THE EFFECT OF PRESENCE OF FEMALES ON SPERMATOGENESIS AND EARLY MATE SEEKING BEHAVIOR IN TWO SPECIES OF *DERMACENTOR* TICKS (ACARI : IXODIDAE) [1]

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ABSTRACT

Dermacentor variabilis and *Dermacentor andersoni* males were each fed in the presence of 0, 1, 2, 3, 6, 12 and 25 females and each group was analyzed for time of voluntary movement to seek females and rate of spermatogenesis. Males migrated earlier when females were present and were more prone to migrate with immature gonadal development in the presence of larger numbers of feeding females. However, spermatogenic rate appeared to be constant whether females were present or not. Since early detachment of *D. andersoni* males was stimulated by fewer females than the same response in *D. variabilis*, it is believed that *D. andersoni* males are more sensitive to the pheromone corroborating the finding of SONENSHINE, et *al.* (1976) indicating greater sensitivity of *D. andersoni* than *D. variabilis* to the sex pheromone.

ZUSSAMMENFASSUNG

Dermacentor variabilis und *Dermacentor andersoni* Männchen wurden in der Gegenwart von 0, 1, 2, 3, 6, 12 and 25 Weibchen gefüttert, und jede Gruppe wurde dann danach untersucht, wann die Männchen anfingen willkürlich die Gegenwart der Weibchen zu suchen und was das Mass der Spermatogenese war. In der Gegenwart von Weibchen suchten die Männchen diese früher und waren ausserdem mehr dazu geneigt, die Weibchen mit noch nicht einmal voll entwickelten Gonaden zu suchen, wenn diese essenden Weibchen in einer grösseren Anzahl vorhanden waren. Trotzdem zeigte es sich aber, dass das Mass der Spermatogenese konstant blieb, ob Weibchen anwesend waren oder nicht. Da die frühe Trennung von *D. andersoni* Männchen durch weniger Weibchen als bei *D. variabilis* hervorgerufen wurde, glaubt man, dass *D. andersoni* Männchen reizbarer für Pheromone sind ; dieses besttäigt aber die Resultate von SONENSHINE, et *al.* (1976) der die grössere Reizbarkeit von *D. andersoni* im Gegensatz zu *D. variabilis* bezüglich des Sex Hormone Pheromones andeutete.

Sex pheromone regulation of mate finding behavior in ticks has been reported by several workers (BERGER et *al*, 1971 ; BERGER, 1972 ; GLADNEY et *al.*, 1974 ; CHOW, et *al.*, 1975 ; WOOD et *al.*, in press ; SONENSHINE et *al.*, 1974 ; SONENSHINE et *al.*, in press). Sex pheromones of insects were defined by JACOBSEN et *al.* (1970) as " chemical substances produced and released by one

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sex to attract or excite the opposite sex for mating ". Recently, studies by SONENSHINE et *al.* (1974) have suggested an extension of this role of the sex pheromone in ticks, namely, as a stimulus for early mate seeking behavior and spermatogenesis. These authors reported that males of *D. variabilis* and *D. andersoni*, when feeding in the presence of females, detached significantly sooner than males feeding alone. Detaching males reattached beside feeding females, with frequent instances of apparent copulation ; many copulating males had been attached only 3 days or less. Relatively large proportions of males were reported to be immature. However, it was not clear whether spermatogenesis may have been accelerated in these ticks.

The possibility that sex speromones may affect the rate of maturation of the opposite sex represents a potentially important new dimension in the role of these chemical regulators and, therefore, worthy of further study. A detailed analysis of the rate of spermatogenesis of *D. variabilis* on hosts within females was done by HOMSHER and SONENSHINE (1972) ; the rate of development in *Dermacentor occidentalis* Marx was studied by OLIVER and BRINTON (1972). New studies reported in this paper provide an opportunity to compare the rate of spermatogenesis in males feeding in the presence of females with the rate in those feeding without them and, consequently, determine the effect of sex pheromones on the male reproductive process.

