NEW FEATURES ON THE MOULTS AND MORPHOGENESIS OF THE HUMAN FILARIA *LOA LOA* BY USING RODENT HOSTS CONSEQUENCES

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Summary :

The development of the human filaria *Loa loa* (Dirofilariinae, Onchocercidae), previously studied in monkeys, was studied using the non permissive hosts-mice and jirds. The development proved to be rapid: moult 3 occurred on day 8 post-inoculation, the adult stage was reached on day 25 and measured at that time 3-3.5 mm in length. As in the other filarioids, the female genital apparatus developed during the fourth stage.

A critical analysis of the studies on the development of Onchocercid species was made. The optimal duration of the stages (i.g. the shortest time) was chosen for the comparison. It appeared that the duration of the stage 3 was a constant character in a given species whatever the experimental conditions, whereas moult 4 might be retarded in a non susceptible host.

Comparison between the 18 developmental cycles of Onchocercidae in the vertebrate host was made. Two biological types could be distinguished: either the moult 3 occurred on day 2-3 and was followed apparently by a late moult 4 (≥ 50 days), or the moult 3 occurred after about one week of development and it was associated with a less long stage 4 (20-40 days). The first group includes *Dirofilaria* and *Onchocerca*, the second group brings together mainly *Loa* and the Onchocercinae of the *Dipetalonema* line and related genera (*Acanthocheilonema, Brugia, Litomosoides*, etc.). The groups thus formed suggest real relationships as they fit with the morphology of the infective stage and the results of a recent molecular analysis of the 5S DNA.

KEY WORDS : *Loa loa*, laboratory mouse, jird, moults, morphogenesis, Onchocercinae, Dirofilariinae, phylogeny.

Résumé : NOUVELLES DONNÉES SUR LES MUES ET LA MORPHOGENÈSE DE LA FILAIRE HUMAINE *LOA LOA*, EN UTILISANT DES RONGEURS. CONSÉQUENCES

Le développement de la filaire humaine Loa loa *(Dirofilariinae, Onchocercidae), antérieurement étudié chez les singes, est précisé chez des souris et des mérions. Il s'avère rapide: la mue 3 s'effectue à J8, le stade adulte est atteint à J25 et mesure alors 3-3,5 mm de long; comme chez les autres filaires, l'appareil génital femelle se développe durant le stade 4.*

Une analyse des travaux sur les développements larvaires des Onchocercidés est effectuée. Les durées optimales des stades (c'est-à-dire les plus rapides) sont choisies pour la comparaison. Il apparaît que la durée du stade 3 est un caractère stable indépendant des conditions expérimentales pour une espèce donnée, alors que la durée du stade 4 peut être plus longue chez de mauvais hôtes.

Parmi les 18 cycles d'Onchocercidae réalisés chez l'hôte vertébré, on remarque qu'il existe deux types biologiques: la mue 3 s'effectue soit à J2-J3 et est suivie apparemment d'une mue 4 tardive (≥ 50 jours), soit après une semaine environ de développement et associée à un stade 4 moins long (20-40 jours). Le premier groupe comprend Dirofilaria *et* Onchocerca*; le second rassemble, entre autres,* Loa *et les Onchocercinae de la lignée* Dipetalonema *et des genres proches* (Acanthocheilonema, Brugia, Litomosoides, *etc). Les groupes ainsi constitués suggèrent de réelles affinités car ils concordent avec la morphologie du stade infectant et les résultats d'une récente analyse moléculaire sur le 5S DNA.*

MOTS CLÉS : Loa loa, *souris de laboratoire, mérion, mues, morphogenèse, Onchocercinae, Dirofilariinae, phylogénie.*

INTRODUCTION

Among the 18 developmental cycles of Onchocercid filariae elucidated in the definitive host (Anderson, 1992 and Table I), that of the human filaria *Loa loa* appears slow: the fourth moult was described to occur at about day 50 post inoculation (Eberhard & Orihel, 1981), as was *Dirofilaria immitis* (Orihel, 1961). This similarity of the biological characters seems satisfactory because the two genera are placed in the Dirofilariinae. However, in the interpretation of the results with *L. loa* obtained in the experimentally infected monkeys there lay a doubt: only one of the two moults has been observed, at day 18; it has been identified as the third moult, although the well advanced organization of the genital apparatus at this date corresponded to what is generally observed in a late filarial fourth stage.

