COMPLEX HYBRIDITY IN *ISOTOMA PETRAEA*

II. COMPONENTS AND OPERATION OF A POSSIBLE EVOLUTIONARY MECHANISM

S. H. JAMES

Botany Department, University of Western Australia

Received 19.ii.69

1. INTRODUCTION

Two evolutionary methods by which complex hybridity may be established in plants have been proposed by students of *Oenothera*. Cleland (1936, 1960, 1962) holds that, in *Oenothera* at least, the system was established by the hybridisation of open pollinating structurally homozygous races which differed from each other by at least six interchanges so that the progeny produced were ring-of-fourteen ($\odot_{14}$) multiple interchange heterozygotes. According to this theory, the ring *subsequently* became stabilised by the evolution of autogamy and a balanced lethal system. In those species of *Oenothera* which are characterised by gametic lethals, it has been proposed (Steiner, 1956, 1957, 1961) that self-incompatibility alleles have contributed to the gametic lethals on the male side. Darlington (1931, 1958), on the other hand, has argued that complex hybridity has arisen gradually, as a response to imposed inbreeding, especially in marginal populations, the system providing a mechanism to conserve genetic hybridity.

In *Isotoma petraea*, complex hybridity has evolved in the extreme south-west corner of the species distributional range and complex hybrids exhibiting $\odot_6$, $\odot_6 + \odot_4$, $\odot_{10}$, $\odot_{12}$, $2\odot_6$, $\odot_8 + \odot_6$, $\odot_{10} + \odot_4$, and $\odot_{14}$ configurations at meiosis have been found (fig. 1). Generally, individual complex hybrid populations are characterised by a single meiotic configuration but some heterogeneity does occur. For example, structural homozygotes as well as the $\odot_6$'s occur in the Pigeon Rock population (Beltran, 1966); structural homozygotes and $\odot_4$'s constitute some 10 per cent. of the otherwise $\odot_{10}$ 3-mile Rock population; both $\odot_6 + \odot_8$ and $\odot_{10} + \odot_4$ configurations are found in the Warralakin (34 miles east of Muckinbudin) population, and the Elachbutting population, initially scored as $\odot_{12}$ on the basis of a single determination, has since yielded $2\odot_6$ plants only. Crossing experiments have established that although interchange is common in the non-complex or floating interchange populations of *petraea* and of its related species, *axillaris* and *anaethifolia*, karyotypic evolution has not occurred to any significant degree: although $\odot_4$'s and rarely $\odot_6$'s are found in some 10 per cent. of plants in such populations, there is a common and primitive chromosome end sequence in all three species. Indeed, the evolution of complex hybridity in *petraea* appears to be associated with the fixation, in a heterozygous condition, of a minimal or near minimal number of interchanges upon this sequence.

The complex hybrids in *petraea* are essentially autogamous, as are the floating interchange populations to the east and north of them, but a residual capacity for crossing still exists. The complex hybrids breed true on selfing,

being stabilised by a balanced lethal system operative at the zygotic level.

The information summarised above has been presented in detail elsewhere (James, 1965) where it was concluded that the pattern exhibited by *petraea* and its complex hybrids was more in harmony with the evolutionary mechanism proposed by Darlington than with that proposed by Cleland.

FIG. 1.—Distribution of *Isotoma petraea* populations in south western Western Australia. The symbol indicates the metaphase-1 chromosome configuration characteristic of the population.

Additional observations on the genetic systems of *Isotoma petraea*, which are discussed in this paper, suggest that, in this species, complex hybridity was in all probability initiated by hybridisation but the hybridisation yielded not large rings, but small rings which have been expanded to include the entire genome. Moreover, Darlington's hypothesis clearly applies insofar as preadaptation of the breeding system is concerned. In addition, however, the evolutionary process has led not merely to a conservation of existing

hybridity, of which there was little, but has involved an active pursuit and capture of new patterns of cumulative hybridity.

2. OBSERVATIONS

(a) *Chromosome morphology.* Root-tip chromosomes from two complex heterozygotes are illustrated in plate I, figs. 1 and 2. Although differences between chromosomes are apparent, the small size of the chromosomes and their relative isobrachy leads to serious equivocation in karyotype analyses. Indeed, it has proved difficult to consistently and unequivocally define the homologues in structural homozygotes let alone trace cumulative changes in the structural heterozygotes. The nucleolar chromosome, however, can be readily identified by the satellite associated with the short arm of that chromosome. In the $\odot_{10}$ 3-mile Rock illustrated one of the nucleolar chromosomes is of the primitive form while the satellite on the other has been greatly enlarged, and the long arm of that same chromosome is noticeably shorter than usual. In the Merredin seedling ($\odot_{14}$ population) the short arm, *i.e.* the arm with the satellite, of one nucleolar chromosome is much enlarged. Thus the morphology of the chromosomes may be greatly altered by interchange, the segments involved need not be equal in size and the points of interchange are not confined to the centromere region. It seems valid to conclude, therefore, that interchange points are randomly distributed within the chromosomes.

(b) *Chromosome behaviour.* The chromatin of interphase nuclei is fully dispersed so that no chromocentres are distinguishable (plate I, fig. 4). During mitotic prophase the terminal regions of the chromosomes remain diffuse for longer than the proximal regions (plate I, fig. 3). If one defines heterochromatic material on the basis of its ability to form chromocentres in interphase nuclei (Heitz, 1928) then these proximally condensed regions cannot be considered to be heterochromatic.

Indeed, the prophase conforms to Lima-de-Faria's (1956) concept of a gradient in coiling proceeding from the centromere. However, the proximal and distal ends of the chromosomes are strikingly different with respect to their coiling behaviours during prophase and consequently, if we follow the modern tendency to define heterochromatin in terms of any allocyclic pattern, the proximal regions might be regarded as heterochromatic in character.

During diplotene also, the distal regions of the chromosomes are distinctly less stained and the chiasmata are strongly restricted to these parts (plate I, fig. 5). The chiasmata are usually fully terminalised at M-1. Only rarely are interstitial chiasmata observed in plants from native populations, but less rarely configurations interpretable as interstitial chiasmata may be observed in synthetic hybrid material. These configurations commonly include "sticky connections" (plate I, figs. 6 and 7), which may or may not represent terminalised chiasmatic connections between essentially non-homologous chromosomes, and occasionally, unequivocal non-terminalised chiasmata (plate I, figs. 8 and 9). The presence of these non-terminalised or interstitial chiasmata shows that it would be possible for crossing-over to take place, at least rarely, in interstitial segments in interchange heterozygotes.

Chiasma frequency is approximately 1.95 per bivalent in structural

homozygotes and but little less in the rings of the interchange heterozygotes, where it is approximately 0.95 per pair of chromosome arms (table 1).

