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Vector competence of *Simulium oyapockense* s.l. and *S. incrustatum* for *Onchocerca volvulus*: Implications for ivermectin-based control in the Amazonian focus of human onchocerciasis, a multi-vector–host system

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ABSTRACT

Although it is now well established that in the Amazonian onchocerciasis focus, straddling between Venezuela and Brazil, the main vectors in the highland (hyperendemic) and lowland (hypoendemic) areas, are respectively *Simulium guianense sensu lato* Wise and *S. oyapockense* s.l. Floch and Abonnenc, investigation of the vectorial role of a third anthropophagic species, *Simulium incrustatum* Lutz has remained inconclusive. Here we compare the vector competence of *S. incrustatum* with that of *S. oyapockense* s.l. by conducting, in the Venezuelan part of the focus, a series of feeding experiments designed to analyze their relative: (a) microfilarial intakes when fed upon the same skin load; (b) proportions of microfilariae (mf) surviving damage inflicted by the cibarial armature (present in both species); and (c) infective (L3) larval outputs. Although the ability of *S. oyapockense* s.l. to ingest mf, for a given microfilaridermia, was markedly higher than that of *S. incrustatum*, the (density-dependent) proportions of those ingested mf that were damaged by the armature were also consistently higher, with the resulting output of L3 larvae being significantly lower in *S. oyapockense* s.l. than in *S. incrustatum*. These results indicate that *S. incrustatum* plays a more important role in onchocerciasis transmission in the Amazonian focus than previously realized. We discuss the implications of our findings for the control and elimination of onchocerciasis with mass administration of ivermectin in this focus, where the three main anthropophagic species often co-occur.

1. Introduction

It is now well recognized that the epidemiology of human onchocerciasis in the Amazonian focus, is highly heterogeneous. Such heterogeneity is due to a complex set of interactions between altitudinal, geological, ecological, anthropological, and entomological factors (Botto et al., 2005; Carabin et al., 2003; Grillet et al., 2001; Vivas-Martínez et al., 1998, 2007). Regarding the latter, there are three main anthropophagic *Simulium* species that have been demonstrated to support the development of *Onchocerca volvulus* microfilariae (mf) to the infective stage (Basáñez et al., 1988; Takaoka et al., 1984). These are, in order of decreasing proportional abundance from the highlands to the lowlands, *Simulium guianense sensu lato* Wise; *Simulium incrustatum* Lutz, and *S. oyapockense* s.l. Floch and Abonnenc, associated with a cline of onchocerciasis prevalence that ranges from hyper- to hypoendemic levels (Basáñez et al., 1988; Grillet et al., 2000, 2001; Vivas-Martínez et al., 1998, 2007). In addition to their variable vector efficiency, their biting and parous rates exhibit marked spatial (by altitude and locality) and temporal (by season and per hour) variation (Grillet et al., 2001, 2005).

Of the three simuliid species, the associations of *S. guianense* s.l. with highland savannahs (and hyperendemic transmission), and of *S. oyapockense* s.l. with lowland forests (and levels of onchocerciasis endemicity that range from low to moderate), have been well established (Basáñez et al., 1988; Shelley, 1988). However, the vectorial role of *S. incrustatum*, which appears to have a more restricted distribution throughout the focus (e.g., man-biting females are seldom collected along the Orinoco river but commonly so along the Ocamo and Putaco rivers in southern Venezuela) has not, to date,

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been investigated in depth. *S. incrustatum* is found between 90 and 920 m above sea level (asl); it is more prevalent in highland forested and mountainous areas than in jungle clearings; and is present in hyperendemic areas (albeit rarely on its own). It has been the fact that *S. incrustatum* possesses a well-developed cibarial armature, a feature in common with *S. oyapockense* (hereafter referred to without explicit reference to its *sensu lato* species complex status) that has placed *S. incrustatum* within the category of relatively inefficient vectors of *O. volvulus* (Shelley et al., 1987).

Although it is true that the cibarial armature (when present) inflicts damage on ingested mf (Reid, 1978) that could jeopardize their chances of developing into infective larvae (Omar and Garms, 1975; Basáñez et al., 1995), the degree of damage exerted by the cibarial teeth could well differ between vector species according to the structure, morphology and size of the spike-like prominences that project towards the foregut lumen (Shelley et al., 1987). Additional factors that could influence and generate inter-species variation in vector competence include, among others, differences in the numbers of ingested mf per fly for a given human microfilaridermia; the size of the flies (and of their respective bloodmeals); their intrinsic susceptibility (determining larval development once the surviving mf have established themselves within the thoracic muscles); their ability to survive, once infected, the parasite's extrinsic incubation period and beyond; the operation of parasite density-dependent mechanisms underlying some of the above; and whether the parasite exploits any of these processes within the vector to enhance its transmission.

In this paper we contrast the vector competence of *S. incrustatum* against that of *S. oyapockense* by quantifying the relative magnitudes of their: (a) microfilarial intakes; (b) proportion of ingested mf that survive the damage inflicted by their cibarial armatures; and (c) L3 output, when flies of both species are fed upon the same (or comparable in skin load) *O. volvulus* carriers whose microfilaridermia levels have been assessed. We present data collected prior to the introduction of mass ivermectin treatment in the Venezuelan part of the Amazonian focus. An investigation of how these three aspects change at various time-points after single and multiple ivermectin treatments will be presented elsewhere. Our overarching goals are those of elucidating the relative vectorial roles of the various anthropophagic simuliid species which often co-occur in localities of the Amazonian onchocerciasis focus, and understanding how the outcome of control/elimination programmes based on regular mass distribution of ivermectin will be influenced, in this multi-vector–parasite system, by the ability of the local vectors to ingest and allow the development of mf into L3 larvae at low levels of human skin infection (Basáñez et al., 2003).

2. Materials and methods

2.1. Study area

Briefly, the study area is situated in the Venezuelan part of the Amazonian focus, sparsely inhabited by the Yanomami Amerindian group, and characterized by dense humid tropical forest, with average temperatures of 26–27 °C, and annual rainfall of 3750–5000 mm distributed between May through August as the rainier months; October through March as the drier months, and April and September as the transitional months between the two seasons (Huber et al., 1984; Grillet et al., 2001). The fly-feeding experiments were carried out in four riverine Yanomami communities in which the man-biting blackfly species composition had previously been established (Grillet et al., 2001; Vivas-Martínez et al., 1998), namely, Mahekoto-theri (Mh; 2°25′N, 64°54′W; 140 m asl), on the Orinoco river and nearly with 100% *S. oyapockense*; Maweti-theri (Mw; 2°55′N, 64°45′W; 140 m asl), on the Ocamo river and nearly with 100% *S. oyapockense*; Awei-theri (Aw; 2°49′N, 64°30′W; 162 m asl), on the confluence of the Ocamo and Putaco rivers and with 5% *S. guianense*; 15% *S. oyapockense*, and 80% *S. incrustatum*; and Pashopeka-theri (Ps; 2°47′N, 64°28′W; 240 m asl), on the Putaco river, and with 5% *S. guianense*; 7% *S. oyapockense*, and 88% *S. incrustatum*. The microfilarial prevalence values of Maweti; Mahekoto; Awei-theri; and Pahopeka were, respectively, 30.3, 40.4, 66.8 and 79.8%; their community microfilarial loads (as defined by Remme et al., 1986) were 0.4, 1.6, 41.4 and 13.5 mf/ss (Vivas-Martínez et al., 1998, 2000a). Detailed descriptions of the focus and of the entomological and parasitological studies conducted at these sites have been presented elsewhere (Botto et al., 2005; Carabin et al., 2003; Grillet et al., 2001, 2005; Vivas-Martínez et al., 2000b, 2007). Awei-theri and Pashopeka are sentinel communities within the National Programme for Onchocerciasis Elimination.

