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Factors Influencing the Developmental Direction of *Strongyloides fuelleborni* First-Stage Larvae

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Abstract

Quantitative studies were carried out on the developmental direction of *Strongyloides fuelleborni* first-stage larvae with regard to the effects of incubation temperature and nourishment in culture system. Effects of temperature on the three developmental directions of free-living generation (namely male, female and filariform larvae) revealed that the temperature range of 30–35°C gave relatively higher total recovery rates with many females and few filariform larvae being formed, whereas at low temperatures such as range 15–20°C, many filariform larvae and few females developed. Concerning males, recovery rates were almost constant regardless of the incubation temperature. Effects of fecal dilution and worm population density on the three developmental directions showed under conditions of good nourishment, many females and a few filariform larvae were formed. Recovery rates of females and filariform larvae were inversely related each other, as with the effect of temperature. Yet, the recovery rate of males was relatively constant under various environment conditions. This report is the first one on *S. fuelleborni* showing that males are predetermined at the egg stage, whereas quantities of females and filariform larvae vary according to environmental conditions.

Key words: *Strongyloides fuelleborni*, development, free-living generations, temperature, nutrient

Introduction

Although *Strongyloides fuelleborni* infects primarily old world primates, there have been several reports of human strongyloidiasis due to this species in Africa and in Papua New Guinea (Wallace *et al.*, 1948; Pampiglion and Ricciardi, 1972; Kelly *et al.*, 1976; Hira and Patel, 1976; Vince *et al.*, 1979; Ashford *et al.*, 1979; Hira and Patel, 1979; Ashford *et al.*, 1992). We have already reported on the factors influencing the developmental direction of *S. stercoralis* first-stage larvae (Shiwaku *et al.*, 1988), which is the most important species of human strongyloidiasis (Beaver *et al.*, 1984). Determination of the factors that affect the developmental direction of *S. fuelleborni* first-stage larvae is important for the control of this disease.

Strongyloides species have a complicated life cycle involving both the parasitic and free-living phases. In the outer environment, eggs deposited from parasitic females in the host intestine develop to filariform larvae (direct development) and eventually to filariform female adults or to males and females (indirect development) at the extra-intestinal environment. It has been reported that parasite genetic factors, the status of host and environmental factors influence either direct or indirect developments of the first-stage larvae (Sandground, 1926; Nishigori, 1928; Kreis, 1932; Faust, 1933; Beach, 1936; Tanabe, 1938a, b; Premvati, 1958; Little, 1962; Abe *et al.*, 1966; Galliard, 1967; Suto *et al.*, 1986a, b; Shiwaku *et al.*, 1988). Concerning *S. planiceps*, *S. ransomi*, *S. papillosus* and *S. stercoralis*, incubation temperature, quantity of nutrient and pH of the culture system have been considered to be important environmental factors (Arizono, 1976a,

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b; Moncol and Triantaphyllou, 1978; Nwaorgu, 1983; Shiwaku *et al.*, 1988). Also the effect of fatty acids on the developmental direction of *S. ratti* first-stage larvae have been reported by Minematsu *et al.* (1989, 1990, 1992). Moreover, the status of host immunity and aging of parasitic females have some effect on the developmental direction of first-stage larvae (Moncol and Triantaphyllou, 1978). On the basis of these reports we examined factors which influence development of *S. fuelleborni* first-stage larvae.

Materials and Methods

S. fuelleborni *infected monkey*

Feces from naturally infected Japanese monkey, *Macaca fuscata* reared at The Primate Institute, Kyoto University in Inuyama, Aichi Prefecture were used as a source of *S. fuelleborni* throughout this experiment. Feces from one uninfected *M. fuscata* of the same institute was used for test tube culture throughout the experiments. Every monkey was reared separately and was fed a commercial pelleted diet 150g per day (Oriental Yeast APF, Oriental Yeast Co. Ltd.) and one sweet potato every other days.

Collection of S. fuelleborni *ova*

Ova were collected from the infected monkey feces by means of sucrose discontinuous density gradient centrifugation technique. Egg containing feces were dissolved in tap water and put onto sucrose density gradient centrifugation tube with specific gravity (s.g.) 1.155 (64 g sucrose in 100ml distilled water) and s.g. 1.078 (32 g sucrose in 100ml distilled water). Centrifugation was carried out at 2,500 rpm for three minutes. The ova concentrated at the s.g. 1.078 sucrose fraction were recovered. This fraction was collected and diluted with tap water, then washed by tap water five times with centrifugation at 2,500 rpm for three minutes. For estimation of the numbers of ova, a droplet of suspension was counted five times by microscope.

