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CYTOGENETIC STUDIES ON NATURAL POPULATIONS OF GRASSHOPPERS WITH SPECIAL REFERENCE TO B CHROMOSOMES

I. *GONISTA BICOLOR**

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Summary

In *Gonista bicolor*
($2n♀ = 22+XX+0{\sim}2B_1+0{\sim}7B_2$, $2n♂ = 22+XO+0{\sim}3B_1+0{\sim}16B_2$)
frequencies of males with B_1's in four natural populations were 29, 35, 14 and 72 per cent, respectively, and those with B_2's were 97, 95, 76 and 92 per cent, respectively.

B_1's were mitotically highly stable in the germ line of the males as well as in the somatic lines of both sexes. The mean numbers of B_1's per male were 0·34, 0·50, 0·14 and 1·00, respectively, in the four populations.

B_2's were mitotically unstable in somatic lines of both sexes. In the germ line of the males the instability of B_2's was a characteristic confined to an early stage of development. Therefore, numbers of B_2's in primary spermatocytes were variable from follicle to follicle within the individuals, though they were usually constant within the follicles. Mean numbers of B_2's per follicle were also different among the four populations, *i.e.* 2·96, 1·77, 1·37, and 2·97, respectively.

Different tissues showed different mean numbers of B_2's per cell; higher numbers in the ovariole walls than in the gastirc caeca (females), and higher numbers in the germ line than in the gastric caeca (males). An estimation of original zygotic numbers of the males indicated that different mean numbers of B_2's between somatic and germ lines and between different somatic lines were accounted for on the basis of tendency to elimination of B_2's in the somatic lines.

1. Introduction

B chromosomes (supernumerary or accessory chromosomes) have been reported in a large number of plant and animal species and extensive literatures have been reviewed by White (1954), Müntzing (1958, 1966), Rutishauser (1960) and Battaglia (1964). There are two kinds of B chromosomes, mitotically stable and unstable. Thus four Acrididian species have stable and five have unstable type of B's (cf. John and Lewis, 1968, p. 49). Additional instances are *Acrida lata* with a stable type of B (Kayano, Sannomiya and Nakamura, 1970), and *Atractomorpha bedeli* with an unstable type of B (Sannomiya and Kayano, 1969). In studying chromosomal variation in an Acrididian grasshopper, *Gonista bicolor* de Haan ($2n♀ = 22+XX$, $2n♂ = 22+XO$), the writer encountered both mitotically stable and unstable types of B's, which are dealt with in the present paper.

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2. Materials and methods

A total of 9 females and 109 males of *Gonista bicolor* were collected at four well separated locations: 35 males at *Gs* (Goshi-mura, Kikuchi-gun, Kumamoto-ken, in 1959), 20 males at *Ks* (Kashii, Fukuoka-shi, Fukuoka-ken, in 1959), 29 males at *Ot* (Ozi-machi, Oita-shi, Oita-ken, in 1961), 9 females and 25 males at *Kg* (Kagamiyama, Karatsu-shi, Saga-ken, in 1970). The testes were fixed with the fluid devised by Newcomer (1953) and squashed in iron-acetocarmine, or stained in alcoholic hydrochloric acid-carmine and squashed in 45 per cent acetic acid (Snow, 1963), a single follicle being squashed in each preparation. Somatic chromosomes were studied in 9 females and 13 males from *Kg*, in which the cells of the gastric caeca or the cells of the ovariole walls (female) were used. The males after removal of testis were injected with 0·02 c^3 of 0·03 per cent aqueous solution of demecolcine for 18-24 hours. To females 0·05 c^3 of the solution was applied. The gastric caeca and ovarioles were fixed with acetic alcohol (1:3) and stained with alcoholic hydrochloric acid-carmine (Snow, 1963).

3. Observation

(i) *Basic complement and B-chromosomes*

The somatic chromosome complement of *G. bicolor* basically consisted of $2n = 22+XX$ in the female and $2n = 22+XO$ in the male. The basic complement showed 11 bivalents plus one X (11 II+X) in the primary

TABLE 1

Pairing of B's at MI in primary spermatocytes

Configurations		Frequency %	Frequency Obs. cells
$2B_1$	1 II	40·6	(115)
	2 I	59·4	(168)
$3B_1$	1 II+1 I	38·1	(16)
	3 I	61·9	(26)
$2B_2$	1 II	80·9	(2002)
	2 I	19·1	(471)
$3B_2$	1 III	1·0	(16)
	1 II+1 I	55·2	(888)
	3 I	43·8	(703)
$4B_2$	1 IV	0·2	(2)
	1 III+1 I	0·9	(8)
	2 II	38·0	(322)
	1 II+2 I	46·5	(395)
	4 I	14·4	(122)
$5B_2$	1 IV+1 I	0·7	(1)
	1 III+1 II	0·7	(1)
	1 III+2 I	1·5	(2)
	2 II+1 I	37·9	(48)
	1 II+3 I	39·5	(51)
	5 I	19·5	(25)
$6B_2$	1 III+3 I	1·3	(2)
	3 II	16·5	(25)
	2 II+2 I	49·7	(75)
	1 II+4 I	28·5	(43)
	6 I	4·0	(6)
$7B_2$	3 II+1 I	9·5	(2)
	2 II+3 I	38·1	(8)
	1 II+5 I	38·1	(8)
	7 I	14·3	(3)
$9B_2$	1 III+6 I	6·7	(1)
	4 II+1 I	13·3	(2)
	3 II+3 I	40·0	(6)
	2 II+5 I	40·0	(6)
$13B_2$	1 IV+3 II+3 I	25·0	(1)
	1 III+2 II+6 I	25·0	(1)
	5 II+3 I	25·0	(1)
	4 II+5 I	25·0	(1)

spermatocytes. Most of the individuals were found to contain B chromosomes ($0\sim3$ B_1's and $0\sim16$ B_2's) in addition to the basic complement. All the B chromosomes were telocentric, as well as the autosomes and the X chromosome (plate I, figs. 1-2). B_1 was medium sized (as large as the 7th or 8th autosomes) with a large heterochromatic block at the proximal region (plate I, fig. 3). Except at pachytene, diplotene, and diakinesis, B_1's were not distinguishable from the autosomes. B_2's were smaller than the smallest A's and were stained like as the proximal region of the A's (plate I, figs. 3-7). In primary spermatocytes no pairing occurred between B_1 and B_2, while B_1's paired into bivalent and B_2's paired into bivalents, trivalent, etc. (table 1; plate I, figs. 3-7).

