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THE CHROMOSOMAL COMPONENT OF REPRODUCTIVE ISOLATION IN THE GRASSHOPPER *CALEDIA CAPTIVA* III. CHIASMA DISTRIBUTION PATTERNS IN A NEW CHROMOSOMAL TAXON

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SUMMARY

An analysis of chiasma distribution patterns among two classes of F_1 hybrids produced by crossing a new chromosomal taxon, Lakes Entrance (LE), to both the Moreton (MAX) and Torresian (TT) taxa, has demonstrated that, when compared to their parental taxa, the (MAX × LE)F_1 hybrids have very different distribution patterns whereas the (LE × TT)F_1 hybrids have similar distribution patterns. Chiasmata in the Lakes Entrance and Torresian taxa, and their F_1 hybrids generally show proximal-distal patterns of localisation in five of the eight largest autosomes although some subtle statistical differences were detected between the F_1 hybrids and the parental taxa in those chromosomes. The highly significant differences in chiasma distribution patterns between the (MAX × LE)F_1 hybrids and their parental taxa in chromosomes 1, 2, 4, 5, 6 and 8 can be directly attributed to pericentric heterozygosity. In these cases most recombination is localised in the interstitial and distal regions of the chromosomes. Although pericentric heterozygosity would be expected to result in a reduced mean cell chiasma frequency, the (MAX × LE)F_1 hybrids have the same mean cell chiasma frequency as both the MAX and TT taxa and the (LE × TT)F_1 hybrids. This appears to be due to the presence of exchanges (scored as chiasmata) within the pericentric re-arrangement region. The data strongly suggest that these exchanges are U-type following straight non-homologous pairing at pachytene rather than the result of crossing over following homologous pairing within an inversion loop. In gross stained meiotic material U-type exchanges were in 15·5 per cent of cells scored. The analysis of chiasma distribution in the F_1 hybrids from crosses between the chromosomally divergent but genically equivalent MAX and LE taxa provides further substantive evidence that the dramatic change in the pattern of recombination in chromosomally heterozygous F_1's disrupts intrachromosomal organisation resulting in the generation of recombinant progeny incapable of completing embryogenesis. In comparison the lack of any noticeable change in the recombination system in the F_1 hybrids from crosses between the genically divergent but chromosomally similar taxa, LE and TT, suggests that the F_2 inviability in this case is most likely a consequence of recombination between genically divergent genomes involving whole chromosome segregation rather than extensive intrachromosomal recombination.

1. INTRODUCTION

The taxonomic species *Caledia captiva* has undergone extensive chromosomal divergence resulting in at least four distinct chromosomal taxa which show various levels of inter taxon reproductive isolation (Daly *et al.*, 1981; Shaw and Wilkinson 1980). There are now many examples of speciation events which are associated with chromosome repatterning and a number of hypotheses have been presented to provide a causal role for chromosomal

re-arrangements in speciation (see Grant 1971; White 1978; Bush 1981; Charlesworth *et al.*, 1982). In the majority of these cases, chromosomal heterozygosity is considered to induce mechanical difficulties during meiosis resulting in reduced fertility and thus potential reproductive isolation. Alternatively Wilson (1975) proposed that chromosomal rearrangements may induce modifications to the regulation of gene expression which could result in the rapid generation of new morphological species. Recently Coates and Shaw (1982), Shaw *et al.* (1982), and Shaw and Coates (1983) have provided substantive empirical evidence that the change in the pattern of recombination which occurs in F_1 hybrids heterozygous for pericentric rearrangements is a major factor in generating post mating reproductive isolation between the Moreton and Torresian taxa of *Caledia captiva*. This evidence was derived primarily from hybridisation studies which demonstrated that even though F_1 hybrids were fully viable and fertile, the F_2 and backcross generations showed a reduction in viability of 100 per cent and 50 per cent respectively. Furthermore, meiosis in the F_1 hybrids generally showed normal bivalent formation (Moran 1980; Shaw and Wilkinson 1980; Coates and Shaw 1982) even though the two taxa are differentiated by at least seven pericentric rearrangements and a complex pattern of heterochromatic bands. The only noticeable effect of chromosomal heterozygosity upon meiosis was the redistribution of chiasmata. Crossing over was totally precluded within the pericentric rearrangement region, and as a consequence of this chiasmata were redistributed into interstitial regions where they normally occur at very low frequencies (Coates and Shaw 1982). Thus the majority of recombinant chromosomes in gametes derived from F_1 parents were novel in terms of their intrachromosomal organisation. In addition, most recombinant chromosomes were shown to be less fit than non-recombinants. It was proposed that this repositioning of chiasmata during F_1 meiosis was responsible for breaking up cis-acting (internally co-adapted) gene complexes which were essential for normal embryonic development. However, in this case, as in all other examples involving chromosomally divergent taxa no clear distinction can be made between the direct role of chromosomal factors and the genic divergence which has accumulated following isolation in allopatry, in generating and maintaining reproductive isolation.

There are numerous examples in flowering plants where reproductive isolation due to F_1 sterility between chromosomally divergent species is not necessarily the result of mechanical difficulties during meiosis (see Grant 1971). In addition the inhibition and breakdown of meiosis during early prophase has been demonstrated in various mammalian interspecific hybrids (Bernischke 1967; Basrur 1969; Chandley *et al.*, 1974). More recently Lining and Pathak (1981) demonstrated the arrest of spermatogenic stages at early prophase in a male hybrid between the extremely chromosomally divergent species the Indian muntjac and the Chinese muntjac. They proposed that the meiotic blockage at pachytene must be due to a physiological disturbance in the germ line. In all these cases hybrid sterility would appear to be due to genic differences which exist between the hybridised species, not the chromosomal differences.

Since the Moreton and Torresian taxa are also genically differentiated (Daly *et al.*) it seems possible, even likely, that at least some of the embryonic inviability in the F_2 and backcross generation may result from incompatible

combinations of genes from the parental taxa. This case is, however, clearly very different to those cited above since the "genic effect" occurs in the F_2 not the F_1. We would therefore appear to have two distinct factors affecting F_2 inviability: (a) Chromosomal: the disruption of *intrachromosomal* organisation resulting from recombination occurring in normally recombination-free regions, as a consequence of chromosomal heterozygosity. (b) Genic: the generation of incompatible genic combinations following *intergenomic* recombination involving two genically divergent taxa.

