Complex hybridity in *Isotoma petraea*. VII. Assembly of the genetic system in the O₆ Pigeon Rock population

S. H. James,
J. F. Sampson and
J. Playford*

Botany Department, The University of Western
Australia, Nedlands, Western Australia 6009.

The Pigeon Rock population of *Isotoma petraea* is highly inbreeding and it is polymorphic with respect to interchange hybridity, allozyme variation and lethal system components. Two thirds of the plants in this population are O₆ interchange heterozygotes and may be considered as N.S complex heterozygotes. The N complexes are two interchanges removed from the standard “axillaris” chromosome end sequence characteristic of *Isotoma* structural homozygotes and they carry the PGM 3 allele. Most of the N complexes are non-transmissible through the pollen and where not so, carry a recessive lethal factor. The associated S complexes in the O₆ interchange heterozygotes carry the standard chromosome end sequence and either PGM 1 or PGM 2. It is suggested that in highly inbreeding species with terminally localized chiasmata the occurrence of balanced deleterious recessives within supergenes results in permanent hybridity and that the yield of fully heterozygous offspring must be very low, following selfing, or very occasional, following rare cross pollination events. Deterministic models incorporating the above observations and deductions indicate that O₆ hybrids should occur at a frequency of approximately 66 per cent under a regime involving approximately 98 per cent recurrent selfing irrespective of whether the N complex is transmissible or not transmissible in the pollen. These values are comparable to those actually found in the Pigeon Rock population. It is concluded that complex hybridity in *I. petraea* had its origin in the Pigeon Rock population, and arose within highly inbreeding lineages in which mutations contributing to an increasingly efficient transmission of permanently heterozygous parental genotypes were selected.

INTRODUCTION

Isotoma petraea (Angiospermae: Lobeliaceae) occurs on granite rocks throughout the Ereman Province of Australia. In its most south westerly populations, the species is characterised by complex hybridity, a diploid genetic system which combines autogamy, multiple interchange hybridity and a balanced lethal system (James, 1965, 1970). This genetic system enables the transmission of a highly heterozygous genotype from parent to sexually produced offspring with high fidelity. The evolution of complex hybridity is readily interpreted as a response to inbreeding in isolated populations (Darlington, 1931; James, 1965, 1970; Carson, 1967; Lewis and John, 1963). Other explanations based on the maintenance of coadapted gene arrays are possible (Cleland, 1972;

Holsinger and Ellstrand, 1983), but unlikely in this case because of the high levels of meiotic non-disjunction associated with multiple interchange hybridity in *Isotoma* (James, 1970).

The Pigeon Rock population of *I. petraea* is thought to be the site of the evolutionary origin of complex hybridity in this species (James, 1965, 1970; Beltran, 1971). The species has a chromosome number of $2n = 14$. It is preadapted to exploit interchange mutations in its populations in that it regularly produces a full complement of terminally localized chiasmata at meiosis. The Pigeon Rock population is cytologically polymorphic and includes a large proportion of true breeding ring-of-six (O₆) interchange heterozygotes. All the Pigeon Rock O₆ complex heterozygotes which have been tested combined one complex two interchanges removed from the standard “axillaris” chromosome end sequence with a complex identical to that sequence. The “axillaris” sequence is the only chromosome end sequence which has been found in *I. petraea* structural homozygotes,

* Present address: Molecular and Population Genetics Group, Research School of Biological Sciences, The Australian National University, Canberra, A.C.T., Australia 2601.

including those of the Pigeon Rock population, and it occurs also in the species' eastern Australian close relatives, namely *I. axillaris* and *I. anaethifolia*. Preliminary analysis also indicated that the Pigeon Rock O₆'s were always PGM 1/3 while the 7II's were PGM 1/1 (James *et al.*, 1983), suggesting that the PGM allozymes may be appropriate markers of chromosome arrangements in this population.