2.2. Microfilarial carriers and ethical considerations

The work took place between 1997 and 1998, prior to the commencement of large-scale ivermectin-based control in the area. Fifteen male volunteer carriers of *O. volvulus* mf (aged ≥ 19 years) agreed to participate in the fly-feeding experiments. Consent was sought from the participants through local interpreters (Yanomami health workers from the communities) both for skin-snipping and fly-feeding. Their microfilarial loads were determined shortly before the fly-feeding experiments as the number of *O. volvulus* mf per mg of skin that emerged after 8–24 h incubation in buffered saline of two skin snips (each from the right and left iliac crests) that were taken with a Holth-type corneoscleral punch and incubated together (Vivas-Martínez et al., 2000a). The resulting microfilaridermias are presented in Table 1. During the study, the subjects were not exposed to fly bites for longer periods than they would have normally been exposed, and ivermectin treatment was administered to all participants after completion of the study.

2.3. Experimental infection methods

In each locality, wild flies of either *S. oyapockense* (at Mh and Mw), or simultaneously of both *S. oyapockense* and *S. incrustatum* (at Aw and Ps) were allowed to feed to satiation on the back and legs of each volunteer by placing an individual plastic tube over the fly when it commenced feeding. (Plastic tubes had been previously prepared as described by Basáñez et al., 1988; Grillet et al., 1994.) When the fly was replete and dislodged itself, the vial was capped and the fully engorged flies (~200–300) collected from each subject were kept inside plastic lunch boxes identified per subject; wrapped in damp kitchen towels, and placed inside insulated (ice) boxes (Takaoka et al., 1984) in a shaded corner of a makeshift field laboratory, usually at the health dispensary of the community. Within the boxes a min/max hygro-thermometer was used to monitor the conditions of the incubation and the boxes were placed on low tables whose legs were immersed in cans containing motor oil to prevent ants from reaching the flies. The identification number of the volunteer, the blackfly species, and the date and time of capture were recorded for each particular fly-feeding experiment. Although wild host-seeking simuliid populations normally consist of a mixture of nullipars and parous flies, previous studies have not detected any significant differences between microfilarial intakes by flies of different reproductive status (Philippon and Bain, 1972).

In the field lab, a group of 15–30 flies per subject (excepting situations when low fly biting density precluded such numbers) were killed with chloroform vapour and dissected at intervals ranging from 0 to 8 h post-engorgement (p.e.) to determine the number

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Table 1
Mean microfilarial intake per fly, *m*, and the proportion of flies, P_m, with microfilariae out of total *Simulium oyapockense* s.l. and *S. incrustatum* examined after 0–8 h post-feeding upon *Onchocerca volvulus* carriers with varying microfilaridermia, *M*, in the Amazonian focus, southern Venezuela, between 1997 and 1998, prior to mass ivermectin distribution

Subject		*Simulium oyapockense* s.l.			*Simulium incrustatum*		
Code	M[a] (number of mf/mg)	$\bar{m}_G$ (range)[b]	P_m (%)[c]	[95% CL][d] for P_m	$\bar{m}_G$ (range)[b]	P_m (%)[c]	[95% CL][d] for P_m
Aw1	0.0	–	–	–	0.8 (0–26)	7/15 (46.7)	[25.8, 73.4]
Mh1	2.2	1.6 (0–27)	6/15 (40.0)	[21.5, 67.7]	–	–	–
Mw2	2.3	2.3 (0–49)	9/15 (60.0)	[35.5, 83.7]	–	–	–
Ps1	6.3	12.0	1/1 (100.0)	[2.5, 100.0]	7.6 (0–130)	18/21 (85.7)	
Ps2	15.2	–	–	–	2.5 (0–21)	13/22 (59.1)	[39.0, 79.3]
Mw3	17.0	0.8 (0–32)	3/15 (20.0)	[9.8, 48.1]	–	–	–
Aw2	23.4	35.7 (23–136)	3/3 (100.0)	[29.2, 100.0]	5.3 (0–117)	15/22 (68.2)	[51.2, 89.3]
Mh2	39.5	3.7 (0–162)	9/15 (60.0)	[35.5, 83.7]	–	–	–
Ps3	64.7	42.7 (35–52)	2/2 (100.0)	[15.8, 100.0]	18.7 (0–377)	19/23 (82.6)	[61.9, 95.0]
Mh3	81.5	27.2 (1–230)	15/15 (100.0)	[78.2, 100.0]	–	–	–
Aw3	100.6	39.6 (10–183)	9/9 (100.0)	[66.4, 100.0]	4.4 (0–165)	17/21 (81.0)	[58.9, 94.6]
Mh4	130.0	34.9 (1–274)	15/15 (100.0)	[78.2, 100.0]	–	–	–
Aw4	133.1	45.6 (16–236)	7/7 (100.0)	[59.0, 100.0]	24.8 (4–115)	7/7 (100.0)	[59.0, 100.0]

[a] *M*, mean number of mf/mg.
[b] $\bar{m}_G$, geometric (Williams') mean number of mf/fly.
[c] P_m, proportion of flies that ingested mf.
[d] [Exact 95% confidence limits]; Mw, Maweti-theri; Mh, Mahekoto-theri; Ps, Pashopeka-theri; Aw, Awei-theri.

of *O. volvulus* mf in the bloodmeal; the proportion of ingested mf lacerated by the cibarial teeth; and the migration of mf to the fly's thoracic muscles. Each fly was placed on a microscope slide and the abdomen of the fly was severed from the remainder with mounted entomological needles, separating the bloodmeal surrounded by the peritrophic matrix in a drop of distilled water to lyse the erythrocytes and facilitate counting of the mf (Demanou et al., 2003). Two smears per slide were prepared for each fly's abdomen (corresponding to the endo- and exo-peritrophic sections) and fixed with cold methyl alcohol, for subsequent staining with 4% methylene blue in 3% acetic acid (Garms, 1985). The smears were examined for enumeration of ingested mf and assessment of damage inflicted by the cibarial armature (Omar and Garms, 1975), scoring mf as undamaged; entire but damaged; and numbers of (cephalic, middle, and caudal) portions; with the numbers of whole mf estimated by dividing the total number of portions by 3 and rounding up (Davies et al., 1997). The remaining insect sections (thorax and head) were fixed individually in vials containing 80% ethyl alcohol (labelled in order to match mf intake with mf/larvae in the thoracic muscles); stained with Mayer's haemalum (Nelson, 1958); and dissected in drops of glycerine on microscope slides at CAICET. The rest of the flies were fed on 30% sucrose solution with antibiotics (Basáñez et al., 1988); kept in captivity at ~27–30 °C and 80–100% relative humidity; checked every 12 h to assess their survival (dead flies were counted, removed, and fixed in 80% ethanol); and on day 8 p.e., all surviving flies were killed and fixed in 80% ethanol for subsequent staining and dissection as described above, in order to ascertain larval maturation up to L3 (Basáñez et al., 1988; Grillet et al., 1994). In CAICET, flies were separated into head, thorax and abdomen and each of these sections was teased apart delicately (to avoid damaging any parasite stage present) in separates drop of glycerine. The location, number and developmental stage of *O. volvulus* larvae were recorded using criteria defined by Duke (1968) and Porter and Collins (1984).

