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# Life Cycle and Mating Behavior of *Hirschmanniella oryzae* (Nematoda: Pratylenchidae) on Excised *Oryzae sativa* Roots

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**Abstract:** The life cycle and mating behavior of *Hirschmanniella oryzae* were observed in vitro on excised roots of *Oryzae sativa* in gnotobiotic culture. Eggs hatched into larvae whose appearance and structure were similar to those of the adults. Larvae grew in size and each larva stage was terminated by a molt. *H. oryzae* had four larva stages, with the first molt occurred in the egg. After the final molt the larvae differentiated into adult males and females. Mating was required for reproduction. After mating, fertilized females began to lay eggs. The life cycle from second stage larva to second stage larva was completed in 33 days.
**Key Words:** *Hirschmanniella oryzae*, rice root nematode, gnotobiotic culture, life cycle, mating behavior

# Kesilmiş *Oryzae sativa* Köklerinde *Hirschmanniella oryzae* (Nematoda: Pratylenchidae) nın Hayat Devri ve Çiftleşme Davranışı

**Özet:** *Hirschmanniella oryzae*'nın hayat devri ve çiftleşme davranışı gnotobiotik kültürde *Oryzae sativa*'nın kesilmiş köklerinde in vitro olarak gözlendi. Yumurtadan çıkan larvalar görünüş ve yapı olarak erginlere benzerlik göstermiştir. Hacim olarak büyümüş larvanın her bir safhası bir kütikül değişimi ile sonlanmıştır. Dört larva safhasına sahip *H. oryzae*'da birinci kütikül değişimi yumurta içinde meydana gelmiştir. Son kütikül değişiminden sonra larvalar, ergin erkek ve dişilere farklılaşmışlardır. Çiftleşmenin

üreme için gerekli olduğu gözlenmiştir. Çiftleşmeden sonra dişiler döllenmiş yumurtalarını bırakmıştır. Hayat devri ikinci devre larvadan ikinci devre larvaya 33 günde tamamlanmıştır.
**Anahtar Kelimeler:** *Hirschmanniella oryzae*, pirinç kök nematodu, gnotobiotik kültür, hayat devri, çiftleşme davranışı

## 1. Introduction

One of the most damaging plant parasitic nematodes in major rice growing areas of all five continents is the rice root nematode, *Hirschmanniella oryzae* (van Breda de Haan, 1902) Luc and Goodey, 1964. It has a high damage potential at relatively low population densities and parasitizes a wide range of hosts among agricultural crops such as rice, cotton, sugarcane and maize [1], but has not been reported from Turkey. *H. oryzae* is a migratory endoparasite of roots [2]. The larvae and adults always enter roots at some distance from the tip, move freely in the air channels between the radial lamellae of the parenchyma and in older roots, may be found anywhere between base and tip; sometimes several have been found in the coleoptile but none in the lower parts of the leaf sheaths [3]. A few days after entry, the female lays eggs which hatch in 4 to 5 days in roots. Under suitable conditions a life cycle is completed in about 30 days [4]. Adults populations reproduce sexually [1].

In this study, life cycle and mating behavior of *H. oryzae* in gnotobiotic culture were investigated.

## 2. Materials and Methods

*Hirschmanniella oryzae* was obtained from infested rice roots and cultured on rice seedlings in clay loam soil in the greenhouse (28 ± 2 °C and % 87-90 relative humidity). Infested samples provided from NISCOM, New Delhi-India. Then a population of *H. oryzae* was established in petri dishes on excised rice roots (*Oryzae sativa* L. cv. Krasnodarsky-424) supported by Gamborg's B5 medium in 1.5 % agar adjusted to pH 5.8 and maintained in darkness at 28 ± 2 °C [5].

To study development, nematode eggs from the in vitro culture of *H. oryzae* were aseptically transferred onto 1 % water agar plates and incubated at 28 ± 2 °C overnight. Hatched second stage larvae ($L_2$) were inoculated onto 9 day-old rice root cultures. Five excised rice roots were cultured on each of four replicate culture dishes and were inoculated with 100-125 $L_2$ of *H. oryzae*. These plates were incubated under

the above conditions. Nematode development and behavior in all replicates were observed daily with dissecting microscope and TZ 240 model Euromex binocular under cold light. The first occurrence of each molt and development stage among the replicates was the criterion used to determine the time periods of the life cycle which reflects a typical time course based on many repeated observations of each event.

Rice root culture dishes were prepared and divided into three groups with 10 replicates each: (1) one molting female fourth stage larva ($L_4$) in each dishes, (2) one molting female $L_4$ and 10 males in each dish, (3) one molting female $L_4$ and 10 males in each dish with males removed after the first egg appeared in the medium in order to prevent further mating. Molting $L_4$ could be sexually differentiated on the basis of body morphology. After the female $L_4$ finished molting and developed into adult, it was considered virgin females and could only be fertilized by the males added to the same dish. These dishes were incubated as described above and nematode behavior was observed daily for 120 days. Once egg deposition in a dish occurred, the eggs were transferred onto corresponding 1.5 % water agar dishes and observed for hatching. Hatched larvae were removed immediately from this agar dish to avoid being mixed with larvae hatching later. The number of eggs produced by each female was also recorded.

