larval aggregation are used together with the functional relationships calculated previously in order to examine the prevalence of flies carrying successful larvae as a function of the mean microfilarial intake.

This procedure provides a way (1) to compare the output of successful larvae (measured as mean numbers per fly and as fractions of potentially infective flies) for different levels of microfilarial input in species possessing and not possessing cibarial teeth, (2) to investigate the resulting patterns and to compare them with those proposed by the hypotheses of limitation or facilitation and (3) since the approximate proportionality between parasite intake and dermal load has already been demonstrated (Campbell *et al.* 1980; Alley *et al.* 1994; Basáñez *et al.* 1994), to advance hypotheses about the efficacy of various control measures in areas with either type of vector.

MATERIALS AND METHODS

Sources of data

Table 1 summarizes the sources of data analysed in this paper and the criteria for success used in the different studies. The definition of the category 'successful larvae' comprises those mff that reach the haemolymph of the insect on their way to the thoracic muscles (haemocoelic mff), those that actually penetrate the muscle cells (thoracic larvae) and those that manage to develop to the infective stage (L3).

Parasitological and entomological procedures

All the results presented here are based on fly-feeding experiments, in which samples of wild flies to the main local vector species were engorged to repletion on subjects harbouring different intensities of skin infection and who, consequently, provided the flies with different mean microfilarial intakes of the local population of *O. volvulus*.

Assessment of the number of mff ingested, in the haemocoele and in the thorax of the flies and of the proportion of flies with larvae. In the different studies, groups of wild flies were fed on particular body regions of the various subjects participating in the investigation in an attempt to reduce heterogeneity in the intakes. The results comprise the figures obtained for different individual carriers and/or different local parasite densities along the body of those carriers. The procedures for fly collection and dissection are the same as those described by Basáñez *et al.* (1994). A subsample of the flies fed on each volunteer was dissected between 0·5 and 12 h post-engorgement (p.e.) in order to score the number of ingested parasites (total microfilarial count within the body of the insect), microfilarial migration out of the bloodmeal (those mff that escaped imprisonment by the peritrophic membrane and were found either in the haemocoele–haemocoelic mff–or in the thoracic muscles of the fly–thoracic mff–), and the fraction of flies harbouring successful mff among those dissected. The period between 0·5 and 12 h p.e. was chosen because a feature present in the published data sets and in our own observations was that mff started to appear in the thorax about 0·5 h after the

Table 2. Successful larvae of *Onchocerca volvulus* from different microfilarial intakes by *Simulium ochraceum s.l.* in Guatemala

Study participants	Mean no. of larvae/fly*					Prevalence of flies with successful larvae (%)
Patient code no.†	No. of flies	Ingested mff (x)	Thor. or Hmcl. mff (y)	No. of flies	L3 larvae (y)	
C1‡	24	1·70	0·00			0·00
				38	0·04	5·26
C2‡	24	2·60	0·00			0·00
				48	0·02	2·08
DIa‡	69	9·00	—			—
				68	0·19	—
C3‡	20	12·50	0·00			0·00
				53	0·02	1·89
C4‡	20	24·20	0·17			15·00
				62	0·17	8·06
C5‡	15	26·90	0·30			20·00
				37	0·12	13·51
C6‡	20	43·10	0·17			20·00
				70	0·17	18·57
C7‡	24	44·70	0·56			12·50
				65	0·17	9·23
C8‡	24	51·10	0·30			25·00
				53	0·51	32·08
C9‡	20	82·20	0·99			40·00
				44	1·04	27·27
O‡	25	91·41	1·41			—
				—	—	—
B2‡§ (Rodolfo)	134	115·99	1·51			39·38
				—	—	—
C10‡	20	117·80	1·76			55·00
				47	1·69	53·19
DII‡	67	170·00	—			—
				99	2·07	—
B3‡§ (Feliciano)	14	222·57	1·69			64·29
				—	—	—
B1‡§ (Aparicio)	221	265·84	2·43			76·49
				—	—	—
DI-b‡	64	390·00	—			—
				70	2·53	—

* See Table 1 for type of mean used.
† D: De León & Duke (1966), B: Bain, Durette-Desset & De León (1974), O: Omar & Garms (1975), C: Collins *et al.* (1977).
Body regions on which flies were fed as follows:
‡ Back, § Fore-arms.

infected meal. In those data sets in which microfilarial intakes were recorded immediately (0 h) after engorgement (*S. oyapockense s.l.* from northern Brazil), it was too early to detect any larvae outside the bloodmeal and therefore the figures shown for thoracic larvae were calculated from different subsamples of flies dissected at 24 h p.e.

Fly maintenance and dissection for the assessment of L3 output. When infective larval loads and proportion of infective flies were to be recorded, the remaining insects collected from each participant were kept alive until the completion of the extrinsic incubation period (within 6–7 days p.e. depending on the conditions under which flies were maintained in captivity). Although the methods of post-prandial transportation and maintenance of flies were slightly different across studies, all shared the common procedures of collection of the simuliids in individual tubes generally lined with filter paper, provision of a single bloodmeal at the beginning of the experiment (the putative infected meal), more regular provision of sugar solution usually with antibiotics, and maintenance under relatively constant conditions of temperature, high humidity and darkness, achieved by keeping the insects inside insulated cages or ice-boxes (Figueroa, Collins & Kozek, 1977; Takaoka *et al.* 1982). The results presented here correspond to mean worm burdens and percentages of flies with total L3 larvae (from head, thorax and abdomen) harboured by specimens dissected from 144 h (6 days p.e.) onwards. It has been observed that

Table 3. Successful larvae of *Onchocerca volvulus* from different microfilarial intakes by *Simulium oyapockense s.l.* in Southern Venezuelan and Northern Brazilian Amazonas

Study participants	Mean no. of larvae/fly*					
Patient code no.†	No. of flies	Ingested mff (x)	Thoracic mff (y)	No. of flies	Thor. larvae or L3 (y)	Prevalence of flies with successful larvae (%)
Carroay-a[4]	41	4·59	0·10			4·88
				46	0·09	4·35
				71	0·05	4·23
Parima[2,3]	18	10·90	0·11	—	—	5·56
Catrimani-a[2]	21	15·70	—			
				25	0·00	0·00
				69	0·27	20·29
Catrimani-b[3]	22	25·09	—			
				11	0·16	9·09
				80	0·13	10·00
Cauamé[2]	26	34·43	—			
				19	0·00	0·00
				37	0·07	8·11
Carroay-b[3]	33	172·46	2·46			48·48
				62	1·41	46·77

* See Table 1 for type of mean used.
Body regions on which flies were fed as follows:
† [2] Shoulders and Back, [3] Iliac zone and Buttocks, [4] Calves.
Catrimani and Cauamé from Shelley *et al.* (1987). Carroay and Parima from this work.

infective larvae can be recruited to the proboscis from any location in the insect during the blood feed (Duke, 1973; Philippon, 1977; Renz, 1987).

