THE EFFECT OF TEMPERATURE ON CHIASMA FREQUENCY

CHARLES G. ELLIOTT
Botany School, Cambridge

Received 1.iv.55

1. INTRODUCTION

In the well-known effect of temperature on crossing-over in *Drosophila*, recombination between loci close to the centromere has a minimum value at 22° C. (Plough, 1917, 1921 ; Stern, 1926 ; Graubard, 1934). Plough's (1917) graph of his results showed two maxima at 13° and 31°, but this was a mistake, as has been pointed out by Smith (1936). In genetical work with other organisms some results similarly show a temperature at which cross-over frequencies are minimal (*e.g.* the fungus *Ustilago hordei* (Hüttig, 1931)), while in other experiments no minimum value was observed over the temperature range studied (*e.g.* another fungus, *Podospora anserina* (Rizet and Engelmann, 1949), and the silkworm, *Bombyx mori* (Kogure, quoted by Tanaka, 1953)). No effect of temperature on crossing-over was found by Clark (1943) in *Habrobracon*.

It is however by no means certain how much of the effect in *Drosophila* is due to crossing-over at meiosis, and how much to crossing-over in preceding oogonial mitoses. Whittinghill (1950) has shown how somatic crossing-over affects subsequent meiotic recombination, especially when the (somatic) cross-over chromatids pass to different cells at the following anaphase. The result of this will be increased recombination in regions proximal to the point of somatic crossing-over. Stern and Rentschler (1936) found that the proportion of mosaics, the result of somatic crossing-over, was lower at 30° than at 25°. Whittinghill (1937) showed that high temperature (33·5°-35·5°) produced somatic crossing-over in male *Drosophila* ; the cross-overs were concentrated in, but not confined to, the region near the centromere. Muller (1925) found that X-rays increased the amount of recombination between loci close to the centromere ; and Whittinghill (1938) was able to show that X-rays increased the amount of oogonial crossing-over.

In view of the uncertainty with which we must now regard the nature of the phenomenon in *Drosophila* because of our increased knowledge of somatic crossing-over, it is important to study the cytological problem of variation in chiasma frequency with temperature, which is distinct from that of variation in genetic recombination. In this connexion the distinction must be made between temperature shocks, that is abnormally high temperatures applied for a short time (*e.g.* Barber, 1941, 1942), and experiments in which the whole of meiosis takes place at one constant temperature. It is to the latter

that we look for results analogous to those of the effect of temperature on crossing-over in *Drosophila*.

The only investigations of this problem are those of White (1934) on three species of Orthoptera : *Chorthippus* (*Stenobothrus*) *parallelus*, *Locusta migratoria*, and *Schistocerca gregaria*. The insects were placed in the temperature chambers 24-72 hours after their last ecdysis, and they remained there three or four days. In all three organisms, at the lowest temperature chiasma frequency was less than at the next higher temperature ; thus White's graphs resemble Plough's (1917). The number of cells scored for each temperature was very small, and we are not told how many animals were used. If more than one was studied, the results for different individuals are not given. In the light of the data to be presented below, White's results appear to be of little value because of this lack of information on variation between replicate animals.

I have carried out experiments on the effect of temperature on chiasma frequency in three species of organisms, an animal and two plants : *Locusta migratoria* (Orthoptera), and *Endymion non-scriptus* (*Scilla non-scripta*) and a diploid variety of *Hyacinthus orientalis* (Liliaceae). In all experiments the chambers of the Low Temperature Research Station, Cambridge, were used.

2. *LOCUSTA MIGRATORIA*

Experimental details.—It is not known if there is any effect of age on chiasma frequency in Orthoptera, but in an experiment such as this, account must be taken of any possible effect by using animals of the same age. This was ensured, as nearly as possible, by placing the insects at controlled temperatures within 24 hours of their last ecdysis. The animals were kept in the dark in large Erlenmeyer flasks, one to each flask. The flasks were lightly stoppered with cotton wool, and the insects provided with grass.

The duration of controlled temperature is a most important consideration. It is essential that all the processes immediately relevant to chiasma formation proceed at the temperature concerned, so the minimum duration of controlled temperature should be from the beginning of leptotene to diplotene. Unfortunately the duration of meiosis is not known in Orthoptera, so that the times for which the insects were kept at controlled temperatures were arbitrary. They were : 37°—3 days ; 25°, 15°—4 days ; 5°, 1°—5 days. By keeping the insects at controlled temperatures for these different periods it was hoped to compensate in some measure for the different rates of cell processes at different temperatures. However, we know nothing of the temperature coefficients of meiosis, so that the experiment was a very rough one. Nevertheless, it was more or less a repetition of White's.

