Evolution of sperm cell number per bundle in insects

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SUMMARY: Spermatozoa of almost all insects are arranged in bundles. The number of spermatozoa per bundle (spz/b) is very constant for a species above the primitive order Odonata.

Spz/b is determined by the number of synchronized mitoses of the definitive spermatogonia, enclosed in a goniocyst. The number of bundles produced is controlled by multiplication of germ cells prior to the definitive gonia. The number of testis follicles depends on the apical apparatuses formed. The total production of spermatozoa depends thus much on the premeiotic cytology of the germ line.

Specialized insects tend to have less spz/b than more primitive insects. It seems possible that reduction of spz/b reflects a general reduction in the sperm production, which in turn may limit genetic variability, sufficiently so to help the population's adaptation to a specialized niche.

INTRODUCTION

After the spermiohistogenesis, the spermatozoa of most insects are arranged in bundles. The number of spermatozoa per bundle (spz/b) is usually constant and specific for a species. Spz/b is determined by the premeiotic cytology of the germ line, which is quite variable between species, although very constant within a species. Cytology of the premeiotic part of the germ line is little studied in insects. Therefore, and because the matter is highly relevant for the present subjetc, a short synopsis seems justified.

The terminology related with the premeiotic cells was very variable until Hannah-Alava (1965) revised it; in this paper, the terminology suggested by her will be used.

There are three categories of spermatogonia in the testes of the insect. *Predefinitive spermatogonia* produce *indefinitive spermatogonia*, which in turn give rise to *definitive spermatogonia*. The history of these categories is, without mentioning all varieties, as follows:

In an early phase of embryogenesis, a number of germinative cells is loosened to circulate freely in the embryo. These are the *pole cells*. Later, when the germinative ridges form in the abdomen, they start to attract and fasten pole cells. Once fixed in the ridges, they are called primordial germ cells. Only those that become attached with the developing testis will survive. Their number is relatively low, less than 30 cells in *Drosophila*.

In the apex of each testis follicle, the primordial germ cells become associated by cytoplasmic connections with a big somatic cell called apical or Verson's cell, to form the so-called apical apparatus. Now the germ cells have become *predefinitive spermatogonia*. These are divided by asynchronous mitoses in such a way that one of the daughter cells retains its connection with the apical cell and is category as a predefinitive gonium, whereas the other on remains free and continues dividing. These unassociated cells are *indefinitive spermatogonia*. This category probably is the most variable quantitatively: in some species it may be absent, in others, the multiplication is enormous.

When an indefinitive gonium associates with a somatic cell, it turns into a *definitive spermatogonium*. The somatic cell divides once or more, enveloping the gonium with a membranous capsule: the goniocyst. The cyst will survive both meiosis and spermiohistogenesis, its name changing accordingly to spermatocyst I, spermatocyst II, and sperm bundle. Within the goniocyst, the gonium starts a series of syncronous divisions. Thus the number of definitive spermatogonia increases according to a geometric series, 2^n. This is the most common system. It is modified especially if predefinitive gonia still arise among the filial cells of the indefinitive or defintive gonia (see Hannah-Alava 1965, p. 166). Anyway, when the mode of multiplication of the definitive gonia is known —and usually it is based on 2^n— it is possible to calculate the number of mitoses occurred by the number of cells in the cyst. This is true still after meiosis, because both meiotic divisions are synchronous within a cyst. For instance, in the case of 256 ($= 2^8$) spermatids per cyst, there have been 6 mitoses of definitive gonia, plus the two meiotic divisions.

