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Research Note

Acid Phosphatase Activity in the Hybrid Microfilariae between *Brugia malayi* and *B. pahangi*

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Brugia malayi and *B. pahangi* are sympatric in West Malaysia (Denham and McGreevy, 1977) and Kalimantan (Borneo), Indonesia (Palmieri *et al.*, 1983). Microfilariae and further developmental stages of *B. malayi* and *B. pahangi* are morphologically similar to each other and the definitive indentification of both species is based on the structural differences in the spicules of the males. Since the diagnosis of human cases of these filariasis rests on the detection of microfilariae in the peripheral blood, an erroneous differential diagnosis may be made in the sympatric areas of both species. Recently, a capability of differentiation of them by the distribution pattern of acid phosphatase (ACP) activity of microfilariae has been reported (Redington, *et al.* 1975; Yen and Mak, 1978). However it is not possible in the developmental stages in the mosquitos (Omar, 1971). Palmieri *et al.* (1983) reported a natural human infection of *B. pahangi* in South Kalimantan by examining ACP activity in microfilariae. Suswillow *et al.* (1978) investigated hybridization potential among *B. pahangi, B. patei* and *B. malayi* and demonstrated that a cross between male *B. pahangi* and female *B. malayi* was infertile although all other crosses were successful in producing microfilariae in the peritoneal cavity of infected jirds.

In the sympatric areas for *B. malayi* and *B. pahangi* there are some possibility of hybridization occurring in nature. Therefore the authors observed ACP activity in hybrid microfilariae between female *B. pahangi* and male periodic *B. malayi* to know whether ACP activity of them shows species characteristic pattern or not. The method of hybridization is principally followed to Suswillow *et al.* (1978). Jirds inoculated with infective larvae of each species in their peritoneal cavities were killed 24 days after the infection of *B. pahangi* and 31 days after that of *B. malayi*. Sexes of the larvae were determined by the body length of the worms, the presence or absence of the curled tail and the spicules. Average body lengths of the larvae on the day of autopsy were 11.13 mm (n = 7, SD = 1.01) in male and 15.32 mm (n = 10, SD = 0.92) in female of *B. pahangi*, and 9.04 mm (n = 5, SD = 0.82) in male and 11.79 mm (n = 8, SD = 0.59) in female of *B. malayi*. Forty two young females of *B. pahangi* thus collected were implanted again into the peritoneal cavity of a naive jird. About 60 days later the absence of microfilariae in the peritoneal cavity was ascertained by examining Hank's balanced solution (10 ml/jird) used for washing the peritoneal cavity of the implanted jird. After confirmation of the absence of microfilariae, 13 *B. malayi* immature males were implanted into the peritoneal cavity of the same jird. ACP staining of hybrid microfilariae was done according to Redington *et al.* (1975). Sucked fluid from the infected jird 6 months post-implantation was smeared on the albuminized glass-slides which had been prepared by dipping in 1% bovine serum albumin solution. The smears were air dried at 5°C for 12 hrs, then they were fixed in chilled absolute acetone for

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1 min and incubated in the coupling solution at 37°C for 60 min. The staining patterns by ACP activity of the hybrid microfilariae are schematically shown (Fig. 1). The proportion of each type to all microfilariae which are observed from the three replications are almost constant.

More than half of the hybrid microfilariae (56.9%: 421/740) stained light pink throughout their entire body (Fig. 2-a) and excretory and anal vesicles were clearly recognized in red. Some of them showed a positive reaction in ACP activity in amphid and/or phasmid areas.

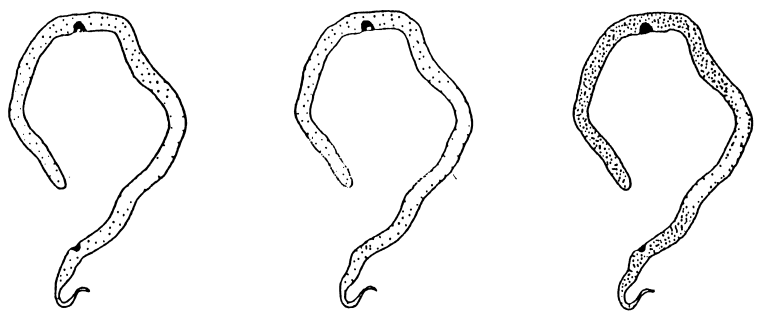

Fig. 1 Illustrated staining patterns of acid phosphatase activity and proportion of each type to all hybrid microfilariae. Figures in parentheses are number of microfilariae observed.