Analysis of data

Measures of the average number of larvae in the bloodmeal, in the haemocoele or thorax and of infective stages per fly. Due to the various ways mean intakes and mean larval loads per fly are reported in the data sources, it was not possible to select a single measure of central tendency. The means most widely used in the published literature were the arithmetic mean (AM), the geometric mean of Williams (WM) and the square root transformed mean (SQRTM). All of them are computed taking into account infected and uninfected specimens. The justification and conditions for their usage, as well as their formulae, have been described by Basáñez *et al.* (1994).

Since the mean numbers of successful larvae appeared to level off along with increasing microfilarial intakes in all data sets examined, the relationship between these two variables was explored by non-linear regression methods (Quasi-Newton and Simplex), with weighted least squares estimation of the parameters (weight = no. flies in each subsample). These analyses were performed with CSS: Statistica® (Complete Statistical System) software.

Prevalence versus intensity data. The results of the non-linear estimations were subsequently used to examine the relationship between the percentage of flies with successful larvae and the intensity of the initial input of mff. The fraction of simuliids harbouring potential or realized infective stages can be related to the mean larval burden per fly (y) by the following expression:

$$\text{Proportion of infective flies} = 1-\{1+y/k\}^{-k}, \quad (1)$$

where k varies inversely with the degree of larval aggregation in the flies. As $k\to\infty$ the distribution tends to Poisson, whilst as $k\to 0$ the distribution is highly contagious or aggregated (variance $\gg$ mean), and can be described in terms of the negative binomial (Anderson & May, 1985).

The estimates of k obtained by this fitting procedure can then be used to predict the prevalence of flies carrying successful larvae as a function of the mean microfilarial intake, assuming different functional relationships between the mean L3 output (y) and the initial average input of mff (x):

$$\text{Proportion of infective flies} = 1-\{1+y(x)/k\}^{-k}, \quad (2)$$

where $y(x)$ are the predictions of the different non-linear regression models aforementioned. The computation of k values and of their asymptotic confidence limits from prevalence-intensity data was performed by maximum likelihood estimation procedures (Cox & Hinkley, 1974; Guyatt *et al.* 1990). Different assumptions about the relationship between the parameter k and the mean were tested by

Table 4. Successful larvae of *Onchocerca volvulus* from different microfilarial intakes by *Simulium damnosum s.l.* in West Africa

Study participants	Mean no. of larvae/fly*				Prevalence of flies with successful larvae (%)
Patient code no.†	No. of flies	Ingested mff (x)	Thor. or Hmcl. mff (y)	L3 larvae (y)	
OCP28	50	0·02	0·00		0·00
OCP37	50	0·30	0·08		6·00
OCP41	50	0·48	0·04		4·00
OCP46	50	0·66	0·00		0·00
OCP56	50	0·84	0·02		2·00
OCP54	50	0·90	0·12		10·00
DD1	93	1·10	—	0·40	—
OCP17	50	1·82	0·14		10·00
OCP55	50	1·96	0·24		14·00
OCP47	50	2·40	0·30		18·00
OCP18	52	2·48	0·13		11·54
OCP57	50	2·80	0·32		24·00
OCP39	50	4·50	0·26		16·00
OCP49	50	5·24	0·30		20·00
OCP36	50	6·12	0·46		26·00
OCP53	50	6·50	0·34		32·00
OCP20	50	7·34	0·46		30·00
OCP34	50	9·56	0·50		38·00
OCP26	50	10·42	0·44		30·00
OCP21	50	12·62	0·44		30·00
OCP33	50	13·54	0·36		22·00
OCP22	50	14·32	0·34		28·00
OCP61	50	14·92	0·28		18·00
OCP29	50	15·94	0·98		50·00
OCP60	50	16·20	1·00		56·00
OCP30	50	16·56	1·06		42·00
OCP58	50	17·16	0·50		30·00
OCP40	50	19·20	0·66		42·00
OCP35	50	21·88	0·68		32·00
PH6[12]	172	22·31	0·53		29·65
OCP23	50	25·76	0·40		28·00
PH6[24]	58	26·03	0·62		31·03
OCP31	49	27·27	0·80		42·86
OVP32	50	30·24	0·64		36·00
OCP38	50	30·32	0·86		42·00
OCP59	50	34·26	1·84		44·00
PH2b[12]	308	38·78	0·56		25·00
OCP19	50	43·68	1·00		44·00
OCP45	50	43·72	1·58		50·00
PB1[24]	270	46·10	1·81		41·26
BA1[12]	102	50·34	1·56		59·80
OCP42	50	50·64	1·12		38·00
OCP43	50	58·34	1·00		46·00
OCP48	49	59·14	1·41		53·06
OCP52	49	70·57	1·39		55·10
OCP44	50	71·52	1·12		38·00
OCP25	50	90·30	1·12		38·00
PB1[12]	462	92·42	1·39		28·57
PH2b[24]	307	100·60	1·14		48·86
OCP27	49	103·51	1·57		57·14
OCP24	49	114·79	1·66		48·98
OCP51	49	120·16	2·57		51·02
PH3b[12]	177	250·69	2·39		39·55
PH3b[24]	40	352·00	1·73		55·00

* See Table 1 for type of mean used.
All flies engorged upon the legs of the volunteers. DD: De León & Duke (1966), BA: Bain (1971), PB: Philippon & Bain (1972) (Subject: Maurice Tiemba, Léraba, raw data provided by Dr Odile Bain), PH: Philippon (1977) (Subjects 3b and 6 from Samandéni, 2b from Léraba), OCP: Onchocerciasis Control Programme (data from Alley *et al.* 1994).
† [12] Flies dissected up to 12 h p.e. [24] Flies dissected at 24 h p.e. All flies from OCP dissected between 4 and 10 h p.e.

