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SPERMATHECA SHAPE VARIATION IN DROSOPHILA AFFINIS

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FEMALE *Drosophila* normally possess paired dorsal seminal receptacles called spermathecae, and the shape of these varies considerably from species to species within the genus (Sturtevant[4]). In a few cases differences of spermatheca shape have been reported to be useful for distinguishing females of closely related forms (Spieth[3], Weeks[5]). Dobzhansky[1] observed significant differences of spermatheca shape between *Drosophila melanogaster* of different mutant genotypes.

This study began as an attempt to find criteria for distinguishing between *Drosophila affinis* females and those of several closely related species. Laboratory strains of *D. affinis* (Florida, Illinois, Massachusetts, Nebraska, Tennessee, Texas), *D. algonquin* (Nebraska), *D. athabasca* (New York), *D. azteca* (Mexico), and *D. narragansett* (Massachusetts) were examined. Camera lucida drawings were prepared of spermathecae considered representative of the strains (Figure 12). It become apparent that *D. affinis* is rather variable as to spermatheca shape, and, although each of the other species was represented by one strain only, it seemed likely that, with the possible exception of *D. narragansett*, the other species were similarly variable. This variation involved the outside proportions of the chitinous capsule, the degree of penetration of the tube into the capsule, the occurrence of a terminal depression, and the nature of this indentation when present. It was not possible to find an altogether reliable criterion for distinguishing spermathecae of *D. affinis* from those of most of the other species. Only *D. narragansett*, with its peculiar broad tube invagination and lined terminal depression (Figure 12*J*), had a spermatheca shape sufficiently distinctive to afford easy recognition.

Among the strains of *D. affinis*, those from Florida (St. Petersburg) and Texas (Alice) showed the greatest difference as to spermatheca shape. The Florida spermathecae (Figure 13*A*) tended to be rather broad and flat, to have relatively much penetration of the tube inside the capsule, and usually had a more or less prominent terminal indentation. The Texas spermathecae (Figure 13*B*) tended to approach a spherical shape, to have a rather small tube penetration, and were never seen with a terminal indentation. Although there was some intrastrain variability, there was not much overlapping of spermatheca shape between the Florida and Texas strains. The other strains, on the other hand, generally had intermediate shapes and overlapped each other considerably. It was decided to investigate the influence on spermatheca shape of crosses involving the Florida and Texas strains. The following is an account of this investigation.

Procedure

It was planned to examine as many as possible of the spermathecae of females derived from a pair mating of each

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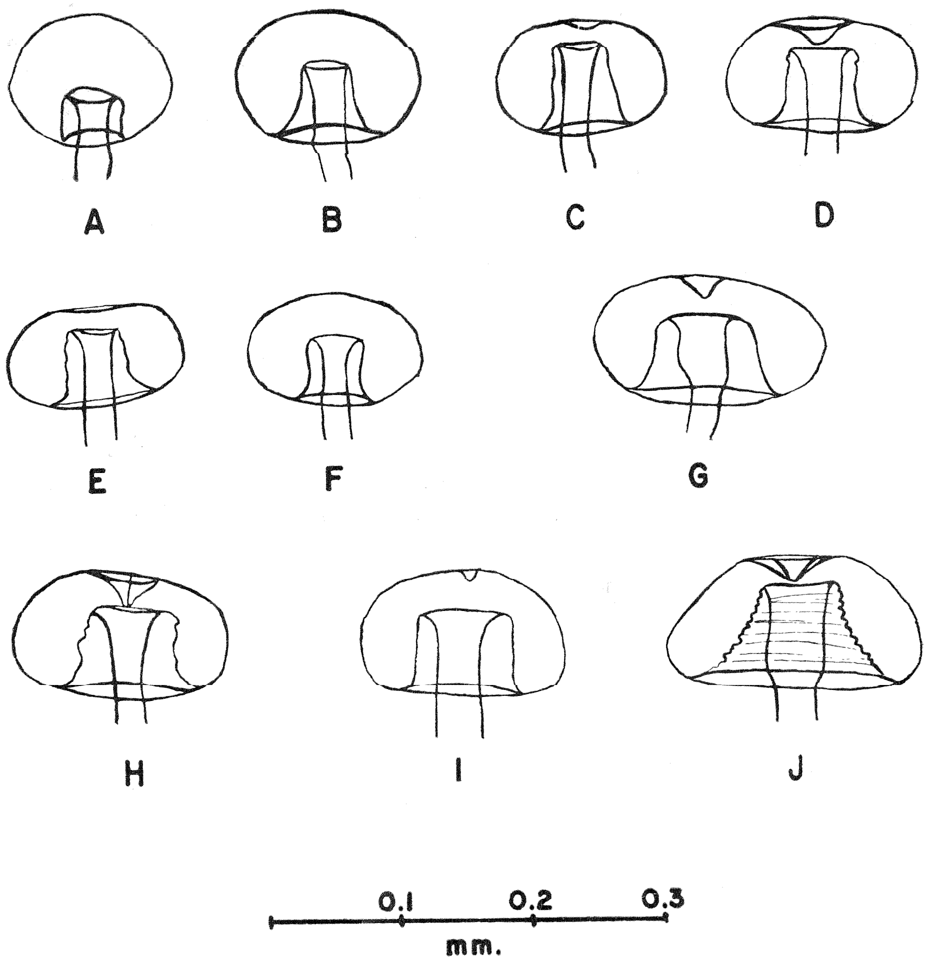