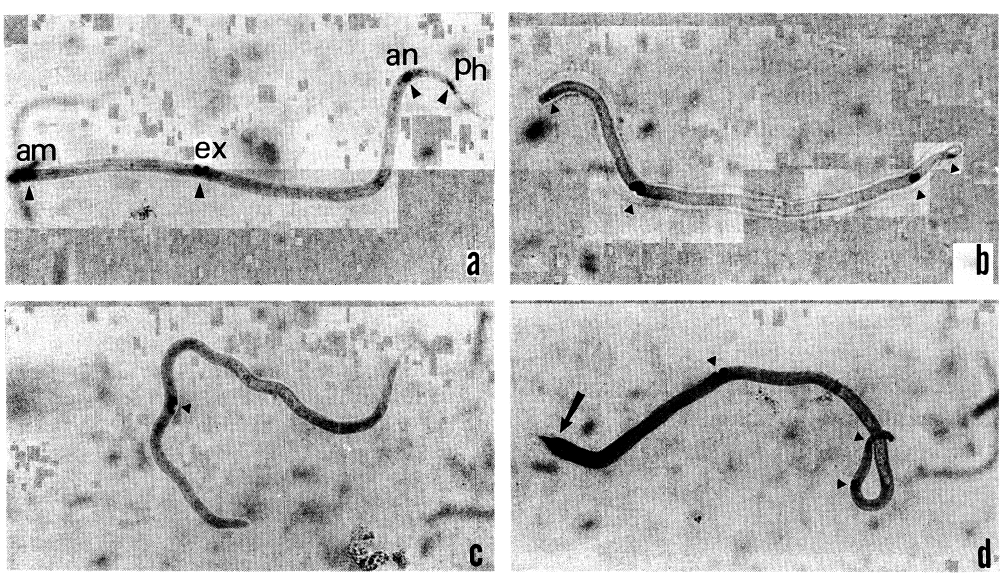

Fig. 2 Acid phosphatase activity patterns of hybrid and *B. malayi* microfilariae (Mf)
a: Hybrid Mf from peritoneal cavity of jird.
am: amphid area, ex: excretory vescicle, an: anal vescicle, ph: phasmid area
b: *B. malayi* Mf from peripheral blood.
c: Hybrid Mf from peritoneal cavity. Note that the anal vescicle does not show ACP activity.
d: Hybrid Mf from peritoneal cavity. Body shows an intense activity in comparison with other hybrid Mf and space between body and microfilarial sheath shows more intense enzyme activity (arrow).

This pattern was completely same to those of *B. malayi* microfilariae (Fig. 2-b). Thirty-five per cent (265/740) stained similar color tone to the former along their entire body but they did not show the enzyme activity in the area of anal and/or excretory vesicles (Fig. 2-c). The rest (7.3%: 54/740) has shown similar pattern of ACP activity to those of *B. pahangi* microfilariae, which have an intense staining along the body (Fig. 2-d). However, the activity of the body was slightly weaker than those of *B. pahangi* if compared with them.

Suswillow *et al.* (1978) made mention of difficulty of differentiation in each hybrid microfilariae because of their uniform staining pattern. The present study has shown that the majority of the hybrid microfilariae have the same ACP activity to *B. malayi* including some variation in enzyme activity and that some hybrid microfilariae (7.3%) could not be differentiated from those of the maternal species (*B. pahangi*). After all, no specific ACP activity pattern characteristic to the hybrid microfilariae is detected. After finishing collection of microfilariae the jird was sacrificed to collect adults for checking the morphological characteristics. All left spicules of the males have shown a spatulate-like structure at the tip which is characteristic to the males of *B. malayi*.

It is also confirmed that the hybrid microfilariae develop to the infective larvae in *Armigeres subalbatus* and that these infective larvae can complete their development to the adults in the peritoneal cavity of the jird. However, no microfilariae are detected from either peripheral blood or exudate of peritoneal cavity at the 8th months postinfection. Of 167 adults collected, only 10 were males. This fact showed a good agreement with the data reported by Suswillo *et al.* (1978).

In the sympatric areas for *B. pahangi* and *B. malayi*, both species has been reported to be differentiated by ACP activity of microfilariae. However, the present study indicates possibility of an erroneous diagnosis if it is carried out by ACP activity alone.

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