MATERIALS AND METHODS

Groups of 10 attached males were confined in a closed environment (7.74 cm^2) on albino rats, *Rattus norvegicus*, with 0, 1, 2, 3, 6, 12 and 25 females. Males which had detached and reattached adjacent to females or were in copula with females were removed and dissected. The reproductive tract was removed and aceto-orcein smears prepared for microscopic evaluation of spermatogenesis. Similarly, where females were absent, the reproductive tracts of males that had detached voluntarily were removed and examined. The most advanced stage of development of the reproductive tissue was recorded. If spermatids were present, a spermatid estimate was made on a scale of 0 (no spermatids) to 10 (spermatids present in at least 10 of 12 fields examined). Identification of spermatogenic stages was based on HOMSHER and SONENSHINE (1972) and OLIVER and BRINTON (1972). Terminology follows that of OLIVER and BRINTON, except that the term " young spermatid " is used for meiotic products up to the point of nuclear elongation and " advanced spermatid " refers to that stage of cellular development wherein the haploid nucleus has elongated and apparently migrated to the lateral margin.

RESULTS

Male *D. variabilis* and *D. andersoni* detached and migrated earlier if females were present in the environment (Tables 1 and 2). The mean period of attachment of *D. variabilis* males declined from 6.19 days when no females were present to as little as 4.26 days when females were present in the environment. The relationship between the duration of male attachment to hosts and the number of females present was found to be highly significant when tested by the method of least squares, using the least squares power equation $y = a^{bx}$ ($r = 0.797$, $p < 0.05$, 6 d. f.). Similar results were obtained with *D. andersoni* males. In this case the duration of male attachment declined from 6.57 days when no females were present to as little as 5.20 days when 25 females were present. Results of the least squares test indicated a highly correlated relationship ($r = 0.868$, $p < 0.05$, 6 d. f.).

TABLE 1. — Evidence of early detaching and migration of *D. variabilis* males in relation to the number of feeding females present in the same closed environment.

No. Days Males Attached	No. males detaching when the number of females present was						
	0	1	2	3	6	12	25
1	0	0	0	0	0	0	1
2	0	0	0	0	0	1	0
3	2	1	3	7	6	6	5
4	0	2	3	4	5	6	6
5	4	3	1	6	5	2	1
6	9	3	8	5	3	6	8
7	2	4	3	2	0	0	2
8	2	1	1	0	0	0	0
9	1	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	1	0	0	0	0	0	0
$\bar{x}$	6.19	5.71	5.42	4.63	4.26	4.29	4.86
± S.D.	1.81	1.44	1.50	1.35	1.10	1.31	1.60

TABLE 2. — Evidence of early detachment and migration of *D. andersoni* males in relation to the number of feeding females present in the same closed environment.

No. Days Males Attached	No. males detaching when the number of females present was						
	0	1	2	3	6	12	25
4	0	2	1	3	1	1	2
5	1	1	1	1	2	4	1
6	2	5	5	5	6	4	4
7	3	1	3	2	0	1	0
8	1	2	0	0	0	0	0
$\bar{x}$	6.57	6.00	6.00	5.55	5.56	5.50	5.20
± S.D.	0.98	1.34	0.94	1.13	0.73	0.85	0.79

TABLE 3. — Spermatogenic development in *Dermacentor variabilis* males in relation to the number of females present.

Stage of Spermatoenesis	No. males and developmental stage in the presence of 0 Fem.	1 Fem.	2 Fem.	3 Fem.	6 Fem.	12 Fem.	25 Fem.
Pre-spermatid Stages	3	1	4	4	10	12	10
Percent	14.3	6.7	21.1	16.7	52.6	57.1	43.5
Young Spermatids	4	4	5	8	2	3	5
Percent	19.0	26.7	26.3	33.3	10.5	14.3	21.7
Advanced Spermatids	14	10	10	12	7	6	8
Percent	66.7	66.7	52.6	50.0	36.8	28.6	34.8
Total Males	21	15	19	24	19	21	23

Table 3 shows the state of spermatogenic development in *D. variabilis* males feeding in the presence of different numbers of females. The number of males migrating with immature gonadal development (e.g., testes with only mitotic, early meiotic, or late meiotic stages) increased when the number of females in the environment increased. This was especially evident when the number of females was 6 or greater. Most males feeding in environments where no females or only 1-3 females were present had advanced spermatids in the testes when they migrated ($\geq$ 50 %), whereas only 28.6 % — 36.8 % of the migrating males had advanced spermatids when 12 or more females were present. Table 4 summarizes the results of a similar study with *D. andersoni*. Presence of female ticks feeding in the same environment as feeding males resulted in an increase in the percentage of males migrating before they were sexually mature. However, in contrast to the results obtained with *D. variabilis*, introduction of even a single female resulted in migration of significantly more males before they completed spermatogenic development. No relationship was found between the state of development of the male gonads when they migrated and the increase in the number of feeding females present.