We have inoculated mice and jirds with *Loa loa* larvae (Table II) and treated most of them with an anti-inflammatory drug (hydrocortisone). The earliest necropsy was performed on day 8 because, in many filarial species, the moult 3 occurs near the end of the first week following larval inoculation. The eight

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Genus	Species	Day M3	Day M4	Host	References	mm M3	mm M4
Loa	*loa*	18	50	monkeys	Eberhard & Orihel, 1981	3	≤ 16.8
		8	***19***	mouse, jird	Present paper.	**2.1**	**3.18**
Pelecitus	*fulicaeatrae*		≤ 20	* *Fulica*	Bartlett & Anderson, 1989		
	scapiceps	6	12	* *Sylvilagus*	Bartlett, 1984	1.4	2.8
Dirofilaria	*immitis*	9	60	* dog	Orihel, 1961		
		≤3	58	* dog	Kotani & Powers, 1982		
		≤3	50	* dog	Lichtenfels *et al.*, 1985	1.2	12.8
		≤3		rat	Sawyer & Weinstein, 1965		
		2		*in vitro*	Sawyer & Weinstein, 1965		
		2		*in vitro*	Lok *et al.*, 1984		
		3		*in vitro*	Lichtenfels *et al.*, 1985		
Litomosoides	*sigmodontis*	***8***	23	* *Sigmodon*	Scott *et al.*, 1951	1.2	≥ 6.4
		8		* *Sigmodon*	Nelson *et al.*, 1982		
		≤10	≤ 28	BALB/c mice	Maréchal *et al.*, 1996	≤ 1.4	
		6 + 3		*in vitro*	Nelson *et al.*, 1982		
Breinlia	*booliati*	***6***	24	* *Rattus*	Singh *et al.*, 1976	≤ 1.5	9.4
Monanema	*globulosa*	***≤10***	25	jird	Bianco *et al.*, 1983	1.1	5.98
	martini	***≤10***	21	* *Lemniscomys*	Wanji *et al.*, 1990	1.4	4.5*
Acanthocheil.	*viteae*	***7?***	≤ 21	* *Meriones*	Chabaud, 1954		
		6	20	hamster	Original	1.8	11.9
		7	23	jird	Johnson *et al.*, 1974		
		6		jird	Eisenbeiss *et al.*, 1994		
Molinema	*arbuta*	≤ 20	≤ 28	* *Erethizon*	Bartlett & Anderson, 1985	2.6	≥ 8.6
	dessetae	20	40	* *Proechimys*	Gayral *et al.*, 1982		
		10	34	* *Proechimys*	Bain *et al.*, 1994	1.35	9*
Brugia	*malayi*	***7***	35	* cat	Edeson & Buckley, 1959	2.2	5.8
		7	29	jird	Ash & Risley, 1970a	1.6	
	pahangui	***8***	23	* cat	Schacher, 1962	2.1	7
		6	18	jird	Ask & Risley, 1970b		
Wuchereria	*bancrofti*	< 14	≤ 42	monkey	Cross *et al.*, 1979	2.3	≤ 4.7
		8		jird	Ash & Schacher, 1971	1.5	
Elaeophora	*schneideri*		≤ 14	* *Odocoileus*	Hibler & Metzger, 1974		≤ 10
Onchocerca	*lienalis*	***2***		* cow	Bianco & Muller, 1982		
		2		*in vitro*	Lok *et al.*, 1984		
		≤3		micro chamber	Bianco *et al.*, 1989	0.5	
	volvulus	5		*in vitro*	Lok *et al.*, 1984		
		≤3		micro chamber	Bianco *et al.*, 1989	0.59	
Splendidofilaria	*picacardina*	< 14	≤ 21	* *Pica*	Hibler, 1963		

Table I. – The 18 cycles of Onchocercidae in the vertebrate host.
Upper part: Dirofilariinae; middle part: Onchocercinae; lower part: Splendidofilariinae. Day M3: the shortest time for moult 3 in days p.i., according to the different authors (italic numbers correspond to the reliable data); day M4: the shortest time p. i. for the male moult 4. Host: * means natural host; micro chamber = micropore chamber containing infective larvae and implanted into diverse hosts. References: a non exhaustive list of the main works. mm M3: maximum length at moult 3, in millimeters; mm M4: maximum length of male larvae at moult 4, in millimeters; * means original data. Acanthocheil: *Acanthocheilonema*.

rodents necropsied from that date to day 25 post inoculation harboured developing worms and we recovered successively larvae undergoing moult 3, moult 4 and young adult worms. Two monkeys, *Cercopithecus leucophaeus*, presenting a patent experimental infection with *L. loa*, were re-inoculated with infective larvae, however no developing larva was recovered 8 and 15 days later, and all results presented here come from the rodent hosts.

MATERIAL

Infective larvae for morphological study. During 1996-1997, the infective larvae were collected by two of us (S.W. & P.E.) from *Chrysops silacea* fed on a volonteer, near Kumba, in Cameroon (n° 295 SE, MNHN-Paris).

