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SOME OBSERVATIONS ON THE LIFE HISTORY OF THE FLY *MEGASELIA SCALARIS* LOEW (PHORIDAE) WITH SPECIAL REFERENCE TO THE ECLOSION PATTERN

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ABSTRACT

Males of a laboratory maintained population of the dipteran *Megaselia scalaris* were observed to eclose approximately four days prior to the females. The peak male eclosion occurred on day three while the female peak occurred on day seven. The smaller male larvae pupated approximately two days earlier than female larvae, and it appeared that male pupae eclosed earlier than female pupae. An excess of male progeny was recovered. The early eclosion of males coupled with delayed mating suggests mechanisms which might reduce the incidence of inbreeding. We also confirm observations that females have greater longevity than do males. The life history traits of larger size, longer developmental time and greater longevity may reflect a greater reproductive burden carried by the females.

INTRODUCTION

The dipteran fly *Megaselia scalaris* is geographically widespread, being reported in 58 countries (James, 1947), and is found in a wide diversity of habitats. It is characterized by a hunch-back appearance and rapid jerky movements (Patton, 1922). There is marked sexual dimorphism, females being approximately twice as large as males (Semenza, 1953). They are known to breed on decaying material (Brunetti, 1912; Patton, 1922), and Patton (1922) suggested they might be a significant medical and veterinary problem in India because they produce myiasis. Priyanond *et al* (1973) have reported a case of urethal obstruction caused by larval infection. Patton (1922), Semenza (1953) and Tumrasvin *et al* (1977) have recorded a number of observations on the basic biology of the fly and Mainx (1964) has reviewed its genetics, but little is known of its natural history. The following study was undertaken to examine the unusual eclosion pattern reported by Semenza (1953) in which the males eclose earlier than the females.

METHODS AND MATERIALS

A laboratory stock of *Megaselia scalaris* was established from pupae collected from the sides of a container used to store anatomical specimens in the basement of the science building at East Tennessee State University. Subsequent generations have been maintained on a yeast, corn meal, sorghum-syrup, agar *Drosophila* medium in one-half pint (232 ml) bottles.

Adult flies were placed on fresh food (approximately 85ml/bottle) at 25°C and allowed to lay eggs for one to four days. The eclosion pattern was determined by collecting newly emerged flies twice daily, morning and afternoon, and recording the number of each sex at each collection. In order to establish the relation of the order of pupation to eclosion the position and date of pupation of individual pupae were marked on the side of the culture bottle, then the date of eclosion was recorded for each pupa as the flies emerged. Additional information on eclosion times and patterns was obtained by collecting white pupal cases and transferring them to fresh food in 8-dram shell vials. White pupal cases were selected because their age was known within four hours as it takes approximately four hours for the pupal case to darken. Female containing pupal cases are larger than male containing cases (Semenza, 1953), but the pupal cases were taken at random without reference to size. The eclosion period was measured from the day the first fly emerged.

RESULTS

For the first two days in the eclosion period only males were recovered (Table 1). The male eclosion reached a peak at day 3 (Figure 1A) and 50% of the total males had eclosed by day 3. Females began to eclose on the third eclosion day (Table 1) and reached their peak on day 7 (Figure 1A). Fifty percent of the total females eclosed by day 8. The total number of males collected was significantly greater than the number of females (Table 1: sex ration 1.19, $P < 0.01$).

TABLE 1: *The number of flies of each sex emerging each day during the eclosion period.*

Day	No. Males	No. Females
1	104	0
2	141	0
3	177	10
4	122	40
5	45	66
6	55	68
7	46	110
8	27	85
9	31	82
10	17	82
11	17	45
12	6	54
13	5	14
14	0	9
15	0	5
16	0	0
Total	793	670

The first individuals to pupate were also the first to eclose. These first pupae were always the smallest and were always males. This shows that male larvae spent a shorter period of time in the larval stage and had a shorter developmental period. The later pupating larger larvae were the females. When white pupal cases were collected from a mature culture in which some flies had already eclosed, but in which new pupae were still forming, the first individuals to eclose were males. These first males appeared at nine days following puparium formation with eclosion extending to day 14. Most of the males had eclosed by day 11. Females began to eclose at day 13 following puparium formation. These results suggest that the females eclosed one to two days later than the majority of the males even when they appeared to pupate at the same time.

