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SPERM REPLACEMENT AND DEPLETION IN THE SPERMATHECA OF THE S AND CS STRAINS OF *ONCOPELTUS FASCIATUS*

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New strains of *O. fasciatus*, reared at 30° C either on seeds of sunflower (strain S) or mixed sunflower and cashew nuts (strain CS), were used. Females of wild (+) or mutant (*wb*) genotype were mated in various sequences with + males (some of them chemosterilized or castrated) or *wb* males. Eggs of each clutch laid were counted and characterized as: infertile, or non-viable (apparently fertile but unable to hatch), or hatching into + or *wb* nymphs. Mating with a second male (except a castrate) causes partial or complete replacement of the spermathecal store of spermatozoa provided by the first male. A model of the replacement mechanism is proposed, which suggests that a volume of semen five times greater than the capacity of the spermatheca must be transferred by the male to cause the complete disappearance of the previous store of sperm.

In the absence of males, or in the presence of castrate males, fertile eggs may be laid for at least 5 weeks but in most cases the store of sperm becomes exhausted sooner. The minimum store of sperm (estimated from the total count of viable eggs) is highly variable (40—400, mean 180). The percent of viable eggs in successive egg-clusters declines proportionately as the sperm store is reduced from 60 to 0.

The fate of spermatozoa after insemination, especially in those insect species in which the female may mate with many different males, is not only of theoretical importance in population biology but may be of practical importance in programs of insect control by male sterilization. The phenomena of sperm precedence and sperm displacement, which ensure that all or most of the eggs laid by a female will be fertilized by sperm provided by the male with which she has most recently mated, have been observed in the coleopterans *Anthonomus grandis* (Lindquist & House, 1967) and *Tribolium castaneum* (Schlager, 1960), in the dipteran *Drosophila melanogaster* (LeFevre & Jonsson, 1962), and in the lepidopterans *Euphydryas editha* (Labine, 1966), *Heliothis virescens* (Flint & Kressin, 1968) and *Spodoptera frugiperda* (Snow *et al.*, 1970). We decided to do a similar study in the hemipteran *Oncopeltus fasciatus* (Dallas) as part of a larger study of male chemosterilization in this species (Economopoulos & Gordon, 1969; Economopoulos, 1970-a).

Mating in colonies of *Oncopeltus* is promiscuous and highly variable both in frequency and in duration (Loher & Gordon, 1968). It is not unusual to find 20 to 30% of the individuals in a colony engaged in copulation, since copulation often

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lasts for many hours and both sexes can copulate more than once a day. Semen is injected directly into the small spermatheca of the female through the very long, fine male penile tube (Bonhag & Wick, 1953). The flow rate is probably very slow, since only copulations lasting longer than 3 hours have a high probability of transferring sperm (Loher & Gordon, 1968). The indirect estimates of Gordon & Gordon (1971) suggest that the number of sperm stored in the spermatheca varies greatly in different females and is usually well below 1000.

The work reported here was designed to explore the effect of successive matings on the number and kind of sperm stored, by making use of both a wild-type and a color-mutant strain and of chemosterilized males to "mark" the spermatozoa, and also of castrate males to examine the effect of mating without sperm transfer.

METHODS

All experiments were performed at 30° C. Two strains of *Oncopeltus* were used. The wild-type (+) strain was the Berkeley CS strain, which had been reared exclusively on a mixture of cashew nuts (*Anacardium occidentale L.*) and hulled sunflower seed (*Helianthus annuus L.*) for more than 15 consecutive generations. At the time this work was done, this was our major experimental strain, but it has since been replaced by its daughter S strain, which now does as well or better on sunflower seed alone. The white-body (*wb*, an autosomal recessive mutant) strain was originally obtained from Dr. P. A. Lawrence (Cambridge, England), who maintains it (like most laboratory strains of *Oncopeltus*) exclusively on milkweed seed (Lawrence, 1970). We crossed it with our S strain and recovered the *wb* homozygotes (now on a sunflower-adapted genetic background) in the F_2. The F_4 was used in our experiments, although its adaptation to sunflower was still imperfect at this stage. Mortality was somewhat higher in the egg, nymph, and adult stages than for the CS or S strains, which had had a much longer period of adaptation to a non-milkweed diet. Although the *wb* males and females used were vigorous individuals, in many crosses 10% or more of the fertilized eggs did not hatch.