In order to estimate the variation between individuals, four insects were kept at each temperature. Only males were used. They were killed, and the testes fixed in 1 : 3 acetic alcohol, within a few minutes of being removed from the constant temperature chambers. Feulgen squashes were made. The chiasma frequency was scored in late diplotene.

Results.—Table 1 gives the mean total chiasma frequency per cell for each animal. This is the chiasma frequency for the autosomes only. Supernumerary chromosomes of the type which vary in number from cell to cell of the one animal (White, 1952 ; Rees and Jamieson,

1954), were present in all but three animals, and their number and the number of chiasmata they formed were recorded for each cell scored. The number of supernumeraries per cell bore no simple relation to the chiasma frequency of the autosomes within most animals, and the number of supernumeraries bore no relation whatever to the mean chiasma frequency of the autosomes when all animals were considered. It was therefore presumed legitimate to ignore the supernumeraries for the present purposes.

TABLE 1

Locusta migratoria. *Chiasma frequencies of different male animals kept at diverse temperatures*

Temperature	Animal no.	No. of cells	Mean total Xta per cell (autosomes)	Temperature means	
				weighted	unweighted
1°	1/4	70	14·00±0·106	14·92	15·04
	1/2	62	14·95±0·179		
	1/1	50	16·16±0·188		
5°	5/3	82	13·51±0·104	14·25	14·47
	5/1	63	14·79±0·145		
	5/4	30	15·10±0·188		
15°	15/3	83	13·75±0·121	14·58	14·44
	15/2	80	14·38±0·188		
	15/1	143	15·19±0·115		
25°	25/4	86	13·77±0·117	14·44	14·28
	25/3	71	13·94±0·153		
	25/1	183	14·40±0·109		
	25/2	179	15·00±0·102		
37°	37/4	90	13·81±0·107	14·86	14·79
	37/1	84	14·49±0·131		
	37/2	103	16·08±0·159		

A sufficient number of cells was not scored in four animals. The results are therefore given for 16 out of the 20 in the experiment. For each temperature we have given in table 1 the weighted mean (the total number of chiasmata divided by the total number of cells) and the unweighted mean chiasma frequency (the sum of the means for each animal divided by the number of animals). The figures do not suggest any marked temperature effect, although the unweighted means show a decrease from 1° up to 25°, followed by an increase between 25° and 37°, which is the sort of result we might expect from what is known of *Drosophila*. An analysis of variance is given in table 2. It will be seen that the variation between individuals is highly significant; that is, at any one temperature they differ significantly in their mean chiasma frequency. The temperature effect is highly significant compared with the variation within individuals, but it is not significantly different from that between individuals ($F = 3{\cdot}60$, P 0·2-0·1).

The present result is quite different from that obtained by White. It should be noted that the results are markedly affected by two individuals, 1/1 and 37/2, which have much higher chiasma frequencies than the other insects, and which alone are responsible for the marked rises in the mean values at the extreme temperatures. This being the case, it is clear that the observed temperature effect is too dependent on the individuals sampled. The proper test of the effect is not that it is greater than the variation within an individual, but that it is not greater than the variation between individuals.

Thus the experiment has failed to demonstrate an effect of temperature on chiasma frequency. It must be emphasised again, however, that it is not conclusive in view of the lack of knowledge of the duration of meiosis, and so the time for which the insects were kept at controlled temperatures was arbitrary.

TABLE 2

Locusta migratoria. *Analysis of variance of chiasma frequencies at different temperatures in different male animals*

	Sum of squares	Degrees of freedom	Variance	F	P
Between temperatures . . .	71·83	4	17·96	11·2	<0.001
Between animals within temperatures	711·17	11	64·65	40·1	<0.001
Error	2330·78	1443	1·61		
Total	**3113·78**	**1458**			

3. *ENDYMION NON-SCRIPTUS*

The serious objection to the use of animal material in investigating the effect of temperature on chiasma frequency is that we cannot observe directly the duration of meiosis, and hence it is not easy to ascertain how long the animals should be kept at controlled temperature to be certain that the cells we observe will really have developed at that temperature. With plant material we can easily see whether the anthers, if we are investigating meiosis in pollen mother cells, contain cells in premeiotic stages in a representative sample of our experimental plants before they are placed at controlled temperatures.