Meiosis in the structural homozygotes is quite regular, leading to almost complete pollen fertility. In plants heterozygous for a single interchange, disjunctional M/A-1 configurations occur in about 90 per cent. of cells. In multiple interchange heterozygotes meiosis is strikingly irregular; non-disjunction is a feature of the division and may be exhibited in a variety of configurations. A number of disjunctional and non-disjunctional cells are illustrated in plate I, figs. 10 to 14, and an analysis of cytological behaviour

TABLE 1
Chiasma frequency in Isotoma petraea

(a) <i>Structural homozygotes</i>							
Plant	No. of cells showing types of bivalents			Total cells	Total bivalents	Total chiasmata	Mean no. chiasmata per bivalent
	7 Rings	6 Rings 1 Rod	5 Rings 2 Rods				
7II Anketell	99	39	3	141	987	1929	1.94
7II Laverton	137	36	3	176	1232	2422	1.96

(b) <i>Multiple interchange rings (bivalents excluded)</i>							
Plant	No. of cells showing types of configurations				Total cells	Total chiasma sites	Mean no. of chiasmata per site
	Ring	1 Chain	2 Chains	3 Chains			
⊙ ₁₄ Merredin	26	11	5	—	42	588	0.96
⊙ ₁₂ Bencubbin	32	20	1	1	54	648	0.96
⊙ ₁₁ 3-mile Rock	16	17	4	—	37	370	0.93

in interchange heterozygotes is presented in table 2 (a). Where, by failure of chiasma formation at two sites, a ring was broken down into two chains and the chromosomes displayed regular alternate segregation, the cell was scored as "disjunctional" even though one could not always be sure of the number of chromosomes in each chain (plate I, fig. 14). This would have led to a slight overestimate of the frequency of disjunction, for presumably half of the cells having two even-numbered chains exhibiting regular alternate chromosome segregation would have been non-disjunctional. Some non-disjunctional orientations lead to numerically unequal segregations and this is reflected at second division. A comparison of the frequency of numerically unequal segregation scored at first and at second division is presented in table 2 (b), and good agreement between the estimates is in evidence. The frequency of non-disjunction increases with ring size; although ⊙₄'s show only 10 per cent. non-disjunction, the ⊙₆'s show about 40 per cent. non-disjunction and this figure rises to the order of 80 per cent. in ⊙₁₄'s. This striking loss of meiotic regularity with increasing ring size is paralleled by a corresponding decrease in pollen fertility (table 2 (c); fig. 2).

Beltran (1970, in prep.) has shown that in a ⊙₆ and in a ⊙₁₂ complex heterozygote, the post meiotic but pre-fertilisation ovule abortion, presumably due to meiotic non-disjunction, is comparable to the pollen abortion. Hence, seed potential is also dramatically reduced in the complex heterozygotes. In a single ring complex heterozygote, the proportion of fertile

Plate I

FIGS. 1-14: cytological characteristics of *Isotoma petraea* 1,2 $\times$ c. 3000; 3-14, $\times$ c. 2000.

- FIG. 1.—Root-tip C-metaphase in $\odot_{10}$ 3-mile Rock showing one nucleolar chromosome with an enlarged satellite and a shortened long arm, *a*.
- FIG. 2.—Root-tip C-metaphase in a Merredin ($\odot_{14}$) seedling, showing one nucleolar chromosome with an enlarged short arm, *b*. In both 1 and 2, the nucleolar chromosome labelled "*p*" has the primitive form.
- FIG. 3.—Root-tip mitotic prophase—note the diffuse terminal regions of the chromosomes.
- FIG. 4.—Root-tip interphase cells—note the absence of chromocentres.
- FIG. 5.—Diplotene in a structural homozygote—note the diffuse terminal regions in which the chiasmata occur.
- FIG. 6.—M/A-1 in 7II (7II Rainy Rock $\times$ 7II "Iron Knob").—Note the "sticky connections" between essentially non-homologous chromosomes as well as the terminalised chiasmata.
- FIG. 7.—M/A-1 in $\odot_{10}$ (!) ($2\odot_6$ Muntagin $\times$ 7II 495.3).—Note the "sticky connections".
- FIG. 8.—M/A-1 in $\odot_6$ ($\odot_{12}$ Mt Stephens $\times$ 7II 495.3).
- FIG. 9.—M/A-1 in 7II [*anaethifolia* 7II $\times$ 7II (*petraea* $\odot_6$ Pigeon Rock $\times$ *axillaris* 7II)]. In both 8 and 9, note the non-terminalised chiasmata (arrowed).

FIGS. 10-14.—M/A-1 in $\odot_{14}$ Merredin.

- FIG. 10.—A disjunctional cell.
- FIG. 11.—Double non-disjunction on opposite sides leading to numerical equality.
- FIG. 12.—Double non-disjunction on the same side leading to numerical inequality (8-6).
- FIG. 13.—Quadruple non-disjunction on the same side, leading to a 9-5 distribution.
- FIG. 14.—This cell would have been scored as disjunctional, but this may not be correct. There are two chains. If there is a *ch3* + *ch11*, it would be disjunctional. If there is a *ch6* = *ch8*, it would have a 50 per cent. chance of being disjunctional. Such cells lead to an overestimate of disjunction frequency.

TABLE 2
Meiotic behaviour in interchange heterozygotes, PMC analysis

M-I configuration Population examined	(a) <i>Disjunction at first division</i>									
	$\odot_4$ Various	$\odot_6$ Pigeon Rock		$\odot_{10}$ 3-mile Rock		$\odot_{12}$ Bencubbin		$\odot_{14}$ Merredin		$\odot_{14}$ Mt.
Plant No.	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	20
Disjunctinal	52 37 31	37 37 30	21 22 9	5 12 9	18 14 14					8
Non-disjunctinal	4 6 3	21 11 11	19 15 22	9 23 22	31 20 25					20
numerically equal	— — —	11 10 9	28 23 17	5 16 11	27 31 24					21
numerically unequal	56 43 34	69 53 50	68 60 48	19 51 42	76 65 63					49
Total Cells	1-332	2-720	7-38	0-538	2-554					16-3
Heterogeneity χ^2	90-2	58-8	29-5	23-2	22-5					46-8
% Disjunction	84-7-94-7	51-1-66-0	22-9-36-5	15-8-31-6	17-0-28-6					7-4-28-5
95% Confidence interval (%)										51-0-77-8

* Significant; $0.05 > p > 0.02$.

(b) *Comparison of numerical equality of segregation scored at first and at second divisions (pooled data)*

M-I configuration Population examined	(b) <i>Comparison of numerical equality of segregation scored at first and at second divisions (pooled data)</i>									
	$\odot_6$	$\odot_{11}$		$\odot_{12}$		$\odot_{14}$		$\odot_{14}$		$2\odot_6$
First Division										
No. cells showing numerically equal segregation	147	108		80		122		129		32
No. cells showing numerically unequal segregation	30	68		32		82		56		8
Second Division										
No. cells showing numerically equality	54	40		36		179		54		52
No. cells showing numerically inequality	8	13		15		111		9		12
Heterogeneity χ^2	0-5633	3-5442		0-1317		0-1853		5-1830		0-0233
p (Id.f.)	0-5-0-3	0-1-0-05		0-8-0-7		0-7-0-5		0-05-0-02 ₅		0-9-0-8

(c) *Pollen fertility (pooled data)*

M-I configuration Population examined	(c) <i>Pollen fertility (pooled data)</i>									
	$\odot_6$ Pigeon Rock	$\odot_{10}$ 3-mile Rock		$\odot_{12}$ Bencubbin		$\odot_{14}$ Merredin		$2\odot_6$ Muntagin		
No. of plants examined	3	1		1		3		1		
Total number of cells analysed	5849	2195		1000		10312		1706		
Pollen Fertility (%)	73-6	57-9		48-0		34-6		37-2		

pollen, as adjudged by its stainability, is consistently higher than that expected from the meiotic data. For example, only 22.5 per cent. of the PMC's are fully disjunctional in the $\odot_{14}$ Merredin, but pollen fertility is about 35 per cent. Such data suggest that some of the non-disjunctional cells yield stainable pollen grains. Eight chromosome pollen grains at PG mitosis have been observed in complex heterozygotes and, in the ovule at least, some disomic spores may be functional since fifteen chromosome plants have been observed in the progenies of crosses involving complex heterozygotes.

FIG. 2.—The relationship between ring type, meiotic behaviour and pollen fertility in *Isotoma* complex hybrids. Note, the relationship in the $2\odot_6$ is reversed (a) as compared with the single ring complex hybrids, unless half the cytologically regular configurations are considered to be non-disjunctional (b).