For some experiments, males of the CS strain were castrated on the first day after the imaginal molt. At this stage the mature sperm in the testis has not yet been transferred into the accessory ducts. Short incisions were made in the right and left side of the second abdominal segment, and the testes (together with the seminal vesicle, but not the mesadenes) pulled out with fine forceps. The wounds were then sealed with liquified beeswax on a heated fine spatula. Mortality from the operation was 30—40%, but the survivors were long-lived and able to mate, as previously shown by Loher & Gordon (1968). They will be referred to as *c* males.

Adult + males of the CS strain were sterilized at 8—10 days of age by topical application of either 1 or 1.8 μg of tretamine in 1 μl of acetone, as described by Economopoulos & Gordon (1969). Both the resulting 20 μg/g and 36 μg/g dosages cause total sterility for a period of 2 weeks or longer, but the spermatozoa

are not inactivated and can penetrate the eggs, but the eggs evidently are not fertilized. Since these *s* males were crossed with *wb* females in the experiments, any non-sterile sperm would be recognizable since it would give rise to a phenotypically + nymph; however, this never occurred. Males treated with 1 μg are referred to as s_1, with 1.8 μg as s_2.

Several experimental designs were used. In the first series, *wb* females 8—14 days of age were paired either with *wb* or + males of about the same age for 6 days. The males were then replaced by males of the other type for a second 6-day period. The second male was removed and the isolated female kept until death. The rearing jars were checked every day. When an egg-cluster was found, the eggs were counted, isolated in plastic cups, and incubated until they hatched. Counts of infertile and fertile eggs were in some cases made on the third day, when infertile eggs are pale-colored (whitish in the *wb* eggs) and shriveled while fertile eggs have become pink or red (Gordon & Gordon, 1971). In many cases, however, a count of infertile eggs was made on the first day, based entirely on the degree of shriveling of the eggs. Later data analysis indicated that such estimates of egg fertility were often too high. Nymph counts on the day of hatching were more accurate, and the *wb* and + phenotypes could be easily distinguished. Since not all eggs hatched on the same day, the early-hatching nymphs were killed and the incubation continued until hatch was completed. For each egg-cluster, therefore, there were as many as four possible types: infertile eggs, fertile but non-viable eggs that failed to hatch, and eggs that hatched either into + or into *wb* nymphs.

In a second series, involving *s* males, sequences of three successive males (either *wb, s, wb* or *s, wb, s*) were used with *wb* females, each male being kept with the female for a 6-day period before being replaced. On the 19th day the last male was removed and the female kept alone until death. Egg-clusters were harvested and counted as in the first series. The presence of sterile sperm in the female was detectable by the high count of fertile but non-viable eggs.

In a third series, mating of a + female with a + male was followed by replacement of the + male by two castrate (*c*) males 5—6 days old. The castrates were kept with the female until her death; any *c* male that died was immediately replaced by another of about the same age. A few matings of virgin females exclusively with *c* males yielded only infertile eggs, in agreement with the earlier results of Gordon & Loher (1968).

RESULTS AND DISCUSSION

Method and examples of analysis of data. Many of the females set up for single-pair studies were infecund or died early, but 87 yielded useful data. The complete set of original data is available (Economopoulos, 1970-a) but much too extensive to be presented here. In order to facilitate evaluation, the numerical values were condensed into a series of "stages" by pooling the values for all egg-clusters produced during each successive stage. The recognizable (although somewhat arbitrary) stages were as follows:

Stage 1. Only infertile eggs laid.

Stage 2. Many fertile eggs and (if the first male in the sequence was not chemosterilized) many hatching into progeny of the first male.

Stage 3. More than half of the fertile eggs in each cluster fertilized by sperm from the first male in the sequence, and the remainder by sperm from the second male.

