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REGENERATION POTENTIALITY OF *PERIONYX SANCIBARICUS* IN CONTEXT OF ORIENTATION

Tanuja Kumari *et al.*

Perionyx sansibaricus

Orientation

Regeneration

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TANUJA KUMARI, KAMLESH PANDEYA, AJAY CHOUDHARY[1] ND M. P. SINHA*
*Department of Zoology, Ranchi University,
Ranchi - 834 008, Jharkhand, INDIA
[1]Department of Zoology, Ranchi College, Ranchi.
E mail: m_psinha@yahoo.com

ABSTRACT

The annelids are the excellent group to investigate the evolution of regeneration abilities. They exhibit qualitative and quantitative variation in regeneration ability, among closely related species. In order to gain insight into the regeneration potentiality with respect to orientation of the earthworm, *Perionyx sansibaricus*, were transected eartworms in different orientation like 25, 50 and 75% anterior portion regenerating posteriorly and similarly 25, 50 and 75% posterior portions regenerating anteriorly and inoculated it into plastic pots containing natural or artificial soil. Measurements were carried after making earthworms straight on ice at an interval of four days for the length regenerated. The ANOVA showed that the regeneration abilities were significantly different between anterior and posterior segments ($p < 0.01$). The ability of anterior segments to regenerate in posterior direction is 36.86 and 75.28% more than posterior segments regenerating anteriorly in natural and artificial soil respectively. Segments in artificial soil showed the same trend showing no effect of habitat change on regeneration. The anterior segments regenerated the tail but posterior segments failed to regenerate the head. The different aspects of regeneration with respect to orientation have been discussed.

*Corresponding author

INTRODUCTION

Animals vary dramatically in their ability to replace lost body parts through regeneration (Goss, 1969; Brusca and Brusca, 2003). Regeneration has remained a central question in biology (Morgan, 1901; Goss, 1969; Elder, 1979; Reichman, 1984). The phylogenetic distribution of regeneration ability across animals implies that this capability has been gained and / or lost many times during evolution. Despite the recent surge in interest in regeneration biology and the clear evidence for the evolutionary liability of regeneration, comparative studies of regeneration are exceedingly rare. To date, regeneration studies have been focused almost exclusively on a few, very distantly related species. Annelids have been studied from different angles of regeneration (Bely, 2006).

In earlier studies on annelids the variation in regeneration has been reported to be of many forms (Hyman, 1940; Berrill, 1952; Herlant - Meewis, 1964). Species may be capable or incapable of regenerating anterior segments and/or terminal asegmental structures. Bely (2006) reported difference in the maximum number of segments that will regenerate (especially anteriorly), in the axial position from which regeneration can take place, and in the overall extent of tissue removal that can be tolerated.

In annelids and particularly earthworms the bodies are composed of repeated segments which largely possess the same structures (segmental nerve ganglia and fibers, musculature, gut, blood vessels, chaetal bundles, nephridia, etc), cuts made at different axial positions along the body result primarily in the removal of different amounts of a given organ system, rather than the removal of different organ systems or unique structures, facilitating comparisons among annelid species.

Despite the promise of annelids for revealing mechanisms underlying the evolution of regeneration, variation in segment regeneration ability in annelids has not been summarized in over half a century. The classical work on annelid regeneration (Hyman, 1940; Berrill, 1952; Herlant - Meewis, 1964) are some important comparative information which however, are silent over presence and absence of segment regeneration ability.

The present paper deals with regeneration potentiality in *Perionyx sansibaricus* in context of orientation under laboratory condition.

MATERIALS AND METHODS

Earthworms for experiment were collected from garbage in the university campus. The worms having been hand sorted from the garbage, were kept in glass jars for a day such that 3/4th part was immersed in water and 1/4th part was exposed, until the gut was cleared. Individuals with any indication of damage by collecting and handling were rigorously rejected and only those which were clitellate were used. The worms were kept in moist soil, taken from the habitat of the worm kept in a plastic pot, for one week. Transections were made under a dissecting binocular microscope using sharp rajor blade exactly across the worm on an intersegmental furrow beginning at 28/29 in one series, at 56/57 in second series and at 84/85 in third series in different orientations. Anterior parts of three series were called as anterior 25%, anterior 50%, anterior 75% and posterior parts as posterior 75%, posterior 50%, posterior 25%. Prior to measurment worms were placed on ice block for thirty seconds to make them straight. Length was taken using divider and scale. After cutting all fragments from the same level were kept together in one pot. Natural soil from habitat and artificial soil were used for experiment (composition of artificial soil was soil: cowdung: rotten saw dust=1: 1: 1). For proper availability of oxygen sufficient holes were made at the lid of the pot. Reading were taken at an interval of four days. During these days proper moisture were maintained in the soil and worms were kept throughout at room temperature (28 ± 2°C).