2.4. Variables of interest and data analysis

2.4.1. Microfilarial intake

For each group of flies fed from each participant (with microfilaridermia *M*, the number of mf/mg), microfilarial intake was measured both in terms of $\bar{m}$, the mean number of ingested mf per fly (for each fly the numbers of mf found in the endo- and exo-peritrophic sections of the fly's abdomen were added), and of P_m, the proportion of flies that ingested mf. We report both the arithmetic mean (AM) and geometric mean (GM) of Williams; the latter obtained after log + 1 transformation of the microfilarial counts (Williams, 1937) for reasons previously described (Basáñez et al., 1994). The AM is one of the parameters of the negative binomial distribution (NBD), typically used to describe the number of parasites per host or vector. This distribution is frequently overdispersed (variance significantly greater than the mean), with most individuals harbouring low numbers of parasites and only a few being heavily infected (Crofton, 1971). Because of this feature, the GM may be useful as a measure of central tendency as it is less sensitive to extreme values than the AM. Exact 95% confidence limits (95% CL) for proportions were computed following Miettinen (1970) as described in Armitage and Berry (1994). Frequency distribution histograms of the number of ingested mf per fly (combining data from all participants) were prepared for each of the two simuliid species under comparison, and the overdispersion parameter (k) and its standard error (S.E.(k)) were estimated by maximum likelihood (ML) (Demanou et al., 2003; Elliott, 1977; Grenfell et al., 1990). The relationship between P_m and both M and $\bar{m}$ was explored and fitted by ML assuming an underlying NBD, and testing for different functional relationships between the overdispersion parameter and microfilaridermia or mean microfilarial intake/fly, $P_m = 1 - [1 + (s/k_s(s))]^{-k_s(s)}$, where k_s can be a constant, or a linear or power function of the mean load of the parasite stage in question ($s = M$ or $\bar{m}$ for, respectively, microfilarial density in skin or flies) (Basáñez et al., 2002; Demanou et al., 2003; Wetten et al., 2007). Nested models were compared using the likelihood ratio statistic, LRS (Clayton and Hills, 1993).

The relationship between microfilarial intake per fly and microfilarial load per mg of skin was explored by assuming a model of the form $\bar{m} = \alpha M^{\beta}$, which can be linearised by taking logs on both sides and where the null hypothesis ($\beta = 1$) indicates proportionality (a linear relationship) between $\bar{m}$ and M, and the alternative hypothesis ($\beta \neq 1$) indicates a nonlinear relationship (with $\beta < 1$ corresponding to negative density dependence, and $\beta > 1$ to positive density dependence). (For further details see Demanou et al., 2003; Soumbey-Alley et al., 2004; Wetten et al., 2007.)

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Table 2
Symbols, definitions, and units of the variables used and parameters estimated in this paper

Symbol	Definition	Units
M	Microfilaridermia	mf/mg
m_b	Number of mf in the bloodmeal of individual flies	mf
$\bar{m}$	Mean microfilarial intake, with $\bar{m}_A$ or $\bar{m}_G$ denoting the arithmetic or the geometric mean, respectively	mf/fly
$\bar{m}'$	Mean intake of unscathed microfilariae, with subscripts as above	Unscathed mf/fly
$\bar{L}$	Mean infective larval load, with subscripts as above	L3/fly
P_m	The proportion of flies that have ingested mf	–
P_d	The proportion of ingested mf that have been damaged by the cibarial armature	–
P_L	The proportion of flies that carry L3 larvae	–
P_Y	Parasite yield or efficiency of conversion from mf to L3	L3/ingested mf or L3/undamaged mf
k_s	Overdispersion parameter of the NBD of parasite stages among flies, with $s = M$, $\bar{m}$, or $\bar{L}$ as appropriate	–
$\log(\alpha)$	The intercept of the linear regression model in the $\log(\bar{m})$ vs. $\log(M)$ relationship	–
β	The slope of the linear regression in the model above	$[\log(M)]^{-1}$
ε	The severity of density-dependent damage inflicted by the cibarial armature on ingested mf	mf^{-1}
δ	The asymptotic proportion of damaged mf as m_b increases	–
ϑ_M, ε_M	Parameters describing initial facilitation in the $\bar{L}$ vs. M relationship (possibly due to density-dependent damage inflicted to mf)	mf^{-1}
c_M	The strength of limitation in L3 output dependent on skin microfilarial density	mf^{-1}

2.4.2. Proportion of ingested mf that are damaged by the cibarial armature

The proportion of damaged mf (the number of entire but damaged mf plus numbers from portions divided by total mf intake) was computed for flies feeding on each subject/microfilaridermia, and denoted as P_d. Plots of individual fly data suggested that the relationship between the proportion of damaged mf and the number of mf in the bloodmeal, m_b, was nonlinear for both these species, approaching a limit as the number of mf increased. Therefore, a model of the form, $P_d = (1-\delta)[\exp(-\varepsilon m_b)] + \delta$ (derived from that presented by Basáñez et al., 1995 for *S. ochraceum* in Guatemala; see also Edwards and Hamson, 1994) was fitted to the data by ML to estimate parameters ε (the severity of density-dependent damage inflicted by the armature) and δ (the asymptotic fraction of damaged mf as m_b increases). The mean number of undamaged mf ingested per fly ($\bar{m}'$) was also calculated (as arithmetic and geometric means) and plotted against microfilaridermia (M).

2.4.3. Larval development in the thoracic muscles of the surviving flies

The number of flies and the number of *O. volvulus* larvae (any stage) found between 12 and 192 h p.e. were tabulated, and the proportion of each larval stage in relation to the total number of larvae recovered for each day was obtained. This permitted to follow the progression of larval development and assess its synchrony within the flies (Jerwood et al., 1984).

2.4.4. Infective larval output

According to the results of larval progression as described above, the mean number of L3 larvae per fly ($\bar{L}$) found between 120 h (day 5) and 192 h (day 8) p.e. was plotted against microfilaridermia (M). Visual inspection of this plot suggested that mean infective larval load saturated for higher microfilarial loads. To incorporate the observation that mf are damaged by the cibarial armature in a density-dependent fashion, the following expression (used by Basáñez et al., 1995 to describe a similar phenomenon in *S. ochraceum*) was fitted to the data by ML, $\bar{L} = \vartheta_M[1-\exp(-\varepsilon_M M)]M/(1 + c_M M)$. Subscript M indicates that the parameters pertain to skin microfilariae, with parameters ϑ_M and ε_M determining the degree of initial facilitation that would derive from the proportion of undamaged, viable mf increasing along with mf density, and parameter c_M measuring the severity of (microfilarial) density-dependent limitation in L3 output that would operate within the flies as microfilaridermia increases. As above, both the AM and GM number of infective larvae per fly are reported. We also plotted mean infective larval output, $\bar{L}$ vs. mean microfilarial intake, $\bar{m}$ and mean number of undamaged mf, $\bar{m}'$. The proportion of infective flies, P_L, was plotted against the mean number of L3 per fly ($\bar{L}$), and the overdispersion parameter, k_L, was estimated by ML. Parasite yield (P_Y) (Pichon et al., 1974) was defined as the proportion of L3/fly developing per mf ingested or undamaged, i.e., $P_Y(\bar{m}) = \bar{L}/\bar{m}$ or $P_Y(\bar{m}') = \bar{L}/\bar{m}'$.

