SOME CAUSES OF SEED STERILITY IN CERTAIN NATIVE AUSTRALIAN PLANTS

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Many of our most beautiful and horticulturally desirable native plants are remarkably seed sterile. The empty cones of so many *Banksia* species and the sparsity of seeds amongst *Verticordia* fruit collections bear witness to this. The phenomenon is not necessarily one imposed to test the patience of plant propagators; it is more probable that natural selection has incorporated sterility into the genetic systems of these plants as part, or as a consequence of, a reproductive strategy that has hitherto proved to be quite successful. Detailed analyses and real understanding of the genetic systems is available in a very limited number of native plant groups. *Isotoma petraea* and *Stylidium* spp. are rather horticulturally unimportant, but they are fascinating plants, and lessons learnt from their genetic systems may well be useful in understanding biological strategies in other plant groups which may be less amenable to investigation.

The sexual cycle in plants. Seed formation is the result of successful sexual reproduction in plants. It requires all the biological miracles of cell division, growth and differentiation leading to the production of flowers on a surviving adult plant. Within the flowers, meiosis takes place in the ovules and anthers leading to the production of the haploid gametophytes. The embryo sac is nurtured in the tissues of the ovule where it ultimately produces an egg. Pollen is released from the anther and is transferred by pollinating vectors to the receptive stigma, where it germinates to produce a pollen tube which grows down through the style and into the ovary, finally delivering two sperm nuclei to the embryo sac within the ovule. One sperm nucleus fuses with the egg to produce the new embryonic plant, the second fuses with two embryo sac nuclei to produce the endosperm. The ovule, now containing a living food storage tissue, the endosperm, and a new plant, the embryo, develops into the seed.

Seed production requires all these steps to be successfully fulfilled. Seed sterility is the result of any one of these steps failing.

The biological role of sexual reproduction. Seed production is not necessary for reproduction. Numerous, perhaps most plants have the capacity to be reproduced vegetatively by cuttings, budding, or grafting, or naturally by means of rhizomes,

corms, bulbils or other asexually produced propagules. Sexual reproduction is the mechanism of generating heritable change. It is a genetic phenomenon, and sexual reproduction, embracing meiosis and fertilization, is the field of the genetic system.

In sexual reproduction, each parent contributes a set of hereditary factors or genes, via the gametes, to the fertilized egg and thus to the progeny individual that develops. This diploid (doubled) set of genes is copied and passed intact and unchanged to each cell of the developing individual, including those cells which undergo meiosis in the anthers and ovules. At meiosis, the diploid set of genes is segregated into a variety of haploid sets, some of which may be identical with the maternal gametic set, some may be identical with the paternal set, but the vast majority will be mixtures of the maternal and paternal sets. If all individuals of a species were genetically pure and identical, this mixing of the parental gene combinations could yield nothing new. The generation of novel gene combinations through the meiotic process requires genetic differences to exist between the maternal and paternal gametic gene sets; i.e., it requires the diploid individual to be heterozygous or hybrid. The mixing of genes into new haploid combinations is termed recombination, and it is an essential component in the genetic system.

Sexual reproduction involving heterozygous individuals leads to the generation of potentially infinite numbers of different gene combinations. Within populations of sexually reproducing species, therefore, the potential exists for the presence of a vast number of different genotypes, but only a relatively limited subset of these genotypes are adapted, and they are naturally selected. Under stable environmental conditions, the tendency in natural populations is for the less fit genotypes to be removed by natural selection and for the population to exhibit an increasing adaptation and fitness over time. Two strategies which enhance fitness may be discerned in natural populations. Firstly, recombination may be limited by various mechanisms. Such conservative devices assure an increased probability that the genotype of the offspring is similar to that of the parent, and hence is likely to be adapted and fit. In some cases, this conservation of parental genotypes is so precise that a lineage loses its capacity to generate new genetic combinations; the ultimate consequence of such folly must be extinction, for the environment will change. Secondly, genotypes will be selected which have enhanced powers of tolerating genetic variation yet achieving the adapted phenotype, and in ever wider ranges of environmental conditions. This inbuilt homeostasis is difficult to conceptualize and explain in exact terms, but its importance and physiological basis is an area of controversy in some aca-

demic circles. It is analogous to, and perhaps identical with hybrid vigour and the reduced phenotypic variance commonly associated with hybrids. It is an important component in the genetic system of all higher organisms, and is especially well serviced by breeding systems which ensure biparental sexuality and the generation of optimal levels of hybridity.

Evolutionary changes in the genetic systems can thus be explained in terms of two phenomena — the pursuit of hybridity and the control of recombination. This approach to genetic system analysis was first developed by Charles Darwin and, in its modern format, by C. D. Darlington. It provides a fascinating insight into a turbulent world of genetic pragmatism beneath the placid surface of our elegant and gracious native plants.