Table 5. Successful larvae of *Onchocerca volvulus* from different microfilarial intakes by *Simulium guianense* in Southern Venezuelan Amazonas

Study participants	Mean no. of larvae/fly*					Prevalence of flies with successful larvae (%)
Patient code no.†	No. of flies	Ingested mff (x)	Thoracic mff (y)	No. of flies	L3 larvae (y)	
Coy-IIa[6]	9	1·61	0·00	—	—	0·00
David-a[6]	30	4·12	0·10	—	—	3·33
Joonafesi[5]	10	4·62	—	11	0·46	18·18
Cipriano[5]	10	4·78	—	10	0·67	40·00
Coy-I[4]	18	8·32	0·24	—	—	16·67
David-b[5]	19	12·77	—	55	1·01	40·00
Coy-IIb[4]	17	21·54	0·70	—	—	35·29
Cecilio-a[3]	9	43·00	—	15	1·69	46·67
Cecilio-b[4]	13	57·91	—	37	1·27	37·84
Mayuba[5]	10	58·01	—	22	1·04	54·55
Coy-III[4]	15	83·33	0·93	—	—	60·00
Cecilio-c[5]	10	85·00	—	46	1·17	41·30
Coy-IV[4]	31	114·95	1·09	—	—	61·29
David-c[4]	31	123·15	1·33	—	—	41·94
David-e[2]	26	165·76	1·11	—	—	46·15
David-f[3]	11	253·05	—	13	1·60	46·15

* See Table 1 for type of mean used.
† Body regions on which flies were fed as follows:
[2] Back, [3] Iliac zone, [4] Buttocks, [5] Calves, [6] Ankles.
David-b and David-f from Takaoka *et al.* (1984*b*). All other volunteers from this work.

means of the likelihood ratio statistic, which is approximately chi-squared distributed with degrees of freedom equal to the difference between the number of parameters in the models being tested (Sokal & Rohlf, 1981; Cox & Oakes, 1984; Armitage & Berry, 1987). The fitting procedure was then repeated with k as a function of microfilarial intake.

RESULTS

Tables 2, 3, 4 and 5 summarize, respectively, the data sets from Guatemala, Northern Brazil, West Africa and South Venezuela.

The examination of raw scatter plots of the mean numbers of successful larvae against mean intakes for the *S. ochraceum s.l.*, *S. damnosum s.l.* and *S. guianense* data sets revealed that in the 3 blackfly species the output of successful larvae tended to reach a plateau as the input of mff increased. As a consequence, the relationship between mean number of successful larvae (y) and mean microfilarial intake (x) was explored by fitting the model $y(x) = ax/(1+cx)$ to all 3 data sets. This function takes the value of zero when no mff are ingested, describes an initially linear relationship with slope a for very small values of x, and encompasses a process of density-dependent saturation in the number of larvae that can successfully develop within the fly (limitation), where c is a measure of the strength of density dependence. Under the null hypothesis ($c = 0$) the model represents a linear relationship (proportionality) between the two variables in question. However, the initial shape of this relationship appeared to be non-linear in *S. ochraceum s.l.* when compared with that in *S. damnosum s.l.* and *S. guianense*. There was a slower and initially exponential increase in the numbers of successful larvae harboured by the Guatemalan simuliid, as opposed to a steeper and convex rise in the African and Venezuelan flies.

In order to test the hypothesis that the initial shape of the relationship between larval output and microfilarial input could be related to the presence or absence of a well-developed cibarial armature in the simuliid foregut, an independent data set, describing the proportion of injured parasites found in bloodmeals with varying microfilarial densities taken up by *S. ochraceum s.l.* (Bain, Durette-Desset & De León, 1974) was analysed. This proportion was observed to decrease with increasing microfilarial intake up to a certain level (approximately up to 40%). This density-dependent reduction of the rupture of ingested parasites by the cibarial teeth could translate into an initially increasing proportion of viable mff capable of migrating out of the abdomen

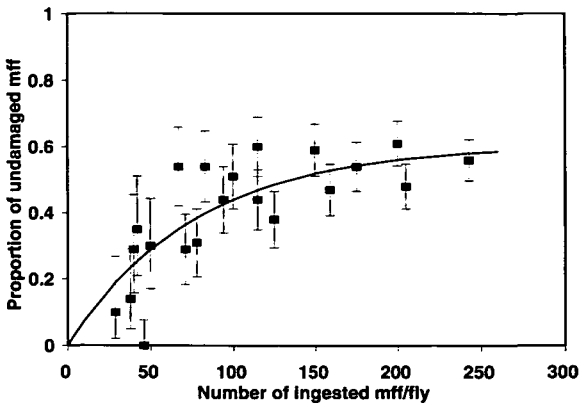

Fig. 1. Scatter plot of the proportion of unscathed microfilariae found in bloodmeals of Guatemalan *Simulium ochraceum s.l.* containing increasing numbers of mff. The line is the non-linear regression fit obtained when the function $z = \alpha(1-\exp(-\beta x))$ was applied to the data points. z is the fraction of uninjured parasites and x the parasite intake. Parameter values are shown in Table 6. Data from Bain, Durette-Desset & De León (1974). The 95% confidence intervals around the observed proportions, p, were calculated using the exact method based on the F distribution for small values of x ($px < 20$) and by the normal approximation ($p \pm 1{\cdot}96\sqrt{p(1-p)/x}$ for larger values of x ($px \geqslant 20$) (Armitage & Berry, 1985).

Table 6. Results of the non-linear model fitted to the data of Fig. 1 describing the fraction (z) of ingested mff that remain unscathed by the cibarial armature of *Simulium ochraceum s.l.* as a function of parasite intake (x) (Data from Bain, Durette-Desset & De León (1974).)

Model	$z = \alpha(1-\exp(-\beta x))$*
n	21
Correlation	0·7917
Proportion of variance explained	0·6268
α	0·6071
S.E. (α)	0·0756
$t(n-2)$	8·0313
P	0·0000
β	0·0129
S.E. (β)	0·0036
$t(n-2)$	3·5273
P	0·0022

* z is the expected proportion of intact mff in the bloodmeal containing x ingested parasites. α is the maximum fraction of unscathed parasites to be attained. The parameter β is a measure of the sensitivity to density dependence in the relationship between microfilarial intake and damage by the cibarial armature.

(initial 'facilitation'), that would be compatible with the pattern observed in the Guatemalan blackfly. As a result, the function $z = \alpha(1-e^{-\beta x})$, describing the expected fraction of unscathed mff, was fitted to the data of Bain (*op. cit.*) plotted as the fraction of mff remaining undamaged by the cibarial armature on their way to the stomach of the insects (Fig. 1). In