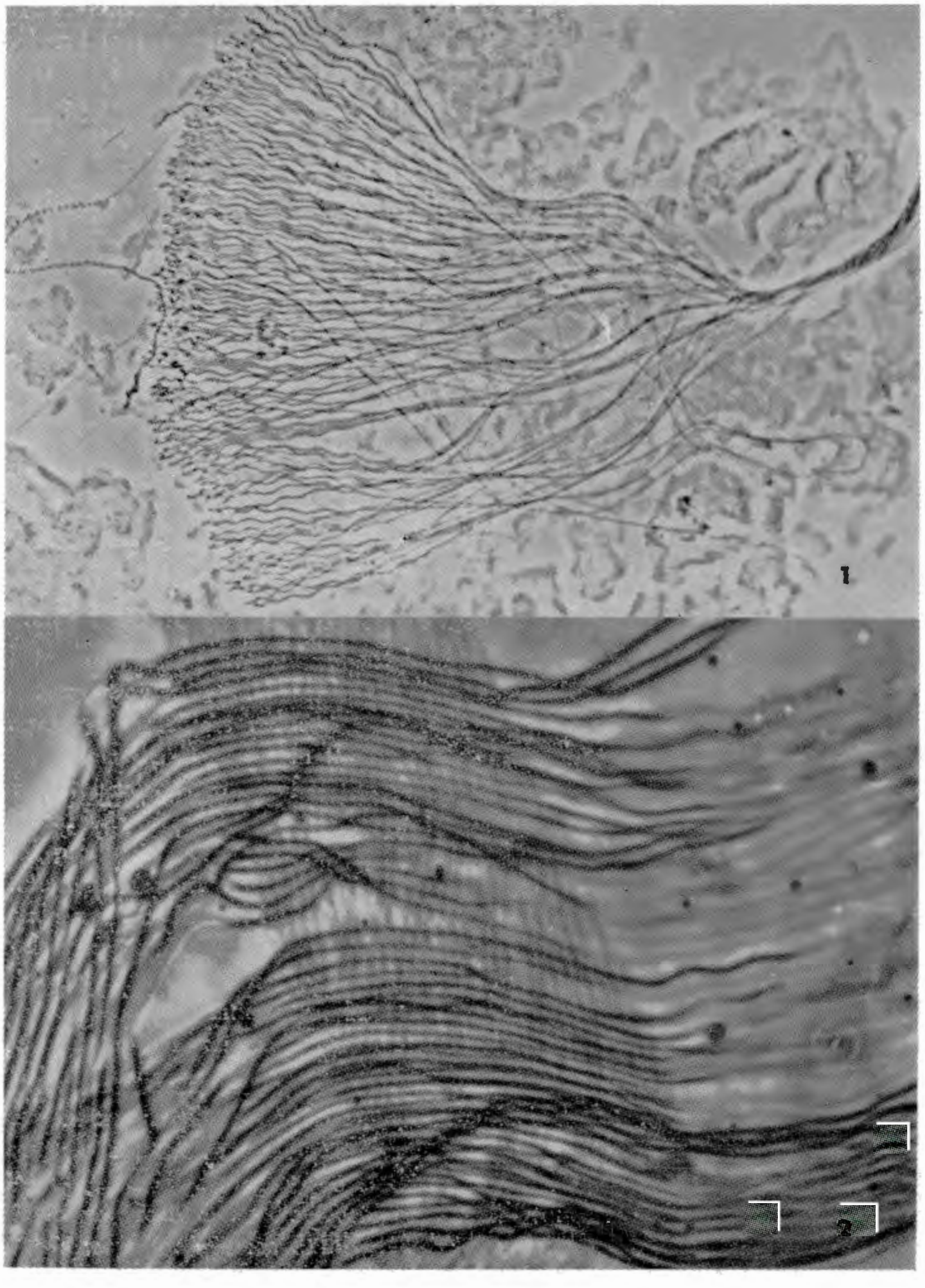
It is to be emphasized that each of the successive "generations" (intermitotic cell populations) of the definitive spermatogonia are subcategories by themselves, characterized by their own volume and other parameters. The general rule is that the size of the definitive spermatogonia diminishes mitosis after mitosis (Omura 1952, 1955, Halkka 1956). It is erroneous to measure or otherwise describe the definitive gonia as a sole category (see Holt and North 1969).

