POPULATION CYTOLOGY OF THE GENUS *PHAULACRIDIDUM*

IV. *PHAULACRIDIDUM MARGINALE* (WALKER)— THE NORTH ISLAND POPULATIONS

M. WESTERMAN

*Department of Genetics and Human Variation, La Trobe University, Bundoora,
Victoria, 3083, Australia*

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SUMMARY

Samples of *Phaulacridium marginale* were taken from the North Island of New Zealand, together with further samples from three South Island populations. Seven of the North Island populations were found to be polymorphic for a small telocentric B chromosome. In only one case was the B chromosome significantly associated with an increased chiasma frequency. All three South Island populations contained B chromosomes. Seven of the nine N.I. populations were also polymorphic for extra segments on the centric end of the S₁₁ chromosome. In six of them this polymorphism co-existed with that for B's. A new polymorphism was encountered in two populations. This was for a large block of heterochromatic material located at the centric end of one of the smaller medium-sized chromosomes which thus becomes acro- rather than telocentric.

It would appear that the New Zealand grasshopper, *Ph. marginale*, is able to tolerate many different polymorphisms for extra heterochromatin. However, this ability is perhaps restricted to central populations. In this context, the high level of occurrence of these polymorphisms in the North Island could be due to the better climatological conditions existing there compared to most of the South Island.

1. INTRODUCTION

POPULATIONS of the New Zealand grasshopper *Phaulacridium marginale* are known to be polymorphic for both B chromosomes and for supernumerary segments on the smaller members of the chromosome complement (Martin, 1970; Westerman and Fontana, 1973; Westerman, 1974, 1975). Seven of 32 populations sampled from South Island over a 3-year period have been found to contain B chromosomes, the frequency of male B carriers in the population varying from 3 to 50 per cent. These frequencies appear to be stable from year to year. Many of the same populations are also polymorphic for supernumerary heterochromatic segments (Westerman, *loc. cit.*). The present paper extends the study of *Ph. marginale* populations to the North Island of New Zealand.

2. MATERIALS AND METHODS

During the 1973-74 meiotic season, population samples were taken from nine different sites on North Island (N.I.) (see fig. 1 and table 1) as well as from three previously sampled South Island (S.I.) sites, Lindis Creek (LC), Otematata (OT) and Areoa Station (AO). Since previous collections have suggested the existence of two morphologically distinct types of individuals on S.I. (Westerman, 1974), all bodies were retained for examination following removal of testes. These were fixed in acetic alcohol (1 : 3) for subsequent cytological investigation.

FIG. 1.—Map of North Island, New Zealand, showing locations of the nine populations sampled. (Land over 1000 m is shaded.)

TABLE 1

Summary of data from the 12 populations sampled in 1974.

The designations of the possible karyotype classes of the S_{11} chromosome are as follows: BB_{11} —basic homozygote; BS_{11} —heterozygous S_{11} ; SS_{11} —structurally homozygous S_{11} .

Population	N	Xa	Var.	OB	1B	2B	qB†	B-type	BB_{11}	BS_{11}	SS_{11}	q_{11} *	Altitude (m)
(a) North Island													
1. South Makara	24	15.75	1.3870	19	4	1	0.125	small telo	23	1	0	0.021	200
2. Pukerua Bay	24	15.31	1.2607	21	3	0	0.063	small telo	23	1	0	0.021	300
3. Kaiparoro	24	15.29	1.4676	24	0	0	0.000	small telo	23	1	0	0.021	200
4. Kuripapango	24	15.62	1.4079	23	1	0	0.021	small telo	24	0	0	0.000	800
5. Gentle Annie	21	16.09	1.8517	21	0	0	0.000	small telo	21	0	0	0.000	1000
6. Urewera	26	15.39	1.3314	22	4	0	0.077	small telo	25	1	0	0.019	800
7. Nuhaka	27	15.58	1.2494	24	3	0	0.056	small telo	23	4	0	0.074	50
8. Sandys Bridge	22	16.05	1.3333	18	3	1	0.114	small telo	21	1	0	0.023	500
9. Black Jack	22	14.97	1.7258	11	7	4	0.341	small telo	20	2	0	0.046	60
(b) South Island													
Lindis Creek	20	17.63	1.7860	18	2	0	0.050	large telo	20	0	0	0.000	915
Orematata	18	16.95	1.8076	15	1	0	0.031	small telo	16	0	0	0.000	915
Areoa Station	33**	15.48	1.1720	26	7	0	0.106	small telo	32	1	0	0.015	180

** One individual heterozygous for segment on S_{10} , three individuals heterozygous for segments on M.

* q_{11} = frequency with which an individual carries an extra segment on S_{11} .