This paper reports on the breeding system of the Pigeon Rock *I. petraea* in terms of stigma protrusion frequencies, and on cytotype and PGM allozyme frequency distributions in the population and in progeny analyses. Deterministic models which incorporate significant population parameters deriving from this study are described.

MATERIALS AND METHODS

Pigeon Rock (29°55' S, 119°16' E) is a large granite outcrop approximately 145 km north of Southern Cross in Western Australia. The population was sampled on nine different occasions during the period 1960 to 1987 and the plants classified by cytological and/or pollen fertility analysis. Three pollen fertility classes exist in the population, corresponding to three cytological forms. Structural homozygotes (7II) exhibit more than 90 per cent stainable pollen. O₆'s exhibit from 60–85 per cent stainable pollen (mean 73 per cent) while O₁₀'s exhibit less than 60 per cent stainable pollen, pollen sterility being due to non-disjunctional chromosome behaviour at meiosis (James, 1970). Potential misclassification of cytotype on the basis of pollen fertility analysis occurs with a frequency less than 1 per cent and is then due to the presence of non-staining or poorly staining pollen as a result of genetic segregation rather than cytological causes.

Sample rootstocks collected in 1982, 1983 and 1987 were transferred and grown under 15 hour day conditions in the glasshouse at The University of Western Australia.

In order to assess the pollination mechanism, the behaviour of flowers on plants of the 1983 and 1987 collections was observed with respect to stigma protrusion through the anther tube under glasshouse conditions. Flowers carrying dried and withered corolla remains were classified according to whether the anther tube had remained intact or split but with no protrusion of the stigma (fig. 3(e) in James, 1965), or whether the anther tube was split and either one or both lobes of the stigma protruded (fig 3(c), (d) in James, 1965).

Selfed progenies were derived from flowers in which the stigma lobes did not protrude and the anther tube remained intact. Controlled cross pollinations were conducted using emasculated flowers covered with short waxed drinking straws as females. The Pigeon Rock materials were often tested against alethal structural homozygotes from the Yackeyackine Soak population (also called "495") which were known to be of the primitive "axillaris" chromosome end sequence (James, 1965). The natural dormancy period of the seed was broken by 24 h imbibition in 1000 ppm gibberellic acid (GA 3) solution.

Cytological and pollen fertility analyses of progenies were conducted on selected plants as they came into flower.

PGM isoenzyme analysis of single young unopened flower buds or whole young seedlings at the two to four leaf stage was conducted using the techniques described in James *et al.* (1983).

OBSERVATIONS

Population composition

I. petraea is an herbaceous perennial which can die down to a rootstock each year and resprout. Plants growing in rock fissures and other protected areas are able to aestivate over drought years with minimal growth. The population varied in number from only a few to thousands of flowering individuals in different years. In 1960, a luxuriant population thrived around the apron of the rock but such abundance has not been seen since. The Pigeon Rock population samples were found to include structural homozygotes (7II) and inter-

Table 1 Cytotypic components in Pigeon Rock *I. petraea* population samples. * = the collection for the year indicated was only a sample of the material available; other data, from Beltran (1971), counted all the flowering specimens that could be found

Year	Number of plants			Total	Per cent O ₆
	O ₆	O ₁₀	7II		
1960*	13			13	
1966*	2		3	5	
1967	45		9	54	83.3
1968	7	1	5	13	53.8
1969	1			1	
1970	50	1	14	65	76.9
1982*	12		11	23	52.2
1983*	75		50	125	60.0
1987*	30	2	23	55	54.5
Total	235	4	115	354	66.4

change heterozygotes (O_6 's and O_{10} 's). Considerable variation in the proportions of the three cytotypes occurred amongst the samples collected during the 1960–87 period. Of 354 plants examined, 32.5 per cent were 7II's, 1.1 per cent O_{10} 's and 66.4 per cent O_6 's (table 1).