Complex hybridity in *Isotoma petraea*. *Isotoma petraea*, the rock isotome, is an herbaceous perennial member of the *Lobelia* family which occurs in rocky habitats throughout the wide central regions of the Australian continent. Over most of its distributional range, it is a fairly conventional species having a pollination mechanism (Figure 1) adapted to promote crossing and a diploid set of 14 chromosomes which form bivalents at meiosis (Figure 2). The species extends to the granite rocks of the eastern wheat belt in Western Australia, where it occurs in discrete populations on individual granite domes. In certain of these populations, high levels of inbreeding became established. This was brought about by a short circuiting of the pollination mechanism so that the stigma became receptive while still contained within the anther tube (Figure 1) making the flowers effectively cleistogamous. Now, self pollination has the result of rapidly eliminating hybridity in sequential inbred generations and is debilitating, both of the inbred plants themselves and of their genetic flexibility and evolutionary potential. As with so many other species of plants which have adopted self pollination, systems which conserve hybridity and resist the debilitating consequences of inbreeding have been incorporated into the genetic system. In this present case, complex hybridity has been evolved. This has been described in some detail elsewhere (2,3,5,6). Briefly, complex hybridity is a genetic system which departs from normality in that recombination at meiosis is virtually totally suppressed (Figure 3); recombinant gametes are generally inviable. In addition, when the two viable parental gamete forms are associated by self pollination, only the heterozygotes survive; the homozygotes are eliminated by a balanced lethal system (Figure 3). The net result, in *Isotoma*, is that 80% of the ovules may be aborted through gametic elimination, while half the remainder are lost following fertilization;

this derived genetic system imposes a seed sterility of up to 90%.
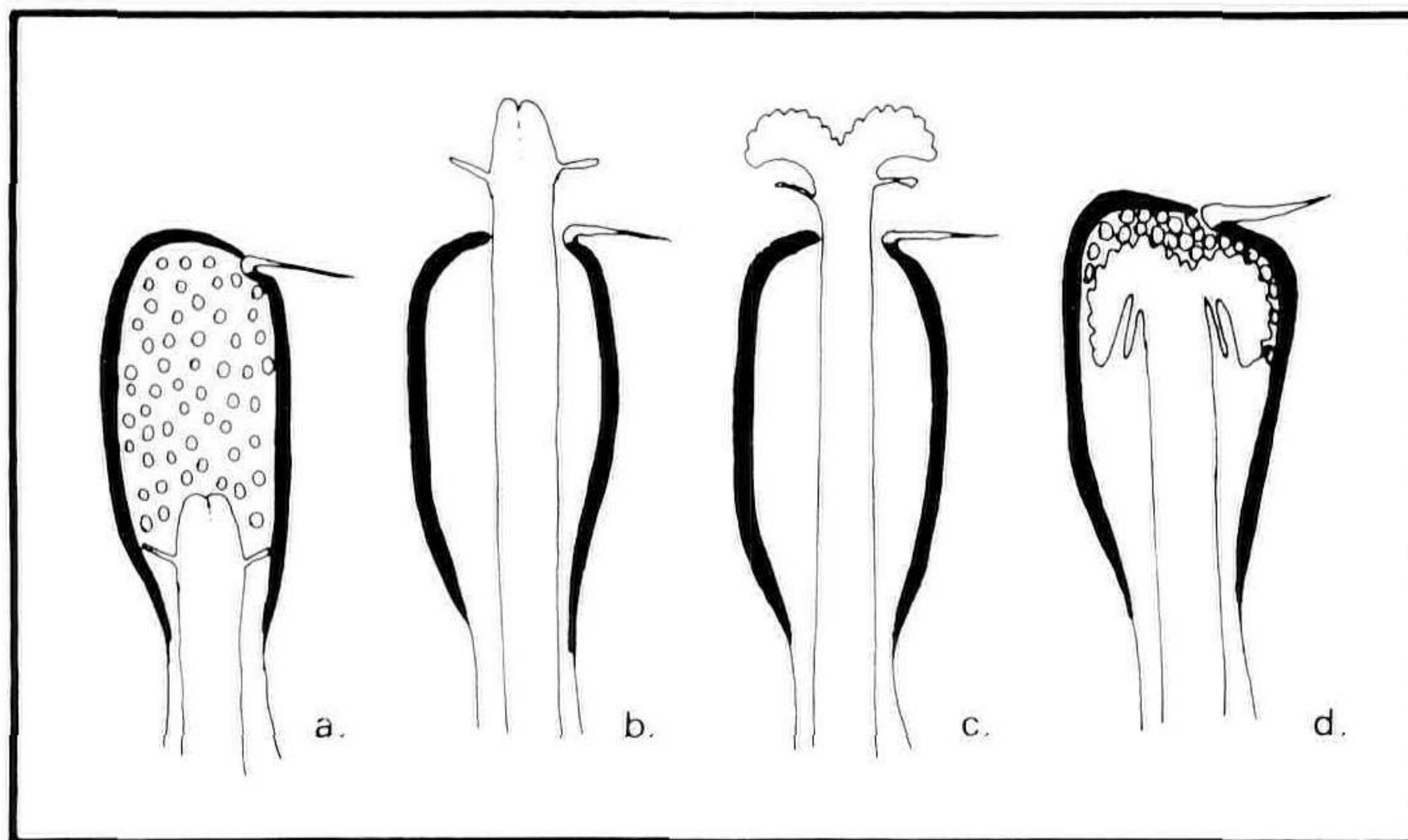

