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Autor:	Karam, M. / Schulz-Key, H. / Remme, J.
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<sup>1</sup> Onchocerciasis Control Programme, Ouagadougou, Burkina Faso
<sup>2</sup> Tropenmedizinisches Institut, Wilhelmstrasse 31, D-7400 Tübingen, Federal Republic of Germany

# Population dynamics of *Onchocerca volvulus* after 7 to 8 years of vector control in West Africa

M. Karam<sup>1</sup>, H. Schulz-Key<sup>2</sup>, J. Remme<sup>1</sup>

### Summary

In an attempt to describe the changing population dynamics of *Onchocerca volvulus* during a period of vector control, nodulectomies were undertaken in 256 patients from ten villages in the Onchocerciasis Control Programme (OCP) and in 74 patients from two villages in an area with ongoing transmission. A total of 1198 nodules were excised and 4350 adult worms were isolated and examined for viability and productivity. In the OCP villages, the worm population is ageing and dying without replacement by new generations of parasites and various findings signal a breakdown of the worm population after about 12 years interruption of transmission. The sexual activity of the worms was significantly reduced. A Productivity Index was developed to measure the microfilariae production at the nodule level. The reduction in this index for the OCP villages correlates closely with the decline over the control period in the community microfilarial loads in the skin. The results show that it is not only the longevity of the parasite which will determine the duration of vector control, but that the reduced productivity of the ageing parasite population is of equal importance.

**Key words:** *Onchocerca volvulus;* Onchocerciasis Control Programme; nodulectomy; longevity; productivity.

# Introduction

The Onchocerciasis Control Programme in West Africa (OCP), launched in 1974, aims at the long-term control of onchocerciasis as a disease of public health and socio-economic importance. The strategy of the programme is to

Correspondence: Dr. M. Karam, OMS/ONCHO, B. P. 549, Ouagadougou, Burkina Faso

interrupt the transmission of the parasite, through control of the vector *Simulium damnosum* s.l., for a sufficiently long period to allow the initial reservoir of infection in man to die out naturally. Once this reservoir has fallen to insignificant levels, the risk of recrudescence of onchocerciasis will be minimal and vector control operations may be scaled down and replaced by less costly activities. The required duration of vector control depends mainly on the longevity and fertility of the adult parasite harboured by the infected human populations.

A first indication of the maximum longevity of infection was given by Roberts et al. (1967) who observed in Kenya the survival of a residual population of living parasites 11 years after the eradication of the vector and the complete disappearance of microfilariae from the skin in the human population after 18 years. Based on these findings, the OCP was initially planned to last for a period of about 20 years. Very little new has been learned since then on the longevity and the fertility of the adult worm and, as the programme was approaching the end of its first decade of control, it became important to arrive at a better understanding of the population dynamics of Onchocerca volvulus. A study of the adult worm population has therefore been undertaken by the OCP in several villages since 1982, using the collagenase technique which enables the routine examination of excised nodules on a large scale. The first aim of the study was to identify reliable criteria and parameters to follow the decrease of the live worm population. The second aim was to compare the reproductivity of the worm population from a non-controlled area with the reproductivity of populations with ageing worms in the programme area using a quantitative assessment of the intra-uterine stages. The results, obtained from various epidemiological situations, are presented in this paper, and show some of the main parasitological trends in the Onchocerciasis Control Programme.

#### Material and Methods

At the start of the OCP, the collagenase technique (Schulz-Key et al., 1977) had not yet been developed and it was not possible to obtain intact adult worms for examination. Baseline data on the composition and the reproductivity of the worm burden in the villages sampled are therefore lacking for a direct comparison with our recent investigations. To make up for the handicap, two hyperendemic villages for onchocerciasis, located outside the OCP in an area with ongoing transmission in Mali, were chosen as controls. These villages are Manambougou, on the left bank of the Niger river, and Missira, at the bend of the Baoule river (see Fig. 1). Nodulectomies were carried out on 74 inhabitants in 1982 and 1983. Ten other villages were selected inside the OCP area in Burkina Faso and northern Ghana where vector control started during the years 1975–1977. Of these four villages, Lamiougou and Bangasse on the upper, Tagou and Kompiembiga on the lower Koulpeolgo river basin, have always had extremely satisfactory vector control with the virtual elimination of the vector population since 1976. Niarba, on the White Volta river basin, has also experienced similar successful vector control since the start of operations in 1976. The villages of Boko and Folonzo, both in the western part of Burkina Faso, had been under vector control since 1975 and light transmission may have occurred as a result of reinvasion of infective flies during the first three years but control was fully achieved afterwards. In Bonga (Burkina Faso), Nakong and Yagaba (Northern Ghana),