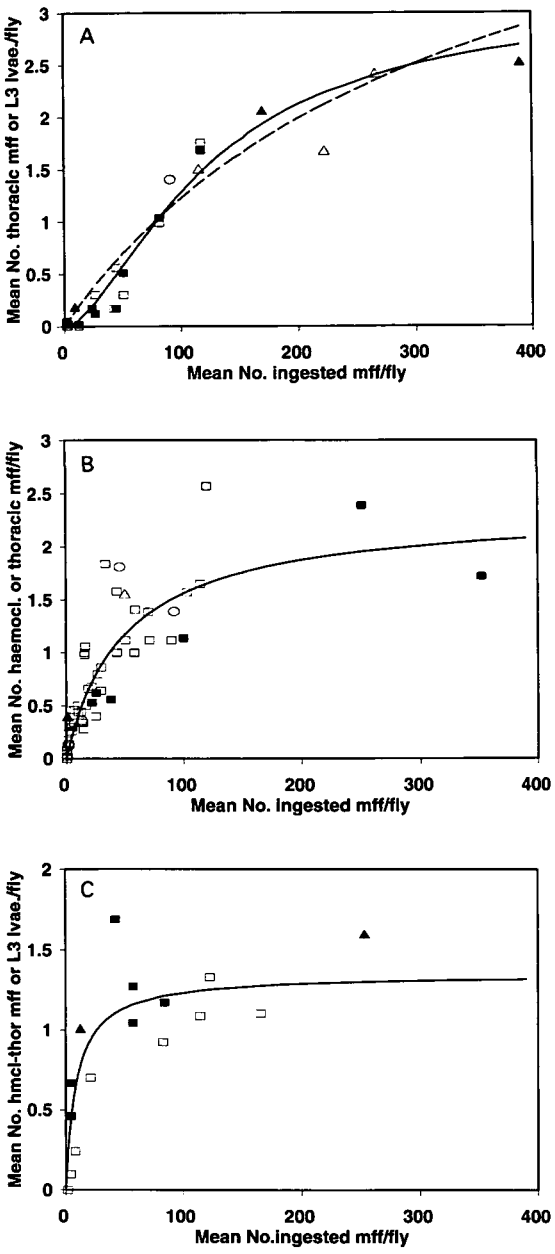

Fig. 2. (A) Scatter plot of the mean numbers of successful larvae (measured as mff escaping from imprisonment by the peritrophic membrane or developing to the infective stage) versus the mean microfilarial intake for *Simulium ochraceum s.l.* from Guatemala. The dotted line corresponds to the limitation model $y = ax/(1+cx)$, whilst the solid line corresponds to the expression $y = a\alpha(1-\exp(-\beta x))$ $x/(1+cx)$, where y is the mean number of successful parasites and x the mean microfilarial intake. The parameter values for the hyperbolic function are: $a = 0{\cdot}0159 \pm 0{\cdot}0017$, $c = 0{\cdot}0030 \pm 0{\cdot}0007$, a/c (saturation level) = 5·3352, $r = 0{\cdot}95$. The parameter values for the sigmoid equation are presented in Table 7. Data points as follows: (▲) L3 larvae, De León & Duke (1966); (△) haemocoelic mff, Bain, Durette-Desset & De León (1974); (○) thoracic mff, Omar & Garms (1975); (■) L3 larvae and (□) thoracic mff, Collins *et al.* (1977). (B) Scatter plot of the mean numbers of successful larvae versus the mean microfilarial intake for *S. damnosum s.s./S. sirbanum* from West Africa. The fitted

Table 7. Results of non-linear regression and correlation analyses of the mean number of successful larvae on mean microfilarial intakes for three *Onchocerca volvulus–Simulium* spp. combinations

	Vector species		
	S. ochraceum s.l.	*S. damnosum s.l.*	*S. guianense*
Cibarial armature	Present	Absent	
Model	$y = \frac{a\alpha(1-e^{-\beta x})x}{(1+cx)}$		$y = \frac{ax}{(1+cx)}$
n	27	54	16
Correlation	0·9730[1]	0·8740	0·8517
	0·9732[2]		
Proportion of variance explained	0·9468[1]	0·7639	0·7255
	0·9471[2]		
a[1]	0·0612	0·0463	0·1406
S.E. (a)	0·0079	0·0097	0·0452
t ($n-2$)	7·7817	4·7563	3·1079
P	0·0000	0·0000	0·0077
c[1]	0·0112	0·0196	0·1040
S.E. (c)	0·0020	0·0067	0·0389
t ($n-2$)	5·5002	2·9521	2·6725
P	0·0000	0·0047	0·0182
$a' = a\alpha$[1]	0·0372		
β	0·0129		
a'[2]	0·0339		
S.E. (a')	0·0098		
t ($n-3$)	3·4718		
P	0·0020		
β[2]	0·0139		
S.E. (β)	0·0034		
t' ($n-3$)	4·1375		
P	0·0004		
c[2]	0·0100		
S.E. (c)	0·0034		
t ($n-3$)	2·7202		
P	0·0119		

[1] The sigmoid model corresponding to the *S. ochraceum s.l.* data was fitted using the parameter values for α and β already estimated from the data shown in Fig. 1 and Table 6, $\alpha = 0{\cdot}6071$ and $\beta = 0{\cdot}0129$.
[2] The model for *S. ochraceum s.l.* was fitted estimating all three parameters $a' = a\alpha$, β and c from the data set shown in Fig. 2A and Table 2.

this expression β is a measure of the degree of density-dependent decrease in the damage to ingested mff caused by the cibarial armature of the vector and α is the maximum proportion of parasites left uninjured as intake increases. When $\beta = 0$, all ingested parasites are lesioned ($z = 0$). When $\beta \to \infty$, a constant, density-independent proportion of mff is left unscathed ($z = \alpha$). The fitting of the model to the data of Fig. 1 provided a value of β which was small but significantly different from zero (Table 6), suggesting the existence of a certain degree of protection against cibarial damage dependent on the density of microfilarial intake. Since under the null hypothesis of no facilitation, $z = \alpha$ for all values of x, the existence of initial non-linearity was tested by plotting the 95% confidence limits around the observed proportions of undamaged mff in Fig. 1. It can be seen that for the lower values of parasite intake the confidence intervals do not include the estimated value of $\alpha = 0{\cdot}6071$ (95% C.L. = 0·4489–0·7653), supporting the hypothesis of initial facilitation. The expression for z was thus incorporated into the hyperbolic function previously described in order to obtain an alternative model for

model is the equation $y = ax/(1+cx)$, where y and x have been defined in (A). Parameter values are given in Table 7. (▲) L3 larvae, De León & Duke (1966); (△) haemocoelic mff, Bain (1971); (○) haemocoelic mff, Philippon & Bain (1972); (■) haemocoelic mff, Philippon (1977); (□) exo-peritrophic mff, Alley *et al.* (1994). (C) Scatter plot of the mean numbers of successful larvae versus mean microfilarial intake for *S. guianense* from South Venezuela. The line corresponds to the expression $y = ax/(1+cx)$ fitted to the data points. Parameter values are indicated in Table 7. (▲) L3 larvae, Takaoka *et al.* (1984*b*); (■) L3 larvae and (□) thoracic mff, this work.