Stage 4. More than half of the fertile eggs fertilized by sperm from the second male, and the remainder by sperm from the first male.

Stage 5. All or nearly all of the fertile eggs fertilized by sperm from the second male, and very few or none by the first male.

Stages 6, 7, and 8 were found when the sequence included a third male, and were equivalent to stages 3, 4, and 5 in the degree of sperm replacement observed.

Stage —1 was usually observed some time after removal of all males. It involved sperm depletion, the percent of infertile eggs tending to increase in successive egg-layings.

Stage —2 followed —1. All (or nearly all) eggs were infertile; when some eggs were sometimes (probably erroneously) judged to be fertile, as a rule very few or no eggs hatched.

Most of the females did not go through every one of the possible stages. Stage 1 was usually absent. Stage 2 was often followed directly by stage 5. There were large individual variations in the patterns of egg production, viability and sperm replacement. The complete stage-analysis tabulation of all experiments is available (Economopoulos, 1970-a) but only 11 selected examples are presented in Table I to illustrate the variety of results obtained. Females 13 and 29 showed immediate, complete, and permanent disappearance of sperm from the first male after his replacement by the second male. Females 14, 24, and 26 showed transitional mixed-sperm stages. Some of these *wb* females (14, 26, and 29) consistently laid a fairly high percentage of fertile but non-viable eggs, possibly an effect of imperfect adaptation to the sunflower seed diet. However, even egg-clusters laid by virgins or by females mated with castrate males sometimes contained 10% of eggs that were judged to be fertile although they never hatched. Such large errors were uncommon but their occurrence lowers the reliability of values for percent of fertile but non-viable eggs.

Females 34 and 67 are representative of a large series of females mated with a sequence of three males, demonstrating that either s_1 or s_2 sperm can replace and be replaced by *wb* sperm. Females 103, 116, and 120 are from a large series that had a + male present during the first few days and two castrate males continuously present thereafter. The castrate males mated, but production of fertile eggs was unaffected. Female 120 had an unusually small store of sperm that was quickly depleted, and then laid a very large number of infertile eggs.

Females 303 and 26 are of interest because the sperm store at the start of depletion (with no males present) was a mixture of *wb* and + sperm. The results indicate that there was a unitary sperm pool, with no marked change in composition

as depletion occurred. There is not, as in *Tribolium* (Schlager, 1960), a hidden reserve store of sperm transferred earlier that can be subsequently utilized by the female. For this reason we use the term "replacement" rather than "displacement", since the latter is ambiguous about the fate of the "displaced" sperm.

Mating causes a permanent loss of previously stored sperm, not a mere dilution. Of the females in our first series (numbers 11 to 29), 11 lived long enough to produce more than 100 nymphs after replacement of the first male by the second male. Of these, 4 produced only progeny of the second male among 1064 nymphs counted, although all had produced eggs fertilized by the first male while he was present. The other 7 produced 136 progeny of the first male and 821 progeny of the second during the 6-day period during which the second male was present and 25 progeny of the first male and 1965 progeny of the second after removal of the second male. The mean number (23, range 4 to 54) of progeny of the first male produced after his removal is only 14% of the mean number (162, range 90 to 272) of progeny of the second male after his removal. That the first male is not less efficient in transfer of sperm is shown by the 100 series, in which the mean number of nymphs produced per female after replacement of the first male by castrate males was 183. We conclude that in each successful sperm transfer there is a permanent loss of previously stored sperm concomitant with the gain of new sperm; this loss is sometimes total but more commonly partial. Lawrence (1970) briefly refers to experiments on *Oncopeltus* with results generally similar to our own.