Cultivation of ova

The filter paper test tube method modified by Arizono (1976a, b) was carried out for the ova culture. 0.2g feces or fecal dilution with tap water from an uninfected monkey were spread on a filter paper strip (2.0×16.0 cm, No. 514A, Toyo Roshi Co. Ltd., Tokyo). A droplet of 200 ova was placed on the filter paper strip. Incubation temperatures and periods were as follows; 24 hr at 40°C, 24 hr at 35°C, 27 hr at 30°C, 40 hr at 25°C, 88 hr at 20°C and 144 hr at 15°C. Following these periods, males, females and filariform larvae developed in the culture system, but no progeny developed from the free-living individuals. After incubation each test tube was filled to the upper edge of the filter paper strip with warm water at 35°C for 20 min. to recover worms. Recovered worms were fixed with formalin solution. Fixed males, females and filariform larvae were collected by centrifugation and counted.

Experiment A

Effects of incubation temperatures 15, 20, 25, 30, 35 and 40°C on the development after hatching were examined. Each experiment was performed in quadruplicate and then repeated three times.

Experiment B

Effects of various fecal dilutions at 30°C were studied at 1:16 (0.2g uninfected monkey feces in 3.2ml of tap water), 1:8, 1:4, 1:2, 1:1 and 1:0. As the control, incubation of eggs with water but no feces was carried out. Each experiment was performed in quadruplicate and repeated three times.

Experiment C

Effects of various worm population densities at 30°C were studied at numbers of 200, 400, 800, 1,600 and 3,200 ova. Each experiment was performed in quadruplicate and repeated three times.

Statistical analysis

All worm recovery rates from ova seeded were expressed as mean±standard error. The differences were estimated by Student's *t* test or Aspin-Welch *t* test against the rate at 30°C on incubation temperature, against the rate at 1:0 on fecal dilution and against the rate at 200 ova on population density. Values of $P<0.05$ were considered significant and expressed as closed circles on the figures.

Results

The effects of incubation temperature on eggs are shown in Fig. 1. As the total recovery rate at 30°C was relatively high, rates of three developmental types and totals at various temperatures were compared with that at 30°C. In the temperature range 30–35°C, there was a higher total recovery rate, whereas temperatures such as 40, 20 and 15°C revealed low total recovery. Males were at all temperatures constantly produced with the exception of 40°C. Recovery rates of females at range 30–35°C were high, but low at critical temperatures such as 40°C, 20°C and 15°C. On the other hand, recovery rates of filariform larvae were inversely proportional to females. The effects of fecal dilution on the three developmental

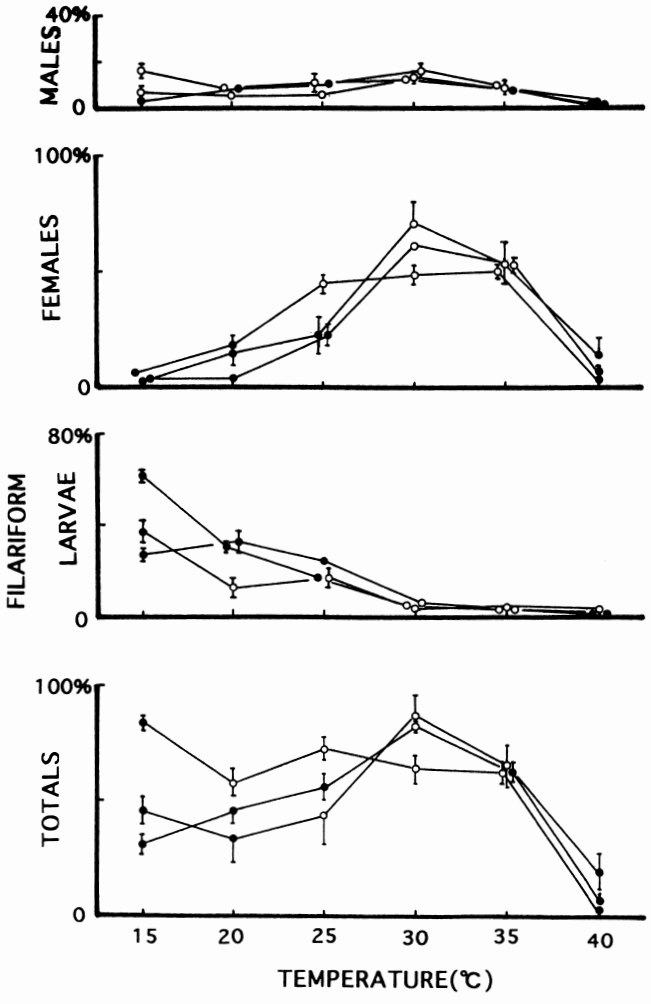

Fig. 1 Effects of temperature on the three developmental types of *Strongyloides fuelleborni* first-stage larvae. Recovery rates were expressed as mean±standard error in each experiment against the number of ova cultured. The differences among recovery rates were expressed as closed circle which was against the rates at 30°C. Feces, 0.2g, of an uninfected monkey were spread on a filter paper strip and 200 ova were seeded.

