Chromosome Behaviour in *Laccifer lacca* (Kerr) Lacciferidae-Coccoidea

T. S. S. Dikshith

Indian Lac Research Institute, Namkum, Ranchi, Bihar, India

Received January 16, 1964

Lacciferidae comprises a relatively uniform and important family of Coccoidea. Although many other members of the Section Lecanoidae have received considerable cytological attention, only very little work has been done on the cytology of Lacciferidae (Brown 1959, Dikshith 1962, 1963, Teotia and Dikshith 1963). *Laccifer lacca* (Kerr) being an economically important coccid due to its versatile resinous secretion—the lac, created much interest to undertake a cytological investigation on the manner of male germ-cell formation. Recent study by Brown (1959) on *Tachardiella* sp. has indicated that he could not follow the complete sequence of chromosomal behaviour with satisfactory clarity.

Materials and methods

Apterous males of the *Rangeeni* strain of *L. lacca* commencing from the second instar up to the time of male emergence were collected from the plantation of Indian Lac Research Institute. They were fixed in Allen's Bouin and Sanfelice fixatives for a period ranging from 4–16 hours. 0.5%, Iron alum haematoxylin and Feulgen, were used as the stains; light-green was used as the counter-stain for the latter. For Feulgen stain reaction, the testicular tissue as well as the smeared slides were hydrolysed in 1N.HCl at 60°C for 5–5½ minutes. All drawings were made with the help of camera lucida (Carl Zeiss Jena) at table level, using the squash and smear preparations. Photomicrographs were taken with the bellow camera using 'Agfa' films. In spite of large numbers of both temporary and permanent preparations it became very difficult to make exact counting of the chromosomes at meiosis, since they were invariably fused together.

Observations

1. Reproductive organs of the male insect

Sexes are separate. The female gives rise to the next progeny of young ones by mating and fertilization. The male lac insect possesses a pair of testes disposed off laterally in the region of abdomen and occupying a major portion of the body cavity. Each testis is an elongated oval structure and runs as a short Vas deferens which joins with the fellow of the other side and forms a common duct—the ducts ejaculatorius. This duct, after running through the rest of the abdominal segments, enters the aedeagus and opens on the penial style (Misra 1924, 1931).

Each testis is made up of many rosettes, each of which in its turn

carries sixteen cells, arranged in four groups of four cells. Individual cells of each group are roughly triangular in shape with their broader ends turning toward the periphery and the narrow ends projecting centrifugally. Each

Figs. 1-16. 1, spermatocytes arranged in groups of four with prominent nuclei. ca. $\times 650$. 2, spermatocyte in resting stage with chromosomes of the heterochromatic set at the nuclear membrane. ca. $\times 650$. 3, chromosomes of the euchromatic set coming up from the chromatin reticulum. ca. $\times 650$. 4, same as Fig. 3. ca. $\times 960$. 5, haploid set of 9 chromosomes of the euchromatic set fully dispersed and the 9 chromosomes of the heterochromatic set in a clumped state. ca. $\times 960$. 6, metaphase I with 18 chromosomal complements (polar view). $\times 650$. 7, metaphase I (side view). ca. $\times 650$. 7A, metaphase I with "accessory structure". ca. $\times 650$. 8 and 9, anaphase I. ca. $\times 650$. 10 and 11, telophase I. Chromosomes of the two sets rearranged. ca. $\times 650$. 12 and 13, chromosomes of the heterochromatic set developing a monopolar spindle and their movement. ca. $\times 960$. 14, heterochromatic set reaching the other pole: cytoplasmic division at telophase II, suppressed. ca. $\times 650$. 15, binucleate spermatid with euchromatic and heterochromatic nuclei. ca. $\times 650$. 16, quadrinucleate spermatid formed by the fusion of sister binucleate spermatids. ca. $\times 650$.

cell possesses a prominent, roughly spherical nucleus and a sheet of dense cytoplasm (Fig. 1).