Sperm transfer and replacement. The frequency of occurrence of different degrees of sperm replacement in all our experiments is summarized in Table II, which also includes values for the effectiveness of sperm transfer to virgin females by the various types of males. In all cases it is the degree of sperm transfer that is being crudely measured, whether it causes replacement of sperm-free fluid in a virgin spermatheca or of sperm-filled fluid in a previously fertilized female. The percentages do not have quite the same meaning, however. As will be shown in the next section, quite small sperm transfers (of the order of 50) to virgin females could cause fertility of 36% or more in a small cluster of eggs laid soon afterward, and transfers of the order of 100 would probably give a fertility above 80%. On the other hand, since we visualize sperm replacement as a "washing out" of the spermathecal contents by the inflow of fresh seminal fluid, it is likely that only a large transfer will cause this. Apparently only about 20 to 30% of all first matings in our strain of *Oncopeltus* are at this level of effectiveness (group A in Table II). However, in another 20 to 45% of the cases this level is attained in a subsequent mating (group B), so that about two-thirds of all the normal males can (during the 6-day period allowed) transfer a large amount of seminal fluid. Chemosterilized males are less effective; the probable cause is a reduction in their mating drive, indicated by abnormally low mating frequency observed both in populations and in a detailed study of many individuals sterilized with tretamine (Economopoulos, 1970-b). Castrate males, on the other hand, seem to be unable to replace stored

TABLE I

Examples of condensed stage-analysis data for various mating sequences

♀ No. (♂ sequence)[a]	Stage	# of EC[b]	# of Eggs	% of eggs that were: [c] INF	FNV	+	wb
13	2	1	52	0	0	0	100
(*wb*, +)	5	9	266	1	3	96	0
	—1	11	300	75	2	23	0
	—2	10	212	89	6	5	0
14	1	1	52	96	4	0	0
(*wb*, +)	2	2	61	0	20	0	80
	3	1	34	0	35	30	35
	4	3	136	0	37	56	7
	5	3	63	11	16	73	0
24	2	3	110	0	2	98	0
(+, *wb*)	3	1	26	0	0	50	50
	4	4	123	0	4	11	85
	5	7	229	2	3	3	92
	—1	2	49	61	4	0	35
	—2	3	37	100	0	0	0
26	2	4	164	7	4	89	0
(+, *wb*)	4	5	149	0	24	6	70
	5	14	384	0	26	3	71
	—1	4	88	48	22	2	28
	—2	1	13	100	0	0	0
29	2	5	133	1	9	90	0
(+, *wb*)	5	12	202	0	12	0	88
	—1	11	229	38	17	0	45
	—2	5	71	79	21	0	0
34	2	3	149	12	9	0	79
(*wb*, s_2, *wb*)	5	6	168	0	100	0	0
	8	15	263	2	2	0	96
	—1	4	210	57	11	0	32
	—2	20	165	87	13	0	0
67	1	3	73	100	0	0	0
(s_1, *wb*, s_1)	2	3	93	3	97	0	0
	4	1	53	0	34	0	66
	5	9	261	7	8	0	85
	6	1	15	0	33	0	67
	8	5	110	1	98	0	1
103	2	20	471	0	4	96	0
(+, *c*)	—1	6	121	52	12	36	0
116	2	10	329	1	0	99	0
(+, *c*)	—1	4	109	32	4	64	0
	—2	12	377	96	4	0	0
120	2	2	35	49	2	49	0
	—1	1	28	93	0	7	0
	—2	28	541	95	5	0	0
303	2	5	89	0	15	0	85
(*wb*, +)	3	3	91	0	0	5	95
	—1	4	93	11	41	3	45
	—2	1	11	91	9	0	0

TABLE II

Frequency of sperm transfer, with and without replacement of previously stored sperm

Male type	No. of males	Type of sperm replaced [1]	Percentage with transfer of degree [2]			
			A	B	C	D
+	30	0		87	13	0
+	15	*wb*	20	40	33	7
wb	19	0		63	27	10
wb	9	+	22	45	33	0
wb	15	s_1	27	33	33	7
wb	9	s_2	33	23	33	10
s_1	10	0		100	0	0
s_1	12	*wb*	17	33	25	25
s_2	8	0		50	13	37
s_2	10	*wb*	20	20	50	10

[1] All females were *wb* except in the first line, which included both + and *wb* females (which gave similar results). Type 0 indicates that the females were virgin. Six-day period of exposure of females to males at 30°.