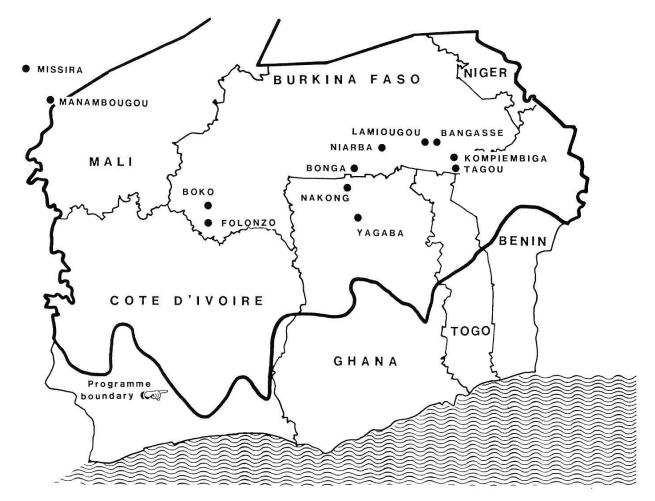


Fig. 1. The OCP area and the locations of the study villages.

vector control had started in 1976, but has not always been completely satisfactory. A local vector breeding outbreak was detected by the entomological evaluation in this area in 1981, and may have resulted in a localized resurgence of transmission.

For each village, a complete physical examination of the inhabitants was undertaken to search for signs of onchocerciasis. Patients older than 15 years among nodule carriers who volunteered underwent nodulectomy which, as a general rule, consisted in the removal of nodules from either the left or the right side of the body leaving one side free to allow lying to sleep at night in minimum comfort. As many nodules as possible were removed however, for ethical reasons, the final decision with regard to the number of sites to be operated respected the patient's wish. The number of patients operated varied from one village to the other and depended on the number of nodule carriers who volunteered. A total of 1198 nodules were surgically removed from 330 patients. The excised nodules were weighed with an accuracy of 0.1 g and their macroscopical characteristics were recorded before and during the digestion of the nodular tissue. The viability and the morphology of each worm were examined and the age grading recorded as described by Schulz-Key et al. (1980). The nodule, rather than the person operated, was taken as the sampling unit and the results of the analysis of the viability and the morphology will be presented as means or proportions per nodule. A special attention was given to the actual phase of reproduction. The female worms were classified into five groups: females with empty uteri, with one-cell-stages, with embryonated stages and living microfilariae, with only dead remnant microfilariae, or with remnant microfilariae and additional oocytes of a new reproductive cycle. Morphologically undamaged female worms were cut into small pieces, the intrauterine stages were squeezed out in a special mortar with an adjusted pestle. The number of developmental stages was then assessed as described by Schulz-Key et al. (1980). All but one of these

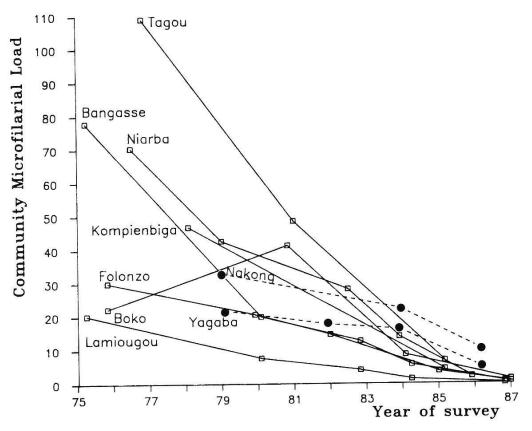


Fig. 2. Trends in the community microfilarial load in the study villages in the OCP area.

ten OCP villages had been, since the beginning of the Programme, regularly followed-up in epidemiological surveys during which one skin biopsy was performed with a Holth corneo-scleral punch at each iliac crest and examined according to the OCP standard methodology. Thus, the results of the worm analysis could be compared with the evolution of the skin microfilarial loads in the villages surveyed.