Recently a new chromosomal taxon, Lakes Entrance (LE), has been found which presents a unique opportunity to partition the relative contributions of both recombinational repatterning, due to chromosomal heterozygosity, and the effects of the genic differences on inviability (Shaw *et al.*, 1982). The Lakes Entrance taxon has an acrocentric karyotype (plate 1) which is identical in gross morphology to the Torresian (TT) taxon. However, the (LE) chromosomes possess interstitial and terminal bands of heterochromatin, similar to but not identical, with those found in the Moreton taxon. In addition the Lakes Entrance and Moreton (MAX) taxa are electrophoretically equivalent whereas Lakes Entrance, like Moreton, is diagnostically different from the Torresian at 5 of the 23 allozyme loci assayed (Daly *et al.*, 1981). Thus (LE) and (TT) are chromosomally equivalent in terms of centromere position, but genically divergent. Therefore we would predict that they would have similar chiasma distribution patterns and there should be no redistribution of chiasmata among their F_1 hybrids. Further, we suggest that any reduction in viability in either the F_2 or backcross generations would arise principally because of genic differences between the (LE) and (TT) taxa. In contrast the (LE) and (MAX) taxa are chromosomally different but genically equivalent and we would therefore predict a significant redistribution of chiasmata in the F_1 hybrids similar to that seen in the (Moreton $\times$ Torresian) F_1 hybrids (Coates and Shaw, 1982). In this case, however, any embryonic inviability among the F_2 and backcross generations should be due principally to recombinational changes induced in the F_1 hybrid. In this paper we present an analysis of the chiasma distribution patterns in the LE, MAX and TT taxa and the (LE $\times$ TT) and (MAX $\times$ LE)F_1 hybrids.

2. Materials and Methods

Samples were collected from the Lakes Entrance population in south east Victoria, a Moreton (MAX) population (Peregian Beach) and a Torresian (TT) population (Childers) both from south east Queensland. These three chromosomal taxa are karyotypically distinct and all chromosomes within each genome can be identified unambiguously (plate 1). Two different classes of F_1 hybrids (MAX $\times$ LE) and (LE $\times$ TT) were generated in the laboratory by crossing field collected and laboratory reared males and virgin females.

Air dried slides of meiotic and mitotic cells were prepared from testicular follicles and embryos respectively, and C-banded using the technique of Webb (1976). Representative C-banded diplotene cells are shown in plates 2–7. Ten cells from 10 individuals were sampled from each population and the F_1 hybrids. Measurements and statistical comparisons of chiasma distributions were carried out as described by Coates and Shaw (1982).

3. Results

(i) *Chiasma frequencies and U-type exchanges*

As demonstrated previously (Coates and Shaw, 1982) the (Moreton × Torresian) F_1 hybrids have a significantly lower mean cell chiasma frequency than either of the parental taxa. This reduction can be directly attributed to pericentric heterozygosity in chromosomes 1, 2, 4, 5, 6 and 8 which results in the complete suppression of crossing over within the limits of pericentric re-arrangement region. In contrast the (MAX × LE)F_1 hybrids show no significant reduction in mean chiasma frequency even though they are characterised by pericentric heterozygosity for the same chromosomes (tables 1 and 2). In fact these F_1 hybrids have a higher chiasma frequency

Table 1

Mean number of chiasmata per bivalent and the mean cell chiasma frequency for the Lakes Entrance population (LE), one Torresian population (TT), one Moreton population (MAX) and three forms of F_1 hybrids

Population	Mean number of chiasmata per bivalent chromosome								Mean cell chiasma frequency*
	1	2	4	5	6	7	8	9	
Lakes entrance (LE)	2·04	2·01	1·75	1·53	1·60	1·30	1·09	1·10	15·42
Torresian (TT)	2·01	2·03	1·59	1·36	1·21	1·21	1·09	1·12	14·62
Moreton (MAX)	1·99	1·84	1·61	1·32	1·14	1·02	1·00	1·08	14·00
LE × TT	2·13	2·08	1·43	1·50	1·00	1·11	1·06	1·04	14·35
MAX × LE	1·90	1·85	1·47	1·20	1·04	1·17	1·14	1·38	14·15
TT × MAX	1·71	1·47	1·12	1·08	1·02	1·05	1·07	1·05	12·57

* Includes chromosomes 10, 11 and 12 which were not measured but always contain a single chiasma. Note the X chromosome is number 3.

Table 2

t test on pairwise comparisons of total cellular chiasma frequencies from the three populations LE (Lakes Entrance), TT (Torresian), MAX (Moreton) and the F_1 hybrids (LE × TT), (MAX × LE) and (TT × MAX). Sample sizes are shown in brackets

	TT (100)	MAX (100)	LE × TT (96)	LE × MAX (89)	TT × MAX (100)
LE (100)	3·63***	6·99***	5·75***	7·19***	22·61***
TT (100)		3·78***	1·71^{ns}	3·35***	17·26***
MAX (100)			2·54*	0·97^{ns}	9·68***
LE × TT (96)				1·94^{ns}	18·15***
MAX × LE (89)					14·23***

than the Moreton population used to generate them. This result is unexpected but can be attributed to the presence of exchanges (scored as chiasmata) within the pericentric re-arrangement region. The diplotene configurations (plates 10, 12 and 13) showing these exchanges may have arisen by crossing-over within an inversion loop following homologous pairing in the inverted region (assuming the pericentric re-arrangements are inversions). However a study of 100 pachytene cells from four (MAX × LE)F_1 hybrids failed to reveal the presence of inversion loops in any of the 6 heterozygous bivalents

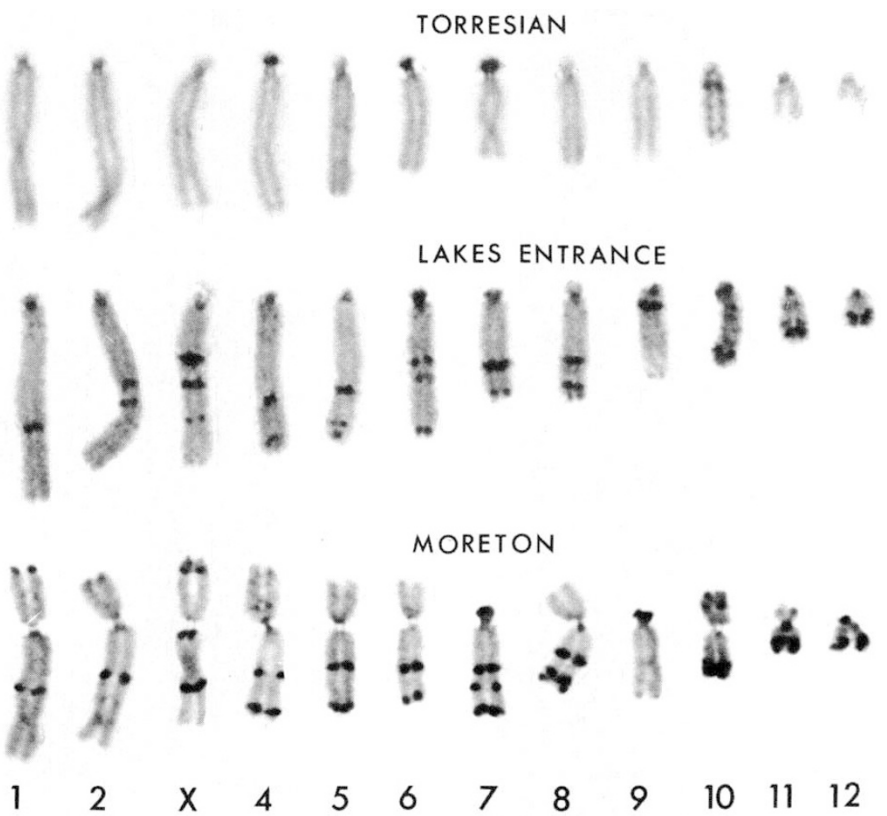

PLATE 1. C-banded karyotypes of the Torresian, Lakes Entrance and Moreton taxa.

