

## Life cycle of the oriental compost worm *Perionyx excavatus* (Oligochaeta)

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In order to exploit the concept of using vermiculture as biotechnology for waste control and protein production, the life cycle of the vermicomposting species, *Perionyx excavatus*, was studied. The development, growth and reproduction of *P. excavatus* were investigated. Urine free cattle manure with a moisture content of 76–83% and a temperature of 25°C was used as substrate. Data were gathered over a period of 300 days. It was found that mating is not a prerequisite for cocoon production, which starts at the mean age of 24 days. Maturation was attained at the age of approximately 21 days. Cocoons were produced at a mean rate of 1,1 cocoons per worm per day. The mean incubation period of cocoons produced by batches of worms was 18,7 days with a mean hatching success of 63,4%. The mean incubation period of cocoons produced by single worms was 20,4 days with a mean hatching success of 40,4%. As a rule only one worm hatched per cocoon. The life cycle of this species is presented diagrammatically.

Ten einde die konsep van vermikultuur as biotegnologie in afvalbeheer en proteïenproduksie te ondersoek, is die lewensiklus van die vermikomposterende erdwurmspesie *Perionyx excavatus* bestudeer. Die ontwikkeling, groei en voortplanting van *P. excavatus* is ondersoek. Urienvrye beesmis met 'n voginhoud van 76–83% teen 'n temperatuur van 25°C is as substraat gebruik. Paring was nie 'n voorvereiste vir kokonproduksie nie. Kokonproduksie het op die ouderdom van 24 dae begin nadat die wurms op 'n ouderdom van gemiddeld 21 dae volwassenheid bereik het. Kokonne is teen 'n gemiddelde koers van 1,1 kokonne per wurm per dag geproduseer. Die gemiddelde inkubasieperiode vir kokonne geproduseer deur wurms wat in groepe aangehou is, was 18,7 dae met 'n gemiddelde uitbroeisukses van 63,4%. Kokonne wat deur enkelwurms geproduseer is, het 'n gemiddelde inkubasieperiode van 20,4 dae en 'n gemiddelde uitbroeisukses van 40,4% gehad. In die reël het slegs een wurm per kokon uitgebroei. Die lewensloop van die spesie word diagrammadies aangebied.

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Very little is known about the general biology of *Perionyx excavatus* (Perrier, 1872) according to Reinecke & Hallatt (1989). Scanty information on the life cycle of this species is contained in various papers, notably Kale, Bano & Krishnamoorthy (1982) and Knieriem-en (1984). This species was described more extensively by Beddard (1886). It was originally described by Perrier (1872). Hanumante (1975) also contributed to our knowledge of this worm species, describing the reproductive system of *P. excavatus*.

This species is considered as a potential waste decomposer by Loehr, Martin, Neuhauser & Malecki (1984), and Reinecke & Hallatt (1989) studied its growth and cocoon production. In order to exploit the concept of using vermiculture as biotechnology for waste control and protein production a fundamental knowledge of the life cycle of all possible vermicomposting species is required. Venter & Reinecke (1988) rendered an extensive description of the life cycle of *Eisenia fetida* under controlled conditions and Viljoen & Reinecke (1989) did the same for *Eudrilus eugeniae*, the African nightcrawler.

No long-term observations on aspects such as survival, growth rate and duration of cocoon production have been made for *P. excavatus* apart from the study by Reinecke & Hallatt (1989). The results published by Kale *et al.* (1982) were not obtained under strictly controlled conditions and although these results provide useful information on various aspects of the growth and fecundity, a complete description of the maturation rate and life cycle in general is still lacking.

The purpose of this study was to determine the life cycle of the oriental compost worm on a favourable organic nutritive source under controlled conditions with regard to moisture and temperature. The growth rate, rate of maturation, cocoon production, the hatching success of cocoons, the incubation period and the number of offspring per cocoon were studied over a period of more than 300 days. Information on the relation between cocoon mass and hatchling size as well as cocoon mass and worm biomass was also obtained. Sufficient data were obtained to make statistically reliable conclusions. Once the basic life cycle under controlled conditions is known, this information can serve as baseline data for experiments to determine the influence of varying biotic and abiotic factors on the growth and reproduction potential of this species.

### Material and Methods

*Perionyx excavatus* was cultured in an environmental control chamber at a temperature of 25°C. The breeding stock originated from cocoons received from Prof. O. Graff of Braunschweig, West Germany, from worms originally obtained from Dr. Kale of Bangalore, India.