Breeding behaviour

Both the structural homozygotes and the O_6 interchange heterozygotes were highly inbreeding (table 2), but stigma protrusion was significantly more frequent in the former (6.2 per cent) than in the latter (3.0 percent) ($\chi^2_{(1)} = 7.8$, $P < 0.01$).

The two O_{10} interchange heterozygotes, however, exhibited a much greater frequency of stigma protrusion (40.0 per cent) than the 7II's or O_6 s.

PGM allozyme polymorphism

Three alleles at the PGM locus were found although PGM 2 had not been recorded in a survey reported earlier (James *et al.*, 1983). The genetic composition of the 7II's and the interchange heterozygotes were distinctly different (table 3). All the interchange heterozygotes were heterozygous for the PGM 3 allele. The 116 O_6 's assayed included 104 PGM 1/3 and 12 PGM 2/3 heterozygotes. Both O_{10} 's assayed were PGM 2/3. No PGM 3 homozygotes occurred in the population, and the PGM 3 allele did not occur in any structural homozygote. The 7II's were polymorphic for PGM 1 and PGM 2. The frequency of PGM heterozygotes amongst the 7II's was significantly less than

Hardy-Weinberg expectations ($\chi^2_{(1)} = 54$, $P < 0.001$) and the value of the Fixation Index (F ; Wright, 1969) for the PGM locus in this cytotype was 0.81.

Progeny analyses

The distributions of PGM genotypes in progeny arrays of usually 20 individuals (range 4–41) from a selection of selfs and crosses involving various Pigeon Rock structural heterozygotes are summarised in table 4. On testing by pollen stainability or cytological analysis, all progeny plants which were PGM 3 heterozygotes were found to be structurally heterozygous while their siblings, not carrying PGM 3, were structurally homozygous. PGM 1/1 or PGM 2/2 homozygotes occurred in 17 of 34 selfed progeny arrays. PGM 3/3 homozygotes did not occur in any of the 34 selfed progeny arrays, in any of the 24 crossed progeny arrays which variously associated 21 PGM 1/3 or PGM 2/3 O_6 heterozygotes, nor in either of two (O_6 (PGM 1/3) $\times$ O_{10} (PGM 2/3)) progeny arrays. When Pigeon Rock O_6 's were crossed to 7II's from Yackeyackine Soak, O_6 and 7II siblings were produced. The O_6 's exclusively carried the PGM 3 allele derived from the Pigeon Rock O_6 . The PGM 3 allele was not transmitted through the pollen in five of six plants tested. In the sixth, the PGM 3 allele was recovered in four of 20 testcross progeny. The PGM 3 allele was freely transmitted through the egg in six plants tested. No evidence of non-transmissibility of other PGM alleles was observed.

Allozymically differentiated twin hybrids were also produced in crosses of the Pigeon Rock O_{10} 's to the Yackeyackine Soak 7II's, but in this case,

Table 2 Breeding behaviour in Pigeon Rock *I. petraea* plants transferred to glasshouse conditions at The University of Western Australia

	No. of plants	No. of flowers	Percentage of flowers with enclosed stigma
7II	43	790	93.8
O_6	72	2283	97.0
O_{10}	2	50	60.0

Table 3 PGM allozyme genotypes in Pigeon Rock *I. petraea* cytotypes

Cytotype	Number of plants with PGM genotype					
	1/1	1/2	2/2	1/3	2/3	3/3
7II	57	6	19			
O_6				104	12	
O_{10}					2	

Table 4 Summary of PGM isoenzyme analyses of Pigeon Rock *I. petraea* selfed and crossed progenies. * = PGM 1 or PGM 2. The vacant column in this table emphasises that no PGM 3 homozygote has ever been found in any progeny. # = Data provided by Mr W. Rochester