In the vast majority of insects, the cyst survives the spermiohistogenesis, that is, the metamorphosis of the spermatids to spermatozoa. The cytoplasm of the ayst is accumulated around one of its nuclei. Probably cytoplasm eliminated from the elongating spermatids cooperates directly or indirectly in the process. In this way, the so-called cap cell forms. Sperm heads become oriented towars it, embedded in its cytoplasm. Only a thin lining of the same cytoplasm surrounds the tails of the sperm cells. *This is the sperm bundle.*

Thus the total progeny of the first definitive spermatogonium stays together, always synchronously in the same phase, until the bundle disintegrates, which

TABLE 1. Sperm cell numbers per bundle encountered in some insect orders. For Coleoptera and Lepidoptera, spz/b = 256 is a typical number; for Heteroptera, probably spz/b = 1024. Note that variation an order is mainly *donward* from the typical number. (x): due to postmeiotic arrangements.

Spz/b *Order*	2^{4} = 16	2^{5} = 32	2^{6} = 64	2^{7} = 128	2^{8} = 256	2^{9} = 512	2^{10} = 1024	2^{11} = 2048	2^{12} = 4096	2^{13} = 8192	2^{14} = 16384	2^{15} = 32768	2^{16} = 65536
Odonata								×	×	×	×	×	×
Orthóptera			×	×	×	×	×	×					
Heteróptera						×	×						
Homóptera (Coccoidea)	(×)	×	×										
Neuróptera			×	×	×								
Coleóptera	×	×	×	×	×	×							
Lepidóptera				×	×								
Díptera		×	×	×	×								



FIGS. 1-2. Immature sperm bundles. 1. *Cacoscelis compta* Erichson: spz/b = 128 (1150X). 2. *Systena s-littera s-littera* L. : spz/b = 64 (2680X).

seldom happens inside of the testis. Only in primitive insects the bundle may be lacking (in family Libellulidae of Odonata, according to Omura 1957).

MATERIAL AND METHODS

My own observations were made during 20 years in preparation made for chromosome studies. A variety of methods were used, the Feulgen and acetocarmine squashes being the most important ones. For some recentmost modifications of technique, see Virkki, 1970.

OBSERVATIONS AND DISCUSSION

1. Sperm cell counts.

Many authors have counted the number of spermatozoa per bundle (spz/b). This information is encountered quite distributed, mostly in articles describing spermatogenesis. Table 1 is a summary of the data presented in an earlier paper of mine (Virkki, 1969).