We specify the type of mean reported as a measure of central tendency using the subscript A or G after the letter denoting the parasite stage, to refer to the arithmetic or the geometric mean, respectively. A value of $P < 0.05$ was considered to indicate statistical

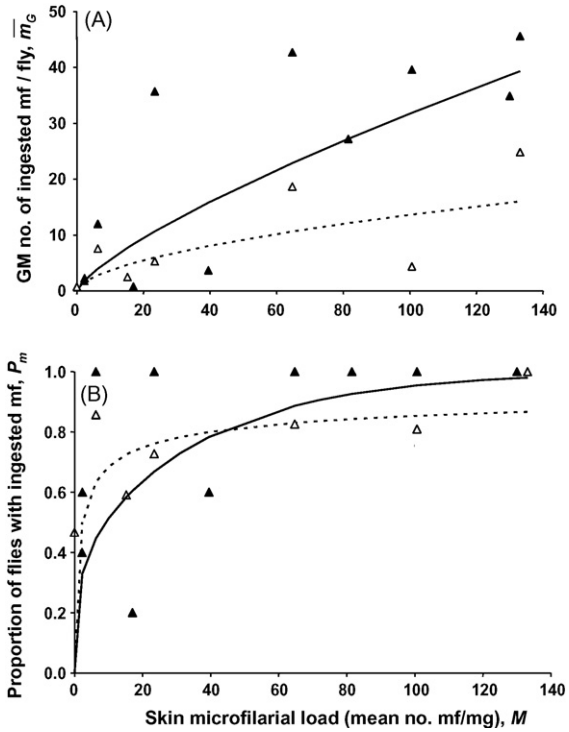

Fig. 1. The observed (markers) and fitted (lines) for: (A) geometric mean microfilarial intake, and (B) proportion of flies that ingested microfilariae, both as function of skin microfilarial load, M, of *Onchocerca volvulus* in *Simulium oyapockense* s.l. (black triangles and solid line) and *S. incrustatum* (open triangles and broken line) in the Venezuelan part of the Amazonian focus of human onchocerciasis. In (A) the model is $\log(\bar{m}_G) = \beta \log(M)$, with $\beta = 0.751$ for *S. oyapockense* and $\beta = 0.567$ for *S. incrustatum*. In (B) the model is $P_m = 1 - [1 + M/k_M(M)]^{-k_M(M)}$ with the overdispersion, k_M parameter displaying a linear relationship with M in the case of *S. oyapockense* ($k_{M_0} = 0.131$; $k_{M_1} = 0.005\ mf^{-1}$) but being independent of M in the case of *S. incrustatum* ($k_{M_0} = 0.337$).

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significance. Table 2 summarises the symbols, definitions, and units of the variables and parameters used in this paper.

3. Results

A total of 1638 *S. oyapockense* flies were engorged on 11 *O. volvulus*–mf carriers in fly-feeding experiments that took place during January 1997 (Mw), February 1997 (Mh), July 1997 and September 1998 (Aw), and September 1997 and September 1998 (Ps). A total of 1475 *S. incrustatum* flies were fed on 7 carriers during July 1997 and September 1998 (Aw), and September 1997 and September 1998 (Ps). The differences in the number of flies obtained by species are in part due to spatial and temporal biting density variation (Grillet et al., 2001).

3.1. The number of microfilariae ingested by flies and the proportion of flies with ingested microfilariae

A total of 112 *S. oyapockense* and 131 *S. incrustatum* were dissected for assessment of microfilarial intake. Table 1 presents the GM number of mf per fly, $\bar{m}_G$, and its range, as well as the proportion of flies that have ingested mf, P_m, and its 95% CL for *S. oyapockense* and *S. incrustatum*. The relationship between $\bar{m}_G$ and skin microfilarial load, *M*, of the carriers upon whom the flies were fed is further depicted in Fig. 1A. For any paired data points (both species feeding on the same person), *S. oyapockense* consistently ingested more mf than *S. incrustatum* for a given value of *M*. When the model was fitted, the estimate of $\log(\alpha)$ for *S. oyapockense* was not significantly different from zero ($\log(\alpha) = 0.093$; 95% CL = −0.649, 0.835), indicating that α would not be statistically different from 1. Therefore the more parsimonious model $\log(\bar{m}_G) = \beta \log(M)$ was used instead, resulting in a value of β that was significantly lower than unity ($\beta = 0.751$; 95% CL = 0.579, 0.923). Likewise, $\log(\alpha)$ for *S. incrustatum*, was indistinguishable from zero (α indistinguishable from 1), and the simpler model had a regression coefficient, β of 0.567 (95% CL = 0.372, 0.762). Therefore, for both species mf intake was negatively density-dependent on microfilaridermia.

Fig. 1B presents the proportion P_m of flies of both species which are positive for mf in their bloodmeal, against skin load (*M*). For a given skin load, the proportion of flies that have ingested mf is generally higher for *S. oyapockense* than for *S. incrustatum*. Confidence intervals, however, are very wide due to the small number of flies dissected to assess mf intake and in most cases overlap between species (Table 1). For *S. oyapockense*, and for the overdispersion parameter fitted to the aggregate P_m vs. *M* data, the best model was that in which $k_M(M) = k_{M_0} + k_{M_1} M$ was a linear function of *M*, with equation k_M and parameter estimates $k_{M_0} = 0.131$ and $k_{M_1} = 0.005$ (log likelihood (LL) = −22.116), in contrast with the model with constant k_M (LL = −25.213; LRS = 6.193, 1 d.f., $P = 0.013$), or the power model, $k_M(M) = k_{M_0} M^{k_{M_1}}$ (LL = −31.326; LRS = 18.420, 1 d.f., $P < 0.001$). This implies that the degree of overdispersion decreases with mean skin density (*k* is an inverse measure of overdispersion), allowing population samples of *S. oyapockense* to be 100% positive for ingested mf. By contrast, for *S. incrustatum*, the

