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PARALLEL POLYMORPHISM FOR SUPERNUMERARY SEGMENTS IN *CHORTHIPPUS PARALLELUS*

V. A NEW POLYMORPHISM IN EUROPE

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1. INTRODUCTION

POLYMORPHISMS for supernumerary segments on the smaller members of the chromosome complement are well known in the Acrididae (White, 1954).

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The situation in *Chorthippus parallelus* (Zetterstedt) has been particularly well studied, for all populations of this species which have been sampled to date, both in Britain (John and Hewitt, 1966a; Hewitt and John, 1968), and in France (Westerman, 1969) have been found to be regularly polymorphic for terminal heterochromatic segments on the two smallest, M_7 and S_8 , chromosome pairs. In addition, the presence of supernumerary segments plays a positive and causative role in the regulation of chiasma frequency, for in all populations studied the mean cell chiasma frequency is significantly higher in those individuals within the population which possess supernumerary segments. In order to investigate further the role and occurrence of these segments, populations of *Ch. parallelus* were sampled through Europe during the 1968 meiotic season.

2. RESULTS AND DISCUSSION

The results of seven of the eight populations sampled are summarised in tables 1 and 2. In all cases the populations were polymorphic for supernumerary segments on the M_7 and S_8 chromosomes. These segments are

TABLE 1

A comparison of the observed karyotype frequencies in the seven European populations sampled in 1968 with the expectations based on Hardy-Weinberg distribution

		Karyotypes								
		Sample size	Observed			Expected			χ^2	P
			BB	BS	SS	BB	BS	SS		
Population										
St Nicklaas	S ₈	27	16	10	1	16.33	9.33	1.33	0.14	0.8-0.7
	M ₇		13	7	7	10.08	12.83	4.08	5.58	0.02-0.01**
Ahrweiler	S ₈	25	23	2	0	23.04	1.92	0.04	0.04	0.9-0.8
	M ₇		12	0	13	5.76	12.48	6.76	25.00	≤0.001***
Irschenberg	S ₈	15	13	2	0	13.07	1.87	0.07	0.08	0.8-0.7
	M ₇		12	3	0	12.15	2.70	0.15	0.19	0.7-0.5
Saalfelden	S ₈	16	15	1	0	15.02	0.97	0.02	0.02	0.9-0.8
	M ₇		5	5	6	3.52	7.97	4.52	2.22	0.2-0.1
Podersdorf	S ₈	21	18	3	0	18.11	2.79	0.11	0.12	0.8-0.7
	M ₇		8	5	8	5.25	10.50	5.25	5.76	0.02-0.01**
Niederstolzen	S ₈	18	15	2	1	14.22	3.56	0.22	3.48	0.2-0.1
	M ₇		6	3	9	3.12	8.75	6.13	7.77	0.01-0.001***
Mont Genève	S ₈	22	13	7	2	12.38	8.25	1.38	0.51	0.5-0.3
	M ₇		15	5	2	13.92	7.16	0.92	2.00	0.2-0.1

similar in position, size and behaviour to those described in other populations of *Chorthippus parallelus* (see John and Hewitt, *loc. cit.*). As in previous collections the behaviour of the M_7 and the S_8 are different. Thus while the observed frequencies of S_8 karyotypes (BB, BS, SS) conform to Hardy-Weinberg expectations (table 1), those of the M_7 do not. In four of the seven populations there is a significant excess of the M_7 homokaryotypes (BB and SS). Again, while the frequency with which the S_8 bears an extra segment does not differ significantly between populations (table 2, $P = 0.2-0.1$) this is not so for the M_7 (table 2, $P = 0.01-0.001$).

In one sample collected from a roadside meadow at Unterloibl (in Austrian Karynthia), a new polymorphism was encountered. Cytological

examination of this population showed that it too was polymorphic for supernumerary segments on the M_7 and S_8 chromosomes but superimposed on this state some individuals also carried an extra segment on one or both M_8 chromosomes (plate I). These segments were distal in location and relatively large, each segment representing an increase of from 30 to 50 per cent. of the material normally present in the M_8 (plate I, b-d) and like the standard M_8 (BB), the extra segment was heteropycnotic from zygotene to diakinesis. Subsequent meiotic behaviour of these distal extra segments on the M_8 chromosome was identical to those on the M_7 and S_8 bivalents in that they invariably divided equationally at anaphase I and reductionally at anaphase II.