(ii) *Mitotic stability and instability of B's*

Numbers of B_1's were variable among individuals but they were generally constant within the individuals in the somatic cells as well as in the primary spermatocytes. There were two exceptions which showed slight variation in the number of B_1's in primary spermatocytes within the follicles as follows:

Case	$2B_1$	$3B_1$	Total cells
1	40	1	41
2	3	18	21

On the other hand, in 62 out of 98 males with B_2's the number of B_2's per cell varied from follicle to follicle within the individuals, while in the remaining 36 males the numbers of B_2's were constant within the individuals (table 2; appendix tables 1-4). In spite of inter-follicular variation, no variation in the number of B_2's was found within the follicles, other than in three exceptional cases out of 683 follicles as follows:

Case	$0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	Total cells
a	48	—	14	3	—	—	—	—	65
b	—	—	—	—	—	26	—	8	34
c	—	—	34	20	—	—	—	—	54

In the cells of the gastric caeca numbers of B_2's varied from cell to cell as well as in the cells of the ovariole walls. In comparison between average number of B_2's per cell of the gastric caeca and that per primary spermatocyte, 10 males showed higher numbers in primary spermatocytes than in the cells of the gastric caeca, two males showed equal numbers, and one male showed a lower number in the primary spermatocytes than in the cells of the gastric caeca (table 3). The mean number of B_2's per primary spermatocyte in 13 males was 2·86 and that per cell of the gastric caeca was 2·09. In comparisons made between gastric caeca and ovariole walls in 9 females, the mean number of B_2's per cell was higher in the ovariole walls (1·97) than in the gastric caeca (1·63) (table 4).

(iii) *Populations*

It was a surprise to find that all the males from *Gs*, *Ks* and *Kg* and the great majority of the males from *Ot* had either B_1 or B_2 or both. Table 5 shows frequencies of B's in the four natural populations (cf. appendix tables 1-4). The frequencies of B_1's are significantly different between the populations ($\chi^2 = 29{\cdot}52$, d.f. = 3, $p < 0{\cdot}01$). Overall means of numbers of B_2's per follicle were different between the populations: 2·96 in *Gs*, 1·77 in *Ks*, 1·37 in *Ot*, and 2·97 in *Kg* ($F^{3}_{105} = 10{\cdot}05$, $0{\cdot}05 > P > 0{\cdot}01$; table 6; cf.

TABLE 2

Number of B_2*'s in males of* G. bicolor *from four populations, Gs, Ks, Ot, and Kg*

Range of no. of B_2's per male	No. of males *Gs*	*Ks*	*Ot*	*Kg*
0	1	1	7	2
1	2	6	5	2
2	4	1	6	1
3	2	—	1	1
4	2	—	2	1
(Sub-total)	(11)	(8)	(21)	(7)
0–1	—	1	—	—
0–2	1	—	—	—
1–2	1	5	7	1
1–3	2	—	—	1
1–4	1	—	—	2
1–5	—	—	—	1
1–9	1	—	—	—
2–3	3	1	1	1
2–4	3	2	—	3
2–5	1	1	—	1
2–6	2	—	—	—
2–13	1	—	—	—
3–4	3	1	—	1
3–5	2	—	—	3
3–6	1	—	—	1
3–7	—	1	—	—
4–6	—	—	—	1
4–7	—	—	—	1
4–9	1	—	—	—
5–6	1	—	—	—
6–8	—	—	—	1
Total males	35	20	29	25
No. of follicles obs.	167	113	96	307
No. of cells obs.	4239	2911	3810	not counted
Mean no. of B_2's per follicle	2·96	1·77	1·37	2·97

appendix tables 1-4). In these populations the numbers of males with variable numbers of B_2's are positively correlated with the overall mean of numbers of B_2's per follicle (table 7).

4. ESTIMATION OF ORIGINAL ZYGOTIC NUMBERS OF B'S

In respect of the mechanism concerned with the maintenance of B_2's in the populations, the original zygotic numbers of B_2's in the *Kg* population were estimated by the following procedure.

First, it was assumed that the predominant follicle type of any male (table 3) represented the original zygotic number of B_2's from which it was developed. Thus original zygotic numbers of B_2's of 13 males were inferred

TABLE 3

Frequencies of B's in the follicles (Foll.) and cells of the gastric caeca (G.C.) of 13 males from the Kg population

