PRELIMINARY OBSERVATIONS ON THE INFLUENCE OF AGE AND MALE SEX ON FECUNDITY IN *DROSOPHILA MELANOGASTER*

It is well known that both life span and fecundity in *Drosophila* are extremely sensitive to a great variety of direct environmental factors such as temperature, light, population density, presence of the other sex, etc. Even the influence of age on fecundity has been shown by Lints and Hoste[1] in *Drosophila melanogaster*, where offspring from old parents have more fecundity than offspring from young parents.

Virgins isolated from Oregon-K strain of *D. melanogaster* aged for five days and fifteen days were employed for the experiments. In one set of experiments both the groups of flies were allowed to mate with five days old males and in another set virgins as such were used. Egg laying capacity was estimated for the next ten days. Fifteen replicates were employed in each case. All the experiments were carried out at 23 ± 1° C.

The data obtained are given in Table I and daily egg laying pattern is depicted in Fig. 1. In mated series of five days old females, the total number of eggs laid were 5266 in contrast to 2892 in fifteen days old flies, whereas in virgin series (unmated) it was 2170 in five days old against 1103 eggs of fifteen days old virgins. Two points emerge out of the data.

TABLE I

Fecundity of 5 days and 15 days old virgin and mated flies of Drosophila melanogaster

Item	Total number of eggs	Number of eggs/female/day
5 days (mated)	5266	35·10
5 days (virgins)	2170	14·46
15 days (mated)	2892	19·20
15 days (virgins)	1103	7·35

One is the influence of male sex on fecundity and the other is age. Less aged flies laid more eggs than the aged flies; even in mated series, five days old flies are more fecund than fifteen days old flies. From Table I, it is also clear that five days mated series is more fecund than five days virgin series. It is also true with the results of fifteen days old flies. This led the authors to conclude that the presence of male alongside with the female, changed the physiology of the female to lay more number of eggs in a given time. Further, these experiments may give an insight into the way of physiological changes in the females to lay more eggs.