Infective larvae for inoculations. They had different origins because the search was performed in different

Host	Inoc. date	Larvae inoculated	D p.i.	N worms recov	Stage	Hydrocortisone
Mouse 24SE	26.11.1991	13 infective larvae	9	4	M3	25 mg × 2/week
Mouse 31SE	05.12.1991	3 larvae 9 day old from 24SE	23	female 1	L4	25 mg × 2/week
Jird 138SE	22.02.1995	100 infective larvae	19	female 1, male 1	L4, M4	0
Jird 140SE	22.02.1995	200 infective larvae	25	female 1, male 1	Ad	25 mg × 2/week
Mouse 310SE.1	23.11.1996	200 infective larvae	8	female 3, male 5	L3, M3	50 mg × 2/week
Mouse 310SE.2	29.11.1996	200 infective larvae	9	female 5, male 7	M3, L4	50 mg × 2/week
Mouse 310SE.3	05.12.1996	200 infective larvae	15	female 4, male 3	L4	50 mg × 2/week
Mouse 310SE.4	15.01.1997	200 infective larvae	22	female 5	L4, M4	50 mg × 2/week

Table II. – *Loa loa* in Swiss mice and *Meriones unguiculatus*: experimental procedures and worms recovered.
Inoc. date: dates of inoculation of larvae into rodents. Larvae inoculated: infective larvae from flies were inoculated, except for 31 SE which was inoculated subcutaneously with the larvae recovered from the mouse 24 SE. D p.i.: time between inoculation of the infective larvae and necropsy (for 34 SE, D 23 = 9 days in 24 SE + 14 days in 31 SE). N worms recov: number of worms recovered. Stage: M, moult; L, larval stage; Ad, adult immature worm. Hydrocortisone: it was administered intraperitoneally just after the larval inoculation twice a week at a concentration of 25 or 50 mg/kg.
Mice 24 SE and 310 SE came from a colony maintained at the Institut Pasteur, Yaoundé; mouse 31 SE and the jirds came from the Biologie Parasitaire laboratory, Paris.

places and times. In 1991, in Cameroon near Campo and in 1995, in Gabon, at the CIRMF in Franceville, the infective larvae were recovered from unfed *C. silacea* and *C. dimidiata* captured with a net and harbouring wild infections, however it is known that these diurnal flies transmit essentially the human strain of *L. loa* (Duke & Wijers, 1958). The flies were dissected individually or according to the technique of Wahl *et al.* (1995). In 1996-1997, at Kumba, the infective larvae originated from flies fed on a volunteer.

Developing larvae. The rodents, the inoculations, the dates of necropsies and the worms recovered are detailed in Table II. Infective and developing larvae were fixed in 5 % formalin for morphological study.

Adult mature worms. One male specimen extracted from a human hydrocoele, fixed in hot 70 % alcohol and sent by Pr C. Ripert (n° 426 HB, collection MNHN-Paris). Four male and four female worms recovered from a monkey exprimentally infected with a human strain of *L. loa* by Eberhard & Orihel, in 1981, fixed in glacial acetic acid and stored in 70 % ethanol plus 5 % glycerine (n° 275 SE, MNHN-Paris).

RESULTS

The dates of the moults, the larval growth and the morphogenesis of *L. loa* are described on a total of 26 worms recovered from rodents.

MOULTS

On day 8 p.i., seven of the eight larvae recovered were undergoing moult 3, and one was retarded; on day 9, nine larvae were moulting, of which three were in the process of exsheathment, and three larvae were at fourth stage. On day 15, the seven larvae recovered were at fourth stage. Moulting 4 of a male worm was observed on day 19 in a jird, and moulting 4 of a female worm on day 22 in a mouse (Table III, Fig. 1 E, F, K; Fig. 3).

GROWTH

The growth was slight. At moult 3 the larvae were 1.9-2.15 mm long, like the infective stage but they were slightly wider (30-39 µm). On day 15 p.i., the male fourth stage larvae were 2.1-2.2 mm long and 31-36 µm wide, the female fourth stage larvae were 2.4-2.77 mm long and 34-43 µm wide; on day 21-22 p.i. the five female fourth stage larvae were 3-3.45 mm long and 40-55 µm wide. The two young female and male adult worms were respectively 3.65 and 3.3 mm long, 42 and 45 µm wide.

MORPHOGENESIS

The evolution of the main structures was analyzed from the infective larva to the adult worm (Figs. 1 and 2, Table III).

• Caudal lappets. Three conspicuous and rounded caudal lappets were present during the whole stage 3 (Fig. 1 B, C, F); they persisted during the stage 4 but they were pointed (Fig. 1 I et K); they decreased at the adult stage: the dorsal lappet disappeared and the lateral lappets were vestigial (Figs. 1 N, P, and 2 Q).

• Papillae of the head. In the infective larvae, the externo-labial papillae were pointed and salient; these papillae and the cephalic ones were arranged as rectangles slightly stretched in the lateral plane (Fig. 2 A, B, C). In the young and mature adult worm, the two groups of papillae were arranged as rectangles stretched in the median plane (Fig. 2 F, G, H).