DISCUSSION

The data obtained in this study are in agreement with the observation of Semenza (1953) that *Megaselia scalaris* males eclose earlier than females. This appears to be due to both a more rapid larval development and a more rapid metamorphosis by the males. For a given population of eggs laid at a specific time the males undergo more rapid larval development and pupate approximately two days earlier than do females. In a pupal population of approximately the same stage of development (white pupal cases) it appears that males eclose one to two days earlier than do females. This means that males develop approximately four days sooner than females, which is the approximate difference between the peak eclosion time of the males and females of the population. It is tempting to speculate that the more rapid development is the reason for the smaller size of the larval male and, in turn, the adult male. Such an interpretation must be viewed with caution, however, because in laboratory *Drosophila* it is recognized that females emerge first, the first females appearing a few hours before the first males, yet the female is larger than the male. Conversely, the smaller size of the male might facilitate more rapid development, but it seems more likely that size and developmental rate are both genetically determined rather than size being determined by developmental rate or the rate of development being a function of the ultimate size of the individual. At present it is not known when during larval development this size difference is first expressed.

In the present study the total time of development

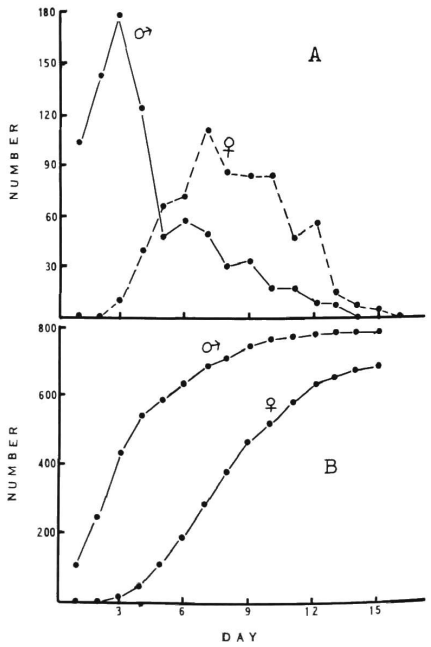

FIG. 1. *Number of progeny of each sex eclosing each day (A) and the cumulative totals for each sex (B).*

was a minimum of 21 days from egg to eclosion of the first adult. This is in close agreement with the observations of Patton (1922) who notes the process "occupied 21 to 27 days." However, Semenza (1953) reports development in 18 days while Mainx (1964) reports that males begin to appear at 18 days and females at 20 days from the time the parents are put in the growth chamber. Tumrasvin *et al* (1977) found that males develop in 15 to 20 days while females require 16 to 22 days. The differences in these results may reflect differences in culture conditions.

Mainx (1964) reports that the optimum temperature for culture is 28°C. Tumrasvin *et al* (1977) reared their cultures at 27°C while Semenza (1953) reports a culture temperature of 23°C. It appears that these temperature differences are not a significant factor in influencing developmental time since the results of Semenza (1953) and Mainix (1964) are in good agreement. All three of these investigators used a protein enriched medium. Mainx (1964) used a mushroom powder additive, Tumrasvin *et al* (1977) cultured on a blood agar medium and Semenza (1953) added a liver extract to a basic *Drosophila* medium. Our cultures were fortified with yeast, but compared to the enriched media used by the other investigators the protein content would be low. A difference in protein content might be a factor in the longer time of development shown in our study, but it did not appear to alter the male-female eclosion pattern. The influence of other environmental factors, such as photoperiod and humidity, have not been investigated. Our collections yielded a significant excess of male flies. Semenza (1953) reports a similar excess, recovering 56% males. At present we can offer no reason for this aberrant sex ratio unless it is associated with the unusual sex determining mechanism which may confer an adaptive advantage to the male (Mainx, 1964).

Flies are neither territorial nor gregarious so this pattern of eclosion would serve to reduce interbreeding between sibling individuals as the males would have eclosed and dispersed by the time female eclosion was beginning. Patton (1922) observed that copulation did not occur until the second day after eclosion. However, Tumrasvin *et al* (1977) reported mating within one day after eclosion and Mainx (1964) states that females copulate early. Delayed development of one sex and any delay in sexual maturity would virtually ensure a low frequency of inbreeding. Outbreeding can be a major mechanism for preserving genetic variability in organisms and in turn maintaining ecological plasticity and evolutionary flexibility (see discussion in Mayr, 1970). *Megaselia* is a cosmopolitan species and shows considerable environmental diversity, feeding on a wide variety of substances ranging from decaying animal matter and a culture of "roast duck and rice" (Brunetti, 1912) to open wounds on animals and humans to decaying plant material and animal wastes (Patton, 1922). This diversity of feeding sites suggests great ecological plasticity. Forced outbreeding would serve to maintain the ability of these flies to exploit a variety of ecological opportunities.