RESULTS AND DISCUSSION

In natural soil maximum and minimum percentage change in length among anterior segments regenerating

Table 1: Percentage change in length of earthworm during regeneration of anterior 25% segment in natural soil

aS.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	20.8	0.0	11.1	11.1	10.75
2	8th	33.3	30.3	50.0	33.3	36.65
3	12th	41.7	45.0	55.5	44.4	46.65
4	16th	54.2	50.0	61.1	55.6	55.23
5	20th	62.5	55.0	77.8	77.8	68.28

Table 2: Percentage change in length of earthworm during regeneration of posterior 75% segment in natural soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	4.9	4.0	3.7	3.6	4.05
2	8th	2.0	12.5	2.7	2.7	12.75
3	12th	6.1	20.0	8.1	2.7	15.78
4	16th	10.2	25.0	10.8	11.1	17.25
5	20th	10.2	25.0	10.8	11.1	17.25

Table 3: Percentage change in length of earthworm during regeneration of anterior 75% segment in natural soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	7.0	10.0	6.3	9.3	8.15
2	8th	10.5	16.0	10.6	13.9	12.75
3	12th	14.0	18.0	14.9	16.2	15.78
4	16th	15.8	20.0	17.0	16.2	17.25
5	20th	15.8	20.0	17.0	16.2	17.25

Table 4: Percentage change in length of earthworm during regeneration of posterior 25% segment in natural soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	0.0	4.5	0.0	0.0	4.5
2	8th	4.5	4.5	4.3	0.0	3.33
3	12th	4.5	4.5	4.3	0.0	3.33
4	16th	4.5	4.5	4.3	0.0	3.33
5	20th	4.5	4.5	4.3	0.0	3.33

Table 5: Percentage change in length of earthworm during regeneration of anterior 50% segment in natural soil

S.N.	Days	Regenerating sets I	II	Mean III	IV	
1	4th	2.00	37.70	8.00	2.30	12.00
2	8th	2.00	53.60	16.00	4.50	19.03
3	12th	2.00	64.20	16.00	6.80	22.23
4	16th	2.00	75.00	16.00	6.80	24.95
5	20th	2.00	75.00	16.00	6.80	24.95

Table 6: Percentage change in length of earthworm during regeneration of posterior 50% segment in natural soil

S. N.	Days	I	II	III	IV	Mean
1	4th	4.20	10.70	80.00	9.50	8.10
2	8th	6.30	32.10	16.00	14.30	17.18
3	12th	8.30	39.30	16.00	19.00	20.65
4	16th	12.50	50.00	16.00	19.00	24.38
5	20th	12.50	53.60	16.00	23.80	26.48

Table 7: Percentage change in length of earthworm during regeneration of anterior 25% segment in artificial soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	17.60	4.30	22.20	13.30	14.35
2	8th	47.10	26.00	44.40	33.30	37.70
3	12th	76.50	47.80	66.60	50.00	60.22
4	16th	105.90	65.20	94.40	63.30	82.20
5	20th	220.00	82.60	122.00	76.60	125.30

Table 8: Percentage change in length of earthworm during regeneration of posterior 75% segment in artificial soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	11.40	6.40	10.80	6.60	8.80
2	8th	20.00	14.90	18.90	16.70	17.62
3	12th	34.30	21.30	32.40	25.00	28.25
4	16th	48.60	31.90	45.90	33.30	39.925
5	20th	62.80	42.60	51.40	43.30	50.02

Table 9: Percentage change in length of earthworm during regeneration of anterior 75% segment in artificial soil

S.N.	Days	I	II	III	IV	Mean
1	4th	33.30	22.50	20.00	9.70	25.27
2	8th	66.70	62.50	62.50	17.10	63.90
3	12th	80.90	70.00	70.00	29.30	73.60
4	16th	95.20	87.50	75.00	29.30	85.90
5	20th	116.00	87.50	75.00	51.20	67.80

Table 10: Percentage change in length of earthworm during regeneration of posterior 25% segment in artificial soil

S.N.	Days	I	II	III	IV	Mean
1	4th	52.4	19.2	30.0	5.8	4.5
2	8th	54.8	23.0	35.0	8.8	3.33
3	12th	54.8	26.9	40.0	8.8	3.33
4	16th	57.0	30.8	40.0	8.8	3.33
5	20th	5.7.0	34.6	40.0	11.8	3.33

Table 11: Percentage change in length of earthworm during regeneration of anterior 50% segment in artificial soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	2.50	3.30	3.60	3.90	3.13
2	8th	5.00	6.00	7.10	4.20	6.03
3	12th	5.00	10.00	10.70	4.30	4.56
4	16th	10.00	13.30	10.70	4.30	11.30
5	20th	17.50	13.30	14.20	4.40	15.00