A reversal of the pollen fertility/meiotic disjunction relationship occurs in the two-ring complex heterozygotes (fig. 2). In $2\odot_6$ Muntagin, the frequency of disjunction is 46.8 per cent. while the pollen fertility is only 37.2 per cent. This suggests that only half of the meiotically regular cells produce genetically competent pollen. This is analogous to the situation where, through failure of two chiasmata, a ring is broken down into two chains each with an even number of chromosomes. As pointed out above, half the apparently regular separations will in fact be non-disjunctional. Thus, in these two-ring complex heterozygotes, the two rings have to be oriented the same way in order to produce viable spores. There is a genetic interdependence which maintains the integrity of the complexes which are here spread over two meiotically independent rings. Note that here the non-disjunctional effects cannot involve the terminal pairing segments, but must result from an interdependence of segments proximal to the pairing segments, comparable to that proposed by Emerson (1936) and Darlington and Gairdner (1938) for the two-ring systems in *Oenothera biennis* and *Campanula persicifolia* respectively.

An observation of considerable interest is the variability exhibited between rings of similar size with respect to their meiotic regularity. Thus, in the Muntagin population, there is heterogeneity within the sample of three

plants studied, two of the plants being much more regular than the third. Furthermore, the 58.8 per cent. disjunction characterising the single $\odot_8$ of the Pigeon Rock complex heterozygote would suggest that two such rings would be together disjunctional in 34.6 per cent. of cells.

This value agrees well with the behaviour of the less regular Muntagin $2\odot_8$ (29.8 per cent. disjunction), but is doubtfully comparable with that of the two more regular Muntagin $2\odot_8$'s (58.3 and 51.3 per cent. disjunction respectively) and the Berringbooding $2\odot_8$ (65 per cent. disjunction).

(c) *Seed abortion.* Examination of the contents of capsules derived by selfing various complex heterozygotes has yielded some useful information concerning properties of the balanced lethal mechanism. Results of a selection of such analyses are presented in table 3. Several classes of inclusions

TABLE 3

Capsule contents of Isotoma petraea complex heterozygotes following self pollination

Race	Capsule no.	No. of seeds normal	No. of aborted seeds	
			Late death	Early death
$\odot_8$ Pigeon Rock	1*	63	37	16
	2*	133	61	49
	3	182	152	
$\odot_{13}$ Bencubbin	1*	231	64	157
	2*	213	63	142
	3	220	179	
$\odot_{10}$ 12 m. W. of Bullfinch	1	198	131	49
	2	218	140	56
	3	204	125	44
$\odot_{10}$ 3-mile Rock	1	306	73	50
	2	213	94	29
	3	290	50	45
$\odot_{14}$ Merredin	1	188	24	39
	2	176	30	31
	3	196	39	20

* Data from Beltran (1970).

may be discerned within the capsule, namely sterile ovules, normal seeds and partly developed seeds. The sterile ovules are very small, white, flattened and tissue-like when the capsule is mature. Such ovules may be expected from either failure to receive a pollen tube (inadequate pollination) or to genetic imbalance of its megaspore. Non-disjunction in the EMC would, of course, contribute to this imbalance. The normal seeds have a characteristic brown pitted testa, and contain a moist oily endosperm and a distinct embryo. The partly developed seeds fail to reach normal mature development and form an almost complete series of intermediate appearances from that of the unfertilised ovule to that of the mature seed. The partly developed seeds are classifiable as "late death" or "early death" according as to whether testa differentiation is apparent or not. The most thoroughly formed "late death" seeds may be very similar to normal seeds except that they are quite hollow and their testas possess a characteristic crystalline

appearance making them readily identifiable. The most extreme "early death" seeds may not develop far beyond the "sterile ovule" appearance; however, they characteristically have a brownish colour, which may be restricted to the micropylar region, and they show no differentiation of the testa.

As can be seen from table 3 the Pigeon Rock $\odot_6$ produces normal and aborted seeds in approximately equal numbers on selfing. More than half of the aborted seeds are of the late death type, and are quite large, approximating the normally developed seeds in size. Many of them are flattened, but quite a large proportion are normally swollen; these are, however, devoid of endosperm and embryo.

The $\odot_{12}$ Bencubbin selfed capsules are characterised by early deaths, and nearly 50 per cent. of ovules showing any development at all are of this class. The $\odot_{10}$ 12 m. west of Bullfinch is more or less intermediate between $\odot_6$ Pigeon Rock and $\odot_{12}$ Bencubbin with respect to capsule contents.

TABLE 4

Composition of the 3-mile Rock population. The sample represents at least 90 per cent. of the population

Number of Plants showing			
High pollen sterility	Low pollen sterility cytologically analysed as		
	$\odot_4$	7II	Not determined
117	6	2	1

Although the frequencies of the various inclusions are comparable in the $\odot_{10}$ and the $\odot_6$, the late death seeds of the $\odot_{10}$ are considerably smaller than those characterising $\odot_6$ Pigeon Rock. In $\odot_{10}$ 3-mile Rock, and $\odot_{14}$ Merredin, the aborted seed class constitutes much less than 50 per cent. of the fraction showing any development, and it is probable that many of the complex homozygous seeds abort to yield products indistinguishable from sterile ovules in these hybrids, but this point has yet to be resolved. It would appear that as the ring enlarges, the lethal system becomes more efficient leading to cessation of development of the complex homozygous combinations at progressively earlier stages. That is, the efficiency of the balanced lethal system increases with increasing ring size.

(d) *Heterogeneity in the 3-mile Rock population.* As well as $\odot_{10}$ complex heterozygotes, structural homozygotes were known to occur in the 3-mile Rock population (James, 1965). Since some $\odot_{10}$'s when crossed to the primitive homozygote ("axillaris" karyotype) yielded $\odot_{10}$ and 7II twin hybrids while others yielded $\odot_{10}$ and $\odot_4$ twin hybrids, it was assumed (*loc. cit.*) that there were two types of $\odot_{10}$'s present in the population, one of them being associated with a redundant interchange.

The population was sampled again, initially by observation of the pollen, and it was found that approximately 8 per cent. of the plants in the population were of low pollen sterility. Cytological analysis showed that the majority of these plants were $\odot_4$'s (table 4).

In order to examine the possibility that the 7II's and $\odot_4$'s might be generated by the complex heterozygotes, a sample of 11 plants, all $\odot_{10}$'s,

were established by means of stem cuttings in the glasshouse; these plants were selfed and one of them, 3MR-1, was crossed to the other ten. Ten progeny plants from each family were scored by means of pollen examination. Low sterility plants occurred in the progeny of 3MR-1 $\times$ 3MR-2 and 3MR-2 selfed, and two of these (from the cross) proved to be $\odot_4$'s. Thus, the association of particular lethals with particular complexes (as defined by their interchange constitution) is not constant within the population. Some alethal complexes must still exist or can be generated within the population, or else the various lethals are transferrable in some plants, at least, from one complex (as defined by interchange constitution) to the other.

An instructive cross is that involving the primitive alethal homozygote 7II495-3 and $\odot_{10}$ 3MR-8. Of the 24 plants from this cross which were analysed cytologically, 10 were $\odot_{10}$'s, 9 were $\odot_4$'s and 5 were 7II's. Thus, recombination within a differential segment must be operative to yield a third gametic chromosome sequence in the parental $\odot_{10}$. A fourth sequence, giving rise to a $\odot_8$ in association with the primitive sequence is also predictable; its lack of occurrence may be due to its possible lethality as a gamete or merely to an insufficient sample size.