[2] Group D, no evidence of sperm transfer by the male. Group C, sperm transfer causing 36—79% egg fertility in virgin females or a sperm mixture consisting of 10—50% of each of the 2 types in previously mated females. Group A-B for virgin females, 80% or higher egg fertility. For previously mated females, Group A showed an immediate decrease (to a level of 0—3%) of the sperm type of the first male, while Group B showed a comparable decrease only after a transitional stage like that of Group C.

sperm by sperm-free semen when they mate. Either the accessory glands alone contribute very little to the volume of seminal fluid, or the whole semen-transfer mechanism is rendered inoperative by the castration injury. If our interpretation of the sperm-replacement phenomenon is correct, this means that there is little or no fluid transfer into the spermatheca when castrates mate.

If the volume of the spermatheca, V, is constant, the influx of fresh seminal fluid must be equal to the efflux of spermathecal fluid. The flux can be expressed as the fraction (k) of V flowing in or out per hour. We cannot estimate the rate of mixing of the seminal and spermathecal fluid, but since spermatozoa are highly motile we can expect rapid mixing of new and old sperm. If we assume that there is no retention (by a filtration or other mechanism) of sperm present in the region of outflow,

Legend of Table I

[a] Females were homozygous *wb* mutants except in the 100 series, where they were wild-type (+). The *c* males were castrated males and the s_1 and s_2 males were males sterilized with tretamine at 20 and 36 mg/kg respectively. Males were kept singly with the females for 6 days, but castrates were kept in pairs with the female until her death.

[b] Number of egg-clusters harvested during the stage.

[c] INF were presumably unfertilized eggs, FNV were apparently fertilized but failed to hatch, while + hatched into phenotypically wild-type nymphs and *wb* hatched into phenotypically *wb* nymphs. Percentage values are rounded off to the nearest integer.

the fraction of the sperm count lost in any brief time interval is equal to the fraction of the total fluid lost. Therefore, if M is the count of old sperm present at any time (t)

$$dM/dt = -k\,M \qquad \text{(Equation 1)}$$

which can be integrated and rearranged to:

$$\log (M_0/M) = 0.435\,k\,t \qquad \text{(Equation 2)}$$

where M_0 is the initial count (at $t = 0$). Whatever the value of k, the value of M will be 0.5 M_0 when about 0.7 V of fresh seminal fluid has been injected into the spermatheca (if $k = 1$ this will require about 0.7 hour), 0.1 M_0 at 2.3 V, and 0.01 M_0 at 4.6 V. If this hypothesis is correct, many spermathecal volumes of semen must be transferred to reduce an initially high count of old sperm to a low value.

The count of new sperm, N, is determined by the constant inflow rate less the outflow rate (which must be exponential like that of the old sperm) and is expressed by the differential equation

$$dN/dt = k\,N_{max} - k\,N \qquad \text{(Equation 3)}$$

which on integration and rearrangement becomes

$$\log (N_{max} / (N_{max} - N)) = 0.435\,k\,t \qquad \text{(Equation 4)}$$

As t increases, N approaches N_{max} (the sperm count of one spermathecal volume of undiluted seminal fluid). The filling of the spermatheca with new sperm is quantitatively like the washing out of old, N being 0.5 N_{max} at 0.7 V, 0.9N_{max} at 2.3 V, and 0.99 N_{max} at 4.6 V.

This simple hypothesis is compatible with what is known so far of mating duration and of sperm transfer, storage, and replacement in *Oncopeltus*. A direct test of the hypothesis would require a large number of sperm counts in virgin spermathecae following copulations of known duration; the minute size and the quite small sperm numbers (100—1000) expected would make this technically difficult.