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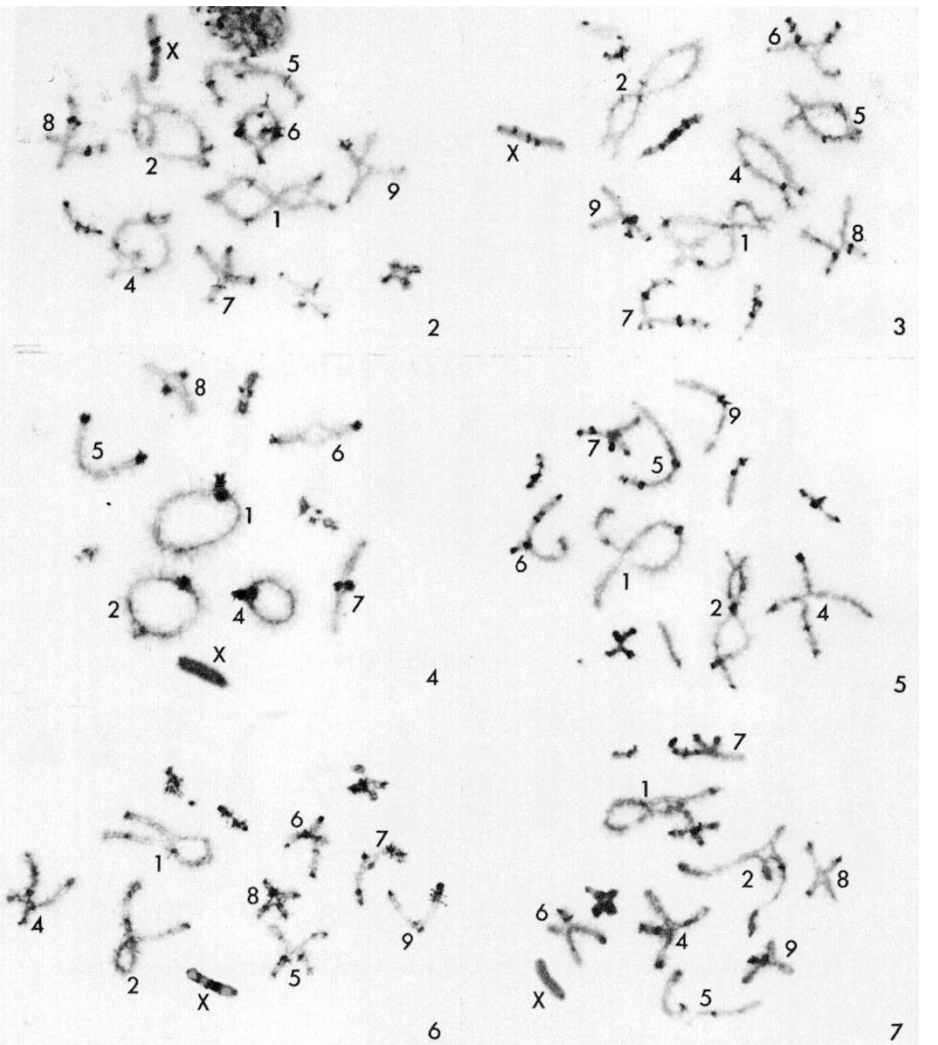
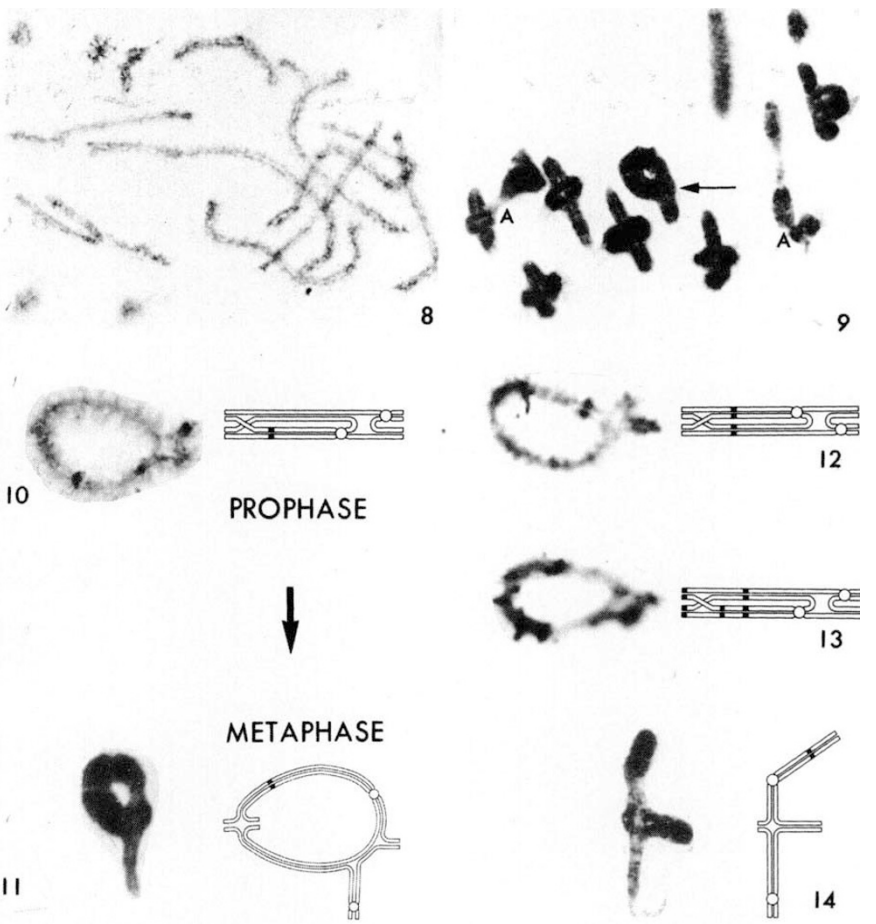


PLATES 2–7. C-banded diplotene cells; plate 2 Lakes Entrance taxon (LE). Plate 3, Moreton taxon acrocentric population (MAX). Plate 4, Torresian taxon (TT). Plate 5, (LE × TT))F_1 hybrid. Plate 6, (MAX × LE)F_1 hybrid. Plate 7, (TT × MAX)F_1 hybrid.



