THE B-CHROMOSOME SYSTEM OF MYRMELEOTETTIX MACULATUS

V. A STEEP CLINE IN EAST ANGLIA

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1. INTRODUCTION

IN British populations of the mottled grasshopper *M. maculatus*, the frequency of individuals containing one or more supernumerary chromosomes is generally correlated with latitude; more northerly populations do not possess the B-chromosome while southerly ones do. There are, however, areas in Southern Britain where some populations do not contain B-chromosomes, and one area that has been studied in some detail is in West Wales near Aberystwyth. Here the frequency of B-chromosomes is negatively correlated with rainfall; the ecologically marginal populations in West Plynlimon mountains do not contain B-chromosomes, but the frequency of the supernumerary increases as one proceeds eastward and westward into areas of lower rainfall (Hewitt and John, 1967). Three of the 5 populations previously examined from East Anglia do not have B-chromosomes (John and Hewitt, 1965) and, since the area is flat and has an even, low rainfall 22-27 inches per annum they present an interesting anomaly which merits further investigation.

In all the populations so far examined the chiasma frequency and its variance in B-containing individuals are significantly elevated above that of its non-B-containing fellows in the populations (John and Hewitt, Hewitt and John, *loc. cit.*). Such an increase in the number of chiasmata is an increase in the recombination potential of that individual and of the population. Consequently the East Anglian populations require examination from this aspect also; particularly since recent work with rye has shown that B-chromosomes can raise the chiasma frequency and variance (Zečević and Paunović, 1969; Jones and Rees, 1967) and also the phenotypic variation in the ensuing generations (Moss, 1966).

2. COLLECTIONS, MATERIALS AND METHODS

During the summer of 1967 and 1968 we extended the collections to include some 27 populations samples of *M. maculatus* from all over East Anglia (see fig. 1 and table 1). The species is found mainly where there is heath-land with many small areas of sandy soil exposed, such as the large Breckland system around Thetford and the considerable coastal strip of East Suffolk, as well as smaller areas in north and central Norfolk. It also occurs in large sand-dune systems which provide a somewhat similar physical environment. All of these major areas have been searched and examined; the rest of the region is predominantly intensively farmed arable land, which accounts for the absence of population samples from certain areas. Between 20 and 25 males were taken from most populations, the testes were dissected

out in saline and fixed in 3 : 1 Alcohol-Acetic. Subsequently squash preparations were made using 45 per cent. lactopropionic orcein. These were examined for B-chromosomes and the chiasma frequency was scored from 10 individuals in each population. The number of B-containing individuals scored for chiasmata was in proportion to the frequency of B-chromosomes in the population. Ten cells at diplotene were scored for chiasma frequency for each individual.

FIG. 1.—A map of East Anglia showing the location of the populations examined and the frequency of B-containing individuals in each population.

Seven of the populations were sampled for two years consecutively and, three of these for a third year (1969), to determine if the B-chromosomes frequencies were stable from year to year.

3. RESULTS

The B-frequencies and chiasma frequencies are presented in table 1. There was no significant difference between years in the seven populations, the figures for these populations are therefore averages and are noted by an

asterisk. All the B-chromosomes were of the mediocentric type (B^m) thus confirming that the submetacentric type is restricted to a more south westerly distribution. They were stable in their behaviour, so that all cells in the testes contained the same number of B-chromosomes (John and Hewitt, 1965). In four of the populations (nos. 17, 18, 20, 21) the frequency of supernumeraries was high enough to provide sufficient individuals with and without B's to allow a comparison of the two classes for chiasma frequency.

TABLE 1

*The location, B-chromosome frequency and mean chiasma frequency of the East Anglian populations. An * indicates that the figure is the mean of 2 years, and ** indicates the mean of 3 years. Brackets () indicate that the sample was small and the exact figure is not reliable*

Population	Grid (O.S. 1" maps)	B-chromosomes %	Population chiasma frequency
1. Kelling Heath	094407	0	—
2. Edgefield Heath	085368	0	14.97
3. Ling Common	655239	37.5	15.14
4. Gt. Massingham Heath	774219	7	14.19
5. Marsham Heath	172234	0	14.30
6. Horsford Heath	187178	0*	14.67
7. Mousehold Heath	243101	0	14.35
8. Horsey Gap	462238	0	14.42
9. Cockley Cley	793044	(40)	—
10. Hopton House	861986	0	13.86
11. Buckenham Tofts	848957	25	15.31
12. Stanford Warren	863938	20	15.10
13. Breckles Heath	932943	0*	14.59
14. Wretham Heath	908883	27	13.60
15. Bridgham Heath	935867	0	—
16. Hargham Heath	032926	18	14.37
17. Lakenheath	746807	45*	14.60
18. Foxhole Warren	735775	46**	14.68
19. Monument	787776	44	—
20. Thetford Heath	844787	50*	14.94
21. Rushford Heath	944809	29**	14.38
22. Knettishall Heath	958805	10	15.23
23. Wortham Ling	088798	0	—
24. Lumpfall Walks	453735	0	—
25. Westleton Heath	452694	0**	—
26. Snape Warren	405580	0	—
27. Sutton Common	312485	0	15.23