• Buccal capsule. It was 6 µm high at the third stage, flattened laterally, hardly sclerotized even at its junc-

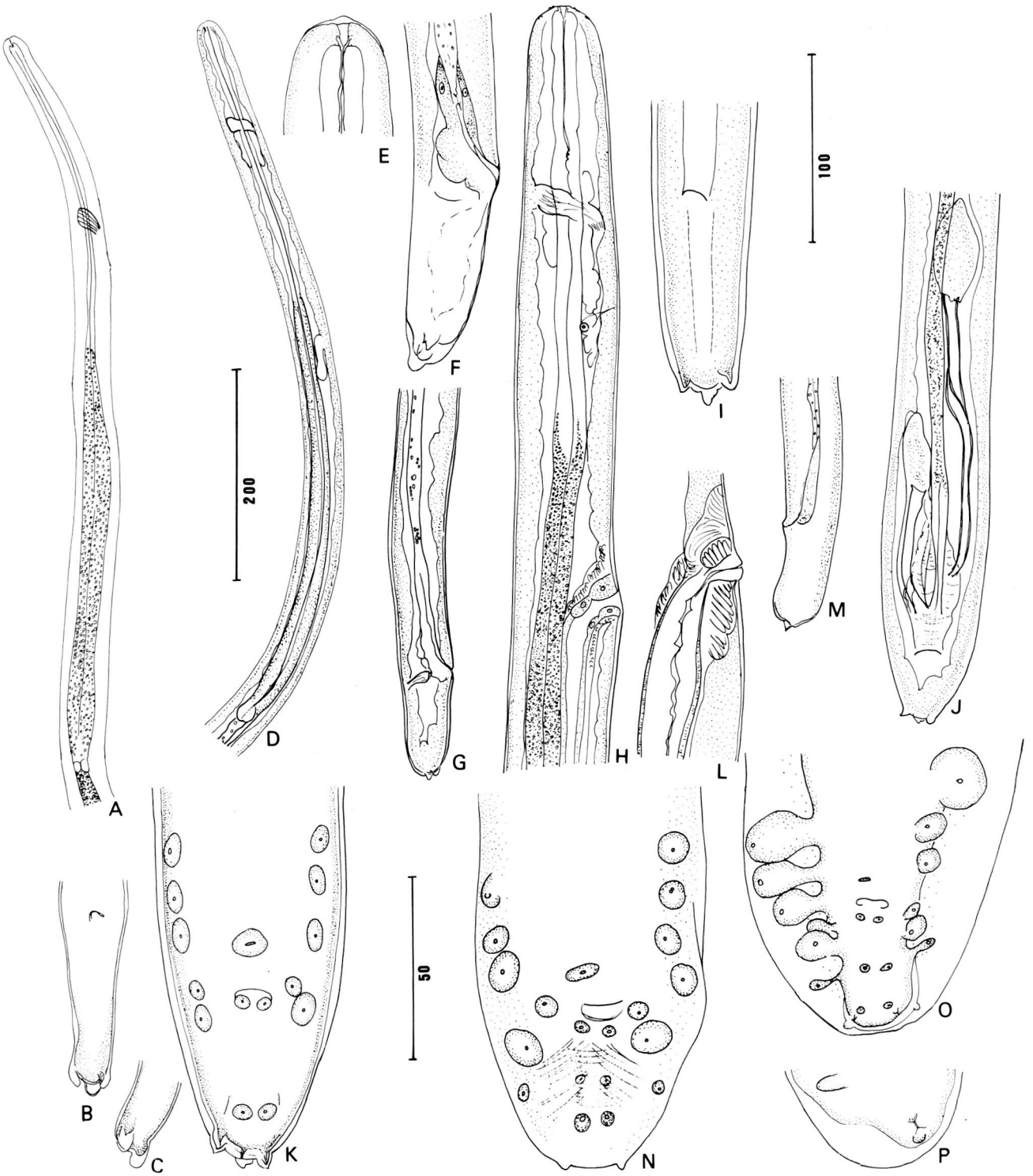

Fig. 1. – *Loa loa* morphogenesis in mice and jirds.
A to C: male infective larva from *Chrysops.* A: anterior region, lateral view (note the post-cephalic constriction); B: tail, ventral view; C: caudal extremity, left lateral view. *D to I,* from mice; *D to G:* female larva, third moult, day 9 p.i.: E: head with the loosened cephalic cuticle of the third stage and the buccal capsules of stages 3 and 4 imbricated; F: tail, with caudal lappets of stage 4 fully developed under the cuticle of stage 3; G: caudal region, lateral view. *H & I:* fourth stage female larva, day 23 p.i., respectively anterior part, in lateral view and tail, in ventral view. *J to P,* from jird; *J & K:* male larva, fourth moult, ventral view, day 19 p.i.; J: posterior part, with spicules; K: caudal papillae under the cuticle of moult 4; *L & M:* female, day 26 p.i.; L: vulva and vagina, lateral view; M: caudal region, lateral view; *N:* male, day 26 p.i., tail, ventral view with incipient caudal alae. *O & P:* mature adult worm from laboratory monkey; O: tail, lateral view; P: extremity, right lateral view. Scales: A, G, J, L, M, O, 100 µm; B, C, E, F, I, K, N, P, 50 µm; D, 200 µm.

