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EFFECTS OF ^{60}Co IRRADIATION ON *AMBLYOMMA HEBRAEUM* KOCH, 1844 (ACARINA: IXODIDAE)

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ABSTRACT

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The effects of ^{60}Co irradiation on *Amblyomma hebraeum* Koch, 1844 were studied by mating normal females to males irradiated to attain dosages of 0, 2, 4, 6 and 8 kilorad. The males of all 5 groups were observed mating 7 days after being placed on the host. The higher the irradiation dosage received by the males, progressively longer were the feeding periods of the females mated with them and fewer completed their engorgement. Furthermore, the mass of the females when engorged was lower, fewer laid eggs and the number of eggs they laid decreased progressively. None of the egg batches produced by females mated with irradiated males hatched. No chromosomal abnormalities or discrepancies in spermiophore formation were found in the 2 and 4 kilorad group males, while no micro- or macroscopical growth of the 6 and 8 kilorad group male testes took place, although accessory gland development appeared normal. *A. hebraeum* males have a chromosome complement of 10 bivalents and a univalent sex chromosome.

Résumé

EFFETS DE L'IRRADIATION AU ^{60}Co SUR AMBLYOMMA HEBRAEUM *KOCH,* 1844 *(ACARINA: IXODIDAE)*

*On a étudié les effets de l'irradiation d'*Amblyomma hebraeum *Koch,* 1844 *par le ^{60}Co en accouplant des femelles normales à des mâles qui avaient reçu des doses de* 0, 2, 4, 6 *et* 8 *kilorad. L'accouplement a été observé chez les mâles de chacun de ces 5 groupes* 7 *jours après qu'on les eut posés sur leur hôte. Plus la dose de rayonnement reçue par ces mâles avait été forte, plus les femelles auquelles ils étaient accouplés ont mis de temps à se nourrir et moins nombreuses ont été les femelles qui ont atteint le stade de réplétion. De plus, la masse des femelles replètes était plus faible, un moins grand nombre ont pondu et le nombre d'oeufs pondus a montré une décroissance progressive. Il n'y a eu aucune éclosion dans les paquets d'oeufs produits par des femelles accouplées à des mâles irradiés. Chez les mâles des groupes* 2 *et* 4 *kilorad on n'a pas observé d'anomalies chromosomiques ou d'irrégularités dans la formation du spermiophore; chez ceux des groupes* 6 *et* 8 *kilorad, malgré un développement apparemment normal des glandes accessoires, les testicules n'ont montré aucune croissance, ni macroscopique ni microscopique. Les mâles d'*A. hebraeum *ont un complément chromosomique de* 10 *bivalents plus un chromosome sexuel univalent.*

INTRODUCTION

Amblyomma hebraeum is of considerable economic importance to farmers in South Africa. This 3-host tick is a vector of *Cowdria ruminantium,* the cause of heartwater in sheep, cattle and goats (Moore, 1912), and *Rickettsia conori,* the tick-bite fever pathogen of man, has been isolated from it (Mason & Alexander, 1939; Gear, 1954). Moreover, the deep wounds caused by its long mouthparts damage hides and are prone to secondary infection which may lead to suppuration and abscess formation (Freer, 1898). Blowflies, notably *Chrysomyia bezziana,* often lay their eggs in these wounds and cause severe maggot infestations in cattle (Lawrence & Cuthbertson, 1934).

A. hebraeum has a very wide host range. The immature stages (larvae and nymphae) feed on birds and various small mammals and frequently also on the same host as the adults, which usually favour the larger animals, including domestic stock and man (Theiler, 1962).

Work on the biology and behaviour of *A. hebraeum* has been done by Lounsbury (1899), Nuttall (1915, 1919), Theiler (1943), Thomas (1962), Norval (1974) and Norval & Capitini (1974). Since the females of this species do not attach to a host in the absence of males but readily do so adjacent to males that have been attached for 4–6 days and are already sexually mature, parthenogenesis apparently does not take place. The males remain on a host for a considerable time during which multiple matings take place. This suggests that *A. hebraeum* would be a likely candidate for sterile male control, as suggested by Norval & Capitini (1974). It has been shown that males of *Amblyomma variegatum* are sterilized by irradiation dosages of less than 8 kilorad (Beuthner, 1975), that egg batches of *Amblyomma americanum* fertilized by males irradiated at 2 kilorad and above do not hatch (Stanley & Oliver, 1975) and that *Rhipicephalus appendiculatus* males are sterilized by only 4 kilorad (Purnell, Dargie, Irvin & Ledger, 1973).

