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Population Cytology of the Genus *Phaulacridium* V.* *Ph. marginale* — The Omarama Population

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Abstract

During the 1975 meiotic season a sample of 105 *Ph. marginale* males was collected from Omarama (South Island, New Zealand). This population is polymorphic for a small metacentric B chromosome (B^M) and for extra segments on the three smallest autosomes. Only 10% of males have the basic karyotype. Previous collections from this population had similar frequencies of the B^M chromosomes and of the extra segments.

One individual carried a small telocentric B chromosome (B^t), unusual at Omarama, as well as the B^M typical of this population. This may be significant for the proposed evolution of B chromosomes in the genus *Phaulacridium*. Both types of supernumerary heterochromatin (B chromosomes and segments) produce a similar increase in mean cell chiasma frequency, and maximal increase is produced by one B chromosome or one segment. There is no evidence for any additive or synergistic action of B chromosomes and segments.

Introduction

In addition to occasional chromosome rearrangements the New Zealand grasshopper *Phaulacridium marginale* is extensively polymorphic for B chromosomes and extra heterochromatic segments (Martin 1970*a*, 1970*b*; Westerman and Fontana 1973; Westerman 1974, 1975*b*). The pattern of distribution of these polymorphisms in North and South Islands of New Zealand suggests that the extra heterochromatin may be better tolerated in ecologically 'central' rather than in 'marginal' populations (Westerman 1975*b*).

Three morphologically distinct types of B chromosomes occur, only one type being present in any one population. These are: a large telocentric (B^T) approximately equal in size to the X chromosome, and morphologically similar to the B^T chromosomes in *Ph. vittatum* and *Ph. nanum*; a smaller telocentric (B^t); and a small metacentric isochromosome (B^M). All three types of B chromosomes are mitotically stable in the male germ line. The frequency of B-carrying males in a population may vary from 4 to 50%. These adult frequencies are stable from year to year with no evidence for either meiotic 'drive' or 'drag'.

At Omarama (South Island) there is a population with an abundance of B^M-containing males (41–50%) and which also has the polymorphisms for heterochromatic segments on the three smallest autosomes. In each of three collections (1972, 1973, 1975) only 10% of males had the basic karyotype. Although analysis of the limited data had not revealed any significant effect of the extra heterochromatin on either

* Part IV, *Heredity*, 1975, **35**, 165–72.

Table 1. Chiasma frequency data of the karyotypes collected from the Omarama population of *Ph. marginale*, 1975

n, Number of individuals seen in each class

Autosomal state[A]			No B chromosome		One B chromosome		Two B chromosomes		Three B chromosomes	
S_9	S_{10}	S_{11}	Mean ± s.d. (and range)	*n*	Mean ± s.d. (and range)	*n*	Mean ± s.d. (and range)	*n*	Mean ± s.d. (and range)	*n*
Nor_9	Nor_{10}	Nor_{11}	16·68 ± 0·40 (14·2–18·6)	10	17·37 ± 0·98 (15·7–19·1)	3	17·13 ± 0·44 (16·0–18·8)	6	17·50 ± 0·25 (17·2–17·3)	2
		Het_{11}	16·67 ± 0·35 (16·0–17·2)	3	17·1	1				
	Het_{10}	Nor_{11}	17·46 ± 0·50 (16·3–19·3)	5	16·37 ± 1·13 (15·5–19·1)	3	18·0	1		
		Het_{11}	18·0	1	17·2	1				
Het_9	Nor_{10}	Nor_{11}	17·50 ± 0·56 (16·4–20·0)	6	17·10 ± 1·12 (15·4–19·2)	3	16·77 ± 0·39 (16·0–17·3)	3		
		Het_{11}	19·1	1	18·1	1				
	Het_{10}	Nor_{11}	16·75 ± 0·41 (14·9–18·4)	8	18·52 ± 0·39 (17·5–20·3)	6	17·70 ± 0·40 (17·3–18·1)	2		
		Het_{11}	16·85 ± 0·25 (16·6–17·1)	2	17·80 ± 1·40 (16·4–19·2)	2				
	Hom_{10}	Nor_{11}	17·47 ± 1·20 (15·1–19·0)	3	17·40 ± 2·20 (15·2–19·6)	2	16·7	1		
Hom_9	Nor_{10}	Nor_{11}	18·23 ± 0·58 (16·8–20·2)	6	17·13 ± 0·69 (16·3–18·5)	3	18·27 ± 0·77 (16·8–19·4)	3		
		Het_{11}	16·7	1	16·8	1				
	Het_{10}	Nor_{11}			18·13 ± 0·68 (17·4–19·5)	3	16·8	1		
		Het_{11}	17·50 ± 1·00 (16·5–18·5)	2						
	Hom_{10}	Nor_{11}	17·85 ± 1·35 (16·5–19·2)	2			18·7	1		
		Het_{11}			18·9	1				