To determine whether the presence of females directly influenced the progress of spermatogenesis, the time (in days) of voluntary migration of males which had achieved each developmental stage was compared with the number of females present. No relationship was found. In *D. variabilis*, sufficient data was available for comparison of 3 stages of development, namely the prespermatid, the young spermatid and the advanced spermatid stages (Table 5). To facilitate this comparison, migrating males in each group were classified as to stage of development and their numbers converted to percent. Total percent feeding days was calculated also. In those males developing only to prespermatid stages, the percentage of migrating males and the percent male feeding days increased as more females were added to the environment. This is indicative of early migration and does not necessarily prove accelerated spermatogenesis. Little change was found in males exhibiting the young spermatid stage. In this case, the total percentage of migrating males was unchanged, but the total percent male feeding days declined only when large numbers of females were present. Finally, in the case of males migrating only

TABLE 4. Spermatogenic development in *Dermacentor andersoni* males in relation to the presence of females in the environment.

Stage of Spermatogenesis	0 Fem.	1 Fem.	2 Fem.	3 Fem.	6 Fem.	12 Fem.	25 Fem.
Pre-spermatid Stages	0	2	2	0	2	1	1
Percent	0.0	18.2	20.0	0.0	22.2	10.0	10.0
Young Spermatids	1	6	3	6	4	3	6
Percent	16.7	54.5	30.0	54.5	36.4	30.0	60.0
Advanced Spermatids	6	3	5	5	3	6	3
Percent	85.7	27.3	50.0	45.5	33.3	60.0	30.0
Total Males	7	11	10	11	9	10	10

TABLE 5. — Proportions of male *D. variabilis* ticks maturing to specific stages of spermatogenesis in relation to duration of attachment and numbers of female ticks present.

Day on which male moved	Prespermatid Stages: % males moving when No. Females present was [1]							Young Spermatid Stage: % males moving when No. Females present was [1]							Advanced Spermatid Stage: % males moving when No. Females present was [1]						
	0	1	2	3	6	12	25	0	1	2	3	6	12	25	0	1	2	3	6	12	25
1	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	5	0	5	4	16	33	13	0	0	0	8	0	0	0	5	7	5	13	11	5	9
4	0	7	11	0	5	19	22	0	7	5	8	11	0	4	0	0	0	8	0	5	0
5	0	0	0	4	5	0	0	5	7	0	13	5	5	4	14	13	5	8	5	19	0
6	0	0	0	4	0	0	4	5	7	11	4	0	5	13	38	13	32	13	16	0	17
7	0	0	0	0	0	0	0	0	0	11	0	0	0	0	5	27	5	8	0	0	4
8	0	0	0	0	0	0	0	5	7	5	0	0	5	0	5	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Percent	5	7	16	16	26	52	47	20	28	32	33	16	15	21	67	60	47	50	32	29	30
Total percent feeding days	15	28	59	56	93	175	155	150	161	202	145	69	95	114	358	353	267	245	154	130	157

[1] Numbers in columns under each of the 3 stages of spermatogenic development represent the percentage of individuals present that achieved that particular stage on the day of voluntary migration.

when they achieved the advanced spermatid stage, both the percent of migrating males and the total percent feeding days decreased. Fully two-thirds (67 %) of the males feeding in the absence of females delayed migration until they achieved this stage of development, whereas only 30 % did so when 25 females were present. Similar findings were made with *D. andersoni*. In the species, sufficient data was available for comparison of 2 stages of development, namely, young spermatids and advanced spermatids. Increases in the number of females from 1 to 25 did not affect the migratory tendency at either stage of spermatogenic development.