SPERMATHECAE OF *D. AFFINIS* AND RELATED SPECIES

Figure 12

Camera lucida drawings of spermathecae of *Drosophila affinis* and several closely related species (the gelatinous, cellular envelope of the capsule is not illustrated). *A-F—D. affinis* (*A*—Texas, *B*—Tennessee, *C*—Nebraska, *D*—Florida, *E*—Illinois, and *F*—Massachusetts). *G—D. algonquin* (Nebraska). *H—D. athabasca* (New York). *I—D. azteca* (Mexico). *J—D. narragansett* (Massachusetts).

of the following kinds: Florida × Florida, Texas × Texas, both reciprocal crosses between Florida and Texas, $F_1 \times F_1$ matings following both reciprocal crosses, and all the possible backcrosses between the F_1 generations and the parent strains. Each mating was made in a culture bottle with standard *Drosophila* medium (corn meal—molasses—agar—Tegosept—yeast) and kept in an incubator at about 23.5°C (±1.5°). After eclosion (always within four days) female offspring were etherized and dissected in Ringer's Solution and the lower reproductive tract removed from each and mounted for microscopic observation. Rows of these reproductive tracts were scanned microscopically with the aid of a mechanical stage. A single spermatheca was selected from each reproductive tract for observation and measurement. It was decided to select the first one of each pair encountered while scanning along the row. However, because of the need of a favorable orientation for making measurements (i.e. so as to present a lateral view), frequently one had to be taken regardless of position or else both had to be rejected. Measurements were made with an eyepiece micrometer, each unit of which corresponded to about 0.0032 mm. at the magnification used (ca. 200×). The following dimensions of the chitinous capsule of the spermathecae were measured: the width (a), the height (b), and the distance of penetration of the tube (c). Each spermatheca was also checked for the presence of a terminal depression, and if one was found, this was classed arbitrarily as small (Figure 12*C* and *E*), medium (Figure 12*D*), or large (Figure 13*A*; as here, terminal depressions classed as large often penetrated the opening of the tube inside the capsule).

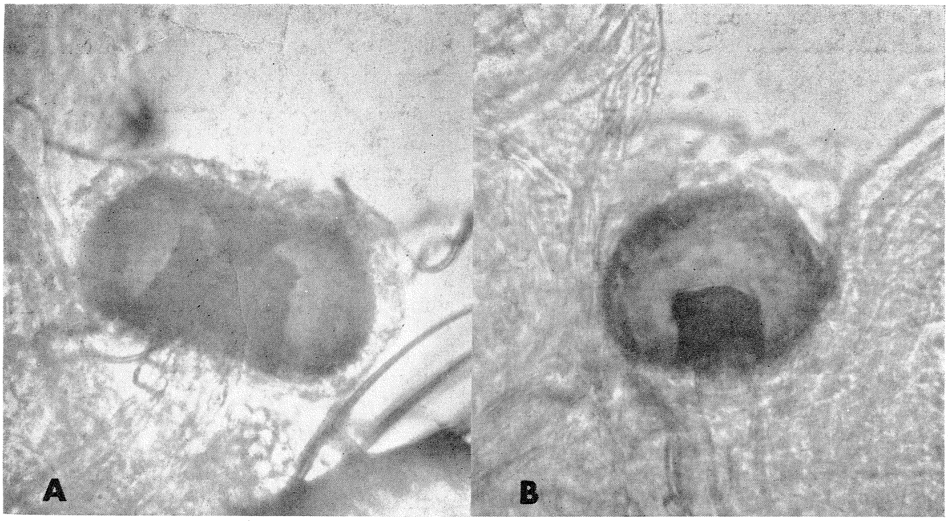
Tables I, II and III were prepared from the data accumulated from the progenies of the pair matings representing the various crosses. The numbers of individuals (N) reflect the different fecundities of the pair matings and the numbers of spermathecae recovered from the progenies. Table I presents the means of the width and height dimensions and the ratio of these (a/b), and Table II the means of the height dimension and the distance of tube penetration and the ratio of these (b/c). Table III shows the variation regarding the terminal depression.