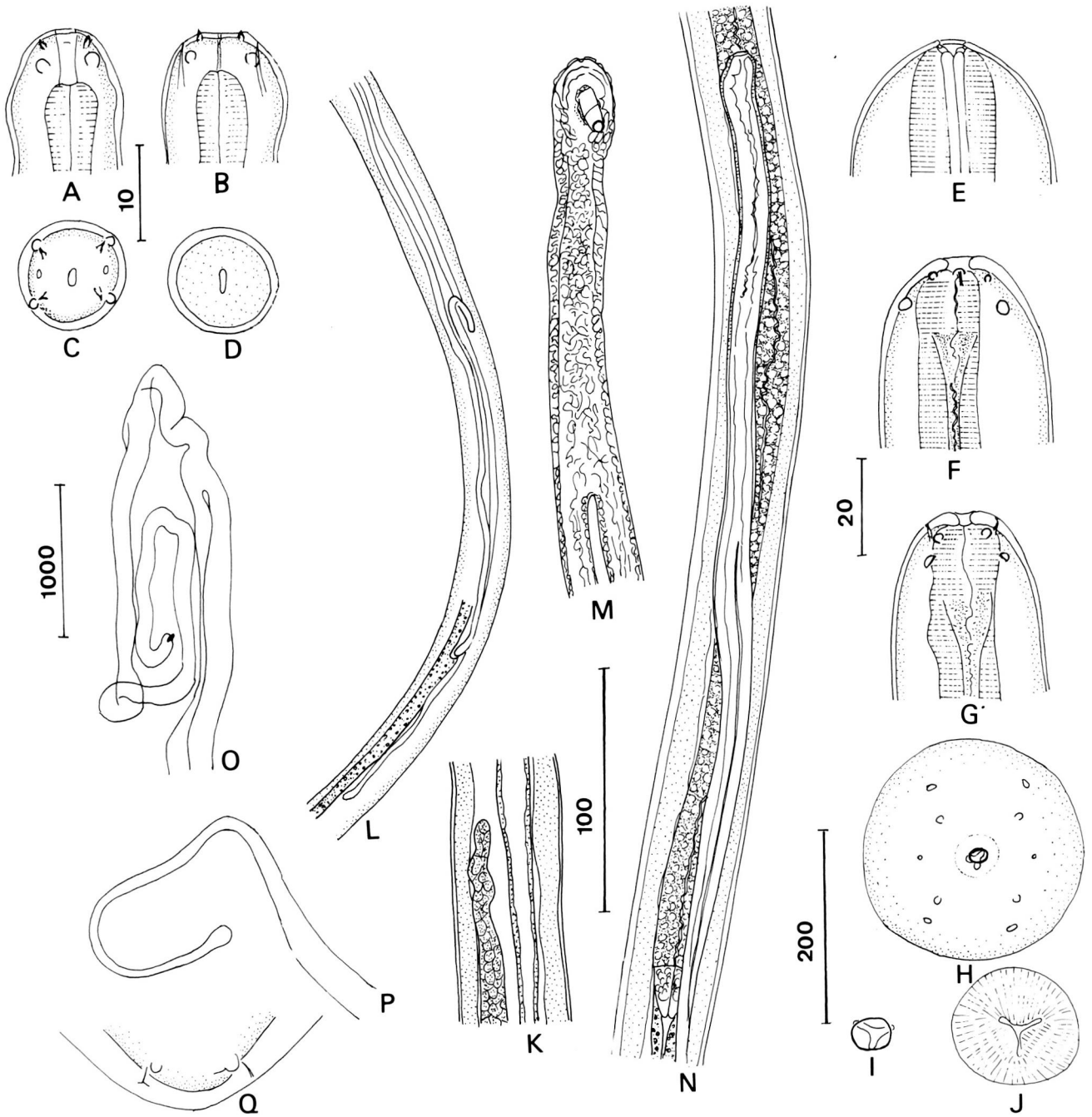

Fig. 2. – Morphogenesis of cephalic and female genital structures of *Loa loa*.
A to D: head, infective stage larva; A & B: lateral and median view; C: apical view; D: optical cross-section at the level of the buccal cavity. E: head, fourth stage larva from jird; *F & G:* head, young adult female stage, lateral and median view; *H to J:* head, mature female adult from monkey; apical view, detail of mouth and base of buccal cavity, optical transversal section of the oesophagus, respectively. *L & M:* female fourth stage larva from mice, ovaries and uteri (intestine drawn along a short distance), vulvar region and ovejector, respectively. *N:* young adult from jird with open vulva in ventral view. *O to Q:* mature adult; O: vulva and ovejector; P: initial portion of an ovary; P: caudal extremity, ventral view. Scales: A to D, 10 µm; E, F, G, M, 20 µm; H, L, P, 200 µm; I, J, K, N, Q, 100 µm; O, 1000 µm.