From these observations, it has been concluded that the cytological heterogeneity of the 3-mile Rock population is attributable to the cytological instability of the complex hybrids themselves, rather than to immigration or persistence of an ancestral form. The basis of the cytological instability, no doubt, is the occurrence of differential segments and recombinational events therein. It seems valid to conclude, therefore, that the 3-mile Rock $\odot_{10}$ is composed of one complex four interchanges removed from the primitive "axillaris" chromosome end sequence, and one complex identical with that sequence. Crossing-over in differential segments, however, is able to generate some variation away from this.

Further evidence for the occurrence of differential segments and of crossing-over therein has been obtained from cytological configurations observed at PMC meiosis in synthetic hybrids derived by crossing plants from different populations (plate I, fig. 7). However, as well as interpreting these configurations on the basis of crossing-over in differential segments, one can always suggest that fortuitous chromosome placements, and/or "stickiness" are involved, and hence, this cytological evidence is noted, but not stressed. The occurrence of triplet hybrids, in lieu of twin hybrids in $\odot_{10}$ 3MR-8 $\times$ 7II495-3 is however, stressed as proof of the existence of differential segments and of crossing over therein.

(e) *Relationship between the $\odot_8$ Pigeon Rock and $\odot_{10}$ 3-mile Rock.* The distribution of the complex hybrids with regard to their ring size, the minimal numbers of interchanges involved in the construction of the rings and the increasing efficiency of the lethal system associated with increasing ring size suggests that the large rings have been formed through a process of fixing interchanges in a heterozygous condition, and in a sequential manner (James, 1965). Thus, the $\odot_8$ Pigeon Rock must, on this hypothesis, represent a more primitive complex heterozygote than does $\odot_{10}$ 3-mile Rock and, presumably, the $\odot_8$ "grew up" into the $\odot_{10}$. Or, more precisely, these two rings must have had a common ancestor which may have been identical with the existing $\odot_8$ Pigeon Rock. Now, both these complex heterozygotes tend to have the new (interchange) arrangements "stacked" into one complex, the other being free (or almost free) of interchanges. Thus, the $\odot_8$

Pigeon Rock is composed of one complex identical with the primitive "axillaris" sequence, and a second two interchanges removed from that sequence (James, 1965). Likewise, as has already been discussed, the $\odot_{10}$ 3-mile Rock consists of two complexes, one identical with the "axillaris" sequence, the second being removed from it by four interchanges.

We might expect, therefore, that if there is indeed an evolutionary relationship between the two complex heterozygotes, the complexes free of interchanges should be related by descent, while the complexes containing the interchanges likewise should be related by descent. Further, we might expect also that any lethal included in the complex free of interchanges in $\odot_6$ Pigeon Rock should be included (though perhaps functioning more efficiently) in the complex free of interchanges in $\odot_{10}$ 3-mile Rock. We might expect a similar relationship in the lethal content of the complexes containing the interchanges in these same two complex hybrids. However, this seems not to be so. One of the twin hybrids derived by crossing $\odot_{10}$ 3-mile Rock with $\odot_6$ Pigeon Rock, and which was identified as a large ring on the basis of high pollen sterility but which was not characterised cytologically was tested by crossing to a primitive alethal 7II and it proved to be composed of the complexes two and four interchanges removed from the primitive sequence. Thus, these complexes, which include all the interchanges, and which are therefore probably related by descent, do not share a common lethal. The evolutionary pathway linking $\odot_6$ Pigeon Rock and $\odot_{10}$ 3-mile Rock includes mechanisms for discarding lethals, generating new ones, and/or for transferring lethals from one complex, as defined by interchange constitution, to the other.

(f) *Genetic architecture of the floating interchange populations.* It has been noted previously that the non-complex, or floating interchange, populations are composed of plants which are generally structurally homozygous but which include some 10 per cent. or so of interchange heterozygotes, usually $\odot_4$'s. Such populations are characterised by the same substantially autogamic pollination mechanism as is found in the complex hybrid populations. These two aspects of the genetic system would appear to be in conflict: the autogamic mechanism should work towards the elimination of heterozygosity, yet the occurrence of interchange heterozygosity may be taken as a marker of maintained genetic heterozygosity. The question thus arises: are the structurally homozygous plants genetically homozygous or heterozygous? In order to pursue this problem further, two populations which differed from each other with respect to flower colour were analysed in terms of both colour and vegetative growth. One of the populations, 495, proved to be uniformly blue flowered, while the other, Rainy Rocks, contained mostly pink flowers, though one small and localised group of blue-flowered plants was found in this population. The F_1 from the pink $\times$ blue interpopulational cross showed blue to be dominant and the F_2 (22 red : 63 blue) established that a single gene was involved. Thus, the Rainy Rocks population is composed essentially of recessive homozygotes with respect to this allelic difference. The few blue-flowered plants, being localised, could well be the progeny of a single immigrant plant from some nearby blue-flowered population. Parental selfed, F_1 and F_2 progenies derived from the original two stocks were grown under glasshouse conditions in a fully randomised plot and the tops (*i.e.* stems and leaves) harvested at three pre-flowering stages and individually dry weighted. The dry weights were expressed as

proportions of the total harvest weight so that the three harvests could be pooled. This pooled data has been expressed as histograms in fig. 3 and it is clear that the hybrids shows positive heterosis for dry weight of tops, a not insignificant component of fitness.

FIG. 3.—Heterosis for dry weight of tops of pre-flowering *Isotoma petraea* aethal structural homozygotes. The points and bars show the mean value and standard errors for the two parent strains, Rainy Rock and "495", the F_1 and the F_2 in relative units. The mid-parent value is shown by the vertical line.

If the results of these preliminary experiments can be regarded as representative, then it follows that:

1. Floating interchange populations are relatively homozygous for different arrays of genes, and
2. Secondary heterosis may be generated by the production of inter-population hybrids.

3. DISCUSSION

(a) *The role of interchanges.* In *Isotoma petraea*, chiasmata are rather strictly localised to the terminal segments of the chromosomes, though this localisation may break down to a perceptible degree in synthetic hybrids. Because this localisation is normally so pronounced, we may consider the bulk of the chromosomes to be internally free of recombination. By ignoring the effects of intra-chromosomal recombination, it is possible to regard the genetic system of *Isotoma petraea* as composed essentially of seven independently assorting entities which may, however, become associated by interchange.

Over and above the genetic effects due to the breakage and reassociation

of possibly significant molecular sequences, structural change has its most important function in influencing recombination. That is, the importance of chromosomal change concerns the "endophenotype" rather than the "exophenotype" (Lewis and John, 1963). Interchanges, in tying otherwise independently assorting chromosomes together, may be useful in two ways:

- (i) in holding adapted gene arrays together irrespective of the breeding system,
- (ii) in the pursuit of hybridity in the face of inbreeding.

In both situations, interchanges will be effective only if strong distal localisation of chiasmata is the rule, since crossing-over in interstitial segments will lead to genetic recombination in spite of the interchange heterozygosity. In *Isotoma petraea* strong distal localisation of chiasmata is the rule, and, as pointed out above, is assumed to be complete for the purposes of this argument. The interchange heterozygosity necessarily imposes a degree of sterility upon its carrier, because of the non-disjunction of adjacent homologous segments in the ring. This effect is minimised by the phenomenon of "directed" segregation which is enhanced by, among other things, the complete terminalisation of chiasmata. In this regard also, *Isotoma petraea* is well pre-adapted to make use of interchanges.

Interchanges and the pursuit of hybridity

Let us suppose that in *Isotoma petraea* (1) genetic heterozygosity and consequent heterosis is a component in the genetic system, and (2) the average number of seeds produced by a structurally homozygous plant is X . Then, with self pollination, the average number of seeds having genetic heterozygosity equivalent to that of the parent (*i.e.* maximum) is $2^{-7}X$ (bearing in mind that intrachromosomal recombination is ignored, and that $n = 7$).

