Int. J. Insect Morphol. & Embryol. 5(1): 51–63. 1976. Pergamon Press. Printed in Great Britain.

NUMBER OF FIRST SPERMATOCYTES IN RELATION TO PHYLOGENY OF *DROSOPHILA* (DIPTERA: DROSOPHILIDAE)

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(*Accepted* 16 *September* 1975)

Abstract—A study of spermatogenesis in relation to phylogeny was carried out by using 78 species from 2 subfamilies, 8 genera and 12 subgenera of the Drosophilidae. Since definitive spermatogonia dichotomously divide a characteristic number of times during the multiplication stage, the number of the first spermatocytes per cyst is indicative of the mitotic divisions of the definitive spermatogonia. Accordingly, the total number of the mitotic divisions can be estimated from cell counts in the first spermatocytal cyst.

The number of the first spermatocytes per cyst of 78 drosophilid species concerned here can be classified into 4 regular classes, $64(2^6)$, $32(2^5)$, $16(2^4)$, $8(2^3)$ and the exceptional ones. There is a clear tendency for the number of first spermatocytes involved in a cyst to be largest at the primitive level, and it decreases with the advancement of evolution in *Drosophila*.

Index descriptors (in addition to those in title): Spermatogenesis.

INTRODUCTION

THE FORMATION of gametes in animals is normally accomplished by a special series of cell divisions. Gonocytes first multiply and give rise to spermatogonia. The latter undergo successively mitotic and meiotic divisions, and a spermiogenic stage.

As reviewed by Courot *et al.* (1970), every animal species usually has a particular number of synchronous divisions during the multiplication stage. Fishes have been reported to have a number of divisions varying from 6 to 13. In birds, on the contrary, the multiplication divisions are much fewer. Numerous investigations on mammals using different tools have shown that the number of the spermatogonial generations varies from 4 to 6.

In insects, Virkki (1969) and Phillips (1970) summarized the range in total number of the multiplication divisions and the number of the spermatozoa, respectively. There are 9–14 in Odonata (Oksala, 1944; Omura, 1955, 1957), 4–9 in Orthoptera (Robertson, 1931; Powers, 1942; White, 1955), 3–8 in Hemiptera (Schrader, 1929; Reuter, 1930; Hughes-Schrader, 1935, 1946, 1948; Halkka, 1956; Nur, 1962; Dikshith, 1966), 4–6 in Neuroptera (Suomalainen, 1952), 5–7 in Coleoptera (Virkki, 1951), 5 in Trichoptera (Lutman, 1920), 5–6 in Lepidoptera (Cretschmar, 1928; Depdolla, 1928; Kawaguchi, 1928; Knaben, 1931; Oksala, 1944; Virkki, 1963) and, 3–6 in Diptera (White, 1946, 1950; Cooper, 1950; Meyer, 1967). The dragonflies appear to be a special group in this respect since the number of the divisions vary both within and between species.

In drosophilid flies, as in most of the other Diptera, there are 2 morphologically different types of spermatogonia, one of which is characterized by undergoing asynchronous mitoses, and the other by undergoing synchronous, dichotomous mitoses in the cysts. The latter

type is called the definitive (or secondary) spermatogonium (Stern, 1941; Cooper, 1950; Kaufmann and Gay, 1963; Seidel, 1963; Hannah-Alava, 1965). Thus the exact generations of the definitive spermatogonia can be determined by merely counting the number of cells enclosed in a cyst of the final generation (Otte, 1906; Cooper, 1950). If 4 synchronous divisions of the definitive spermatogonia occur, the first spermatocytes enclosed in the cyst, which are derived from a single definitive spermatogonium, must number 16 (2^4). Likewise, when the divisions take place 3 times, 8 spermatocytes should be produced.

A rather small number of reports concerning the number of the mitotic divisions in *Drosophila* has so far been published. Cooper (1950) summarized this. *Drosophila melanogaster* has 4 divisions (Pontecorvo, 1944; Tihen, 1946). Dobzhansky (1934) reported that both *D. pseudoobscura* and *D. miranda* have 5 divisions. Meyer (1967) indicated that 3 divisions were observed in *D. hydei*. The present article deals with the number of the mitotic divisions of the definitive spermatogonia and its phylogenic meaning in 78 drosophilid species covering several taxonomic groups.

MATERIALS AND METHODS

Two subfamilies, 8 genera, 12 subgenera, including 78 species in total were examined for spermatogenesis. More than 20 adult males in each species were dissected for observation. Live adult males coming from both laboratory stocks and natural habitats were dissected in Ephrussi-Beadle's Ringer solution. After removing the testicular wall on a glass slide with needles, a few drops of Ringer solution were added and the preparation was gently covered with a coverslip. A phase contrast microscope was used for observation in every case.

RESULTS AND DISCUSSION

64-cell class

Sixty-four first spermatocytes per cyst is the largest number encounted in this investigation. This number indicates that the definitive spermatogonia have divided 6 times during the multiplication stage (2^6). Only 3 species, *D. coracina*, *D. throckmortoni*, and *D. bryani*, all belonging to the subgenus *Scaptodrosophila*, are involved in this class (Table 1). Figure 1

TABLE 1. NUMBER OF FIRST SPERMATOCYTES PER CYST IN 78 SPECIES OF DROSOPHILIDAE (ASTERISKS IMPLY SLIGHT VARIATION IN NUMBER).