#### Results

# Changes in skin microfilarial loads inside the vector-controlled area

Fig. 2 gives, for the follow-up villages, the evolution of the community microfilarial load (CMFL), which is the geometric mean number of microfilariae emerging per skin snip for a cohort of adults who have been examined at each survey (Remme et al., 1986). In seven of the villages the reduction in the CMFL was more than 90% at the last examination. Two villages in northern Ghana, Nakong and Yagaba, for which no baseline data are available for 1976, showed only a slight reduction in the microfilarial loads between 1979 and 1984 followed later by a more accelerated decrease. In Boko at the Comoe river basin in Burkina Faso a remarkable increase of the microfilarial densities was observed in 1980 followed by a considerable reduction in 1984 and 1986. This village was initially analysed separately, but, since the results of the worm analysis were not significantly different from those for the neighbouring village

Village	No. of excized	Geom. mean nodule	Number of non-	Male worms		Female worms		Sex ratio of living
	nodules	weight (grams)	calcified nodules	total	% dead	total	% dead	worms (M:F)
Manambougou	158	0.67	155	258	1.9	442	12.0	1:1.54
Missira	116	0.77	116	197	4.6	307	10.1	1:1.47
Boko	82	0.88	81	121	5.0	214	24.3	1:1.41
Folonzo	70	0.56	70	102	6.9	181	28.2	1:1.37
Yagaba	110	0.54	110	144	15.3	241	33.6	1:1.31
Nakong	94	0.48	94	126	14.3	229	45.0	1:1.17
Bonga	45	0.55	44	59	10.2	103	35.0	1:1.26
Tagou	104	0.59	103	152	10.5	261	36.4	1:1.20
Kompiembiga	183	0.49	177	221	17.2	398	45.7	1:1.18
Niarba	118	0.54	114	137	35.0	219	43.4	1:1.39
Lamiougou	70	0.48	65	50	42.0	100	67.0	1:1.14
Bangasse	48	0.50	41	26	61.5	62	66.1	1:2.10
Total	1198		1170	1593		2757		

Table 1. Number of excised nodules per village and general caracteristics of the adult worms examined

of Folonzo, they were combined and presented together. Following the pattern so far observed in the OCP, the decrease in the prevalence of microfilariae carriers is, in all villages, remarkably slower than that of the microfilarial loads in the skin.

### Observations on nodules and adult parasites outside the vector-controlled area

*Nodules:* The geometric mean weight of the 274 excised nodules was 0.7 g (Table 1). There was no difference in relation to sex of the inhabitants, but there was a significant increase of the nodule weight in patients between 30 and 50 years old followed by a significant decrease in patients over 50 years. Only 1.1% of the nodules were calcified.

Adult worms: Fig. 3A shows that 71% of the nodules contained living male worms (range 1 to 17), and 90% of the nodules harboured living female worms (range 1 to 20). Degenerated male worms were only occasionally found in the form of calcified fragments or disintegrated non-calcified worms (Table 1). 11.2% of the female worms were degenerated and were found in 28% of the nodules. Three quarters of these females were calcified or partially calcified. Less than 10% of the worms were old, brownish with a heavily coated cuticle. Two-thirds of the females were relatively young, more or less transparent and had an almost clean cuticle. About 3% of the female worms were very small, less than 1 cm in length and did not contain oocytes. They represented the immature adults. Immature male and female worms were always associated with