Discussion of Results

The tables show that the progenies of the Florida × Florida and the Texas × Texas matings were quite distinct as to spermatheca shape. These progenies differed significantly (P less than 5 percent) as to the means of the a/b and b/c ratios (according to the t test, which was also applied to other differences of means), and the terminal indentation was generally present in the Florida spermathecae, regularly absent in those from Texas. However, there was some overlapping; the ranges of both ratios overlapped slightly, and two out of the 46 spermathecae of the Florida

TABLE I. ***D. affinis*** **Florida (St. Petersburg) and Texas (Alice) and generations derived from crossing these; each progeny obtained from a pair mating. Spermatheca width (a) and height (b) means; a/b ratio means (with standard errors), limits (in parentheses beneath means), and standard deviations (with standard errors)**

Parents	N	Mean a	Mean b	a/b $\bar{x}$	a/b s
Florida × Florida	46	22.0	13.9	1.59±0.07 (1.33—2.00)	0.50±0.05
Texas × Texas	73	18.3	15.1	1.22±0.01 (1.06—1.39)	0.07±0.01
Fla. ♀ × Tex. ♂	55	21.1	13.7	1.55±0.02 (1.33—1.83)	0.12±0.01
Tex. ♀ × Fla. ♂	62	21.2	14.3	1.49±0.02 (1.19—1.69)	0.14±0.01
F_1 (Fla. ♀ × Tex. ♂)	69	20.0	13.7	1.47±0.02 (1.19—1.77)	0.15±0.01
F_1 (Tex. ♀ × Fla. ♂)	40	20.6	14.2	1.47±0.03 (1.12—1.83)	0.17±0.02
F_1 (F. ♀ × T. ♂) ♀ × F. ♂	66	21.8	13.2	1.66±0.01 (1.40—1.92)	0.11±0.01
F. ♀ × F_1 (F. ♀ × T. ♂) ♂	39	22.0	13.9	1.58±0.02 (1.31—1.92)	0.14±0.02
F_1 (T. ♀ × F. ♂) ♀ × F. ♂	64	22.0	13.6	1.62±0.01 (1.33—1.92)	0.12±0.01
F. ♀ × F_1 (T. ♀ × F. ♂) ♂	20	22.3	13.2	1.69±0.03 (1.50—1.92)	0.14±0.02
F_1 (T. ♀ × F. ♂) ♀ × T. ♂	59	19.6	14.1	1.40±0.02 (1.13—1.75)	0.15±0.01
T. ♀ × F_1 (T. ♀ × F. ♂) ♂	32	19.0	14.5	1.32±0.03 (1.06—1.83)	0.17±0.02
F_1 (F. ♀ × T. ♂) ♀ × T. ♂	65	19.6	14.4	1.38±0.02 (1.00—1.75)	0.18±0.02
T. ♀ × F_1 (F. ♀ × T. ♂) ♂	60	19.2	14.5	1.33±0.02 (1.13—1.62)	0.13±0.01



D. AFFINIS SPERMATHECAE

Figure 13

A and *B* shows photomicrographs of spermathecae of *D. affinis*: *A*—Florida (St. Petersburg); *B*—Texas (Alice).

progeny were without a terminal indentation.

The progenies resulting from crossing the Florida and Texas strains and the generations derived from these show the influence of hereditary factors contributed by both strains. However, the rather continuous nature of the variation and the considerable overlapping of the spermatheca shapes of the different generations make it difficult to determine the kinds of factors involved. The situation is probably also complicated by genetic heterogeneity of the Florida and Texas strains. Nevertheless, certain tentative conclusions may be drawn from the available data. These are indicated to a large extent by the general pattern of spermatheca shape variation as revealed by the a/b and b/c ratios and the frequencies of terminal indentations of the several kinds; as is pointed out below, differences of ratio means and of frequencies of depressions, though sometimes suggestive, are not always statistically significant.