Figure 1. Pollination mechanisms in *Isotoma petraea*. Pollen is shed into a tube formed by the fused anthers and released onto visiting vectors through a pore opened by depressing the large terminal bristle (a.). The style elongates, pushing the still non-receptive stigma through the anther tube (b.) whereupon the stigma becomes receptive to pollen (c.) delivered by insect vectors. (a.) to (c.) represent the primitive cross pollination system. In (d.) the stigma becomes receptive within the anther tube and self pollination is effected. Since the stigma is not exposed, cross pollination is not possible in flowers exhibiting this behaviour.

We have inquired into the consequences of the evolution of this genetic system by means of two investigations summarized below, but which will be described in detail elsewhere.

We examined the distribution of variation within the two parts of the species, the one, primitive, characterized by normal chromosome behavior, the second, derived, characterized by ring formation. This was done by measuring some nine items on 5 samples from some 868 glasshouse-grown plants which were derived as seed from native plants in 4 primitive and 3 ring or complex hybrid populations. The hierarchical arrangement of the investigation is illustrated in Figure 4. Statistical analyses permitted the allocation of components of variation to various levels within the hierarchy, and the average results are illustrated in Figure 5. From this figure it is clear that the evolution of complex hybridity has been associated with a redistribution of variation within *Isotoma* populations. The derived complex hybrids exhibit much more variation between populations than within populations, and quite importantly, the variation between samples within individual plants is less, in the derived complex heterozygotes, than in the primitive forms. Indeed, the enhanced robustness of the complex hybrids was

a.