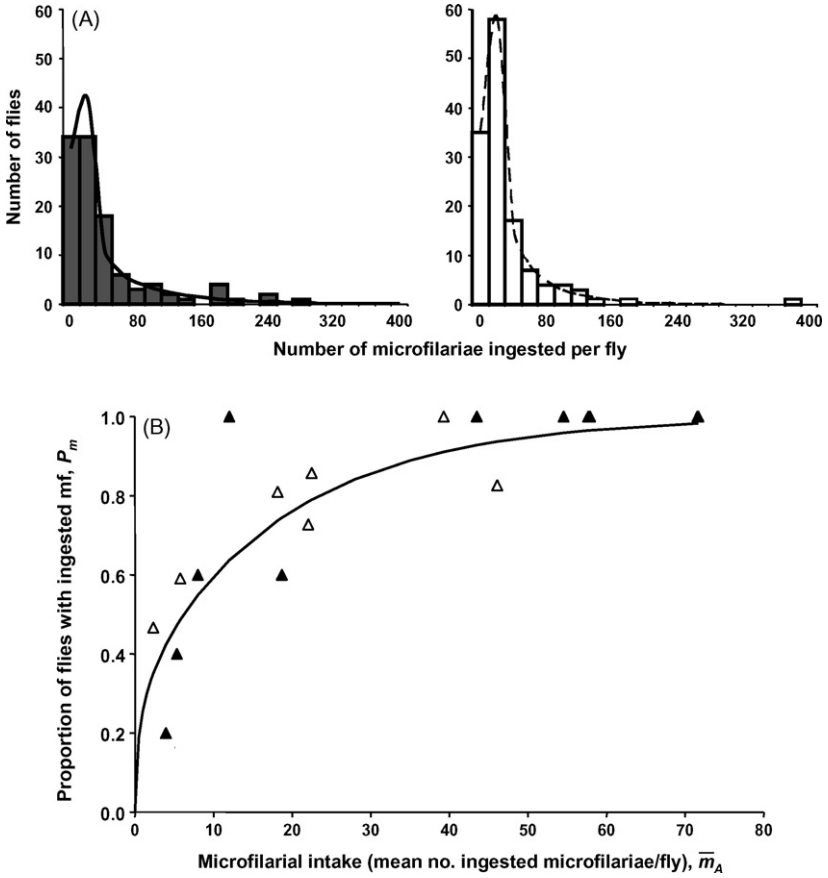

Fig. 2. The distribution among flies of the number of ingested microfilariae per fly, *m*, of *O. volvulus* in feeding experiments conducted in the Venezuelan part of the Amazonian focus: (A) frequency histogram distributions of observed data (bars) and fitted negative binomial distribution (lines) for *S. oyapockense* (dark bars and solid line, with arithmetic mean of 33.2 mf/fly, $k_m = 0.254$) and *S. incrustatum* (open bars and broken line, 21.6 mf/fly, $k_m = 0.308$), and (B) relationship between the observed (markers as in Fig. 1) and fitted (line) proportion of flies that ingested microfilariae and mean microfilarial intake ($P_m = 1 - [1 + \bar{m}/k_m(\bar{m})]^{-k_m(\bar{m})}$) with a linear relationship between k_m and $\bar{m}$ for both simuliid species ($k_{M_0} = 0.130$; $k_{M_1} = 0.011\ \mathrm{mf}^{-1}$).

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most parsimonious, yet adequate model was that with constant $k_{M_0} = 0.337$ (LL = −26.927), and P_m tended to level off at around 90% (Fig. 1B).

A frequency distribution histogram of the number of flies with ingested mf among the participant *O. volvulus* carriers confirmed marked overdispersion, with mean and parameter k equal to $\bar{m}_A = 33.2$ mf/fly (range = 0–274), $k_m = 0.254$ (S.E.(k_m) = 0.041) for *S. oyapockense*, and $\bar{m}_A = 21.6$ mf/fly (range = 0–377), $k_m = 0.308$ (S.E.(k_m) = 0.044) for *S. incrustatum*. On average, *S. oyapockense* ingested more mf than *S. incrustatum*, with little difference in the degree of clumping when the NBD was fitted to the overall distribution of the combined data (Fig. 2A).

In Fig. 2B, P_m is plotted against the (AM) microfilarial intake, $\bar{m}_A$ The data points for both species can now be described by a single relationship, suggesting that the degree of aggregation in mf intake is similar for both species (as suggested by Fig. 2A). The best functional form for $k_m(\bar{m})$ was the linear model with $k_{m_0} = 0.130$ and $k_{m_1} = 0.011$.

3.2. The proportion of ingested microfilariae that are damaged by the cibarial armature and the relationship between the mean number of unscathed mf and microfilarial intake

Fig. 3A plots the fraction of mf that has been injured by the armature, P_d, as a function of the number of mf in the bloodmeal, m_b for individual flies who ingested ≥10 mf (to ensure comparability with the *S. ochraceum* data plotted in Basáñez et al., 1995). The estimated values of the parameters governing this relationship were $\delta = 0.60$ and $\varepsilon = 0.087$ mf^{-1} for *S. oyapockense*, and $\delta = 0.21$ and $\varepsilon = 0.043$ mf^{-1} for *S. incrustatum*. Parameter δ measures the asymptotic proportion of damaged mf found in the bloodmeal as the number of ingested mf increases, and parameter ε measures the degree of density dependence operating in this process. Therefore, whilst both *S. oyapockense* and *S. incrustatum* can damage nearly all ingested mf when intakes are low, the former can damage up to 60% and the latter only up to ∼20% when mf intakes are high. Given the marked difference between both simuliid species regarding the fraction of parasites that would be available for continuing development within the fly (arguably the number of unscathed mf), Fig. 3B plots the mean number of undamaged mf ($\bar{m}'$) per fly against mean mf intake, $\bar{m}$. The mean number of viable mf is nearly three times greater in *S. incrustatum* than in *S. oyapockense*. In summary, although for a given microfilaridermia *S. incrustatum* ingests a lower number of mf, and the proportion of flies that ingest mf is also lower, its cibarial armature damages a smaller proportion of the ingested mf, resulting in a larger number of viable parasites in the bloodmeal that would be available for migrating to the thorax and progressing towards development.

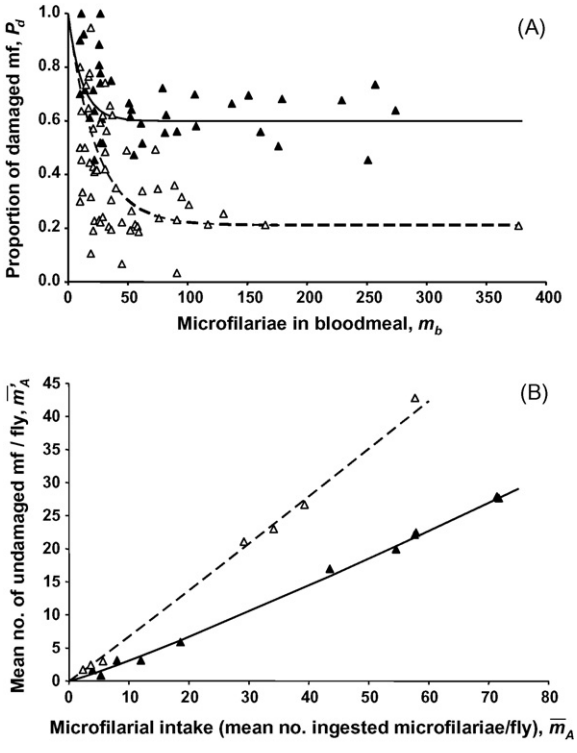

Fig. 3. The damage inflicted to ingested microfilariae of *O. volvulus* by the cibarial armature of the two Amazonian simuliid species under comparison: (A) the observed (markers) and fitted (lines) proportion of damaged microfilariae, P_d, as a function of the number of microfilariae in the bloodmeal, m_b, and (B) the mean number of undamaged microfilariae $\bar{m}'$ as a function of mean microfilarial intake $\bar{m}$ for *S. oyapockense* (solid triangles and line) and *S. incrustatum* (open triangles and broken line). In (A) the model fitted is $P_d = (1-\delta)[\exp(-\varepsilon m_b)] + \delta$, with $\delta = 0.60$, $\varepsilon = 0.087$ mf^{-1} for *S. oyapockense*, and $\delta = 0.21$, $\varepsilon = 0.043$ mf^{-1} for *S. incrustatum*. In (B) the model is the best fit trendline ($\bar{m}' = a\bar{m}^b$) with $a = 0.26$, $b = 1.09$ ($R^2 = 0.94$) for *S. oyapockense* and $a = 0.63$, $b = 1.03$ ($R^2 = 0.99$) for *S. incrustatum*.