Type of progeny	Number of progenies including PGM genotypes			
	3/3 3/* */*	3/* */*	3/* */*	*/*
O_6 (PGM 3/*) Selfed		15	17	
O_{10} (PGM 3/*) Selfed		2		
O_6 (PGM 3/*) $\times$ O_6 (PGM 3/*)		20	4	
O_{10} (PGM 3/*) $\times$ O_6 (PGM 3/*)		2		
7II (PGM*/*) $\times$ O_6 (PGM3/*)		1		5
O_6 (PGM 3/*) $\times$ 7II (PGM*/*)		6		
O_{10} (PGM 3/*) $\times$ 7II (PGM*/*)		1*		

both were O_6 's. O_6 's in progenies derived from the Pigeon Rock O_{10} 's provide the only instances of structural heterozygotes not carrying the PGM 3 allele observed in these materials.

DISCUSSION

The chromosome arrangement marked by the PGM 3 allele may be defined as the "N" complex. Non-recovery of the PGM allele in some crosses indicates that the N complex is usually not transmissible in the pollen, that is, it carries a pollen lethal. However, this is not true for some plants, but since no PGM 3 homozygotes occurred in the population samples nor in any of the progenies observed, it may be concluded that the N complex also carries a recessive zygotic lethal gene. All N complexes tested were found to be two interchanges removed from the standard "axillaris" chromosome end sequence. Apparently, all N complexes share a common chromosome end arrangement, the common PGM 3 allozyme marker and similar lethal system components. It is probable that they had a monophyletic origin in which the three common elements of the complexes were associated in a single series of mutational events.

The N complex was associated with a complementary "S" complex in the interchange heterozygotes. The S complex carried either PGM 1 or PGM 2. In the O_6 's tested, the S complex was always of the standard sequence and, in its most primitive form, may be considered to have been a standard ax genome. The extant S complexes may be considered to be evolved ax genomes which have coadapted with the N complexes with which they are associated. In the O_{10} 's, this genome was two interchanges removed from standard and may be designated S^* . Some S complexes carried recessive lethals which eliminated the S.S homozygotes from selfed progenies while others did not.

No evidence of non-transmissibility of the S complex has been obtained. The Pigeon Rock structural heterozygotes are therefore N.S complex hybrids, heterozygous for multiple interchanges and for balanced or unbalanced lethal systems which may be composed of both gametic and zygotic lethal elements.

Complex hybridity in plants is invariably associated with inbreeding. A very high level of inbreeding in the Pigeon Rock *I. petraea* population is indicated by its pollination mechanism. The stigmas protrude from only a small proportion of the flowers on these plants. The frequency of flowers which do not protrude the stigma must

represent a minimum estimate of the frequency of recurrent self pollination in the population. The $7II$'s and O_6 's, with 93.8 per cent and 97.0 per cent stigma retention respectively, must reproduce almost entirely by self pollination. The O_{10} 's with 40 per cent stigma protrusion, are at least potentially capable of much higher levels of outbreeding than the $7II$'s and O_6 's, but they constitute a very minor component of the population.

A very high level of inbreeding is reflected by the paucity of heterozygotes and the high value of F at the PGM locus in the $7II$ component of the population. This pattern, however, is not reflected amongst the O_6 's which, according to their stigma retention frequencies, may be even more inbred than the $7II$'s. Rather, the fixed hybridity at the PGM locus in the O_6 's reflects the operation of the complex hybrid genetic system.

The accumulation of deleterious mutations in the absence of recombination is a well documented possibility (Muller, 1964; Felsenstein, 1974; Haigh, 1978; de Waal Malefijt and Charlesworth, 1979). The phenomenon would be most likely in a relatively small isolated population of haploid asexuals subject to bottleneck effects. In such a system, stochastic processes may at any time eliminate the individuals with the fewest deleterious mutations. This would increase the minimum number of deleterious mutations in the succeeding population. Deleterious genes may therefore be expected to accumulate through the operation of this "ratchet" (Muller, 1964). Sexual reproduction, on the other hand, provides a mechanism whereby daughter genomes with a reduced number of deleterious mutations may be generated. This capability requires biparentality and recombination (Maynard Smith, 1978; Chao, 1988; Redfield, 1988). In sexual diploids, biparentality may be suppressed by self pollination, and recombination within chromosomes may be suppressed by terminal chiasma localization. Terminal chiasma localization converts a whole chromosome into a single supergene or recon within which there is no recombination. Recombination between these recons, however, is possible through independent assortment at meiosis.

