Differential length of Onchocerca volvulus infective larvae from the Cameroon rain forest and savanna

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Abstract

Infective larvae from the savanna strain of *Onchocerca volvulus* are significantly longer (662.6 ± 66.8 µm; n = 99) than those from the forest strain (624.2 ± 62.0 µm; n = 236). The length of the infective larvae is not influenced by the size, species or age post infectionem (pi) of the *Simulium damnosum s. l.* vectors nor by their localization in the fly's head, thorax or abdomen, the worm load per fly or the thoracic volume per larva. However, the lengths of infective larvae within one individual fly have a conspiciously low variance.

Introduction

Human onchocerciasis differs as regards clinical manifestations in the savanna and rain forest of West Africa (Anderson et al., 1974). Microfilariae of the putative savanna strain are more pathogenic, than those from the forest strain, if injected into the eyes of rabbits (Duke and Anderson, 1972). The two strains also differ in antigenic (Lobos and Weiss, 1985) and isoenzyme patterns (Flockhart et al., 1986) and in the acid phosphatase activity of skin dwelling microfilariae (Omar et al., 1982). The length of the uterine microfilariae was longer in the savanna than in the forest (Botto et al., 1988).

Therefore it might be expected, that the infective larvae of different strains of *O. volvulus* can be identified by simple morphometric methods. The length of the infective larvae is of particular interest, as it is commonly used to differentiate *O. volvulus* from infective larvae of animal origin in wild caught *Simulium* flies (Nelson et al., 1962; Duke, 1967; Renz et al., 1989). Infective larvae of *O. volvulus* from the Cameroon forest and savanna strain were produced by intrathoracic injection of microfilariae into different *Simulium* species, in order to obtain reference samples from both regions. Intrathoracic injection was chosen as forest microfilariae did not develop in *Simulium* flies from the savanna following ingestion by a blood meal and vice versa (Duke et al., 1966).

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Table 1 Length of the infective larvae (forest strain) in different vector species, classified according to setae color in wing tufts, postcranium and scutellum, antenna compression, number of pale antenna segments and thorax length/antenna length ratio (–?– designs flies with intermediate morphology)

Host fly species	∑ flies	∑ L3	mean µm	SD µm
"*S. mengense*"	16	61	638.5	60.5
–?–	3	5	652.0	41.4
"*S. squamosum*"	8	20	612.5	74.9
–?–	8	21	642.0	67.8
"*S. damnosum s. str.*" /"*S. sirbanum*"	18	68	611.0	52.7

Material and methods

Skin biopsies were taken from the iliac crest and calves of highly infected volunteers from Barombi Mbo (near Kumba, rain forest) and Sora Mboum (near Touboro, Sudan savanna), and incubated in RPMI 1640 medium. The microfilariae isolated were injected intrathoracically into pupae hatched black flies (as described by Eichner et al., in prep., Bianco et al., 1989-). The flies were maintained individually at 21.5 ° ± 2 °C in constant darkness at high humidity and were fed on cotton balls soaked with a 10% (w/v) sugar solution containing 0.2% (w/v) nipagine® (= methyl-4hydroxybenzoate).

Dead or moribund flies were collected every 24 hours and examined for their morphological features: those having only pale postcranial, scutellum and wing tuft setae were classed 'pale', accordingly those with dark setae as 'dark' and all the others as 'mixed' flies. The ratio of the thorax length divided by the length of the antenna, the number of pale basal segments of the antenna and the compression of its segments 4–7 were noted. Head, thorax and abdomen of the dead flies were separately dissected at magnification of 20 × in a drop of demineralized water. The length of the infective larvae was measured under the dissecting microscope (at magnification 50 ×).

The length distribution of the infective larvae of both, savanna and forest strain, were tested for their fit to normal distributions by a chi square test. Their variances were compared by a F-test and their means by a Student t-test. Subsequently the variances were compared by a Bartlett test and the means by one way ANOVA to dinstinguish the larvae lengths from different parts of the body (head, thorax, abdomen) or from morphologically classified flies. The Spearman rank correlation test was used to investigate, if the age pi, the length of thorax, the fly's worm load or the thoracic volume per parasite influences the length of the infective larvae (thoracic volume ≈ $1/2$ (length of thorax)^3). Before using this test the average length of the larvae of each individual fly was calculated.