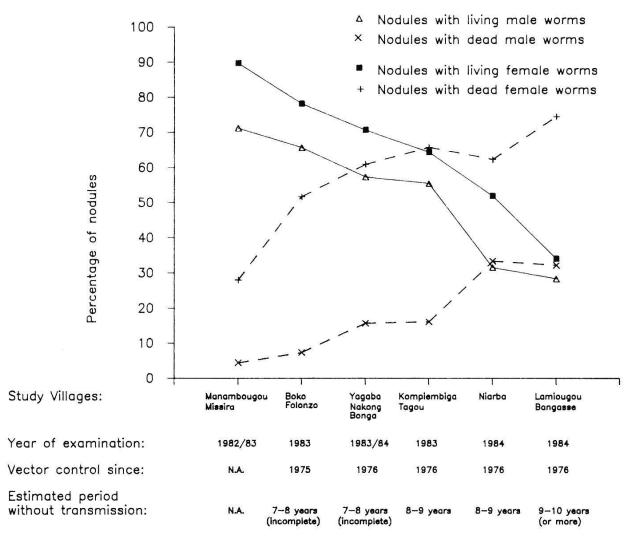


Fig. 3A. Percent ages of nodules with living or dead worms in the different village clusters.

old or even degenerated worms in the nodules. The reproductive activities of the female worms are recorded in Table 2 and are once more in accordance with our previous conclusions of the periodical, asynchronous release of microfilariae (Schulz-Key and Karam, 1986).

# Observations on nodules and adult parasites inside the vector-controlled area

*Nodules:* The geometric mean weight of the excised nodules was significantly lower (15-31%), with the exception of Boko at the Comoe river basin, where the nodules were, on the average, even bigger than in the control villages (Table 1). The number of fibrous and caseous nodules had increased at the same time. The portion of calcified ones was around 3%. In Bangasse, even one out of every sixth nodule was calcified.

Adult worms: Fig. 3A shows a trend of reduced number of living adult worms with a longer period of interrupted transmission; only 30% of the nodules contained living adult worms in the area with no transmission for 9–10 years compared to 90% in the two control villages.

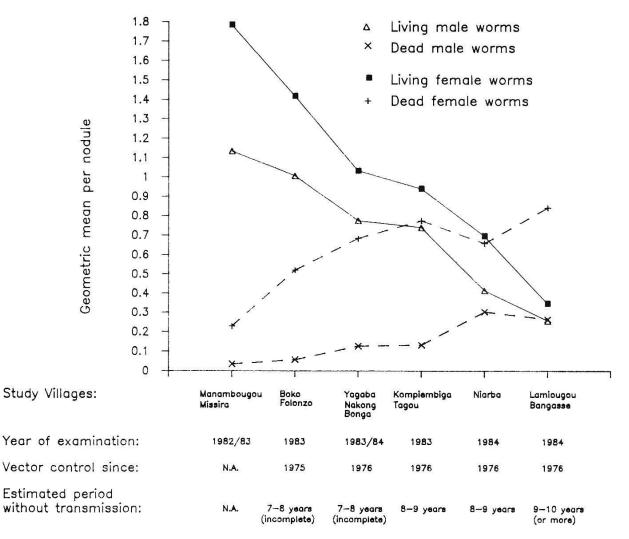


Fig. 3B. Mean number of living and dead worms per nodule in the different village clusters.

The living worms showed distinct signs of age, which we have already described in a previous paper (Schulz-Key et al., 1980). These were the consistence of the surface coat of female filariae, its colour, inclusions in the body cavity and in the organs, iron pigments and beginning of calcification. Nearly all the worms had become more or less opaque with the exception of several worms found in northern Ghana and at the Comoe river system.

The surface of the male worms was occasionally found charged with incrustations indicating a reduced migration from one nodule to another and therefore a reduced sexual activity. Up to five living male worms were found associated in nodules with only fragments of dead female worms. The sex ratio of living worms approached a more balanced ratio at the same time in all villages with the exception of the villages of Niarba and Bangasse, where the portion of dead worms was extremely high suggesting an imminent breakdown of the parasite population. Fragments of degenerate male worms were more difficult to detect than those of females, because they were often very small and sometimes mixed with the numerous fragments of calcified female worms.