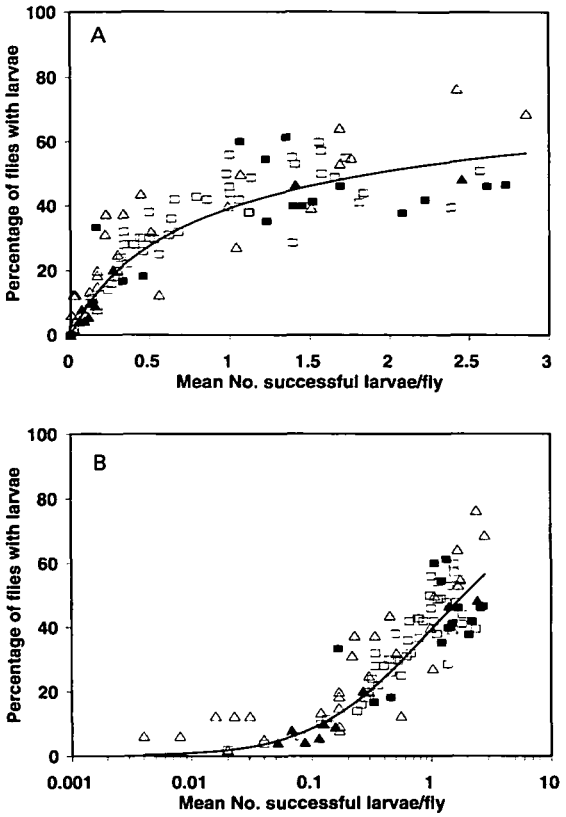

Fig. 3. (A) Scatter plot of the percentage of flies harbouring successful larvae versus the mean larval load per fly for all simuliid species included in this study. The line is the maximum likelihood fit applied to this prevalence versus intensity data set assuming the relationship in equation (1): proportion of potential or realized infective flies $= 1-[1+y/k]^{-k}$, where y is the mean number of successful larvae and k an inverse measure of the degree of larval aggregation ($k = 0{\cdot}3950$, see text). Data points are: (△) *Simulium ochraceum s.l.*; (▲) *S. oyapockense s.l.*; (□) *S. damnosum s.l.*; (■) *S. guianense*. (B) Percentage of flies with successful larvae plotted against the logarithm of the mean number of larvae per insect. Line and data points are as described in (A).

S. ochraceum s.l., in which $y(x) = a\alpha(1-e^{-\beta x})x/(1+cx)$. The inclusion of a positive feed-back operating at low densities in otherwise hyperbolic expressions has led to satisfactory sigmoid fittings of observed functional responses in other biological situations (Hassell, Lawton & Beddington, 1977).

Two approaches were taken to estimate the parameters of the sigmoid model fitted to the Guatemalan blackfly. In the former, the values of the parameters α and β, obtained from the fit applied to the data shown in Fig. 1, were fed into the alternative equation describing the relationship between successful and ingested larvae for *S. ochraceum s.l.*, whilst in the latter, all the parameters in this equation were freely estimated from the data set depicted in Fig. 2A. The proportion of the variance explained by the hyperbolic limitation model was then compared with that resulting from the sigmoid function including initial facilitation.

The scatter plots of the data points corresponding to *S. ochraceum s.l.*, *S. damnosum s.l.* and *S. guianense*, and the curves fitted to them are shown respectively in Fig. 2A, B and C. A visual inspection of the curves in Fig. 2A suggests that the sigmoid function confers a better fit to the Guatemalan data set than the hyperbolic expression. The proportion of the variation accounted for by the limitation model alone (0·9024), did increase when the expression for facilitation was added to the function (0·9468 in the case of α and β being estimated from Fig. 1, and 0·9471 when all parameter values were computed from Fig. 2A). In addition, a better behaviour of the residuals was observed with the sigmoid function, and the fitting of the hyperbolic equation to *S. ochraceum s.l.* provided an upper limit of 5·34 successful larvae per fly, well in excess of observed values. As a consequence, we concentrated on the model with initial facilitation for *S. ochraceum s.l.* and with limitation alone for *S. damnosum s.l.* and *S. guianense*. These models, the parameter values, and associated statistics are presented in Table 7. It can be seen that for *S. ochraceum s.l.*, the two procedures used to fit the sigmoid model provided very similar results. The 95% C.L. around the parameter β were 0·0052–0·0205 and 0·0070–0·0209 in each case.

The crucial test for detecting density-dependent limitation of the numbers of larvae that succeed in developing within the 3 blackfly species, in the framework of the chosen functions, depends on the parameter c being significantly greater than zero. The 95% confidence intervals around this parameter did not include zero in any of the data sets examined. The limits were 0·0063–0·0330 for *S. damnosum s.l.* and 0·0205–0·1875 for *S. guianense*. In the sigmoid models fitted to *S. ochraceum s.l.* the values were: 0·0070–0·0153 when the information derived from Fig. 1 was used, and 0·0024–0·0175 when all the parameters of the proposed equation were estimated from the data points in Fig. 2A. The fitted expressions predict a maximum number of larvae equal to a/c in the case of *S. damnosum s.l.* (2·35) and *S. guianense* (1·35) and equal to $a.\alpha/c$ for *S. ochraceum s.l.* (3·32).

Fig. 3A and B show the percentage of simuliids carrying successful larvae plotted against the mean larval burden per fly in arithmetic and logarithmic scales, respectively, for all blackfly species included in this study. The line is the maximum likelihood fit to these data using the relationship in equation (1) with k independent of the mean larval load (y). The fit provided an estimate of $k = 0{\cdot}3950$ with 95% asymptotic confidence limits = 0·3579–0·4367. The likelihood ratio statistic showed no significant difference between the model with constant k and those with k as a linear ($k(y) = \delta+\phi y$), power ($k(y) = \delta y^{\phi}$)