Experimental details.—All the plants used in these investigations came from Madingley Wood, near Cambridge. They were dug up on 18th October 1952 and placed in the constant temperature chambers on 20th October. While in the temperature chambers the bulbs were kept in pots of soil, the pots being watered occasionally. Several plants examined when collected were found to have the pollen mother cells differentiated but not yet rounded off, and in no case did they contain anything but resting nuclei. It is thus certain that the whole of meiosis took place at the controlled temperatures, and that the effect of constant temperature and not temperature shocks was studied. The dates of fixation were :—1°—19th February 1953; 5°—13th January; 10°—20th November 1952; 15°—28th November; 20°—18th November. Inflorescences were fixed in 4 : 3 : 1 chloroform alcohol acetic acid for twenty-four hours, and then transferred to 60 per cent.

alcohol. After allowing time for the material to soften (about two weeks), aceto-carmine squashes were made of the anthers. Chiasma frequencies were scored at Metaphase I in temporary preparations.

All the plants at 10° were fixed at too early a stage to score chiasma frequencies. At 20° only one plant contained cells at Metaphase I.

Results at 1°-15°.—The data are given in table 3. It appears that chiasma frequency is minimal at 5°, and increases at higher and lower temperatures. To test the significance of this result an analysis

TABLE 3

Endymion non-scriptus. *Chiasma frequencies of pollen mother cells in plants grown at various temperatures*

Temperature	Plant no.	No. of cells	Mean total Xta per cell	Temperature means	
				weighted	unweighted
1°	52/1/2	40	17·68±0·331	18·17	18·24
	52/1/1	40	18·48±0·235		
	52/1/3	20	18·55±0·540		
5°	52/5/2	40	16·38±0·267	16·93	16·94
	52/5/3	40	16·93±0·283		
	52/5/1	40	17·50±0·349		
15°	52/15/6	40	16·30±0·218	18·24	18·37
	52/15/5	40	17·30±0·258		
	52/15/1	40	17·65±0·375		
	52/15/4	40	18·18±0·336		
	52/15/7	40	19·30±0·296		
	52/15/3	20	19·50±0·380		
	52/15/2	40	20·35±0·387		

of variance has been done ; the temperatures are compared in pairs (table 4). In each case the between plant variance is much greater than that within plants, so plants differ significantly in their chiasma frequencies. The difference between 1° and 5° is just significantly greater at the 5 per cent. level than the variation between plants. However the temperature variance for the difference between 5° and 15° is not significant compared with the plant difference. If more plants could have been scored at 1° and 5° it is doubtful whether the difference between these would have been significant. It will be noticed that the range in mean chiasma frequency of the seven plants at 15° covers all six plants at 1° and 5°.

Results at 20°.—The mean total chiasma frequency per cell of the plant at 20° is only 2·17±0·193. Many cells have only univalents. There are eight pairs of chromosomes, and the numbers of cells containing 0 to 8 bivalents are given in table 5. The expected numbers of cells with different numbers of bivalents are given by the terms of the binomial expansion $[p+(1-p)]^8$, where p is the probability of any one pair of chromosomes forming a bivalent. This assumes

that p is the same for all chromosomes, which is unlikely in view of their range in length. The maximum likelihood estimate of p is 0·230, and it will be seen that the calculated values fit the observed

TABLE 4

Endymion non-scriptus. *Analysis of variance of chiasma frequencies at different temperatures*

	Sum of squares	Degrees of freedom	Variance	F	P
Between 1° *and* 5°					
Between temperatures . .	83·42	1	83·42	8·01	0·05-0·01
Between plants within temperatures	41·73	4	10·43	2·85	0·05-0·01
Error	781·85	214	3·65		
Total	**907·00**	**219**			
Between 5° *and* 15°					
Between temperatures . .	139·14	1	139·14	2·47	0·2-0·1
Between plants within temperatures	459·92	8	56·24	12·79	< 0·001
Error	1626·83	370	4·40		
Total	**2216·89**	**379**			

very poorly : $\chi^2_{[3]} = 22·68$. The Poisson distribution based on the mean of 1·840 bivalents per cell gives a slightly better, but still very poor fit : $\chi^2_{[3]} = 9·71$, P 0·05-0·02. There are too many cells with 16 univalents, and correspondingly few with one, or two bivalents.

TABLE 5

Endymion non-scriptus. *Bivalent frequency at 20° (one plant)*

No. of bivalents per cell .	0	1	2	3	4	5	6	7	8
No. of cells observed .	25	22	20	19	8	4	1	1	0
No. expected (binomial) .	12·36	29·53	30·87	18·44	6·89	1·65	0·25	0·02	0·00
No. expected (Poisson) .	15·88	29·23	26·88	16·49	7·58	2·80	0·86	0·23	0·05

Barber (1941, 1942) showed that heat shocks given during the pairing process in *Uvularia* and *Fritillaria* resulted in proximal localisation of chiasmata, which was interpreted as due to pairing being incomplete before the time limit for pairing expired. The present results can also be interpreted in terms of a time limit. At constant high temperatures the time available for pairing is greatly reduced, and before the time limit has expired either a large number of chromosomes, or none, has paired.