Genus	Subgenus	Species gr.	Species	No. of Div.-Spc.
Subfamily Steganinae				
Amiota	*Amiota*		*dispina*	4–16
	Phortica		*variegata*	4–16
Leucophenga	*Leucophenga*		*magnipalpis*	4–16
			ornatipennis	4–16
			maculata	4–16
	Paraleucophenga		*argentosa*	4–16
Subfamily Drosophilinae				
Drosophila	*Scaptodrosophila*		*coracina*	6–64
			throckmortoni	6–64
			bryani	6–64
Microdrosophila	*Microdrosophila*		*purpurata*	5–32
			cristata	5–32
Chymomyza			*procnemis*	4–16
Drosophila	*Sophophora*	*obscura* gr.	*obscura*	5–32
			bifasciata	5–32
			imaii	5–32
			pseudoobscura	5–32
			miranda	5–32
		melanogaster gr.	*suzukii*	4–16
			pulchrella	4–16

TABLE 1 (continued)

Genus	Subgenus	Species gr.	Species	No. of Div.-Spc.
			melanogaster	4–16
			simulans	4–16
			ananassae	4–16
			bipectinata	4–16
			takahashii	4–16
			lutea	4–16
			rufa	4–16
			auraria	4–16
			pectinifera	4–16
			kikkawai	4–16
			panjabiensis	4–16
			ficusphila	4–16
Liodrosophila			*area*	4–16
Drosophila	*Hirtodrosophila*		*confusa*	5–32
			fascipennis	5–32
			quadrivittata	5–32
			alboralis	5?–24
			sexvittata	3–8
			denticeps	3–8
Scaptomyza	*Scaptomyza*		*graminum*	4–16
			consimilis	4–16
	Parascaptomyza		*pallida*	4–16
Drosophila	*Dorsilopha*		*busckii*	4–16
Zaprionus			*vittiger*	4–16
			tuberculatus	4–16
Drosophila	*Drosophila*	*immigrans* gr.	*curviceps*	5?–24
			annulipes	5?–18
			quadrilineata	4–16
			immigrans	4–16
			albomicans	4–16
			sulfurigaster	4–16
			hypocausta	4–16
		melanderi gr.	*makinoi*	4–16
		testacea gr.	*testacea*	4–16
		bizonata gr.	*bizonata*	4–16
		histrio gr.	*sternopleuralis*	4–16
			histrio	4–16*
		grandis gr.	*acutissima*	4–16
			tenuicauda	4–16
		quinaria gr.	*brachynephros*	3–8
			nigromaculata	3–8
			kuntzei	3–8
		annulimana gr.	*daruma*	4–16
		virilis gr.	*ezoana*	3–8
			virilis	3–7, 8
		melanica gr.	*pengi*	4?–12*
		tumiditarsus gr.	*tumiditarsus*	4?–14
		robusta gr.	*moriwakii*	4–16
			sordidula	4?–14
			lacertosa	3–7*
		repleta gr.	*repleta*	4–16
			stalkeri	4–16
			mercatorum	4–16
			hydei	3–8
		funebris gr.	*maculinotata*	5?–24
			funebris	3–8
			multispina	3–8
			macrospina	3–8
			subfunebris	3–8

shows several cysts of the first spermatocytes of *D. bryani* which are separated from each other by optically clear borders. Sixty-four cells can be counted in every cyst. A later stage of an isolated cyst is shown in Fig. 2. Figure 3 shows telophase of the first meiotic division in the same species. All cells are simultaneously becoming second spermatocytes. Figure 4 illustrates the spermatid cells after the meiotic divisions. The cell number, 256 ($2^6 \times 4$), counted in this cyst must be 4 times as many as that calculated in the premeiotic stage. Each sperm bundle presumably consists of the same number of spermatozoa as spermatids (Fig. 5). Individual definitive spermatogonia, particularly in the earlier stages of these species, cannot clearly be identified because the cells are very small in size.

The subgenus *Scaptodrosophila* is the only group including species with an unreduced sixth sternite in the male (Throckmorton, 1962). Species having this type of sternite can be placed in a more primitive position in the phylogeny of *Drosophila* than those having a reduced one (Wheeler, 1960). Furthermore, most species of this subgenus have primitive genital organs, namely, elliptical testes, primitive vasa in males, very short ventral receptacles in females, etc. (Okada, 1956; Throckmorton, 1962). These characteristics of the internal and external organs can adequately support the supposition that species having the higher number of first spermatocytes per cyst can be considered to be more primitive. Thus, the fact that all 3 species, *D. coracina*, *D. throckmortoni* and *D. bryani*, commonly have 64 cells may support Throckmorton's opinion that this group might have arisen from the most basal stem of the *Drosophila*.

32-cell class

Several species from 3 different groups are included in this class (Table 1). *Microdrosophila cristata* and *M. purpurata* of the genus *Microdrosophila; D. confusa*, *D. fascipennis*, and *D. quadrivittata* of the subgenus *Hirtodrosophila*; and *D. obscura*, *D. bifasciata*, *D. imaii*, *D. pseudoobscura* and *D. miranda*, all belonging to *obscura* group of the subgenus *Sophophora*, have commonly 32 first spermatocytes per cyst. The mitotic divisions in these species would have been repeated 5 times (2^5) during the multiplication stage. Figures 6 and 7 illustrate various stages of the spermatogenesis of *D. bifasciata*. Thirty-two first spermatocytes can be recognized in each cyst. The different stages of the spermatid and the spermatozoa, which are definitely bordered by the cyst wall are also shown (Fig. 7). The cell number of the spermatid per cyst can be expected to be 128 ($2^5 \times 4$). These cells are, in general, larger in size than those seen in species having 64 cells.

Both *Microdrosophila cristata* and *M. purpurata* are known to have primitive genital organs, such as testes with a low degree of coiling in males and conical egg-guides and short ventral receptacles in females (Okada, 1960, 1968a, b). The species *M(I). incisurifrons* (not studied here) also has the primitive, fusiform testis. Thus the genus *Microdrosophila* may also occupy the basal branch in the phylogeny of *Drosophila*.

There is considerable variation in the number of the first spermatocytes for the species of the subgenus *Hirtodrosophila*. Three species, *D. confusa*, *D. fascipennis*, and *D. quadrivittata*, have 32 cells, while 2 species, *D. sexvittata* and *D. denticeps*, have only 8 cells. The remaining one, *D. alboralis*, appears to have 24 cells, and so belongs to the exceptional number class. This cell number suggests that the mitotic divisions would have occurred 5 times as for the species having 32 cells, and some of the spermatogonial cells have supposedly disappeared during the successive mitoses. The first 3 species, representing the higher number of the cells, have primitive testes and shorter ventral receptacles as compared with the latter 2 (Okada, 1956, 1967). Moreover, females of *D. quadrivittata* have a single sternite-like egg-guide