This study was undertaken as a preliminary to investigating the possibility of using sterile *A. hebraeum* males to improve the control of this problem species.

MATERIALS AND METHODS

Five groups, each containing 100 1-week-old *Amblyomma hebraeum* males, were subjected to an irradiation dosage of 120 kilorad (kR) per hour for varying lengths of time to attain dosages of 0, 2, 4, 6 and 8 kR, respectively. Irradiation was done by the Atomic Energy Board at Pelindaba Research Institute using a ^{60}Co source. Breeding experiments commenced 4 days after irradiation had taken place. Eighty males from each group were fed in bags fixed on the backs of sheep with a contact adhesive. A corresponding number of normal females was added 4 days after the males had been placed on the animals. In the course of daily observations, engorged, detached females were collected, mass measured and kept in an incubator (26 °C day, 18 °C night; 60 % RH throughout) to record preoviposition, oviposition and incubation periods, total egg production per female and percentage hatch per egg batch.

Percentage hatch was used as the criterion for determining the reduction of fertility induced in the males by the amount of irradiation received. Observations on the host ceased on Day 42 after the initial male infestation when no more live males could be found in the 8 kR group.

The remaining 20 males from each group, likewise fed on sheep, were used to study the effect of irradiation on spermatogenesis. At least 2 males from each group were removed daily and dissected, and squashes made of the testes using aceto-orcein stain (La Cour, 1941). The coverslips were sealed with nail varnish before the slides were examined.

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TABLE 1 Initial percentage attachment of males and females to the host. Females were placed on the host 4 days after the males

Irradiation group kR	Days on host									
	1st		2nd		3rd		4th		5th	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	84	0	97	30	100	63	100	94	100	94
2	91	0	98	35	100	71	100	97	100	97
4	72	0	96	26	96	81	96	91	96	96
6	94	3	100	40	100	92	100	96	100	100
8	82	0	96	31	98	75	98	90	98	96

Observations and Results

Breeding experiments

Attachment

The initial attachment of the males was not affected by irradiation and all viable males in both the control and irradiation groups had attached within 3 days of infestation (Table 1). There was no noticeable difference in the rate of attachment of females feeding with normal and those feeding with irradiated partners and all viable ticks had attached within 8 days of the initial male infestation (Table 1).

Feeding and detachment

Irradiation dosage had a proportional effect on the rate of female feeding and detachment. Females feeding with 2 and 4 kR group males appeared to engorge at the same rate as the controls (0 kR), but females with 6 and 8 kR group males fed more slowly, the first of these females only completing their engorgement and detaching 5 days after the first females of the 0, 2 and 4 kR groups. The greater the irradiation dosage of the male partners the lower were the initial numbers of detached females and later, the higher the numbers towards the end of the drop phase (Fig. 1). The majority of females feeding with irradiated males thus experienced a prolonged feeding period corresponding to the higher dosage received by their partners.

In all 5 groups, the longer the females remained attached to the host the lower was the mean mass of engorged females (Fig. 2).

The mean mass of engorged females from the irradiated groups was lower in each case than that of the control group and, with the exception of the 8 kR group, it decreased with the increased irradiation dosage (Table 2).

The yield of engorged females in relation to the original infestation differed considerably among the 5 groups (Table 2).

TABLE 2 Mean mass of engorged females, yield of engorged females from original infestation and mean preoviposition period of the 5 groups studied

Irradiation group kR	Mean engorged mass (g)	% Yield	Mean preoviposition period (d)
0	1,48	51,25	25,8
2	0,99	31,25	28,8
4	0,85	27,50	22,6
6	0,68	13,75	15,0
8	0,94	21,25	16,6

Preoviposition period

The mean preoviposition periods of the 5 groups are shown in Table 2. Shorter periods were recorded for females feeding with the 6 and 8 kR group males. A tendency for females with a higher mass to have shorter preoviposition periods than those with a lower mass was shown by the normal and 2 kR group females (Fig. 3). Females from the 4, 6 and 8 kR groups did not show this tendency (Fig. 4).

Oviposition period

The percentage of engorged females that oviposited in each group is shown in Fig. 5. Increased irradiation of their partners resulted in progressively fewer females ovipositing.