Patton (1922) observed that mating occurred on the second day after eclosion, but females did not lay eggs until the third day after mating. Tumrasvin *et al* (1977) reported a two day delay in egg deposition. The seven males involved in Patton's study all died by the seventh day after eclosion. Six of the females lived an average of 29 days (one having escaped) and produced an average of 298 eggs from the single mating. Tumrasvin *et al* (1977) reported that their nine females lived an average of only 5.2 days and produced an average of only 32 eggs. Their males lived an average of 2.7 days. Our preliminary data from virgin male and female flies kept in well yeasted food bottles shows that males can live as long as 32 days, but 50% mortality occurs by day 12. Individual females lived to approximately 40 days with 50% mortality occurrring at the 23rd day (unpublished results). These results are consistent with those of Patton (1922) and Tumrasvin *et al* (1977) in suggesting that females have greater longevity than males. These life history traits, greater time of development, the greater size and the greater longevity, may be a reflection of a greater reproductive burden carried by the female. At present it is not known if the female mates more than once in her lifetime, or if the male mates with more than one female. Mainx (1964) states that the fertility of both sexes is limited to a short period after eclosion. More knowledge of these factors might shed light on the relative roles of the various factors in the life history which are involved in reproductive effort and success.

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EFFECTS OF ULTRAVIOLET LIGHT ON *HYMENOLEPIS DIMINUTA* OVA AND CYSTICERCOIDS

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The ova and cysticercoids of *Hymenolepis diminuta* were exposed to a 2537 Å wave length of ultraviolet light for various time periods. Development was extremely impaired in the cysts which had been irradiated for 30 and 60 minutes. When these were administered to the final host no tapeworms developed.

From 113 intermediate host beetle larvae fed with irradiated ova, only three cysticercoids were recovered. Development was impaired in both cases and the infective rate of irradiated ova and cysts of the least exposed groups was lower than that of the controls.

INTRODUCTION

A number of investigations have been conducted involving the effects of ultraviolet radiation upon parasitic worms. Several of these studies have involved the ova of nematodes (Hollaender, Jones and Jacobs, 1940; Hollaender and Jones 1944; Shalimov, 1935; Wright and McAlister, 1934); however, there is little information concerning the possible effects of ultraviolet radiation upon the ova and cysticercoids of cestodes.

The purpose of this study was to investigate the effects of ultraviolet light at a wave length of 2537 Å on the ova and cysticercoids of *Hymenolepis diminuta* at a distance of 5 cm. from the light source. The ova and cysts were exposed to irradiation for various time periods. Attempts were made to determine (1) the most effective time exposures required to produce the most damage, (2) the infective rate of the irradiated ova and cysticercoids, and (3) the effects of irradiation on fecundity of the adult tapeworm in the final host.

MATERIALS AND METHODS

The Norway rat, *Rattus norvegicus*, (Muridae), served as the definitive host for the adult tapeworm, and the grain beetle, *Tenebrio molitor*, (Tenebrionidae), was the intermediate host for the cysticercoids used in this experiment.

Eggs were taken from several mature proglottids of *Hymenolepis diminuta* and placed upon plain agar filled Syracuse watch glasses which were covered with a thin film of .75% physiological saline. About 2,000 ova were placed in each dish in a single layer to insure similar exposure to radiation. Eggs used as controls were kept in the saline solution for time periods equal to each radiation exposure.

A Fisher lamp with a wave length of 2537 Å was used as the source of ultraviolet radiation. Constant intensity was maintained by keeping the material 5 cm. from the lamp. During irradiation, the ova and cysts were kept moist by spraying a mist of .75% saline solution over the surface with an atomizer.

Eggs were irradiated for 3, 7, 15, 30, and 60 minute periods prior to immersion in saline at the end of each radiation exposure period and then transferred by means of a Pasteur pipette to a sterile centrifuge tube. The suspension was centrifuged at 2,000 rpm for one minute and the supernatant liquid was poured off. The remaining small amount of saline with the treated ova was mixed with a small amount of flour and fed to about 40 or 50 young beetle larvae which had been starved for a five-day period prior to the experiment (Tan, 1965). The same procedure was followed with the control eggs.

Fifteen days after exposure to feeding on irradiated or control eggs, the beetle larvae were dissected and the cysticercoids were placed in a Syracuse watch glass containing saline. Two albino laboratory rats (2 months old) were infected with cysticercoids which developed from irradiated eggs, and later the rats were sacrificed and dissected to determine cyst viability.

To test the effects of irradiation on cysticercoids, normal cysticercoids were teased with dissecting needles from the body cavity of the intermediate host. Approximately 40 were placed in each Syracuse watch glass and treated in the same manner described for the ova. Cysticercoids were irradiated for only 15, 30, and 60 minute periods since they are more resistant than eggs.