The average chiasma frequency of the B-containing members of a population was higher in all cases than that of their compatriots without B-chromosomes (table 2). There was, however, no correlation between the chiasma frequency and the B-frequency of the populations. Nor was there any correlation between the chiasma frequency and the location of the population, be it a Northerly to Southerly comparison or a central to marginal comparison. But there is a distinct relationship between the location of the population and its B-chromosome frequency (fig. 1). In the north and east of the region the populations are devoid of supernumeraries while in the south and west the populations contain 40-50 per cent. of the individuals with B-chromosomes as do the populations of the adjoining Midlands and Southern England.

As has already been mentioned high B-chromosome frequency is correlated in Britain with sunny, dry and thus more favourable habitats for

grasshoppers, and at first sight it is difficult to explain this East Anglian distribution. There is no land over 300 feet, the rainfall is uniformly low and the insolation over the whole area is high. In order to investigate this more thoroughly the gross climate of the region was obtained from the Climatological Atlas of the British Isles (H.M.S.O.) and from consultation with the Meteorological Officers at Mildenhall and Bracknell (to whom we are most grateful). Careful comparison of this gross climate and B-chromosome distribution indicates that there is little correlation between the winter climate and the distribution of B-chromosomes in East Anglia. It is a very different matter with summer climates, however, here the mean daily maximum temperature, mean daily range of temperature mean relative humidity, mean saturation deficit all appear to have some correlation, with the distribution of the B-chromosomes. This is not the case with the mean rainfall and mean daily duration of bright sunshine since these are uniform

TABLE 2

A comparison of the chiasma frequency of non-B and plus-B individuals in four populations where the B-frequency was high enough to give sufficient numbers to make the comparison possible. Five +B and five -B individuals were scored in each population

Population	B %	$\bar{X}a$ frequency		P. "t" test
		-B	+B	
17	45	14.18	14.58	0.1-0.05
18	44	14.34	15.10	0.002-0.001
20	40	14.04	15.08	0.001
21	25	14.02	15.42	0.001

for the region. Thus it appears that temperature is the main correlative factor (R.H. and S.D. both being dependent on temperature) and this is illustrated by the isotherms for the average means of daily maximum temperature for June (1901-30) (fig. 2).

Mr H. H. Lamb of the Meteorology Office informs us that the distribution of isotherms in the region is due to the coastal winds blowing off the sea during the spring and summer months and penetrating considerable distances inland. These are usually cooler than the south westerly winds which predominate over the west and centre of the region, and bring with them sea fog and low cloud particularly in early summer. The insolation and temperature toward the coast is thus considerably lower than in land. The inland populations will consequently enjoy less stringent conditions than the more marginal ones.

The absence of B-chromosomes from these more marginal populations is thus in agreement with the general situation in Britain and in particular with the detailed investigation around Aberystwyth (Hewitt and John, 1967). A similar argument may apply to the Cornish and Welsh peninsulas.

In addition there is a quite sudden change from populations with a high supernumerary frequency to those with none at all. This is particularly marked in the Thetford area, where the B-chromosomes frequency changes from around 40 per cent. to 0 per cent. in as little as 2 miles to 5 miles. Over the rest of its distribution the B-chromosome is present at frequencies around 40 per cent. to 50 per cent. most commonly. This "Quantum" jump is also apparent in the Aberystwyth populations, where the B-frequency

changes from around 40 per cent. to 0 per cent. in a few miles, but the sudden change in frequency is more remarkable in East Anglia because of the relatively uniform terrain, while in Wales the cline is up the western slopes of the Plynlimon mountains.

FIG. 2.—The isotherms for average mean daily maximum temperature for June (°F).

4. DISCUSSION

No single climatic factor is responsible for the distribution of B-chromosomes in Britain, rather the selective forces acting differentially on the supernumeraries in marginal and in more central environments are dependent upon the overall level of stringency or favourability of the environment. In Wales it is cloud and rainfall which are the predominant factors, in East Anglia it is onshore winds, coastal cloud and summer temperature.

A similar general situation has been noted in three plant species, *Festuca pratensis* (Bösemark, 1965), *Centaurea scabiosa* (Fröst, 1958) and *Phleum phleoides* (Bösemark, 1967), which have been studied extensively in Sweden

and neighbouring regions. In each case the B-chromosomes are most frequent in the populations which are living in the most favourable habitats

FIG. 3.—The overall distribution of B-chromosomes in British Populations.

for the species. Such "favourability" has been expressed in terms of clay content of the soil (*F. pratensis*), the low relative humidity of a continental climate (*C. scabiosa*), or the temperature and drainage afforded by light calcareous soils (*P. phleoides*). It may be that the distribution of B-chromo-

somes in rye (*Secale cereale*), also fits into this pattern, for commercial varieties in Europe possess few or no B-chromosomes, whilst in primitive strains from Asia they are very common (Müntzing, 1954). In all these studies the B-chromosome is most frequent in central populations, and not in marginal populations as Darlington (1963) suggested might be the case. Any increase in variation that they introduce, therefore, is for these central populations and not available in the marginal ones. It may be that the marginal populations are more prone to "bottle-necking" by more frequent reductions to a small number of individuals and consequently the B-chromosomes may be lost more readily by chance. This type of situation has been discussed in *Clarkia* for chromosomal interchanges (Wedberg, Lewis and Venkatesh, 1968).