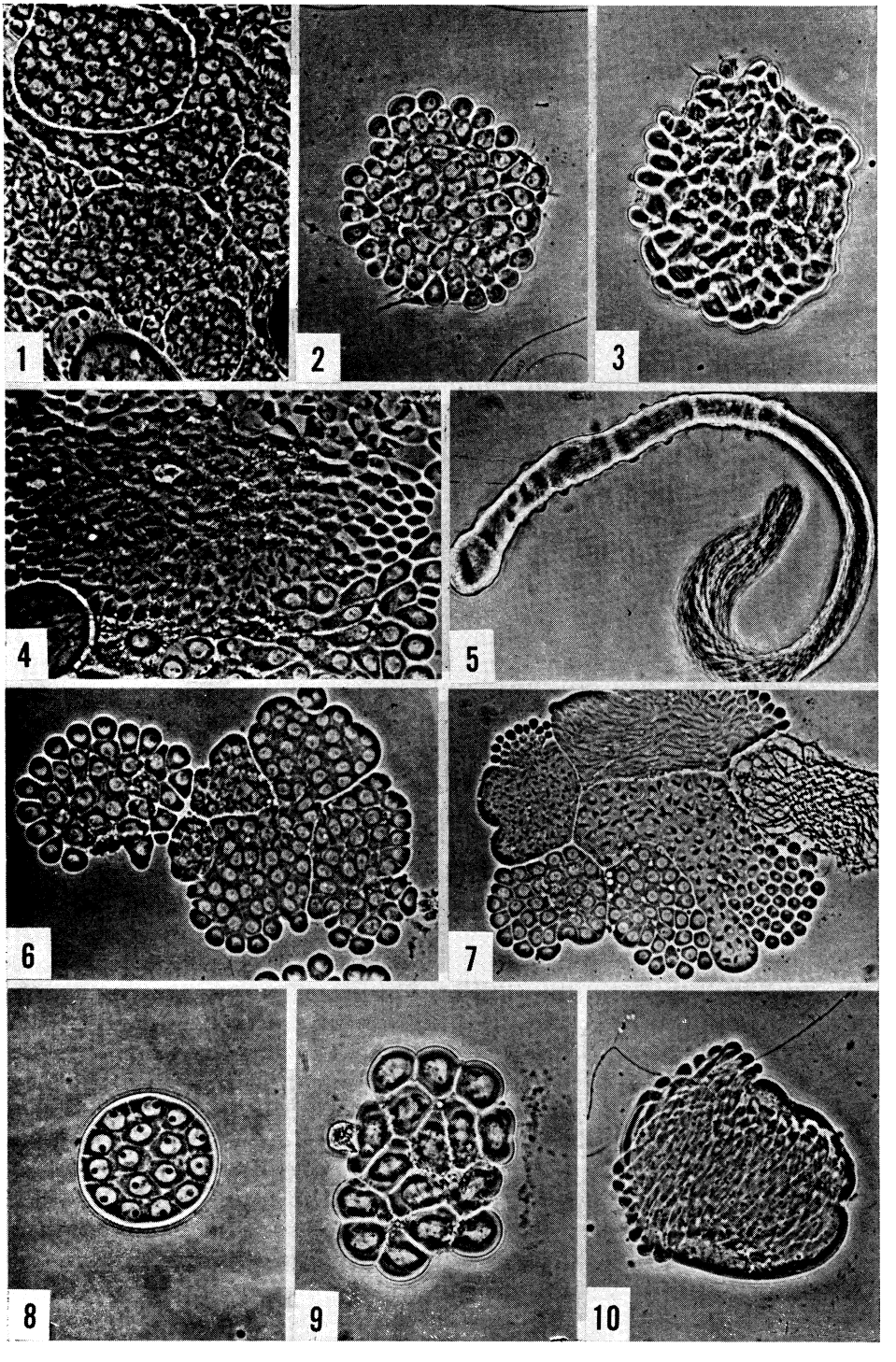
which is also one of the most primitive characters (Okada, 1967). On the other hand, 2 species, *D. sexvittata* and *D. denticeps*, having 8 first spermatocytes, show highly coiled testes and very long ventral receptacles (Okada, 1967). *Drosophila alboralis* has testis with 2 inner and 3·5 outer coils, and the ventral receptacle shows about 6 loops (Momma and Takada, 1954). The shape and size of these organs observed in the last species are intermediate between the former 2 types. This nicely coincides with the cytological observation that *D. alboralis* has 24 cells, which is intermediate in number between 32–8, though Okada (1971) has proposed that the *denticeps* species group can be placed on a branch derived most basally from the hypothetical ancestor of the *Hirtodrosophila*.

In spite of the fact that most members of the subgenus *Sophophora* commonly show 16 cells per cyst, all 5 species of the *obscura* group consistently show the same number, 32, regardless of whether their distribution is from the Palaearctic or the Nearctic region. This group is characterized by having the most primitive morphological traits among the subgenus *Sophophora* (Okada, 1956). The cell number 32, observed in this group, is the same as that observed in the genus *Microdrosophila* and in some members of the subgenus *Hirtodrosophila*. In addition to the facts explained by Throckmorton (1962), the cytological evidence also supports the possibility that the subgenus *Sophophora* might have arisen during the time immediately following the separation of the *Scaptodrosophila* stem population.

16-cell class

About 60% of the species dealt with, covering different subfamilies, genera and subgenera, commonly appeared to have 16 first spermatocytes per cyst (Table 1). The cell divisions in the definitive spermatogonia would supposedly be 4 (2^4). Six species from 2 different genera, *Amiota* and *Leucophenga*, both belonging to the subfamily Steganinae, commonly appeared to have 16 cells per cyst. In the subfamily Drosophilinae, a number of species from the genera, *Liodrosophila*, *Chymomyza*, *Zaprionus* and *Scaptomyza*, and from the subgenera, *Sophophora* and *Drosophila*, all appeared to have the same number of the cells. The fact that all 5 species from the 2 genera, *Scaptomyza* and *Zaprionus*, commonly appeared to have 16 cells may support the conclusion by Throckmorton that these genera have substantially similar characters in the external and internal organs, and hence they might be separated from a common stem of the phylogeny. Throckmorton (1962) mentioned that the genus *Chymomyza* and the subgenus *Sophophora* have common characteristics in both males and females. The number 16 of first spermatocytes, is also common to both groups, although only one species of American *Chymomyza* was examined here. Some of the *Hirtodrosophila* and the *Scaptomyza* possibly show similarity in their external morphology, particularly in the genital organs (Okada, 1967), but they are not similar cytologically.