2. The chromosome cycle

Chromosome number is the same in both the sexes, the diploid number being eighteen. No sex chromosomes have been demonstrated. Behaviour of these 18 chromosomal complements in the male is quite interesting and is in consonance with the "Lecanoid system" (Brown 1959, Hughes-Schrader 1935). The resting nuclei of the male always carry a heterochromatic body which is not present in the cells of the female insect (Fig. 2). Clumping together of the chromosomal complements—a common feature among the members of Lecanoidae, does not allow for an easy and satisfactory observation. It was, however, encountered several times during the observations that haploid set of nine chromosomes was spreading out quite clearly, while the other set of nine chromosomes was lying in a fully clumped state. The two sets of chromosomes have been named as the Euchromatic and Heterochromatic sets, respectively (Figs. 3 to 5).

Spermatogonial stages are very rare and this could be attributed to their quick divisions and brief halts during the division cycle. However, the spermatocyte stages after mid-prophase and onwards are quite numerous. The nucleus at mid and late prophase I, presents a pycnotic knot of chromatin attaching itself to the nuclear membrane and the chromosomes of the euchromatic set scattered all over the nuclear cavity in the form of chromatin reticulum (Fig. 2). Later on, individual chromosomes of the euchromatic set come up by and by from the chromatin reticulum (Figs. 3 to 5). At metaphase I, for reasons unexplainable, this characteristic feature of the heterochromatic set vanishes; the chromosomes of the two sets are indistinguishable (Fig. 6 and 7). The first division is an equational one with the formation of a typical bipolar spindle and the chromosomes separate out equationally (Figs. 8, 9, 10, 11).

At late telophase I (Figs. 10, 11), the chromosomes of each group segregate once again into two sets: euchromatic set and heterochromatic set. This stage presents a fine structure of interzonal fibres which, as the division advances forms a fine cytoplasmic stalk (Fig. 10). No interphasic nuclei have been observed here also as in *Phenacoccus* (Hughes-Schrader 1935). At late metaphase II, a monopolar spindle is developed from the chromosomes of the heterochromatic set while the chromosomes of the euchromatic set do not show any such division apparatus (Figs. 12, 13). As the division progresses, the fibres of the monopolar spindle become shortened and the chromosomes of the heterochromatic set move poleward (Fig. 14). There is no cytoplasmic division of the cell after the separation of the two sets of nuclei and this suppression of the cytoplasmic division results in the formation of the binucleate spermatids (Fig. 15). Sister binucleate spermatids fuse together to form a quadrinucleate spermatid (Fig. 16). A detailed investigation

on the formation of the spermatozoon from the spermatid has been already discussed elsewhere (Dikshith 1963).

Figs. 17-20A. 17, same as in Figs. 1 and 2. ca. $\times 560$. 18, metaphase I, anaphase I. ca. $\times 560$. 19, telophase I. ca. $\times 1200$. 20, binucleate spermatid. ca. $\times 800$. 20A, quadrinucleate spermatid forming the sperm. ca. $\times 650$.

During the course of this investigation it was also observed at one place what has been called the "accessory structure" by Brown (1959). It was

encountered with metaphase I, of the division cycle (Fig. 7A). This structure took the Feulgen stain quite well which indicates that the structure appears to be chromatin in nature. It is not known whether this structure has got any bearing with the meiotic cycle alone, since it was not encountered during somatic mitosis. Any kind of conclusion is, however, unwarranted till further researches prove the correct occurrence of this structure.

Discussion

The unorthodox cytological system in *Pseudococcus nipae* (Mask) was unravelled for the first time by Schrader (1921). Later studies of Schrader (1929) and Hughes-Schrader (1935, 1948) resulted in classifying the peculiar behaviour of chromosomes of these mealy bugs as "Lecanoid", which has been characterized by the facultative heterochromatization of one set of chromosomes in the male embryo and its exclusion from the genetic continuum during spermatogenesis without participating in gamete formation. Hughes-Schrader (1948) assuming this set as a genetically inert one declared the condition of the male Lecanoids as one of "physiological haploidy" and further stated that this set has been paternally derived.