In a single interchange hybrid, the average number of seeds would be reduced to dX where d is the frequency of disjunction at meiosis. Also, since the number of independently assorting units is reduced to six, the average number of seeds having maximum genetic heterozygosity would be $2^{-6}dX$, and these must all be structural heterozygotes.

The increase in the number of such seeds, as compared with the structural homozygotes, would be $\Delta X = 2^{-7}X(2d - 1)$.

The value of ΔX is positive provided d is greater than 0.5; *i.e.* provided segregation of the ring is directed.

In general, with autogamy, any new non-redundant interchange will increase the number of seeds produced which have genetic heterozygosity equivalent to the parent, provided that the acquisition of the new interchange does not affect the frequency of disjunction at meiosis by a factor equal to or less than 0.5. If heterosis is a component of fitness, such interchanges will be adaptive.

If we accept behaviour at PMC meiosis to reflect adequately that in the EMC, and preliminary data suggest that comparable frequencies of non-disjunction obtain in both sexes, then it is clear that the meiotic behaviour in the multiple interchange heterozygotes, though very poor, is still sufficiently regular to permit an effective pursuit of genetic hybridity in the selfed progeny (fig. 4).

FIG. 4.—Non-disjunction as a limitation on the role of interchange hybridity. The percentage disjunction for various rings (see table 2, (a)) is plotted as open circles with bars representing the 95 per cent. confidence interval for estimate. The lower curve (+-symbols) represents the minimum level of disjunction for rings of various sizes necessary to permit an effective pursuit of genetic hybridity in the selfed progeny and is based on the expression $d = (\frac{1}{2})^i$ where i is the minimum number of interchanges involved in the ring. The third curve (closed circle symbols) represents the minimum level of disjunction of rings of various sizes necessary to permit an effective maintenance of adapted gene arrays in interpopulational F_2 's and is based on the expression $d = [2 \times (\frac{3}{4})^m - (\frac{1}{2})^m]$ where m is the number of independently assorting entities ($m = 7 - i$).

Disjunctional behaviour is greater than the minimum requirement for the pursuit of hybridity but less than the minimum requirement for maintaining adapted gene arrays for all rings except perhaps for the O_4 .

Interchanges and the maintenance of adapted gene arrays

Let us suppose that the genomes of two separate autogamic, and therefore genetically homozygous, populations are each specifically and uniquely integrated to function as internally balanced unitary entities. If population (1) is homozygous for the genome $A_1B_1C_1D_1E_1F_1G_1$ while population (2) is homozygous for the genome $A_2B_2C_2D_2E_2F_2G_2$ then any rare interpopulational hybrid will be heterozygous $A_1A_2 B_1B_2 C_1C_2 D_1D_2 E_1E_2 F_1F_2 G_1G_2$. It is clear that, in the selfed progeny of these hybrids, only a small proportion of the offspring will have one or both genomes intact. In fact, if intrachromosome recombination is ignored, the number of seed produced which contain at least one parental genome is $X[2 \times (\frac{3}{4})^m - (\frac{1}{2})^m]$ where X is the seed potential and m is the number of independently assorting units at meiosis. Where $m = 1$, the number of such seeds is X ; and where $m = 7$, the number of such seeds is $0.2506X$.

It would seem that multiple interchange heterozygosity would again be useful here, in tying chromosomes together and thus reducing the number of independently assorting units. However, the effects of non-disjunction are again important. In *Isotoma petraea*, the $\odot_{14}$ has a frequency of disjunction of approximately 22.5 per cent. and because of this meiotic irregularity alone, the seed potential of such a plant is reduced to something below the yield of genetically adequate seeds that would be achieved, on the above assumptions, in a structural homozygote.

In 1931, Darlington outlined three possible methods by which complex hybridity could be evolved, and these methods are illustrated in fig. 5.

Darlington favoured the second method. He considered the other two methods, particularly the third, to be highly improbable, since natural selection would have been acting on the individual differentiated lines, adapting them, whereas their hybrids, not being the product of natural selection, would be less adapted. He conceded, however, that "where two hybrids are formed between gametes equally distinct physiologically, one having a potential ring of fourteen and the other a potential seven pairs, the former will be vastly more fertile than the latter. Ignoring non-disjunction . . . the former will produce two kinds of gametes, each identical with a viable and functional parental type; the latter will produce 2^7 types of gametes of which two will be of the viable and functional parental types, the rest (according to the rule, which we may make, that chance combinations are less successful than those which have survived natural selection) will be less successful". A consideration of the high degree of non-disjunction associated with multiple interchange heterozygosity denies even this concession to the biological utility of large rings of cataclysmic origin in *Isotoma petraea*. However, as its shown in fig. 4, the directed segregation of the small rings in *Isotoma petraea* is sufficient to allow such utility; and it is possible that in floating interchange populations the small rings may be adaptive in both the pursuit of hybridity and in the maintenance of adapted gene arrays.

(b) *The utility and consequences of two-ring complex heterozygosity.* The two-ring system characterises 8 of the 20 populations of complex heterozygotes so far examined. The high incidence of pure breeding two-ring complex heterozygote populations is a feature unique to the *Isotoma* situation. It is possible to deduce a plausible explanation for the existence of such plants.

If indeed the two complexes in a $2\odot_6$ hybrid are spread across six

chromosomes each, as was suggested earlier, being thereby arranged to give two meiotically independent rings with a consequent 50 per cent. loss of gametes through segregation effects in meiotically regular cells, the yield of functional gametes may still exceed that of a comparable $\odot_{12}$ complex heterozygote because of the much greater non-disjunction of the latter. Thus,

FIG. 5.—The three possible methods of $\odot_{14}$ origin (after Darlington, 1931). The symbols indicate interchange constitution and conform with the key provided to fig. 1, except that here the $\odot_4$ symbol (vertical half-bar) and the $\odot_8$ symbol (solid semicircle) are used. Solid connecting lines represent unchanged gametes; broken connecting lines represent new interchanges. Methods 1 and 3 depend upon outbreeding and hybridisation between segmentally differentiated lines to produce progressively larger rings (in 1) or a large ring at one stroke (in 3). Method 2 depends upon the sequential fixation of interchanges in the heterozygous condition as a response to inbreeding.

the $2\odot_8$ Muntagin has a disjunction frequency of 46.8 per cent.; it is just as fertile as a $\odot_{12}$ with 23.4 per cent. disjunction and more fertile than a $\odot_{12}$ with a lower frequency of disjunction. Indeed, the Berringbooding $2\odot_8$ has a gross disjunction frequency of 65 per cent., according to the estimate presented in table 2 (a) and, allowing for a 50 per cent. loss through genetical interdependence of the rings, a net disjunction frequency of 32.5 per cent. This is better than the 3-mile Rock $\odot_{10}$. The Berringbooding $2\odot_8$ may well have the advantages of a $\odot_{12}$, with regard to its maintained hybridity, but only the disadvantages of a $\odot_{10}$, with regard to its meiotic behaviour and

consequent ovule wastage. It seems reasonable to assume that if highly non-disjunctional rings could be broken down, by the incorporation of redundant interchanges into the system, to yield more regular but genetically interdependent small rings, then, other things being equal, selection would favour the more fertile form. On this basis, as high degrees of meiotic regularity are achieved in the larger rings, through the incorporation of interchanges conferring enhanced "mechanical stability" of the rings or through the acquisition of genetic backgrounds having a regularising effect on meiosis, then the possibility of the existence of two-ring complex heterozygotes diminishes. It is of interest to note that in *Oenothera*, in which the large rings are much more regular than in *Isotoma*, two-ring complex heterozygotes are virtually absent from natural populations.

