Acta Ecologica Sinica 31 (2011) 197–204

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

The regeneration capacity of an earthworm, *Eisenia fetida*, in relation to the site of amputation along the body

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ARTICLE INFO

Article history:
Received 1 January 2010
Revised 18 March 2011
Accepted 12 April 2011

Keywords:
Earthworm
Eisenia fetida
Amputation
Site
Regeneration length
Survival rate

ABSTRACT

It is well known that parts of earthworms can survive if they are cut off. Our aim was to link the regeneration capacity of an earthworm, *Eisenia fetida* (Oligochaeta, Annelida) with the site of the amputation, so we amputated earthworms at different body segment locations along the length of the body to examine the different survival rates and regeneration lengths of the anterior, posterior, and medial sections.

The greatest survival rates occurred for earthworms with the most body segments remaining after amputation. The anterior regeneration lengths were of two types. The lengths of regeneration of amputated from body segment 6/7 to further down the body posteriorly increased gradually (Type L_I). However, the regeneration lengths of earthworm which were amputated behind the 23rd segment, with less than a quarter of the total segments remaining, did not increase until the blastema and tail bud formation (Type L_{II}). These treatments were not completely regeneration. There were significant differences in both survival rates and lengths of regeneration lengths between immature earthworms and clitellate adult earthworms during the early stages of regeneration, but not at later stages of regeneration. The immature earthworms had a greater regeneration potential than clitellate adults amputated at the same segment. The survival rates of earthworms were correlated significantly with the number of body segments remaining after amputation, but not with the position of the amputation. The relationships between the survival rates and the numbers of remaining segments could be described by linear regressions. The anterior regeneration lengths were correlated with the position of the amputation, but not with the number of remaining segments; the posterior regeneration lengths, were not correlated with the number of segments remaining nor the amputation position. The anterior regeneration length was not related to the survival rates for all earthworm amputations after 30 days but was related in this way after 60 days.

1. Introduction

Earthworms are extremely important in soil formation, maintenance and structure and turnover of dead organic matter so the factors that affect their survival are important in their ecology [9]. Because of their availability and rapid regenerative power, annelids have been used commonly to study regeneration mechanisms ([21,4,3]). For example, some fresh water oligochaetes and polychaetes, can replace segments amputated along the body rapidly and become virtually indistinguishable from normal adults [21,2]. Among annelids, the lumbricid earthworm, *Eisenia fetida*, which is important in breaking down organic wastes, has been used commonly for research into regeneration, because it is easy to culture and handle in the laboratory [8,9]. This species, and other lumbricid species, are characterized by being triploblastous with a true coelom, metamere, a closed vascular system, centralized brain ganglia and a nervous system with longitudinal cords and sense organs. Lumbricid earthworms are often cut into sections by predators or soil cultivators; hence for the survival of these earthworms it is important to know the regeneration capacity of such decapitated earthworms.

Whether the regeneration capacity of lumbricid earthworms depends on the number or position of the lost body segments is an interesting question. Gates [10] reported that the regeneration capacity decreased, as more segments were amputated from the earthworm *E. fetida*. Moment [15] showed that the rate of regenerative growth was faster when the position of amputation along earthworm was closer to the anterior end. Liebmanm [12] showed the rate of head regeneration began to fall slightly after the

Abbreviations: CF, Clitellum Factor; A, anterior part of amputated body; M, medial part of body retained; P, posterior part of body amputated.

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doi:10.1016/j.chnaes.2011.04.004

removal of nine segments; the decrease becoming more pronounced behind the seventh segment, with each additional metamere removed decreasing the degree of regeneration.

The regeneration capacity can also be influenced by environmental factors such as temperature and nutrition. Liebmanm [12] reported earthworms cultured at 25 °C regenerated faster than those kept at 30 °C and 20 °C. He also stated that sexual activity can also influence rates of regeneration, and this influence decreases progressively, the farther posterior from the sexual organs is the point of amputation. Although regeneration is so important in earthworm ecology, little research on earthworm regeneration has been reported since the 1980s. The ways in which the regeneration capacities of earthworms depend on the numbers or position of amputation, has not been demonstrated in any detail to date.

The main aims of the present study were: (i) to determine the effects of the numbers and position of the amputations along the earthworm body on the lengths of regeneration and survival rates; (ii) to compare regeneration lengths and survival rates between immature and adult earthworms; and (iii) to analyze correlations between regeneration lengths and survival rates of earthworms having the same position of amputation.

2. Materials and methods

Earthworms, *E. fetida* (Annelida: Oligochaeta) were reared in cow manure at 20 ± 1 °C, in a laboratory climate chamber for several generations. All earthworms that hatched from cocoons after 20 days were regarded as immature until they developed a clitellum and were used for the immature regeneration experiments. Reproductive adult earthworms, which developed a clitellum about 60 days after hatching, were selected for the adult experiments. The adult earthworms had a well-developed clitellum consisting of seven segments with 26–32 segments before the clitellum and 76 segments behind it. During hermaphrodite reproduction, the clitellum passes over the earthworm's head and hardens to form a cocoon containing one or more eggs.