The species groups in the subgenus *Sophophora* are compact as compared with those in the subgenus *Drosophila*. As described above, most of the species belonging to this subgenus consistently show 16 cells, with the exception of the *obscura* group. The fact that all members belonging to *melanogaster* group consistently show 16 cells is suggested. Figure 8 shows the first spermatocytal cyst isolated from the testicular follicle of *D. auraria*. The dividing cells in the telophase of the first meiotic division are illustrated in Fig. 9. Figure 10 shows metamorphosing spermatids. The cytological evidence revealed here, incidentally, emphasizes the supposition by Throckmorton that this subgenus seems to have a monophyletic origin.



(Figures 1–10)

A considerable number of species having 16 cells also appears in the subgenus *Drosophila* (Table 1). The members of *immigrans* group, with the exception of 2, *D. annulipes* and *D. curviceps*, commonly have 16 cells. *Drosophila testacea*, *D. bizonata*, *D. makinoi*, *D. acutissima*, and *D. tenuicauda*, all from the *quinaria* section, and, *D. repleta D. stalkeri*, *D. mercatorum*, from the *repleta* group have 16 cells in common.

The various stages of the spermatogenesis of *D. immigrans* are shown in Figs. 11–13. The first spermatocytes and the number of spermatids per cyst can be calculated as 16 and 64, respectively. From the standpoint of the cell number, the supposition by Throckmorton (1962) that the *immigrans* group might have arisen directly from the basal stem population for the subgenus *Drosophila*, and hence might have characters resembling those of the *Sophophora* is also supported. The species included in this class seemed to be good material for cytological observation. Cell size is large and the configuration is readily demonstrable during mitoses.

8-cell class

Several species from different subgenera and species groups are included in this class (Table 1). Of the 6 species examined, both *D. sexvittata* and *D. denticeps* of the subgenus *Hirtodrosophila* appeared to have 8 first spermatocytes per cyst. The number of the mitotic divisions is thus assumed to be only 3 (2^3). The cell number in the subgenus *Drosophila* is quite variable between and within species and species groups. Some of the species have 16 cells (see former class), the same as those of the subgenus *Sophophora*, while others have 8 and the exceptional numbers.

The *quinaria* section consists of 2 different classes of cell numbers, one of which shows 16 cells (see former class) and the other shows 8 cells, and includes all 3 species of the *quinaria* group. *Drosophila histrio*, which usually has 16 cells, appears to show a slight amount of variation. The species of the *funebris* group appeared to show 8 cells, excepting the Japanese member of *D. maculinotata* showing 24+. Only the species, *D. hydei* from the *repleta* group has 8 cells.

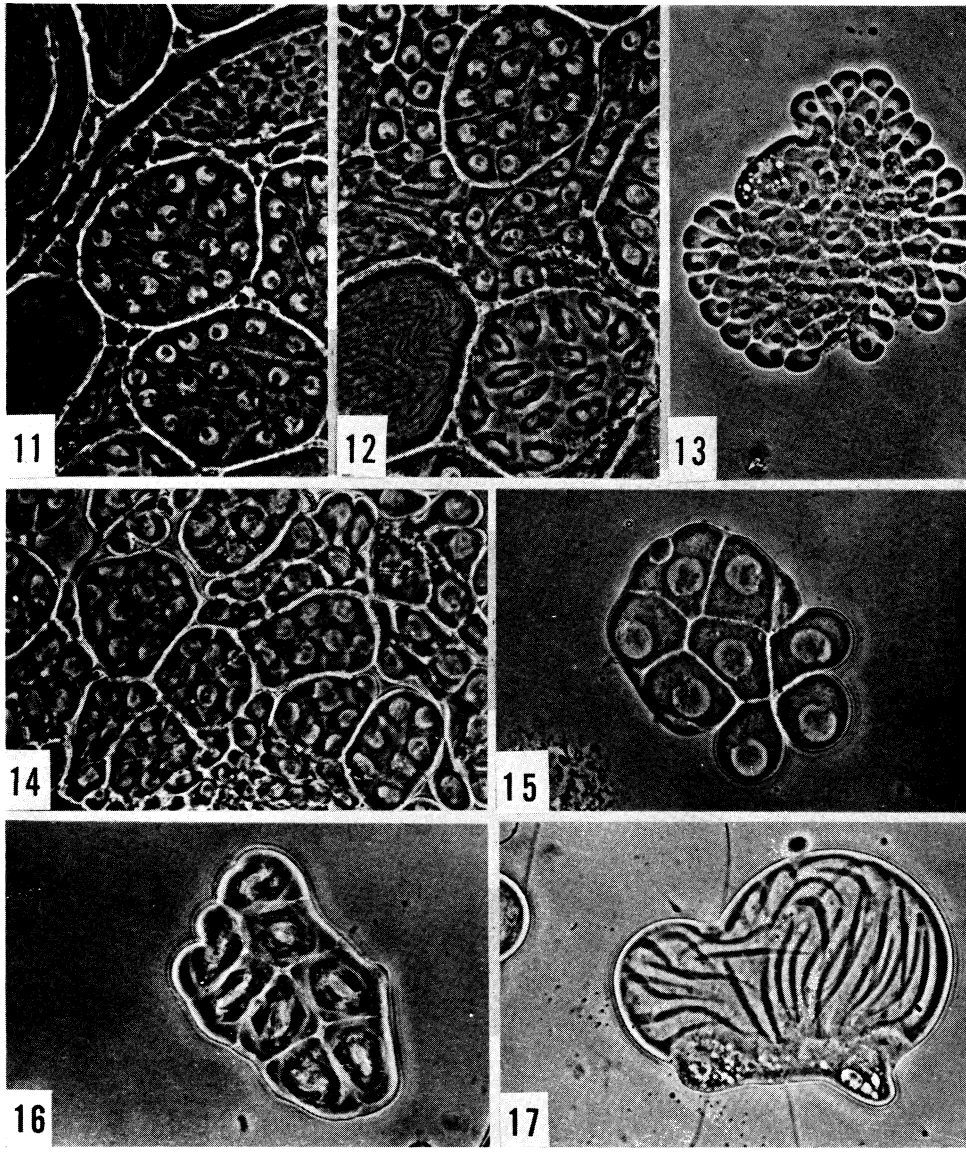
Several cysts, including the first spermatocytes of *D. virilis*, are shown in Figs. 14–17. *Drosophila virilis* and *D. ezoana* usually show 8 cells per cyst, but the former sometimes appeared to have cysts involving 7 and 8 cells within a follicle (Fig. 14). The 14 secondary spermatocytes which might have arisen from 7 first spermatocytes can accordingly be observed in *D. virilis*. The 28 spermatids per cyst are also observable. Eight dividing cells with elongated mitochondria at anaphase of the first meiotic division are shown (Fig. 16). The cells that are going to be spermatozoa can be calculated as 32 ($2^3 \times 4$) (Fig. 17). The coexistence of 2 kinds of cysts involving different number of the cells is also seen in some species of the subgenus *Drosophila* other than *D. virilis*.