b.
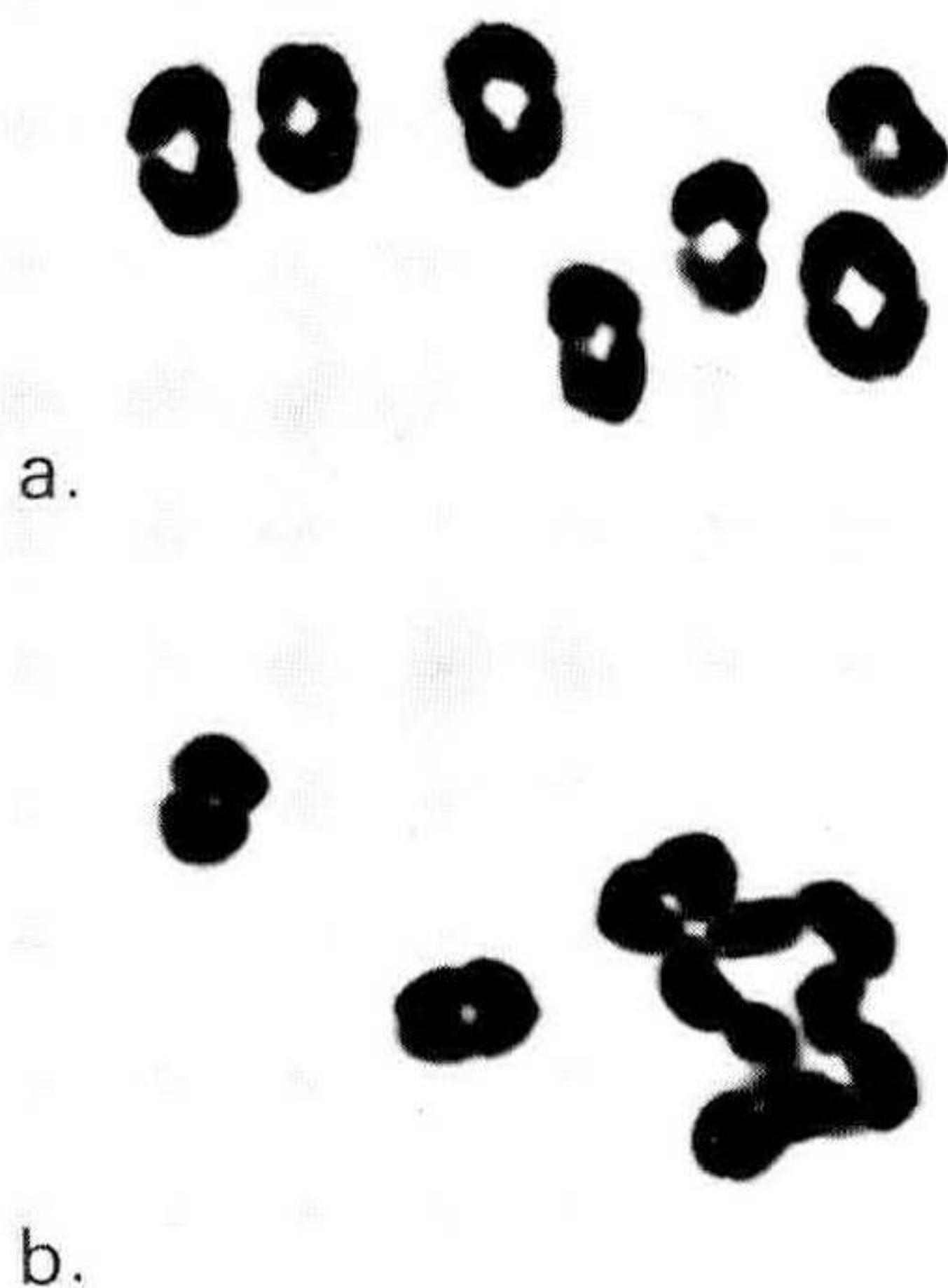
Figure 2. Photographs of chromosomes at first metaphase of meiosis in *Isotoma petraea*. (a.) - the primitive condition in which the fourteen chromosomes form seven bivalents, each being two chromosomes held together by terminal chiasmata. (b.) - a derived complex heterozygote in which eight of the fourteen chromosomes form a ring association (⊙ 8) and six form three bivalents. Complex heterozygotes may exhibit ring associations of 6 (and 4 bivalents) through to 14 (with no bivalents). The effect of ring formation is to prevent the independent assortment of genes carried on the different chromosome pairs.

