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SEX DETERMINATION IN THE SNAKE MITE

OPHIONYSSUS NATRICIS (GERVAIS)

(ACARINA : DERMANYSSIDAE) ¹

BY

J. H. OLIVER Jr. ², J. H. CAMIN ² and R. C. JACKSON ³

Although comparatively little has been written on sex determining mechanisms in the Acarina, arhenotoky, thelytoky and sex chromosomes have all been reported. According to OPPERMANN (1935) sex is determined by sex chromosomes in Argas columbarum, in which the male is heterogametic. Thelytoky was reported in Ornithodoros moubata by DAVIS (1951) and in four species of Anoetidae by HUGHES & JACKSON (1958). Arhenotoky is the most common mode of sex determination that has been reported in mites, but most of these reports are based only on rearing data (SkaLy & Hayes, 1949; Camin, 1953; Ubertalli, 1955; Hughes & Jackson, 1958; & Oliver, unpublished) and do not contain cytological confirmation. However, COOPER (1937), working with Siteroptes graminum, demonstrated that the cells of parthenogenetic eggs contained three chromosomes and those of fertilized eggs contained six and Hughes and Jackson (1958) found that the haploid number is four in Anoeetus laboratorium. No similar cytological research has been conducted on the dermanyssid mites except for some rather inconclusive studies on Dermanyssus gallinae by Warren (1940).

The purpose of the present investigation was to establish the mode of sex determination in Ophionyssus natricis utilizing cytological methods.

MATERIALS AND METHODS.

Our stock colony of Ophionyssus natricis was started in 1955 with mites obtained from the Lincoln Park Zoo in Chicago. Since that time new additions to the gene pool have frequently been introduced by placing snakes, infested with mites from

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². Department of Entomology, University of Kansas.

³. Department of Botany, University of Kansas.

other sources, into the colony. Stock colonies are maintained in various kinds of containers, ranging from cardboard drums to wooden cages with screen tops and bottoms and glass fronts. One or more snakes, depending on size and availability, are kept in the containers at all times as a ready source of blood for the mites.

Adult males frequently locate, climb on, and ride deutonymphal females. This behavioral trait was utilized in distinguishing between the sexes in the immature stages. Mites with males riding on them were removed from the stock colony and placed individually in plastic zipper vials after removing the males. Upon molting, these mites emerged as adult virgin females. After being starved for several days, each virgin female was placed separately into the ear cavity of a lizard (*Anolis carolinensis*), and the ear opening was covered with a fine mesh bolting cloth fastened with Pliobond Cement (available from W. J. Ruscoe Co., Akron, Ohio, U.S.A.). This allowed for ventilation and made possible the examination of the feeding mite without disturbing it. Upon reaching repletion, the mites were removed from the ears and placed separately into zipper boxes containing a strip of moist filter paper and kept at room temperature (65-80°F). The time of oviposition was noted and, as they were laid, the eggs were removed and stored in separate containers until they were used for chromosome studies. Upon completion of oviposition after each meal, the females were fed again and isolated as described above. This procedure was continued as long as the mites lived.

Mated females were obtained by placing males and virgin females together and also by randomly removing recently engorged adult females from the stock colony. In employing the latter procedure, if the first two or three eggs produced were haploid, the female was discarded. If any diploid eggs were produced, the female was isolated and subsequent eggs collected for chromosome studies.

All of the chromosome determinations were made on early cleavage divisions. The squash technique with aceto-orcein and aceto-carmine stain was utilized.

Figs. 1 and 2. — Haploid chromosome complement at mitotic metaphase; chromosomes in Fig. 2 have been outlined.
RESULTS.

The mites did not always feed each time they were placed in the ear cavity. Some of the failures were probably due to the molting of the lizards, a condition which would affect the distance that the mite's mouthparts would have to penetrate in order to reach blood.

The chromosome complement of this mite was found to be \( n = 9 \) (figures 1 & 2) and \( 2n = 18 \). They ranged from 1.2 to 2.8 \( \mu \) in length, averaging 1.8 \( \mu \). The karyotype will be discussed in detail in a subsequent paper in which the karyotypes of several dermanyssid species will be compared.