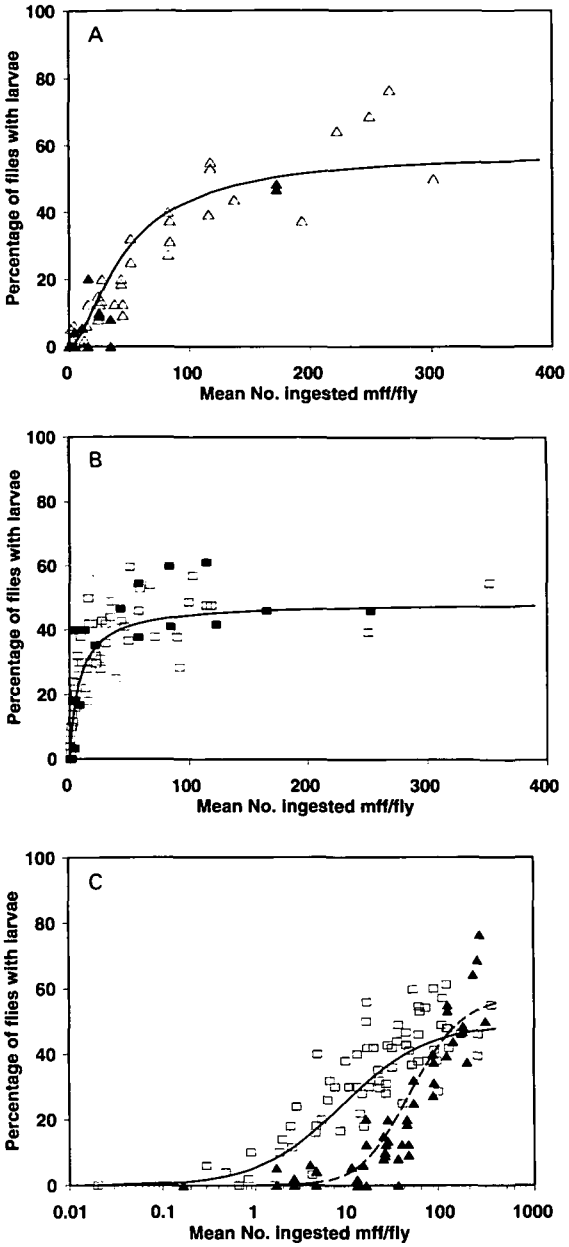

Fig. 4. (A) Percentage of flies with successful larvae plotted against mean microfilarial intake in simuliid species possessing a well-developed cibarial armature. The line was obtained by applying equation (2): proportion of infective flies $= 1-[1+y(x)/k]^{-k}$, where y (the mean no. of successful larvae) is a function of x (parasite intake). The function is described in Fig. 2(A); (solid line); parameter values are those for *Simulium ochraceum s.l.* (Table 7), and k value is that of Fig. 3. (△) *S. ochraceum s.l.*; (▲) *S. oyapockense s.l.* (B) Percentage of flies with successful larvae versus mean microfilarial intake in simuliid species with unarmed cibarium. Line as described in (A) where $y(x)$ follows the expression in Fig. 2B and C. Parameter values are those for *S. damnosum s.l.* (Table 7) and k value is that of Fig. 3. (□) *S. damnosum s.l.*; (■) *S. guianense*. (C) Percentage of flies with successful larvae plotted against the logarithm of the mean parasite intake in armed and unarmed blackflies. The logarithmic scale permits a better appreciation of the difference between both kinds of vectors particularly at low microfilarial intakes. Dotted line is as described in (A) solid line as in (B). (▲) Simuliids with cibarial armature; (□) simuliids without armature.

and exponential ($k(y) = \delta e^{\phi y}$) function of the mean worm burden per fly, so the results of these models are not presented. A common value of k was computed to measure the degree of aggregation of potentially or realized infective larvae in both armed and unarmed simuliids. Our argument in favour of a common k was based upon the fact that this parasite stage has already evaded the effect of the cibarial armature when present, and also because a shared k value provided a better description of the proportion of flies with successful larvae as a function of the mean microfilarial intake per fly (see below). The low value of k suggests a high degree of larval aggregation which was also observed in the distributions fitted to the direct larval counts per insect. The latter did not depart significantly from the negative binomial when tested with the goodness of fit chi-squared test (data not shown). The average k in this case was 0·3334, ranging from 0·1811 to 0·5381, in agreement with the value estimated from equation (1).

The values of k thus obtained were used to fit the relationship between the percentage of infective flies and the mean microfilarial intake (x) by means of equation (2) and the sigmoid expression $y(x) = a\alpha(1-e^{-\beta x})x/(1+cx)$ for the species with cibarial armature (*S. ochraceum s.l.* and *S. oyapockense s.l.*, Fig. 4A), whilst equation (2) plus the hyperbolic function $y(x) = ax/(1+cx)$ were used for the unarmed species (*S. damnosum s.l.* and *S. guianense*, Fig. 4B). In the case of the data set combining the species with cibarial teeth, the parameter values of $y(x)$ were those of *S. ochraceum s.l.* For the data concerning the species without armature the parameter values of *S. damnosum s.l.* were chosen (the figures estimated for *S. guianense* tended to underestimate the proportion of infective flies). Fig. 4C compares on a logarithmic scale the data and fitted lines of the percentage of flies with successful larvae as the numbers of mff ingested increase in the armed and unarmed simuliids. This proportion increased more slowly and tended to level off between 50 and 60 % for flies ingesting more than 150 mff on average in the former group of flies, whilst the increase was faster and the maximum (between 40 and 50 %) reached earlier (intakes of 50 mff upwards) in the latter group.

DISCUSSION

Basáñez *et al.* (1994) found little evidence of density dependence in the acquisition of skin microfilariae by flies feeding on *O. volvulus* carriers in 3 different endemic areas where *S. ochraceum s.l.*, *S. damnosum*

s.l. and *S. guianense* were the predominant vectors. The evidence for density dependence seems to be stronger when the fate of the ingested parasites is examined in the same blackfly species. The mean numbers of larvae succeeding in reaching the thoracic muscles of the flies and undergoing larval development to the infective stage reached a plateau of about 1–3 larvae per fly in the 3 simuliid species. This non-linearity was statistically significant in all data sets examined. Limitation had already been demonstrated in *S. damnosum s.l.* (Pichon, 1974; Bain, 1976; Boussinesq, 1991) but not studied in detail in any other blackflies. Apart from species-specific differences in vector competence, the lower saturation value found for *S. guianense* may be due to the fact that, in general, the Venezuelan flies dissected in order to detect thoracic mff were examined probably too early (average time ranging from 1·15 to 5·11 h p.e.), with the result of lowered larval loads being observed. The Guatemalan and West African specimens were dissected between 4 and 10 h p.e., when most, if not all, microfilarial migration would have taken place (Laurence, 1966; Philippon, 1977). This conjecture finds some support in the better description of the relationship between the proportion of flies with larvae versus mean intake which was achieved when the parameter values obtained for the African species were used instead of those derived from the Venezuelan simuliid in equation (2).

The numerical investigation of the patterns of limitation, facilitation and proportionality occurring in several vectors of human and animal filariases has, so far, been based upon the statistical examination of the relationship between the so-called 'parasite yield' (ratio of successful larvae to microfilarial intake, y/x), or the inverse of the parasite yield (x/y), and the microfilarial intake (x). In species with limitation y/x tends to decrease with x and there is a linearly increasing relationship between x/y and x. The opposite is to be expected in species with facilitation. Proportionality translates into either the parasite yield or its inverse being independent of x (Pichon, 1974; Prod'hon *et al.* 1980; Southgate & Bryan, 1992). However, since the variable x appears in both axes, the interpretation of the trends followed by the above-mentioned ratios is not necessarily straightforward on statistical grounds alone (Dr C. Dye, personal communication). Another, more general consideration to be taken into account when drawing conclusions about the patterns emerging from the analyses of variables that constitute mean values, is that a great deal of the variation in the data sets explored may be concealed, i.e. both the average number of successful larvae and the mean microfilarial intake per fly are subject to random variation.

The hyperbolic function fitted to the African data set is simpler than the empirical models previously used by Alley *et al.* (1994), Pichon (1974), and Plaisier *et al.* (1991), yet it fits the data well and the estimation of the saturation level (around 2–3 larvae per fly) is very similar. The more complex functional form of the relationship between microfilarial input and infective larval output advocated by Plaisier *et al.* (1991) was found, however, to be necessary to allow those authors to explore the dynamics of recrudescence after cessation of vector control and the impact of ivermectin on transmission at low dermal parasite loads (Alley *et al.* 1994).