A hypothesis of somewhat different form could explain sperm replacement in species (such as *Drosophila*) where the sperm are deposited in the vagina and swim into the spermatheca. If conditions make it possible for the spermatozoa to swim into the spermatheca, it should be equally possible for those inside to swim out. The exchange between the spermatheca and the external semen deposit would be described by the equation

$$N_1 + N_2 = (V \cdot d_1 + W \cdot d_2)\,(V/(V + W))$$

where N_1 is the residual count of any sperm originally present in the spermatheca, N_2 the count of fresh sperm in the spermatheca introduced by a new insemination, d_1 the original sperm density in the spermathecal fluid, d_2 the sperm density in the seminal fluid of the new insemination, V the spermathecal volume, and W the volume of external seminal fluid. The equation indicates that storage of new sperm would be increased by a high sperm density in the external seminal fluid, while extraction of old sperm would be increased by a large volume of external seminal

fluid. The effect is generally similar to that produced by the direct insemination mechanism of *Oncopeltus* but less efficient.

Sperm depletion and minimum viable sperm store. Since many of the females in our experiments showed sperm depletion (either in the absence of normal or in the presence of castrate males), we analysed the data in essentially the same way described by Gordon & Gordon (1971). However, great variability was found when the percentage of presumably fertilized eggs was plotted against the estimated minimum sperm store (the total count of all presumably fertilized eggs that were laid in the cluster and all clusters laid subsequently). The reason for this is probably incorrect identification of many infertile eggs as fertile. We therefore changed the parameters to the percentage of eggs hatching and the minimum viable sperm store (the total count of eggs hatching), numbers which are far more reliable. The graphic data for the depletion stage of 29 females (15 with castrate males present and 14 without males) are shown in Figure 1. There is greater variability than in the data of Gordon & Gordon (1971). However, the latter made use of weekly egg collections, which would average out the variations in several egg-clusters, while our data are for individual clusters. Nevertheless, the tendency of egg hatchability to decline roughly linearly with the minimum number of viable sperm is clear. The presence of castrate males seems to have no effect on the

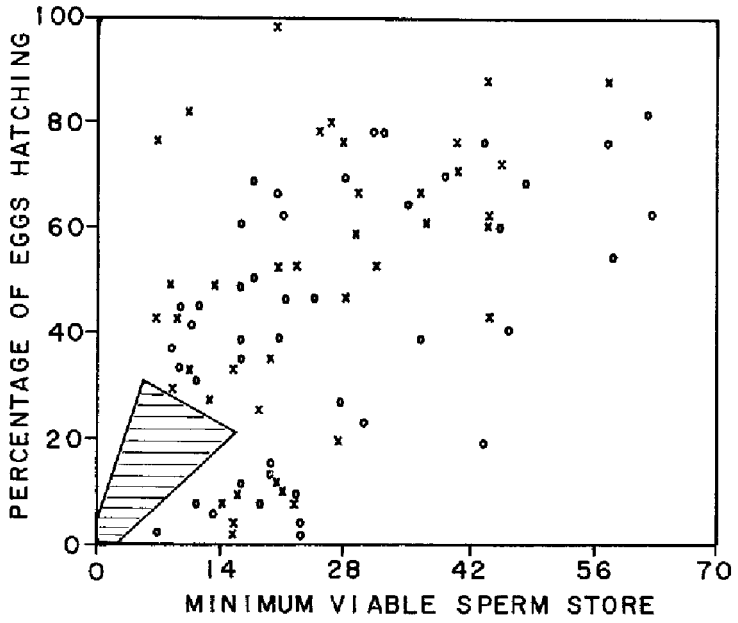

Fig. 1. Relation between the percentage of eggs hatching from single clusters and the minimum viable sperm store at the time of egg-laying (measured by the total number of hatchable eggs to be laid during the subsequent depletion period). x are layings by *wb* females in the absence of males, o are layings by + females in the presence of two castrate males. Cross-hatched area represents a dense concentration of 11 x and 25 o points.
Total number of females is 29.

course of depletion. Egg hatchability during the pre-depletion stage was high but variable in individual clusters, ranging from 80 to 100%. Figure 1 indicates that the decline in percent hatch starts at a minimum viable sperm store of about 60. This is lower than the minimum sperm store of 100 found for a milkweed-reared strain at room-temperature by Gordon & Gordon (1971). Part of the discrepancy may lie in the omission of the fairly large fraction (sometimes as high as 20%) of non-viable but nevertheless fertilized eggs produced by our new strains reared on non-milkweed diets. Since our study was carried out at 30°, it is also possible that higher sperm motility makes egg fertilization highly probable even when the sperm count is at a level that would allow only a 60% probability at lower temperature.