† qB = frequency with which an individual carries a B chromosome.

3. RESULTS AND DISCUSSION

Morphologically all individuals from the nine N.I. populations sampled, as well as those from AO were of the same type and corresponded to the "larger" individuals from S.I. (see Westerman, 1974). The 1974 samples from LC and OT were still wholly of the "small" type of individual. The data available therefore suggest that the "small" individuals of *Ph. marginale* are restricted to the Mackenzie Plains-Cromwell region of South Island. Transects of two areas in this region where populations of "large" and "small" individuals are found close together will be completed in the coming season to ascertain the degree of spatial separation between them.

Seven of the N.I. populations had heterochromatic B chromosomes in from 4 to 50 per cent of the males sampled ($q_B = 0.02-0.34$, see table 1). In every case the B chromosome was of the same morphological type—a small telocentric element about one-third of the size of the X chromosome. The X is itself telocentric and is the second largest member of the complement of *Ph. marginale*. Like the X chromosome of the related species *Ph. vittatum* (Westerman, unpublished observation), the heterochromatic X of *Ph. marginale* is often seen to have a small, lighter staining region close to the centromere (plate I, a). A similar constriction is often seen on the B chromosome (see plate 2, e, Westerman, 1975).

The meiotic behaviour of the B chromosomes was identical to that already described for the S.I. populations (see Westerman, 1975), the segregation of the B at anaphase I being at random relative to the X. The B chromosome of the AO population, as in the 1973 collection, was also a "small telocentric" type. This was true also of the B found in the OT population and recorded here for the first time. The B chromosome of the LC population was still of the "large telocentric" type recorded in 1973. Thus, like the large B chromosome of *Ph. vittatum*, that of *Ph. marginale* is stable. As has been previously argued, the large B chromosomes of *Ph. marginale* (and of *Ph. vittatum*) may well have arisen by non-disjunction from the X chromosome (see Westerman, 1975). These large telocentric B's may subsequently evolve into the "small telocentric" type by loss of distally located chromatin, and then into metacentric isochromosomes by centromeric misdivision. Although the origin of B's in this way may be frequent and even geologically recent events, the widespread occurrence of the small telocentric "derived" B chromosomes indicates that the polymorphism is indeed quite old unless the evolutionary step from "large" to "small" telocentric type occurs rapidly following inception of the B. To date no populations of *Ph. marginale* have been encountered in which more than one type of B chromosome has been found. Although this could perhaps be related to the relatively small sample sizes used in the study (approx. 25 males), it is of interest to note that in a similar sized sample taken of one population of the related species *Ph. vittatum*, males have been observed containing B's of both sorts (Westerman, unpublished observation).

In the six N.I. populations in which more than one B carrier was found, analysis of the chiasma frequency data showed that in only one case (SB) was the B chromosome significantly associated with high mean cell chiasma frequency. The effect of the B chromosome in this population was to raise the mean cell chiasma frequency by 1.75 chiasmata. No effect on mean

Plate I

The meiotic behaviour of a supernumerary heterochromatic segment in *Ph. marginale*.

- a Normal early diplotene cell showing proximal constriction on the X chromosome.
- b Diplotene cell showing the extra segment on a medium-sized chromosome.
- c Random orientation of M segment and X at Metaphase I. Note relatively uncoiled nature of the segment at this stage.
- d Diakinesis-MI showing similarity of coiling cycle of X and M segment. Note also relatively uncoiled nature of centromere on the other M homologue (arrow).
- e Segregation of M segment relative to X and B chromosomes.
- f Metaphase I showing "short arms" (arrows) on one of the M bivalents.
- g, h g, Anaphase I and h, Metaphase II cells showing acrocentric nature of the segment bearing M chromosome (line = 10 μ).

log variance was observed. These results are in agreement with the S.I. data (Westerman, 1975).