[A] Nor = normal; hom = homozygote; het = heterozygote.

mean cell chiasma frequency or on between-cell variance, it was felt that this particular population offered a unique opportunity to examine the possible role of extra heterochromatin on chiasma indices. At the same time it should be possible to determine possible modes of interaction of B chromosomes and segments when present in the same individual.

Materials and Methods

During the 1975 meiotic season a sample of 105 young adult males was collected from Omarama (for location see Figure 1, Westerman 1974). Since the population is not very large, more extensive collecting is not possible if the population is to be preserved for future analysis. For this reason no sample of females was taken in order to minimize loss to the reproductive capacity of the population. After removal of the testes, which were fixed in 1 : 3 acetic alcohol, each cadaver was kept for subsequent morphological examination. A total of 100 individuals was subsequently karyotyped and from each individual a sample of 10 diplotene cells was scored for chiasma frequency.

Table 2. Frequencies of B chromosomes and segments in the Omarama population of *Ph. marginale*, 1969–1975

q_x, Frequency with which a chromosome carries an extra segment

(*a*) B chromosomes

Year	No B	1 B	2 B	3 B	B-containing males	B chromosomes per male
1969	7	3	4		0·5000	0·7857
1972	10	3	2	2	0·4117	0·7647
1973	14	8	5		0·4815	0·6667
1975	50	30	18	2	0·5000	0·7200
Totals	81	44	29	4	0·4873	0·7215

(*b*) Extra segments

Year	BB_9	BS_9	SS_9	q_9	BB_{10}	BS_{10}	SS_{10}	q_{10}	BB_{11}	BS_{11}	SS_{11}	q_{11}
1972	11	5	1	0·21	5	10	2	0·41	15	2	0	0·04
1973	20	5	2	0·17	13	11	3	0·31	25	2	0	0·06
1975	36	40	24	0·44	53	37	10	0·29	83	17	0	0·09
Totals	67	50	27	0·36	71	58	15	0·31	123	21	0	0·07
	$\chi^2_4 = 15·7981$ $(0·001 < P < 0·01)$				$\chi^2_4 = 3·4270$ (n.s.)				$\chi^2_2 = 1·6922$ (n.s.)			

Results

The Karyotypes

The karyotypes of the 100 scoreable males are shown in Table 1. The polymorphisms for extra heterochromatin were as extensive as previous collections at Omarama had indicated. Thus 50 individuals were seen to carry one or more B chromosomes in all their germ line cells (Table 2). The frequency of B-containing males was consistent with previous estimates (Table 2*a*). Almost all B chromosomes scored were of the type previously described for Omarama, i.e. B^M, and up to three B chromosomes could be present in the same cell. One individual, however, was observed to carry two different B chromosomes—one B^M and one B^t (see Fig. 1). The revelance of this individual to a proposed evolution of B chromosomes in the genus *Phaulacridium* will be discussed below.

The sample was also extensively polymorphic for extra segments on the three smallest autosomes. The possible presence of extra heterochromatin on neither (BB), either (BS) or both (SS) homologues of the S_9, S_{10} and S_{11} chromosomes means that 27 different karyotypes can be present in the population with respect to these segments.