types are summarized in Fig. 2. Total recovery rates were the highest at dilutions of 1:0 and 1:1. Males were recovered in constant numbers except for a low value at 1:16. Recovery rates of females and filariform larvae were inversely related such that at low fecal dilution many females developed and few filariform larvae were formed, whereas at high fecal dilution many filariform larvae and few females were seen. The effects of population density on the three developmental directions were slight (Fig. 3). Total recovery rates were relatively constant compared with other experiments. Male recovery rates were constant. Recovery rates of females and filariform larvae were inversely related, but the effect was not so pronounced as that seen in experiments A and B.

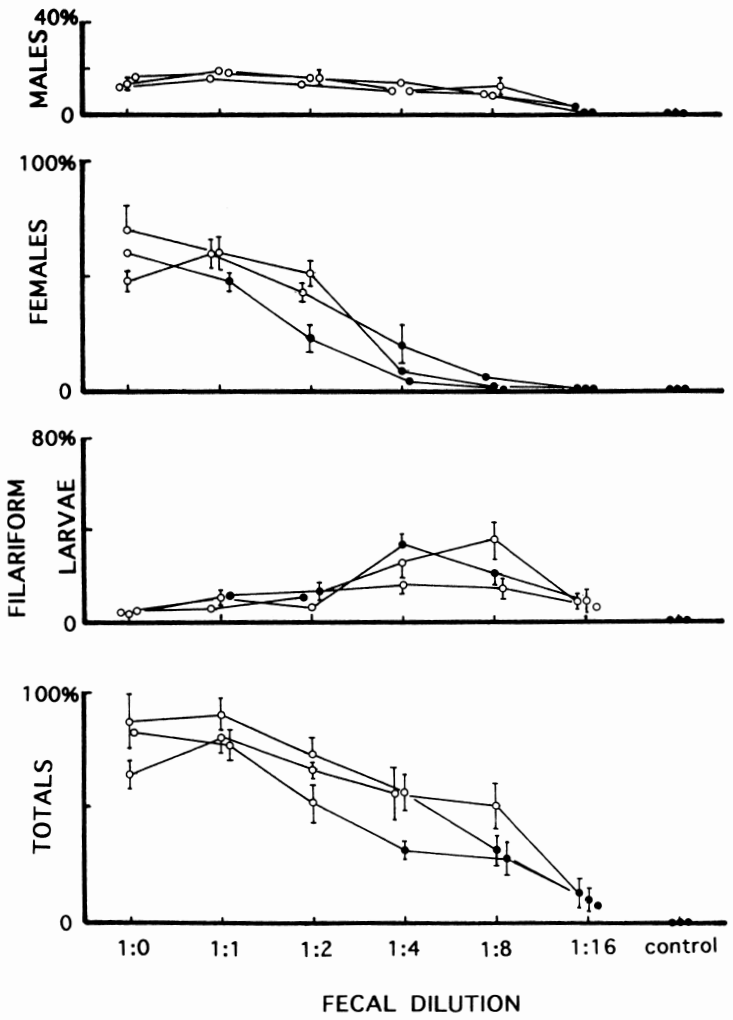

Fig. 2 Effects of fecal dilution on the three developmental types of *Strongyloides fuelleborni* first-stage larvae at 30°C. Recovery rates were expressed as mean±standard error in each experiment against the rate at fecal dilution 1:0. The differences among recovery rates were expressed as closed circle which was against the rates at fecal dilution 1:0. Feces or fecal dilution, 0.2g, of an uninfected monkey were spread on a filter paper strip and 200 ova were seeded. Incubation of ova with water but no feces was as in the control.