Seven of the N.I. populations were also found to be polymorphic for supernumerary heterochromatic segments on the S_{11} chromosome, six of them being simultaneously polymorphic for the B's described above. The extra segment was located at the centric end of the S_{11} chromosome, making it sub-metacentric. In no case was there any significant deviation of observed karyotype classes from Hardy-Weinberg expectations, and the frequency of segments (q_{11}) varied from 0.02 to 0.07 (see table 1). These low frequencies together with the lack of significant effect of S_{11} heterochromatin on chiasma frequency are in agreement with those recorded from S.I. populations (see tables 3, 5 and 6, Westerman, 1975).

A new polymorphism was encountered in two populations (South Makara and Areoa Station). This consisted of an extra block of heterochromatic material located at the proximal end of one of the smaller medium-sized chromosomes making it acro- rather than telocentric. In all five individuals (two from SM and three from AO) containing the extra M segment, this was present in all germ-line cells and in early prophase was visible as a positively heterochromatic body at the centric end of one of the medium-sized chromosomes (see Plate I, b). The bivalent carrying the extra segment was therefore "unequal" in that there was from four to five times more heterochromatin at the centric end of one homologue as compared with the other. The acrocentric nature of the segment bearing chromosome could be clearly seen at anaphase I and metaphase II (see plate I, g, h).

In spite of the heterochromatic nature of the extra material and the similarity of its coiling cycle to that of the X, no association was seen at any stage between this segment and the X chromosome. One of the two individuals from South Makara also contained a heterochromatic B chromosome in all germ-line cells. Once again there was no association between the segment and this B chromosome. This behaviour is also true of a heterochromatic supernumerary segment (K_{10}) and a B chromosome in Maize (Snope, 1967). With respect to the rest of the complement, the M segment appeared slightly negatively heterochromatic at late diakinesis and metaphase I, as did the X and B chromosomes (plate I, c). Indeed, it was observed that the centric end of the non-segment carrying homologue was also slightly under-condensed at the same time (plate I, d). The anaphase behaviour of the unequal bivalent was such that it divided reductionally at AI and equationally at AII. The extra segment appeared to segregate at random with respect to both the X chromosome (table 2, a) and the B chromosome (table 2, b, and plate I, c, e) at anaphase I.

The lack of association between this large heterochromatic segment and either the X or B chromosomes suggests that it has not arisen as a result of a translocation between one of these and an autosome (see also Snope, 1967; John, 1973). Rather, like the segments on the S_9 , S_{10} and S_{11} chromosomes of *Ph. marginale* and on the S_{11} of *Ph. vittatum* (Rowe and Westerman, 1974), this extra material has most probably arisen by tandem duplication of pre-existing material. This could be at the distal end of the chromosome (S_9 and S_{10}) or at the centric end (S_{11} and M chromosome). The latter region is known in many organisms to be rich in highly repeated sequence DNA (Pardue and Gall, 1972; Gall *et al.*, 1973; Hearst *et al.*, 1973)—material which often appears to increase in a saltatory fashion. Some

highly repeated sequences may also be located at the distal ends of chromosomes (Rae, 1972; Brown and Wilmore, 1974). The centric region of the chromosomes of *Ph. marginale* are also positively heterochromatic during early meiosis. This may explain the coiling behaviour of the S_{11} and M segments which occur in this region. That the M segments (and S_{11} segments) appear as short arms suggests that the previous classification of

TABLE 2

Segregation behaviour of the segment on the M chromosome at Anaphase I with respect to the X and B chromosomes in two individuals from the South Makara population

(a) *Segregation with respect to X chromosome*

Individual 16	X+segment : 0	X : segment
Observed No. of cells	73	65

$$\chi^2_1 \leq 1.0 \text{ n.s.}$$

(b) *Segregation behaviour with respect to X and B chromosomes*

Individual 4	B+X : seg.	B : seg.+X	B+seg. : X	B+seg.+X : 0
Observed No. of cells	19	16	13	16

$$\chi^2_3 = 1.1250 \text{ n.s.}$$

these chromosomes as strict telocentrics may in fact be incorrect. Occasionally in non-segment-bearing individuals one of the smaller M chromosomes is seen to possess what may well be "short" arms (see plate I, f). This is true both of *Ph. marginale* and *Ph. vittatum*; however, further work is needed to clarify the nature of these chromosomes and their centromeres.