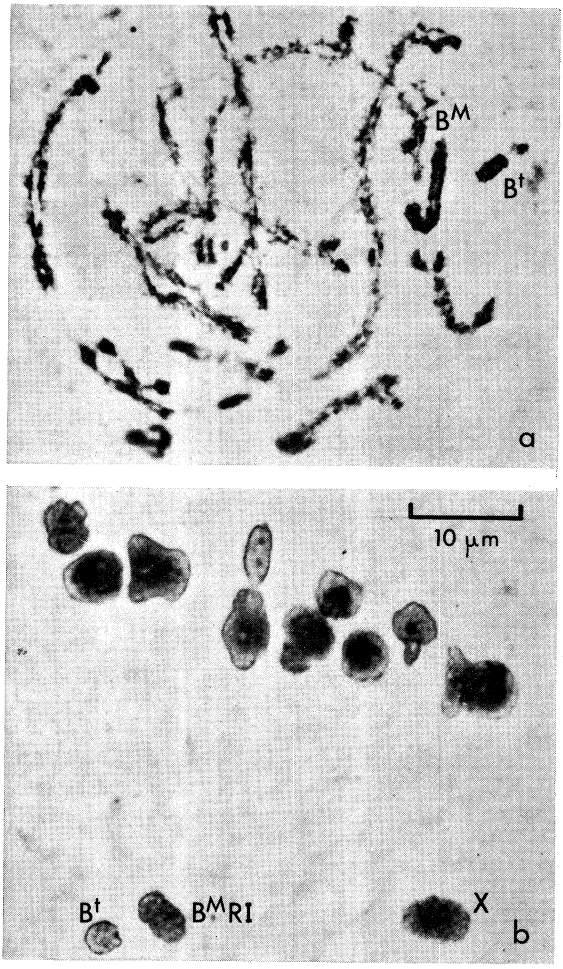

Fig. 1. Meiotic cells from a *Ph. marginale* individual carrying a metacentric isochromosome (B^M) together with a small telocentric B chromosome (B^t). (*a*) Pachytene cell. (*b*) Metaphase I, showing B^M present as ring univalent.

Since 39% of the individuals carrying supernumerary segments also carry one or more B chromosomes (up to three) then a grand total of 108 different karyotypes could be expected in the Omarama population. To date 38 of these possibilities have been encountered. This 'superabundance' of karyotypic classes within the one population poses certain difficulties in the analysis of the effects of B chromosomes and segments and their interaction because numerically small populations such as Omarama cannot

generate the very large sample sizes necessary. As with the B chromosomes, the extra segment polymorphisms were present from year to year (Table 2*b*) and the observed numbers of individuals present in each category—basic homozygote (BB,) segment heterozygote (BS) and segment homozygote (SS)—were in agreement with Hardy–Weinberg expectations. Although the contingency χ^2 tests on the S_{10} and S_{11} chromosomes showed no difference between years, in the case of the S_9 chromosome there were significant differences. This result is probably due to the large number of individuals homozygous for segments on the S_9 in 1975 (24 observed, cf. 19 expected) and fewer heterozygotes (40 observed, 49 expected).

Table 3. Analysis of variance of the effect of extra heterochromatin on mean cell chiasma frequency in *Ph. marginale*

Data are from Table 2

Item	Degrees of freedom	Mean square	Variance ratio	*P*
	(*a*) Means			
Between-B classes	3	0·7714	0·4719[A]	n.s.
Between-segment classes	5	2·6699	1·6332[A]	n.s.
B × segment interaction	8	0·6105	0·3522	n.s.
Error sum of squares	83	1·7335		
	(*b*) Log variances			
Between-B classes	3	0·0737	1·0935[B]	n.s.
Between-segment classes	5	0·0276	0·4095[B]	n.s.
B × segment interaction	8	0·0430	0·6169	n.s.
Error sum of squares	83	0·0697		

[A] Pooled error mean square = 1·6348. [B] Pooled error mean square = 0·0674.

Chiasma Frequencies

In order to ascertain whether this supernumerary heterochromatin had any effect on the level of recombination in the population, and hence on rate of release of variability, chiasma scores were made for 10 diplotene cells per individual. The results were analysed using a DEC system-10 program (courtesy Dr D. Hay) (for details see Westerman 1975*a*). The analyses are shown in Tables 3*a* and 3*b*. It can be seen that neither B chromosomes nor segments significantly affect either mean cell chiasma frequency or between-cell log variances. Similar analysis of the data from individuals having B chromosomes but no segments at all would again suggest that B chromosomes do not significantly affect chiasma frequency ($t_{19} = 0{\cdot}99$, $P > 0{\cdot}05$). Such results agree with previous findings for both *Ph. marginale* (Westerman and Fontana 1973; Westerman 1974, 1975*b*) and for *Ph. vittatum* (Rowe and Westerman 1974; John and Freeman 1974, 1976).