TABLE 2

A comparison of basic chromosome frequencies of the S_8 and M_7 from the seven populations in table 1, $q_B = 1$ means that all individuals in a population have a BB karyotype

Population	Sample size	S_8	q_B	M_7	S_8	q_B in angles	M_7
St Nicklaas	27	0.7778		0.6111	61.9		51.4
Ahrweiler	25	0.9600		0.4800	78.5		43.9
Irschenberg	15	0.9300		0.9000	75.0		71.6
Saalfelden	16	0.9688		0.4687	79.9		43.2
Podersdorf	21	0.9286		0.5000	74.6		45.0
Niederstolzen	18	0.8889		0.4166	70.5		40.2
Mont G�n�vre	22	0.7500		0.7955	60.0		63.2
		χ^2_8			8.8177		20.1179
Total	144	P		0.2-0.1	0.01-0.001***		

Since in this particular population, supernumerary segments may be borne on one or both homologues of the M_8 , M_7 and S_8 bivalents, 27 distinct karyotypic classes are theoretically possible. Of these, 11 were observed in the small sample studied (see table 3). For the M_8 and S_8 chromosomes, the observed frequencies of the three karyotypes (BB, BS, SS) are not significantly different from the expectations based on a Hardy-Weinberg distribution (table 4, S_8 , $P = 0.2-0.1$; M_8 , $P = 0.3-0.2$). The frequencies (p_B) with which the two chromosomes bear an extra segment do however differ markedly (S_8 $p_B = 0.1875$; M_8 $p_B = 0.5625$). In contrast, the observed frequencies of the M_7 karyotypes do not agree with Hardy-Weinberg expectations ($\chi^2_1 = 3.9825$, $P = 0.05-0.02$). Although this result is of borderline significance, it none the less conforms with the situation already known in British and other European populations; *i.e.* there is an excess of the two homokaryotypes (BB and SS). The frequency with which the M_7 is observed to carry an extra segment ($M_7 p_B$) is 0.5625.

Previous studies on the role of supernumerary heterochromatic segments in populations of *Ch. parallelus* have shown that mean cell chiasma frequency is increased when extra heterochromatin is present. The exact mode of increase is not clear however. The pooled data from eight distinct French populations (Westerman, *loc. cit.*) appear to suggest a dosage effect, whilst in a large sample taken at Ashurst (John and Hewitt, 1969), a threshold effect would appear to be operating; that is, differences in chiasma frequency between karyotypes result primarily from the addition of one extra segment, others above this making little difference. In order to test the situation in

Plate I

a-d. Supernumerary heterochromatic segments on the M_6 chromosome. *a*, diakinesis cell showing BS M_6 . *b-d*, the three possible karyotypes of the M_6 : *b* BB M_6 ; *c*, BS M_6 ; *d*, SS M_6 . (Arrows denote extra segment.)

TABLE 3

The distribution of karyomorphs and mean cell chiasma frequency of the individuals collected at Unterloibl. (The designation of karyotypic classes is that of Hewitt and John, 1968, table 1. Numbers in parenthesis are numbers of individuals in each class)

M ₆ bivalent	M ₇ bivalent	S ₈ bivalent BB	BS	SS	Number of individuals	Totals
BB	BB	14.1, 14.6, 15.2 (3)	(0)	(0)	3	
	BS	15.3, 15.6 (2)	(0)	(0)	2	
	SS	16.2 (1)	(0)	(0)	1	6
BS	BB	(0)	14.9, 15.5 (2)	15.0, 16.1 (2)	4	
	BS	14.9, 15.1 (3)*	(0)	(0)	3	
	SS	16.4, 16.5 (2)	(0)	(0)	2	9
SS	BB	(0)	(0)	(0)	0	
	BS	16.3 (1)	15.6 (1)	(0)	2	
	SS	15.5, 15.7, 16.0 (5) 16.5 16.7	17.2, 17.4 (2)	(0)	7	9
Totals		17	5	2	24	

* One individual was karyotyped but there were not enough cells to score Xa frequency.