3.3. Intrinsic susceptibility: larval development until the infective stage of those parasites established within the thoracic muscles

We proceed to investigate the fate of those parasites that establish themselves successfully within the thorax of the flies. A total of 1526 flies were dissected for *S. oyapockense*, whereas 1344 flies of *S. incrustatum* were dissected to assess larval development. The (AM) mean number of larvae (of any stage) per fly examined was 0.045 (69/1526), ranging from 0 to 7 larvae per fly (the latter corresponding to a fly with 7 L1 larvae) in *S. oyapockense*, and 0.051 (69/1344), ranging from 0 to 5 larvae per fly (the latter corresponding to a fly with 5 L3 larvae) in *S. incrustatum*.

Table 3 presents, for the total number of larvae obtained on each day p.e., the percent distribution of larvae according to stage. Infective larvae appear for the first time in both species on day 5 p.e. Larval development within *S. oyapockense* may appear to be more asynchronic than in *S. incrustatum*; of a total of 69 larvae recovered for each species from days 1 to 8, only 10 (14.5%) of them were L3 in *S. oyapockense* in comparison with 39 (56.5%) in *S. incrustatum*, whilst 56 (81.2%) in the former but only 23 (33.3%) in the latter were L1. However, the number of flies dissected each day p.e. was different between the two species, reflecting their respective survivorships up to day 8 p.e. The number of *S. oyapockense* flies dissected earlier is higher than that of *S. incrustatum* because more of them died earlier, leading to a higher recovery of L1 larvae in the former. For days 5–8 p.e. the mean numbers of L3/fly harboured by *S. oyapockense* were, respectively, 0.009, 0.044, 0.048 and 0.077. The corresponding values for *S. incrustatum* were 0.045, 0.023, 0.036 and 0.074. Regarding migration of the infective larvae to the cephalic capsule of the fly, there was again no difference between the species, with 5 out of 10 (50%) L3 in *S. oyapockense* reaching the head, and 19 out of 39 (49%) in S. *incrustatum*. However, fly survival is also a component of vector competence, and the higher survivorship of *S. incrustatum* translates into a higher net number of L3 larvae available for transmission.

Since L3 were present from day 5 onwards, Table 4 summarises the results concerning dissections that were conducted between 120 and 192 h to ascertain larval development to the infective stage (with those flies dissected on day 8 p.e. being alive). Of the 11 microfilarial carriers listed in Table 1 on whom *S. oyapockense* flies were fed, only 6 (in order of microfilaridermia: Ps1, Aw2, Mh2, Ps3, Aw3, and Aw4) resulted in successful infectivity. The number of flies that were dissected from day 5 onwards for the remaining sub-

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Table 3
The proportional distribution of *Onchocerca volvulus* larvae into L1 (sausage stage), L2 (pre-infective stage), and L3 (infective stage) according to day post-engorgement (p.e.) within *Simulium oyapockense* s.l. and *S. incrustatum* after being fed on untreated microfilarial carriers in the Amazonian focus, southern Venezuela

Day p.e.	*Simulium oyapockense* s.l.					*Simulium incrustatum*				
	Number of flies	L1 (%)	L2 (%)	L3 (%)	Total larvae	Number of flies	L1 (%)	L2 (%)	L3 (%)	Total larvae
1	395	13 (100)	0 (0)	0 (0)	13	197	8 (100)	0 (0)	0 (0)	8
2	348	6 (100)	0 (0)	0 (0)	6	129	4 (100)	0 (0)	0 (0)	4
3	311	21 (100)	0 (0)	0 (0)	21	136	3 (100)	0 (0)	0 (0)	3
4	186	9 (100)	0 (0)	0 (0)	9	153	4 (57)	3 (43)	0 (0)	7
5	114	5 (71)	1 (14)	1 (14)	7	111	1 (11)	3 (33)	5 (56)	9
6	91	2 (25)	2 (25)	4 (50)	8	131	1 (25)	0 (0)	3 (75)	4
7	42	0 (0)	0 (0)	2 (100)	2	138	0 (0)	0 (0)	5 (100)	5
8	39	0 (0)	0 (0)	3 (100)	3	349	2 (7)	1 (3)	26 (90)	29
Total (%)	1526	56 (81)	3 (4)	10 (15)	69 (100)	1344	23 (33)	7 (10)	39 (57)	69 (100)

jects were: 17 from Mh1; 10 from Mw2; 87 from Mw3; 14 from Mh3 and 9 from Mh4, with none harbouring L3. All 7 microfilarial carriers on whom *S. incrustatum* flies were fed resulted in flies becoming infective. Fig. 4A shows the relationship between the mean number of L3 per fly ($\bar{L}$), and the skin microfilarial load (M) upon which the flies were fed. (Although the AM number of L3 is shown, the pattern resulting from plotting the GM values (presented in Table 4) is very similar; not shown.) The values for the parameters estimated from fitting the model to data from both species were $\vartheta_M = 0.021\ mf^{-1}$; $\varepsilon_M = 0.020\ mf^{-1}$; and $c_M = 0.261\ mf^{-1}$ for *S. oyapockense*, and $\vartheta_M = 0.014\ mf^{-1}$; $\varepsilon_M = 0.035\ mf^{-1}$; and $c_M = 0.122\ mf^{-1}$ for *S. incrustatum*. These values suggest that the degree of negative density dependence in larval development operating within *S. oyapockense* (a smaller fly) is twice as high as that operating within *S. incrustatum*. The inset in Fig. 4A illustrates the relationship between $\bar{L}$ and $\bar{m}'$ (a similar shape ensues when plotting $\bar{L}$ vs. $\bar{m}$; not shown). The curve depicted is the best (exponential) trendline, shown only to highlight (rather than to describe through a realistic biological model) that mean infective larval load is higher in *S. incrustatum* than in *S. oyapockense* even when the effect of the cibarial armature is controlled for by using undamaged mf. The values of parasite yield presented in Table 4 demonstrate that this is true also for crude mean mf intake. (An exception is participant Ps1, for whom only 10 flies were dissected; again patterns were similar irrespective of whether AM or GM were used.)

Fig. 4B presents the proportion of infective flies P_L as a function of $\bar{L}$ for both species analyzed together; the fitted curve assumes a NBD of the number of L3 larvae among flies. The most parsimonious model was the one in which the degree of overdispersion is constant, and the estimated value of parameter k_L was 0.329.