Recent studies of Bennet and Brown (1958), Brown and Nelson-Rees (1961, 1962) on some Lecanoids with X-ray radiation have demonstrated that the heterochromatic set of chromosomes, which are being eliminated during spermatogenesis, are of paternal origin and they also have shown that the male haploidy in these mealy bugs is a resultant of chromosome elimination during embryogeny. The same appears to be a true condition with the lac insect, *Laccifer lacca*. It has been observed at all places during this investigation that the heterochromatization of one set of chromosomes appears only in the case of male tissues and never in the female tissue. There is thus a close association of heterochromatization and the male insect and this characteristic feature manifests from the blastula stage onwards of the male.

Stevens (1905) while discussing the theory of Paulmer and Montgomery on the function of heterochromosomes, states that the heterochromatin is a degenerating chromosome. According to him, Montgomery further considers them as follows: "chromosomes that are in the process of disappearance in the evolution of higher to a lower chromosome number". Although the chromosomes of the heterochromatic set in *Laccifer lacca* are ultimately to degenerate, it is very difficult at this stage to state as to how far they are contributing in the reduction of chromosome number. In the male *Laccifer lacca*, the chromosomes of the heterochromatic set exhibit from the beginning to end indications of abnormality and finally of their degeneration (Dikshith 1963). It is also not clearly understood whether the causes that are responsible for the heterochromatization of one haploid set of chromosomes in the males of *Laccifer lacca* are genetic or otherwise.

Nelson-Rees (1960) has reported that possibly the environmental stimuli may induce the chromosome differentiation into euchromatic and heterochromatic sets and thus govern the physiological haploidy of males. He advocates that a change in the constitution of the ovarioles, viz. the stage of female meiosis and the nature of the egg cytoplasm at the time of fertilization as they are aged, may be responsible for the induction of heterochromatization of paternal chromosomes soon after fertilization. Since the genetic equipment of

the oocyte at meiosis and the structure of cytoplasm at fertilization control the differentiation, it appears both environmental and genetic factors are responsible for the phenomenon of heterochromatization.

Bennett and Brown (1953) observed that sex is determined or rather predetermined by factors governing chromosome elimination. According to them an individual embryo will develop either into male or female according to the elimination or non-elimination of the paternal set; and this might have been influenced by environmental or genetic factors or both kinds. They have stated that the physiological condition within the ovary is responsible for predetermining the sex of the offspring. It is possible that the same condition may be true in the case of the lac insect, *L. lacca* also.

Researches of James (1937, 1938) with *Pseudococcus citri* (Risso) have also shown that the environment plays a marked role on the sex-ratio. However, it becomes necessary to know that, when the sex has been controlled and determined by the environment, how no "intersexes" have been observed in spite of the variable effects of the environment. A clear picture of the phenomenon of heterochromatization and also of sex determination in the members of Lecanoidae is thus wanting and further researches alone could throw light on these lines.

It may be desirable to refer to recent accounts on the "chromosome number and spermatogenesis in the male *Laccifer lacca* (Kerr)" as reported by Tulsyan (1963). These findings seem to be completely erroneous in view of the following facts:—

Tulsyan (1963) has wrongly used the terms like "spermatocytes" and "spermatids". What has been called as the "secondary spermatocyte" (Ref. to his Fig. 2) is in reality an advanced stage of the quadrinucleate spermatid and this is a common structure met in the males of all Lecanoids (Brown 1959, Brown and Nelson-Rees 1961, Dikshith 1963, Hughes-Schrader 1935, Nelson-Rees 1960, Schrader 1921, 1929). Further the proximal end of the nuclear papilla (cytoplasmic anlage) has been wrongly termed as the "spermatid" and the distal end as the "sperm". It has not been mentioned by Tulsyan (1963) anywhere how a mature sperm looks like, since the same has not been figured, although the structure of the mature sperm* of *L. lacca* has been studied in detail by Dikshith (1962, 1963). On the contrary, the just formed nuclear papilla has been wrongly termed both as a spermatid and a sperm (Ref. Tulsyan, 1963, Fig. 4).