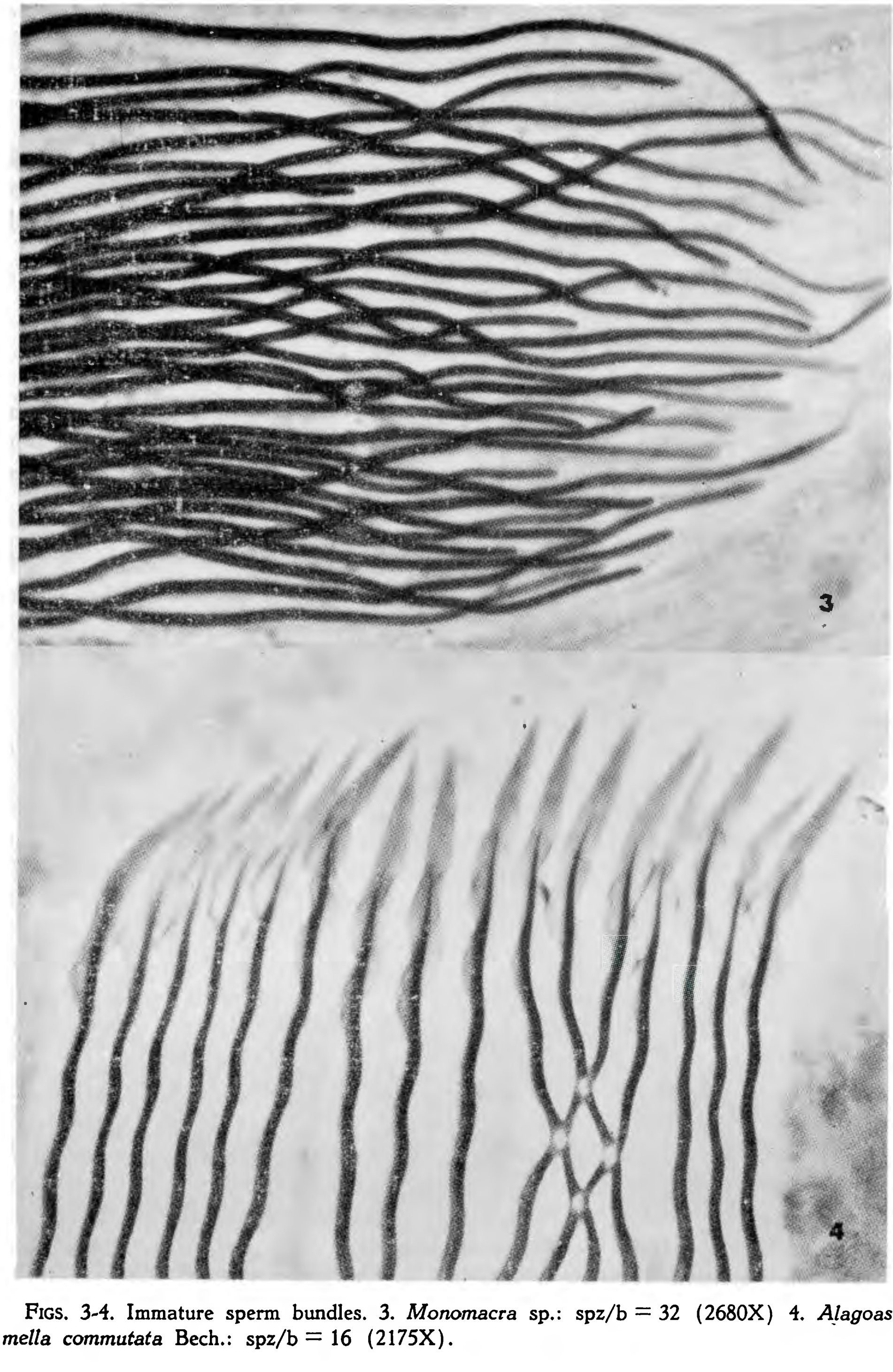
The order Odonata is characterized by a great variation in spz/b, which, in addition, can occur within one and the same specimen. The species-specific spz/b has not emerged yet. As mentioned above, the family Libellulidae has no bundles whatsoever.

In Orthoptera, spz/b is already fixed per species, although some species may show two alternative numbers, an instability that can perhaps develop in any part of the insect system. White (1955) has counted many spz/b in grasshoppers, finding the highest numbers (2048) in the primitive family Eumastacidae, and very low numbers (64) in Cyrtacanthacrididae.

Heteroptera has relatively high numbers, but the very specialized homopterous family Coccoidea has very low numbers, inclusive the low record, spz/b = 16, which, however, is the result of certain postmeiotic arrangements that lower the original number of 64 spermatids per cyst through 32 to 16 (Nur 1962, Dikshith 1956).

Higher numbers than spz/b = 512 have not been found among holometabolous insects. In Neuroptera, a primitive family, Chrysopidae, has the lowest counts.