Measurements	Female				Male		Measurements
	day 9; moult 3	day 22; stage 4	day 19; stage 4	day 25; adult	day 25; adult	day 19; moult 4	
Length	2,130	3,280	3,500	3,650	3,300	3,180	Length
Width	35	50	50	42	45	55	Width
Nerve ring	110	110	110	115	110	105	Nerve ring
Excretory pore	220	180	–	–	–	175	Excretory pore
Oesophagus	760	730	630	790	630	650	Oesophagus
(musc)	(280)	(250)		(350)	(220)		(musc)
Vulva-apex	350	340	340	330	145 (60)	160 (50)	Left spicule (shaft)
Genital length	45	2,030	1,300	1,740	95	70	Right spicule
(ovej)		(90)		(210)			
Tail	55	62	60	55	50	50	Tail

Table III – Detailed measurements of *Loa loa* worms recovered from mice, on day 9 and 23, and jirds, on day 19 and 25.
musc: muscular; ovej: ovejector. All measurements are given in µm.
For comparison, mature adult female worms are 50-70 mm long and 500 µm wide and mature adult male worms are 2,7 mm long and 350 µm wide with left and right spicules 180-120 µm long (Eberhard & Orihel, 1981 and present study); spicules are 123-88 µm long according to Brumpt (1949).

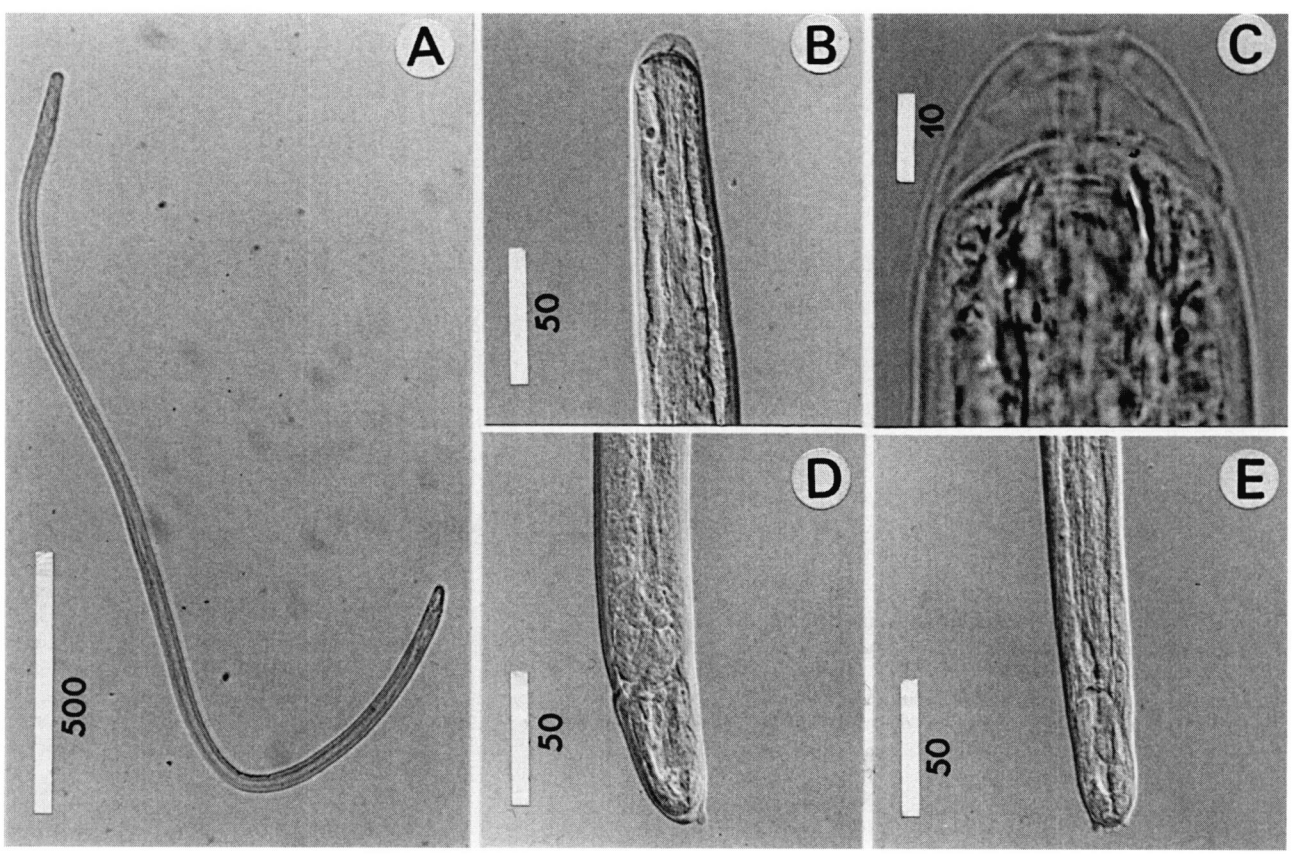

Fig. 3 - Third moulting of *L. loa*, eight days after inoculation. A: general aspect of a larva. B, C: anterior extremity; D, E: posterior extremity, lateral and median views (Scale: A, 500 µm; B, D, E, 50 µm; C, 10 µm).

tion with the oesophagus (Fig. 2 A to D). It was reduced in the stage 4 (Fig. 2 E) and absent in the adult stage (Fig. 2 G).

• Female genital apparatus. During the stage 3, the genital anlage hardly evolved compared to the infective larva (Fig. 1 D). The differentiation of the genital apparatus occurred during the stage 4 (Fig. 2 L, M). The vagina remained simple in shape, without any curve or sphincter, but the muscular ovejector became very long in the mature adult (Fig. 2 O).