Urine free cattle droppings were used as substrate to maintain hatchlings and growing worms. The substrate was prepared by adding distilled water to sun-dried, sieved manure until a moisture content of 76–83% was reached. The manure was ground in a liquidizer and sieved as described by Viljoen & Reinecke (1989). This particle size (500 µm) was found to be favourable for the

raising of young worms of the species *Eisenia fetida* (Venter & Reinecke 1988) and *Eudrilus eugeniae* (Viljoen & Reinecke 1989). The moisture level of the substrate was easily maintained in the environmental chamber where a relative humidity of 80% prevailed. The dampened substrate was left for at least 48 h to stabilize before it was used in experiments.

For the growth study newly hatched specimens were gathered from repli dishes containing the distilled water in which cocoons had been placed to hatch. The biomass of each worm was determined before it was placed into the substrate. Worms were raised singly and in batches of five. Single worms were placed in flasks containing 10 g of the moistened substrate while the batches of five worms each were placed in larger flasks containing 50 g of the moistened substrate. Every five days the biomass of all the worms was determined until they were 50 days old, after which the biomass of the worms and the moisture content of the substrate in each of the flasks were determined every 10 days. The worms were fed by adding fresh cattle manure to every container on a regular basis in quantities related to the number of worms in the container. This was repeated approximately every 10 days over a period of 250 days.

All worms were examined individually on a daily basis from day 14 onwards in order to determine the onset of clitellum development. In order to determine the onset of cocoon production the substrate was examined under a magnifying glass. This was done every second day as soon as clitellate worms were observed in the substrate of a particular flask. This continued until day 80 after which the medium was searched for cocoons every ten days over a further period of 120 days.

The cocoons were counted and weighed, following which they were placed into repli dishes in distilled water and incubated at 25°C in an environmental chamber. The viability of the cocoons as well as the number of offspring were determined.

The number of worms used and the number of replications are referred to in the accompanying tables and figures. The experiments started out with many replications (see tables) but the worm numbers were reduced later on when it became clear that statistically reliable results could be obtained by using fewer worms.

## Results and Discussion

### Growth rate and maturation

The growth rate of *P. excavatus* on cattle manure is presented by Reinecke & Hallatt (1989). Their study showed, as was confirmed by this study, that *P. excavatus* grows well on this organic substrate with a growth rate of 3,5 mg per worm per day over the first 30 days. The worms reached a mean biomass of approximately 600 mg after 250 days. As shown by Reinecke & Hallatt (1989) this species has a slower growth rate than other vermicomposting species such as *Eudrilus eugeniae* (Viljoen & Reinecke 1989) and *Eisenia fetida* (Venter & Reinecke 1988).

The first indication of clitellum development appeared between the 14th and 21st day after hatching with more

than 90% of the worms being clitellate by the 28th day (Table 1). Neuhauser, Kaplan & Hartenstein (1979) pointed out that food availability and population density determined sexual maturation in earthworms. In this

**Table 1** Maturation (clitellum development) of *Perionyx excavatus* kept in batches of five worms each at 25°C in cattle manure

Age of worms (days)	No. of clitellate worms	% Clitellate worms
17	9	19,1
20	26	55,3
21	28	59,6
22	33	70,2
24	34	72,3
25	36	76,6
26	41	87,2
28	43	91,5
30	44	93,6
35	45	95,7
40	46	97,9
45	47	100,0
$\bar{x}$ 27,8		

**Table 2** Cocoon production of *Perionyx excavatus* kept in batches of five worms each at 25°C in cattle manure

Age of worms (days)	Mean no. of cocoons	Cumulative mean	Mean no. of cocoons/worm/day
19	0,3	0,3	0,02
20	0,5	0,8	0,04
22	1,2	2,0	0,09
25	2,1	4,1	0,16
27	2,3	6,4	0,24
28	2,5	8,8	0,32
30	4,9	13,8	0,46
35	6,2	20,0	0,57
40	6,0	26,0	0,65
45	8,4	34,4	0,76
50	11,2	45,6	0,91
60	27,7	73,3	1,22
70	24,0	97,3	1,39
80	25,1	122,4	1,53
90	15,6	138,0	1,53
100	15,5	153,6	1,54
110	16,2	169,8	1,54
120	21,7	191,5	1,59
130	21,5	212,9	1,64
140	17,8	230,7	1,65
150	16,8	247,5	1,65
160	15,2	262,6	1,64
170	14,0	276,6	1,63
180	12,6	289,2	1,61
190	10,3	299,5	1,58
200	14,7	314,2	1,57
			$\bar{x}$ : 1,1 ± 0,12

study the worms in batches grew at basically the same rate as the single worms. Food availability was never a limiting factor.