borne out in the second experiment (Table 1) in which a replicate of the first was grown under rather adverse conditions in the garden in which approximately 50% mortality was in evidence. In this experiment, 92% of the complex hybrids survived, while only 25% of the primitive forms survived. It is clear from these investigations that the evolution of complex hybridity has resulted in the production of a very superior lineage, able to withstand environmental vissicitudes much more effectively than their progenitors, and capable of passing their superior genotype unchanged to their offspring. Even though the genetic system demands a 90% seed sacrifice, the accountancy of nature has proven the benefits. But, the cost-benefit analysis is short-sighted, and the *Isotoma* complex hybrids have renounced their genetic flexibility. Complex hybrids are known in only three, perhaps 4, other groups of plants; the rarity of the genetic system bears witness to its ultimately limited utility, and we may confidently conclude that the *Isotoma*
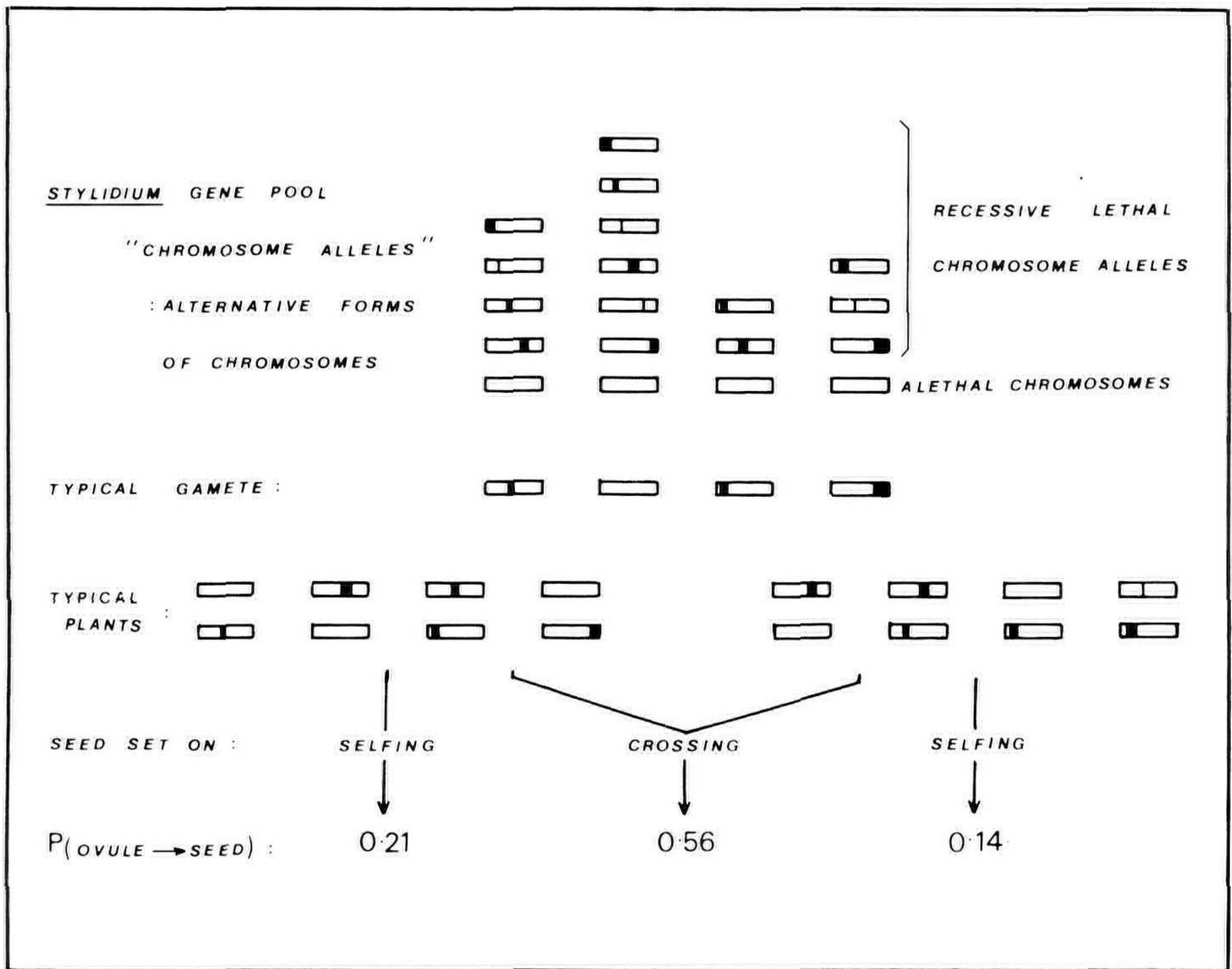
Figure 3. Causes of seed sterility in *Isotoma petraea* complex hybrids. All complex hybrids exhibit a 50% seed abortion due to their zygotic lethal system. The degree of gametic inviability depends upon the number of chromosomes included in the complex. The 80% estimate for gametic inviability here refers to complex hybrids with all 14 chromosomes in the ring.

Table 1. Survival among garden-grown *Isotoma petraea* plants.

Genetic System	Survived	Died	Total	P(survival)
7II	25	75	100	0.25
⊙	69	6	75	0.92
Total	94	81	175	0.54

$X^2_{(1)}$ Homogeneity $= 77.5$

p ————> 0.0

complex hybrids are doomed to extinction.