No effect on the mean oviposition periods of the 5 groups could be seen (Table 3). The relationship between the mass of the engorged female and number of eggs produced was linear for the normal group (Fig. 6), but no such relationship could be established for females mated with irradiated partners.

The mean number of eggs produced by females feeding with irradiated partners decreased with the increased irradiation dosage (Table 3).

TABLE 3 Mean oviposition period, range and mean number of eggs produced by females of the 5 groups studied

Irradiated group kR	Mean oviposition period (d)	Range (d)	Mean numbef of eggs produced
0	49,52	30–60	9 956
2	45,25	23–61	5 429
4	49,17	33–59	2 414
6	47,32	31–58	800
8	46,40	27–60	1 040

Incubation and hatch

Egg batches laid by normally mated females produced a mean of 93% viable larvae after a mean incubation period of 65 days, while no hatch was recorded from eggs laid by females mated with irradiated partners.

Spermatogenesis, mating and chromosome complement

A. hebraeum males have a chromosome complement of 10 bivalents and a univalent sex chromosome (Fig. 7). Spermatogenesis and spermiophore formation in the male (Fig. 8–11) proceed in the same manner as that described for *Boophilus decoloratus* by Londt & Spickett (1976), and no difference was observed between the control, 2 kR and 4 kR group males. No cell division, spermiophore formation or testes growth took place in the 6 and 8 kR group males.

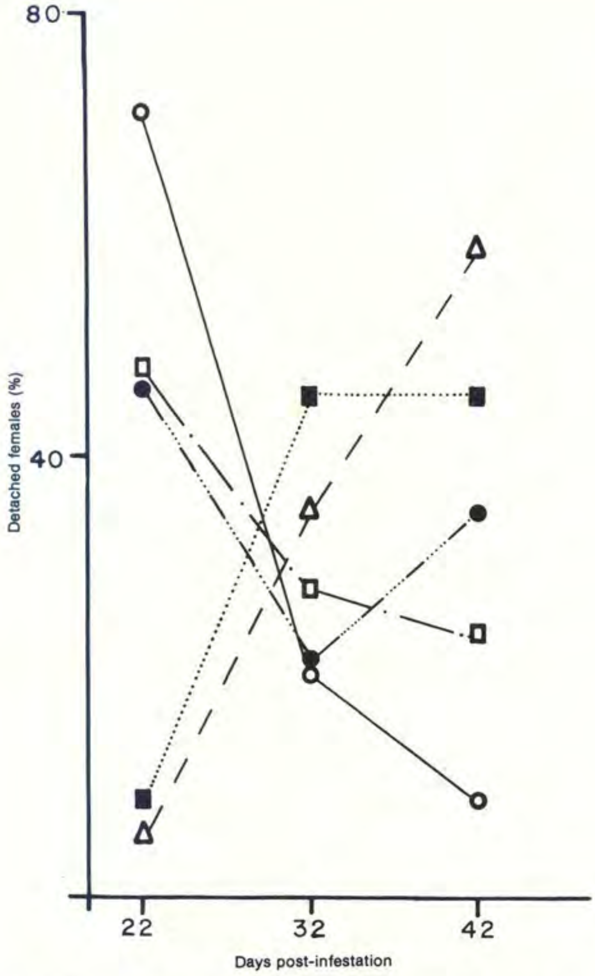

FIG. 1 Percentage drop of females within the 5 groups during the drop phase. ○ kR = 0, 2 kR = □, 4 kR = ●, 6 kR = ■, 8 kR = △

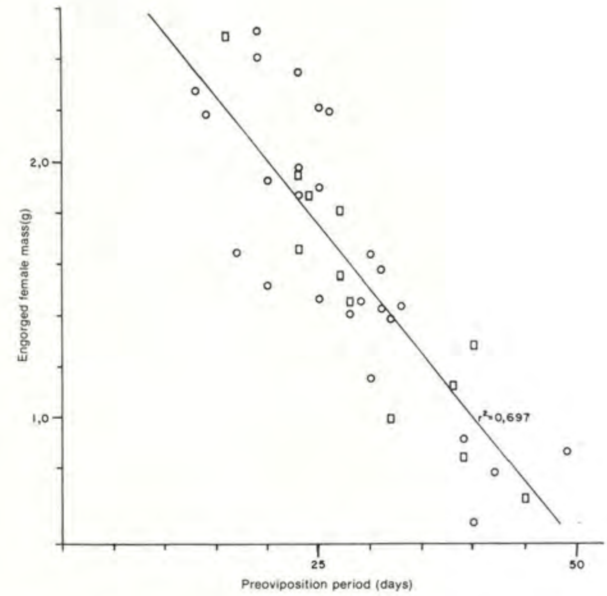

FIG. 3 The relationship of engorged female mass to preoviposition period of 0 kR (○) and 2 kR (□) group females. Linear regression fitted for 0 kR group, r^2=0,697