In spite of these apparently non-significant results, however, it is evident from inspection of the Omarama data from the three samples (1972, 1973, and 1975) that in each year a trend is apparent for B chromosomes and for segments on the S_9 and S_{10} chromosomes (see Table 4)—i.e. addition of one piece of extra heterochromatin *does* lead to a rise in mean cell chiasma frequency.

The 1975 Omarama data were therefore re-analysed as shown in Table 5. Here mean cell chisma frequency is listed for a series of classes of individual; no distinction is made between B chromosomes and segments, each being regarded as a 'piece of heterochromatin'. The overall one-tailed *t*-test shows that the mean cell chiasma

Table 4. Effect of supernumerary heterochromatin on mean cell chiasma frequency in the Omarama population of *Ph. marginale*

BB_{10} denotes basic homozygote for S_{10}, BS_{10} denotes structural heterozygote for S_{10}, SS_{10} denotes structural homozygote for S_{10}

Year	B chromosomes				Extra segment on S_{10}			Extra segment on S_9		
	None	1	2	3	BB_{10}	BS_{10}	SS_{10}	BB_9	BS_9	SS_9
1972	15·67		17·75	17·33	15·50	16·90	19·40	15·50	16·20	
1973	15·37	17·30	16·50		15·37	17·50	17·27	15·37	17·70	
1975	16·68	17·37	17·13	17·50	16·68	17·46		16·68	17·50	18·23

Table 5. Effect of supernumerary heterochromatin on chiasma frequency in *Ph. marginale*

One B chromosome or one segment is regarded as one piece of heterochromatin

Pieces of hetero-chromatin	1975 Mean ± s.d. (and range)	*n*	1973 Mean ± s.d. (and range)	*n*	1972 Mean ± s.d. (and range)	*n*
0	16·68 ± 0·40 (14·2–18·6)	10	15·37 ± 0·33 (14·7–15·7)	3	15·67 ± 0·20 (15·3–16·0)	3
1	17·32 ± 0·29 (15·7–20·0)	17	17·29 ± 0·49 (15·3–20·0)	9	16·67 ± 0·57 (16·0–17·8)	3
2	17·32 ± 0·26 (14·9–20·2)	29	17·18 ± 0·28 (15·8–18·7)	11	18·90 ± 0·91 (17·8–20·2)	3
3	17·54 ± 0·13 (15·1–20·3)	23	16·90 ± 0·90 (16·0–17·8)	2	16·78 ± 0·50 (15·5–17·8)	4
4	17·79 ± 0·32 (15·2–19·6)	17	16·95 ± 0·05 (16·9–17·0)	2	16·98 ± 0·71 (15·2–18·5)	4
5	16·75 ± 0·05 (16·7–16·8)	2				
6	18·80 ± 0·10 (18·7–18·9)	2				

Table 6. One-tailed *t*-tests for differences between means of heterochromatin-containing and standard karyotype individuals for the three samples (1972, 1973, 1975) from the Omarama population of *Ph. marginale*

d.f., Degrees of freedom

Year	Chiasma frequency (mean ± s.d.) Basic karyotype	Heterochromatin	*t*	d.f.	*P*
1975	16·68 ± 0·4041	17·49 ± 0·0416	1·94	98	0·05–0·025
1973	15·37 ± 0·4392	17·18 ± 0·2147	2·69	25	0·01–0·005
1972	15·67 ± 0·3432	17·26 ± 0·3188	1·82	15	0·05–0·025

frequency of individuals carrying extra heterochromatin is significantly higher than that of individuals with standard karyotypes ($t_{98} = 1·94$, $0·02 < P < 0·05$). The same is also true for the 1972 and 1973 data (see Table 6). In all three years the increase is of the plateau type seen with the B chromosomes of *Myrmeleotettix*

maculatus (John and Hewitt 1965), i.e. one piece of extra heterochromatin (B chromosome or segment) raises the chiasma frequency, subsequent pieces having little extra effect. There is thus no evidence for any additive or synergistic interaction of B chromosomes and segments on chiasma frequency when both are present in the same individual male.