• Male genital apparatus. During the stage 3, the genital anlage was at mid body and it increased in size; it was 60-130 µm long and 7 µm wide at moult 3; its anterior extremity was rounded (10-12 µm) and shortly flexed. No coelomocyte, as described by Bartlett (1984) with *Pelecitus scapiceps*, was observed near this extremity. The spicular primordia of the fourth stage larvae were 55 µm long; the left spicular pouch at moult 4 was 215 µm long.

• Caudal papillae and alae of the male worm (Fig. 1 K, N, O). There was no important change between moult 4 and maturation, except the development of the lateral alae. However it was noted that the presence of four precloacal papillae on the left side of a young adult male (Fig. 1 N) suggested a primitive scheme of ten pairs of caudal papillae (Chabaud & Petter, 1961), rapidly reduced to nine pairs. The papillae had a size and were arranged according to a very constant scheme (see also Fig. 12 *in* Eberhard & Orihel, 1981). They formed a subventral set of three equidistant pairs (the median pair has not been seen in the young adult) and a set of six latero-ventral pairs: three large precloacal pairs, one small paracloacal, one large and one small post-cloacal pairs.

• Cuticular bosses. Consistent with the description of Eberhard & Orihel (1981), they were absent in the young adult worm. They appeared during the maturation and formed one of the anti-sliding apparatus necessary during mating, which have diverse origins and shapes in filarioids (Bain & Chabaud, 1988).

DISCUSSION

No mature adult worms of *L. loa* were recovered in mice and jirds, even following immune depletion with hydrocortisone. However, larval development until the young adult stage was obtained and the diverse experiments performed presented very constant results. Compared to the development in humans or susceptible simian species, the growth and consequently the moults might have been expected to be retarded. Thus the features observed in rodents seem reliable. *L. loa* makes moults 3 and 4 as early as day 8 and day 19 respectively, its growth is very slight during the stage 4. Studies of the larval development of the Onchocercids have been performed in different experimental conditions and a critical analysis of the results is needed before comparing the duration of the stages as between species. The moulting is a long process and it is necessary to agree on the term moult: it is only when the caudal extremity and the buccal capsule of the following stage are formed under the cuticle that the exuviation is impending; these two last phases are called the moult. In addition, moults are not perfectly synchronous for a given species, particularly when the host or experimental conditions are not suitable. For example, moult 3 of *O. volvulus* began on day 3 and continued through day 14, in surrogate primate and rodent hosts (Abraham *et al.*, 1993). However, a reliable datum is the optimal development, that is the more rapid and with the greater growth. Thus we have based the comparison on the shortest times of stages (Table I).

The developments to the adult stage have been examined in the natural or surrogate hosts inoculated with infective larvae. The stage 3 has been also studied under artificial conditions: infective larvae placed in micropore chambers implanted in the sub-cutaneous tissue of hosts, and *in vitro* cultures of infective larvae or of larvae which have begun their development in the vertebrate host.

The duration of the stage 4, which corresponds to the genital development, appeared dependent upon the experimental conditions. Moult 4 might be retarded in resistant hosts. In fact, in many species, the time of moult 4 is not known because it did not occur, even when the survival of the larval worms lasted a long time, such as *Wuchereria bancrofti* in jirds (Ash & Schacher, 1971).

On the other hand, it appeared that the optimal duration of the stage 3 was a stable character, as well as its size, not depending upon the experimental conditions. For example, in the resistant B10D2 mice, the size of *Litomosoides sigmodontis* larvae at moulting 3 and the date of moulting were similar to those obtained in the susceptible BALB/c mice (Maréchal *et al.*, 1996), in *Meriones unguiculatus* and in the natural host, *Sigmodon hispidus*.

The *in vitro* trials are particularly interesting. The first ones, performed with *Dirofilaria immitis*, gave results different to those in the *in vivo* observations: *in vitro* the moult 3 was seen at day 3 (Yoeli *et al.*, 1964) when it was said to occur between day 9 and day 12 in the dog (Orihel, 1961). Later, Sawyer & Weinstein (1965) compared the development of *D. immitis* in the dog, the young rat and *in vitro* and they noted that, in all cases, the moult occurred at days 2-3. These features were confirmed by the other authors (see review *in*

Lichtenfels *et al.*, 1985 and Table I) and the moults observed by Orihel corresponded probably to retarded larvae.

Wong *et al.* (1982) compared *Brugia pahangi* with *D. immitis in vitro* and demonstrated that the first species moulted seven days later than the other, that is after nine days of development, just as it does in the host.

The use of the mixed protocol, *in vivo* followed by *in vitro*, performed with *B. pahangi* by Chen & Howells (1979) and *L. sigmodontis* by Nelson *et al.* (1982) showed that the total *in vivo* + *in vitro* duration of stage 3 was equal to that observed in the natural conditions.