The diploid chromosome number of *L. lacca* is 18 in both sexes. In this respect *L. lacca* falls in line with other Lecanoids (except *Puto*). Brown (1959) states that "in the Lecanoid system the chromosome number is the same in the males and females". Nelson-Rees (1960, 1962) also in his study on the ovarian modification in *Planococcus citri* (Risso) states that "in the Lecanoid system both sexes are diploid". Again Brown and Nelson-Rees (1961) discussing the radiation analysis of a Lecanoid genetic system very clearly state that "in the Lecanoid system both males and females are dip-

* Recent studies of Dikshith (in press) have further revealed that the structure which appeared as the 'sperm' is really the 'sperm bundle' carrying within it 16 sperms.

loid'. Further, as it has been described (Tulsyan 1963), the division is neither 'usual' nor there are the 'formation of 32 binucleate secondary spermatocytes'. No singular instance has been recorded so far wherein the Lecanoid genetic system would present the 'formation of 32 binucleate secondary spermatocytes'. Tulsyan (1963) has entirely mistaken a quadrinucleate spermatid for a binucleate secondary spermatocyte. So far only binucleate spermatids have been recorded (Dikshith 1963, Hughes-Schrader 1935, 1948, Schrader 1921, 1929).

The characteristic feature of male Lecanoid and also of *L. lacca* in possessing two kinds of chromosomes viz. euchromatic and heterochromatic has been totally ignored by Tulsyan 1963, since the heterochromatic nuclei have been misnamed as 'nuclear matter' and with no reference to the euchromatic nuclei which alone give rise to the sperms (Bennett and Brown 1958, Brown 1959, Dikshith 1963, Hughes-Schrader 1935, 1948, Schrader 1921, 1929). Moreover while studying the 'spermatogenesis' Tulsyan (1963) has not taken into account the important features of the Lecanoid system such as i) the division of the gonial cells ii) the formation of the monopolar spindle iii) suppression of the second cytoplasmic division iv) formation of binucleate and quadrinucleate spermatids v) fate and role of the heterochromatic nuclei and their contribution in the restoration of the physiological haploidy of the male *L. lacca*.

References cited (Tulsyan 1963, ref. 3 to 8) have got no bearing either to the cytology of *L. lacca* or to any other Lecanoids though there are numerous references available on the cytology of Lecanoids. It has been stated (Tulsyan 1963) that the sex mechanism of *L. lacca* falls in line with that of *Puto*. It appears that the author, Tulsyan, (1963) does not seem to have seen the original work of Hughes-Schrader (1944) on *Puto* sp. where it is described that '*Puto* sp. of the family Pseudococcidae reveals a primitive chromosome cycle probably archetypal for coccids'. Further she states that 'its relatively generalized chromosome cycle is most nearly approached by the more primitive species of the Llaveine tribe of the family Margarodidae' and not by the Lecanoids. Once again Hughes-Schrader (1948) while discussing the Lecanoid type very clearly writes that 'with the single exception of *Puto* all species of Lecanoids thus far studied possess a unique type of male meiosis characterized by i) segregation without prior synapsis ii) differentiation in the two haploids (euchromatic and heterochromatic) of chromosomes of distinctive behaviour and coiling cycles and iii) the suppression of sperm formation in half of the gametic nuclei'. Researches carried out in this laboratory on *L. lacca* (Dikshith 1962, 1963) fully agree with the works of Brown (1959) and Hughes-Schrader (1935, 1948) on other Lecanoids.

Tulsyan (1963) has not noticed that in *Puto* four normal sperms are formed from each quadrinucleate spermatid without any degeneration of nuclei, a condition unavailable either in *L. lacca* or in any other Lecanoid.

Summary

The diploid chromosome number of *L. lacca* is eighteen in both sexes. Males of *L. lacca* maintain, from an early embryonic stage, one haploid set of chromosomes in a condensed form and this heterochromatic set later on at second division develops a monopolar spindle and reaches the other pole of the spindle.

Cytoplasmic division is suppressed and this results in the formation of binucleate spermatids which later fuse to form the quadrinucleate spermatids.