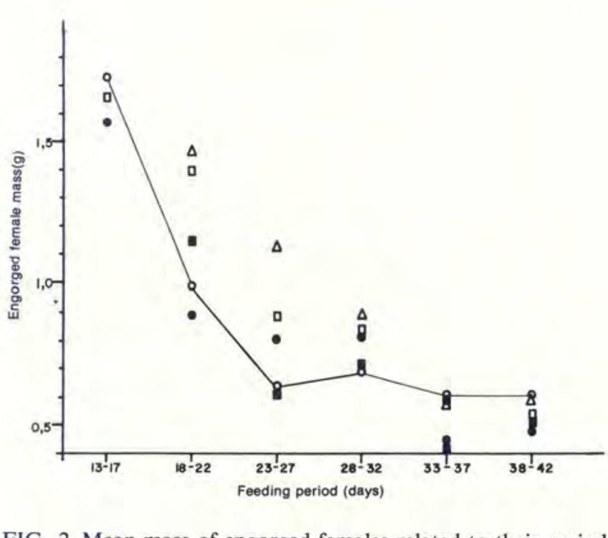

FIG. 2 Mean mass of engorged females related to their period of feeding. Symbols as in Fig. 1

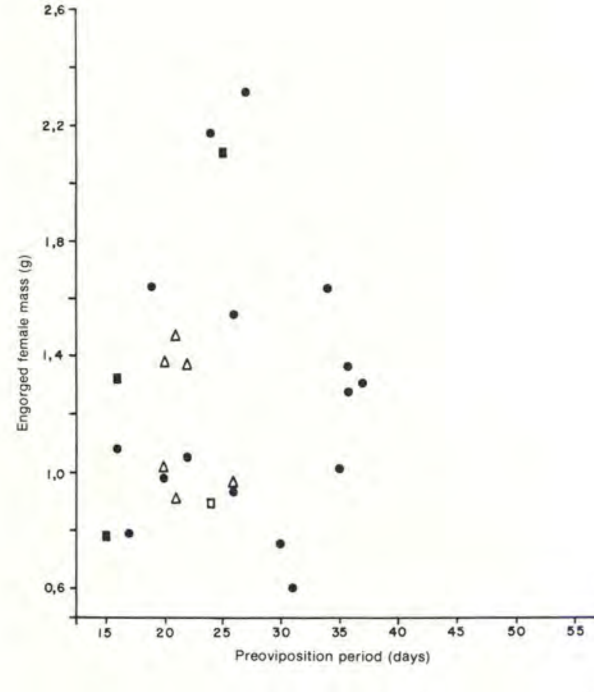

FIG. 4 The relationship of engorged female mass to preoviposition period of 4 kR (●), 6 kR (■) and 8 kR (△) group females

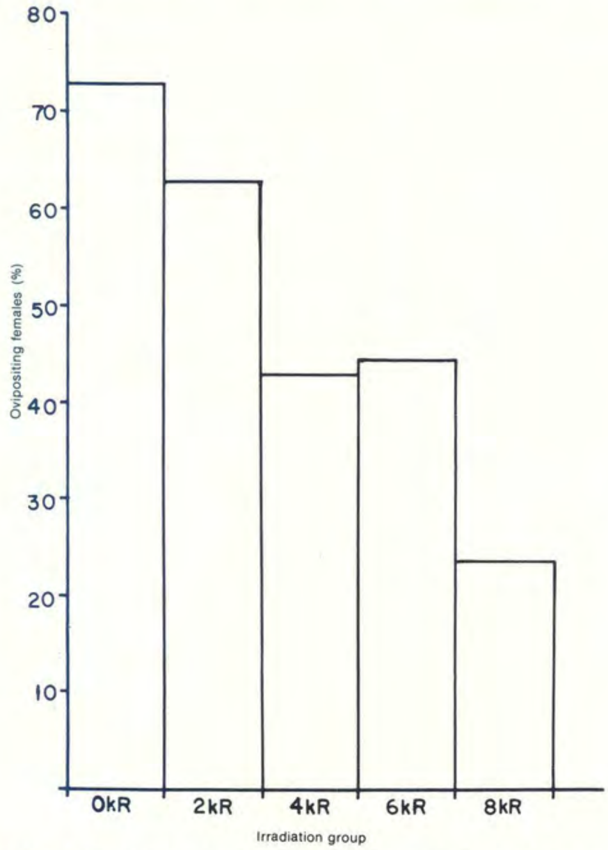

FIG. 5 Histogram showing the percentage of engorged females that laid eggs in each group

