MEIOSIS IN THE GRASSHOPPER

III. CHIASMA FREQUENCIES IN FEMALES AFTER ELEVATED TEMPERATURE

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SUMMARY

The effect of high temperature on chiasma formation during oogenesis has been studied in the grasshopper *Melanoplus femur-rubrum*. Prolonged heat treatment (40° C.) during mid-prophase of meiosis causes a reduction in the mean chiasma frequency per cell. Only those bivalents in which more than one chiasma occurs are affected by the heat. The pattern of chiasma frequency response to heat is similar to that which occurs in males of the same species.

1. INTRODUCTION

THE sequence of events occurring during meiosis has been the subject of many recent investigations. Most experimental results have been or can be interpreted to indicate that genetic crossing-over occurs during pachytene of meiosis (for review see Henderson, 1970). For technical reasons, most investigators have studied male meiosis, *i.e.* spermatogenesis or microsporogenesis. The assumption is made that similar meiotic events occur in both sexes. However, Grell and Chandley (1965) obtained evidence that in *Drosophila melanogaster* females genetic recombination most likely occurs at or near the premeiotic DNA synthetic period. No information is available concerning the time of genetic recombination or chiasma formation from females of other species. Where female meiosis has been studied differences have been observed compared to the male meiotic process. Those differences include, for example, the diffuse diplotene stage in females (Callan and Lloyd, 1960) and differences in chiasma frequency and localisation (see John and Lewis, 1965, for review). Thus, the possibility remains that there are distinct differences between the sexes concerning the time of chiasma formation. A problem in pursuing this reasoning is the technical difficulties encountered in studying the cytology of female meiosis. However, I have determined that the ovaries of nymphal stages of a grasshopper *Melanoplus femur-rubrum* display some diplotene cells from which chiasma frequencies can be obtained. In addition to the technical reasons, *Melanoplus femur-rubrum* females were chosen for the study because information is available from similar studies carried out in the male of this species (Church and Wimber, 1969) and a basis for comparison between the sexes is available.

The present report describes the chiasma frequency response to prolonged heat treatment in *M. femur-rubrum* females. The results indicate that chiasma formation occurs at or following mid-prophase. These mid-prophase events can be disrupted by prolonged heat treatment. Furthermore, the chiasma frequency response to high temperature is remarkably similar to that which occurs in males of the same species.

2. METHODS AND MATERIALS

Melanoplus femur-rubrum female nymphs aged 1 to 3 weeks were used in the experiment. For details on collecting, hatching and rearing see Church (1972).

Prior to the beginning of the experiment, chiasma frequencies were obtained for 30 cells in diplotene. Ovaries were extirpated and placed in a drop of 2 per cent lacto-propio-orcein for 5 minutes. Squash preparations with very light thumb pressure were made. The coverslips were rimmed with fingernail polish. Immediately after each slide was prepared it was scanned for cells in diplotene from which an accurate chiasma frequency could be obtained. Obtaining 30 analysable cells in diplotene is a monumental task for the following reasons. Subsequent to pachytene of meiosis the bivalents form a typical diplotene configuration. However, this stage must be a relatively short one since it is present in only a few of the ovarioles and when it is present only three or four cells display the stage. Following this typical diplotene stage, the chromosomes elongate into a lampbrush stage and can no longer be visualised using conventional techniques. When diplotene cells are present in a preparation, they cannot always be analysed. Thus approximately 125 animals were sacrificed in order to obtain chiasma frequencies for 30 cells in diplotene.

Having established a normal chiasma frequency for *M. femur-rubrum* females, nymphs were then injected with $2.6 \mu\text{C}$ of H^3 thymidine (Sp. act. 6.7 C/mM) and placed in incubators at $40^\circ \pm 0.5^\circ \text{C}$. The grasshoppers were sampled every 24 hours for 6 days. Ovaries were extirpated and squash preparations in 2 per cent lacto-propio-orcein were made. The slides were immediately scanned for cells in diplotene. Chiasma frequencies were obtained for 10 cells per daily sample. Again, many animals had to be sacrificed each day to obtain 10 analysable cells.

Ovaries from four nymphs from each daily sample were prepared for autoradiography. Freshly extirpated ovaries were squashed in 45 per cent glacial acetic acid. The coverslips were removed by the dry ice method and dipped in Kodak NTB liquid emulsion. The slides were exposed for 2 weeks and processed for autoradiography. The autoradiographs were stained through the emulsion with Delafields hematoxylin.

3. RESULTS

The grasshoppers were injected with H^3 thymidine in order to label a block of cells in the pre-meiotic DNA-synthetic period at the beginning of the heat treatment. Meiotic development was determined autoradiographically by daily sampling (table 1). At 40°C ., the time required for cells to pass from the premeiotic DNA synthetic period through the preleptotene spiral stage (plate I, fig. A) to leptotene-zygotene of meiosis (plate I, fig. B) is approximately 2 days. Labelled cells appeared in pachytene at day 3 (plate I, fig. C). It requires approximately 5 days for cells labelled during pre-meiotic S to reach diplotene (plate I, fig. D).

Chiasma frequencies are presented in text-figs. 1 and 2. The normal mean chiasma frequency for this population of *M. femur-rubrum* females is 15.233 per cell (text-fig. 1). Three or four bivalents usually display two or three chiasmata (plate I, figs. E and G), and the majority of the cells con-

TABLE 1
Timing of meiosis

Days at 40° C. after injection with H3 TdR	Latest meiotic stage labelled					
	Interphase	Pre- leptotene spiral	Leptotene	Zygotene	Pachytene	Diplotene
0	2*	—	—	—	—	—
1	2	2	—	—	—	—
2	—	1	1	—	—	—
3	—	—	—	1	2	—
4	—	—	—	—	2	—
5	—	—	—	—	2	1

* Number of individual nymphs observed.

tained 14 or more chiasmata (text-fig. 1). Significant drops in chiasma frequencies occurred on days 2 and 3 following the beginning of heat treatment (text-fig. 2). The chiasma frequency remained low throughout the experiment. The majority of the cells from samples obtained of days 2, 3, 4, 5 and 6 displayed 12 or 13 chiasmata (plate I, fig. F; text-fig. 2). The minimum chiasma frequency per cell observed was 12. Univalents were not observed in any of the timed samples.