Ten irradiated cysticercoids were randomly chosen and injected into the digestive tract of each of six 4-month old albino rats by means of a stomach tube. There were two rats in each of three test groups.

The final hosts infected with irradiated cysts were sacrificed 44 days later by ether asphyxiation and then dissected. The intestines were opened with forceps and the worms removed and counted.

To determine viability of the ova produced by these adult worms, the last three or four gravid proglottids were removed, mascerated on a piece of filter paper, covered with 100-150 starved beetle larvae and another sheet of filter paper. Flour was added after the larvae had fed on the mascerated proglottids for a three-day period. At the end of 15 days, the larvae were dissected and examined for cysts.

RESULTS

Irradiation of *Hymenolepis* inhibited the development of cysts from irradiated eggs. Only three cysts were recovered from 113 beetle larvae fed irradiated ova; 135 cysts were recovered from 25 control larvae, (Table I). Of the three recovered cysts, one appeared abnormal. To test the viability of the other two cysts which had developed from irradiated eggs, two albino rats, age two months, were each infected with one of the two cysticercoids.

TABLE I: *Effect of ultraviolet exposure on cyst development of* Hymenolepis diminuta.

Group	Time Irradiated in minutes	No. of Cysts Recovered No. of Larvae Examined	Percent of Cyst Recovery
1	3	1/25	4.0
2	7	1/20	5.0
3	15	*1/9	11.1
4	30	0/14	.0
5	60	0/14	.0
Totals		3/113	2.6
Controls		135/25	

*abnormal

TABLE II: *Adult* Hymenolepsis diminuta produced by *cysticercoids irradiated with ultraviolet light.*

Group	Time Irradiated in minutes	No. of Worms Recovered No. of Implanted Cysts	Percent of Worms Recovered
1	15	1/20	5.0
2	30	0/20	.0
3	60	3/20	15.0
Totals		4/60	6.6
Controls		15/20	75.0

Upon autopsy, one mature tapeworm was found in each rat, thereby confirming viability of the cysts. The effect of irradiation on normal cysts is shown in Table II. There is obvious reduction in the numbers of adult tapeworms developing from irradiated cysts. In the experimental groups, 4 worms were recovered from a total of 60 implanted cysts; in the controls, 15 out of 20.

To determine the fecundity of adult worms developed from irradiated cysts, three gravid proglottids from each group were fed to starved beetle larvae. The dissection of 40 beetle larvae showed that the ova in the adult worm exposed to 15 minutes of radiation as a cysticercoids had formed 327 cysts. The ova exposed to 60 minutes of radiation as cysticercoids had formed 154 cysts.

DISCUSSION

Ultraviolet radiation with a wave length of 2537 Å is absorbed by and produces effects on living material (Hollaender and Carlson, 1944). Giese (1945) showed that this action spectra, 2500 to 2800 Å, causes ciliary reversal, immobilization and vesiculation of *Paramecium*. When eggs of the tobacco budworm and the bollworm were exposed to 2537 Å, there was a decrease in the percentage of egg hatch and no hatch after twenty minutes (Guerra, Ouije, and Bullock, 1968). Hollaender, Jones and Jacobs (1940) showed that the ova of *Enterobius vermicularis* have increased sensitivity to radiation from 2280 Å to 2805 Å. Stoll, Ward and Mathieson (1945) found that all *Entamoeba histolytica* cysts were destroyed by 10 minutes of 2537 Å exposure.

The region of maximal absorbtion by nucleic acids is a wave length of 2600 Å. Apparently as a result of the high absorption by nucleic acids, the experimental wave length of 2537 Å produces striking effects on living cells (Hollaender and Carlson, 1944). This may be the main cause for the decrease in viability of the irradiated ova and cysticercoids in the present study. Muller (1954) indicated that, even when nucleic acid is the absorber of ultraviolet radiation, much of the action is transferred to surrounding substances (even water), which in turn react upon the genes. This is probably explained by the fact that hydroxyl radicals often react to form hydrogen peroxide and its derivatives in water irradiated with ultraviolet light. These peroxides have been shown to be conductive to mutagenesis.

In 1934, Wright and McAlister showed that some of the irradiated ova of both *Toxocara canis* and *Toxascaris leonina* developed partially, but development was definitely arrested with various irregularities occurring in the developmental stages. These malformations included vacuoles in the cytoplasm, degeneration and redistribution of cytoplasm into various areas within the shell, abnormalities in blastulation and gastrulation, and partly formed embryos. In the present study, a majority of the oncospheres from the 30 and 60 minute exposures showed similar irregularities. One of the three cysticercoids that developed from the irradiated ova was aberrant. Ultraviolet radiation produced detrimental effects on ova and cysticercoids of *H. diminuta*.

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