Village	No. of live females examined	% of total number of living female worms						
clusters		immature females	mature, empty uteri	mature, one-cell stages only	mature, living mf and embr. stages	mature, remnant mf and oocytes	mature, remnant mf only	
Manambougou/Mis-								
sira (control villages)	634	2.7	20.8	17.4	53.0	4.1	2.0	
Boko/Folonzo Yagaba/Nakong/	292	0.0	25.8	19.7	45.4	8.1	0.1	
Bonga	353	0.0	30.8	19.9	32.8	12.9	3.6	
Kompiembiga/Tagou	382	0.0	30.4	15.3	40.3	10.4	3.6	
Niarba	124	0.0	36.4	28.9	16.5	9.9	3.6	
Lamiougou/Bangasse	54	0.0	46.5	13.8	27.6	12.1	0.0	

Table 2. Content of the uter	i of the live Onchocerca	volvulus females per village cluster
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The mean number of living worms per nodule had distinctly decreased (Fig. 3B) as compared to the non controlled areas (Schulz-Key et al., 1985). The percentage of reduction was as high as 77% for the male and 80% for the female worms in the Koulpeolgo basin. The reproductive activities of the female worms had changed remarkably. The portion of females just developing embryonated stages or microfilariae had decreased, whereas the portion of females with empty uteri had increased up to 47% (Table 2). The reproductive capacity of old worms decreased on average. The mean number of intra-uterine stages (embryonic stages and microfilariae) in gravid worms was reduced by up to 50% in some of the villages (Table 3). At the same time the portion of abnormal intra-uterine stages increased. The uteri were often filled with waste products. Sometimes they were even blocked with calcified particles or with big solid inclusions making a quantitative assessment in the suspensions more difficult.

The portion of male worms with only spermatids in the testes, i. e. of those which had just delivered sperms, was decreasing, whereas the portion of males with undelivered sperms increased. Male worms with completely empty testes became more frequent. They always represented old brownish worms, but their portion remained low.

#### Discussion

#### Nodules as sampling unit

Estimates of the worm load per person as an index in our analysis of the population dynamics of *Onchocerca volvulus* was not used as it seems inappropriate due to the following reasons: first, the total nodule load in patients cannot be excised by ambulatory nodulectomies, because the portion of unpalpable,

Village	Geom. mean	Proportion	Geom. mean	Productivity	% of reduction in	
	no. of live female worms per nodule (x)	of gravid among live female worms (y)	no. of embryonic stages and mf in gravid female worms (z) (in 1000)	Index per nodule (x) (y) (z)	Productivity Index	CMFL
Manambougou						
and Missira	1.75	0.53	159	147.4	0.0	_a
Boko	1.49	0.45	101	67.0	54.6	59.6 <sup>b</sup>
Folonzo	1.31	0.47	100	60.9	58.7	69.2
Yagaba	1.02	0.37	152	57.7	60.8	77.0°
Nakong	0.98	0.27	73	19.5	86.8	68.8 <sup>c</sup>
Bonga	1.14	0.32	113	42.0	71.5	_a
Tagou	1.16	0.45	96	49.6	66.4	76.1
Kompiembiga	0.78	0.37	111	31.9	78.4	66.3
Niarba	0.67	0.17	74	8.1	94.5	84.3
Lamiougou	0.32	0.18	91	5.1	96.5	93.6
Bangasse	0.29	0.42	83	10.1	93.2	93.1

Table 3. Reduction in Productivity Index and in community microfilarial load within the OCP area

<sup>a</sup> No longitudinal data

<sup>b</sup> compared to second survey

<sup>c</sup> between 1979 and 1986

deep lying nodules can be considerable and still remains unknown (Duke, 1970). Second, even subcutaneous nodules may be difficult to palpate as reported by Hamilton et al. (1974), who found that considerably more nodules were detected by the same observer during a second examination after one year as result of increased experience. The third and major limitation is that the average number of worms per person in a village depends largely on the intensity of transmission during the pre-control period (Remme et al., 1986). It is well known that the intensity of transmission varies greatly between villages and the confounding effect of this variability would severely complicate any direct comparisons between the OCP villages and the non-controlled villages.