**3. Results and Discussion**

The $L_2$ to $L_2$ life cycle of *H. oryzae* was completed under gnotobiotic conditions at 28 ± 2 °C in 33 days (Table). The $L_2$ moved to the root tips and began feeding within 1.2 hour after inoculation. Feeding lasted for 12 to 24 hours, then the $L_2$ became immobile and remained positioned like a “C” or a closed circle. The second molt ($M_2$) started 3 days after inoculation. The most significant change during molting occurred in the oesophageal region. During the first 12 to 24 hours of molting, the stylet shaft, oesophageal lumen and median bulb became invisible. Only the stylet cone remained discernible. Twelve hours later, the new cuticle became visible inside the old one, followed by the appearance of the new stylet shaft. Then the oesophageal lumen and the median bulb emerged and gradually became more distinctive. The larva body progressively elongated until it was confined by the old cuticle. At this time the new stylet began to probe the old cuticle at the rate of once every 5 to 15 seconds, associated

with contraction of the median bulb once every 4 to 6 probings. The nematode finally broke through the old cuticle and migrated out. This molting period ($M_2$) lasted for 2 days. The third stage larva ($L_3$) began feeding again. At 9 days after inoculation the $L_3$ entered the third molting ($M_3$) period, which lasted for 3 days and resulted in the emergence of the fourth stage larva ($L_4$). The $L_4$ started feeding on the roots again, followed by the fourth molting period. Larvae that developed into males started the fourth molt ($M_4$) 19 days after inoculation. By the end of the 6 days molting period, the male gonad, the spicules and the caudal alae had formed and the male migrated out of the old cuticle 25 days after inoculation. Larvae that developed into females started $M_4$ at 19 days after inoculation, which lasted for 6 days. By the end of the molting period, the female gonads and the vulva had formed. The female migrated out of the old cuticle 25 days after inoculation. Faster development of males than females has been observed with other nematodes, e.g., *Heterodera schachtii* [6] and *Belonolaimus longicaudatus* [7] in gnotobiotic cultures.

Males of sting nematode, *Belonolaimus longicaudatus* Rau. approached females soon after the females finished the last molt. Often two or more males surrounded a female, which caused more competition for mates [7, 8, 9]. These findings were similar with *H. oryzae*. The males seemed to be directly attracted by the females and gathered around them quickly. The males moved around the female and began to intensely rub the side of the female body with the lateral side of their lip region. The rubbing movement of the male head was perpendicular to the axis of the female body. In the meantime one of the males moved toward the female head so that its bursa finally touched the female body. This male would move farther ahead, continuously rubbing the female body until its bursa reached the vulva region of the female. The male then moved back and forth and the female also twisted its body until finally the spicules penetrated through the vulva with the bursa covering the area around the vulva. Then, the body movement of both nematodes slowed down. Mating in this manner lasted for 6 to 10 minutes, during which fertilization presumably took place. The male then withdrew its spicules and both nematodes moved away. This mating behavior was observed at least 6 times and each time the mating occurred on the surface of the culturing medium. It was not determined whether a female mated more than once during its life.

After mating, both females and males fed on the surface of the culturing medium. Meanwhile, eggs began forming within female uteri and were clearly visible. Before females began to lay eggs, they stopped feeding and moved slowly within or on the surface of the medium. The eggs in the uterus were pushed toward the vagina. The eggshell was very flexible and was squeezed to pass the shallow lumen of the vagina and delivered through the vulva. Egg deposition was completed in approximately 3 minutes, during which the female did not move. The egg resumed its shape outside the female body. The first eggs were laid 27 days after inoculation. All larva stages as well as the adult stages of both genders fed on the host.

Elmiligy and Norton [10] found no evidence that females of rice root nematode could reproduce in the absence of males. The present study allowed examination of this hypothesis in more detail and confirmed that sexual reproduction was obligatory.

In vitro culture of plant nematodes allows continuous observation of the nematodes and has been utilized in nematological studies in the 1950s [11]. It has proven to be helpful in studying nematode life cycle and host-parasite relationships. By means of this technique, the life cycle of *Heterodera glycines*, *H. zeae* and *Helicotylenchus multicinctus* have been described in detail [12,13,14]. However, only a few strictly ectoparasitic phytonematodes such as *Criconemella xenoplax* [15] and *H. multicinctus* [14] have been successfully cultured on excised roots. This technique not only provided sterile nematode inocula for well controlled host-nematode relationship studies but also allowed direct observation of the nematode behavior without the interference of soil flora and fauna [16]. Results must be interpreted with caution however, since the metabolic response of the host might be quite different from that of an intact host. However, no obvious changes were observed during the course of this study in terms of behavior and parasitism.

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**Table. Life cycle period of *Hirschmanniella oryzae***

| LIFE STAGES (Female) | LIFE PERIOD (Day/Days) | L[a] (mm) | LIFE STAGES (Male) | LIFE PERIOD (Day/Days) | L (mm) |
|---|---|---|---|---|---|
| First stage larva ($L_1$) | 1 | 0.38 | First stage larva ($L_1$) | 1 | 0.30 |
| First molt ($M_1$) | 1 | | First molt ($M_1$) | 1 | |
| Second stage larva ($L_2$) | 3 | 0.57 | Second stage larva ($L_2$) | 3 | 0.50 |
| Second molt ($M_2$) | 2 | | Second molt ($M_2$) | 2 | |
| Third stage larva ($L_3$) | 4 | 0.71 | Third stage larva ($L_3$) | 4 | 0.62 |
| Third molt ($M_3$) | 3 | | Third molt ($M_3$) | 3 | |
| Forth stage larva ($L_4$) | 7 | 1.06 | Fourth stage larva ($L_4$) | 7 | 0.92 |
| Fourth molt ($M_4$) | 6 | | Fourth molt ($M_4$) | 6 | |
| Mating<br>Egg-laying }<br>Feeding | 3 | 1.39[b] | Mating<br>}<br>Feeding | 6 | 1.12[c] |
| Embryo | 3 | | | | |

**a:** Total Body Length (Data are means of five replicates for each stage) **b:**Adult Female **c:**Adult Male