Male no.		No. of B_1	Frequency (%) of follicles or cells with $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	$8B_2$	$9B_2$	$10B_2$	$11B_2$	$16B_2$	Total no.	Mean no. of B_2's
25	Foll.	3	100	—	—	—	—	—	—	—	—	—	—	—	—	12	0·00
	G.C.	3	100	—	—	—	—	—	—	—	—	—	—	—	—	36	0·00
10	Foll.	1	—	100	—	—	—	—	—	—	—	—	—	—	—	20	1·00
	G.C.	1	—	100	—	—	—	—	—	—	—	—	—	—	—	50	1·00
9	Foll.	1	—	100	—	—	—	—	—	—	—	—	—	—	—	10	1·00
	G.C.	1	37·5	25·0	25·0	12·5	—	—	—	—	—	—	—	—	—	8	1·13
1	Foll.	0	—	11·1	55·6	22·2	11·1	—	—	—	—	—	—	—	—	9	2·33
	G.C.	0	9·1	63·6	27·3	—	—	—	—	—	—	—	—	—	—	11	1·18
12	Foll.	1	—	22·2	44·5	22·2	11·1	—	—	—	—	—	—	—	—	18	2·22
	G.C.	1	29·1	51·6	12·9	4·8	1·6	—	—	—	—	—	—	—	—	62	0·98
13	Foll.	1	—	66·7	13·3	6·7	—	13·3	—	—	—	—	—	—	—	15	1·80
	G.C.	1	23·5	67·6	5·9	3·0	—	—	—	—	—	—	—	—	—	34	0·88
14	Foll.	1	—	—	90·9	9·1	—	—	—	—	—	—	—	—	—	11	2·09
	G.C.	1	9·7	19·3	64·5	6·5	—	—	—	—	—	—	—	—	—	31	1·68
15	Foll.	1	—	—	18·2	72·7	9·1	—	—	—	—	—	—	—	—	11	2·91
	G.C.	1	1·2	37·4	21·7	18·1	8·4	7·2	1·2	1·2	—	3·6	—	—	—	83	2·53
16	Foll.	1	—	—	—	100	—	—	—	—	—	—	—	—	—	14	3·00
	G.C.	1	—	7·1	40·5	31·0	14·2	2·4	2·4	2·4	—	—	—	—	—	42	2·81
17	Foll.	1	—	—	—	22·2	66·7	11·1	—	—	—	—	—	—	—	9	3·89
	G.C.	1	—	—	41·7	16·7	25·0	8·3	8·3	—	—	—	—	—	—	12	3·25
18	Foll.	1	—	—	—	11·8	82·3	5·9	—	—	—	—	—	—	—	17	3·94
	G.C.	1	—	13·0	34·8	21·8	17·4	8·7	4·3	—	—	—	—	—	—	23	2·87
24	Foll.	2	—	—	—	—	9·1	18·2	54·5	18·2	—	—	—	—	—	11	5·82
	G.C.	2	10·4	27·6	31·0	17·3	3·4	6·9	—	3·4	—	—	—	—	—	29	2·14
7	Foll.	0	—	—	—	—	—	—	11·1	55·6	33·3	—	—	—	—	9	7·22
	G.C.	0	—	—	4·6	—	7·0	23·3	18·6	16·3	11·6	7·0	7·0	2·3	2·3	43	6·67
Mean	Foll.	1·08	7·7	23·1	17·1	20·5	14·6	3·7	5·0	5·7	2·6	—	—	—	—	—	2·86
	G.C.	1·08	17·0	31·7	23·8	10·1	5·9	4·4	2·7	1·8	0·9	0·8	0·5	0·2	0·2	—	2·09

to be $0B_2$ (1), $1B_2$ (3), $2B_2$ (3), $3B_2$ (2), $4B_2$ (2), $6B_2$ (1) and $7B_2$ (1), with the mean of the zygotic number 2·77 per male (number of males in parentheses, table 8). In contrast, the original zygotic numbers of B_2's of the 13 males were inferred to be $0B_2$ (1), $1B_2$ (6), $2B_2$ (5) and $5B_2$ (1) on the basis of the frequencies of cell types in the gastric caeca (mean number of B_2's per male 1·62, table 8).

TABLE 4

Frequencies of B's in cells of the ovariole walls (O.W.) and cells of the gastric caeca (G.C.) of 9 females from the Kg population

Female no.		No. of B_1	Frequency (%) of cells with $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	Cells obs.	Mean no. of B_2's
1	O.W.	1	1·0	99·0	—	—	—	—	—	—	100	0·99
	G.C.	1	7·5	91·2	1·3	—	—	—	—	—	80	0·94
2	O.W.	0	32·0	24·0	44·0	—	—	—	—	—	100	1·12
	G.C.	0	78·7	18·8	2·5	—	—	—	—	—	80	0·24
3	O.W.	2	1·0	96·0	3·0	—	—	—	—	—	100	1·02
	G.C.	2	18·8	76·2	3·7	1·3	—	—	—	—	80	0·88
4	O.W.	1	22·0	76·0	2·0	—	—	—	—	—	100	0·80
	G.C.	1	35·0	51·3	10·0	3·7	—	—	—	—	80	0·83
5	O.W.	1	—	14·0	64·0	21·0	1·0	—	—	—	100	2·09
	G.C.	1	10·0	42·5	38·8	5·0	3·7	—	—	—	80	1·50
6	O.W.	2	1·0	1·0	24·0	49·0	16·0	8·0	1·0	—	100	3·06
	G.C.	2	—	25·0	30·0	36·2	7·5	—	1·3	—	80	2·31
7	O.W.	1	—	2·0	39·0	43·0	14·0	2·0	—	—	100	2·75
	G.C.	1	—	3·7	35·0	47·5	11·2	1·3	1·3	—	80	2·75
8	O.W.	2	—	2·0	23·0	40·0	26·0	6·0	1·0	2·0	100	3·22
	G.C.	2	—	17·5	43·8	26·2	7·5	3·7	—	1·3	80	2·41
9	O.W.	1	—	—	29·0	65·0	4·0	2·0	—	—	100	2·69
	G.C.	1	—	1·2	30·0	56·2	10·0	1·3	—	1·3	80	2·85
Mean	O.W.	1·22	6·3	34·9	25·3	24·2	6·8	2·0	0·2	0·2	—	1·97
	G.C.	1·22	16·6	36·4	21·7	19·6	4·4	0·7	0·3	0·3	—	1·63

Second, frequencies of different types of sperms to be produced in the males were calculated on the basis of frequencies of different follicle types and frequencies of pairing configurations of B_2's at MI. If, for example, the cells with 1 II of B_2's gave rise to sperms all with $1B_2$ and those with 2 I's gave rise to the sperms with $0B_2$, $1B_2$ and $2B_2$'s in the ratio 1:2:1, the follicles with $2B_2$'s (17·1 per cent) would produce sperms with 0, 1 and 2B's in the frequencies 0·817, 15·467 and 0·817 per cent, respectively, since in the follicles with 2B's 80·9 per cent were cells with 1 II and 19·1 per cent were cells with 2 I's (cf. table 1). On such assumptions relationships between follicle types and the resulting gametic types were calculated as shown in table 9. Then, the frequencies of the gametes produced in this population was calculated to be 21·36 ($0B_2$), 38·98 ($1B_2$), 23·20 ($2B_2$'s), and so forth (table 9).