PLATES 8–14. Non-sister U type exchanges in (MAX × LEF$_1$ hybrids; plate 8, pachytene cell in an individual heterozygous for 6 pericentric re-arrangements. Plate 9, Metaphase I cell showing a bivalent (arrowed) with a configuration indicating a non-sister U type exchange in the pericentric re-arrangement region plus a distal chiasmata. Note there are also two non-homologous associations (A). Plate 10, C-banded diplotene bivalent (chromosome 1) with a distal chiasmata and a non-sister U type exchange in the pericentric re-arrangement region. Plate 11, gross stained metaphase I bivalent with a configuration resulting from the same exchange events as in plate 10. Plate 12, C-banded diplotene bivalent (chromosome 2), with the same configuration as in plate 10. Plate 13, C-banded diplotene bivalent (chromosome 5) with the same configuration as in plate 10. Plate 14, gross stained metaphase I bivalent with the configuration due to a single U type exchange in the pericentric re-arrangement region.

(Plate 10). This was also the case in other studies which have shown that although pericentric polymorphisms are common in Orthoptera, reverse looping is extremely rare (Weissman 1976; Hewitt 1978). Alternatively the diplotene configurations could have resulted from U-type exchanges following straight, non-homologous pairing which has also been demonstrated previously in Moreton × Torresian F_1 hybrids. In addition, straight pairing is characteristic of individuals which are heterozygous for up to 8 pericentric rearrangements, in a number of Moreton and south-east Australian populations (Shaw 1976; Coates unpublished data). As will be discussed later this evidence does not entirely preclude the possibility that inversion loops may be present at early pachytene and are followed by synaptic adjustment leading to straight non-homologous pairing (Moses 1977; Moses *et al.*, 1978). However the available data strongly suggest that the observed diplotene configurations (plates 10, 12 and 13) are most likely the result of non sister U-type exchanges following straight pairing in the pericentric rearrangement region.

Non sister U-type exchanges were observed in chromosomes 1, 2 and 5 (plates 10, 12 and 13) in C-banded diplotene cells and probably occur in all chromosomes in the (MAX × LE)F_1 hybrids which show pericentric heterozygosity. In orcein stained material these exchanges could be detected relatively easily at diplotene and metaphase I (plates 8–14). By far the most frequent configuration found during diplotene and metaphase I resulted from a U-type exchange within the pericentric rearrangement region and a normal crossover in the distal region (plates 10–13). Occasionally bivalents were found with a single U-type exchange within the pericentric rearrangement region resulting in the metaphase configuration shown in plate 14. U type exchanges were never detected outside the pericentric rearrangement region. The mean frequency of U-type exchanges per individual in four (MAX × LE)F_1 hybrids was 15·5 per cent (table 3). This value would appear

TABLE 3

Frequencies of U type exchanges within the pericentric re-arrangement region and a pair of univalents (chromosome 6) in metaphase cells from 4 (MAX × LE)F_1 hybrids

		Cells with			
Individual	Normal cells	U Type exchanges	Univalents (Chromosome 6)	Both	Total
1	37	6	10	4	57
2	50	9	14	0	73
3	21	5	1	1	28
4	30	5	11	2	48
Total	138	25	36	7	206
%	(67·0)	(12·1)	(17·5)	(3·4)	

to account for the discrepancy in mean cell chiasma frequency between the (MAX × LE) and (MAX × TT)F_1 hybrids.

In addition to U-type exchanges other meiotic anomalies such as univalent formation (table 3) and associations between non-homologous chromosomes (plate 9) were observed in the (MAX × LE) F_1 hybrids. A

single pair of univalents, mostly involving chromosome 6, was observed in 20·9 per cent of the cells scored.

In the (LE×TT)F_1 hybrids there is no pericentric heterozygosity and this is reflected in a higher number of chiasmata per bivalent for the larger chromosomes (table 1) and also a mean cell chiasma frequency which although significantly lower than the (LE) taxon is not significantly different from the (TT) taxon.

The (LE) taxon has a significantly higher mean cell chiasma frequency than either the (TT) or (MAX) taxa. A substantial proportion of this increase seems to be due to the considerably higher mean number of chiasmata per bivalent for chromosomes 4, 5 and 6.

(ii) *Chiasma distribution patterns*

The frequency distribution of chiasma position along individual bivalents for autosomes 1–9 of the Lakes Entrance population (LE), the Torresian population (TT), the Moreton population (MAX) and the two classes of F_1 hybrids, (LE×TT) and (MAX×LE), have been compared statistically using the Kolmogorov–Smirnoff two sample test (tables 4 and 5) and a summary is provided in table 6. Typical frequency distributions for chromosomes 2, 4 and 5 are shown in figs. 15–20.

Comparisons of chiasma distribution patterns between each of the two classes of F_1 hybrids with their parental taxa (figs. 15–20, tables 4 and 5) clearly show that the (MAX×LE)F_1 hybrids have very different distribution

TABLE 4

Comparison of chiasma distribution patterns in the autosomes of the Lakes Entrance population (LE) and the F_1 hybrid (LE × TT) using the Kolmogorov–Smirnoff test. Note that for two chiasmate bivalents the distributions for the proximal and distal chiasmata are compared separately. A similar situation obtains in the case of three chiasmate bivalents

Chromosome number	No of chiasmata	Populations		D	$P(D(M, N) < D)$
		M (LE)	N (LE×TT)		
1	2	92	78	0.15357	NS
1	2	92	78	0·37291	***
1	3	8	13	0·46154	NS
1	3	8	13	0·36538	NS
1	3	8	13	0·39423	NS
2	2	97	77	0·22520	*
2	2	97	77	0·22854	*
4	1	26	54	0·31197	NS
4	2	78	41	0·48311	***
4	2	78	41	0·21513	NS
5	1	49	46	0·25776	NS
5	2	55	49	0·14434	NS
5	2	55	49	0·26865	*
6	1	43	94	0·40524	***
7	1	74	86	0·18856	NS
7	2	30	9	0·30000	NS
7	2	30	9	0·34444	NS
8	1	95	88	0·08002	NS
9	1	94	92	0·30712	***

TABLE 5

Comparison of chiasma distribution patterns in the autosomes of the Lakes Entrance population (LE) and the F_1 hybrid (MAX × LE) using the Kolmogorov-Smirnoff test