Table 2 Comparison of the length of infective larvae of *O. volvulus* published by different authors

Author (s)	Origin	Length	Measured in	
In this study	**Cameroon**			
by intrathoracic	**– rain forest**	**624 µm**	**aqua dest.**	
injection	**– savanna**	**663 µm**	**aqua dest.**	
Bain	Upper Volta			
1969	– savanna	650 µm	bouin fixed	
Blacklock	Sierra Leone		aqua dest. or	
1926	– rain forest	622 µm	haemalaun stain	A
Duke	Cameroon			
1967	– rain forest	635 µm	glycerol	F
	– savanna	625 µm	glycerol	
Mc Call and Trees	Mali			
1989	– savanna	687 µm	saline	
Philippon	Upper Volta			R
1977	– savanna	655 µm	aqua dest.	
Renz	Cameroon			I
in prep.	– rain forest	603 µm	aqua dest.	
	– savanna	655 µm	aqua dest.	
Schibel	Cameroon			C
pers. comm.	– savanna	670 µm	aqua dest.	
Sechan	Mali			A
1984	– savanna	648 µm	glycerol	
Nelson et al. 1962	Uganda	600 µm	glycerol	
Collins	Guatemala	600 µm	0.85% saline	
1979			+ 10% glycerol	A
Lok et al.	Guatemala	657 µm	?	
1980				M
Porter & Collins	Guatemala	657 µm	physiol. saline	
1984			+ 10% dimethyl-sulfoxide	E
Takaoka et al.	Venezuela	553 µm	70% ethanol	
1984 a				R
Takaoka et al.	Venezuela	474 µm	70% ethanol	
1984 b			5% Giemsa	I
Takaoka et al.	Guatemala	566 µm	0.9% saline	
1984 c				C
Takaoka et al.	Venezuela	549 µm	0.9% saline	
1986		517 µm		A
Takaoka et al.	Ecuador	490 µm	70% ethanol	
1988			Giemsa	

Table 3 Ranges of length to be considered for differentiating *O. volvulus* infective larvae in wild caught flies from those of animal *Onchocercidae*

O. volvulus	60% of larvae	95% of larvae
Savanna	607 µm ≤ length ≤ 720 µm	532 µm < length ≤ 794 µm
Forest	572 µm ≤ length ≤ 676 µm	503 µm ≤ length ≤ 746 µm

The variance of the lengths of a group of n larvae isolated from the same fly was compared to the expected value, had the sample been taken at random from a normal distribution, using the Kolmogorov-Smirnov test.

Results

O. volvulus larvae from the Sudan savanna were significantly longer than those from the rain forest (662.6 ± 66.8 µm and 624.2 ± 62.0 µm respectively; $\alpha \leq 0.001\%$; Fig. 1). To test the hypothesis whether the length of the infective larvae was influenced by the species of the host fly, forest strain microfilariae were inoculated into savanna flies. However the infective larvae obtained were within the same length range as in forest flies. If forest and savanna flies were classed according to their morphology into the prevailing vector groups (*S. mengense, S. squamosum, S. damnosum s. str. / S. sirbanum*), no significant influence on the length of the larvae