TABLE 4

A comparison of the observed karyotype frequencies with the expectations based on Hardy-Weinberg distribution

Bivalent	Karyotypes						χ^2_1	P
	Observed			Expected				
	BB	BS	SS	BB	BS	SS		
M ₆	6	9	9	4.594	11.812	7.594	1.3605	0.3-0.2
M ₇	7	7	10	4.594	11.812	7.594	3.9835	0.05-0.02*
S ₈	17	5	2	15.844	7.312	0.844	2.4001	0.2-0.1

TABLE 5

The effect of supernumerary heterochromatic segments on mean cell chiasma frequency

Number of segments	Number of individuals	Karyotype observed			Mean cell Xa frequency $\pm$ sd	Mean cell Xa frequency per type $\pm$ sd
		BB ₆	BB ₇	BB ₈		
0	3	BB ₆	BB ₇	BB ₈	14.63 $\pm$ 0.1825	14.63 $\pm$ 0.1825
1	2	BB ₆	BS ₇	BB ₈	15.45 $\pm$ 0.2760	15.45 $\pm$ 0.2760
2	1	BB ₆	SS ₇	BB ₈	16.20 $\pm$ 0.3887	
	2	BS ₆	BB ₇	BS ₈	15.20 $\pm$ 0.2248	15.32 $\pm$ 0.1677
	2	BS ₆	BS ₇	BB ₈	15.00 $\pm$ 0.2616	
3	2	BS ₆	BB ₇	SS ₈	15.55 $\pm$ 0.1983	
	2	BS ₆	SS ₇	BB ₈	16.45 $\pm$ 0.1983	16.06 $\pm$ 0.1382
	1	SS ₆	BS ₇	BB ₈	16.30 $\pm$ 0.3000	
4	1	SS ₆	BS ₇	BS ₈	15.60 $\pm$ 0.2667	
	5	SS ₆	SS ₇	BB ₈	16.12 $\pm$ 0.1799	16.03 $\pm$ 0.1576
5	2	SS ₆	SS ₇	BS ₈	17.30 $\pm$ 0.3000	17.30 $\pm$ 0.3000

TABLE 6

Analysis of variance on Xa frequency data from the Unterloibl population

Item	d.f.	M.S.	V.R.	P
(a) <i>Mean cell Xa frequency</i>				
Between segment groups	5	2.0774	7.7371	< 0.001***
Within segment groups	17	0.2685	—	—
(b) <i>Between cell variance</i>				
Between segment groups	5	0.6303	1.6862	> 0.1
Within segment groups	17	0.3738	—	—

TABLE 7

Analysis of the regression of mean cell chiasma frequency on segment number

Item	d.f.	M.S.	V.R.	P
Regression	1	3.5843	28.1342	0.01-0.001***
Remainder	4	0.1274	—	—

FIG. 1.—Influence of increasing segment number on Xa frequency. $\bar{x} \pm s.d.$

the Unterloibl population, 10 cells per individual where possible were scored at diplotene-diakinesis. The results are shown in tables 3 and 5. As with the French populations, the amount of chromosomal recombination as expressed by mean cell chiasma frequency appears to be increased when extra heterochromatin is present. An analysis of variance (table 6 (a)) showed significant differences exist between mean cell chiasma frequency when different numbers of segments are present. The effect is the same irrespective of which bivalent(s) carry the extra segment(s).

In contrast to this effect on mean cell chiasma frequency, supernumerary segments had no observable effect on between cell variance (table 6 (b)). This result is in agreement with earlier findings.

A regression analysis of chiasma frequency on segment number was carried out and the result was highly significant (see table 7 and fig. 1)—the slope (b) of the regression line being 0.4526. It would thus appear that in our small sample of 23 individuals, a dosage effect is operating in an additive manner, but the numbers of individuals involved are small and the situation needs to be examined in terms of larger samples. If the dosage effect on the continent is a real one it would suggest that while these polymorphisms are old and stable, they are operating in a different manner in different parts of the species range. Moreover, this new European polymorphism described at Unterloibl suggests that the species may contain still further polymorphisms within its range.

3. SUMMARY

1. A new polymorphism for large supernumerary heterochromatic segments on the M_6 bivalent of *Chorthippus parallelus* is described in one population from Austria.

2. Since the M_6 polymorphs co-exist with equivalent polymorphisms for the M_7 and S_8 chromosomes, 27 possible karyotypic classes exist within the population. Eleven of these were found in a population sample of 24.

3. The presence of extra segments increased mean cell chiasma frequency but had no observable effect on between cell variance.

4. The limited data obtained suggests that the action of these extra segments is cumulative in this population.

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