4. *HYACINTHUS ORIENTALIS*

The differences in chiasma frequency between temperatures observed in *Locusta* and *Endymion* are of doubtful significance since this variation is small compared with the variation between replicate individuals at the different temperatures. To obtain a satisfactory result this variation between individuals must be very small and not significant, and the best chance of obtaining this is to use clonal material. Since the varieties of garden hyacinths are reputedly clones, this material was studied.

Experimental details.—The diploid variety Yellow Hammer was used in these experiments. Bulbs were obtained specially early in the season from a firm of commercial growers, and on 14th July 1952 the stem apex was found to have completed the initiation of leaves, and the primordia of the lowest flowers were just evident. The optimum conditions for the development of the inflorescence and its elongation have been stated by Blaauw (1924), and in accordance with his findings the bulbs were placed at 25° C., and left there until 22nd August. Several bulbs were dissected on this date, and it was found that the lowest flowers of the inflorescence had slightly elongated anthers, but that pollen mother cells were not differentiated, and it is probable that another premeiotic mitosis had yet to take place. In the highest flowers the anthers were still very small and spherical in shape. Bulbs were transferred to temperatures of 1, 5, 10, 15 and 20° C., and some were left at 25°, and they remained there till the date of fixation. Throughout all this time the bulbs were kept dry in stout paper bags.

Meiosis was not observed at 1° and 25°. Four bulbs were examined from each of the temperatures 10, 15 and 20°, and six bulbs at 5°. Two or three flowers were examined from each bulb. The dates of fixation were: 20°—4th October 1952 (2 bulbs), 7th October (2 bulbs); 15°—30th September; 10°—20th October; 5°—7th January 1953 (4 bulbs), 14th January (2 bulbs). Chiasma frequencies were scored at Metaphase I.

Chromosomes of diploid hyacinths.—The chromosomes of hyacinths have been described by Darlington (1926), Stone and Mather (1932), and Darlington, Hair, and Hurcombe (1951). The diploid number is 16, and this comprises 4 pairs of long chromosomes, designated L, with median centromeres; 2 pairs of medium-sized chromosomes, designated M, with sub-terminal centromeres; 2 pairs of short chromosomes, designated S, one with a sub-terminal and the other with a sub-median centromere. At Metaphase I, 4 L, 2 M, and 2 S bivalents can be distinguished, but cannot be further differentiated. The mitotic lengths of the chromosomes were measured by Stone and Mather from Darlington's illustrations. The mean lengths of each chromosome in the several classes are given by them as L 21 μ, M 9 μ, S 5 μ. I have measured the chromosomes in a few cells and agree with these figures.

Results.—The results for the four temperatures are summarised in table 6. The highest chiasma frequency is attained at 20°, it is slightly lower at 15°, lower still at 10°, and very much lower at 5°. At 5° univalents are frequent in all chromosome classes, and 7·0 per cent. of cells had 16 univalents.

TABLE 6

Hyacinthus orientalis (*var. Yellow Hammer*). *Chiasma frequencies in pollen mother cells of plants stored at various temperatures*

Temperature	20°	15°	10°	5°
Mean total Xta per nucleus of replicate plants	18·27±0·181 18·80±0·177 19·10±0·197 19·91±0·195	17·27±0·189 18·23±0·181 18·30±0·201 18·83±0·204	15·81±0·203 15·86±0·188 16·72±0·205 17·31±0·290	7·13±0·383 7·79±0·350 8·56±0·400 8·79±0·332 10·10±0·378 10·49±0·361
Weighted means . .	18·96	18·16	16·36	8·81
Total no. of cells . .	375	400	371	600
Mean Xta per pair of chromosomes				
L	3·285	3·155	2·808	1·470
M	1·758	1·654	1·509	0·898
S	1·150	1·115	1·051	0·568

TABLE 7

Hyacinthus orientalis (*var. Yellow Hammer*). *Analysis of variance of total chiasma frequency per cell for plants at each temperature*

Temperature		Sum of squares	Degrees of freedom	Variance	F	P
20°	Between plants . .	119·34	3	39·78	4·34	0·1 -0·05
	Between flowers of plants	36·64	4	9·16	2·81	0·05-0·01
	Error . . .	1194·42	367	3·26		
	Total . . .	**1350·40**	**374**			
15°	Between plants . .	126·55	3	42·18	11·75	0·05-0·01
	Between flowers of plants	14·36	4	3·59		
	Error . . .	1480·17	392	3·78		
	Total . . .	**1621·08**	**399**			
10°	Between plants . .	132·27	3	44·09	5·01	0·05-0·01
	Between flowers of plants	35·21	4	8·80	2·37	0·05
	Error . . .	1349·56	363	3·72		
	Total . . .	**1517·04**	**370**			
5°	Between plants . .	841·22	5	168·24	9·28	0·01-0·001
	Between flowers of plants	163·09	9	18·12	1·15	>0·2
	Error . . .	9192·03	585	15·71		
	Total . . .	**10196·34**	**599**			