Again following the analysis of Gordon & Gordon (1971), the frequency distribution of values of the minimum viable sperm store is presented in Table III. There is a scattering of values comparable to that in the milkweed strain at lower temperature, but all in a much lower range, with means less than half those of the previous study. The quantitative difference can only be partly attributed to the omission of non-hatching but fertilized eggs in Table III. The remaining consid-

TABLE III

Frequency distribution of the minimum viable sperm store in wb *females (S strain) and* + *females (CS strain) of* Oncopeltus *at 30°.*

Number of nymphs [1]	wb ♀♀		+ ♀♀
	A[2]	B[3]	A[2]
0— 50	1	0	1
50—100	3	1	3
100—150	2	5	5
150—200	3	1	5
200—250	3	3	2
250—300	1	0	0
300—350	0	0	3
350—400	0	0	2
400—450	0	0	1
No. of ♀♀	13	10	22
Mean No. of eggs hatched	157	145	183
Std. deviation	70	52	113

[1] The numbers of nymphs (viable eggs) produced during the depletion period are ordered in groups of ± 25 eggs to simplify the distribution, but statistics are based on ungrouped data.

[2] A values are for periods that ended in total depletion of viable sperm (0% hatch).

[3] B values are for periods that ended in partial depletion (5%—50% hatch) and are corrected by adding estimated residuals of less than 60, as in the study of Gordon & Gordon (1971). There were so few such values in the + female data that they are not included.

erable difference could be dismissed as a physiological difference between strains maintained on very different diets, but there is some evidence that the duration of mating is shorter at higher temperature (Andre, 1934). One would not expect sperm flow to be much faster, since the viscosity of aqueous media is decreased only 20%

by a temperature rise from 20 to 30°. It is therefore possible that smaller volumes of semen are transferred in matings at 30°; if so, sperm replacement should be more efficient at lower temperatures. A third possibility is that the density of spermatozoa in the semen is lower at high temperature, if this should cause more active secretion of carrier fluid. Whatever the cause of the quantitative differences between our results and those of Gordon & Gordon (1971), there is substantial agreement in the basic phenomena.

RÉSUMÉ

*LA SUBSTITUTION ET L'ÉPUISEMENT DES SPERMATOÏDES DANS LA SPERMATHÈQUE DE DEUX LOTS (S) ET (CS) D'*ONCOPELTUS FASCIATUS

En vue d'analyser les conséquences d'accouplements successifs chez *Oncopeltus fasciatus*, on a utilisé des femelles de deux souches : l'une de type sauvage (+), l'autre correspondant à un mutant à corps blanc (*wb*, mutant autosomique récessif). Ces femelles subissent des croisements alternés avec les mâles de l'une ou l'autre souche; en outre, certains mâles utilisés étaient soit castrés chirurgicalement (effet de l'accouplement sans transfert de sperme), d'autres avaient subi une application topique d'un chimiostérilisant (tetramine) pouvant donner des œufs fécondés, mais non viables.

L'accouplement avec un second mâle (sauf un castré) provoque le remplacement partiel complet dans la spermathèque du stock de spermatozoïdes issu d'un premier mâle. Un modèle du mécanisme de remplacement est proposé selon lequel un volume de sperme 5 fois plus grand que la capacité de la spermathèque devrait être introduit par le second mâle pour assurer la disparition complète du dépôt précédent.

En l'absence d'accouplement renouvelés, ou en présence de mâles castrés la ponte d'œufs fécondés peut durer au moins 5 semaines, mais dans la plupart des cas le dépôt de spermatozoïdes s'épuise plus tôt. Le stock minimum de spermatozoïdes, estimé d'après le nombre total d'œufs viables, est très variable (40—400, moyenne 180). Le pourcentage d'œufs viables dans les pontes successives diminue proportionnellement à la réduction de ce stock au-dessous d'un minimum de 60.

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