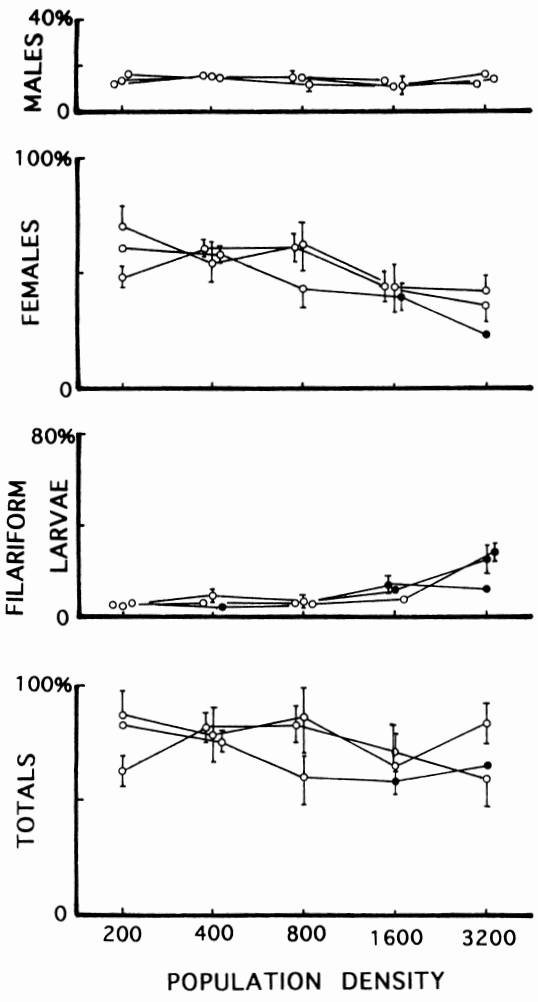

Fig. 3 Effects of population density on the three developmental types of *Strongyloides fuelleborni* first-stage larvae at 30°C. Recovery rates were expressed as mean±standard error in each experiment against the rate at population density 200. The differences among recovery rates were expressed as closed circle which was against the rates at population density 200. Feces, 0.2g, of an uninfected monkey were spread on a filter paper strip and 200, 400, 800, 1,600, 3,200 ova were seeded.

Discussion

Premvati (1958) indicated that temperatures between 20 and 30°C were favorable for *S. fuelleborni* indirect development, *i.e.* many males and females were formed; however, few filariform larvae developed. The present study revealed that the optimal temperature for this species is 30°C, not range 20–25°C. Generally, the recovery rates of males were constant regardless of the incubation temperature. Recovery rates of filariform larvae were inversely related to those of females. This indicates, as other investigators have suggested, that free-living females, parasitic females and filariform larvae originate from the ova or first larval stage with the same genetic character. As Minematsu *et al.* (1990) clearly showed on *S. ratti* that only eggs that would develop into filariform larvae were able to change into females.

The optimum temperature for development was 30°C for *S. fuelleborni*. However, this varied by species. Regarding the effects of temperature, Arizono (1976b), Nwaorgu (1983) and Shiwaku *et al.* (1988) reported quantitative observation in *S. planiceps, S. papillosus* and *S. stercoralis*, respectively. As compared with their results, *S. fuelleborni* was different from the other three species in the effects of incubation temperature. The optimum temperature for development of females was range 28–32°C in *S. planiceps*, 35°C in *S. papillosus*, range 20–30°C in *S. stercoralis* and range 30–35°C in *S. fuelleborni* (present authors), while the optimum temperature for development of filariform larvae was range 12–16°C in *S. planiceps*, 20°C in *S. papillosus*, range 35–40°C in *S. stercoralis* and range 15–20°C in *S. fuelleborni*. The reason for this difference among the four species of *Strongyloides* is not clear. It seems that the temperature 20–30°C, which corresponds to the mean temperature in tropical and subtropical areas (National Astronomical Observatory, 1993), is well suited to produce numerous progeny of *S. stercoralis* by indirect development (Shiwaku *et al.*, 1988).

In cases of autoinfection of *S. stercoralis*, all or some of the rhabditoid larvae in lumen of the intestine molt to the second rhabditoid stage, then metamorphose into filariform larvae *en transit* down the bowel. They may reinfect by invading the mucosa of the lower portion of the ileum and the colon (Beaver *et al.*, 1984). Although these data on *S. planiceps, S. papillosus, S. stercoralis* and *S. fuelleborni* were determined *in vitro*, not *in vivo* of the representative host animal, only *S. stercoralis* has an optimum temperature for development of filariform larvae at

range 35–40°C, similar to the body temperature of mammals. When discussing autoinfection, many factors such as host's immune state and the parasite's reproductive mechanisms (Beaver *et al.*, 1984) must be considered. Therefore, the fact that only *S. stercoralis* has an optimum temperature for development of filariform larvae *in vitro* at range 35–40°C still leaves room for discussion about autoinfection.

Regarding the effects of food availability, *e.g.* fecal dilution and population density on the three developmental directions, our results confirm earlier reports (Premvati, 1958; Arizono, 1976a; Moncol and Triantaphyllou, 1978; Shiwaku *et al.*, 1988) that eggs of the genus *Strongyloides* developed into many females with a few filariform larvae in low population density cultures with a sufficient supply of feces as food; and, in adverse conditions females became few in number whereas, many filariform larvae were formed.

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