CORRELATION BETWEEN THE MOLT CYCLE AND LIMB REGENERATION IN THE CRAYFISH ORCONECTES OBSCURUS (HAGEN) (DECAPODA, ASTACIDEA)

BY

J. ROSS STEVENSON and BARBARA A. HENRY 1)
Dept. of Biological Sciences, Kent State University, Kent, Ohio 44240, U.S.A.

INTRODUCTION

There are four major periods in a complete crustacean molt cycle (Carlisle, 1960). (1) Ecdysis, the actual shedding of the old cuticle. (2) Metecdysis, or postmolt (stages A, B, and C of Drach, 1939), the period of cuticular thickening and hardening and the return to “normal” physiological conditions. (3) The intermolt period (stage C4). This period may be of relatively long duration (anecdysis) or imperceptible (diecdysis). During intermolt the cuticle is of maximum thickness and hardness. (4) Proecdysis, or premolt (stage D), the preparation for molt. The old cuticle is partially reabsorbed and the new cuticle partially secreted beneath it.

Autotomy is the breaking off of a limb at a preformed breakage point (Wood & Wood, 1932; Bliss, 1960). A single membrane extends across the appendage internally, through which only blood vessels and nerves pass. Because no muscles pass across the plane, the amount of tissue damage is small (Bliss, 1960). The appendage so removed regenerates. The rate of growth of the regenerating limb bud may be controlled by the same factors that control internal tissue growth. Internal tissue growth, in turn, is known to be related to the stages of the molting cycle. This paper describes an investigation of the relationship between the growth of regenerating limb buds and the molting cycle in the crayfish. The investigation was undertaken to resolve some apparent discrepancies observed by Stevenson et al. (1968) between the limb regeneration patterns of the crayfish and those of other crustaceans.

MATERIALS AND METHODS

Collections of Orconectes obscurus in their first year of growth were made from the West Branch of the Mahoning River in Portage County, Ohio north of Ohio Route 59.

The crayfish were maintained in aerated plastic aquaria supplied with slowly running tap water. The bottom of each aquarium was covered with oyster shell as

1) Present address: Cuyahoga Community College 7300 York Rd., Parma Heights, Ohio 44130, U.S.A.
a calcium source, and the animals were fed canned "Puss n' Boots" cat food every other day. Water temperature was controlled by thermoregulators to 20 ± 1.5°C. A daily photoperiod of 14 hr. was maintained, using a 25 watt bulb behind each aquarium as the source of light. Each of the large aquaria contained six smaller compartments. After its first molt, each crayfish was isolated in a smaller compartment containing a flat rock supported by a smaller rock to provide shelter. These conditions were designed to duplicate as closely as possible the normal summer environment of these animals.

After each laboratory molt, the carapace length was measured and the crayfish was forced to autotomize a left or right rear walking leg by grasping the leg with forceps at a point distal to the autotomy plane. Autotomy can be induced readily by this method within two days after molting, but is difficult at any other time. The molt stage was determined every two days according to methods described by Stevenson (1968) and Stevenson et al. (1968). Proecdysial stages $D_1'$, $D_1''$, and $D_1'''$ were identified by holding the uropods and telson ventral side down on a glass slide secured to the stage of a microscope. The development of new setae, upon which recognition of these stages depends, could be seen with 100 × magnification.

The regenerating limb buds were also measured at least every two days with calipers. The length of the regenerating limb, from the autotomy plane to the tip of the limb bud, was recorded and converted to an R value as first described by Bliss (1956). The R value is used for comparisons among individuals of different sizes to compensate for the dependence of limb length upon total body size. It is calculated as follows:

$$R = \frac{\text{length of limb bud}}{\text{length of carapace}} \times 100$$

Thirty-one individuals, 17 males and 14 females, from 13.7 mm to 21.0 mm carapace length, were isolated after their first molt and studied.

RESULTS

Within a day after autotomy, light brown scar tissue had covered the breakage plane. No further change was observed for several days, characteristic of the lag period described by Durand (1960). The range of this lag period was 2 to 15 days, with a mean of 6.5 ± 0.48 days ($N = 40$) (mean value + standard error).

All animals reached stage C before limb growth began. Over half were in stage $C_0$ or $C_1$ when growth began, including those having cycles of both short and long duration.

The limb bud first appeared as a colorless protuberance from the tissue at the autotomy plane. In some cases continuous growth was observed until ecdisis, and in others growth halted (growth plateau) for either short or prolonged periods. A correlation was noted between length of the plateau period and length of the cycle. The coefficient of correlation was 0.8 ($P<0.1\%$). Only half the cycles shorter than 38 days included plateau periods, and all these plateaus were fewer