Direct measurement of the rate of spermatogenesis was hindered by migration of some of the males before they had completed this process. Therefore, sampling of attached males at regular intervals to determine gonadal development was not possible. However, it was possible to estimate the approximate time required to produce advanced spermatids in *D. variabilis* (based on HOMSHER and SONENSHINE, 1972) once the reproductive status of each individual was known. Similarly, the same estimates were used for *D. andersoni*; consequently, males which detached voluntarily were dissected and their reproductive tracts were examined to determine the progress of spermatogenesis in these individuals. The additional time that would have elapsed if each of these males had been allowed to produce advanced spermatids was estimated, based on HOMSHER and SONENSHINE (1972). Subsequently, the mean duration to achieve advanced spermatids was determined for each group. Table 7 shows the results of this analysis. In the case of *D. variabilis*, it is obvious that no significant change in the rate of spermatogenesis occurred as the number of females increased. In the case of *D. andersoni*, no consistent trend was found.

DISCUSSION

Little attention has been given to the rate of spermatogenesis in ixodid ticks. Aside from the work of OLIVER and BRINTON (1972) on *D. occidentalis* and HOMSHER and SONENSHINE (1972) on *D. variabilis*, we were unable to find evidence of other studies of the time required for the various phases of the process in hard ticks. OLIVER (1974) refers to unpublished studies concerning timing of spermatogenesis and spermiogenesis in *Ixodes holocyclus*, but no details are given. In this same article, OLIVER notes that feeding and the acquisition of sufficient nutrient material are critical factors in determining the onset of spermatogenesis and probably stimulate the neurosecretory activity that mediates this process.

The results of the present study suggest that spermatogenesis, at least in the 2 species of *Dermacentor* ticks studied, is initiated by the nutritional stimulus and proceeds at a more or less constant rate, irregardless of the presence of feeding females. However, some differences between *D. variabilis* and *D. andersoni* were noted, e.g., even a single *D. andersoni* female induced early detaching of the homologous males in contrast to 6 or more females required for this effect in *D. variabilis*. The reason for this difference is unknown, but may be related to the greater sensitivity of *D. andersoni* males to the sex pheronome. SONENSHINE et *al.* (1976) noted that *D. andersoni* males responded to as little as 0.5 nanograms of 2,6 dichlorophenol, whereas *D. variabilis* responded to as little as 2.0 nanograms of this compound.

Our findings suggest that the effect of the sex pheromone, at least in the 2 species studied, is limited to attraction of the opposite sex.

TABLE 6. — Proportions of male *D. andersoni* ticks maturing to specific stages of spermatogenesis in relation to duration of attachment and numbers of female ticks present [1].

Day on which male moved	Young Spermatids: % males moving when no. Females present was							Advanced Spermatids: % males moving when no. Females present was						
	0	1	2	3	6	12	25	0	1	2	3	6	12	25
4	0	0	0	9	0	0	0	0	18	0	18	0	0	10
5	14	0	0	9	11	20	40	0	0	0	0	0	20	0
6	0	45	20	36	27	10	20	29	0	30	9	33	30	20
7	0	9	10	0	0	0	0	43	0	20	14	0	10	0
8	0	0	0	0	0	0	0	14	9	0	0	0	0	0
Total %	14	54	30	54	38	30	60	86	27	50	41	33	60	30
Total % of feeding days	70	333	190	297	217	160	320	587	144	320	224	198	350	160

TABLE 7. — The effect of the presence of feeding females on the rate of spermatogenesis in the ticks *D. variabilis* and *D. andersoni* as determined by comparison of the projected mean time (hours) for complete maturation of the testes [1].

	Mean time (hours) to complete maturation of the testes when the number of females present was:						
	0	1	2	3	6	12	25
A. *D. variabilis*							
Range	72-292	72-194	72-202	72-250	113-203	113-194	72-204
Mean	153.0	141.1	150.4	135.5	148.4	160.1	159.7
± S.D.	47.1	37.0	30.1	44.3	30.5	27.4	42.6
B. *D. andersoni*							
Range	143-193	95-218	136-167	89-160	136-218	112-168	89-150
Mean	158.9	155.2	150.9	128.5	152.0	137.3	130.8
± S.D.	17.6	39.8	13.2	29.7	34.2	18.0	19.0

1. Numbers in columns under each of the 2 stages of spermatogenic development represent the percentage of individuals present that achieved that particular stage on the day of voluntary migration.

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