An analysis of variance for the plants and flowers at each temperature is given in table 7. The variances for the three highest temperatures are homogeneous, and we can make the following combined estimates :—

	Variance	D.f.	F	P
Between plants at each temperature .	42·02	9	5·85	0·01-0·001
Between flowers of a plant . .	7·18	12	2·00	0·05-0·01
Between cells (error) . . .	3·59	1122	...	...

It will be seen that variation between flowers of a plant is significant compared with variation between cells, except when the 15° data are analysed separately (table 7). 15° is the nearest temperature to that found by Blaauw (1924) to be the optimum for cell extension, *viz.* 17°. This suggests that the heterogeneity is the result of variation in development and differentiation at abnormal temperatures. But at 15°, as in the data as a whole, variation between plants is significant compared with variation between flowers of a plant, suggesting that in addition to environmental variation, and despite the supposedly clonal nature of the material, the plants are different in their chiasma-forming properties. Nevertheless, because of the magnitude of the temperature effect, the results for the different plants have been combined for each temperature. The variation between temperatures is greater than variation between plants at each temperature, except in the case of 15° and 20°. We note that at 5° both the variation between plants and the variation between cells are much greater than at the higher temperatures.

Table 8 gives the numbers of bivalents per cell for each of the length classes of chromosomes for two plants, one at 5°, the other at 10°. If p is the probability that any one chromosome of its respective class will form a bivalent with its homologue, the expected numbers of cells with different numbers of bivalents will be given by the terms of the expansion of $[p+(1-p)]^4$ for the L chromosomes, and of $[p+(1-p)]^2$ for the M and S chromosomes. The maximum likelihood estimates of p and the expected numbers calculated therefrom are given in table 8. It will be seen that at 5° the observed frequencies agree very poorly with those expected from a binomial distribution : $\chi^2_{[2]} = 27{\cdot}66$, $P < 0{\cdot}001$ for the L chromosomes, and $\chi^2_{[1]} = 8{\cdot}29$ and 10·30 for the M and S respectively, P 0·01-0·001. There are too many cells with either all univalents or all bivalents. We may say that within the time limit for pairing all the chromosomes or none of them will have made contact with their homologues and paired to a sufficient extent to form a chiasma. On the other hand, at 10° the observed frequencies of numbers of bivalents per cell agree very well with

those calculated from the binomial expansion. The L chromosomes have the highest probability of forming a bivalent, the S chromosomes the least.

Fig. 1 shows the relationship between chromosome length and the chiasma frequency per pair of chromosomes, that is, the quantity C/n, where C is the total number of chiasmata and n the number of pairs of chromosomes in a given chromosome length class for all the cells concerned. The relation is strictly linear for 10° and 15° and nearly so for 20°. If chromosome length is x, the values of C/n for

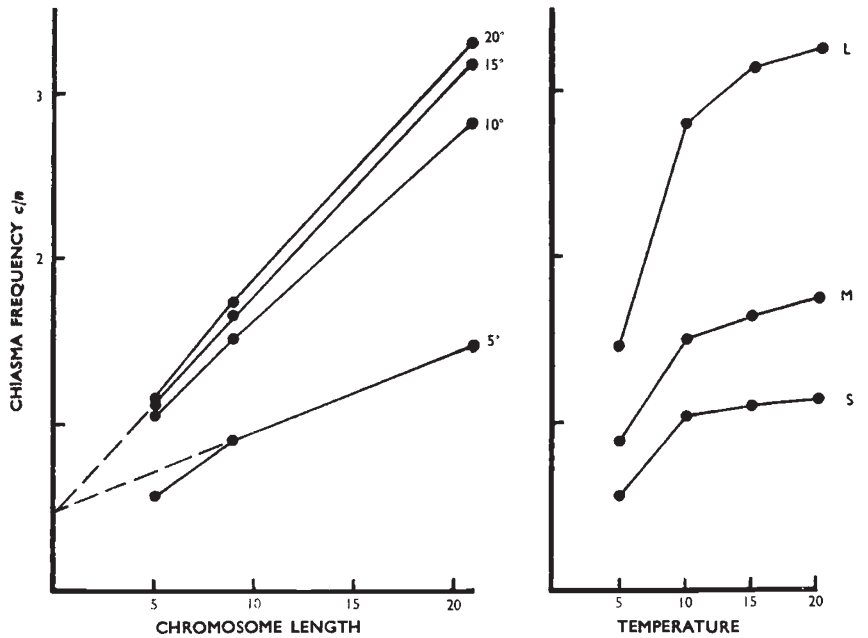
TABLE 8

Hyacinthus orientalis (*var. Yellow Hammer*). *Numbers of bivalents per cell for one plant at each of two temperatures. Values of* p, *the probability that two homologous chromosomes will form a bivalent, are substituted in the binomial expansions to give expected frequencies*