Chromosome number	No. of chiasmata	Population *M* (LE)	Population *N* (MAX × LE)	*D*	*P*(*D*(*M*, *N*) < *D*
1	2	92	71	0·73071	***
1	2	92	71	0·26500	**
2	2	97	74	0·76679	***
2	2	97	74	0·14140	NS
4	1	26	52	0·48077	***
4	2	78	40	0·74551	***
4	2	78	40	0·14295	NS
5	1	49	74	0·61224	***
5	2	55	18	0·76061	***
5	2	55	18	0·28586	NS
6	1	43	89	0·44578	***
7	1	74	75	0·20414	NS
7	2	30	17	0·39020	NS
7	2	30	17	0·26667	NS
8	1	95	81	0·40650	***
9	1	60	94	0·23475	*

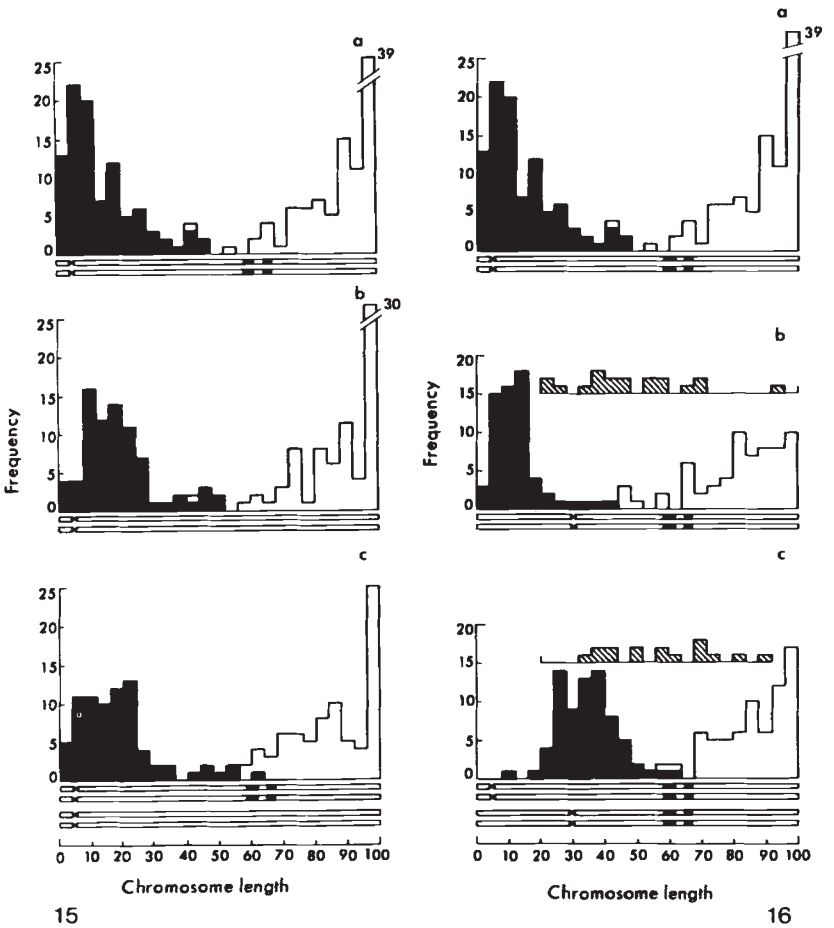
TABLE 6

Percentage proportion of Kolmogorov–Smirnoff comparisons which are significantly different between the Lakes Entrance population (LE), the Moreton population (MAX), the Torresian population (TT) and the F_1 hybrids (LE × TT) and (MAX × LE)

	TT	MAX	LE × TT	MAX × LE
LE	66·7	57·9	36·8	62·5
TT		61	38·9	68
MAX				68·8
LE × TT				68·8

patterns, for all chromosomes, from those of the parental taxa. In contrast the (LE × TT)F_1 hybrids have quite similar distribution patterns to both of the parental taxa for chromosomes 1, 2, 5, 7 and 8 (table 4).

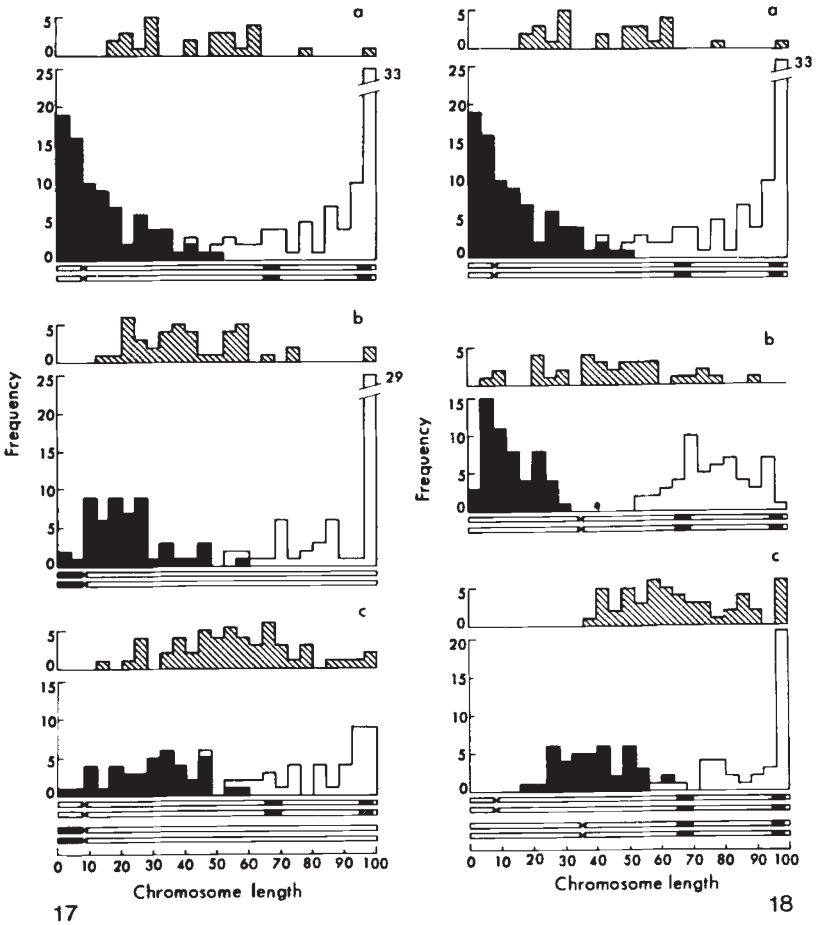
The difference in chiasma distribution patterns between the (MAX × LE)F_1 hybrids and the (LE) and (MAX) taxa in chromosomes 1, 2, 4, 5, 6 and 8 (table 5) can be directly attributed to pericentric heterozygosity. This is very similar to the effect reported previously for the (Moreton × Torresian)F_1 hybrids (Coates and Shaw, 1982). In addition, even though there is no pericentric rearrangement difference on chromosome 9, there is a significant difference in the overall chiasma distribution pattern for this chromosome between these F_1 hybrids and the parental taxa. A similar difference also occurs between the (LE × TT)F_1 hybrid and the LE taxon. It is not clear whether this effect is due to genotypic differences, heterochromatin differences or undetected chromosome rearrangements such as paracentric inversions.