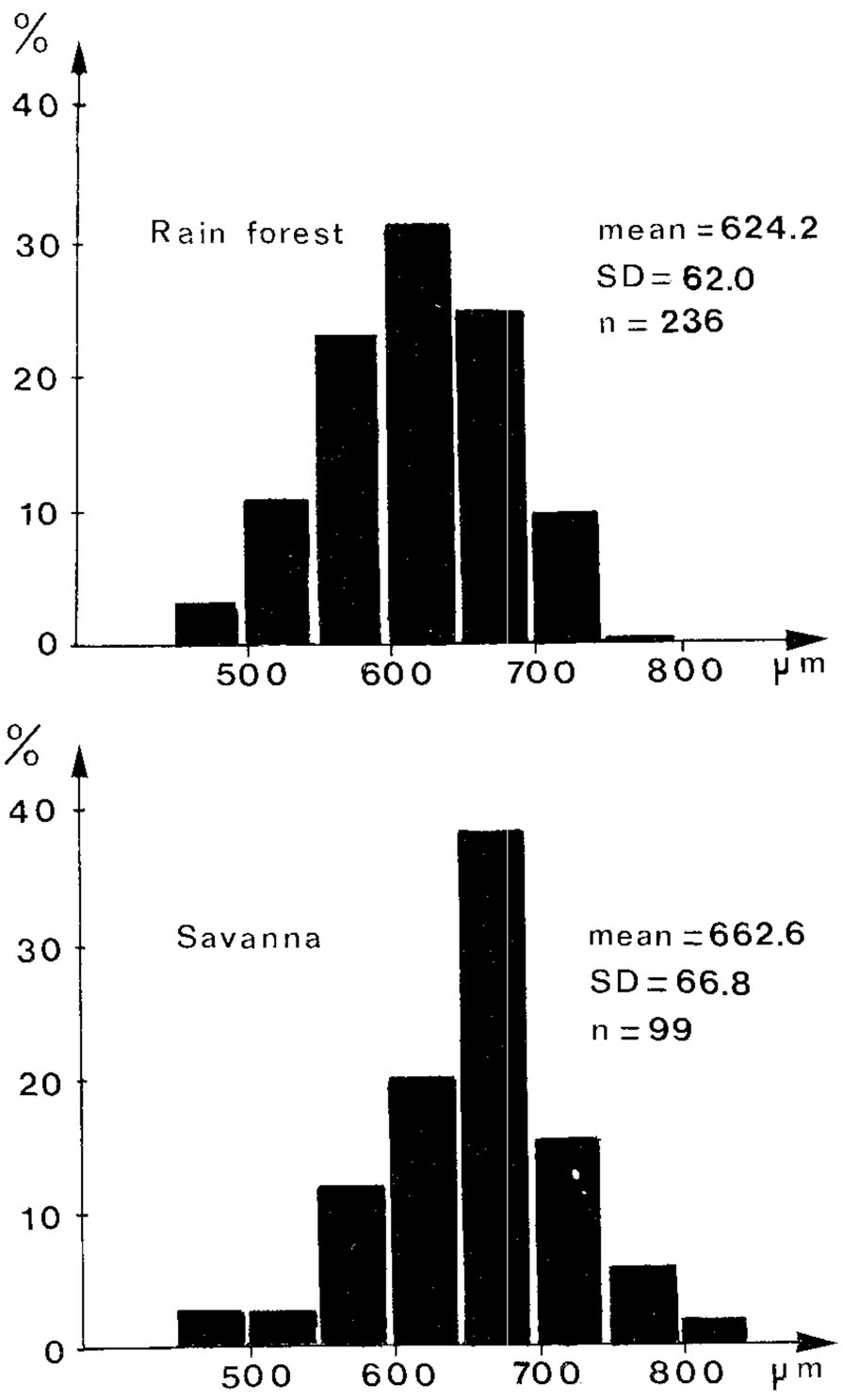

Fig. 1 Length distributions of the infective larvae of the savanna and rain forest strain of *O. volvulus*

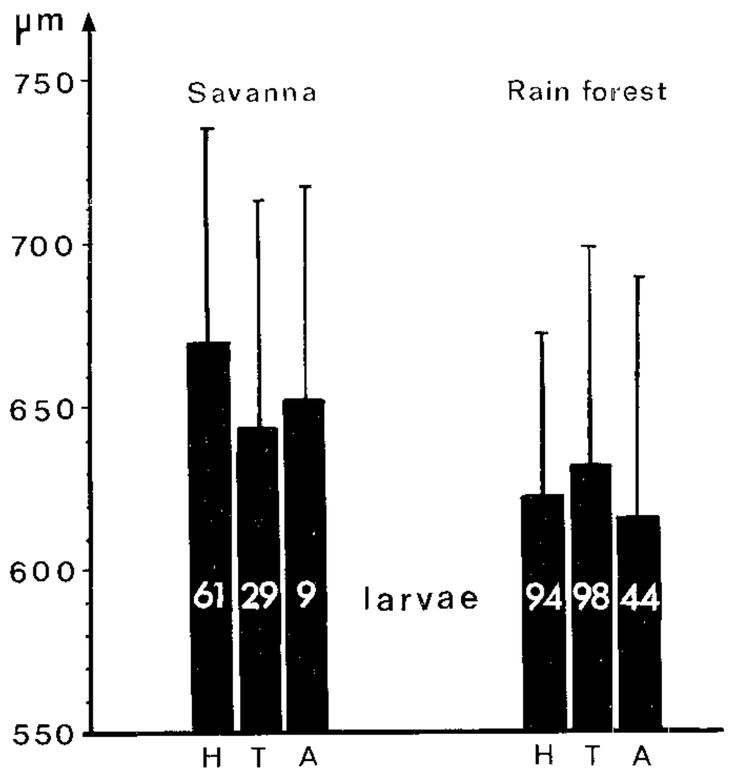

Fig. 2 Length of the infective larvae in head (H), thorax (T) and abdomen (A) of the vector flies with standard deviation