Temperature		L chromosomes					M chromosomes			S chromosomes		
		0	1	2	3	4	0	1	2	0	1	2
5°	Obs.	9	7	18	25	41	26	35	39	46	32	22
	Exp.	*0·76*	*7·24*	*25·95*	*41·34*	*24·70*	*18·92*	*49·15*	*31·92*	*38·44*	*47·12*	*14·44*
	p	0·705					0·565			0·380		
10°	Obs.	...	...	...	2	98	...	6	94	3	28	69
	Exp.	...	...	*0·06*	*3·88*	*96·06*	*0·09*	*5·82*	*94·09*	*2·89*	*28·22*	*68·89*
	p	0·990					0·970			0·830		

$x = 0$ (the chiasma frequency of a very small chromosome, or the probability of forming a chiasma in a very short segment of a long chromosome) calculated from the regression equations are $0{\cdot}512 \pm 0{\cdot}031$ for 10°, $0{\cdot}495 \pm 0{\cdot}026$ for 15°, and $0{\cdot}528 \pm 0{\cdot}026$ for 20°. These values agree very well. The relation between chiasma frequency and chromosome length is not linear at 5°. However the line through only the L and M points cuts the line $x = 0$ at $C/n = 0{\cdot}467 \pm 0{\cdot}049$. Hence the probability of forming a chiasma in a short segment is the same for the L and M chromosomes at 5° as for all the chromosomes at the higher temperatures. The point for the S chromosomes at 5° lies below the extrapolation of the L-M line. The value for $x = 5$ on the L-M line is $0{\cdot}707 \pm 0{\cdot}036$. The observed chiasma frequency of the S chromosomes is $0{\cdot}568 \pm 0{\cdot}079$. For the difference between these $t = 3{\cdot}45$, $P < 0{\cdot}001$.

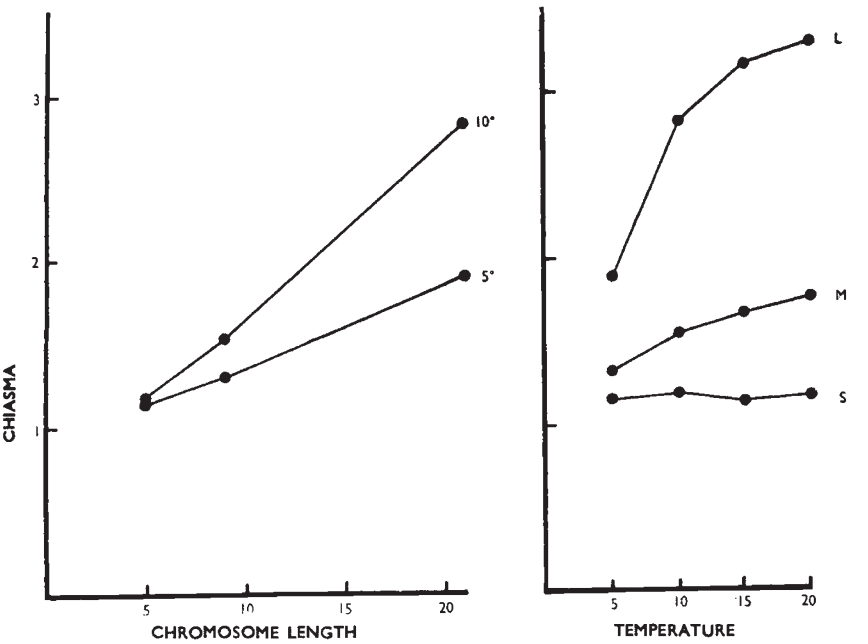
If there are n_0 univalent pairs among the total of n pairs of chromosomes of a given size class in all the cells, $C/(n-n_0)$ is the chiasma frequency of the bivalents only. The relation between this quantity and chromosome length has been investigated, and it is linear at both 5° and 10° (fig. 3); that is, when univalents are



FIGS. 1-2.—*Hyacinthus orientalis* (var. Yellow Hammer).

FIG. 1.—The relation between chiasma frequency per pair of chromosomes (including univalent pairs) (C/n) and chromosome length (scale in microns at mitotic metaphase) for each temperature.

FIG. 2.—The relation between C/n and temperature for the L, M and S chromosomes.