FIGS. 1–5. Various stages of spermatogenesis of *D. bryani*. 1. First spermatocytal cysts consisting of 64 cells in each. × 200 2. Later stage of a cyst isolated from a testicular follicle. × 200 3. Telophase of first meiotic division. × 200 4. Spermatids consisting of 256 cells. × 200 5. Sperm bundle. × 200

FIGS. 6, 7. Various stages of spermatogenesis of *D. bifasciata*. 6. First spermatocytal cysts consisting of 32 cells in each. × 200 7. Cysts representing various stages, spermatids or spermatozoa consisting of 128 cells per cyst. × 200

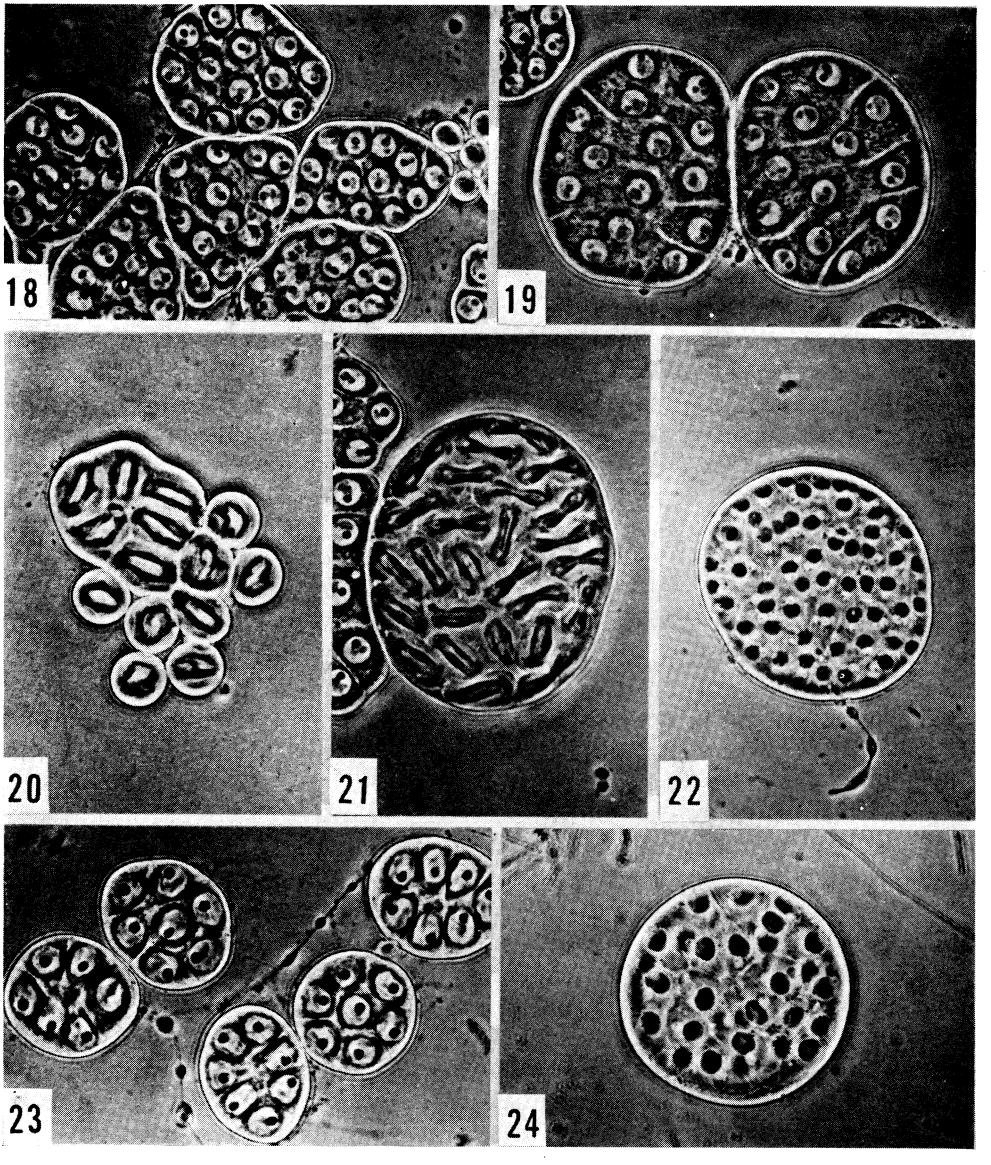
FIGS. 8–10. Various stages of spermatogenesis of *D. auraria*. 8. Single cyst involving 16 first spermatocytes. × 200 9. 16 dividing first spermatocytes at telophase of first meiotic division. × 200 10. Metamorphosing spermatids. × 200

The 3 species of the *robusta* group mutually appear to have different numbers of the cells per cyst. *Drosophila moriwakii*, *D. sordidula*, and *D. lacertosa* show the numbers, 16, 14, and 7, respectively (Figs. 18–24). Figures 18 and 19 illustrate several cysts of *D. sordidula*, which involve commonly 14 first spermatocytes. The 14 dividing cells at telophase of the first



FIGS. 11–13. Various stages of spermatogenesis of *D. immigrans*. 11. First spermatocytal cysts involving 16 cells in each. × 200 12. Telophase of first meiotic division. × 200 13. Spermatids consisting of 64 cells. × 200

FIGS. 14–17. Various stages of spermatogenesis of *D. virilis*. 14. Several first spermatocytal cysts. Cysts usually involve 8 but sometimes 7 cells in this species. × 200 15. Singly isolated cyst with 8 first spermatocytes. × 200 16. Telophase of first meiotic division. × 200 17. Spermatids just going to be metamorphosed. × 200