While deleterious mutations may be purged from (*mutant/normal*) heterozygous recons by autogamous sexuality, balanced deleterious mutations within a single recon cannot be eliminated from an autogamous diploid system; such recons are fixed into permanent hybridity.

Terminal chiasma localization is characteristic of *I. petraea* (James 1965, 1970). If deleterious recessive mutations arise in both alleles of a super-

gene in a completely inbreeding lineage, then that supergene will be fixed into permanent hybridity. The accumulation of deleterious recessives, and ultimately lethals, at all supergenic loci would seriously reduce the reproductive fitness of such individuals. Indeed, only 2^{-m} of the selfed progeny, where m is the number of independently segregating supergenes, will be as heterozygous as the parent plant while the $(1-2^{-m})$ complete or partial homozygotes will be incapable of contributing to the posterity of the lineage. In *Isotoma* structural homozygotes, m is likely to equal 7, the haploid chromosome number. Thus, if heterozygous deleterious recessives are present in all of its supergenes, it may be expected that only one in 128 of the selfed progeny is potentially capable of surviving in the field, the remainder being homozygous for one or more supergenes and, presumably, competitively disadvantaged. Selection would favour lineages with a more efficient production of offspring. Most obviously, the number of

independently segregating supergenic units carrying deleterious recessives may be reduced, for example by interchange hybridity.

A simple model which aims to demonstrate the adaptive utility of interchange hybridity in the Pigeon Rock population as envisaged above is summarized in fig. 1. In this model, the genome is considered to be composed of seven supergenes in 7II's and five supergenes in O_6 's. All the products of selfs must be as heterozygous as the parent to survive and all partial homozygotes are considered lethal. All progeny from crosses are considered to be fully viable. The complexes are considered to be equally transmissible in the ovules, but the N complex is not transmissible through the pollen and because of this, N.N homozygotes cannot occur in the population. The initial population was modelled as consisting of 99 per cent 7II's and 1 per cent O_6 's and cycled across 50 non-overlapping generations (the rare O_{10} 's being ignored). At each generation, there was the proportion s of selfing

					MODEL 1			MODEL 2		
Female Parent		Pollination Type/Freq.	Male Parent		Relative Contribution to Next Generation			Relative Contribution to Next Generation		
Type	Freq.		Type	Freq.	Total	Types		Total	Types	
						N.ax	ax.ax		N.ax	ax.ax
N.ax	P	s	N.ax	1	$D \times \frac{1}{32}$	1		$D \times \frac{1}{32}$	1	
		1-s	N.ax	P	$D \times 1$	$\frac{1}{2}$	$\frac{1}{2}$	$D \times \frac{3}{4}$	$\frac{2}{3}$	$\frac{1}{3}$
			ax.ax	1-P	$D \times 1$	$\frac{1}{2}$	$\frac{1}{2}$	$D \times 1$	$\frac{1}{2}$	$\frac{1}{2}$
ax.ax	1-P	s	ax.ax	1	$\frac{1}{128}$		1	$\frac{1}{128}$		1
		1-s	N.ax	P	1		1	1	$\frac{1}{2}$	$\frac{1}{2}$
ax.ax	1-P		1		1	1	1		1	

Figure 1 Explanatory diagram of the Pigeon Rock population models. See text for details. N = the N complex, two interchanges removed from the standard chromosome end sequence. ax = the standard chromosome and sequence. N. ax = O_6 . ax. ax = 7II. P = the frequency of N. ax. D = the disjunction frequency in O_6 's = 0.73 = the mean O_6 pollen fertility. s = the recurrent selfing rate.