Third, frequencies of zygotic types in next generation were calculated assuming that the frequency distributions of B_2's in the eggs were the same as in the sperms, and matings took place at random. Then, among 13 zygotes 0·59 would be individuals without B_2, 2·17 with $1B_2$, 3·27 with $2B_2$'s 2·90 with $3B_2$'s and so on (table 8). These values are rather close to those of the

TABLE 5

Frequencies of B's in natural populations of G. bicolor

1. *Gs*

	No. of males with						Mean no. of B_1's per male
	$0B_1$	$1B_1$	$2B_1$	$3B_1$	Total	(%)	
No. of males without B_2's	—	1	—	—	1	(2·9)	—
No. of males with B_2's	25	7	2	—	34	(97·1)	—
Total	25	8	2	—	35	(100·0)	0·34
(%)	(71·4)	(28·6)			(100·0)	—	—

2. *Ks*

	No. of males with						Mean no. of B_1's per male
	$0B_1$	$1B_1$	$2B_1$	$3B_1$	Total	(%)	
No. of males without B_2's	—	1	—	—	1	(5·0)	—
No. of males with B_2's	13	4	1	1	19	(95·0)	—
Total	13	5	1	1	20	(100·0)	0·50
(%)	(65·0)	(35·0)			(100·0)	—	—

3. *Ot*

	No. of males with						Mean no. of B_1's per male
	$0B_1$	$1B_1$	$2B_1$	$3B_1$	Tstal	(%)	
No. of males without B_2's	7	—	—	—	7	(24·1)	—
No. of males with B_2's	18	4	—	—	22	(75·9)	—
Total	25	4	—	—	29	(100·0)	0·14
(%)	(86·2)	(13·8)			(100·0)	—	—

4. *Kg*

	No. of males with						Mean no. of B_1's per male
	$0B_1$	$1B_1$	$2B_1$	$3B_1$	Total	(%)	
No. of males without B_2's	—	1	—	1	2	(8·0)	—
No. of males with B_2's	7	11	5	—	23	(92·0)	—
Total	7	12	5	1	25	(100·0)	1·00
(%)	(28·0)	(72·0)			(100·0)	—	—

	No. of females with						Mean no. of B_1's per female
	$0B_1$	$1B_1$	$2B_1$	$3B_1$	Total	(%)	
No. of females without B_2's	—	—	—	—	—	(0·0)	—
No. of females with B_2's	1	5	3	—	9	(100·0)	1·22
(%)	(11·1)	(88·9)			(100·0)	—	—

TABLE 6

Mean numbers of B_2's per follicle of 109 males from four populations

Gs		Ks	Ot		Kg	
0·00		0·00	0·00		0·00	
1·00		0·86	0·00		0·00	
1·00		1·00	0·00		1·00	
1·00	3·00	1·00	0·00		1·00	
1·33	3·13	1·00	0·00		1·80	
1·60	3·17	1·00	0·00		1·86	
2·00	3·25	1·00	0·00		2·00	
2·00	3·33	1·00	1·00		2·00	
2·00	3·50	1·20	1·00		2·09	
2·00	3·60	1·20	1·00	1·50	2·22	
2·00	4·00	1·29	1·00	2·00	2·33	
2·14	4·00	1·67	1·00	2·00	2·73	
2·20	4·00	1·75	1·17	2·00	2·91	
2·25	4·00	2·00	1·25	2·00	3·00	3·94
2·50	4·33	2·50	1·33	2·00	3·05	4·00
2·60	4·50	2·75	1·33	2·00	3·42	4·31
2·67	5·20	3·00	1·40	2·33	3·89	4·67
3·00	5·33	3·10	1·40	3·00	3·92	5·00
	5·50	3·33		4·00		5·82
	6·33	4·67		4·00		7·22
(Overall mean 2·96)		(Overall mean 1·77)	(Overall mean 1·37)		(Overall mean 2·97)	

present generation inferred from frequencies of follicle types than those inferred from frequencies of cell types in the gastric caeca (table 8).

5. DISCUSSION

(i) *Factors affecting mitotic stability of B's*

Though both B_1 and B_2 of *G. bicolor* are highly stable during spermatogonial cell divisions they are different in that B_1 is mitotically stable but B_2 is unstable in the germ line and also in the somatic lines. Coexistence of two types of B's, mitotically stable and unstable, is known in *Allium cernuum* (Grun, 1959) and *Lilium callosum* (Kayano, 1962). These indicate that the ability or the inability to undergo non-disjunction is primarily an inherent function of the type of B chromosomes. However, this function is affected by some factors within the cells. Thus, in *G. bicolor* the same B_2's are variable in number within certain individuals but not within others (table 2); they are unstable in the germ line at an early stage of development but stable at

TABLE 7

Correlation between frequency of intra-individual variation and mean number of B_2's per follicle

Population	(I) Males with variable no. of B_2's	(II) Total males with B_2's	$\frac{(I)}{(II)}$	Mean no. of B_2's per follicle
Ot	8	22	0·364	1·37
Ks	12	19	0·632	1·77
Gs	24	34	0·706	2·96
Kg	18	23	0·783	2·97

TABLE 8

Estimation of original zygotic numbers of B_2's in 13 males from the Kg population

	Frequency of										Mean no. of B_2's
	$0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	$8B_2$	Total	
% follicles in the males*	7·7	23·1	17·1	20·5	14·6	3·7	5·0	5·7	2·6	100·0	2·86
% gametes produced by the males†	21·36	38·98	23·20	9·87	5·12	1·16	0·26	0·05	—	100·0	—
Zygotes estimated	0·59	2·17	3·27	2·90	1·99	1·18	0·57	0·23	0·10	13·0	2·85
Males inferred from follicle types	1	3	3	2	2	—	1	1	—	13·0	2·77
Males inferred from cells of G.C.	1	6	5	—	—	1	—	—	—	13·0	1·62

* Cf. table 3.
† Cf. table 6.