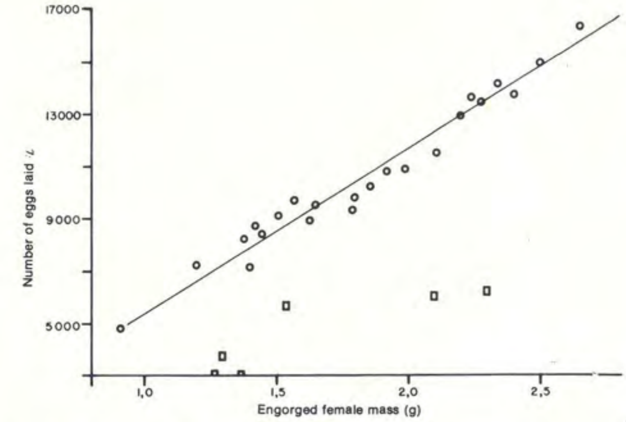

FIG. 6 The relationship of engorged female mass to number of eggs produced. 0 kR = 0, 2 kR = □

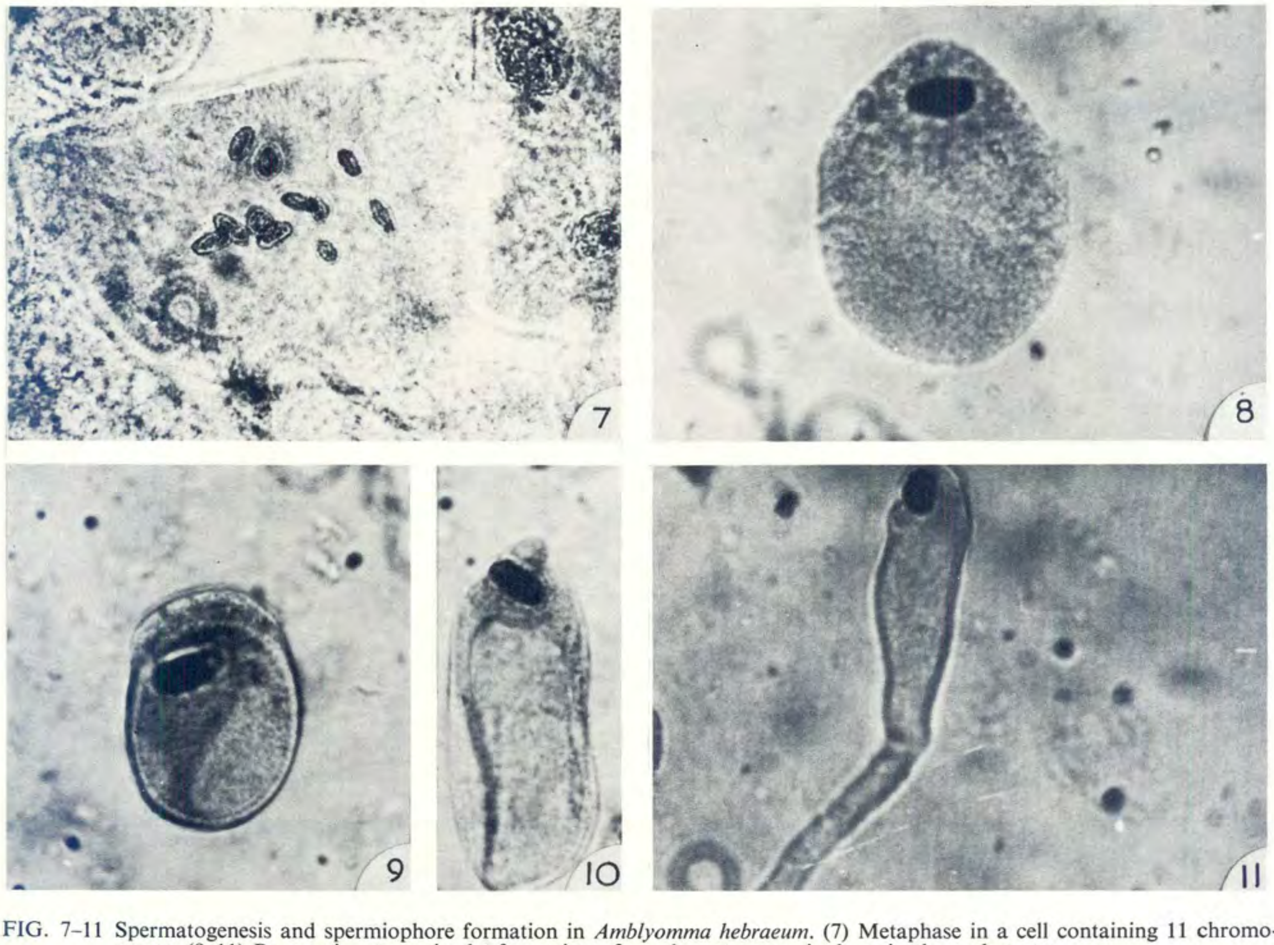

FIG. 7–11 Spermatogenesis and spermiophore formation in *Amblyomma hebraeum*. (7) Metaphase in a cell containing 11 chromosomes. (8–11) Progressive stages in the formation of an elongate spermiophore in the male testes

No chromosomal abnormalities were detected in any of the irradiated males examined. The 2 and 4 kR group males were slow to undergo meiosis but spermiophore formation and capacitation proceeded as in normal males, except that mature spermiophores were evident 1 day later than in normal males. Although no macro- or microscopical development of the 6 and 8 kR male testes was observed, accessory gland development appeared normal and males of all 5 groups were observed mating 7 days after being placed on the host.

Dissection of 4 engorged detached females from both the 6 and 8 kR groups revealed the presence of 2 spermiophore capsules in the seminal receptacles of each female but no evidence of spermiophores. Spermiophore capsules filled with spermiophores, however, were present in the seminal receptacles of engorged females from the 0, 2 and 4 kR groups.

Discussion

A. hebraeum females are attracted by males already attached to the host (Norval, 1974), so it is important when considering the possible application of sterile male control that attachment and subsequent feeding should not be influenced unduly by the sterilizing procedure. In these experiments irradiation of males had no apparent effect on the attachment of either sex, and mating took place in all 5 groups 7 days after the initial male infestation. Referring to *A. hebraeum,* Norval (1974) mentions that "completion of spermatogenesis is accompanied by physiological and behavioural changes, viz., the production of a sex pheromone and the response to the presence of a female". In these experiments males receiving 6 and 8 kR dosages