The F_1 generations were characterized by intermediate means of the a/b and b/c ratios (Tables II and III), but closer to the corresponding Florida means than to those of Texas. In fact, the a/b ratio mean of the progeny of the Florida female mated to a Texas male was not significantly different from the Florida mean of this kind, although the other F_1 ratio means were significantly different from the corresponding means of the parent strains. Regarding both ratio means, each F_1 was somewhat closer than the other one to the progeny representing the strain of its female parent, and the differences are statistically significant (less complete data were responsible for the report of Miller[2] of no influence of direction of cross). The F_2 means of the a/b and b/c ratios were shifted in the direction of the corresponding Texas values (though only the Florida female $\times$ Texas male F_1-F_2 differences were significant), and the two F_2 generations were alike as to these means. The backcrosses to the Florida and Texas strains gave progenies with means of the a/b and b/c ratios invariably displaced in the directions of the strains used in backcrossing, especially those to the Florida strain. The backcrosses to Texas (but not to Florida) showed slightly greater shifts of ratio means toward the parental strain where a female of this strain was used in backcrossing, but these differences are not significant. It may be concluded that Florida factors influencing the a/b and b/c ratios show some dominance over those of Texas. In addition, the difference between the F_1 progenies and those gotten from backcrosses to Texas suggest a possible maternal effect on spermatheca shape.

Regarding the terminal indentation of the spermatheca (Table III), the F_1 generations were intermediate to Florida and Texas, but more similar to Texas than to Florida. The F_2 generations were still more similar to Texas than the F_1's, but application of the chi-square test to the frequencies of sperma-

TABLE II. *D. affinis* Florida (St. Petersburg) and Texas (Alice) and generations derived from crossing these; each progeny obtained from a pair mating. Spermatheca height (b) and distance of tube penetration (c) means; b/c ratio means (with standard errors), limits (in parentheses beneath means), and standard deviations (with standard errors)

Parents	N	Mean b	Mean c	b/c $\bar{x}$	b/c s
Florida × Florida	46	13.9	8.9	1.58±0.02 (1.30—2.00)	0.10±0.01
Texas × Texas	73	15.1	5.5	2.84±0.07 (2.00—5.33)	0.60±0.05
Fla. ♀ × Tex. ♂	55	13.7	8.3	1.66±0.03 (1.30—2.50)	0.21±0.02
Tex. ♀ × Fla. ♂	62	14.3	7.6	1.90±0.03 (1.60—2.50)	0.22±0.02
F_1 (Fla. ♀ × Tex. ♂)	69	13.7	7.2	1.96±0.05 (1.44—3.20)	0.40±0.04
F_1 (Tex. ♀ × Fla. ♂)	40	14.2	7.5	1.96±0.08 (1.44—3.40)	0.49±0.06
F_1 (F. ♀ × T. ♂) ♀ × F. ♂	66	13.2	8.8	1.51±0.02 (1.20—2.00)	0.16±0.01
F. ♀ × F_1 (F. ♀ × T. ♂) ♂	39	13.9	8.4	1.67±0.02 (1.40—2.00)	0.13±0.01
F_1 (T. ♀ × F. ♂) ♀ × F. ♂	64	13.6	8.8	1.56±0.02 (1.30—2.00)	0.15±0.01
F. ♀ × F_1 (T. ♀ × F. ♂) ♂	20	13.2	8.2	1.62±0.02 (1.44—1.86)	0.10±0.02
F_1 (T. ♀ × F. ♂) ♀ × T. ♂	59	14.1	6.6	2.25±0.10 (1.44—5.33)	0.74±0.07
T. ♀ × F_1 (T. ♀ × F. ♂) ♂	32	14.5	6.2	2.46±0.11 (1.50—3.75)	0.62±0.08
F_1 (F. ♀ × T. ♂) ♀ × T. ♂	65	14.4	6.8	2.22±0.07 (1.44—3.75)	0.56±0.05
T. ♀ × F_1 (F. ♀ × T. ♂) ♂	60	14.5	6.3	2.41±0.07 (1.56—3.75)	0.51±0.05