FIGS. 15 and 16. The distribution of chiasmata along chromosome 2 in one-chiasmate and two-chiasmate bivalents in (15(a)–(c)) the Lakes Entrance taxon (LE), the Torresian taxon (TT) and the (LE×TT)F_1 hybrids respectively and (16(a)–(c)) the Lakes Entrance taxon (LE) the Moreton taxon (MAX) and the (MAX×LE)F_1 hybrids respectively. Fixed (solid shading) and polymorphic (diagonal lines) heterochromatic regions are indicated.

In the (LE) taxon the majority of bivalents involving chromosomes 1, 2 and 4 have two chiasmata which show marked proximal/distal localisation. Both the (MAX) and (TT) taxa also show proximal-distal localisation patterns, but in the latter case the proximal chiasmata tend to be located more interstitially than those in the (LE) taxon (figs 15 and 17). In contrast when (LE) and (MAX) are compared, it is the distal chiasmata in (MAX) which are shifted towards the interstitial regions (figs. 16 and 18).

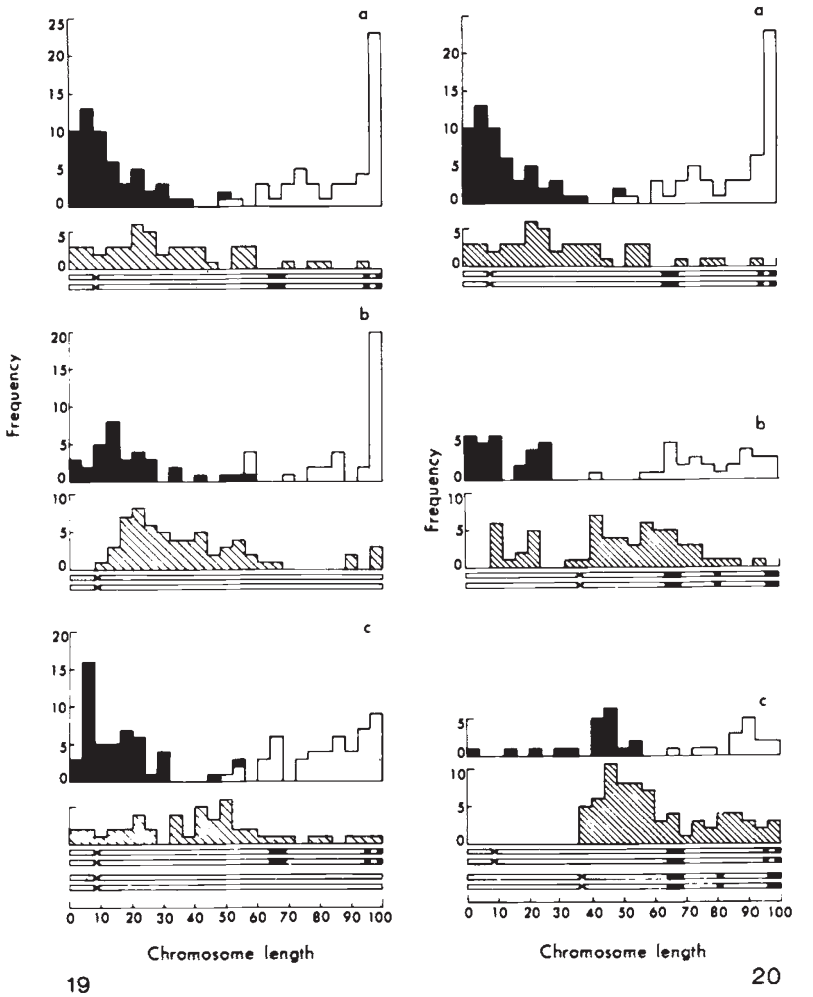
Chiasmata in chromosomes 1 and 2 in the (LE×TT)F_1 hybrids also show strong proximal-distal localisation with only minor differences in distribution patterns when compared to the parental taxa. In chromosome 4, however, the proximal chiasmata show a marked shift in position towards interstitial regions and there is also a significant increase in the number of monochiasmate bivalents (fig. 17). This effect is similar to that found pre-



FIGS. 17 and 18. The distribution of chiasmata along chromosome 4 in one-chiasmate and two-chiasmate bivalents in (17(a)–(c)) the Lakes Entrance taxon (LE), the Torresian taxon (TT) and the (LE × TT)F_1 hybrids respectively and (18(a)–(c)) the Lakes Entrance taxon (LE), the Moreton taxon (MAX) and the (MAX × LE)F_1 hybrids respectively. The differences in chromosome structure between the taxa are shown below the distributions as in figs. 15 and 16.

viously in bivalents heterozygous for a pericentric rearrangement in both the (Moreton × Torresian)F_1 hybrids (Coates and Shaw, 1982) and (MAX × LE)F_1 hybrids. Although there is no pericentric heterozygosity in this case, the (TT) chromosome 4 carries a completely heterochromatic short arm which, when heterozygous appears to reduce chiasma formation in the regions adjacent to the centromere in the long arm.

In contrast to the (LE × TT)F_1 hybrids, chiasma distribution on chromosomes 1, 2 and 4 in the (MAX × LE)F_1 hybrids tend to be localised in interstitial and distal regions. These differences in chiasma distribution pattern between the (LE × TT) and (MAX × LE)F_1 hybrids also occur in chromosomes 5, 6 and 8 even though in these smaller chromosomes the number of bi-chiasmate bivalents is considerably reduced (*e.g.*, figs 19 and 20).



FIGS. 19 and 20. The distribution of chiasmata along chromosome 5 in one-chiasmate and two-chiasmate bivalents in (19(a)–(c)) the Lakes Entrance taxon (LE), the Torresian taxon (TT) and the (LE × TT)F_1 hybrids respectively and (20(a)–(c)) the Lakes Entrance taxon (LE), the Moreton taxon (MAX) and the (MAX × LE)F_1 hybrids respectively. The differences in chromosome structure between the taxa are shown below the distributions as in figs. 15 and 16.

In summary, while the chromosomes in all three parental taxa have chiasma distribution patterns which in many cases appear superficially very similar, statistical analysis does reveal more subtle differences. For instance chromosomes with bi-chiasmate bivalents generally show extreme proximal-distal localisation in (LE), less proximal and extreme distal localisation in (TT) and extreme proximal and less distal localisation in (MAX). The chiasma distribution patterns in most chromosomes of the (LE × TT)F_1 hybrids appear very similar to the parental taxa. All chromosomes in the (MAX × LE)F_1 hybrids, including chromosome 9 which is not characterised by pericentric heterozygosity, have chiasma distribution patterns which are

very different to those in the parental taxa. This is clearly demonstrated in table 6 which shows that only 37 per cent of the Kolmogorov–Smirnoff comparisons are significantly different when the (LE × TT)F_1 hybrids are compared with their parental taxa, whereas 66 per cent show significant differences when the parental taxa are compared with the (MAX × LE)F_1 hybrids.