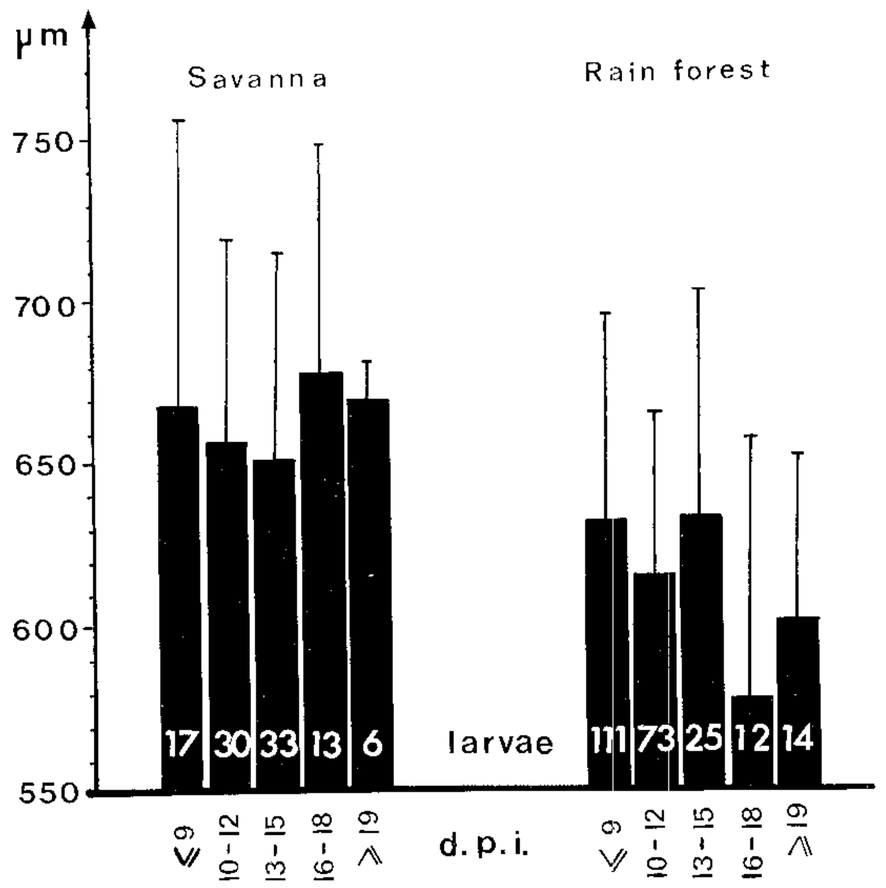

Fig. 3 Length of the infective larvae in dependance of the age p. i. with standard deviation

was observed (Table 1). Even in *S. hargreavesi*, which is not known to feed on man, forest *O. volvulus* larvae reached their normal length (mean 622 µm, SD = 48.6 µm, n = 12).

Infective larvae (savanna and forest strain) in the flies' head were not longer than those found in the thorax or abdomen (Fig. 2). There was no effect of the age pi (7 to 20 days pi; Fig. 3) or of the size of the fly on their length nor did the worm load and the thorax volume per larva influence the larvae length (forest strain). For the savanna strain the length of the infective larvae increased with the number of larvae in the host fly (1 to 7 larvae/fly; $\alpha = 3.4\%$).

The length variances of n ($2 \leq n \leq 7$) larvae, that developed together in the same fly were usually too small, when compared to the variance expected for n values chosen at random from normal distributions (Fig. 1). For the forest strain this differences proved to be significant in two cases (n = 5 and 6; $\alpha \leq 5\%$).

Discussion

The observed differences in the length of larvae provide additional evidence for separate strains within the species *O. volvulus*. Infective larvae of the savanna strain, which is highly pathogenic to the eye, are longer than those from the forest (Fig. 1). This parallels the measurements of microfilariae lengths (Botto et al., 1988). Striking differences have also been described in the length of infective larvae from Central and South America and from Africa (Tab. 2). However the methods of fixing and measuring the larvae were not identical in the various studies. Since they were also based on measurements of larvae from wild-caught flies, infective larvae of animal origin, otherwise indistinguishable from *O. volvulus*, might have interfered with the results. For the practical purpose of differentiating *O. volvulus* infective larvae in wild caught flies from those of animal origin the ranges of length shown in Table 3 should be considered.

The lengths of the larvae, that developed in the same fly, have too small variancs. This may be interpreted as an influence of the vector on the larvae or a mutual influence of the larvae. This effect has also been observed in experimentally blood infected and wild infected flies (A. R.).

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