Seventeen virgins yielded 227 eggs and chromosome counts were obtained from 179 of these. All except four of the 179 eggs were haploid. These four diploid eggs, which showed the \( 2n \) number of 18, were laid by three females and were preceded and followed by haploid eggs. The virgin that laid two diploid eggs produced a total of 32 eggs, the diploids being numbers 18 and 20. If she had become fecundated, more diploid eggs should have been laid.

Chromosome counts were made on 226 eggs from 20 mated females. One hundred three eggs were haploid and 123 were diploid. This approximates a 1:1 ratio as confirmed by a Chi square test for goodness of fit (.10 < \( P < .20 \)).

DISCUSSION.

The data indicate that the haplo-diploid type of sex determining mechanism is operative in Ophionyssus natricis. This is further supported by a comparison of our cytological data with CAMIN's (1953) rearing data. He isolated several hundred eggs, obtained from more than 20 virgins, and all developed parthenogenetically into males. Eggs laid by mated females developed into both males and females. These rearing data compare well with our cytological observations except for the four diploid eggs which were produced by virgins. Although the four diploid eggs were viable at the time they were sacrificed, this does not indicate that they would have developed into viable adults, nor does it indicate what sex they would have been had they reached maturity.

Such parthenogenetic diploid eggs could have been formed in one of two ways. At an early cleavage division, cytokinesis may have occurred while karyokinesis did not go to completion. That is to say, the chromosomes formed chromatids which subsequently separated but did not migrate to the poles. After this division, karyokinesis might have become synchronized with cytokinesis, resulting in subsequent cells with the \( 2n \) number. The second possibility is that two haploid nuclei fused. Possibly during oögenesis, immediately after the reduction division, the two nuclei fused instead of one of them being cast off. Thereafter, mitosis occurred normally.
At present, we are unable to explain the mechanism in fecundated females which allows half the eggs to become fertilized and half to remain unfertilized. Although haploid and diploid eggs were frequently laid alternately, no regular or cyclical alternation could be demonstrated. The data gathered from the eggs of three mated females were tested by means of the non-parametric Runs Test and no significant deviation from a random distribution was demonstrated at the .05 level.

The haplo-diploid mode of sex determination does not usually favor the production or maintenance of a 1 : 1 sex ratio. In any inbred population, even with differential sexual mortality, one sex frequently outnumbers the other. In animals with life cycles similar to those of the dermanyssid mites, the sex ratio probably shifts and is constantly in a state of flux. CAMIN (1953) noticed a shift in sex ratio in the snake mite and stated that "at times males would be very numerous and females comparatively scarce. In a few days, at 25°C., a shift would take place and the sexes could be collected in almost equal numbers. The shift would continue, however, and after a week or more the females became numerous and the males were relatively scarce." The explanation of this shift seems obvious and logical. If there is an abundance of males present, most of the females will be fecundated. If males are scarce or absent, most of the females will remain virgins and produce parthenogenetic haploid eggs (i.e. males).

Fecundated females laid approximately 50% of each type of egg (n and 2n) during the period of observation. It is not known whether they produce eggs in this proportion throughout their lifetimes. There is the possibility that later in life the female may become depleted of sperm and produce only haploid eggs, although OLIVER (unpublished) found this not to be the case in another dermanyssid species. If, on the other hand, fecundated females continue to produce haploid and diploid eggs in approximately equal numbers, the shift in sex ratio should be theoretically different from that described by CAMIN (1953). It seems likely that the ratio in a small inbred population, while constantly shifting, would shift from an abundance of males to one of about equal sex ratio and then shift back to more males. It seems improbable that the shift would ever reach the stage where females greatly outnumber the males unless there is a drastic differential mortality between the two sexes. This has not been demonstrated but cannot yet be ruled out.

**Summary.**

1. Chromosome determinations were made on early cleavage divisions. The squash technique with aceto-orcein and aceto-carmine stain was utilized and it was established that the chromosome number is n = 9 and 2n = 18 in Ophionyssus natricis.

2. All but four of 179 eggs obtained from virgins were haploid. The four diploid eggs were laid by three females and were preceded and followed by haploid eggs. Chromosome counts were made on 226 eggs from 20 mated females and 103 were haploid and 123 were diploid. This approximates a 1 : 1 ratio (.10 < P < .20).
3. Our cytological data and rearing data (Camin, 1953) indicate that sex is determined in *Ophionyssus natricis* by the haplo-diploid mechanism.

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