In Coleoptera, spz/b = 256 is rather common, but 512 has been found in the primitive scarab genus *Pleocoma.* In the scarab tribe Aphodiini, *Aphodius* has spz/b = 128, whereas a more primitive genus *Psammodius,* has 256 spz/b. Some 25 species of Passalidae studied recently (Virkki and Reyes-Castillo 1972) have all 128 spz/b. Among Coleoptera, lower numbers than this are solely due to the family Alticidae. In Alticidae, some very primitive genera survive, like *Forsterita.* On the other hand, the family comprises some of the most "modern" Coleopterans: the tribe Oedionychini. Figs. 1-4 show sperm bundles of some Alticidae. *Forsterita* still has a very typical Coleopteran count: 256 spz/b. 128 spz/b is very rare, but has been found in two primitive or systematically isolated genera: *Cacoscelis* and *Ocnoscelis.* Species of the primitive and cosmopolitan genus *Altica* have 64 spz/b.



FIGS. 3-4. Immature sperm bundles. 3. *Monomacra* sp.: spz/b = 32 (2680X) 4. *Alagoasa mella commutata* Bech.: spz/b = 16 (2175X).

The subtribes of Oedionychini differ in spz/b. Genera of Disonychina have 32 spz/b, whereas all genera of Oedionychina have only 16 spz/b. This is the true low record known up to date among all insects. The number of spermatocytes I is 4 per cyst, that of spermatocytes II being 8, and that of spermatids, 16 per cyst (Virkki 1969, p. 20). Thus no postmeiotic arrangements are involved.

Turning to Table 1, the order Lepidoptera is very constant, 256 spz/b being the characteristic number. However, in a very specialized case, a leaf miner *Tischleria,* only 128 spz/b were found.

The "high" orde Diptera has relatively low counts, the lowest, spz/b = 64 and spz/b 32 being encountered in the specialized family Drosophilidae.

The impression one obtains from this comparison is that the higher orders are characterized by lower spz/b than the more archaic ones, and within an order or a lower taxon, the most specialized species have lower numbers than the less specialized ones. In other words, there seems to be a tendency comparable with the so-called Dollo's law, which attributes an irreversible reduction of metameric characteristics like number of ocelli, or hairs, to the derivation (see Crowson 1970).

2. Volumen of testis, spermatocyst, and spermatocytes in relation with spz/b

Let us consider these relevant characteristics. If the number of spermatocytes per cyst is reduced, the volume of the cyst is reduced correspondingly, and so could be the volume of the testis follicles also. There are two compensatory factors, however: 1) increase of the spermatocyte volume and 2) increase of the number of cysts, owing to proliferation of indefinitive and/or predefinitive spermatogonia. Both factors operate indeed. It is uncertain whether reduction of spz/b is often, or always, accompanied by an increase of the spermatocyte volume, but there are some good examples of this anyway: Oedionychina in Alticidae, Aphodiini in Scarabaeidae, and certain species of *Drosophila.* Apart from insects, it is known to occur also in Ostracoda (Dietz 1958, 1968). Despite the elimination of most of the cytoplasm during the spermiohistogenesis, the spermatozoa of these animals also grow very long. I have just measured the length of the spermatozoon of *Oedionychus bicolor* L. to be 4.4 mm, which is close to the body length. Meyer (1968) found the spermatozoa of *Drosophila melanogaster* (spz/b = 64) and *D. hydei* spz/b = 32) to be 1.8 mm and 6.6 mm long, respectively. In Ostracoda, spermatozoa up to 1 cm long have been reported (Bauer 1940). These are very markant cases, principally due to an enormous diplotenic growth. But also in less dramatic cases there could be a slight increase of the initial volumen of spermatocytes I as a result from a lowered number of cells per cyst. This is so because one or more of the cell-size-reducing mitoses of definitive gonia have been omitted, or have turned to budding, like in Oedionychina (Virkki 1972).

As a good example for the other compensatory factor we have the Passalidae: the number of sperm cells is reduced to 128 per bundle, but the number of bundles has been enormously increased, the volume of testis follicles being also moderate or big.