Considering these reasons, we looked for other, more robust, parameters to describe the population dynamics of *Onchocerca volvulus*. The nodule, rather than the patient, was chosen as the sampling unit. This would not only be a better reflection of the actual practice in the field, but the results, if expressed per nodule, would be less susceptible to individual or local variations. This assumption is based on the finding that in both our present and previous studies (Schulz-Key and Albiez, 1977), the geometric mean number of living worms per nodule was rather similar and around 1.1–1.3 for male worms and 1.7–1.9 for female worms in villages without vector control. In addition, the nodule load itself proved to be relatively stable even several years after vector control. A similar observation was also reported by Parow (1983). However, one limita-

tion to consider is the fact that old nodules are shrinking and losing some weight when the stimulus of living parasites is ceasing (Schulz-Key et al., 1980) thus making it more difficult for these nodules to be detected. Consequently, bias towards the detection of larger nodules during the control period is introduced. It is also thought that in the final phase of vector control, only completely calcified nodules will persist. This may explain the high proportion (15%) of calcified nodules in Bangasse.

# Parasitological changes observed in the central area of the OCP

*Changes in comparison with the non-controlled area:* With the interruption of the transmission, no new parasites are being introduced into the final host. This resulted in a disequilibrium in the dynamics of the parasite population leading to the ageing and death of existing parasites without any replacement by young worms. In the non-controlled areas, almost 3% of the female worms were immature while none were found in the controlled area. In the controlled regions, there were clear signs of a senile population. The proportion of dead females was always higher wherever there was vector control. We do not know how long it takes for dead worms to be resorbed, nor do we know why some worms calcify completely whereas others do not show any signs of calcification. Calcified fragments obviously persist for a longer period and accumulate in the nodules to a certain extent. Therefore, during the first years of vector control, the reduction of the total number of worms might be caused mainly by the resorption of non-calcified ones.

Variations within the vector controlled area: Though there is a clear difference between the results obtained in the OCP and those from the non-controlled area, the difference is not homogeneous. In the eastern front of the OCP, the results from the 4 villages of the Koulpeolgo river basin show that the ageing of the parasite population was much more advanced than in the central and western area. Most of the worms were either very old or dead and calcified. The longitudinal data on microfilarial loads in the skin also indicate an onset of interruption of transmission in the East of Burkina Faso before vector control activities of the OCP. Two elements may account for this observation: first, the drought of the early seventies that has particularly affected this area, and second, its protection from reinvasion by the vector from adjacent western zones where vector control had started two years earlier. Thus these factors could have substantially reduced or interrupted the transmission already 2 to 4 vears before the beginning of OCP vector control operations. In the two villages on the Comoe on the western flank of the initial Programme area the parasite population is clearly younger. These villages are located in the vicinity of the zone initially affected by reinvasion of infective simulids (Walsh et al., 1979) and a possible transitory transmission can not be excluded although the evolution of the community microfilarial load has been quite satisfactory for Folonzo throughout the control period and for Boko since the second follow-up survey.

In northern Ghana, on the Sissili and on the Kulpawn river basin, the presence of a few obviously young worms was an indication of some residual transmission after the start of the control operations. This is not surprising given the results of the entomological evaluation which showed that the vector density, though greatly reduced by vector control, had remained high enough for a localised transmission between 1980 and 1983, and particularly in 1981. The heterogeneity of the results from the zone under vector control reflects the diversity of the epidemiological patterns after the start of the vector control activities as well as the situation prior to these operations.