Lethal systems in *Stylidium*. Triggerplants, of the genus *Stylidium* have a sensitive column or trigger which bears, in sequence, the anthers and then the stigma. This sensitive column arches back and may be triggered by visiting insects so
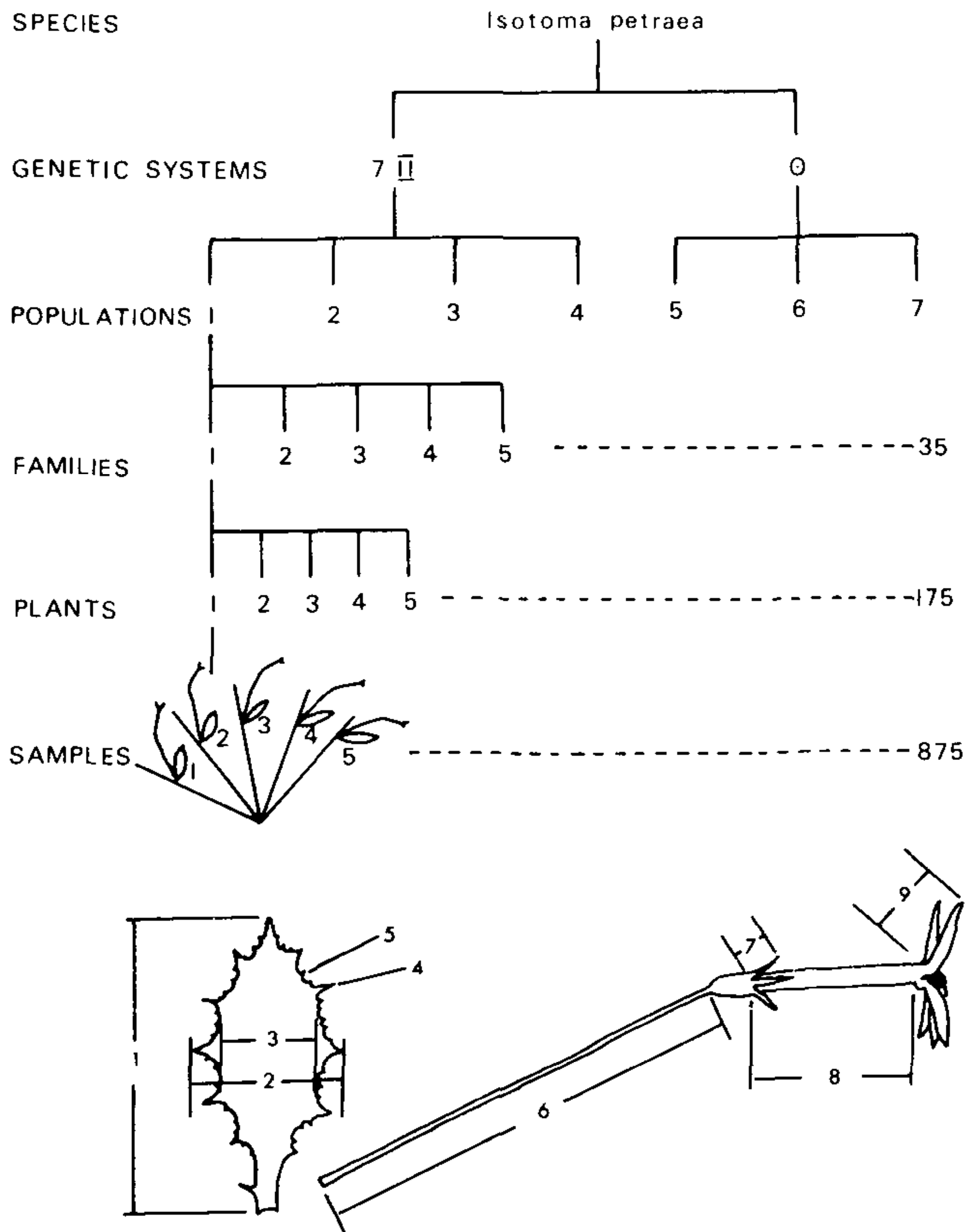
Figure 4. The hierarchical design for the analysis of variance of *Isotoma petraea*. See text for details. Of the possible 875 samples, 868 were measured for each of the nine characters indicated.