(iii) *Recombination in the F_1 hybrids*

The chiasma distributions have also been used to determine the pattern of recombination occurring in specific regions of the chromosomes in the two different classes of F_1 hybrids (fig. 21). The chromosomes have been subdivided into diagnostic regions using the pericentric rearrangements and heterochromatic bands to delineate the boundaries.

With the exception of chromosome 4, recombination in the (LE × TT)F_1 hybrids is localised in the proximal and distal regions of the chromosomes. In contrast there is substantially less recombination in proximal regions, and a corresponding increase in interstitial regions, in chromosomes of the (MAX × LE)F_1 hybrid. This reduction in recombination in the proximal regions is primarily due to pericentric heterozygosity. These marked differences in the pattern of recombination between the two classes of F_1 hybrids provides a further basis for investigating the relationship between recombination change induced by pericentric heterozygosity and F_2 and backcross inviability.

4. Discussion

(i) *Recombinational repatterning in the F_1 hybrid and F_2 inviability*

The analysis of the patterns of recombination in both the (Moreton × Torresian)F_1 and (Moreton × Lakes Entrance)F_1 hybrids, have shown that chromosomal heterozygosity has a dramatic effect upon the distribution of chiasmata with almost complete suppression of crossing over within the pericentric rearrangement region. In contrast, the analysis of the (Lakes Entrance × Torresian)F_1 hybrids, where both the parental taxa have similar karyotypic structure, has revealed that only minor changes occur in the pattern of recombination. Shaw *et al.* (1982) suggested that approximately 46 per cent of the F_2 embryonic breakdown in a (Moreton × Torresian) cross may arise solely from the effects of chromosomal heterozygosity upon recombinational repatterning. This was based upon the following important observations:

(i) Since the F_1 generation is fully viable and fertile, the Moreton and Torresian chromosomes are normally functional when together in unrecombined states.

(ii) Pericentric heterozygosity induces chiasma formation in chromosome regions which normally only undergo low levels of recombination and leads to the generation of novel recombinant chromosomes.

(iii) Those zygotes which successfully complete embryogenesis do not contain a random sample of gametes derived from the F_1 hybrid parent.

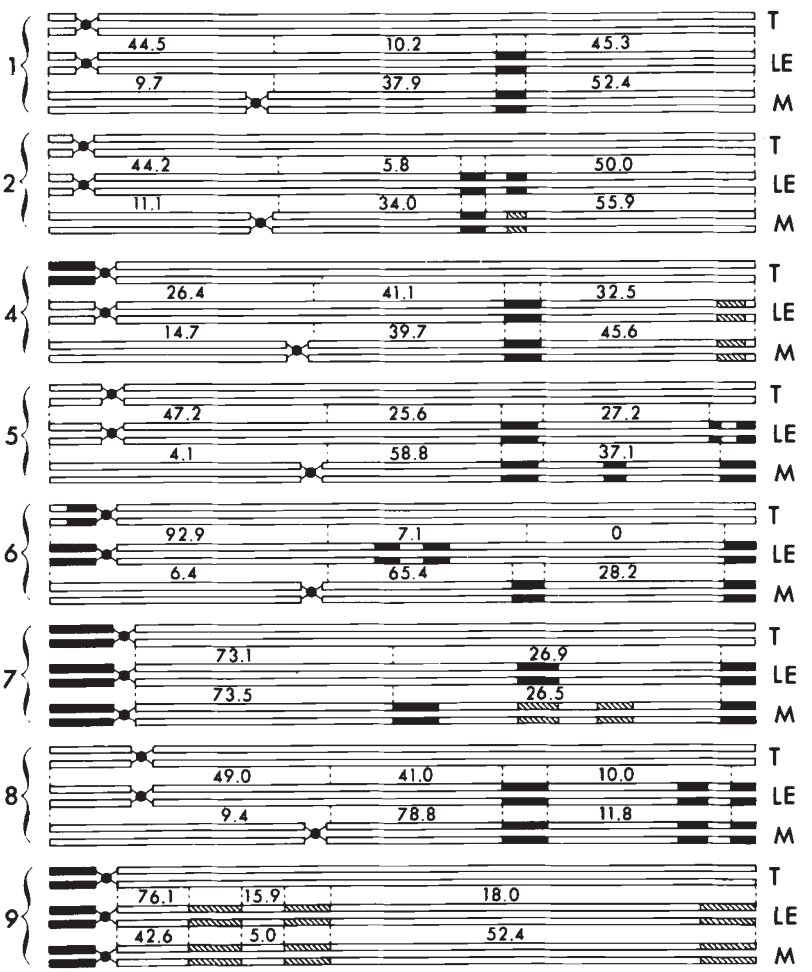


FIG. 21. The frequency of recombination events which occur in each chromosomal region in autosomes 1–9 (note 3 = X) in the F_1 hybrids (LE × TT) and (MAX × LE). The upper chromosome in each triplet represents the Torresian taxon the middle chromosome the Lakes Entrance taxon and the lower chromosome the Moreton taxon. The upper and lower values in each triplet represent the frequency of recombination calculated from the F_1 hybrids (LE × TT) and (MAX × LE) respectively. Heterochromatic regions are indicated as in figs. 15 and 16. Note there is substantially reduced recombination within the limits of the pericentric re-arrangement region in the (MAX × LE)F_1 hybrids which is compensated for by a striking increase in recombination values in the interstitial regions.

(iv) There are no significant pair-wise interactions between non-homologous chromosomes, thus all the observed viability effects can be attributed entirely to *intra-* rather than *inter*-chromosomal interactions.

(v) In the (Lakes Entrance × Torresian) F_2 generation there is 54 per cent inviability which compares with 100 per cent among the (Moreton × Torresian) crosses.