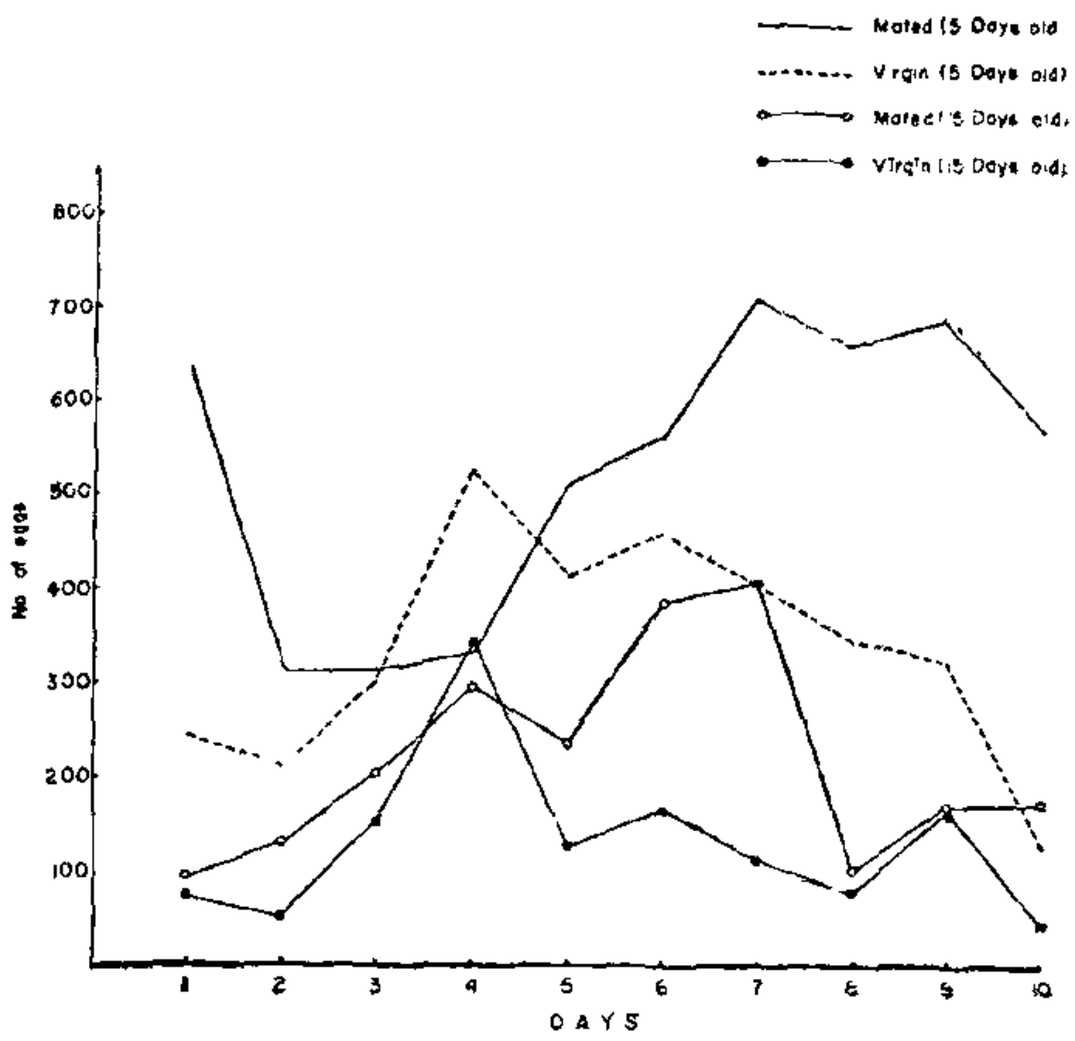

FIG. 1. Pattern of egg laying in 5 days and 15 days old mated and virgin flies.

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INDUCTION OF STERILITY IN *ZABROTES SUBFASCIATUS* (BOH.) (BRUCHIDAE: COLEOPTERA) BY THE USE OF GAMMA RADIATION

SEVERAL species of stored grain pests have been treated with gamma radiation for the production of sterile males and females which could be used for the self-destruction of the pests[1–6]. Such observations pertaining to bruchids are due to Wiendl[1,2] who studied the effect of gamma radiation on the survival, longevity and reproduction of *Zabrotes subfasciatus* (Boh.). Szentsi[3] studied the effect of gamma and X-rays on the mortality of developmental stages of *Acanthoscelides obtectus* and Pajni[4] exposed the pupae of different ages for the induction of sterility in the adults of *Callosobruchus analis*. The present communication deals with gamma radiation doses required by the

pupae of different ages for production of the desired type of sterile males and females in *Zabrotes subfasciatus*. This bruchid is a serious pest of stored *Phaseolus lunatus* (*Rajmash*) in this country, and the investigations at hand are expected to provide basic information for the ultimate control of the pest through sterile-male technique.

The seeds containing pupae of different ages were taken from a culture maintained on '*Rajmash*'. The rate of 89·31 r/minute at a source sample distance of 60 cm were placed in separate vials and were allowed to metamorphose and copulate with the normal individuals of their opposite sex. The fate of the eggs laid by four random females under different combinations was watched and the complete failure of the eggs to hatch was considered to indicate the total sterility in the individuals of the exposed sex used for copulation.

TABLE I

Showing the behaviour of the adults of Zabrotes subfasciatus *Boh. that emerged from pupae of different ages exposed to different doses of gamma rays*

Age of pupae		Dosage					
		1000 r	1500 r	2000 r	2500 r	3000 r	Control
One Day	Number of Pupae exposed	60	60	60	60	60	60
	Mortality	10%	8·3%	20%	30%	95%	Nil
	* reduction in egg hatch						
	(i) Sterile Males	66·7%	44·5%	75%	100%	..	2·5%
	(ii) Sterile Females	36·4%	13·4%	35·5%	80%	..	Nil
Two Day	Number of Pupae exposed	60	60	60	60	60	60
	Mortality	..	20%	20%	30%	50%	..
	* reduction in egg hatch						
	(i) Sterile Males	14%	100%	100%	100%	100%	4–5%
	(ii) Sterile Females	23·5%	37·5%	55·6%	42·3%	66·5%	Nil
Three Day	Number of Pupae exposed	60	60	60	60	60	60
	Mortality	5%	Nil	Nil	Nil	Nil	Nil
	* reduction in egg hatch						
	(i) Sterile Males	75%	78%	100%	100%	100%	2·2%
	(ii) Sterile Females	12·5%	37·5%	52·2%	16·7%	50%	Nil
Four Day	Number of Pupae exposed	60	60	60	60	60	60
	Mortality	10%	13·7%	10%	15%	5%	Nil
	* reduction in egg hatch						
	(i) Sterile Males	57·8%	48%	87·2%	40%	69·6%	2%
	(ii) Sterile Females	15·8%	79·1%	42·9%	63·7%	100%	Nil

* Average of four females.

advent of the pupal stages is indicated by the appearance of a circular 'Cap' or 'Window' on the surface of the seed, marking the position of the emergence hole of the adult. The pupal stage lasts for five days at 30° C and 70% R.H. One to four-day old pupae, contained in the seeds, were exposed separately in batches of 20 as a mixed population of males and females, with three replications, to five different doses of gamma radiation (1000 r, 1500 r, 2000 r, 2500 r and 3000 r). The use of $Cobalt^{60}$ (Theratron 60) source in the P.G.I., Chandigarh, was made possible through the courtesy of Dr. T. K. Dutta of Radiotherapy Department. The pupae exposed to a dose

The results of the above trials (Table I) indicate that in the case of one-day old pupae, complete sterility in the males is produced by a dose of 2500 r, the lower doses bringing about a maximum of 75% sterility while the higher dose (3000 r) resulting in almost total mortality of the pupae, with a few survivors badly damaged. The resulting sterility in the males was, however, discouraging on account of the appreciable mortality in the treated pupae. None of the doses produced a complete sterility in the females in this experiment. Likewise, the treatment of two-day old pupae also produced cent per cent sterility in the males with the four higher doses of radiation but in all the

cases, the total sterility was accompanied by a rather high rate of mortality (20% to 50%) in the treated pupae. The sterility in the females was again far short of 100%, the highest of 66·5% occurring only with a dose of 3000 r.