TABLE 9

Frequency of sperms to be produced by 13 males shown in table 3

Spermatocytes		Sperms (%)									
		$0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	$8B_2$	Total
$0B_2$		7·700	—	—	—	—	—	—	—	—	7·70
$1B_2$		11·550	11·550	—	—	—	—	—	—	—	23·10
$2B_2$	1 II	—	13·834	—	—	—	—	—	—	—	17·10
	2 I	0·817	1·633	0·817	—	—	—	—	—	—	
$3B_2$	1 III	—	0·103	0·103	—	—	—	—	—	—	20·50
	1 II+1 I	—	5·658	5·658	—	—	—	—	—	—	
	3 I	1·122	3·367	3·367	1·122	—	—	—	—	—	
$4B_2$	1 IV	—	0·010	0·010	0·010	—	—	—	—	—	14·60
	1 III+1 I	—	0·033	0·066	0·033	—	—	—	—	—	
	2 II	—	—	5·548	—	—	—	—	—	—	
	1 II+2 I	—	1·697	3·394	1·697	—	—	—	—	—	
	4 I	0·131	0·526	0·788	0·526	0·131	—	—	—	—	
$5B_2$	1 IV+1 I	—	0·004	0·009	0·009	0·004	—	—	—	—	3·70
	1 III+1 II	—	—	0·013	0·013	—	—	—	—	—	
	1 III+2 I	—	0·007	0·021	0·021	0·007	—	—	—	—	
	2 II+1 I	—	—	0·701	0·701	—	—	—	—	—	
	1 II+3 I	—	0·184	0·551	0·551	0·184	—	—	—	—	
	5 I	0·023	0·113	0·225	0·225	0·113	0·023	—	—	—	
$6B_2$	1 III+3 I	—	0·004	0·016	0·024	0·016	0·004	—	—	—	5·00
	3 II	—	—	—	0·825	—	—	—	—	—	
	2 II+2 I	—	—	0·621	1·243	0·621	—	—	—	—	
	1 II+4 I	—	0·089	0·356	0·534	0·356	0·089	—	—	—	
	6 I	0·003	0·019	0·047	0·062	0·047	0·019	0·003	—	—	
$7B_2$	3 II+1 I	—	—	—	0·271	0·271	—	—	—	—	5·70
	2 II+3 I	—	—	0·272	0·815	0·815	0·272	—	—	—	
	1 II+5 I	—	0·068	0·339	0·679	0·679	0·339	0·068	—	—	
	7 I	0·006	0·045	0·134	0·223	0·223	0·134	0·045	0·006	—	
$8B_2$*	4 II	—	—	—	—	1·300	—	—	—	—	2·60
	8 I	0·005	0·041	0·142	0·284	0·356	0·284	0·142	0·041	0·005	
Total		21·36	38·98	23·20	9·87	5·12	1·16	0·26	0·05	—	100·00

* Pairing configurations of B_2's in spermatocytes of the follicles was not analysed.

later stages. In *Festuca pratensis* (Bosemark, 1956) the rate of mitotic non-disjunction of the B's in pollen grain is affected by interaction between the B's, and in *Lilium callosum* coexistence of the f_l type of B's enhance the rate of non-disjunction of another type of B's, f_s (Kayano, 1962). In *G. bicolor* the frequency of males with varying number of B_2's is higher in the population with higher mean number of B_2's per follicle (table 7), suggesting that interaction between the B_2's enhance the rate of their mitotic non-disjunction. The same type of interaction of B's has been reported in *Scilla scilloides* (Haga, 1961).

(ii) *Difference in number of B_2's between somatic and germ lines*

Nur (1963) suggested originally that variation in the number of B's due to non-disjunction would be maintained in association with an accumulation mechanism. This is the case in *Camnula pellucida* (Nur, 1969) and *Locusta migratoria* (Nur, 1969; Kayano, 1971), in which the mean numbers of B's per primary spermatocyte were higher than the means per cell of the gastric caeca. In *G. bicolor*, however, mitotic instability of B_2's in the germ line is not likely to be associated with an accumulation mechanism because increase and decrease of number of B_2's tend to be balanced within the population and the mean number of B_2's in the primary spermatocytes is very close to the mean of the estimated original zygotic number. Therefore, lower mean numbers of B_2's per cell in the somatic line than in the germ line is very likely to indicate true elimination of B_2's from the somatic line.

(iii) *Difference between populations*

In spite of different frequencies of B_1's and B_2's between populations of *G. bicolor* both types of the B's occur in all four localities, suggesting that the populations have descended from a common ancestral population but nowadays they are more or less isolated from one another (cf. Hewitt and John, 1970; Kayano, Sannomiya and Nakamura, 1970). Evans (1960) has reported that two populations of *Helix pomatia* are different in the frequency of individuals with B's (mitotically unstable) and the cause is ascribed to historical difference between the populations. In *Myrmeleotettix maculatus* climatic differences and differences in transmission rates between the populations affect equilibrium frequencies of B's (Hewitt and Brown, 1970; Hewitt, 1973). Some of these explanations may be applicable to the population differences in *G. bicolor*. However, another explanation is also possible that the population may be different for the genotypes affecting tolerance to any harmful effect of the B's (cf. Östergren, 1947).