It seems obvious that the actual size of testis depends also on the sperma-

TABLE 2. Body length and certain measures related with spermatogenesisin four fleabeetles of different degrees of specialization.

	Body length (mm)	*No. of follicles per testis*	*Diameter of testis (mm)*	*Volume of testis* (mm^3)	*Volume of spermatocyte I* (u^3)	*Volume of spermatocyst I* (u^3)	*Spz/b*
Forsterita sp.	4.7	4	1.5	2.4	5,000 ×)	1,280,000	256
Altica occidentalis Suffr.	4.0	4	0.8	0.3	9,496	611,800	64
Altica jamaicensis F.	8.0	4	2.2	5.7	15,986	1,003,100	64
Oedionychus bicolor L.	5.8	4	1.8	3.0	104,770	1,676,300	64

×) A rough estimate from squashed cells.

TABLE 3. Total sperm production as a function of premeiotic cytology in two scarab genera, *Pleocoma* and *Lichnanthe*.

	Number of testes	*Follicles per testis*	*Sperm bundles per follicle*	*Spz/b*	*Total spermatozoa*
Pleocoma	2	18	360 ×)	512	6,635,520
Lichnanthe	2	9	168	256	774,144

×) Approximate number.

togenetic stage. Also the mode of the course of spermatogenesis affects it: a process that takes place in one big wave, needs a bigger testis than a slow process.

Furthermore, the size of the testis correlates positively with the size of the body. To mention a dramatic example, there are big dynastines (small spermatocytes !) that have much bigger testis follicles than the total body volume of many *Aphodius* species (very big spermatocytes !). Presumably, the factors controlling body size, pleiotropically affect all or almost all organs, inclusive testes. The mechanism of their action in the testis could be based 1) on the increase of the number of apical apparatuses (and hence of testis follicles), 2) on the numerical increase of the spermatogonia in one or more of the three categories, and 3) on increase of the spermatocyte volume.

Actually, I do not know any case where the big size of the testis would be accompanied by an increased volumen of spermatocytes, or by an increased number of spermatocytes per cyst. But there is the family Passalidae, where the follicle size certainly has been influenced by the proliferation of gonia prior to gonia definitiva.

Let us still see how certain measures taken from Alticids concord with these considerations (Table 2). The diameter of testis follicles corresponds well with the length of the body. The two species of *Altica* have both 64 spz/b. Relation of the volumes of the spermatocyte I approximates to 1:2, relation of the volumes of the follicles being 1:19, respectively. The discrepancy between the volume relationships must be due to increased number of cysts, just like in Passalidae as compared with most other Scarabaeoids. Increase of the volume of spermatocyte I by 20 times from *Forsterita* to *Oedionychus,* with simultaneous radical reduction of spz/b, does not affect much the volume of the spermatocyst I nor that of the testis follicles. Probably there has been no essential change in the number of sperm bundles produced; in that case, the total production of spermatozoa has been reduced to 1/16 (= 16/256).

I think this is enough to show that the size of the testis does not reflect truly its capacity of sperm production, nor the size of the germ cells, nor spz/b. Many factors are involved in the control of sperm production.

3. Total production of spermatozoa

It is not easy to determine the total sperm production of an insect. I think I was able to do it with some exactness in two scarab genera, *Pleocoma* and *Lichnanthe* (Virkki 1967). The male meiosis of these beetles proceeds in one big wave, which facilitates counting of all spermatocysts in carefully squashed testis follicles. In the testis of *Lichnanthe rathvoni* Leconte, in comparison with that of *Pleocoma,* all phases of germ cell multiplication are reduced simultaneously: 1) the number of apical apparatuses (expressed in number of follicles) lowered, 2) the number of predefinitive and/or indefinitive gonia (expressed in number of sperm bundles produced) lowered, 3) the number of mitoses of definitive gonia (expressed in spz/b) also lowered (Table 3).