Discussion

It has been suggested (Westerman 1975*a*) that the B^T chromosome of *Ph. marginale* and *Ph. vittatum* may have originated from the X chromosome. Recently a morphologically similar B^T chromosome has been found in another species, *Ph. nanum* (Westerman, unpublished observation). Subsequent to its origin, the B^T chromosomes may have evolved further in both *Ph. marginale* and *Ph. vittatum* by loss of material to give rise to the B^t type and by further misdivision of the centromere of a B^t to give the metacentric isochromosome B^M of *Ph. marginale*. More direct evidence for such a scheme is unavailable since use of the various chromosome-banding techniques has yielded little useful information. Thus although the B chromosome, like the X and the autosomes, has a proximal C band, no further morphological characteristics are revealed either by G banding or by fluorescence microscopy—B and X both fluoresce brightly along their whole length.

However, some support for such a scheme comes from two observations. Firstly, in one population of *Ph. vittatum* which is characterized by B^T chromosomes, occasional individuals which contain the B^t type are found. It is unlikely that these have migrated in from surrounding populations carrying B^t chromosomes as this type of B is rare in *Ph. vittatum* and has never been observed to be the only type of B chromosome found in any population. Secondly, the individual from Omarama described in the results above (B^M and B^t) may also be of importance in that the B^t chromosome could be interpreted as representing the original B^t chromosome from which the B^M was derived and which is still present in the population, albeit at very low frequencies (the B^M being the predominant type at Omarama). It is, however, also possible that this B^t chromosome is itself the product of misdivision of a B^M isochromosome since evolution of the B chromosome in *Phaulacridium* is unlikely to end with formation of the B^M. If this is so then further collections may reveal other morphological types of B chromosomes at Omarama. Active evolution of B chromosomes may well be continuing in *Phaulacridium*.

The data available from the Omarama population of *Ph. marginale* as well as from other populations suggest that the frequencies of all types of B chromosomes are stable over a number of years (0·66–0·79 B chromosomes per male at Omarama for 7 years). The same is generally true for the extra segments on chromosomes S_9, S_{10} and S_{11}. Robinson and Hewitt (1976) have shown that stable frequencies of B chromosomes in a population over long time periods may well mask a whole range of selective changes in B frequency within particular generations. Thus the B-containing individual in a population may be selected for at some times of the life history and selected against at others. These authors have shown that in the British grasshopper *Myrmeleotettix maculatus* there are three periods of differential selection for and against B chromosomes in the egg stage and through to early adult. Similarly, Rees and his colleagues have shown that in *Secale cereale* and *Lolium perenne*, B-containing individuals are at a selective advantage under some experimental conditions (e.g.

sowing densities) and at a disadvantage at others (Rees and Hutchinson 1973; Hutchinson 1975). The two species above differ in their response. More recently Teoh *et al.* (1976) have shown that not only is there a marked selective advantage of *Lolium* plants with B chromosomes under conditions of high sowing density but that this advantage changes with age of the population. It is therefore possible that B frequency equilibrium in natural populations could be promoted by selection operating for B chromosomes in one part of the habitat but operating against B chromosomes in other parts.

The findings of Westerman and Dempsey (1977) that B frequency in adult males of *Ph. vittatum* changes during the summer and that this change coincides with changes in mean daily temperature suggest that there may be some differential selection acting on B-containing individuals at the adult stage. Maximum B frequency in the population of *Ph. vittatum* coincides with the warmest driest conditions of the season and again lends support to the suggestion (Westerman 1975*b*) that extra heterochromatin in the genus *Phaulacridium* may be better tolerated in ecologically 'central' rather than in 'marginal' populations. The patterns of distribution of extra heterochromatin are similar in populations of both *Ph. marginale* (Westerman 1975*b*) and *Ph. vittatum* (Rowe and Westerman 1974; John and Freeman 1976).