The discovery of a sudden change over a few miles from the high B-chromosome frequency which exists in populations in southern Britain to the zero B-chromosome frequency in East Anglia (fig. 1) is of considerable interest. Several other workers have examined and discussed steep clines for genetically controlled characters. In the butterfly, *Maniola jurtina* the distribution of hindwing spot frequencies changes abruptly from East Cornish to Southern English stabilisations over the width of a hedge (Creed *et al.*, 1959). An equally sudden change is seen in *Agrostis tenuis* (and other grasses) for heavy metal tolerance across the boundaries of spoil heaps from metal mines in Wales. Here the interaction of gene flow and selection for tolerance have been elegantly investigated (Jain and Bradshaw, 1966). Thirdly, a melanic form of the moth, *Amanthes glareosa* occurs in a cline through Shetland which is quite steep in parts. However, the Tingwall valley which is the most obvious block to gene flow does not coincide with the steepest part of the cline (Kettlewell and Berry, 1969). Interestingly populations of *Amanthes* from different parts of the island are locally adapted in terms of their flight activity (Kettlewell *et al.*, 1969). Whilst these situations are very different, they all demonstrate the complex interaction of selection and dispersal, and a number of processes can exist which may be interacting to produce such a steep cline for B-chromosomes in *M. maculatus*.

- (i) The most obvious possibility is that the presence and absence of B-chromosomes is the result of the various advantages and disadvantages that it confers on the species which are ultimately determined by climate. However, the climate gradient across the transition zone does not appear to be steep enough to be simply causing the change in B-frequency; this is apparent from the gross meteorological data (fig. 2) and also from our preliminary micro-meteorological records of five localities spanning the area. Consequently it is necessary to invoke a threshold effect or some third factor to explain this situation.
- (ii) One factor that could contribute to this steep cline is an accumulation mechanism. No such mechanism can be demonstrated in mitosis and meiosis in the male, and the female also has a somatically stable B-chromosome. However, it is possible that some form of preferential involvement of B-containing gametes in fertilisation could be occurring. Because of such a mechanism the frequency of B-chromosomes would be boosted to a high level in populations which possessed them.

Just why this level is 40 per cent. to 50 per cent. is probably due to detrimental effect of two or more B-chromosomes in an individual. Indeed in the rare 3B individuals which have been collected, there has been mitotic instability. Thus higher frequencies of B-chromosomes will reduce the population fitness.

- (iii) It is possible that the genetic architecture of the B and non-B populations is so different as to form a barrier to effective B-chromosome flow. For example, the progeny of a B-containing migrant and a non-B resident may have reduced fitness, and the steep cline in B-frequency may represent a "hybrid zone" between two races or sub-species.
- (iv) Whilst the frequency of B-chromosomes has been stable over the last 3 years in the populations examined it is worth considering the consequences of a slowly moving distribution. The B-chromosome distribution may be advancing into more marginal populations because the supernumerary is beneficial or has some intrinsic mechanism for relatively rapid increase when it is present in the population; but its progress is impeded by slow grasshopper dispersal between populations, or by the genetic inertia produced if the recipient non-B population genotype is not co-adapted with the B-chromosome. These situations will give a steep clinal front to the supernumerary advance. If the front is retreating, and this may be likely in view of our deteriorating climate (Lamb, 1965), then a combination of accumulation and population bottle-necking may furnish the explanation.

These possibilities for the distribution of B-chromosomes in *M. maculatus* are amenable to experimental testing and this is in hand.

5. SUMMARY

1. The B-chromosomes in East Anglian populations of the grasshopper, *Myrmeleotettix maculatus* raise the chiasma frequency, as has been reported for other areas of Britain.

2. Populations from the north and east of the region do not contain B-chromosomes while those from the south and west do.

3. This distribution is correlated with the cooling effects of on shore winds from the North Sea. It is argued that this maritime effect makes the localities nearer the coast ecologically more marginal than the central ones which enjoy a more continental climate.

4. This correlation between high B-frequency and favourability of the environment is found in other organisms and may be a general phenomenon.

5. The change from a frequency of 40 per cent. B-chromosome in populations to 0 per cent. B's takes place in a few miles, forming a very steep cline.

6. Possible mechanisms for the production and maintenance of this steep cline are discussed, and some testable hypotheses are advanced.

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