### Cocoon production

The cocoon production of *P. excavatus* is presented in Tables 2 and 3. Mating is not a prerequisite for cocoon production (Reinecke & Hallatt 1989) as seems to be the case for *Eisenia fetida* (Venter & Reinecke 1988). *P. excavatus* started producing cocoons at the mean age of 24 days. This is quite early when compared with other vermicomposting species. The rate of cocoon production was 1,6 cocoons per worm per day after 80 days. The mean number of cocoons per worm per day over the whole observation period of 200 days was 1,1. This production rate is much higher than that of *E. fetida* of 0,5 cocoons per worm per day under similar conditions (Venter & Reinecke 1988). It is very similar to that of *Eudrilus eugeniae* (Viljoen & Reinecke 1989).

### Incubation and hatching success

Three hundred randomly selected, newly produced cocoons produced by worms that were kept together,

**Table 3** Cocoon production of *Perionyx excavatus* kept singly at 25°C in cattle manure

Age of worms (days)	Mean no. of cocoons	Cumulative mean	Mean no. of cocoons/worm/day
29	0,6	0,6	0,02
35	4,4	5,0	0,14
44	9,2	14,2	0,32
50	6,6	20,8	0,42
60	17,9	38,7	0,64
70	12,6	50,2	0,72
80	15,2	60,9	0,76
90	12,9	73,7	0,82
100	12,9	86,5	0,87
110	16,7	103,2	0,94
			$\bar{x}: 0,57 \pm 0,10$

**Table 4** Incubation period of cocoons of *Perionyx excavatus* at 25°C

Ages of worms (days)		% Cocoons hatched		Incubation period (days) $\pm$ SE	
Batches	Singles	Batches	Singles	Batches	Singles
25	35	43,2	44,5	19,0	21,5
26	36	59,3	19,0	19,6	20,0
28	44	60,0	51,3	17,2	17,7
30	60	55,6	72,3	18,0	16,2
32	70	57,1	27,3	18,9	21,0
129	108	76,0	28,2	19,1	25,6
211	-	80,0	-	18,5	-
300	-	76,0	-	18,8	-
$\bar{x}$		63,4	40,4	18,7 $\pm$ 0,26	20,4 $\pm$ 1,34

were used to determine the incubation period and hatching success. The mean incubation period was 18,7  $\pm$  0,26 days (see Table 4). From Table 6 it can also be seen that the hatching success of cocoons varied with the age of the parental worms. Cocoons produced by worms between the ages of 25 and 32 days had a mean hatching success of 55% while cocoons produced by worms between the ages of 129 and 300 days had a mean hatching success of 77%. The incubation periods of these two groups of cocoons did not differ. The mean hatching success of all the cocoons was 63,4%.

The mean incubation period of cocoons produced by single worms that were not allowed to mate, was 20,4  $\pm$  1,31 days (Table 4). The mean hatching success of these cocoons was 40,4% and no difference was observed between cocoons produced by younger and older parental worms. The low hatching success of the cocoons produced by single worms indicates that this alternative mode of reproduction could be less efficient than the sexual mode. If the mode of reproduction of single worms is in fact parthenogenesis or self-fertilization it provides for an alternate life history strategy in *Perionyx excavatus*. It should, however, be borne in mind that the worms in batches could also have produced some cocoons by this alternative mode, which could also explain the relatively low hatching rate of 63,4% when compared with other sexual reproducers, such as *Eisenia fetida* (Venter & Reinecke 1988).

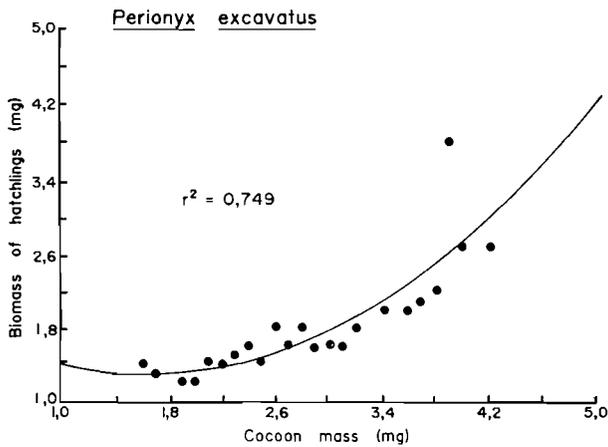
### Fecundity

The inherent ability of a population to increase (natality) can be measured in terms of the new individuals that hatch. In the present study the conditions were probably close to ideal so that a theoretical maximum production of new individuals could be expected. Earthworm fecundity is often expressed in various ways. The rate of cocoon production, the hatching success of these cocoons, the duration of incubation and the number of offspring emerging from each cocoon are all factors determining the number of new individuals produced per unit of time for a specific population of reproductive individuals. The reproductive rate is naturally also influenced by factors such as age and population density when physical factors are not limiting. This was shown in the present study where the hatching success of the cocoons of older worms was better than that of younger worms, as indicated above. This will in turn affect the specific growth rate of a population.