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6. References

BATTAGLIA, E. 1964. Cytogenetics of B-chromosomes. *Caryologia*, *17*, 249-299.

BOSEMARK, N. O. 1956. On accessory chromosomes in *Festuca platensis*. III. Frequency and geographical distribution of plants with accessory chromosomes. *Hereditas*, *42*, 189-210.

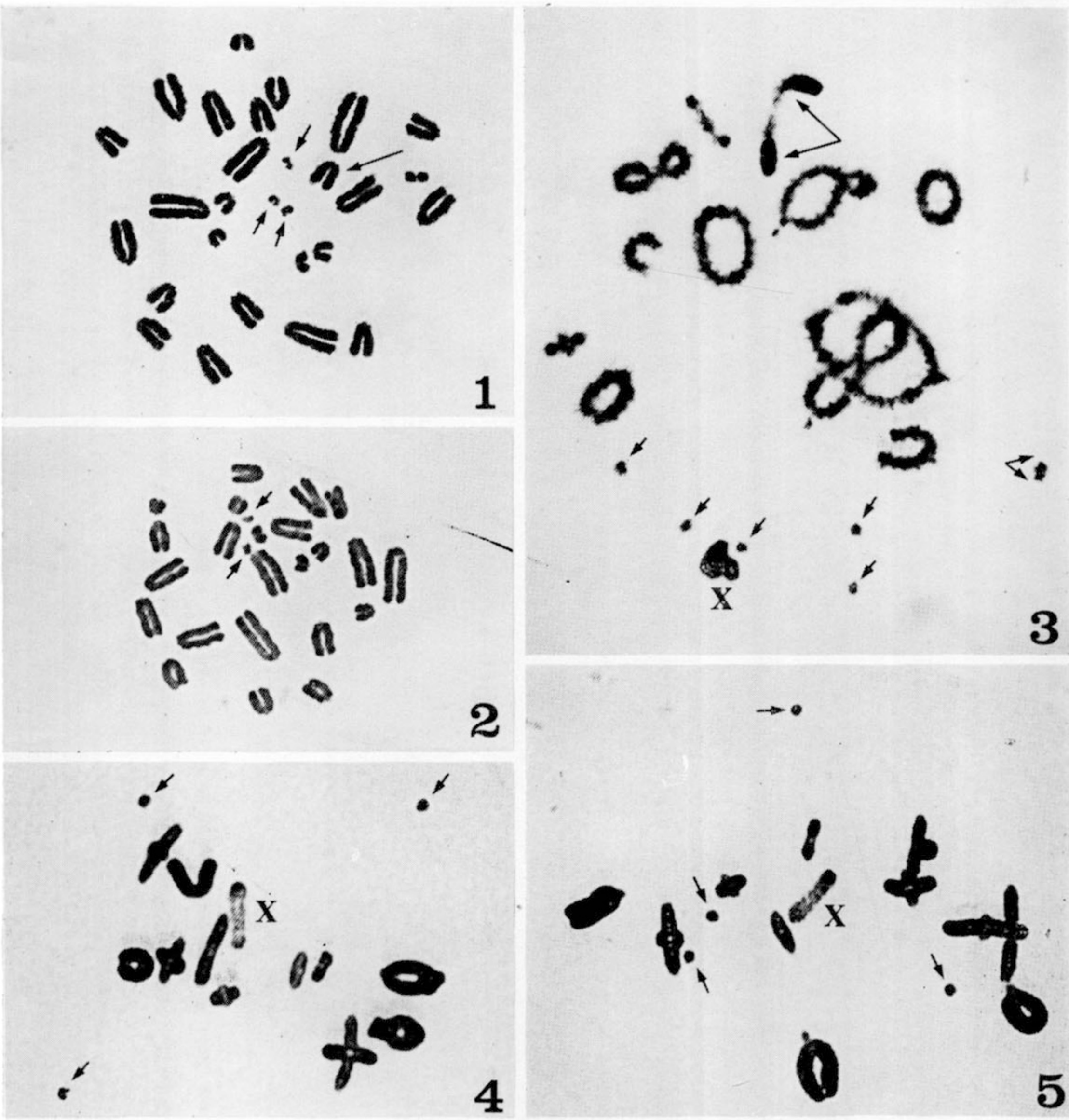
EVANS, H. J. 1960. Supernumerary chromosomes in wild population in the snail *Helix pomatia* L. *Heredity*, *15*, 129-138.

Plate I

(All figs. $\times 1000$)

Chromosomes of *G. bicolor*. Long and short arrows indicate B_1 and B_2, respectively.

FIG. 1.—A cell of a gastric caecum from a female, $2n = 22+XX+1B_1+3B_2$.
FIG. 2.—A cell of a gastric caecum from a male, $2n = 22+XO+2B_2$.
FIG. 3.—A primary spermatocyte at diplotene, showing
$$11\ II+X+1\ II(B_1)+1\ II+5\ I\ (B_2).$$
FIGS. 4-7.—MI's of primary spermatocytes from different follicles of a male; $3B_2$, $4B_2$, $5B_2$, and $7B_2$, respectively.


1
2
3
X
4
X
5
X
6
X
7
X

GRUN, P. 1959. Variability of accessory chromosomes in native populations of *Allium cernuum*. *Amer. J. Bot.*, *46*, 218-224.

HAGA, T. 1961. Intra-individual variation in number and linear patterning of the chromosomes. I. B-chromosomes in *Rumex*, *Paris*, and *Scilla*. *Proc. Japan Acad.*, *37*, 627-632.

HEWITT, G. 1973. A variable transmission rates of B-chromosome in *Myrmeleotettix maculatus* (Thunb.) (Acrididae: Orthoptera). *Chromosoma*, *40*, 83-106.