FIGS. 3-4.—*Hyacinthus orientalis* (var. Yellow Hammer).

FIG. 3.—The relation between chiasma frequency per bivalent (excluding univalent pairs) ($C/(n\text{-}n_0)$) and chromosome length for each temperature.

FIG. 4.—The relation between $C/(n\text{-}n_0)$ and temperature for the L, M and S chromosomes.

omitted the chiasma frequency of the S chromosomes at 5° agrees with that expected on the basis of length and the number of chiasmata in the L and M bivalents. The omission of univalents is thus a test of the hypothesis that the univalents are due to failure of pachytene pairing in the main, and that this mostly affects the S chromosomes.

The quantity $C/(n-n_0)$ for the S chromosomes shows no systematic variation with temperature (fig. 4), and the systematic increase of C/n with temperature (fig. 2) must be due to the increased probability of forming a bivalent at the higher temperatures. Because of their length the S chromosomes are at a disadvantage compared with the M and L chromosomes in finding their partners and pairing to a sufficient extent to form a chiasma. At low temperatures a greater amount of the total time available for pairing is taken up by the first of these processes, and this reduces the probability of forming a chiasma in a very short segment when all the S chromosomes are considered together.

The relation between C/n and chromosome length, at least for the longer chromosomes, presents a series of lines radiating from a point, with increasing slope for increasing temperature. This means that at the higher temperatures more complete pachytene pairing is facilitated. The extent to which the chromosomes become so paired with their homologues is proportional to their total length up to 15°. At 20° the point for the M chromosomes lies above a line through the S and L points. The M chromosomes, with sub-terminal centromeres, are thus paired to a greater extent, relative to their length, than the L chromosomes, which have median centromeres. A situation similar to this is known in *Fritillaria* (Bennett, 1938 ; Frankel, 1940).

Deficient pachytene pairing at low temperatures (5° and 10°) is indicated at Metaphase I not only by the numerous univalents, but also by the proximal localisation of chiasmata, which is associated with procentric pairing in other Liliaceæ (Darlington, 1937 ; Barber, 1941, 1942). Even at higher temperatures (15° and 20°), pairing is often incomplete in the L chromosomes, as shown by proximal localisation of chiasmata.

5. DISCUSSION

A pronounced effect of temperature on chiasma frequency was found in diploid *Hyacinthus*, but this is shown to be due to an effect of temperature on the process of chromosome pairing prior to pachytene. Probably the result can be interpreted entirely in terms of such an effect, without need to invoke any other phenomena. These experiments, and the effect of high temperature (20°) in *Endymion*, illustrate the profound physiological effect of temperature on chromosome pairing, which was also strikingly shown by the heat shock experiments of Barber (1941, 1942).

No conclusive evidence has been found of an effect of temperature

on chiasma frequency within paired regions of chromosomes. There was some indication of a temperature at which chiasma frequency has a minimum value in *Locusta* and *Endymion*. The difference in unweighted mean chiasma frequency in *Locusta* between 1° and 25° is about 0·8. The three smallest bivalents have invariably one chiasma ; this leaves eight bivalents among which the effect if real is shared. The most extreme loci showing any temperature effect would then differ in recombination frequency by $(0{\cdot}8 \times 50)/8 = 5$ per cent. This is a similar value to the difference in recombination between *b* and *pr* in *Drosophila*, which is 6·3 units over the range 9° to 22° (Smith, 1936). However the result in the present experiments is not statistically significant because of the great variation in chiasma frequency between replicate individuals at the one temperature. Heterogeneity in chiasma frequency between randomly sampled individuals of a population is a widespread and important phenomenon (Elliott, 1953). The reputedly clonal material of an old-established variety of hyacinths failed to satisfy the condition that replicate individuals should be homogeneous in chiasma frequency. Possibly the members of a newly-established clone would be less heterogeneous. For future work on this problem it would be essential to establish a population homogeneous in chiasma frequency under fixed conditions.

6. SUMMARY

1. The conflicting results of previous experiments on the effect of temperature on crossing-over, or the uncertainty of their meaning in terms of an effect on chiasma frequency, makes necessary an investigation of the cytological problem of the effect on chiasma frequency of different temperatures constant throughout the whole of meiosis.

2. No effect of temperature on chiasma frequency could be established in *Locusta migratoria* (Orthoptera) over the range 1°-37° C., or in *Endymion non-scriptus* (*Scilla non-scripta*) (Liliaceæ) over the range 1°-15°, since the replicate individuals at each temperature were heterogeneous in chiasma frequency, and the variation between temperatures was insignificant compared with variation within temperatures.

3. High temperature (20°) in *Endymion* leads to reduced chiasma frequency as a result of failure of chromosome pairing at pachytene.