The spermatid nuclei formed by the chromosomes of the heterochromatic set degenerate while those formed by the euchromatic set give rise to typical coccid sperms.

Acknowledgment

Grateful acknowledgement are made to Mr. Y. Sankaranarayanan, Officiating Director of the Institute, for his keen interest during this investigation and to Mr. P. Das for making drawings and for the preparation of photomicrographs.

Literature cited

- Bennett, F. D. and Brown, S. W. 1958. Life history and sex determination in the diaspine scale *Pseudaulacaspis pentagona* (Targ) (Coccoidea). *Canad. Ent.* 90: 317-325.
- Brown, S. W. 1959. Lecanoid chromosome behaviour in three more families of the coccoidea (Homoptera), *Chromosoma* 10: 278-300.
- and Nelson-Rees, W. A. 1961. Radiation analysis of a lecanoid genetic system. *Genetics* 46: 983-1007.
- Dickson, R. C. 1941. Inheritance of resistance to hydrocyanic acid fumigation in the California red scale. *Hilgardia* 13 (9): 513-522.
- Dikshith, T. S. S. 1962. A living sperm of *Laccifer lacca*, *Curr. Sci.* 31: 73-74.
- 1962 (a). Chromosome number of *Laccifer lacca* (Kerr) (Kusmi strain) Homoptera-Coccoidea. *Curr. Sci.* 31: 383-384.
- 1963. Spermiogenetic studies in *Laccifer lacca* (Kerr) Homoptera-Coccoidea. (Abst) Ind. Sci. Congress (Delhi, 1963) *Proc.* 50: 470.
- Hughes-Schrader, Sally, 1935. The chromosome cycle of *Phenacoccus* (Coccidae). *Biol. Bull.* 69: 462-468.
- 1944. A primitive coccid chromosome cycle in *Puto* sp. *Biol. Bull.* 87: 167-176.
- 1948. Cytology of coccids (Coccoidea-Homoptera). *Adv. Genetics* 2: 127-203.
- James, H. C. 1937. Sex ratios and the status of the male in Pseudococcinae (Hemiptera-Coccidae). *Bull. Ent. Res.* 28: 429-461.
- 1938. The effects of the humidity of the environment on sex-ratios from over aged ova of *Pseudococcus citri* (Risso) (Hemiptera-Coccidae). *Proc. Roy. Ent. Soc. London, Ser. A*, 13: 73-79.
- Misra, A. B. 1924. On the genitalia of the male lac insect (*Tachardia lacca*, Kerr) with a note on its method of copulation. (Abst) Ind. Sci. Congress (Bangalore-1924) *Proc.* 11: 119.
- 1931. On the internal anatomy of the male lac insect, *Laccifer lacca* (Kerr) (Homoptera-Coccidae) *Proc. Zool. Soc. London, Part 4* 1: 1359-1381.
- Nelson-Rees, W. A. 1960. A study of sex predetermination in the mealy bug, *Planococcus citri* (Risso) *J. Exptl. Zool.* 144: No. 2, 111-137.

- 1962. The effects of radiation damaged heterochromatic chromosomes on male fertility in the mealy bug, *Planococcus citri* (Risso). *Genetics* 47: 661-683.
- Schrader, F. 1921. The chromosomes of *Pseudococcus nipae*. *Biol. Bull.* 40: 259-270.
- 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: 149-179.
- Stevens, N. M. 1905. Studies in spermatogenesis with special reference to the "accessory chromosome". Publication, 36, Carnegie Institution of Washington.
- Teotia, T. P. S. and Dikshith, T. S. S. 1963. Utility of phloroglucinol in the chromosome studies of *Laccifer lacca* (Kerr) (*Rangeeni* strain). *Homoptera-Coccoidea. Indian. J. Ent.* 25: 263-264.
- Tulsyan, G. P. 1963. Studies on the chromosome number and spermatogenesis in the lac insect, *Laccifer lacca* (Kerr), *Curr. Sci.* 32: 374.
-