Table 12: Percentage change in length of earthworm during regeneration of posterior 50% segment in artificial soil

S.N.	Days	Regenerating sets I	II	III	IV	Mean
1	4th	5.00	6.70	3.60	4.30	5.10
2	8th	7.50	6.70	3.60	8.70	5.90
3	12th	10.00	16.70	7.10	8.70	11.27
4	16th	15.00	33.30	7.10	13.00	18.46
5	20th	15.00	33.30	7.10	13.00	18.47

Table 13: Two way ANOVA showing impact of duration and orientation on anterior segments in natural soil

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	2.17705	5	0.43541	23.42593	3.19E-05	3.325835
Columns	24.88413	2	12.44207	669.4082	2.24E-11	4.102821
Error	0.185867	10	0.018587			
Total	27.24705	17				

Table 14: Two way ANOVA showing impact of duration and orientation on posterior segments in natural soil

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.499161	5	0.099832	5.386954	0.01164	3.325835
Columns	13.07948	2	6.539739	352.8848	5.32E-10	4.102821
Error	0.185322	10	0.018532			
Total	13.76396	17				

Table 15: Two way ANOVA showing impact of duration and orientation on anterior segments in artificial soil

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	9.762111	5	1.952422	7.19675	0.004229	3.325835
Columns	28.30301	2	14.15151	52.16333	5.12E-06	4.102821
Error	2.712922	10	0.271292			
Total	40.77804	17				

Table 16: Two way ANOVA showing impact of duration and orientation on posterior segments in artificial soil

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3.911561	5	0.782312	7.803959	0.003135	3.325835
Columns	13.05448	2	6.527239	65.1125	1.84E-06	4.102821
Error	1.002456	10	0.100246			
Total	17.96849	17				

posteriorly are shown by anterior 25% and anterior 75% on 20th day. The significant values are 68.28 and 17.25 % growth with respect to the original length. The two way ANOVA analysis showed that the result is significant [F=23.42, df 5, 2, p<0.01; F=669.41, df 5, 2, p<0.01] (Table 1, 3 and 13). Posterior 50 % and posterior 25% have shown maximum and minimum percentage change in length towards anterior direction. The corresponding values were 26.48 and 3.33 % which was significant [F=7.20, df 5, 2, p<0.01; F=352.88, df 5, 2, p<0.01] (Table 5, 6 and 14).

In artificial soil anterior 25% and anterior 50% directed to grow posteriorly shown maximum and minimum percentage change in length which were found significant [F=7.20, df 5,2 p<0.01 , F=52.16, df 5,2 p<0.01] (Table 15). On 20th day anterior 25% showed 125.30 % change and anterior 50% showed 15% change (Table 7 and 11).

The posterior segments (75%) regenerating anteriorly showed maximum growth on 20th day. Similarly the posterior 25% portion regenerated anteriorly showed minimum growth on the same date. Two way ANOVA showed significant values that are 50.02 and 3.33% [F=7.80, df 5, 2, p<0.01; F=65.11, df 5, 2, p<0.01] (Table 8, 10 and 16). In the case of change in percent anterior 25% and anterior 50% showed maximum and minimum values both in natural and artificial soil. However, among posterior segments regenerating anteriorly minimum values in both types of soil were exhibited by different segments.

The ability to regenerate posteriorly appears to be nearly universal in the annelids (Bely, 2006). In the present experiment anterior segments showed maximum regeneration towards posterior direction (68.28 %). However, ability to regenerate in anterior direction by posterior segments is less (31.42 %). In natural soil anterior 25% showed more regeneration (68.28%) in comparison to anterior 50% (24.95%) and anterior 75% (17.25%). Among posterior segments least regeneration was seen in posterior 25% in both type of soil. It means regeneration towards posterior direction decreases successively. Gates (1826) reported similar results that all the substrates with cut surfaces at levels from 8/9 anteriorly regenerated. At level behind 54/55 no regeneration took place. The ability to regenerate posteriorly appears to be nearly universal in the annelids. The ability to regenerate anteriorly is common but less wide spread (Bely, 2006). The result showed that in *Perionyx sansibaricus* regeneration towards anteriorly is not widespread. Anterior segments regenerated tail but posterior segments failed to regenerate the head that might be due to complexity of head because of which ganglia are unable to regenerate soon. This is in accordance with Painter (1938) which found in successively more posterior segments, the capacity of regeneration of characteristic head structure diminishes. This difference of regeneration shown by anterior and posterior segments may be due to absence of brain and supraesophageal ganglion in posterior segments. A stimulatory factor or growth factor from the brain is probably required for release of a regenerating factor from subesophageal ganglia or nerve cord neurosecretor cell (Bedate and Sequers, 1984).

Regenerating segments in artificial soil has shown the same trend as found in natural soil showing no effect of habitat on regeneration.

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