(c) *The source of the hybridity being pursued in the Isotoma complex heterozygotes.* It has been suggested (James, 1965) that the peripheral occurrence of the complex heterozygotes in *Isotoma petraea* lend support to Darlington's (1958) view that "any species forced to inbreed at the edge of its range, and having the right type of chromosomes and of meiosis, will make use of interchange hybridity and fix it to preserve general hybridity and to make itself into a permanent hybrid". The suggestion was also made that in *Isotoma* the change to inbreeding had forced the species to either eliminate heterosis from its genetic system, or conserve it; and that complex hybridity was clearly a mechanism for preserving heterosis. The inference clearly made was that the heterosis, or hybridity, being preserved was an ancestral or primitive hybridity engendered by the primitive Lobeliaceous cross pollination mechanism. There is no doubt in the mind of the author that the normal out-crossing Lobeliaceae include heterosis as a component in their genetic systems, a point to be discussed in a future publication, and that the change to autogamy in *Isotoma petraea* must have been associated with compensating changes in genetic architecture. However, the preponderance of structural homozygotes in the autogamic floating interchange populations would suggest that in these populations heterosis has been effectively eliminated, although the beneficial effects of gene hybridity may well be reflected in the occurrence of a relatively high frequency of structural heterozygotes within the populations, and in the heterosis exhibited by the interpopulational hybrids. It is possible, therefore, that the hybridity being conserved in the complex hybrids is of secondary origin, and is capable of being generated by crossing relatively homozygous, but genetically dissimilar individuals, to yield heterotic interpopulational hybrids. Furthermore, if sequential interpopulational crossings and the conservation of this secondary heterosis by complex hybridity is invoked as the evolutionary strategy, then the combined resources of the various populations become available for the genesis of the system. It is no longer necessary to invoke an endogenous origin for the multiplicity of interchanges, or for the lethal modifying (improving) genes within single autogamic lineages. And this agrees very well with the progressive elaboration of the system, both in terms of the size of the complexes and in the efficiency of the lethal mechanism, as one moves from Pigeon Rock in a southwesterly direction through Western Australia. It may be argued that Darlington's criticism that such hybrids, not being the product of selection, would be less adapted than the parental lines, would be valid. However, if each existing complex hybrid represents the product of a cross between a native structural homozygote (or floating interchange hetero-

zygote) and an immigrant complex heterozygote, the former could supply an adapted genome, part of which would be preserved in the homozygous condition outside the ring, and the latter, a genome which engenders substantial heterosis in combination with the former, part of which will be preserved, along with the residue of the native genome, within the ring. In other words, the native would supply the necessary internally balanced genome of the inbreeder (Mather, 1943), while the immigrant genome confers a bonus of relational balance: the total balance being governed by the size of the ring.

On the basis of these speculative considerations the following model has been formulated to account for the evolution of complex hybridity in *Isotoma petraea*. In the model, mutational events are invoked but minimally. The routine operation of the genetic system, involving the rules of chromosome behaviour and of the breeding system as described above, together with natural selection and chance, provides all that is required to piece the system together. It must be stressed also, that it is a *model* which is described below, and the identification of chromosome segments and sequences is arbitrary and does not imply the existence of any such specifically identified sequence in nature. However, the model does reflect qualitatively the situation as it occurs in nature.

(d) *Description of the model.* The primitive "axillaris" chromosome end sequence occurs right across Australia, and predates the separation of *petraea* from the related species *axillaris* and *anaethifolia*. Although no evidence has been obtained for the fixation of new interchange arrangements in the homozygous condition, there is no need to suppose that interchange homozygotes never exist, or that interchanges are inherently recessive lethals. Recessive lethal interchanges could, however, be generated by the occurrence of two interchange events involving the same chromosomes, but different loci, and recombination between them thus:

Primitive: A 1 2.3 4 B C 5 6.7 8 D E 9 10.11 12 F ...

Interchange (1): A 1 2.3 8 D C 5 6.7 4 B

Interchange (2): A 1 2.7 8 D C 5 6.3 4 B

Plants heterozygous for both interchanges may yield recombinant gametes, e.g.

A 1 2.3 8 D C 5 6.3 4 B (i)

which is duplicate for segment 3 and deficient for segment 7.

Small duplications seem to be fairly readily tolerated by many organisms, and for the purposes of this discussion, they will be assumed to have no significant effect on viability. Deletions, on the other hand, frequently have profound effects, and for the purposes of this discussion, it will be assumed that such deletions are either recessive lethals or gametic lethals, depending upon the segments concerned. Let us assume here that segment 7 deficiency is a recessive lethal; genome (i) is thus a recessive lethal interchange arrangement.

Similarly, plants heterozygous for the interchanges

Interchange (3): C 5 6.7 11 12 F E 9 10.8 D

Interchange (4): C 5 10.11 12 F E 9 6.7 8 D

may yield the recombinant gamete

C 5 6.7 11 12 F E 9 6.7 8 D (ii)

which is duplicate 6, 7 and deficient 10, and may therefore be considered to include a recessive lethal gene.

The combination of (i) and (ii) yields a $\odot_6$ which will be true breeding on selfing, being stabilised by a balanced lethal system. Although the construction of such complex hybrids as the naturally occurring $\odot_6$ Pigeon Rock may have come about by sequential mutational events within a single autogamic lineage, it seems equally, if not more, likely that the necessary components could have been produced in separate lineages and combined by hybridisation. Although crossing is no doubt infrequent in *Isotoma petraea*, it is possible under natural conditions, particularly in cooler weather when the stigmatic surfaces may protrude from the anther tube. If the recessive lethal interchanges were put together by interpopulational hybridisation following dispersal, the product may have been heterotic as well. And by conserving at least part of this heterosis in the $\odot_6$, the newly formed complex hybrids and their autogamically produced progeny would have a selective advantage over the homozygous natives. The coincidence of these various events is no doubt highly improbable, but complex hybridity is itself a highly improbable genetic system, recorded in only some half a dozen groups of plants, and hence, its generation may well be due to the coincidence of a highly improbable set of circumstances.

The $\odot_6$ complex hybrid composed of the genomes (i) and (ii) includes two segments in triplicate, namely segments 3 and 6. Because of this, various synaptic patterns would be possible. Now, although distal localisation of chiasmata is pronounced in *Isotoma petraea*, crossing-over in the proximal regions is not entirely excluded, and indeed crossing-over in a differential segment must readily take place in the naturally occurring $\odot_{10}$ 3-mile Rock. Crossing over in differential segments will yield new chromosome end sequences, and the following illustrates some important consequences of this behaviour:

Genome (i): A 1 2.3 8 D C 5 6.3 4 B E 9 10.11 12 F

Genome (ii): A 1 2.3 4 B C 5 6.7 11 12 F E 9 6.7 8 D

Synaptic associations

and

yield the two recombinant gametes indicated with the bolder lines, namely

$$\underline{F\ 12\ 11\ 7.6\ 5\ C}\quad \underline{E\ 9\ 6.3\ 4\ B}\quad \underline{A\ 1\ 2.3\ 8\ D}\quad (iii)$$

which is duplicate 3, 6 and deficient 10, and

$$\underline{E\ 9\ 10\ 11.12\ F}\quad \underline{C\ 5\ 6.3\ 8\ D}\quad \underline{B\ 4\ 3.2\ 1\ A}\quad (iv)$$

which is duplicate 3 and deficient 7, which may be combined in this autogamic plant to yield a new complex heterozygote with one complex being equivalent to the primitive form with respect to chromosome end sequence, the other being two interchanges removed from the primitive "*axillaris*" sequence. This is the situation which obtains in $\odot_6$ Pigeon Rock.