Model 1 assumes non-transmissibility of the N complex in pollen. In this case, the frequency of N. ax in the next generation is given by

$$P' = \frac{PsD/32 + PtD/2}{PsD/32 + PtD + P^2tD/2 + PQtD/2 + Qs/128 + Qt}$$

where $t = 1 - s$ and $Q = 1 - P$.

Model 2 assumes equal transmissibility of the N and S complexes in pollen and recessive lethality of N. In this case, the frequency of N. ax in the next generation is given by

$$P' = \frac{PsD/32 + P^2tD/2 + PQtD/2 + PQt/2}{PsD/32 + P^2tD/2 + PQtD/2 + PQt/2 + P^2tD/4 + PQtD/2 + Qs/128 + PQt/2 + Q^2t}$$

and $(1-s)$ of crossing. The fecundity of the O_6 's relative to the $7II$'s, due to meiotic nondisjunction, was taken to be 0.73, equivalent to their average pollen fertility (James, 1970). During the course of 50 generations, the model population achieved a stable frequency of $7II$'s and O_6 's, and the nature of the stable population was dependent upon the value of the recurrent selfing rate. The model was run for values of s ranging from 0 to 1 at intervals of 0.05. It can be seen, from fig. 2, that stable significant frequencies of the O_6 structural heterozygotes were achieved only with very high levels of recurrent self pollination. A stable O_6 frequency of 66.4 per cent, as is characteristic of the Pigeon Rock population, required a recurrent selfing rate of over 98 per cent. Stigma protrusion frequencies, coupled with the probability of geitonogamy in crosses, suggest that these values may well be achieved in this population.

Male transmissibility of the N complex occurs in some Pigeon Rock plants. If the model is adjusted to permit equal transmissibility of the complexes in the pollen and to assume that individuals homozygous for the N complexes from ($O_6 \times O_6$) crosses are inviable because of a common zygotic lethal carried by all N complexes, it is found that comparable levels of recurrent selfing are needed to maintain the polymorphism. With this adjusted model, a stable O_6 frequency of 66.4 per cent required a recurrent selfing rate of 97.6 per cent. Thus, the transmissibility or non-transmissibility of the N complex in pollen appears to be of little consequence in the selective elevation of N. ax O_6 heterozygotes to high frequency in this population; what is essential, however, is an extraordinarily high level of inbreeding in the population.

Figure 2 O_6 frequencies after 50 generations achieved in the model populations summarised in fig. 1 for various values of s the recurrent selfing rate.

It is of interest to note that the O_{10} 's in the Pigeon Rock population could be even more efficient than the O_6 's in transmitting the parental genotype on selfing, but they occur at an insignificant frequency in the population. The O_{10} 's may not exhibit sufficient levels of inbreeding to replace $7II$'s in the same way as did the O_6 's. However, as no O_6 's devoid of PGM 3 were found in the population, it appears that the interchanged S^* complex is presently confined to the O_{10} cytotype and has not been distributed into the population by outbreeding. It remains to precisely identify the biological parameters which render the O_{10} 's uncommon in the population.

We conclude that O_6 complex hybridity on Pigeon Rock is likely to have arisen from progenitors exhibiting extreme levels of inbreeding and chiasma localization, and permanent hybridity for balanced deleterious recessives in several or all of their seven supergenes. It is possible that the suppression of normal sexuality in the ancestral structural homozygotes allowed a ratchet driven establishment of permanent hybridity, creating the conditions in which complex hybridity could evolve. Within this primed population, natural selection favoured a doubly interchanged genome which was conveniently marked at the PGM locus, pollen non-transmissible and recessively lethal, and elevated it to high frequencies. The nascent complex hybrids so established represent forms which significantly improved the yield of parentally heterozygous genotypes in the seed produced by selfing.

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