### Longevity of the parasite

The longevity of the parasite can only be reliably determined when the epidemiological variations mentioned are considered. Information on the level of the transmission before 1975, as well as the data reflecting the entomological situation during the control period are therefore of great importance for the analysis. We have attempted to estimate the actual period of interruption of transmission using all the available information for the different OCP areas. In Figs. 3A and B the examined villages are grouped by geographical clusters with similar estimates for the period without transmission. The reduction of 80% in the number of living worms per nodule after 9-10 years of interruption of transmission in Lamiougou and Bangasse may be the most important information on the longevity of Onchocerca volvulus. It indicates an imminent breakdown of the worm population in the near future which is supported by recent findings in one of the neighbouring villages which, though initially mesoendemic, did not show a single infected individual during the last follow-up skin snip examination in 1986. In previously mesoendemic villages near Dedougou (Black Volta, Burkina Faso, under vector control since 1975) we found prevalences of microfilariae carriers of less than 10% and only six nodule carriers out of 300 palpated inhabitants in 1985.

Although we lack sufficient epidemiological precontrol data, our results indicate that the average longevity is considerably shorter than the maximum longevity of infection of 18 years as observed by Roberts et al. (1967). The conclusion of Parow (1983) that vector control should continue for 20 years seems too pessimistic. It is based on observations of nodules excised in Burkina Faso in an area of OCP where the community microfilarial load decreased only after a delay of several years while wherever the vector control was satisfactory, this index started to decline in the early stages of the interruption of the transmission. The particular evolution of the parasitological trend in this area reflects a special situation, and underlines the importance of considering various epidemiological situations in the attempt to estimate the longevity of the parasite. The decrease of microfilarial loads in the skin (Fig. 2) and our observations on adult worms indicate a breakdown of the worm population 11–12 years after the beginning of vector control.

### Sex ratio and reproductivity

The clear predominance of female worms in nodules gradually disappeared in the controlled areas as also observed by Büttner et al. (1983). A longer life expectancy of male worms might be suggested. But the male worms leave the nodules regularly to look for female worms in neighbouring nodules (Schulz-Key et al., 1980) and are in turn, often not recovered by nodulectomy. The observed shift in the sex ratio may be only the result of reduced migration of male worms. In very old worm populations (Niarba or Lamiougou and Bangasse) this reduced mobility may also explain the dramatic increase in the proportion of dead male worms in nodules.

The frequency of the reproductive cycles had slowed down and was closely related to the behaviour of the male worms (Kläger et al., 1985). The proportion of females with empty uteri increased (Table 3). More often worms with remnant intra-uterine microfilariae could be found. They had recently finished the release of microfilariae and remained then inactive for a longer period. Although microfilariae could not be observed in dead worms with certainty, female worms seem to be potentially reproductive until they die. Old females, still alive, but with a calcified tail, showed remnant microfilariae. The observed decrease in the productivity is not only due to ageing of the parasites but also to the reduced frequency of the cycles.

#### The productive potential of Onchocerca volvulus

The decrease in the initial number of live adult worms, as a result of the natural death of *Onchocerca volvulus*, and the reduced reproductivity of the surviving worms, are the two major factors which determine the fall in the total microfilarial production and in the microfilarial density in the human host population during the control period. The "productivity index" which measures the combined effect of both factors at the level of our sampling unit, the nodule, is used to demonstrate this phenomenon (Table 3). The reduction in the productivity index correlated with the observed reduction in the community microfilarial load during the control period. The good agreement with the results obtained independently (skin snips collected longitudinally) supports the validity of our approach to the worm analysis for the description of the population dynamics of *Onchocerca volvulus* during vector control activities.

The longevity of the adult parasite was considered to be the determining factor for the duration of vector control. However, the present study suggests that the ceasing reproductivity of the superannuated worm population plays a similarly important role for the control programme. Its major contribution to the depletion of the parasite reservoir will also help reduce the risk of transmission in case of a local outbreak of *Simulium damnosum* breeding. After an estimated period of 12 years without transmission in the upper Koulpeolgo river basin, this reservoir is nil or insignificant for some villages in 1986,

indicating that in such areas, vector control could theoretically be suspended without risking resurgence. In practice, the high probability of recontamination of such areas by infected Simulium from neighbouring places, where the CMFL has not yet decreased below a safety threshold, requires OCP to maintain its vigilance.

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