Presently the only contradictory case concerns *Onchocerca volvulus* which, according to Bianco *et al.* (1989) and Abraham *et al.* (1993), did its first moult 3 slightly earlier than day 3 in the micropore chambers and, according to Lok *et al.* (1984 *b*), at day 5 *in vitro*. The first data is thought to be the most reliable. It is similar to that of *O. lienalis* for which all the authors agree (Bianco & Muller, 1982; Lok *et al.*, 1984 *b*; Bianco *et al.*, 1989).

Taking into account these remarks, the 18 developmental cycles of Onchocercidae elucidated in the definitive host were compared. They are distributed in three subfamilies (Table I), the Dirofilariinae: two genera and three species; the Onchocercinae: nine genera and 13 species; the Splendidofilariinae: one genus, one species. However the data are sometimes unsufficient; it is the case for two species: *Pelecitus fulicae-atrae* and *Elaeophora schneideri* which are thus eliminated from the analysis.

One feature became apparent by consulting the whole data of the Table I: moult 3 occurred either very early, at day 2-3, or after about a week of development. The case of *Molinema dessetae* needs a comment; moult 3 was said to occur on day 20 (Gayral *et al.*, 1982) however we have maintained this species over many years and observed the exsheathment of moult 3 much earlier, on day 10 (Bain *et al.*, 1994). In *M. arbuta*, studied by Bartlett & Anderson (1985), fourth stages were recovered together with moulting third stages on day 20, strongly suggesting that in this species too the first moults 3 occurred earlier.

The distribution of the genera based on the duration of the stage 3 does not agree with the classification of Anderson & Bain (1976). However, the regrouping made according to that character did allow association of some genera which seemed to have other features in common, such as the duration of the stage 4 and the morphology of the infective larva. Taken together, they could well illuminate real relationships.

The group presenting an early moult 3 includes two genera, which are respectively the types of the Dirofilariinae and Onchocercinae subfamilies: *Dirofilaria*, of which *D. immitis* is the only cycle elucidated, and *Onchocerca*, with two elucidated cycles, *O. lienalis* (Bianco & Muller, 1982; Bianco *et al.*, 1989) and *O. volvulus* (Bianco *et al.*, 1989). As far as we know, moult 4 has not been determined in *Onchocerca* spp. (Abraham *et al.*, 1992) although the micropore chamber technique allowed recovery of a young adult male worm at day 100 (Strote, 1985). Moult 4 of *D. immitis* does not occur before day 50 (Orihel, 1961; Kotani & Powers, 1982; Lichtenfels *et al.*, 1985). The infective larvae of both genera have a short tail and tiny caudal lappets (Bain & Chabaud, 1986).

The second group, with a late moult 3, includes – the seven other genera studied in the Onchocercinae, *Litomosoides, Breinlia, Monanema, Acanthocheilonema, Molinema, Brugia* and *Wuchereria*; they belong to the *Dipetalonema* line or are closely related to it (Chabaud & Bain, 1976), – the two other genera studied in the Dirofilariinae, *Loa* and *Pelecitus* (*sensu* Bartlett & Greiner, 1986), – and the Splendidofilariinae *Splendidofilaria* (Table I). In this second group, moult 4 occurs well before day 50. However this group appears heterogeneous as *Pelecitus* becomes adult as soon as the second week following inoculation while the other genera become adult in 20-40 days. The infective larvae of *Loa* and the *Dipetamonema* line *sensu lato* have a long tail with conspicuous caudal lappets. *Pelecitus* and *Splendidofilaria* have different morphologies. The affinities which are suggested between *Loa* and the *Dipetalonema* line of the Onchocercinae on one hand, and between *Dirofilaria* and *Onchocerca* on the other, agree with the results of an analysis of molecular phylogeny, based on the 5S DNA spacer, perfomed by Xie *et al.* (1994) with seven of these filarial genera, and *Mansonella*.

CONCLUSION

The rodent models, even when they are poor hosts, appear interesting for elucidating the morphogenesis and the larval biology of filarioids in the definitive host. The knowledge of this phase of the cycle so far is poor as data have been published for only 13 out of about 80 genera which compose the Onchocercids.

The determinations made on the dates of the *L. loa* moults and the distinction of two biological types of filariae according to the long or short duration of stage 3 are of practical interest for the simian models of loiasis (Pinder *et al.*, 1994) and other filarial models. Moult 3 corresponds to important changes in the composition of the cuticle (reviewed by Behnke *et al.*, 1992) and some authors give this phase of the cycle

a predominant role in the establishment of host protection against filarial infection (Eisenbeiss *et al.*, 1994; Daniel *et al.*, 1996).

These biological features seem also to present a phylogenetic interest. Morphology is poor and convergences important in the filarioids, due to their tissue invading habits, and new characters are necessary.

The genus *Loa* is presently placed in the Dirofilariinae on the basis of adult characters. The larval biology, the morphology of the infective larva and some sequences of DNA do not seem to confirm its links with *Dirofilaria*, which itself might be closely related to *Onchocerca*. The features presented here suggest that *Loa* should be brought nearer to the Onchocercinae of the *Dipetalonema* line *sensu lato*. We do not suggest however that these hypotheses should bring about any formal systematic modifications as yet.

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