Anteriorly, the cerebral ganglion is a bi-lobed structure, which occurs dorsally in segment 3 and segment 4, and contains the sub-oesophageal ganglion [9,25]. The earthworm *E. fetida* has typically 108 segments. The influence of the clitellum on the process of regeneration could be observed by amputating earthworms between segment 26 and segment 32 where the clitellum began (Fig. 1). Segments 32/33, 60/61 and 90/91 were selected as amputation sites to represent 1/4, 1/2 and 3/4 of the total body length, respectively.

Three types of amputation were used including: (i) anterior parts of earthworms whose posterior segments were removed (A), (ii) medial parts of earthworms whose anterior and posterior segments were removed (M) and (iii) posterior parts of earthworms whose anterior segments were removed (P). These manipulations were designed to observe the effects of position of amputation on lengths of regeneration and survival. A total of 18 different sections of body segments were amputated to examine the effects of numbers of segments remaining on the rates of regeneration of the earthworm (Fig. 1). Five types of amputation were done on the anterior segments Fig. 1a, eight on medial segments Fig. 1b, and five on posterior segments (Fig. 1c) (Table 1). Controls were earthworms with no amputations. All amputation treatments were replicated three times with 20 earthworms per treatment, each kept in a 200 ml plastic container with cow manure.

Before amputation, each earthworm was rinsed with de-ionized water, placed on a wet block of ice, and viewed under a dissecting microscope. This cooling down slowed the activity of the earthworm, to allow accurate counting of segments and to facilitate precise amputations at particular segments [22]. Earthworms were amputated quickly with a sharp scalpel, and after amputation were cultured in polyethylene plastic containers (200 ml) with cow manure, and kept moist by sprinkling with water weekly in a climate chamber at 20 ± 1 °C. The survival rates of amputated earthworms were checked every 5 days for 110 days. To do this, the regenerating sections of each earthworm were placed on a wet block of ice and the length of regeneration measured (mm/individual), under a dissecting microscope. Two indices, the survival rates (%) and the length of regeneration (mm/individual) were recorded, to examine the regeneration capacities of earthworms under different conditions.

The data were analyzed using the SPSS program (Standard Version 10.0, SPSS Inc.). Group means of parameters were compared, using ANOVA, to determine the significant differences between treatments ($P = 0.05$). Pearson Correlation was used to analyze correlation coefficients between the survival rates and lengths of regeneration by the control earthworms and those amputated. The probability level used for the statistical significance was $P < 0.05$.

3. Results

3.1. Survival rates of earthworms amputated at different segments

The survival rates of the *anterior segments of immature earthworms* after removal of different lengths of posterior segments (Fig. 1a) are summarized in Fig. 2. The survival rate-time curves can be classified into three types. Amputation treatments A90 and A60, had a high survival rate during the process of regeneration, and they survived up to 91.67% after 50 days (Type S_I). The survival rates A32 and A25 amputation treatments, decreased gradually during the process of regeneration (Type S_{II}). The A6 amputation treatment had the lowest survival rates and all of these earthworms died within 5 days (Type S_{III}).

The survival rates of the anterior segments of immature earthworms increased when more segments remained after amputation. Correlations between the survival rates (Y) and the numbers of remaining segments (X) after 30 days of amputation for immature earthworms could be described by a regression equation: $Y = 1.083X + 12.183$ ($R^2 = 0.809$, $F = 12.761$, $df = 4$, $P = 0.037$).

The survival rate-time curves, occurring after anterior amputations of adult clitellate earthworms, were similar (Fig. 3). The regression equation of the survival rates (Y) and the numbers of remaining segments (X) for adult earthworms with anterior segments amputated after 30 days could be described by the equation: $Y = 0.978X + 11.014$ ($R^2 = 0.774$, $F = 10.311$, $df = 4$, $P = 0.048$).

In all of the experiments involving the removal of anterior segments of earthworms, the immature earthworms survived longer than the adult clitellate earthworms. Significant differences in survival rates were observed between immature and clitellate adult earthworms 15 days after treatment ($F = 1556.579$, $df = 1, 4$), but not after 30 days ($F = 2.507$, $df = 1, 4$, $P = 0.129$) and 60 days ($F = 0.215$, $df = 1, 4$, $P = 0.647$).

Three patterns of survival rate-time curves were recorded for survival of the *posterior segments of immature earthworms after the anterior parts* (Fig. 1c) were amputated (Fig. 4). 100% of earthworms amputated at the 6/7 segment (P7 treatment Type S_1) survived up to 60 days. The survival rate of earthworms in the P33, P26 and P61 amputation treatments (Type S_{11}), decreased gradually to 55%, 48.3% and 43.3% respectively during the process of regeneration, after 60 days. All the earthworms with the P91 amputation treatment (Type S_{111}) died by 15 days. The regression equation on the survival rates (Y) and the numbers of remaining segments (X) posterior segments of immature earthworms from