TABLE III. *D. affinis* Florida (St. Petersburg) and Texas (Alice) and generations derived from crossing these; each progeny obtained from a pair mating. Numbers and percentages (in parentheses beneath) of spermathecae with no terminal indentation and with terminal indentations classed as small, medium, and large

Parents	N	Indentation: None	Small	Med.	Large
Florida × Florida	46	2 (4.3)	3 (6.5)	14 (30.4)	27 (58.7)
Texas × Texas	73	73 (100)	0	0	0
Fla. ♀ × Tex. ♂	55	42 (76.4)	13 (23.6)	0	0
Tex. ♀ × Fla. ♂	62	50 (80.6)	10 (16.1)	2 (3.2)	0
F_1 (Fla. ♀ × Tex. ♂)	69	68 (98.6)	1 (1.4)	0	0
F_1 (Tex. ♀ × Fla. ♂)	40	37 (92.5)	2 (5.0)	1 (2.5)	0
F_1 (F. ♀ × T. ♂) ♀ × F. ♂	66	7 (10.6)	31 (47.0)	15 (22.7)	13 (19.7)
F. ♀ × F_1 (F. ♀ × T. ♂) ♂	39	6 (15.4)	11 (28.2)	7 (17.9)	15 (38.5)
F_1 (T. ♀ × F. ♂) ♀ × F. ♂	64	11 (17.2)	18 (28.1)	18 (28.1)	17 (26.6)
F. ♀ × F_1 (T. ♀ × F. ♂) ♂	20	3 (15.0)	8 (40.0)	6 (30.0)	3 (15.0)
F_1 (T. ♀ × F. ♂) ♀ × T. ♂	59	57 (96.6)	2 (3.4)	0	0
T. ♀ × F_1 (T. ♀ × F. ♂) ♂	32	32 (100)	0	0	0
F_1 (F. ♀ × T. ♂) ♀ × T. ♂	65	59 (90.8)	4 (6.2)	2 (3.1)	0
T. ♀ × F_1 (F. ♀ × T. ♂) ♂	60	60 (100)	0	0	0

thecae with and without indentations (i.e. by combining the different classes of depressions) shows only the shift of the Florida female × Texas male F_2 to be statistically significant. The backcrosses to Florida and Texas regularly produced offspring resembling the strain used in backcrossing, especially the backcrosses to Texas. Some dominance of Texas factors preventing terminal indentations is suggested by the F_1 generations and the more complete return to the parental type accompanying the Texas backcrosess. The fact that the F_2 progenies resembled Texas more than did the F_1's (contrary to the expectation based on Texas dominance) may be due to a dependence of terminal indentation formation on other aspects of spermatheca shape (as indicated by the a/b and b/c ratios) under the influence of somewhat dominant Florida factors.

Conclusions

D. affinis may be described as polymorphic as to spermatheca shape, with genetic factors contributing to this polymorphism. A rather complex genetic basis for spermatheca shape variation seems likely, although the possibility of a small number of major factors with variable effects cannot be excluded.

The dissimilarity of the Florida and Texas strains suggests that which sometimes exists between different taxonomic groups—e.g. subspecies, even species. However, there was no detectable reproductive isolation between these strains (e.g. their hybrids were fully fertile), and, moreover, the various other strains of *D. affinis* exhibited spermatheca shapes of a more or less intermediate nature and varied so as to overlap each other broadly. The available strains do not suggest a very simple geographical distribution of spermatheca shape variation. It is, of course, not possible to conclude whether any of the strains are very representative of the wild populations from which they originated. It would be especially desirable to investigate further the variation of spermatheca shape in southern Florida and southern Texas populations of *D. affinis*.

Summary

Spermatheca shape was investigated in *Drosophila affinis* and several similar species (*D. algonquin, athabasca, azteca,* and *narragansett*). With the exception of *D. narragansett,* none of these other species had spermathecae easily distinguishable from those of *D. affinis.* Considerable intraspecific variation in spermatheca shape was revealed within *D. affinis.* Laboratory strains of *D. affinis* from Florida and Texas were found to be especially different in this respect. The Florida spermathecae tended to be rather broad and flat, to have relatively much penetration of the tube inside the capsule, and usually had a more or less prominent terminal indentation. The Texas spermathecae tended to approach a spherical shape, to have rather little tube penetration, and these were never found to have a terminal indentation. Crosses between the Florida and Texas strains and the offspring of these yielded progenies with more or less intermediate spermatheca shapes, with some evidence of dominance of both Florida and Texas factors and of a slight maternal effect.

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