that they are struck by the rapidly moving column. The released trigger then resets and awaits the next visitor. In this way pollination is achieved. It is obviously a mechanism to promote cross pollination. But, cross pollination between flowers on the one plant is also possible and this is equivalent to self pollination. Most *Stylidium* species in Western Australia can discriminate between crosses and selfs so that few seeds are set following self pollination, while seeds are set much more freely following cross pollination, especially if the parents involved come from closely adjacent but different populations. This behavior imposes very substantial seed sterility upon *Stylidium*. The discrimination between crossing and selfing is achieved by a widespread occurrence of recessive lethal genes in the *Stylidium* gene pools (Figure 6) The relevant information has
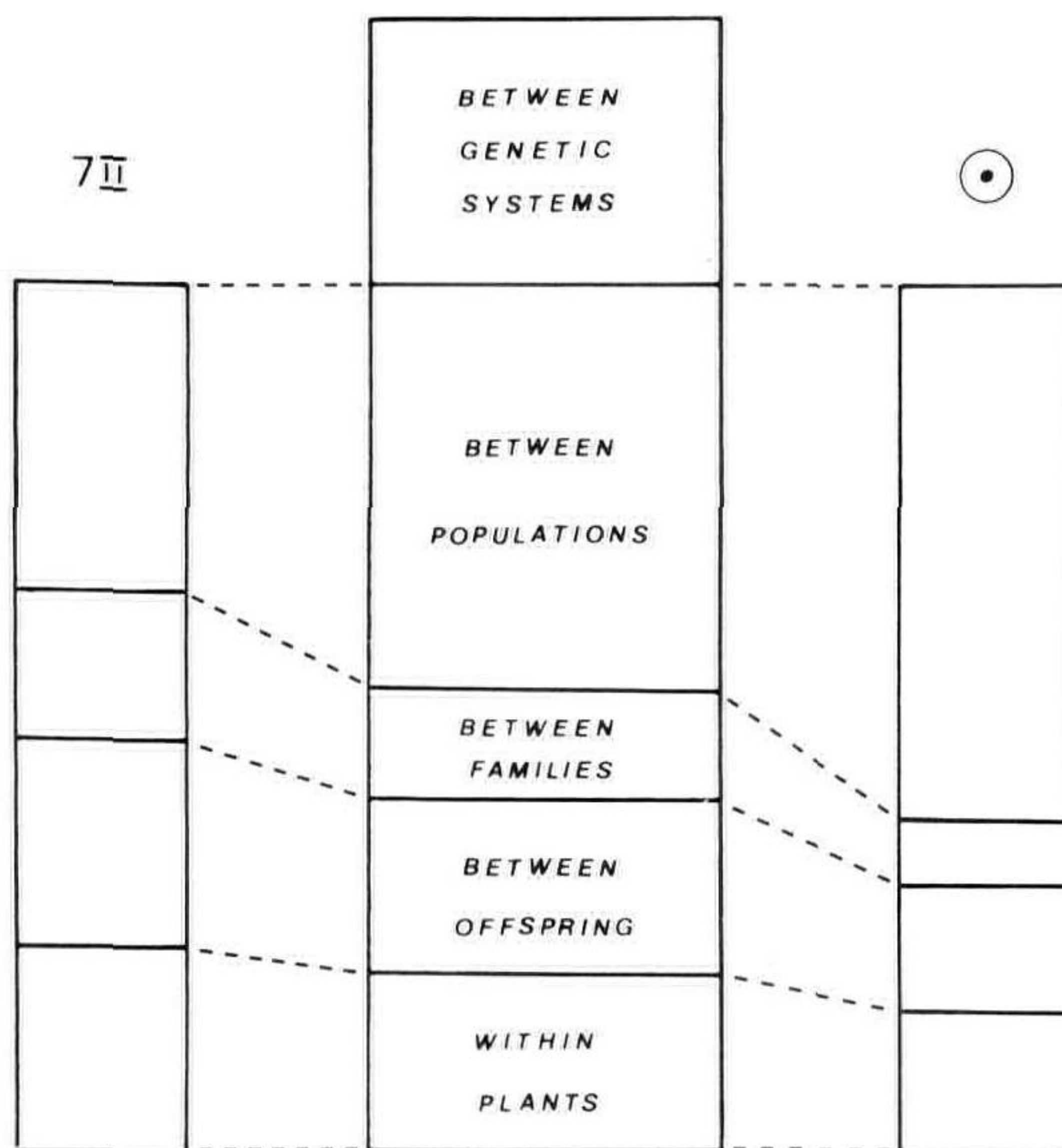
Figure 5. The distribution of variation in *Isotoma petraea*. The relative estimates of variation at each level of the hierarchical experimental design were estimated by relatively adjusting and linearly averaging the unbiased estimates of variance components calculated for each of the 9 measured parameters, indicated in Fig. 3, by the unequal sample size method of Snedecor and Cochran (1967: 291-294). See text for general interpretation.

been described elsewhere (1,7) or is being prepared for publication.