The results of the feeding studies analysed here, in which a single bloodmeal was provided at the beginning of the experiment, indicate that the average numbers of mff that reach the haemocoele or the thorax of the insects are generally good predictors of the numbers of L3 larvae to be found in the flies once the extrinsic incubation period is completed. This suggests that there is probably little larval loss during intra-thoracic development in susceptible flies, as has previously been reported by other authors (Duke, 1962*a*; Collins *et al.* 1977; Philippon, 1977). However, comparative analyses with natural infection rates and larval burdens are necessary. On the one hand, naturally infected females will have had the opportunity to ingest blood more than once (it has been shown that *Onchocerca* larval development is dependent on blood intake, Ham & Gale (1984)), but also will have experienced some larval loss through feeding (Duke, 1973; Philippon, 1977; Renz, 1987) and different survival rates. On the other hand, these analyses could also throw some light on the relevance of possible mechanisms of acquired resistance by the biting fly population in natural settings. Until now, the presence of such mechanisms has only been demonstrated in experimental *Onchocerca–Simulium* systems based on intra-thoracic inoculation of mff (Ham, 1986, 1992), although there is an early observation describing that in wild host-seeking females, the presence of a previous infection in the fly seems to reduce the number of parasites that can develop from a subsequent infection (Duke, 1968).

The most important regulatory processes directly affecting *Onchocerca* survival and establishment within the flies (i.e. not considering indirect effects such as parasite-induced vector mortality), seem to be taking place early in the parasitic phase that occurs in the vector, namely, during the migration of the mff out of the mid-gut. The peritrophic membrane, which is secreted in response to the ingestion of blood, has been considered to act as an effective barrier to microfilarial migration in simuliids (Lewis, 1950, 1953; Duke & Lewis, 1964; Bain & Philippon, 1969; Reid & Lehane, 1984; Eichner *et al.* 1991). However, the evidence is scarce and the mechanisms remain largely unknown. Bain *et al.* (1976) have proposed that density-dependent changes in the thickness and rate of formation of the peritrophic membrane may explain the limitation observed in

the numbers of ingested parasites that gain access to the thoracic muscles in savanna members of the *S. damnosum* complex. Other possible explanations include the activation of insect defences, such as the lectin-like molecules acting at the level of the midgut epithelium that have been claimed to interfere with microfilarial migration in other filaria–culicid systems (Phiri & Ham, 1990). This activation may be dependent on the density of the microfilariae.

The hypothesis that the level of saturation found in the system *Onchocerca–Simulium* is the result of processes acting early upon microfilarial migration rather than of some 'crowding effect' due to the overcoming of a larval carrying capacity, as described in other filaria–vector combinations such as *Wuchereria–Aedes* (Rosen, 1955) and *Brugia–Mansonia* (Wharton, 1957*a*, *b*), is supported by the results of experimental injections of mff directly into the thorax of the flies. This procedure permits the circumvention of the barriers associated with the infection *per os* and has resulted in the demonstration of a nearly proportional relationship between larval output and microfilarial input in intrinsically susceptible flies (Lok *et al.* 1980; Ham & Bianco, 1983; Eichner *et al.* 1991).

Although limitation, acting at high parasite intakes, was common to the 3 blackfly species studied (expressed in the term $1/(1+cx)$ of the models fitted and in the statistical significance of the parameter c), the pattern of initial exponential increase in the numbers of mff reaching the haemocoele or thorax of the flies present in the Guatemalan data set, is more compatible with initial 'facilitation'. Bain *et al.* (1974) interpreted their data on *S. ochraceum s.l.*, plotted on logarithmic scales on both y and x axes, as evidence of proportionality. Our analysis suggests that density-dependent damage of the ingested mff by the cibarial armature in *S. ochraceum s.l.* may produce an initial positive feed-back between microfilarial intake and the number of parasites which are viable and hence capable of migrating out of the bloodmeal.

The hypothesis of an association between the presence of a cibarial armature in the insect host and the pattern of initial facilitation requires further investigation in other filaria–vector combinations. So far, the causal mechanism that has been claimed to operate in relation to facilitation involves histological changes in the abdominal epithelium (Bain & Brengues, 1972). However, it is interesting to note that facilitation has solely been reported for vectors possessing cibarial teeth, such as *Anopheles* mosquitoes of the subgenus *Cellia* (McGreevy *et al.* 1978), including *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* (Brengues & Bain, 1972; Southgate & Bryan, 1992), although the statistical evidence for its demonstration in some of these cases has recently been questioned (Dye, 1994). Table 8 summarizes the patterns reported in the literature for several vector–filaria combinations concerning the relationship between microfilarial input and larval output. It can be seen that only limitation or proportionality have been described for vectors with poorly developed armatures or totally unarmed cibaria (*Culex*, *Aedes*, *Mansonia*, *S. damnosum s.l.*).

A possible explanation for the decline in the proportion of ingested mff that are injured by the cibarial armature as intake increases, is that it may be more likely for a few mff all to be ruptured by the teeth as they are pumped in with the bloodmeal, whilst as more larvae are ingested, the ones that become entangled may protect the remainder. A similar decrease in the fraction of ingested parasites ruptured by the cibarial teeth has been found in *Wuchereria bancrofti–An. funestus*, but not in *W. bancrofti–An. gambiae s.l.* (Bryan & Southgate, 1988; Bryan, McMahon & Barnes, 1990).

Although the proportion of the variance in the Guatemalan data explained by the function incorporating density-dependent damage by the cibarial armature of *S. ochraceum s.l.* is very high, we can only suggest that this mechanism may contribute to the pattern observed without the exclusion of other possible underlying processes. More probably, the phenomena of 'facilitation' and 'limitation' are the result of the combination of a variety of factors whose net effect is still to be elucidated. Among others, these factors include the interaction of the ingested mff with the cibarial teeth, with the peritrophic membrane, clotting agents and digestive enzymes acting in the bloodmeal, cellular reactions in the epithelium of the stomach, and insect defences (Denham & McGreevy, 1977; Townson & Chaithong, 1991). For example, Bryan *et al.* (1990) have reported that not all the undamaged mff of *W. bancrofti* observed in the bloodmeals of anopheline vectors in East Africa were able to reach the thoracic muscles of the mosquitoes.