The three-day old pupae, when exposed to different doses, produced 100% sterility in the males with the doses of 2000 r, 2500 r and 3000 r. Moreover, in all the three cases, there was no mortality in the pupae. Apparently, the ideal type of sterile males, satisfying the requirements of the desired type of sterility, are produced when the three-day old pupae are exposed to any of the three higher doses of gamma radiation. With regard to females all the doses failed to produce 100% sterility in the three-day old pupae. In fact, 100% sterility of the females was witnessed only in a single case when the four-day old pupae were exposed to a dose of 3000 r and, fortunately, the accompanied mortality of the pupae had a maximum of 50%.

It is evident from the above, that the complete sterility in the males and females of *Zabrotes subfasciatus* can be induced by exposing three-day old pupae to doses of 2000 r, 2500 r and 3000 r and four-day old pupae to a dose of 3000 r respectively. The trials with other bruchids show, that the sterile males of *Callosobruchus analis*[4] are produced on irradiating the two-day old pupae by 1000 r and its sterile females by exposing three-day old pupae to 1500 r. In the case of *Callosobruchus chinensis*[6] the adult insects required an excessively high dose of about 42000 r for the production of sterile males and females.

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HISTOCHEMICAL LOCALIZATION OF STEROIDOGENIC SITES IN THE OVARY OF THE LIZARD, *PSAMMOPHILUS DORSALIS* (GRAY)

THE capacity of the reptilian ovary to synthesize various steroid hormones is well documented[1, 2]. Histochemical localization of steroidogenic enzymes has been considered to indicate the cellular sites of steroid biosynthesis[3]. Ovarian components like granulosa cells, thecal cells, cortical ooplasm of ovarian follicles, corpora lutea, atretic follicles and interstitial cells of ovarian stroma have been identified as sites of steroidogenesis in reptiles[4–8]. The present study describes the distribution of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH), 17β-hydroxysteroid dehydrogenase ((17β-HSDH), glucose-6-phosphate dehydrogenase (G-6-PDH), reduced nicotinamide adenine dinucleotide diaphorase ($NADH_2$ diaphorase) and lipids in the ovary of the lizard, *Psammophilus dorsalis*.

Frozen cryostat sections of ovary from sexually mature female lizards collected during breeding season (May–August) were incubated for HSDHs according to Baillie *et al.*[3]. Pregnenolone and dehydroepiandrosterone (DHA) were used as substrates to localize Δ^5-3β-HSDH, estradiol and testosterone were used as substrates to localize 17β-HSDH. Similarly, G-6-PDH and $NADH_2$ diaphorase were demonstrated as per methods of Altman[9] and Chayen *et al.*[10]. Sudan black B method of Pearse[11] was employed to localize lipids. Suitable control sections were also incubated in appropriate media without the substrate or nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NAD/NADP). After incubation, sections were washed, fixed in 10% neutral formalin and mounted in glycerol jelly or PVP mounting medium.

Histochemically, Δ^5-3β-HSDH and 17β-HSDH were localized in follicular granulosa cells, thecal cells, peripheral ooplasm of the developing and vitellogenic follicles (Fig. 1) and luteal cell mass. DHA as substrate gives more intense reaction than pregnenolone for Δ^5-3β-HSDH but there is no substrate specificity for 17β-HSDH. The distribution of G-6-PDH, $NADH_2$ diaphorase (Fig. 2) and lipids is similar to that of hydroxysteroid dehydrogenases.

Δ^5-3β-HSDH and 17β-HSDH are important steroidogenic enzymes which play a key role in steroid biosynthesis. The former is involved in oxidative conversion of Δ^5-3β-hydroxysteroids to Δ^4-3 ketosteroids and the latter is involved in oxidative interconversion of sex steroids (androgens and estrogens). In mammals these enzymes have been identified in granulosa of atretic follicles, theca interna of developing follicles, interstitial tissue and corpora lutea[3]. Presence of Δ^5-3β-HSDH and 17β-HSDH in thecal cells, granulosa cells peripheral ooplasm and corpora lutea of *Psammophilus dorsalis* suggests these as principal sites of steroid metabolism. The distribution of G–6–PDH is used as an indirect histochemical evidence to identify steroidogenic sites since it is one of the potential generators of reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) which is involved in hydroxylation reaction in steroid biosynthesis. The presence of $NADH_2$ diaphorase which