did not undergo spermatogenesis, nor was there any growth of the testes, but mating did result in the introduction into the female of a spermatophore, although this did not contain any spermiophores. This suggests that the production of a sex pheromone is related, not to spermatogenesis as such, but possibly to attachment and feeding, release being triggered by the ability to mate, that is, the ability to produce a spermatophore, a function of the male accessory gland, which appeared to develop normally in the 6 and 8 kR group males.

The fact that none of the eggs laid by females mated with irradiated males hatched indicates that the lowest irradiation dosage tested, namely, 2 kR, will sterilize *A. hebraeum* males. This is the dosage that Stanley & Oliver (1975) found effective for *Amblyomma americanum* males. Norval (1974) found that *A. hebraeum* males remained attached to a host for up to 132 days and that some were able to mate with a maximum of 8 females. In these experiments all 8 kR group males died within 42 days. No attempt was made to determine whether a shortening of lifespan occurred in males receiving 6, 4 and 2 kR dosages.

Other aspects requiring investigation are the mating potential of irradiated males, female preference for normal and irradiated males and the possible loss of sterility by irradiated males after a lapse of time.

In *A. americanum,* Stanley & Oliver (1975) found a positive correlation between chromosome damage and increased radiation. No such correlation could be found for *A. hebraeum* with the techniques used, possibly because of the dosage tempo used and the stage at which radiation was applied.

The chromosome complement for *A. hebraeum* males, 2n=21, is the commonest type amongst ixodid ticks. If it is assumed that sex determination is by the xx:xo mechanism, as in most species of Metastriata (Oliver & Bremner, 1968), a chromosome complement of 2n=22 is suggested for *A. hebraeum* females.

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