A summary of the known polymorphisms for supernumerary heterochromatin in populations of *Phaulacridium marginale* is given in table 3. Although the population data available from North Island are to date less extensive than that from South Island, it would appear that polymorphisms for extra heterochromatin are more common in the former. Thus eight out of nine populations from N.I. carry them, compared with 11 out of 32 from S.I. The difference between the two islands may be even more marked when cognisance is taken of the fact that in South Island, with the exception of the SU and AJ populations, all others known to be polymorphic for extra heterochromatin are to be found in two regions—the Mackenzie Plains–Cromwell area and the Awatere Valley. The climate in these regions, characterised by warmer summers and a low annual rainfall, is similar to that experienced by many N.I. populations and may represent ecologically less marginal environments.

Phaulacridium marginale is found throughout New Zealand below 1000 m and may therefore be intolerant of excessive cold. The species' tolerance of excess moisture may well be greater as populations are found in regions of very high annual rainfall, e.g. Milford Sound has more than 6400 mm rainfall per year. The pattern of distribution of the extra material suggests that it can be better tolerated in "central" than in "marginal" populations in the sense of ecological rather than geographical marginality.

Mayr (1963) notes that studies on phenotypically polymorphic species "reveal almost invariably that the degree of polymorphism decreases towards the border of the species and that the peripheral populations are

TABLE 3

The polymorphisms for supernumerary heterochromatin encountered in populations of Phaulacrididium marginale

Type of heterochromatin	Population	Frequency of carriers in population		
		1972	1973	1974
I. <i>B chromosomes</i>				
(a) <i>Telocentric</i>				
(i) Similar in size to X	Lindis Creek ¹	—	0.09	0.05
(ii) Half size of X	Tekapo Pukaki ¹	—	0.27	—
	Otematata ¹	—	—	0.03
	Sumner ¹	—	0.42	—
	Awatere ¹	—	0.17	—
	Areoa Station ¹	—	0.03	0.11
	South Makara ²	—	—	0.13
	Pukerua Bay ²	—	—	0.06
	Kuripapango ²	—	—	0.02
	Urewera ²	—	—	0.08
	Nuhaka ²	—	—	0.06
	Sandys Bridge ²	—	—	0.11
	Black Jack ²	—	—	0.34
(b) <i>Metacentric</i>				
(i) Iso-chromosome	Omarama ¹	0.26	0.33	—
II. <i>Supernumerary segments on—</i>				
(a) Medium chromosome	Areoa Station ¹	—	—	0.05
	South Makara ²	—	—	0.04
(b) S ₉	Omarama ¹	0.21	0.17	—
	Skippers Saddle ¹	—	0.02	—
(c) S ₁₀	Omarama ¹	0.41	0.32	—
	Lake Pukaki ¹	—	0.02	—
	Areoa Station ¹	—	—	0.02
(d) S ₁₁	Omarama ¹	0.06	0.04	—
	Tekapo Pukaki ¹	—	0.03	—
	Lake Ohau ¹	—	0.03	—
	Skippers Saddle ¹	—	0.10	—
	Areoa Station ¹	—	0.03	—
	Ariki Junction ¹	—	0.05	—
	South Makara ²	—	—	0.02
	Pukerua Bay ²	—	—	0.02
	Kaiparoro ²	—	—	0.02
	Urewera ²	—	—	0.02
	Nuhaka ²	—	—	0.07
	Sandys Bridge ²	—	—	0.02
	Black Jack ²	—	—	0.05

¹ South Island.