4. In a diploid variety (Yellow Hammer) of *Hyacinthus orientalis* (Liliaceæ) decreasing temperature below 20° results in a progressive reduction in chiasma frequency, but this effect is shown to be due largely, if not entirely, to failure of chromosome pairing at pachytene.

Acknowledgments.—I wish to thank Professor D. G. Catcheside, to whom I am indebted for suggesting the problem, and Dr H. W. Howard, for their encouragement, advice and criticism.

Present address : Department of Genetics, University of Glasgow.

7. REFERENCES

BARBER, H. N. 1941. Chromosome behaviour in *Uvularia*. *J. Genet.*, *42*, 223-257.

BARBER, H. N. 1942. The experimental control of pairing in *Fritillaria*. *J. Genet.* *43*, 359-374.

BENNETT, E. S. 1938. The origin and behaviour of chiasmata. XIV. *Fritillaria chitralensis*. *Cytologia*, *8*, 443-451.

BLAAUW, A. H. 1924. The results of the temperature during flower-formation for the whole hyacinth (first part). *Verh. Akad. Wet. Amst.* (2e sect.), *23* (4).

CLARK, A. M. 1943. Linkage relations in *Habrobracon* and the effects of temperature, X-radiation, and age on crossing over. *Proc. Pennsylvania Acad. Sci.*, *17*, 47-64.

DARLINGTON, C. D. 1926. Chromosome studies in the Scilleæ. *J. Genet.*, *16*, 237-251.

DARLINGTON, C. D. 1937. *Recent Advances in Cytology*. London.

DARLINGTON, C. D., HAIR, J. B., AND HURCOMBE, R. 1951. The history of the garden hyacinths. *Heredity*, *5*, 233-252.

ELLIOTT, C. G. 1953. Variation in chiasma frequency in natural populations. *P. Int. Gen. C.* (*9*) (in the press).

FRANKEL, O. H. 1940. The causal sequence of meiosis. I. Chiasma formation and the order of pairing in *Fritillaria*. *J. Genet.*, *41*, 9-34.

GRAUBARD, M. C. 1934. Temperature effect on interference and crossing over. *Genetics*, *19*, 83-94.

HÜTTIG, W. 1931. Über den Einfluss der Temperatur auf die Keimung und Geschlechterverteilung bei Brandpilzen. *Zeit. Bot.*, *34*, 529-577.

MULLER, H. J. 1925. The regionally differential effect of X-rays on crossing over in autosomes of *Drosophila*. *Genetics*, *10*, 470-507.

PLOUGH, H. H. 1917. The effect of temperature on crossing over in *Drosophila*. *J. exp. Zool.*, *24*, 147-209.

PLOUGH, H. H. 1921. Further studies on the effect of temperature on crossing over. *J. exp. Zool.*, *32*, 187-203.

REES, H., AND JAMIESON, A. 1954. A supernumerary chromosome in *Locusta*. *Nature*, *173*, 43-44.

RIZET, G., AND ENGELMANN, C. 1949. Contribution à l'étude génétique d'un ascomycète tétrasporé : *Podospora anserina*. *Rev. Cytol., Paris*, *11*, 201-304.

SMITH, H. F. 1936. Influence of temperature on crossing over in *Drosophila*. *Nature*, *138*, 329-330.

STERN, C. 1926. An effect of temperature and age on crossing over in the first chromosome of *Drosophila melanogaster*. *P.N.A.S.*, *12*, 530-532.

STERN, C., AND RENTSCHLER, V. 1936. The effect of temperature on the frequency of somatic crossing over in *Drosophila*. *P.N.A.S.*, *22*, 451-453.

STONE, L. H. A., AND MATHER, K. 1932. The origin and behaviour of chiasmata. IV. Diploid and triploid *Hyacinthus*. *Cytologia*, *4*, 16-25.

TANAKA, Y. 1953. Genetics of the silkworm, *Bombyx mori*. *Advanc. Genet.*, *5*, 239-317.

WHITE, M. J. D. 1934. The influence of temperature on chiasma frequency. *J. Genet.*, *29*, 203-215.

WHITE, M. J. D. 1952. Cytogenetics of Orthopteroid insects. *Advanc. Genet.*, *4*, 267-330.

WHITTINGHILL, M. 1937. Induced crossing over in *Drosophila* males and its probable nature. *Genetics*, *22*, 114-129.

WHITTINGHILL, M. 1938. Oogonial crossing over in *Drosophila melanogaster* (Abstract). *Genetics*, *23*, 175-176.

WHITTINGHILL, M. 1950. Consequences of crossing over in oogonial cells. *Genetics*, *35*, 38-43.