The value of the clumping parameter k, obtained from the proportion of flies with successful larvae versus mean larval burden, indicates a high degree of larval aggregation in the insect host. The magnitude of this aggregation ($k = 0{\cdot}395$) appears to be greater than that computed for ingested mff by means of the same kind of analysis ($k = 0{\cdot}588$–$0{\cdot}632$, Basáñez *et al.* (1994)), reflecting the fact that not all the flies that ingest mff become subsequently infected with developing and developed larvae. This high degree of contagion is compatible with an overdispersed distribution such as the negative binomial which was found to be an adequate description of the distribution of the raw larval counts (data not shown). The trend towards a decline in the value of k with increasing age of infection in the flies has also been observed when the negative binomial distribution has been fitted to the numbers of larvae per fly found in naturally infected *S. damnosum s.l.* populations (Cheke, Garms & Kerner, 1982; Garms & Cheke,

Table 8. Patterns observed for the relationship between microfilarial input and larval output in several vector–filaria combinations

Vector spp.	Parasite spp.	Pattern	Cibarial armature in the vector	Reference
Aedes aegypti	*Wuchereria bancrofti*	Limitation	Absent	Bain (1971) Brengues & Bain (1972); Pichon (1974)
	Setaria labiatopapillosa			Bain (1971) Bain & Chabaud (1974)
	Skrjabinofilaria skrjabini			Bain (1976) Chabaud *et al.* (1986)
	Dipetalonema dessetae			Bain (1976)
Aedes polynesiensis	*W. bancrofti*			Rosen (1955) Pichon (1974)
Aedes togoi	*Brugia malayi*			Southgate & Bryan (1992)
Culex quinquefasciatus (=*C. pipiens fatigans*)	*W. bancrofti*		Very poorly developed	Jordan & Goatly (1962) Pichon (1974) Southgate & Bryan (1992)
Simulium damnosum s.l.	*O. volvulus*		Absent	Bain (1971) Philippon & Bain (1972) Pichon (1974)
Mansonia dives/ bonneae (=*M. longipalpis*)	*Brugia malayi*	Proportionality	Absent	Wharton (1957) Southgate & Bryan (1992)
Anopheles gambiae s.s.	*W. bancrofti*	Facilitation	Present	Brengues & Bain (1972) Southgate & Bryan (1992)
An. arabiensis				Southgate & Bryan (1992)
An. funestus				

1985). The opposite, a lower degree of aggregation, is to be expected in the presence of density-dependent parasite-induced vector mortality (Anderson & Gordon, 1982). However, this assumes that this process operates uniformly at all stages of larval development, lopping the tails of skewed distributions. We have evidence that *Onchocerca*-induced *Simulium* mortality acts predominantly during the early phases of infection, i.e. during microfilarial ingestion. The aspects of density-dependent mortality of infected vectors and parasite aggregation, in both human and fly hosts, will be the subject of subsequent publications.

The non-linear regression models describing larval output as a function of microfilarial input in the cases of the simuliids with and without cibarial armature, were used together with this value of k in order to investigate the relationship between the

fraction of flies with successful larvae and the average microfilarial intake. The fits obtained appeared to be reasonable descriptions of the data in both cases. The sigmoid equation derived for the Guatemalan blackfly species seemed adequate to explain the data of *S. ochraceum s.l.* and *S. oyapockense s.l.*, whilst the hyperbolic model was suitable for *S. damnosum s.l.* and *S. guianense*. The results show that simuliids with armed foreguts require higher average parasite intakes than those by unarmed species in order to acquire equivalent levels of effective infection, measured as mean larval burdens per fly and as percentage of flies with larvae. Nevertheless, these same indicators suggest an increased vectorial efficiency of the armed species over the species without armature at heavier microfilarial intakes, in agreement with the remarks made by Shelley (1988, 1991). These differences will be further explored in the light of parasite-induced vector mortalities elsewhere. Elevated fly mortalities, following the ingestion of large numbers of parasites, have been reported to affect the species without the 'protection' afforded by the cibarial teeth (Lewis, 1953; Duke, 1962*a*, 1966; Omar & Garms, 1977; Takaoka *et al.* 1984*b*), in contrast to the lower death rates recorded for species with toothed fore-guts (De León & Duke, 1966; Collins *et al.* 1977; Takaoka *et al.* 1984*a*; Shelley *et al.* 1987). However, the amount of parasite-related vector loss which really takes place in endemic areas will depend on how frequent is the acquisition of high parasite intakes in the field.

In conclusion, our analyses suggest the existence of 'initial facilitation' in blackfly species like *S. ochraceum s.l.* and *S. oyapockense s.l.*, and confirm the occurrence of 'limitation' in the savanna members of *S. damnosum s.l.*; the latter also being found in *S. guianense*. The sigmoid nature of the relationships depicted in Figs 2A and 4A, is compatible with previous claims that facilitation may provide a threshold for the interruption of transmission in areas where vector species with this pattern prevail (Pichon *et al.* 1974; Bain, 1976; Webber, 1991; Southgate & Bryan, 1992). Given that microfilarial intakes are roughly proportional to skin loads (Campbell *et al.* 1980; Alley *et al.* 1994; Basáñez *et al.* 1994), it is possible to postulate that control campaigns based solely on the reduction of the parasite reservoir in the human host are likely to be more successful in foci where the principal vectors have cibarial armatures, whilst a combination of both, measures against the vector and against the parasite, may be necessary in endemic areas maintained by blackfly species without such armatures (Shelley, 1991, 1994). The results of ongoing chemotherapy programmes with the microfilaricide ivermectin seem to support this conjecture (Remme *et al.* 1990; Cupp, 1992).

However, any speculation on the existence of a transmission threshold must be based on formal stability analysis of dynamic models that explicitly incorporate the features described above in the equations for the parasite in the vector host, as well as reasonable assumptions about the processes affecting the parasite in the human host (Dye, 1994; Dye & Williams, 1994). The existing onchocerciasis models have been constructed for the epidemiological situations arising in West and Central African savanna regions (with density-dependent limitation of the number of parasites developing in the flies (Dietz, 1982; Plaisier *et al.* 1990, 1991, Alley *et al.* 1994), or in forest areas (proportionality is assumed by Davies (1993)). A model for the Latin American scenarios is greatly needed and is currently being developed. The practical relevance of theoretical breakpoints in the complex life-cycles of helminth parasites has been shown to depend on the nature of the frequency distributions of the numbers of worms per definitive and intermediate host (Macdonald, 1965; May, 1977; Anderson, 1980). In the case of highly overdispersed distributions, the parasite intensities at which unstable equilibria occur (below which the system is driven to extinction), may be too low to have a meaningful epidemiological impact (Anderson & May, 1985).

The non-linearities described above need to be evaluated in the context of most field situations, where overdispersion translates into only a few individuals of the host populations harbouring the parasite intensities at which density-dependent constraints are likely to play a role. This applies to mff in the skin of the human host (Basáñez & Yarzábal, 1989; Boussinesq, 1991) as well as to larvae in the simuliid vector (Cheke *et al.* 1982; Garms & Cheke, 1985; Renz, 1987; Wenk, 1991). The question of whether the events comprising the life-cycle of the nematode in the vector are occurring in parasite ranges where the relationships are practically linear, will be explored in a subsequent publication in the light of the frequency distributions of worm burdens per human and per vector host.

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