FIGS. 18–22. Various stages of spermatogenesis of *D. sordidula*. 18. First spermatocytal cysts consisting of 14 cells in each. × 200 19. Isolated first spermatocytal cysts. × 200 20. Telophase of first meiotic division. × 200 21. Telophase of second meiotic division. × 200 22. Spermatids. × 200

FIGS. 23, 24. Various stages of spermatogenesis of *D. lacertosa*. 23. Isolated cysts involving 7 first spermatocytes in each. × 200 24. Spermatids. × 200

meiotic division are observed in Fig. 20. The 28 cells dividing at the secondary meiotic division, and the 56 spermatids are seen in Figs. 21 and 22, respectively. Seven first spermatocytes represented normally in *D. lacertosa* are the fewest among all the species dealt with (Fig. 23). There is evidence that every first spermatocytal cyst apparently enclose only 7 cells, and that the spermatids can easily be counted as 28 (Fig. 24). This suggests that

decrease in the cell number has occurred in the mitotic phase and not in the meiotic one. If these spermatids produce altogether functional spermatozoa, 28 should be the lowest number among drosophilid species. The cytological configuration of the species involved in this class is very identifiable, because every cell is really large in size and observable.

Exceptional cell number class

The species representing the "exceptional cell number" are much more frequent in the subgenus *Drosophila* than in the subgenus *Sophophora*, and they appear in various species groups especially in the former. There are many exceptional cell numbers, such, 24, 18, 14, 12 and 7 (Table 1). *Drosophila alboralis* is the only member showing the exceptional number 24 in the subgenus *Hirtodrosophila*. The Japanese species, *D. maculinotata* of the *funebris* group and *D. curviceps* of the *immigrans* group are both characterized by having 24 cells. The number of mitotic divisions in these species can accordingly be estimated as 5. Only *D. annulipes* of the *immigrans* group appears to show 18 cells. Both *D. sordidula* (Figs. 18–22) and *D. tumiditarsus* usually show 14 cells. Each spermatid cyst of these species, accordingly, consists of 56 cells (Fig. 22), with a few exceptional cases showing 54. As far as we have observed, only *D. pengi* of the *melanica* group characteristically shows 12 cells. *Drosophila virilis* sometimes appeared to have cyst with 8 or 7 cells, while *D. lacertosa* appeared to show ordinarily the least number 7 (Figs. 23, 24).

It has been known that the number of spermatozoa in mammals is, in general, smaller than the theoretically expected one. In fact, cellular degeneration occurs at a given stage in the spermatogenic line (Courot *et al.*, 1970). In this connection, whether the stem-cell method is reliable or not in *Drosophila* is of great concern for cytologists and geneticists. Hannah-Alava (1965) mentioned that a corollary of the stem-cell hypothesis is that the time of origin of the new predefinitive (stem-cell) spermatogonium can be predicted on the basis of counts of the number of definitive spermatogonia, or spermatocytes of common origin. Since the definitive spermatogonia multiply dichotomously, if the new predefinitive spermatogonium is isolated in the first division, the number of definitive spermatogonia of common origin will be a geometric multiple of 2 (i.e., $2^n = 4, 8, 16, 32, 64$ etc., depending upon the total number of definitive divisions). If the stem-cell is isolated after the second definitive division, the number of spermatogonia derived from its 3 sister cells will be a geometric multiple of 3 (i.e., 6, 12, 24, 48 etc.), and after the third division a geometric multiple of 7 (i.e., 14, 28, 56 etc.) as shown in the model by Clermont and Leblond (1953). However, whether or not some species with an "exceptional cell number" can be explained by the above models is uncertain. Although the cause of the exceptional numbers has not yet been determined, the fact that the species representing these numbers are conspicuously seen in the subgenus *Drosophila* is interesting. Hypothetically, this could be indicative of the fact that these species are transitional between the ordinary types, such 32 and 16, 16 and 8, and, 8 and 4(?), respectively.

CONCLUSION

The definitive spermatogonia undergo a particular number of dichotomous divisions and produce first spermatocytes. Since the first spermatocytes undergo in common 2 meiotic divisions and produce 4 times as many spermatozoa as first spermatocytes, the final number of spermatozoa per bundle depends on the number of mitotic divisions during the multiplication stage (Fig. 25).

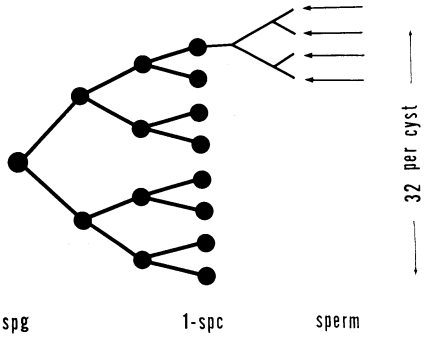


FIG. 25. Scheme of spermatogenesis of certain species of *Drosophila*. A single definitive spermatogonium dichotomously multiplies 3 times, and gives rise to 8 first spermatocytes per cyst. Every first spermatocyte subsequently divides twice during meiotic phase and gives rise to 4 spermatozoa, respectively. spg: definitive spermatogonium, spc. first spermatocytes.

The number of the first spermatocytes per cyst of the 78 species of the Drosophilidae can usually be classified into the following 5 classes: $64(2^6)$, $32(2^5)$, $16(2^4)$, $8(2^3)$, and the exceptional ones. Thus, the number of divisions of the definitive spermatogonia can be presumably classified into 4 types: 6, 5, 4 and 3, respectively. The cell number counted in the cyst seems to be generally consistent within taxa, such as, species groups, subgenera and genera (Table 1).