Of the 300 cocoons that were incubated four produced two hatchlings and one produced three hatchlings each. All the other cocoons that hatched successfully (63%) produced only one hatchling each.

### Relation between biomass of cocoons and hatchlings

In Figure 1 the relation between the cocoon mass and biomass of hatchlings emerging from these cocoons is illustrated. The certainty that a relation of this kind did exist, is indicated by the  $r^2$  value of 74,9%.



**Figure 1** The relation between the biomass of hatchlings of *Perionyx excavatus* and the mass of the cocoons from which they emerged after incubation at 25°C.

It can therefore be deduced that bigger worms developed from the heavier cocoons in most ( $\pm 75\%$ ) cases and that smaller hatchlings emerged from the smaller cocoons.

#### Relation between worm biomass and cocoon mass

Specimens of *P. excavatus* kept singly or in small batches of worms, have been shown to have a different growth rate from the age of about 170 days (Reinecke & Hallatt 1989). The worms kept singly attained a higher biomass during an earlier stage of development. From the above-mentioned study it was also apparent that sexual maturity was attained earlier by worms kept in batches. They started producing cocoons at an earlier age than the single worms. This is also evident from the present study shown in Tables 5 and 6. From the results it can also be seen that heavier cocoons were produced by larger, heavier worms and in the group of singly kept worms, where the highest biomass was attained.

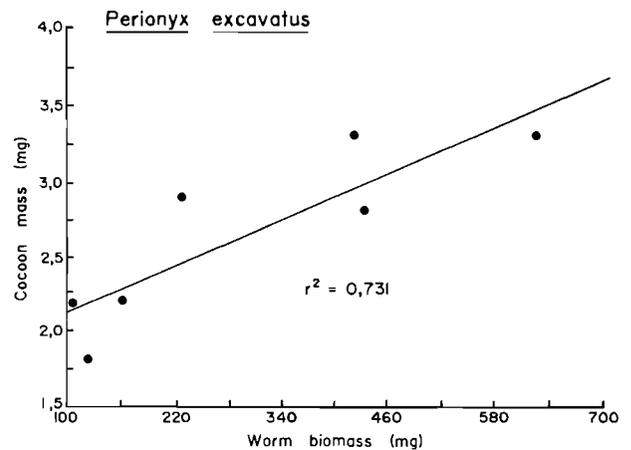
A relation does seem to exist between worm mass and mass of cocoons for *P. excavatus* as illustrated in Figure 2 (single worms) and Figure 3 (batches of worms). In Figure 2 the regression line presents the relation between the biomass of worms kept singly and the mass of their cocoons. The  $r^2$  value in this case was 73%, indicating that the trend did emerge. If, however,

**Table 5** Relation between worm biomass and the mass of cocoons produced for *Perionyx excavatus* kept in batches at 25°C

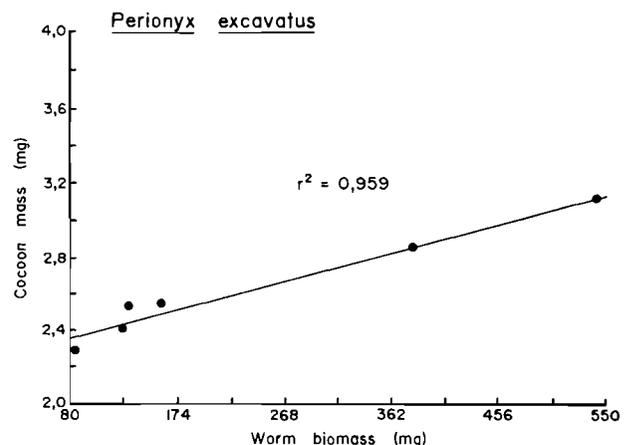
Age of worms (days)	No. of worms	Worm biomass (mg) $\pm$ SE	No. of cocoons	Mass of cocoons (mg) $\pm$ SE
20	70	84,4 $\pm$ 3,72	4	2,3 $\pm$ 0,85
25	30	125,7 $\pm$ 6,19	36	2,4 $\pm$ 0,70
30	72	130,4 $\pm$ 4,09	39	2,5 $\pm$ 0,68
35	48	157,4 $\pm$ 4,34	16	2,6 $\pm$ 0,05
130	30	381,7 $\pm$ 15,7	134	2,9 $\pm$ 0,03
210	28	539,8 $\pm$ 27,0	58	3,1 $\pm$ 0,07