HEWITT, G. M., AND BROWN, M. F. 1970. The B-chromosome system of *Myrmeleotettix maculatus*. V. A steep cline in East Anglia. *Heredity*, *25*, 363-371.

HEWITT, G. M., AND JOHN, B. 1970. The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.). IV. The dynamics. *Evolution*, *24*, 169-180.

JOHN, B., AND LEWIS, K. R. 1968. The chromosome complement. *Protoplasmatologia*, *4*. Springer-Verlag.

KAYANO, H. 1962. Cytogenetic studies in *Lilium callosum*. IV. Transmission and multiplication of a small supernumerary B chromosomes. *Evolution*, *16*, 86-89.

KAYANO, H. 1971. Accumulation of B chromosomes in the germ line of *Locusta migratoria*. *Heredity*, *27*, 119-123.

KAYANO, H., SANNOMIYA, M., AND NAKAMURA, K. 1970. Cytogenetic studies on natural populations of *Acrida lata*. *Heredity*, *25*, 113-122.

MÜNTZING, A. 1958. Accessory chromosomes. *Trans. Bose Res. Inst.* (Calcutta), *22*, 1-15.

MÜNTZING, A. 1966. Accessory chromosomes. *Bull. Bot. Soc. Bengal*, *20*, 1-15.

NEWCOMER, E. H. 1953. A new cytological and histological fixing fluid. *Science*, *118*, 161.

NUR, U. 1963. A mitotically unstable supernumerary chromosome with an accumulation mechanism in a grasshopper. *Chromosoma*, *14*, 407-422.

NUR, U. 1969. Mitotic instability leading to an accumulation of B-chromosomes in grasshoppers. *Chromosoma*, *27*, 1-19.

ÖSTERGREN, G. 1947. Heterochromatic B-chromosome in *Anthoxanthum*. *Hereditas*, *33*, 261-296.

RUTISHAUSER, A. 1960. Zur Genetik überzäliger Chromosomen. *Arch. J. Klaus-Stift. Vererb. Sozial. Rass.* (Zürich), *35*, 440-458.

SANNOMIYA, M., AND KAYANO, H. 1969. Local variation and year-to-year change in frequencies of B-chromosomes in natural populations of two grasshopper species. *Japan J. Genetics*, *44*, Suppl. *1*, 84-92.

SNOW, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. *Stain Technol.*, *2*, 116-117.

WHITE, M. J. D. 1954. *Animal Cytology and Evolution*, 2nd ed. Cambridge Univ. Press, Cambridge.

7. Appendix tables

Appendix table 1

Frequencies of B's in the Gs population of G. bicolor

No. of B_1	Male no.	Number of follicles with $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$9B_2$	$13B_2$	No. of folls.	No. of cells	Mean no. of B_2's per follicle
$0B_1$	1	—	5	—	—	—	—	—	—	—	5	169	1·00
	2	—	7	—	—	—	—	—	—	—	7	409	1·00
	3	—	7	1[a]	1	—	—	—	—	—	9	309	1·33
	4	—	1	2	1	—	—	—	—	—	4	32	2·00
	5	—	1	—	—	—	2[b]	1	1	—	5	107	5·20
	6	—	—	2	—	—	—	—	—	—	2	66	2·00
	7	—	—	1	—	—	—	—	—	—	1	27	2·00
	8	—	—	3	—	—	—	—	—	—	3	24	2·00
	9	—	—	6	1	—	—	—	—	—	7	264	2·14
	10	—	—	3	1	—	—	—	—	—	4	84	2·25
	11	—	—	3	1	1	—	—	—	—	5	125	2·60
	12	—	—	1	3	2	—	—	—	—	6	211	3·17
	13	—	—	2	4	1	1	—	—	—	8	95	3·13
	14	—	—	1	—	2	2	1	—	—	6	87	4·33
	15	—	—	1	2	—	—	2	—	—	5	117	4·00
	16	—	—	—	9	—	—	—	—	—	9	312	3·00
	17	—	—	—	1	—	—	—	—	—	1	32	3·00
	18	—	—	—	3	1	—	—	—	—	4	130	3·25
	19	—	—	—	2	3	—	—	—	—	5	77	3·60
	20	—	—	—	2	2	—	—	—	—	4	91	3·50
	21	—	—	—	1	6	1	—	—	—	8	126	4·00
	22	—	—	—	1	3	—	2	—	—	6	145	4·50
	23	—	—	—	—	7	—	—	—	—	7	94	4·00
	24	—	—	—	—	1	—	—	—	—	1	24	4·00
	25	—	—	—	—	1	—	2	—	—	3	87	5·33
$1B_1$	26	3	—	—	—	—	—	—	—	—	3	114	0·00
	27	—	2	3	—	—	—	—	—	—	5	117	1·60
	28	—	1	3	—	1	—	—	—	—	5	120	2·20
	29	—	—	4	—	—	—	—	—	—	4	150	2·00
	30	—	—	2	—	1	—	—	—	—	3	96	2·67
	31	—	—	1	—	1	—	—	—	1	3	47	6·33
	32	—	—	—	5	—	1	—	—	—	6	134	3·33
	33	—	—	—	—	—	1	1	—	—	2	23	5·50
$2B_1$	34	1	5	1	—	—	—	—	—	—	7	81	1·00
	35	—	—	2	2*	—	—	—	—	—	4	113	2·50
Total	—	4	29	42	40	33	8	9	1	1	167	—	Mean 2·96)
No. of cells	—	130	973	1087	1046	682	113	189	15	4	—	4239	—

[a], [b] Each indicates the follicle with variable number of B_2's given in the text.

* Indicates inclusion of a follicle with variable number of B_1's given in the text as case 1.