It is of course possible that the lethal interchanges, genomes (i) and (ii), had no independent existence outside complex heterozygotes. There would clearly be strong selective pressure operating against such sequences occurring singly within autogamic lineages devoid of relational balance and, indeed, it is possible to generate lethals following the combination of three interchanges in an *appropriate* heterotic individual. Thus, the alethal interchanges, (2) and (4) defined above, may be associated to yield the synaptic arrangement

which may yield the recombinant genome

$$\underline{A\ 1\ 2.7\ 8\ D}\quad \underline{B\ 4\ 3.6\ 9\ E}\quad \underline{C\ 5\ 10.11\ 12\ F}$$

This alethal genome may become associated, in a heterotic interpopulation hybrid, with the alethal interchange

$$\underline{A\ 1\ 2.3\ 4\ B}\quad \underline{C\ 5\ 6.11\ 12\ F}\quad \underline{E\ 9\ 10.7\ 8\ D}$$

In this hybrid, the synaptic arrangement

may yield the two recombinant genomes

A 1 2.3 4 B C 5 10.7 8 D E 9 10.11 12 F

and

A 1 2.7 8 D B 4 3.6 9 E C 5 6.11 12 F

which, if united by autogamy, would yield a $\odot_8$ complex heterozygote comparable to the $\odot_8$ Pigeon Rock, for one complex is identical to the “*axillaris*” sequence and includes a recessive lethal, deficiency 6, while the other complex is two interchanges removed from “*axillaris*” and includes a different lethal, deficiency 10.

Now, the newly formed $\odot_8$ complex heterozygote may itself be dispersed to another floating interchange population and, since it maintains heterozygosity within the ring and is therefore heterotic and superior to the native homozygotes, it could become established. Furthermore, crossing could occur between this complex heterozygote and the natives, again yielding heterotic interpopulational hybrids which would then continue to propagate autogamically. Again, interchange heterozygosity would preserve genetic heterozygosity in the face of inbreeding, particularly if associated with recessive lethals. If the interpopulational cross involved the $\odot_8$ complex heterozygote and an alethal interchange, it is possible that $\odot_8$'s may result in the progeny. The possibilities here are interesting too.

Consider the combination of (iii), which is two interchanges removed from the primitive sequence and includes duplications for segments 3 and 6, and is deficient for segment 10, and a new alethal interchange, *e.g.*,

Interchange (5): A 1 14.15 16 H B 4 3.2 13 G

The following are two of the several synaptic associations possible:

and these may yield the recombinant gametes indicated by the bolder lines, namely,

A 1 14.15 16 H G 13 2.3 4 B D 8 3.6 9 E F 12 11 7.6 5 C (v)

and

A 1 2.3 8 D G 13 14.15 16 H B 4 3.6 5 C E 9 10.11 12 F (vi)

which may be combined together by autogamous reproduction of the original interpopulational hybrid. Now, since (v) is deficient for segment 10, and (vi) is deficient for 7, this derived $\odot_8$ is a true breeding complex hybrid.

Note that the deficiency for 7 has been *generated* in the interpopulational $\odot_8$ hybrid; it was not introduced with the original combination of gametes.

Further interpopulational hybridisations could in a similar fashion continue to expand the ring by a process of interchange capture. Of course, interchanges which do not increase the ring size, *i.e.*, redundant interchanges, may also be captured, and these lead to the genesis of *new* lethals. For example, if the genome (v) was combined, by hybridisation, with the alethal arrangement

Interchange (6): A 1 2.3 D C 5 6.7 8 4 B

the following synapctic arrangement

could yield the recombinant gamete indicated, namely

F 12.11 7.6 5 C B 4 3.6 9 E G 13 14.15 16 H A 1 2.3 D (vii)

which is duplicate 3, 6 and deficient 8, 10. This second deficiency, of segment 8, may also act as a recessive lethal, and the two lethals together could well be expected to operate earlier in the post-fertilisation development of the homozygote than does deficiency 10 alone. Thus, with this model, there exists a built-in mechanism for the improvement of lethal efficiency, and, in nature, it is found that improved lethal efficiency is associated with increasing ring size in the complex heterozygotes.

A second synapctic arrangement possible in this (v)/Interchange (6) hybrid, *viz.*

yields the recombinant gamete

F 12 11 7.6 5 C E 9 6.7 8 4 B G 13 14.15 16 H A 1 2.3 D (viii)

which is duplicate 6, 7 and deficient for 10. It is interesting to note that this combination, which has the less efficient lethal, deficiency 10 only, has exactly the same end sequence as (vii). Where both such genomes exist in a population, it is reasonable to assume that, other things being equal, genome (vii) would have a selective advantage over (viii) since, with the earlier termination of the post fertilisation development in the (vii)/(vii) homozygotes, the carriers of this complex expend less energy in the construction of non-profitable abortive products.

If genome (vii) is combined, by interpopulational crossing, with an alethal interchange such as

Interchange (7): E 9 10.13 G H 16 15.14 11 12 F

the synaptic arrangement

may yield the recombinant gamete

$$\underline{A\ 1\ 2.3\ 4\ B}\ \underline{C\ 5\ 6.7\ 8\ D}\ \underline{E\ 9\ 6.7\ 11\ 12\ F}\ \underline{G\ 13\ 14.15\ 16\ H}\quad (\text{ix})$$

which has the primitive chromosome end sequence, as does genome (iv), but, unlike genome (iv) which is deficient for segment 7, this genome, (ix), is deficient for segment 10, as is genome (iii). Thus, if we take the combination of genomes (iii) and (iv) to yield a complex hybrid similar to the naturally occurring $\odot_6$ Pigeon Rock (see above), then by a series of interchange captures and recombinations, we have reconstituted one of the Pigeon Rock complexes, as far as end sequence is concerned, but now with the lethal characteristic of the other.

As has been pointed out above, after a consideration of the association of interchanges and lethals in the complexes of $\odot_6$ Pigeon Rock and $\odot_{10}$ 3-mile Rock, the evolutionary mechanism operative in the elaboration of complex hybrids in *Isotoma* must include a mechanism for discarding, generating and/or transferring lethals from one complex to another (if we define the complexes in terms of their interchange constitution), and such a mechanism is available in this model.

Finally, the genome

$$\underline{A\ 1\ 14.15\ 16\ H}\ \underline{G\ 13\ 2.3\ 8\ D}\ \underline{B\ 4\ 3.6\ 9\ E}\ \underline{F\ 12\ 11\ 7.6\ 5\ C}\quad (\text{x})$$

may be constructed in the hybrid involving genome (v) and the alethal interchange (6) through recombination in a synaptic association figured previously, and may be perpetuated in a complex heterozygote. If this genome becomes associated, through hybridisation, with the alethal genome

$$\text{Interchange (8): } \underline{A\ 1\ 2.3\ 4\ H}\ \underline{G\ 13\ 14.15\ 16\ B}$$

a ring of six association will result, for these genomes have the chromosome A.H in common. However, the middle segments of these chromosomes are quite distinct. Since the bivalent and the $\odot_6$ are, for the most part, meiotically independent, four different gametes may normally be produced. These are the two parental genomes, (x) and interchange (8), and the recombinants

$$\underline{A\ 1\ 2.3\ 4\ H}\ \underline{G\ 13\ 2.3\ 8\ D}\ \underline{B\ 4\ 3.6\ 9\ E}\ \underline{F\ 12\ 11\ 7.6\ 5\ C}\quad (\text{xi})$$

and

$$\underline{A\ 1\ 14.15\ 16\ H}\ \underline{G\ 13\ 14.15\ 16\ B}\ \underline{C\ 5\ 6.7\ 8\ D}\ \underline{E\ 9\ 10.11\ 12\ F}\quad (\text{xii})$$