**Table 6** The relation between worm biomass and the mass of cocoons produced for *Perionyx excavatus* kept singly at 25°C

Age (days)	No. of worms	Biomass of worms (mg)	No. of cocoons	Mass of cocoons (mg) $\pm$ SE
29	15	104,6	7	2,2 $\pm$ 0,07
35	11	124,8	9	1,8 $\pm$ 0,16
44	11	160,4	31	2,2 $\pm$ 0,06
60	10	225,8	16	2,9 $\pm$ 0,06
140	6	431,6	47	2,8 $\pm$ 0,06
150	10	417,3	22	3,3 $\pm$ 0,06
250	9	622,2	39	3,3 $\pm$ 0,07

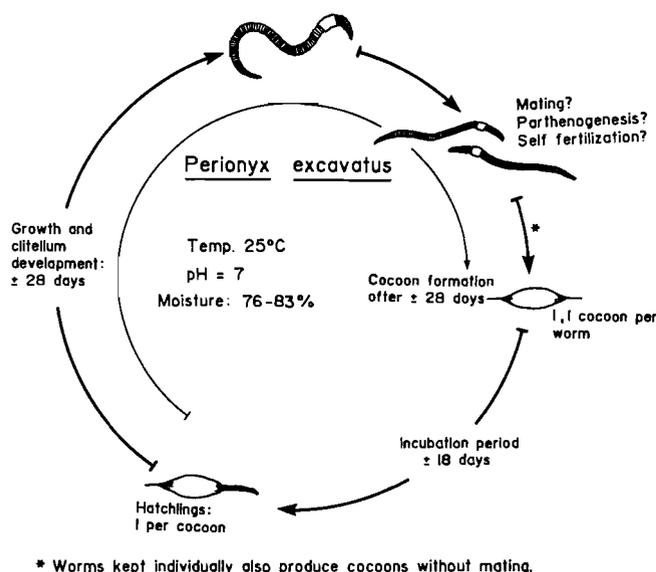


**Figure 2** The relation between worm biomass and cocoon mass for *Perionyx excavatus* kept singly at 25°C.



**Figure 3** The relation between worm biomass and cocoon mass for *Perionyx excavatus* kept in batches at 25°C.

compared with the regression line for results obtained with worms kept in batches (Figure 3), with a  $r^2$  value of 96% it seems that the biomass of the worms which were allowed to copulate and cross fertilize related stronger with cocoon mass, the larger worms forming heavier cocoons and those weighing less producing the lighter ones.



**Figure 4** Diagram of the life cycle of *Perionyx excavatus* at 25°C in cattle manure with a moisture content of 73–86%.

### Conclusion

The life cycle of *Perionyx excavatus* is now confirmed by experimental work on all the various stages. The life cycle is rendered diagrammatically in Figure 4. Cocoon formation starts at the mean age of 28 days irrespective of whether the worms have mated or not. Cocoon production can be sustained for an extended period of time. In the present study observations were terminated after the worms reached the age of 250 days. Cocoons are produced at a mean rate of 1,1 cocoon per worm per day. The mean incubation period of cocoons at 25°C is 18 days and usually only one hatchling emerges from each cocoon. Under ostensibly favourable conditions such as prevailed during the present study the offspring will attain sexual maturity within 28 days after hatching.

Should the life cycle of *Perionyx excavatus* be compared with that of other compost-dwelling earthworms such as *Eudrilus eugeniae* and *Eisenia fetida*, it has a very fast maturation rate (Reinecke & Hallatt 1989) and starts producing cocoons at a much earlier stage. *P. excavatus* is also capable of producing cocoons without copulation being a prerequisite.

The potential utilization of *P. excavatus* as waste decomposer or as a source of protein is, amongst others, dependant on its growth rate as well as its reproductive rate. This species has a lower mean growth rate than *E. eugeniae* (Viljoen & Reinecke 1989) and *E. fetida* (Ven-

ter & Reinecke 1988). It also produces fewer cocoons and hatchlings per cocoon. It does, however, have a much shorter life cycle than these species. Furthermore the ability to reproduce without copulation could be a great advantage under certain circumstances.

Being a tropical species, its ability to survive and reproduce under varying environmental conditions needs further investigation before the vermicomposting potential of this species can be compared to that of *Eisenia fetida* which seems to cope well under widely differing environmental conditions.

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