FIG. 1.—Frequency distributions of chiasma frequencies obtained from animals prior to the beginning of heat treatment.

Based on autoradiographic evidence, it can be estimated that the cells in diplotene on day 2, the time when a reduction in chiasma frequency occurred, were most likely in pachytene when heat treatment was begun. Labelled cells did not reach diplotene until day 5. Thus the heat sensitive period is separated from the premeiotic DNA synthetic period by at least 3 days.

4. DISCUSSION

Prolonged heat treatment during mid-prophase of meiosis results in a lowering of the chiasma frequency in *Melanoplus femur-rubrum* females. The nature of the temperature-induced chiasma-frequency response is most likely

FIG. 2.—Frequency distributions of chiasma frequencies obtained from daily samples (D₁-D₆) collected after the beginning of heat treatment.

a complex one. Henderson (1970), using a high temperature pulse regime has determined that heat specifically affects at least four discrete stages in the meiotic process in the males of *Shistocerca gregaria* and other Acridid grasshoppers. The latest meiotic stage affected was pachytene and this was assumed to be the stage when the actual cross-over event occurred (Henderson, 1970).

The present experiments allow me to conclude that disruption of the formation of some chiasmata occurs in female grasshoppers when meiotic cells in mid-prophase are subjected to temperatures of 40° C. The first cells in diplotene to show a chiasma-frequency reduction occurred approximately 2 days after the heat treatment was started. The latest stage labelled in these samples was leptotene. Labelled cells did not reach diplotene until day 5. Thus, these experiments have shown that a temperature-sensitive stage marked by the latest induction of a chiasma-frequency response (day 2) occurs 3 days following the completion of chromosomal replication (day 5). Whether the temperature is affecting the actual cross-over event or events which occur in preparation for cross-over is unknown. Whatever these events are, they are occurring during mid-prophase of meiosis.

The results of this investigation are strikingly similar to those obtained from similar experiments performed with males of the same species (Church and Wimber, 1969). In both sexes chiasma frequencies were lowered in response to high temperatures. The pattern or response was also similar. Although the chiasma frequency was lowered by prolonged heat treatment, univalents were never observed in either sex. These results differ from those obtained by Henderson (1966) for the locust *Shistocerca gregaria* (males), and by Peacock (1968) for the grasshopper *Goniaea Australasiae* (males). Univalents were readily produced by prolonged heat treatment during prophase of meiosis in the males of these species. In *M. femur-rubrum* males and females only bivalents containing chiasmata in excess of one appear to be affected by the high temperature. Thus chiasmata respond differently to high temperatures between bivalents and within bivalents. This suggests that there may be more than one mechanism involved in chiasma formation in both sexes of this species.

The time required for a cell to pass from premeiotic DNA synthesis to diplotene of meiosis at 40° C. is shorter in females than in males. Church and Wimber (1969) determined this time to be 8 days in males as compared to 5 days observed for females in the present investigation. However, the experiments are not strictly comparable since they were carried out on male adults and female nymphs. There is some evidence (Church, unpublished) that meiotic prophase is shorter in length in male nymphs than in male adults.

Chiasma frequencies per cell are higher in females than in males. The mean chiasma frequency per cell in *M. femur-rubrum* males was approximately 13.5 (Church and Wimber, 1969). The mean chiasma frequency observed for females was approximately 15.2 per cell. However, males contain only 11 bivalents and the univalent X chromosome, whereas the females display 12 bivalents. Therefore, the mean chiasma frequency per bivalent is approximately the same in both sexes.

My investigations of male and female meiosis in *M. femur-rubrum* were specifically initiated to compare basic meiotic mechanisms between the sexes of the same species. The striking observation is the remarkable similarity

between the sexes. Both display a preleptotene spiral stage (Church, 1972), chiasma frequencies are similar, and the pattern of chiasma frequency response to high temperatures is similar.

Two lines of evidence suggest that chiasma formation in *M. femur-rubrum* females occurs during prophase of meiosis and not during the pre-meiotic DNA synthetic period as occurs in *Drosophila melanogaster* females (Grell and Chandley, 1965). *M. femur-rubrum* females display a preleptotene spiral stage (Church, 1972) following premeiotic DNA synthesis. All chromosomes become visible and no evidence for homologous chromosome association is observed. This observation places the time of chiasma formation as a post DNA synthesis event. Furthermore, the evidence presented in this report suggests that temperature sensitive events are occurring during mid-prophase which are involved in chiasma formation.

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Plate I

- FIGS. A-D.—Autoradiographs of cells from nymphs sampled on days 1, 3, 4, and 5 respectively after injection with H^3 -TdR and the beginning of heat treatment. Figs. A-D show labelled cells ($\times 2000$) in the pre-leptotene spiral stage, zygotene, pachytene and diplotene respectively.
- FIGS. E. and G.—Cell in diplotene ($\times 2000$) and enlarged bivalents from a cell in diplotene. Cells were obtained from animals prior to the start of heat treatment. Both cells contained bivalents showing more than one chiasma.
- FIG. F.—Cell in diplotene ($\times 1700$) from animal, sampled on day 3 following the beginning of heat treatment. A minimum chiasma frequency of 12 is observed.