APPENDIX TABLE 2

Frequencies of B's in the Ks population of G. bicolor

No. of B_1	Male no.	Number of follicles with $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	No. of folls.	No. of cells	Mean no. of B_2's per follicle
$0B_1$	1	1	6	—	—	—	—	—	—	7	101	0·86
	2	—	9	—	—	—	—	—	—	9	118	1·00
	3	—	6	—	—	—	—	—	—	6	91	1·00
	4	—	7	—	—	—	—	—	—	7	229	1·00
	5	—	4	—	—	—	—	—	—	4	82	1·00
	6	—	5	2	—	—	—	—	—	7	205	1·29
	7	—	1	2	—	—	—	—	—	3	117	1·67
	8	—	1	3	—	—	—	—	—	4	68	1·75
	9	—	—	7	—	—	—	—	—	7	163	2·00
	10	—	—	1	3	1	—	—	—	5	225	3·00
	11	—	—	6	1	—	1	—	—	8	279	2·50
	12	—	—	—	2	1	—	—	—	3	35	3·33
	13	—	—	—	1	2	2	—	1	6	161	4·67
$1B_1$	14	6	—	—	—	—	—	—	—	6	165	0·00
	15	—	4	—	—	—	—	—	—	4	78	1·00
	16	—	3	—	—	—	—	—	—	3	87	1·00
	17	—	—	1	3	—	—	—	—	4	27	2·75
	18	—	—	1[c]	7	2	—	—	—	10	544	3·10
$2B_1$	19	—	6	1	—	—	—	—	—	5	90	1·20
$3B_1$	20	—	4	1*	—	—	—	—	—	5	46	1·20
Total	—	7	54	25	17	6	3	—	1	113	—	(Mean 1·77)
No. of cells	—	174	1142	613	699	201	61	—	21	—	2911	—

[c] Indicates the follicle with variable number of B_2's given in the text.

* Indicates the follicle with variable number of B_1's given in the text as case 2.

APPENDIX TABLE 3

Frequencies of B's in the Ot population of G. bicolor

No. of B_1	Male no.	No of follicles with $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	No. of folls.	No. of cells	Mean no. of B_2's per follicle
$0B_1$	1	4	—	—	—	—	4	193	0·00
	2	3	—	—	—	—	3	84	0·00
	3	4	—	—	—	—	4	134	0·00
	4	6	—	—	—	—	6	172	0·00
	5	2	—	—	—	—	2	72	0·00
	6	1	—	—	—	—	1	39	0·00
	7	3	—	—	—	—	3	163	0·00
	8	—	3	—	—	—	3	86	1·00
	9	—	5	—	—	—	5	254	1·00
	10	—	3	—	—	—	3	131	1·00
	11	—	2	1	—	—	3	235	1·33
	12	—	3	2	—	—	5	178	1·40
	13	—	3	1	—	—	4	112	1·25
	14	—	3	2	—	—	5	187	1·40
	15	—	2	1	—	—	3	253	1·33
	16	—	5	1	—	—	6	269	1·17
	17	—	—	1	—	—	1	20	2·00
	18	—	—	5	—	—	5	201	2·00
	19	—	—	3	—	—	3	104	2·00
	20	—	—	7	—	—	7	359	2·00
	21	—	—	2	—	—	2	50	2·00
	22	—	—	2	1	—	3	83	2·33
	23	—	—	—	2	—	2	73	3·00
	24	—	—	—	—	2	2	40	4·00
	25	—	—	—	—	1	1	56	4·00
$1B_1$	26	—	2	—	—	—	2	62	1·00
	27	—	2	—	—	—	2	124	1·00
	28	—	1	1	—	—	2	39	1·50
	29	—	—	4	—	—	4	37	2·00
Total	—	23	34	33	3	3	96	—	(Mean 1·37)
No. of cells	—	857	1603	1164	90	96	—	3810	—

APPENDIX TABLE 4

Frequencies of B's in the Kg population of G. bicolor

No. of B_1	Male no.	Number of follicles with† $0B_2$	$1B_2$	$2B_2$	$3B_2$	$4B_2$	$5B_2$	$6B_2$	$7B_2$	$8B_2$	No. of folls.	Mean no. of B_2's per follicle
$0B_1$	1*	—	1	5	2	1	—	—	—	—	9	2·33
	2	—	—	18	—	—	—	—	—	—	18	2·00
	3	—	—	3	3	4	2	—	—	—	12	3·42
	4	—	—	—	18	1	—	—	—	—	19	3·05
	5	—	—	—	—	7	—	—	—	—	7	4·00
	6	—	—	—	—	2	—	1	—	—	3	4·67
	7*	—	—	—	—	—	—	1	5	3	9	7·22
$1B_1$	8	19	—	—	—	—	—	—	—	—	19	0·00
	9*	—	10	—	—	—	—	—	—	—	10	1·00
	10*	—	20	—	—	—	—	—	—	—	20	1·00
	11	—	1	4	1	—	—	—	—	—	6	2·00
	12*	—	4	8	4	2	—	—	—	—	18	2·22
	13*	—	10	2	1	—	2	—	—	—	15	1·80
	14*	—	—	10	1	—	—	—	—	—	11	2·09
	15*	—	—	2	8	1	—	—	—	—	11	2·91
	16*	—	—	—	14	—	—	—	—	—	14	3·00
	17*	—	—	—	2	6	1	—	—	—	9	3·98
	18*	—	—	—	2	14	1	—	—	—	17	3·94
	19	—	—	—	2	10	1	—	—	—	13	3·92
$2B_1$	20	—	1	6	—	—	—	—	—	—	7	1·86
	21	—	—	5	4	2	—	—	—	—	11	2·73
	22	—	—	—	3	6	1	3	—	—	13	4·31
	23	—	—	—	—	3	7	3	—	—	13	5·00
	24*	—	—	—	—	1	2	6	2	—	11	5·82
$3B_1$	25*	12	—	—	—	—	—	—	—	—	12	0·00
Total	—	31	47	63	65	60	17	14	7	3	307	(Mean 2·97)

* Indicates 13 males given in table 3.

‡ Follicle type was determined by the observation of over 20 cells.