Figure 26 shows the phylogenic relationship of subfamilies, genera, subgenera, species groups and species all included in this study. The phylogenic tree indicates only the general sequence of origin of the different groups, because it is mostly based on the Throckmorton's opinion, which is derived from morphology (Throckmorton, 1962, 1965), and ecology and geographical distribution (Throckmorton, 1975, in press). It can generally be recognized

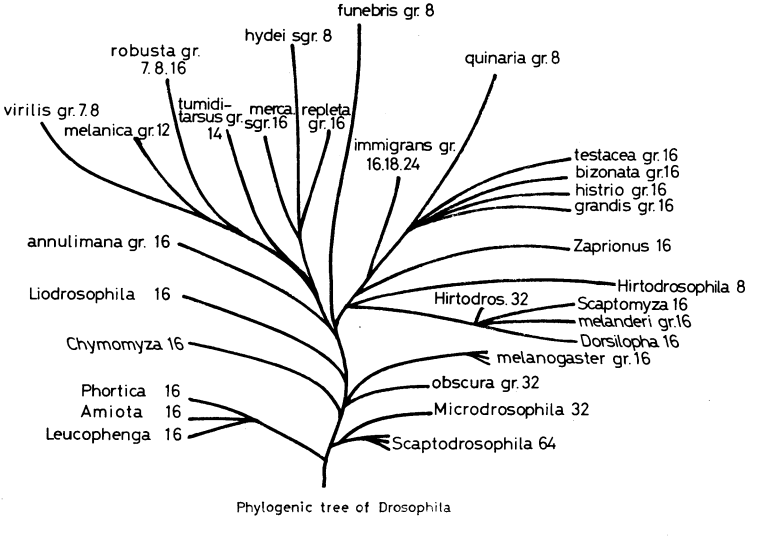


FIG. 26. Phylogenic tree of Drosophilidae with numbers of first spermatocytes in a cyst. Figures mean cell numbers of first spermatocytes per cyst found in species or taxonomic groups.

that the mitotic divisions during the multiplication stage are fewer in the subgenus *Drosophila* than in the subgenus *Sophophora*. The former group consists of morphologically more advanced forms as compared with the latter (Okada, 1955, 1956; Throckmorton, 1962, 1965). This coincides with a tendency revealed here that the number of first spermatocytes per cyst tends to be reduced in more advanced forms. There is also clear tendency that the spermatocytes increase in volume as the cells decrease in number. The size of spermatozoa is, thus considered to be largest in the members having fewest number of the spermatocytes (Hihara and Kurokawa, in preparation). These facts can be supported by data summarized by Virkki (1969) that the number of the spermatozoa per bundle decreases with the advancement of evolution of insects. Generally, archaic groups appear to have many more spermatozoa, whereas the advanced or specialized groups have fewer ones. This has been demonstrated in Orthoptera (White, 1955), Hemiptera (Virkki, 1969), and Coleoptera (Virkki, 1969).

It seems that the reduction of the first spermatocytes has taken place several times during evolution of *Drosophila*: at least once in the subfamily Steganinae, at least once in the subgenus *Sophophora*, and probably twice in the subgenus *Drosophila*. This suggests some important adaptive significance.

Acknowledgement—We are grateful to Prof. L. H. Throckmorton, Department of Biology, The University of Chicago for his critical reading of the manuscript and comments. This work was partly supported by a Grant (964163) from the Ministry of Education of Japan.

REFERENCES

CLERMONT, Y. and C. P. LEBLOND. 1953. Renewal of spermatogonia in the rat. *Amer. J. Anat.* **93**: 475–82.

COOPER, K. W. 1950. Normal spermatogenesis in *Drosophila*, pp. 1–61. *In* M. DEMEREC (ed.) *Biology of Drosophila*, Wiley, New York.

COUROT, M., M. T. HOCHEREAU-DE REVIERS and R. ORTAVANT. 1970. Spermatogenesis, pp. 339–432. *In* A. D. JOHNSON, W. R. GOMES and N. L. VANDEMARK (eds.) *The Testis*, Vol. I, Academic Press, New York.

CRETSCHMAR, M. 1928. Das Verhalten der Chromosome bei der Spermatogenese von *Orgyia thyellina* Btl. und *antigua* L. sowie eines ihrer Bastardte. *Z. Zellforsch.* **7**: 290–399.

DEPDOLLA, P. 1928. Die Keinzellenbildung und die Befruchtung bei den Insekten, pp. 825–1116. *In* C. SCHRADER (ed.) *Handbuch der Entomologie*, Vol. I, Fischer, Jena.

DIKSHITH, T. S. S. 1966. Spermatogenesis in *Laccifer lacca* (Kerr) (Lacciferidae-Coccidea). *Cytologia* **31**: 302–08.

DOBZHANSKY, Th. 1934. Studies on hybrid sterility. I. Spermatocytes in pure and hybrid *D. pseudoobscura*. *Z. Zellforsch. Mikroske. Anat.* **21**: 169–223.

HALKKA, O. 1956. Studies on mitotic and meiotic cell division in certain Hemiptera under normal and experimental conditions. *Ann. Acad. Sci. Fenn. A* IV **32**: 1–80.

HANNAH-ALAVA, A. 1965. The premeiotic stage of spermatogenesis. *Adv. Genet.* **13**: 157–226.

HESS, O. and G. F. MEYER. 1968. Genetic activities of the Y chromosome in *Drosophila* during spermatogenesis. *Adv. Genet.* **14**: 171–223.

HUGHES-SCHRADER, S. 1935. The chromosome cycle of *Phenacoccus* (Coccidae). *Biol. Bull.* (*Woods Hole*) **69**: 462–68.

HUGHES-SCHRADER, S. 1946. A new type of spermiogenesis in iceryine coccids, with linear alignment of chromosomes in the sperm. *J. Morphol.* **78**: 43–84.

HUGHES-SCHRADER, S. 1948. Cytology of coccids (Coccidea-Homoptera). *Adv. Genet.* **2**: 127–203.

KAUFMANN, B. P. and H. GAY. 1963. Cytological evaluation of differential radio-sensitivity in spermatogeneous cells of *Drosophila*, pp. 375–408. *In* F. SOBEL (ed.) *Repair from Genetic Radiation Damage*, Macmillan (Pergamon), New York.

KAWAGUCHI, E. 1928. Cytologische Untersuchungen am Seiden-spinner und Seinen Vervandten. I. Gametogenese von *Bombyx mori* L. und *Bombyx mandarina* M. und ihrer Bastardte. *Z. Zellforsch.* **7**: 519–52.

KNABEN, N. 1931. Spermatogenese bei *Tischeria angusticolella* Dup. *Z. Wiss. Biol.* **83**: 290–323.

LUTMAN, B. F. 1910. The spermatogenesis of the caddisfly (*Platyphylax designatus* Walker). *Biol. Bull.* (*Woods Hole*) **19**: 55–72.