Since the LE and TT taxa are chromosomally very similar (plate 1) one might expect no significant changes in the patterns of recombination in their F_1 hybrid and indeed this has been confirmed in this study. As a result, it appears that the inviability in the (LE × TT)F_2 cross arises primarily as a consequence of the genic divergence which exists between these two taxa. However it is important to stress that this inviability occurs only after recombination between the LE and TT genomes, although in this case disruption of any internal chromosome organisation is likely to be minimal as the (LE × TT)F_1 hybrids show strong proximal-distal chiasma localisation in most bivalents. Since intrachromosomal recombination is generally restricted in this F_1, most of the F_2 inviability would appear to be the result of intergenomic recombination involving whole chromosome segregation. This mixing of these two divergent genomes seems to generate inviable genic combinations. Yet it remains a puzzle why the "genic effect" on inviability occurs following intergenomic recombination when the F_1 between these two subspecies, although containing two genically divergent haploid genomes, is fully fertile and viable. If intergenomic recombination as described above is a major factor contributing to F_2 inviability in the (LE × TT) cross we might expect significant interchromosomal interactions. However, this is contrary to evidence from the (Moreton × Torresian) backcross analysis (Shaw *et al.*, 1982) although it is likely that any such effect on viability would be extremely difficult to separate from the very significant intrachromosomal interactions demonstrated in that study. Effects on fitness due to epistatic interaction between chromosomes have been demonstrated in the grasshopper *Keyacris scurra* (Lewontin and White 1960) and in addition Wallace (1955) detected both intra and interchromosomal effects on fitness after crossing different populations of *Drosophila pseudoobscura*. The prediction that viability in the (LE × TT)F_2 is due primarily to inter chromosomal interactions can be readily tested and is currently under investigation.

In comparison the Lakes Entrance and Moreton taxa are genically equivalent and considered to represent different populations within the same subspecies. However, they are chromosomally very different and the F_2 inviability can be primarily attributed to the disruption of intrachromosomal organisation following the very significant effect chromosomal heterozygosity has on the recombination pattern in the F_1 hybrid. This result is similar in certain respects to that obtained by Vetukhiv (1953) and Brncic (1954) from tests designed to demonstrate co-adaptation (see Dobzhansky 1950; Wallace 1955) in *Drosophila pseudoobscura*. In these studies the F_1 interpopulational hybrid showed a higher viability, fecundity and longevity than the parents while the F_2 showed lower values for these measures. From these data Brncic (1954) emphasised the importance of intrachromosomal organisation and postulated that recombination in the F_1 disrupted balanced gene combinations within chromosomes. However, it is implicit in the coadaptation hypothesis that F_1 heterosis and F_2 breakdown between populations would occur in any environment. Yet Vetukhiv and Beardmore (1959) found that in *D. pseudoobscura* the effect does not necessarily occur in all environments; it is more pronounced in stringent environments and may not even be detected under optimal conditions.

From the data in this and previous studies it is apparent that, in F_1 hybrids pericentric, heterozygosity in the majority of chromosomes has such

a marked effect on the recombination system that embryonic viability in the progeny is significantly reduced. Clearly in this situation the recombination system plays an integral role in maintaining the functional unity of the chromosome and this functional unity is essential for successful embryogenesis. As stated earlier (Shaw *et al.*, 1982) it seems that the important differences between the Moreton and Torresian taxa relate to the sequence of gene expression during development rather than the differences in structural genes *per se*, and this sequence of gene expression requires that certain chromosomal regions retain their linear gene association intact.

The hypothesis proposed by Shaw and Wilkinson (1980) and tested in this and the two previous papers (Coates and Shaw 1982; Shaw *et al.*, 1982) provides an alternative explanation for the role of chromosomal re-arrangements in the generation and maintenance of reproductive isolation between species. As mentioned previously one of the major criticisms of many of the previous studies in which chromosome re-arrangements are considered to play a primary role in initiating and maintaining reproductive isolation between taxa is that no critical distinction is possible between the role of chromosomal and genic factors. We have shown in previous studies that there is 100 per cent inviability in the (MAX × TT)F_2 (chromosomal + genic differences) and 54 per cent inviability in the (LE × TT)F_2 (genic differences) and we would predict 46 per inviability in the (MAX × LE)F_2 (chromosomal differences). A recent study has shown that there is in fact 42 per cent inviability in the (MAX × LE)F_2 generation (Shaw *et al.*, in prep.) Thus at present we estimate that 46 per cent of the embryonic inviability in the (Moreton × Torresian) cross is due to chromosomal effects with the remainder being due to the genic differences which exist between those two taxa. Further investigations are currently underway to obtain more accurate estimates of these chromosomal and genic components of reproductive isolation.

(ii) *U-type exchanges in the pericentric re-arrangement region*

The relatively high frequency of U-type exchanges within the pericentric re-arrangement region of bivalents in the (MAX × LE)F_1 hybrids was entirely unexpected since no exchanges were previously observed in studies on (Moreton × Torresian)F_1 hybrids where the same pericentric heterozygosity occurs. As mentioned earlier the diplotene U-type configurations could have arisen following crossing over within an inversion loop, but no inversion loops were observed at pachytene which showed straight and non-homologous pairing. However since early pachytene is extremely difficult to interpret, only middle to late pachytene cells were analysed and there remains the possibility that synaptic adjustment (Moses, 1977; Moses *et al.*, 1978) may be occurring. Moses (1977) postulated that the phenomena of synaptic adjustment functioned during late pachytene to produce straight non-homologous pairing in heterologous chromosome regions. Recently, however, Gillies (1983) has presented evidence that synaptic adjustment may not be a general phenomenon and that, at least in maize prophase, it may be the exception rather than the rule. In addition Nur (1968) provided a clear demonstration of inversion loops in the grasshopper *Camnula pellucida* at late pachytene. It therefore seems likely that the meiotic configurations we observed were due to U-type exchanges following non-homologous pairing in the pericentric re-arrangement region.

U-type exchanges have been identified in a variety of plant and animal species and have usually been detected because they result in anaphase I bridge and fragment configurations which cannot be attributed to crossing-over within reverse pairing loops of a paracentric inversion (Walters, 1950, Lewis and John, 1966; Newman, 1966; Jones, 1968, 1969; John, 1976). Similar exchanges have also been demonstrated previously in *Caledia* in (Daintree × Moreton)F_1 hybrids (Shaw and Wilkinson 1978).

There is now strong evidence that U-type exchanges represent aberrant crossover exchanges and arise as errors of chiasma formation (Lewis and John, 1966; Jones, 1968, 1969; Jones and Brumpton, 1971). This argument is supported by the evidence revealed in this study. It has been shown in other organisms that U-type exchanges commonly occur in genetically unusual types, such as F_1 hybrids, and usually as part of a syndrome of errors reflecting a breakdown in the control of meiotic processes. In *Caledia*, U-type exchanges have only been detected in F_1 hybrids and are usually associated with other meiotic anomalies such as associations between non-homologous chromosomes and univalent formation. In addition, the distribution of U-type exchanges is closely correlated with the pattern of chiasma distribution. Since the U-type exchanges observed in the (MAX × LE)F_1 hybrids involve those regions of the parental chromosomes in which there is a high frequency of chiasma formation, it seems highly probable that they arise as errors during chiasma formation in regions of non-homology. However, if this is the case, this does not explain why they occur at relatively high frequencies in the (MAX × LE)F_1 hybrids but are totally precluded in the (Moreton × Torresian)F_1 hybrids where apparently the same type of pericentric heterozygosity exists.

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