We can anticipate that when a strong tendency, or need, of reduction of the sperm cell production sets in, all these three levels are affected. When the tendency is less, the first two methods may be sufficient. It seems namely conceivable that the number of definitive mitoses is the most conservative in the whole system of gonia, and obviously so because the meiotic situation is introduced in the cells during these divisions. Intervention in such a delicate system must be a risk. Being less sensitive, earlier divisions are more suitable for regulation —up and down— of the total number of cells produced.

4. A hypothesis.

As a result of these observations and considerations, a tripartite hypothesis can be established: 1) spz/b tends to be lowered in specialization, 2) reduction of spz/b tends to reflect a radical reduction taken place in the total sperm cell production, 3) adjusment —up or down— of total sperm cell production is preferably arranged regulating multiplication of germ cells prior to definitive spermatogonia.

5. Production of gametes and evolution

What adaptative value should adjustments of sperm cell production have for an insect population? The basic requirement is that enough gametes are produced to assure the survival of the population. Thus the ratio of spermatozoa to eggs usually reflects the risks of fertilization (Davis 1966).

Probably it deals with a control of genetic variability also. Obviously, a population well adapted to occupy a slightly variable niche benefits from an arrest of genetic variability, whereas another population, conquering or holding a very variable niche, benefits from an increased genetic variability.

The total mathematical potential of genetic recombination can be adjusted in many ways: by adjustment of chromosome number, by control of chiasma frequency, sometimes reduced to O (*Drosophila* males, Lepidopteran females), by chiasma distribution (in the case of extreme localization of chiasmata, practically the whole length of the chromosome is excluded from crossing over). Additional control of the effective recombination is provided by regulation of the total production of gametes. Examples of an extreme reduction of gamete production are *Copris* (Scarabaeidae), where the total production of eggs is 5 per female and lifetime (Matthews 1969), and *Miastor* (Cecidomyidae), with total production of 1024 spermatozoa per male (White 1946). The less is the number of gametes produced per population, the less are the changes for rarer mutations and recombinations to pass to the next generation. This should help in their elimination, especially in small populations.

6. Big cells for large chromosomes.

Worth mentioning before I conclude is an interesting evolutionary application of the big size of spermatocyte (which, as discussed above, seems frequently if perhaps not always to be associated with a reduced spz/b. I am referring to the spermatocytes of Alticidae: Oedionychina. The cell size supposedly controls the size of chromosomes (White 1954). The sex chromosomes of these fleabeetles are so large, up to 50 microns in length, that they would not fit in spermatocytes of conventional size. Everybody who has collected these beetles in tropical foliage, must be impressed by the competence of this subtribe to occupy specialized niches. The sort of karyotype they possess seems to be very advantageous genetically. But the prerequisite for its evolution has been the large size of the cells, especially of the spermatocytes I, where the peculiar orientation and segregation of the giant sex chromosomes takes place (Virkki 1970).

Resumen

Los espermatozoides de casi todos los insectos están arreglados en fascículos. El número de espermatozoides por fascículo (espz/f) es muy constante para la especie, excepto en el primitivo orden Odonata.

El espz/f es determinado por el número de las mitosis sincronizadas de las espermatogonias definitivas. El número de los fascículos es controlado por la multiplicación de las células germinativas anteriores a las gonias definitivas. El número de fascículos testiculares depende del número de aparatos apicales formados. Consecuentemente, la producción total de espermatozoides depende grandemente de la citología, premeiótica de la línea germinativa.

Los insectos especiaizados tienden a tener menos espz/f que los insectos más primitivos. Parece posible que la reducción de espz/f refleje reducción en la producción total de espermatozoides, que a su vez puede limitar la variabilidad genética y así ayudar a la población a adaptarse para nichos especializados y poco variables. Tales ajustes son reversibles, aunque el espz/f puede que sea muy conservador, cambiándose sólo en caso de necesidad extrema.

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