Reproductive Maturation in the Slug, *Limax maximus*,
and the Effects of Artificial Photoperiod

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Summary. Maturation of the reproductive tract of *Limax maximus* was studied in a natural population using a reproductive organ-to-body weight index. Male-phase maturation, denoted by enlargement of the hermaphrodite gland (gonad), took place mainly in June and July. Female-phase maturation, signaled by growth of the albumen gland, occurred primarily in September. When slugs were subjected to artificial photoperiods consisting of long (LD 16:8) or short (LD 8:16) days, female-phase maturation was not found to be significantly affected by photoperiod (Fig. 4). In contrast, male-phase maturation appeared to be induced by a short-day to long-day transition (Fig. 7). Implications of this finding for normal seasonal maturation are discussed and a maturation sequence involving both photoperiodic and endocrine control mechanisms is suggested.

Introduction

The development of the complex reproductive system of pulmonate slugs has been studied in a variety of species with respect both to the morphological changes that take place (Lüsis, 1961; Kugler, 1965; Smith, 1966; Runham and Laryea, 1968) and to the endocrinological factors influencing sexual maturation (La-violette, 1954; Pelluet and Lane, 1961; Pelluet, 1964; Gottfried et al., 1968; Gottfried and Dorfman, 1970; Runham et al., 1973; Wattez, 1975; Wijdenes and Runham, 1976). Little is known, however, about the environmental factors that influence seasonal maturation in these hermaphrodites.

As pointed out by Menaker (1971), photoperiod (daylength) is employed by many species of plants, animals, and birds as a seasonal cue to control the annual reproductive cycle. The adaptive advantage gained by the use of this factor appears to be that it serves as a reliable predictor of seasonal change. Thus, reproductive activity can be initiated at an appropriate time to insure the availability of nutrients for early rapid growth or to guarantee that the proper developmental stage will have been reached to withstand recurrent harsh climatic conditions such as winter cold or summer drought.

As is the case for other seasonally reproducing slugs, *Limax maximus* appears to be distinctly protandrous. We have found evidence for three general reproductive stages in a naturally occurring *Limax* population: (1) in the male phase there is enlargement of the gonad and the initiation of sperm production in the spring; (2) in the female phase growth and development of the female accessory glands takes place in the summer; (3) fertilization and oviposition occur in the fall accompanied by gonadal regression.

We report here not only on the characterization of normal seasonal maturation but also on our efforts to influence the reproductive cycle of *Limax* by manipulating photoperiod. Our results suggest that photoperiod can affect general growth, but does not significantly affect reproductive development in already maturing female-phase animals. In contrast, we have found that male-phase maturation can be significantly influenced by photoperiod, with long days promoting and/or short days inhibiting the normal spring-time enlargement of the hermaphrodite gland.

Methods

Slugs (*Limax maximus*) were used that were either wild-caught (at a single location in the vicinity of Baltimore, Maryland) or raised from eggs in environmentally-controlled chambers. The chambers were kept at 15 ± 0.5°C and 80-90% relative humidity throughout all experiments. Light dark (LD) cycles were controlled by external timers arranged to provide either 8 h of light per day ("short" days; LD 8:16) or 16 h of light ("long" days; LD 16:8). Lighting was provided by 6, 25-W incandescent bulbs in each
chamber. Slugs were housed either singly or in groups of from 10 to 50 animals (depending upon size and the nature of the experiment) in individual refrigerator storage boxes provided with wet paper toweling. Food consisted of ground rat pellets (Wayne Lab-Blox, Allied Mills, Chicago) supplemented with sand, calcium carbonate, and vitamins (Vionate Vitamin-Mineral Powder for Pets, E.R. Squibb), and moistened with a 0.15% solution of Tegosept (Goldschmidt Chemical Co., New York) to inhibit growth of mold. Containers were cleaned and fresh food was supplied weekly in the light phase of the LD cycle.

Animals in different experimental groups were matched as closely as possible for body weight at the beginning of an experiment and weighed again just prior to dissection. When possible the entire reproductive system was removed and weighed, followed by separation and weighing of the separate parts such as the hermaphrodite gland (gonad), albumen gland, common duct, etc. In addition detailed notations were made of the appearance of the various reproductive structures (swelling, coloration) and of the presence or absence of sperm in the hermaphrodite duct. In some cases it was not possible to obtain the separate weights of individual organs in immature reproductive systems owing to their small size. Therefore, only the total reproductive tract weight and that of the hermaphrodite gland were recorded.

Various statistical procedures were used to analyze the data including linear regression, student's t-test, and Kolmogorov-Smirnov two-sample test. Where applied, these methods are noted in the text or in figure legends. Means in text and tables are expressed as Mean ± S.D.; bracketed values in figures indicate S.E.M.

Results

Description of Developmental Indices and the Course of Normal Seasonal Maturation

In order to assess rapidly and reliably the general reproductive state of individual slugs it was necessary to establish a quantitative index for describing the stages of development of the reproductive tract without recourse to histological techniques. Previous workers have, to a large degree, formulated staging categories for reproductive maturation based upon detailed morphological descriptions of reproductive tissue (Lüsí, 1961; Kugler, 1965; Smith, 1966; Runham and Laryea, 1968). While useful, such categories are often difficult to quantify reliably, although attempts have been made to measure the degree of gonadal differentiation in slugs by egg counts (Pelluet and Lane, 1961; Pelluet, 1964).

Preliminary observations made on wild-caught Limax suggested that enlargement of the female accessory glands took place mainly in mid-to-late summer whereas gonadal size increased one to two months earlier with maximum enlargement appearing to coincide with the initiation of sperm release. We therefore chose to examine separately growth of gonads and albumen glands in order to chart male and female developmental stages throughout the Limax reproductive season.

In order to compare animals having very different body weights at any given time of the year, we computed the ratio of organ size to total body weight for each individual. The appropriateness of this simple index is illustrated in Figure 1A in which gonad weight is plotted against body weight for wild-caught slugs collected at two different times of the year. In general, we found a strong correlation between body weight and gonad weight for animals collected within a given 1 week period. A similar relationship was found between albumen gland size and body weight (Fig. 1B). In spite of marked variability in both organ size and body weight within cohorts of slugs collected at various times of the year, the average, normalized sex organ size appeared to offer a reliable index of the state of the organs within a population.

Slugs were collected locally over the period July 12 through October 30, 1976 and May 20-July 6, 1977 and were immediately examined to determine gonad and albumen gland size. When gonad and albumen gland indices were plotted as a function of collection time, the seasonal course of sexual maturation became clearly evident (Fig. 2). The gonad index began to increase in early-to-mid June reaching a maximum in early-to-mid August and declining steadily thereafter. The albumen gland index began increasing in mid-to-late August and appeared to level off by the end of October.