Again, this curious genetic system is best explained in terms of a pursuit of hybridity. Zygotes homozygous for particular recessive lethal genes die virtually at their conception; only those zygotes heterozygous for at least a substantial proportion of their genotype survive. All this has consequences upon the distribution of variation with *Stylidium* species. Individual species, e.g. *S. crossocephalum* (4) have their distributional areas subdivided into local areas in which the lethal systems tend to be relatively homogenous, while different suites of lethals characterize different units of the distributional mozaic. As in *Isotoma*, the pursuit of hybridity has lead to discontinuities in the variation pattern, at least at the chromosomal level, concentrating differences between local areas. More than 100 species of *Stylidium* occur in Western Australia, and it appears that this rich and extensive speciation is a consequence of the aberrant genetic systems exploited by the group.
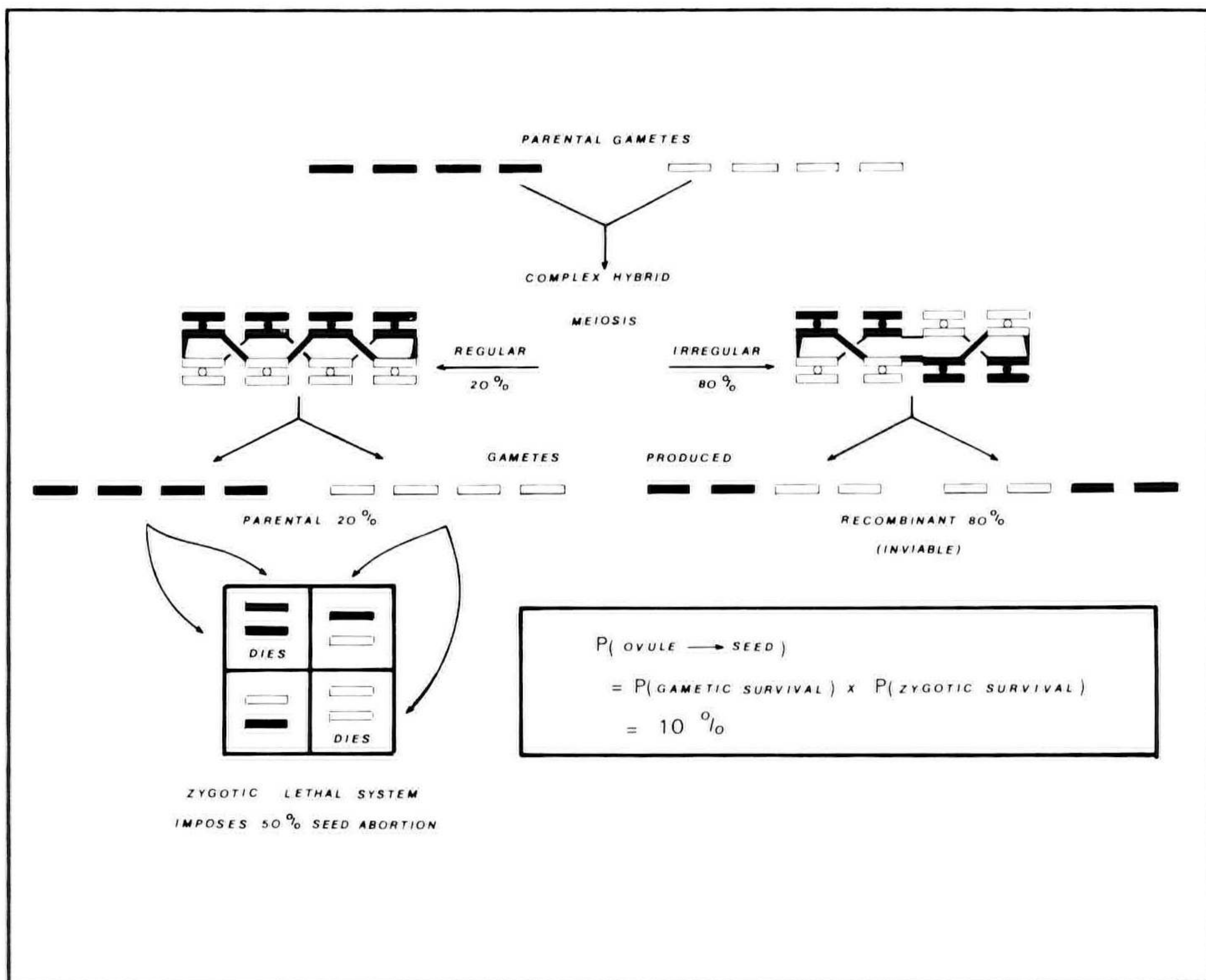
Figure 6. The architecture of a *Stylidium* species' gene pool. Within populations, each chromosome exists in a number of forms; an alethal form, and several to many forms carrying one (or more) recessive lethal genes. Each gamete must carry one chromosome drawn from each stack, and each plant has a genotype produced by the union of two gametes so that it is effectively made up of two chromosomes from each stack. If the two chromosomes from any one stack are carrying identical lethals, the plant, being homozygous for that recessive lethal gene, dies soon after its conception. The seed set probability for the typical plants given is readily reduced from elementary Mendelian principles; the first plant, on selfing, produces ($3/4 \times 3/4 \times 3/4 \times 1/2 \times 3/4 = {}^{27}/_{128} = 0.21$) surviving seeds, the second produces ($3/4 \times 1/2 \times 3/4 \times 1/2 = {}^{9}/_{64} = 0.14$) surviving seeds, while crossing between the two plants produces ($1 \times 3/4 \times 3/4 \times 1 = {}^{9}/_{64} = 0.56$) surving seeds. (The identity of particular recessive lethals may be determined visually in this diagram.)

Other richly speciated groups in the Australian flora may also owe their diversity to an exploitation of conservative genetic systems. This is quite probably the case for the Styphelieae (Epacridaceae) (9) and the Chamaelauciinae (Myrtaceae) (8) in which aberrant genetic systems have been described. At present, a great deal of research needs to be done before a really satisfactory understanding of the role of the genetic system in

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the evolutionary biology of native plant groups can be claimed. However, a general principle which seems to be emerging is that where poor levels of seed set are encountered amongst native plants, there is a high probability that sophisticated genetic systems which ensure a high quality of seed, in terms of their genetic content, will also be found. It is also becoming apparent that these derived genetic systems, if conservative, are also restrictive, and though they may contribute to taxonomic diversity, they almost surely limit the long term evolutionary potential of their lineages.

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