In spite of the presence of extensive and stable polymorphisms for extra heterochromatin (B chromosomes and segments) in populations of *Ph. marginale*, there is still little evidence of what the possible selective advantage may be. Inspection and measurements on cadavers from all population samples have consistently failed to show any direct effect of B chromosomes or segments on any external morphological characters. Analysis of the 1973 data had suggested that the B chromosome frequency of populations of *Ph. marginale* is positively correlated with mean cell chiasma frequency ($r = 0{\cdot}413$, $0{\cdot}01 < P < 0{\cdot}05$). Within any one population sample, however, there was no evidence of a significant effect of either B chromosomes or extra segments on chiasma indices. This also appeared to be the case for B chromosomes in *Ph. vittatum* (John and Freeman 1974; Rowe and Westerman 1974). Westerman and Dempsey (1977) have shown, however, that in the La Trobe campus population in 19 samples out of 20 taken over a two-year period in which B chromosomes were present, the B-containing individuals consistently had higher recombination levels than those without B chromosomes. Thus B chromosomes do appear to significantly raise mean cell chiasma frequency in *Phaulacridium*. This finding reinforces the analysis of the Omarama data reported here which also suggests that extra heterochromatin in *Phaulacridium* is associated with an increase in chiasma frequency. The increase caused by one B or one segment is equivalent and additional material above this level causes no further rise. Whilst this effect of supernumerary heterochromatin in increasing the chiasma frequncy of individuals carrying it may not be as marked in the genus *Phaulacridium* as in some other organisms, e.g. *Myrmeleotettix maculatus* (John and Hewitt 1965), *Stethophyma* (Shaw 1971), *Secale cereale* (Zécévič and Paunovič 1969) and *Listera ovata* (Vosa and Barlow 1972), it is nonetheless significant and sufficient to suggest one possible adaptive role in natural populations.

This influence of extra heterochromatin on the endophenotype, i.e. its transchromosomal effect on chiasma frequency, is of no advantage *per se* to the individual carrying B chromosomes or segments. Rather, the advantage would be to the population, through the release of more variability in the next generation (Darlington

1956; Hewitt and John 1970). This greater variability amongst the offspring would lead to a greater potential of populations with B chromosomes or segments to accommodate changes in the environment or to exploit new niches in non-marginal areas of the species' range. Increased chiasma frequencies mediated by supernumerary heterochromatin could be expected to facilitate the break-up of co-adapted gene complexes not otherwise available for recombination. This may be a strong reason why such extra material is found mainly in ecologically non-marginal populations of many organisms (see Jones 1975).

In both *Ph. marginale* and *Ph. vittatum* the increased chiasma frequencies are due to the presence of extra crossovers in the long and medium bivalents. In these *Phaulacridium* species, as in other acridids (see Shaw and Knowles 1976 for references), cells with low chiasma frequencies tend to have the chiasmata of long and medium bivalents located largely in distal or proximal positions or both. The relative lack of interstitial chiasmata would tend to favour build-up of 'supergene' complexes particularly in the interstitial regions of bivalents. When chiasma frequency is raised by extra heterochromatin, this is usually the result of introduction of chiasmata into regions of bivalents not normally having them (for example the interstitial regions of L-bivalents) and this would then be expected to break up such gene complexes. It should be noted that increased chiasma frequencies do not depend solely on presence of B chromosomes or extra segments since many populations of 'small' individuals have very much higher chiasma frequencies than those of 'large' individuals (see Westerman 1974). In either case these extra chiasmata would be expected to lead to the release of some of the variability embodied in regions not normally recombined.

As Shaw and Knowles (1976) point out 'if there is a valid correlation between patterns of chiasma positions and genic recombination, then significant differences in patterns of chiasma frequency and distribution must represent different adaptive strategies to the production of genetic variability under different ecological regimes.' Rees and Dale (1974) have shown that in *Lolium* and *Festuca* populations there is a significant relationship between low variability of phenotypes and high chiasma frequencies. A similar relationship would appear to hold also for *Ph. marginale* where significent negative correlations have also been found between variance of some morphological characters and population chiasma frequencies (Westerman, unpublished data).

Ecologically non-marginal populations of *Ph. marginale* containing extra heterochromatin and having higher chiasma frequencies do appear to show a lower phenotypic variability than do more marginal ones. This lower variability may reflect a greater adaptation to the environment at the centre of the species' range and the fixation of more genotypes. It could equally reflect the less stringent selective forces acting in these non-marginal populations. It has been claimed that in ecologically more marginal environments the levels of genetic heterozygosity appear to be higher than in more central ones and perhaps remain higher as no particular genotypes are favoured for very long (Lewontin 1974). If this is true also for the genus *Phaulacridium* then it is perhaps surprising that the presence of extra heterochromatic material with its property of raising mean cell chiasma frequency, as well as other possible deleterious effects, can be tolerated only in ecologically non-marginal populations. Reductions in population heterozygosity caused by the break-up of interstitial gene complexes may well be selected against in more marginal environments.