4. Discussion

4.1. The relative efficiency of S. oyapockense and S. incrustatum as hosts for O. volvulus

The transmission success of onchocerciasis among its human and vector hosts is influenced by the operation, at various points in the life-cycle of *O. volvulus*, of a variety of density-dependent processes, whose overall contribution to the regulation of parasite abundance depends on the distribution of parasites in human and fly populations (Churcher et al., 2005). This paper examined three such processes which determine vectorial efficiency within simuliids that possess well-developed cibarial armatures, namely, the ingestion of microfilariae from a given skin load; the proportion that survive the damage inflicted by the armature, and the development of the surviving parasites to the infective stage. Unlike other species, such as the savannah members of the *S. damnosum* complex in West Africa, *S. guianense* s.l. in the Amazonian focus (both with unarmed cibaria), and *S. ochraceum* s.l. in Meso America (armed), for which microfilarial intake is almost or linearly proportional to microfilaridermia (Basáñez et al., 1994), the relationship between the numbers of mf ingested by *S. oyapockense* and *S. incrustatum* and those in the skin of the human host was significantly nonlinear and negatively density-dependent. The magnitude of the parameter quantifying departure from proportionality (β) was lower in *S. incrustatum* (0.57) than in *S. oyapockense* (0.75) (albeit with overlapping CLs). These stronger constraints on mf intake by *S. incrustatum* translated into lower numbers of ingested mf and lower proportions of flies with ingested mf for given microfilaridermias than those recorded for *S. oyapockense* (Fig. 1). Our results are reminiscent of those obtained by Duke (1962) for

Table 4
Development of *Onchocerca volvulus* microfilariae into infective larvae within *Simulium oyapockense* s.l. and *S. incrustatum* dissected between 120 h (5 d) and 192 h (8 d) p.e. after feeding on untreated subjects in the Amazonian focus, southern Venezuela

Subject code	*Simulium oyapockense* s.l.				*Simulium incrustatum*			
	$\bar{L}_G$ (range)[a]	P_L (%)[b]	[95% CL][c] for P_L	$P_Y(\bar{m})$ (%)[d], $P_Y(\bar{m}')$ (%)[e]	$\bar{L}_G$ (range)[a]	P_L (%)[b]	[95% CL][c] for P_L	$P_Y(\bar{m})$ (%)[d], $P_Y(\bar{m}')$ (%)[e]
Aw1	–	–	–	–	0.024 (0–5)	2/103 (1.9)	[0.2, 6.8]	3.00, 4.17
Ps1	0.148 (0–3)	1/10 (10.0)	[0.3, 44.5]	1.23, 4.77	0.018 (0–1)	3/116 (2.6)	[0.5, 7.4]	0.24, 0.55
Ps2	–	–	–	–	0.012 (0–2)	1/92 (1.1)	[0.03, 5.9]	0.48, 0.78
Aw2	0.025 (0–1)	2/55 (3.6)	[0.4, 12.5]	0.07, 0.20	0.022 (0–2)	4/141 (2.8)	[0.8, 7.1]	0.42, 0.81
Mh2	0.042 (0–1)	1/17 (5.9)	[0.1, 28.7]	1.14, 3.43	–	–	–	–
Ps3	0.048 (0–2)	2/38 (5.3)	[0.6, 17.7]	0.11, 0.29	0.054 (0–4)	7/122 (5.7)	[2.3, 11.5]	0.29, 0.46
Aw3	0.012 (0–1)	1/56 (1.8)	[0.05, 9.6]	0.02, 0.09	0.022 (0–5)	2/116 (1.7)	[0.2, 6.1]	0.50, 1.16
Aw4	0.080 (0–1)	3/27 (11.1)	[2.4, 29.2]	0.18, 0.48	0.095 (0–3)	4/38 (10.5)	[2.9, 24.8]	0.38, 0.79

[a] $\bar{L}_G$, the geometric mean number of infective, L3, larvae per fly.
[b] P_L, the proportion of flies with L3 larvae among those examined.
[c] [Exact 95% confidence limits].
[d] $P_Y(\bar{m}) = \bar{L}_G/\bar{m}_G$, the proportion of ingested mf that reach the L3 stage.
[e] $P_Y(\bar{m}') = \bar{L}_G/\bar{m}'_G$, the proportion of unscathed microfilariae that reach the L3 stage. Abbreviations of localities as in Table 1.

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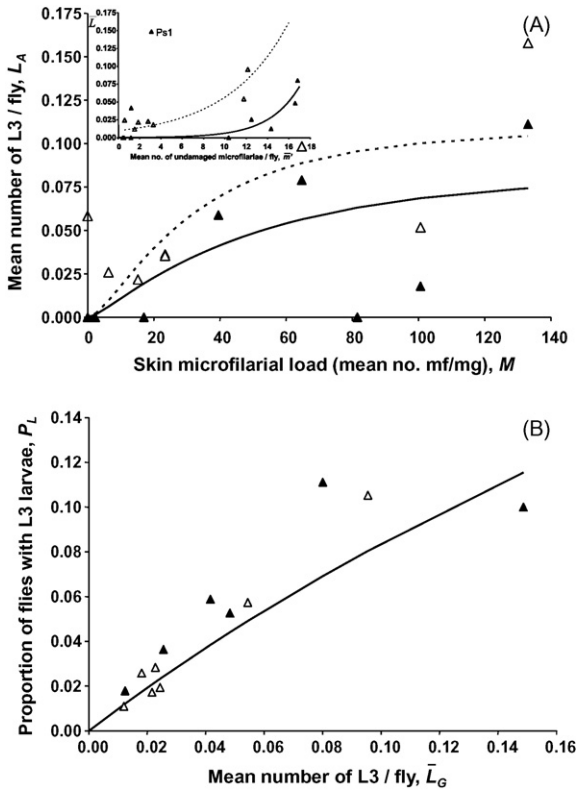

Fig. 4. The development of microfilariae to the infective stage in the Amazonian onchocerciasis vectors *S. oyapockense* and *S. incrustatum*: (A) the observed (markers) and fitted (lines) mean number of L3 larvae per fly, $\bar{L}$ as a function of skin microfilarial load, *M*, and (B) the proportion of flies harbouring infective larvae 8 days post-feeding as a function of mean L3 larval load. In (A) the fitted model is $\bar{L} = \{\vartheta_M[1 - \exp(-\varepsilon_M M)]M\}/(1 + c_M M)$ with solid triangles and line for *S. oyapockense* ($\vartheta_M = 0.021\ mf^{-1}$; $\varepsilon_M = 0.020\ mf^{-1}$; and $c_M = 0.261\ mf^{-1}$), and open triangles and broken line for *S. incrustatum* ($\vartheta_M = 0.014\ mf^{-1}$; $\varepsilon_M = 0.035\ mf^{-1}$; and $c_M = 0.122\ mf^{-1}$). The inset depicts the relationship between L3 load and undamaged mf intake with best fit (exponential) trendline shown only for illustrative purposes. In (B) the model is $P_L = 1 - (1 + \bar{L}/k_L)^{-k_L}$ with $k_L = 0.329$ for both simuliid species analyzed together.

S. damnosum from West Cameroon, where flies were fed on subjects who had signs of skin lichenification; this limited mf intake as microfilaridermia, and associated cutaneous pathology increased. Demanou et al. (2003) compared, from published literature, several species of the *S. damnosum* complex in their ability to ingest mf from a given skin load and concluded that in general, savanna *Onchocerca–Simulium* combinations showed higher mf intakes than forest combinations. This, they argued, was possibly related to parasite strain-specific differences in the depth of mf in the skin (Bain et al., 1986; Vuong et al., 1988). Vivas-Martínez (1999) found, in our study areas of the Amazonian focus that the risk of lichenified and chronic papular onchodermatitis increased significantly with skin mf density after adjusting for host age. She also found that although skin depigmentation was not associated with mf infection, it was probably caused by insect bites, and particularly *S. oyapockense*, which is found biting in large numbers in the hypo- and mesoendemic communities (Vivas-Martínez et al., 1998; Grillet et al., 2001). It is therefore possible that onchocercal and non-onchocercal skin pathology is involved in the patterns of mf transmission from the human hosts to the simuliid vectors reported here. In addition, differential microfilarial concentration by the two species under investigation, probably due to the existence of chemo-attractant factors in the blackfly saliva recruiting mf to the site of the bite (Stallings et al., 2002) may also play a role (Shelley et al., 1979). It has been demonstrated that simuliid species in the American continent differ in the vasodilator activity of their salivary secretions (Cupp and Cupp, 1997), with *S. ochraceum* (armed) exhibiting higher levels of such activity than *S. metallicum* (unarmed).