MEYER, G. F. 1967. Spermiogenese in normalen und Y-defizienten Mänchen von *Drosophila melanogaster* und *D. hydei*. *Z. Zellforsch.* **84**: 141–75.
MOMMA, E. and H. TAKADA. 1954. *Drosophila* survey of Hokkaido I. Description of a new species, *Drosophila alboralis* sp. nov. (Subgenus *Hirtodrosophila*). *Annot. Zool. Japon.* **27**: 97–101.
NUR, U. 1962. Sperms, sperm bundles and fertilization in a mealy bug, *Pseudococcus obscurus* Essig. (Homoptera: Coccidea). *J. Morphol.* **111**: 173–99.
OKADA, T. 1956. *Systematic study of Drosophilidae and allied families of Japan.* Gihodo, Tokyo, Japan.
OKADA, T. 1960. The genus *Microdrosophila* Malloch from Japan (Diptera, Drosophilidae). *Kontyu* **28**: 211–22.
OKADA, T. 1967. A revision of the subgenus *Hirtodrosophila* of the old world, with descriptions of some new species and subspecies (Diptera, Drosophilidae, *Drosophila*). *Mushi* **41**: 1–36.
OKADA, T. 1968a. Taxonomic treatment of the correlative characters in the genus *Microdrosophila* (Diptera, Drosophilidae). *Proc. Jap. Soc. Syst. Zool.* **4**: 1–7.
OKADA, T. 1968b. Addition to the fauna of the family Drosophilidae of Japan and adjacent countries (Diptera). I. Genera, *Stegana*, *Amiota*, *Leucophenga*, and *Microdrosophila* with discussion on the homology of phallic organs. *Kontyu* **36**: 303–23.
OKADA, T. 1971. Systematic and biogeographical analyses of the *denticeps* group, with description of two new species (Diptera, Drosophilidae). *Bull. Biogeogr. Soc. Jap.* **26**: 29–38.
OKSALA, T. 1944. Zytologische Studien and Odonaten. II. Die Entstehung der meiotischen Prökozität. *Ann. Acad. Sci. Fenn. Ser. A* IV **5**: 5–33.
OMURA, T. 1955. A comparative study of the spermatogenesis in the Japanese dragonflies. I. *Biol. J. Okayama Univ.* **2**: 95–135.
OMURA, T. 1957. A comparative study of the spermatogenesis in the Japanese dragonflies. II. *Biol. J. Okayama Univ.* **3**: 1–86.
OTTE, H. 1906. Samenreifung und Samenbildung von *Locusta viridissima*. I. Die Samenreifung. II. Samenbildung. *Zool. Anz.* **30**: 529–35 und 750–54.
PHILLIPS, D. M. 1970. Insect sperm: Their structure and morphogenesis. *J. Cell Biol.* **44**: 243–77.
PONTECORVO, G. 1944. Synchronous mitoses and differentiation sheltering the germ tract. *Drosophila Inform. Ser.* **18**: 54–55.
POWERS, P. B. 1942. Metrical studies of spermatogenial chromosomes of the Acrididae (Orthoptera). *J. Morphol.* **71**: 523–76.
REUTER, E. 1930. Beiträge zu einer einheitlichen Auffassung gewisser Chromosomenfragen. *Acta Zool. Fenn.* **9**: 1–484.
ROBERTSON, W. R. B. 1931. Chromosome studies. II. Synapsis in the Tettigidae with special reference to the presynaptic split. *J. Morphol.* **51**: 119–45.
SCHRADER, F. 1929. Experimental and cytological investigations of the life cycle of *Gossyparia spuria* (Coccidae) and their bearing on the problem of haploidy in males. *Z. Wiss. Zool.* **134**: 149–79.
SEIDEL, S. 1963. Experimentelle Untersuchungen über die Grundlagen der Sterilität von Transformer (Tra) Mänchen bei *Drosophila melanogaster*. *Z. Vererbungsl.* **94**: 215–41.
STERN, C. 1941. The growth of testis in *Drosophila*: I, II. *J. Exp. Zool.* **87**: 113–58 and 159–80.
SUOMALAINEN, E. 1952. Localization of chiasmata in the light of observations on the spermatogenesis of certain Neuroptera. *Ann. Zool. Soc.* "*Vanamo*" **15**: 1–104.
THROCKMORTON, L. H. 1962. The problem of phylogeny in the genus *Drosophila*, pp. 207–343. *In* M. R. WHEELER (ed.) *Studies in Genetics*, Vol. II, Univ. Texas Pub., Austin Texas.
THROCKMORTON, L. H. 1965. Similarity *versus* relationship in *Drosophila*. *Syst. Zool.* **14**: 221–36.
THROCKMORTON, L. H. 1975. The phylogeny, ecology and geography of *Drosophila*. *In* R. C. KING (ed.) *Handbook of Genetics*, Vol. 3 (in press), Plenum Pub., New York.
TIHEN, J. A. 1946. An estimate of the number of cell generations preceding sperm formation in *Drosophila melanogaster*. *Amer. Nat.* **80**: 389–92.
VIRKKI, N. 1951. Zur Zytologie einiger Scarabaeiden (Coleopter). *Ann. Zool. Soc.* "*Vanamo*" **14**: 1–104.
VIRKKI, N. 1963. Gametogenesis in the sugarcane borer moth, *Diatraea saccharalis* (F.) (Grambidae). *J. Agric. Univ. Puerto Rico* **47**: 102–37.
VIRKKI, N. 1969. Sperm bundles and phylogenesis. *Z. Zellforsch.* **101**: 13–27.
WHEELER, M. R. 1960. Sternite modification in males of the Drosophilidae (Diptera). *Ann. Entomol. Soc. Amer.* **53**: 133–37.
WHITE, M. J. D. 1946. The cytology of the Cecidomyidae (Diptera). The chromosome cycle and anomalous spermatogenesis of *Miaster*. *J. Morphol.* **79**: 323–70.
WHITE, M. J. D. 1950. Cytological studies on gall midges (Cecidomyidae). *Univ. Texas Pub.* **5007**: 1–80.
WHITE, M. J. D. 1955. *Animal Cytology and Evolution.* Cambridge Univ. Press, Cambridge.