Note, however, that the recombinant genomes are very strongly duplicate/deficient. Thus, (xi) is deficient for segments 14, 15 and 16 as well as 10, while (xii) is deficient for segments 2, 3 and 4. If any of these segments include genes which are necessary for gametophyte development (*e.g.* genes concerned in mitotic activity), the spores bearing the genomes would be unable to function. Thus, if these deficiencies are accepted as gametic

lethals, the parental gametes only would be viable. That is, although the $\odot_6$ and the bivalent are meiotically independent, they are genetically interdependent, and only those meiocytes in which the two associations are oriented the same way would give rise to viable products. The interchange (8) genome is alethal. However, the recessive lethal, deficiency 7, can be regenerated and a properly balanced lethal system reconstituted by association of the genomes

A 1 2.3 4 H B 16 15.14 13 G C 5 6.7 11 12 F D 8 7.6 9 E (xiii)

which is deficient 10, and

A 1 14.15 16 H B 4 3.6 5 C E 9 10.11 12 F G 13 2.3 8 D (xiv)

which is deficient 7, both being produced by recombination in the following synaptic association possible in the (x)/interchange (8) heterozygote.

It is clear that larger genetically interdependent rings may be formed by the association of appropriate interchanges and their recombination to yield complex heterozygotes such as the $\odot_6 + \odot_4$, $2\odot_6$ and other such types as occur naturally in *Isotoma petraea*.

Continued evolution along these lines would progressively build up the ring size until all the chromosomes become incorporated into the complexes. Thus, following each interpopulational hybridisation, a variety of unstable autogamic lineages will be initiated. The instability would come about because of the synaptic possibilities associated with the duplicated segments scattered throughout the differential segments of the immigrant complex hybrid genome. Autogamy would thus lead to the segregation of a variety of lines, and of these, the most fit would ultimately displace the others. Here, fitness includes general vigour, determined by the heterotic interaction of the internally balanced native genome and the genetically dissimilar immigrant genome, fertility, determined largely by meiotic regularity, and economy in terms of minimal photosynthate expenditure on abortive seeds. Thus, the resultant complex hybrid would be expected to be considerably superior to the initial interpopulational hybrid in many ways, including meiotic regularity: the two complexes combined in the resultant hybrid exhibiting a maximum co-adaptation in terms of both their genetical and cytological interactions. The meiotic instability characterising synthetic interpopulational hybrids, on this basis, would reflect an expected lack of cytological co-adaptation, and this in itself, would be an adaptive feature of the system.

4. SUMMARY

In this and a preceding paper, significant aspects of the genetic systems of *Isotoma petraea* have been defined. These include:

1. In Western Australia, the species occurs in small, isolated populations between which migration and gene exchange must be limited, though obviously possible.

2. Primitive cross-pollination has been replaced by autogamy, although a residual capacity for crossing obtains.

3. The individual populations are relatively homozygous for different gene arrays so that interpopulational hybrids may exhibit heterosis.

4. The species is cytologically pre-adapted to make use of interchange in that distal localisation and terminalisation of chiasmata is pronounced.

5. Interchange heterozygotes occur in virtually all populations of *petraea* and of the related species *axillaris* and *anaethifolia*, and interchange constitutes a longstanding component in their genetic systems.

6. Interchange points are randomly distributed along the chromosomes.

7. The three species possess a common and primitive chromosome end sequence which has not been replaced to any significant degree by any other arrangement in the homozygous condition.

8. Complex hybridity has evolved in the southwestern corner of the species range, by a process of sequential fixation of interchanges, in the heterozygous condition, upon the primitive chromosome end sequence.

9. Meiosis in the complex hybrids is highly irregular, the large rings exhibiting non-disjunction in some 80 per cent. of PMC's, and comparable behaviour occurs in the EMC's.

10. Two-ring complex heterozygotes occur in which the rings exhibit genetic interdependence. It is suggested that in such hybrids, spreading the complexes over two rings results in enhanced fertility through greater meiotic regularity.

11. Instability in one complex hybrid population has been shown to be dependent upon the existence of differential segments and of frequent crossing-over therein. Synthetic interpopulational hybrids frequently exhibit cytological configurations interpretable as resulting from crossing-over in differential segments.

12. The association of lethals with chromosome end arrangements in the complexes is not always as would be expected. The same lethal may or may not be associated with complexes of comparable interchange constitution.

13. The efficiency of the lethal system is positively correlated with ring size.

A model is presented which suggests that, providing chance associates a requisite sequence of interchanges together in a heterotic individual, the action of the genetic system and chance will inevitably bring about the evolution of $\odot_{14}$ complex heterozygotes via a sequence of intermediate types comparable to those which have been described in the species.

Acknowledgments.—This paper includes materials submitted, in a rather modified form, as part of a Ph.D. Thesis to the University of Sydney. The bulk of the work has been supported by University of Western Australia Research Grants and by Australian Research Grants; the author is grateful to the committees administering these funds. The author also wishes to thank Messrs J. Courtney, R. Cohen and R. Harrison for their technical assistance at various times and to Dr B. John (Birmingham, England) for his helpful criticism of the draft manuscript.

5. REFERENCES

- BELTRAN, I. C. 1970. Embryology of *Isotoma petraea* and the operation of lethals in two complex hybrids. (In preparation.)
 CLELAND, R. E. 1936. Some aspects of the cytogenetics of *Oenothera*. *Bot. Rev.*, 2, 316-348.
 CLELAND, R. E. 1960. A case history of evolution. *Proc. Indiana Acad. Sci.*, 69, 51-64.
 CLELAND, R. E. 1962. The cytogenetics of *Oenothera*. *Adv. in Genetics*, 11, 147-237.

- DARLINGTON, C. D. 1931. The cytological theory of inheritance in *Oenothera*. *Jour. Genet.*, 24, 405-474.
- DARLINGTON, D. C. 1958. *The Evolution of Genetic Systems*. Oliver and Boyd, London.
- DARLINGTON, C. D., AND GAIRDNER, A. E. 1938. The variation system in *Campanula persicifolia*. *Jour. Genet.*, 35, 97-128.
- EMERSON, S. 1936. A genetic and cytological analysis of *Oenothera pratincola* and one of its revolute leaved mutations. *Jour. Genet.*, 32, 314-342.
- HEITZ, E. 1928. Das heterochromatin der Moose, I. *Jahrb. wiss. Bot.*, 69, 762-818.
- JAMES, S. H. 1965. Complex hybridity in *Isotoma petraea*. I. The occurrence of interchange heterozygosity, autogamy and a balanced lethal system. *Heredity*, 20, 341-353.
- LEWIS, K. R., AND JOHN, B. 1963. *Chromosome Marker*. J. & H. Churchill Ltd., London.
- LIMA-DE-FARIA, A. 1956. The role of the kinctochore in chromosome organisation. *Hereditas*, 42, 85-160.
- MATHER, K. 1943. Polygenic inheritance and natural selection. *Biol. Review*, 18, 36-64.
- STEINER, E. 1956. New aspects of the balanced lethal mechanism in *Oenothera*. *Genetics*, 41, 486-500.
- STEINER, E. 1957. Further evidence of an incompatibility allele system in the complex heterozygotes of *Oenothera*. *Amer. J. Bot.*, 44, 582-585.
- STEINER, E. 1961. Incompatibility in the complex heterozygotes of *Oenothera*. *Genetics*, 46, 301-315.