² North Island.

not infrequently monomorphic". The accuracy of this generalization is improved, as Lewontin (1974) points out, if a distinction is made between geographically peripheral and ecologically marginal populations. As with the European grasshopper *Myrmeleotettix maculatus*, where "the selective forces acting differentially upon supernumeraries . . . are dependent upon the overall level of stringency or favorability of the environment" (Hewitt and Brown, 1970; see also Hewitt and Ruscoe, 1971), so too in *Ph. marginale* there is a general correlation of B chromosomes (and extra segments) with

local environmental conditions. The North Island, in those regions which remain relatively undisturbed by man's activities, may be regarded as climatologically more favourable for grasshoppers. Although populations of *Ph. marginale* occur throughout South Island and even on Stewart Island, these habitats can be regarded as ecologically more marginal because of their higher latitude. The area inhabited by a species is however generally a mosaic of favourable and unfavourable habitats. The Mackenzie Plains and Awatere Valley areas of South Island can be regarded as being less unfavourable and therefore less marginal.

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4. REFERENCES

- BROWN, A. K., AND WILMORE, P. J. 1974. Localization of repetitious DNA in the chromosomes of the Desert Locust (*Schistocerca gregaria*). *Chromosoma (Berl.)*, 47, 379-383.
- GALL, J. G., COHEN, E. H., AND ATHERTON, D. D. 1973. The satellite DNA's of *Drosophila virilis*. *C.S.H.S.Q.B.*, 38, 417-421.
- HEARST, J. E., CECI, T. R., MARK, K., ROSENFELD, A., AND ALLEN, J. R. 1973. Characterization of the rapidly renaturing sequences in the main CsCl density bands of *Drosophila*, Mouse and Human DNA. *C.S.H.S.Q.B.*, 38, 329-331.
- HEWITT, G. M., AND BROWN, F. M. 1970. The B-chromosome system of *Myrmeleotettix maculatus*, V. A steep cline in East Anglia. *Heredity, Lond.*, 25, 363-371.
- HEWITT, G. M., AND RUSCOE, C. 1971. Changes in microclimate correlated with a cline for B-chromosomes in the grasshopper *Myrmeleotettix maculatus* (Thunb.) (Orthoptera: Acrididae). *J. Anim. Ecol.*, 40, 753-765.
- JOHN, B. 1973. The cytogenetic systems of grasshoppers and locusts, II. The origin and evolution of supernumerary segments. *Chromosoma (Berl.)*, 44, 123-146.
- LEWONTIN, R. C. 1974. *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York and London.
- MARTIN, J. 1970. The cytogenetics of some New Zealand grasshoppers (Acrididae). M.Sc. Thesis, Victoria University, Wellington.
- MAYR, E. 1963. *Animal Species and Evolution*. Harvard, Belknap Press, Cambridge, Mass.
- PARDUE, M. L., AND GALL, J. G. 1972. Molecular genetics. In *Molecular Genetics and Developmental Biology*: Symp. Soc. Gen. Physiol., Woods Hole, Mass. 1971, ed. M. Sussmann. Prentice Hall.
- RAE, P. M. M. 1972. The distribution of repetitive DNA sequences in chromosomes. In *Advances in Cell and Molecular Biology*, 2, 109-150.
- ROWE, H. J., AND WESTERMAN, M. 1974. Population cytology of the genus *Phaulacridium*, I. *Phaulacridium vittatum* (Sjöst)—Australian mainland populations. *Chromosoma (Berl.)*, 46, 197-205.
- SNOPE, A. J. 1967. The relationship of abnormal chromosome 10 to B-chromosomes in Maize. *Chromosoma (Berl.)*, 21, 243-249.
- WESTERMAN, M. 1974. Population cytology of the genus *Phaulacridium*, II. *Phaulacridium marginale* (Walker); Chiasma frequency studies from South Island, New Zealand. *Chromosoma (Berl.)*, 46, 207-216.
- WESTERMAN, M. 1975. Population cytology of the genus *Phaulacridium*, III. *Phaulacridium marginale* (Walker); Polymorphisms for extra heterochromatin. *Heredity*, 34, 11-28.
- WESTERMAN, M., AND FONTANA, P. G. 1973. Polymorphisms for extra heterochromatin in *Phaulacridium marginale*. *Heredity*, 31, 223-229.