The degrees of parasite aggregation estimated for mf and L3 among simuliids of both species (Figs. 2 and 4B) are similar to those estimated for *S. damnosum* s.l., *S. soubrense* B, *S. guianense* s.l., *S. exiguum* (Cayapa form) and *S. ochraceum* s.l. (Basáñez et al., 1994, 1995; Demanou et al., 2003; Wetten et al., 2007), confirming strong overdispersion and providing values that can be used to parameterize onchocerciasis models for specific *Onchocerca–Simulium* combinations, both to estimate the overall contribution of within-vector density dependence to parasite population regulation, and quantify the prevalence of infected and infective flies that would ensue from given mf loads in a range of settings.

In the Amazonian focus, the role of *S. incrustatum* in onchocerciasis transmission had not been clarified partly because of the scarcity of natural and experimental infection entomological data (Basáñez et al., 1988) and partly because of the well established ecological correlations between hypoendemicity and *S. oyapockense* on the one hand, and between hyperendemicity and *S. guianense* on the other (Shelley, 1988; Shelley et al., 1997). In fact, the study by Vivas-Martínez et al. (1998) had found a negative association between the biting rate of *S. incrustatum* and onchocerciasis prevalence along the altitudinal gradient studied. In this paper, we have shown that although for a given microfilaridermia the mf intakes of *S. incrustatum* are lower than those of *S. oyapockense*, the intakes of undamaged, viable mf are in fact higher given the smaller proportion of mf that are lacerated by the cibarial armature (Fig. 3). This, in turn, results in higher infective larval outputs for a given microfilaridermia (Fig. 4A). However, the difference in infective larval output between the two species is not just determined by the availability of undamaged mf; the intrinsic susceptibility of *S. incrustatum* as a host for *O. volvulus*, and its survivorship once infected seem also to be greater. Even after controlling for the damage inflicted by the armature, the means (and ranges) of L3 loads per fly were higher (Table 4 and inset to Fig. 4A), as was the parasite yield (Table 4) and the rates of larval progression, with proportionally more larvae reaching the infective stage (Table 3), although this is possibly due to higher survival rates. These differences are unlikely just to be due to the size of the flies, which averages 2.3 mm for *S. incrustatum* and 2.1 mm for *S. oyapockense* (Shelley et al., 1997). Regarding vector mortality, Basañez et al. (1998) evaluated the survivorship in captivity of these two species after *O. volvulus* infection, finding that the expectation of infective life of *S. incrustatum* was longer than that of *S. oyapockense*. However, in the field, parous rates of *S. oyapockense* can be very high (Grillet et al., 2001).

4.2. Epidemiological significance of our findings for the control of onchocerciasis

Both, the nonlinear relationship between the proportion of mf damaged by the cibarial armature of *S. oyapockense* and *S. incrustatum*, and the low probability of successful development of mf into L3 for low mf densities, particularly within *S. oyapockense*, suggest that initial facilitation, followed by subsequent limitation may be operating within these two simuliid species; hence the sigmoid nature of the curve fitted to infective larval output as a function of mf input (Fig. 4A). Facilitation, or positive density dependence (when the rate of a population process increases with density) may derive from the fact that at higher intakes, the mf that get entangled in the armature projections protect the remainder from damage (Basáñez et al., 1995). The epidemiological importance of positive density dependence (the mating probability of the dioecious adult worms being another example) is

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the possibility of unstable equilibria arising in the host–parasite population system, leading to the existence of transmission breakpoints (Macdonald, 1965). Potential breakpoints due to vectors with armed cibaria have been identified in the case of *O. volvulus*–*S. ochraceum* s.l. in Mexico and Guatemala (Basáñez and Ricárdez-Esquinca, 2001). However appealing (the parasite would be driven to local elimination if mf density could be sustainably reduced below such thresholds), the true operational significance of transmission breakpoints in control programmes will also depend on the magnitude of the vector biting rate (Basáñez and Rodríguez, 2004), and the degree of parasite overdispersion (May, 1977; Churcher et al., 2006) among other factors (Duerr et al., 2005). In general, the higher the density of vectors biting on humans, and the stronger the degree of overdispersion (of the distribution of parasites and/or vectors among humans), the lower the critical parasite density below which extinction may be possible and the more difficult to attain it.

Basáñez et al. (2002) estimated that the critical or threshold biting rate for onchocerciasis endemicity in areas where highly anthropophagic *S. ochraceum* s.l. is the main vector would be of the order of 8000–10,000 bites person^{-1} year^{-1} (see also Wada, 1982). For values of vector density above such threshold biting rate, the basic reproduction ratio (R_0) of the parasite would be greater than 1, ensuring introduction and persistence of the infection. Although it would be expected that the threshold biting rate for *S. oyapockense* (and possibly *S. incrustatum*) is higher than that for *S. ochraceum* (given their respective vector competences), biting rates of *S. oyapockense* can be extremely large in the lowlands of the Amazonian focus (up to 8000 flies person^{-1} h^{-1}; Grillet et al., 2001). In the study localities investigated in this paper, daily biting rates for *S. oyapockense* of ~4300 (Maweti), and ~1500 (Mahekoto) have been reported (Vivas-Martínez et al., 1998). In these lowland areas, and despite the poorer performance of *S. oyapockense* as a host for *O. volvulus*, transmission is probably maintained by virtue of such high biting rates. Nevertheless, mass administration of ivermectin is anticipated to have a greater impact on onchocerciasis transmission as *S. oyapockense* is virtually the only species present and reductions of microfilaridermia are expected to render repeatedly treated humans practically non-infective to flies. The situation is probably more complex in Aweitheri and Pashopeka, where both species are present, with ~100 bites person^{-1} day^{-1} due to *S. oyapockense* and ~600 due to *S. incrustatum* in the former, and ~20 for *S. oyapockense* and ~300 for *S. incrustatum* in the latter, *S. guianense* (the most efficient vector of the three) also being present (Vivas-Martínez et al., 1998; Grillet et al., 2001). In these areas, it is anticipated that as regular ivermectin treatment reduces microfilaridermia levels, the contribution of *S. oyapockense* to transmission will decrease fastest (biting rates already probably closer to critical levels), followed by *S. incrustatum*. However, as microfilarial densities decrease, negative density-dependent processes will be relaxed, and the per microfilaria probability of transmission from humans to vectors may increase, particularly in the cases of *S. incrustatum* and *S. guianense*. Assessment of the impact of ivermectin treatment in those localities with greater diversity of vector species in the Amazonian focus will require the collection of robust longitudinal data in humans and flies, and the aid of mathematical models to evaluate the relative contributions to transmission of this multi-vector–host system.

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