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References

Darlington, C. D. (1956). 'Chromosome Botany'. (Allen and Unwin: London.)

Hewitt, G. M., and John, B. (1970). The B-chromosome system of *Myrmeleotettix maculatus* (Thumb). IV. The dynamics. *Evolution* **24**, 169.

Hutchinson, J. (1975). Selection of B-chromosomes in *Secale cereale* and *Lolium perenne*. *Heredity* **34**, 39.

John, B., and Freeman, M. (1974). B-chromosome behaviour in *Phaulacridium vittatum*. *Chromosoma* **46**, 181.

John, B., and Freeman, M. (1976). The cytogenetic structure of Tasmanian populations of *Phaulacridium vittatum*. *Chromosoma* **53**, 283.

John, B., and Hewitt, G. M. (1965). The B-chromosome system of *Myrmeleotettix maculatus* (Thumb). II. The statics. *Chromosoma* **17**, 121.

Jones, R. M. (1975). B-chromosome systems in flowering plants and animal species. *Int. Rev. Cytol.* **40**, 1.

Lewontin, R. C. (1974). 'The Genetic Basis of Evolutionary Change'. (Columbia University Press: New York.)

Martin, J. M. (1970*a*). The cytogenetics of some New Zealand grasshoppers (Acrididae). M.Sc. Thesis, University of Wellington, Victoria, N.Z.

Martin, J. M. (1970*b*). The use of grasshopper chromosomes to demonstrate meiosis. *Tuatara* **18**, 1.

Rees, H., and Dale, P. J. (1974). Chisamata and variability in *Lolium* and *Festuca* populations. *Chromosoma* **47**, 335.

Rees, H., and Hutchinson, J. (1973). Nuclear DNA variation due to B-chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **38**, 175.

Robinson, P. M., and Hewitt, G. M. (1976). Annual cycles in the incidence of B-chromosomes in the grasshopper *Myrmeloetettix maculatus* (Acrididae : Orthoptera). *Heredity* **36**, 399.

Rowe, H. J., and Westerman, M. (1974). Population cytology of the genus *Phaulacridium*. I. *Ph. vittatum*—Australian mainland populations. *Chromosoma* **46**, 197.

Shaw, D. D. (1971). The supernumerary segment system of *Stethophyma*. II. Heterochromatin polymorphism and chiasma variation. *Chromosoma* **34**, 19.

Shaw, D. D., and Knowles, G. R. (1976). Comparative chiasma analyses using a computerized optical digitiser. *Chromosoma* **59**, 103.

Teoh, S. B., Rees, H., and Hutchinson, J. (1976). B-chromosome selection in *Lolium*. *Heredity* **37**, 207.

Vosa, C. G., and Barlow, P. W. (1972). Meiosis in *Listera ovata* (Orchidaceae). *Caryologia* **25**, 1.

Westerman, M. 1974. Population cytology of the genus *Phaulacridium*. II. *Ph. marginale* (Walker)—chiasma frequency studies from South Island, N.Z. *Chromosoma* **46**, 207.

Westerman, M. (1975*a*). Population cytology of the genus *Phaulacridium*. III. *Ph. marginale* (Walker)— polymorphisms for extra heterochromatin. *Heredity* **34**, 11.

Westerman, M. (1975*b*). Population cytology of the genus *Phaulacridium*. IV. *Ph. marginale* (Walker)—the North Island populations. *Heredity* **35**, 165.

Westerman, M., and Dempsey, J. (1977). Population cytology of the genus *Phaulacridium*. VI. Seasonal changes in the frequency of the B chromosome in a population of *Ph. vittatum*. *Aust. J. Biol. Sci.* **30**, 329.

Westerman, M., and Fontana, P. G. (1973). Polymorphisms for extra heterochromatin in *Phaulacridium marginale*. *Heredity* **31**, 223.

Zécévič, L., and Paunovič, D. (1969). The effect of B-chromosomes on chiasma frequency in wild populations of rye. *Chromosoma* **27**, 198.

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