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Genomic Imprinting in Endosperm: Its Effect on Seed Development in Crosses between Species, and between Different Ploidies of the Same Species, and Its Implications for the Evolution of Apomixis

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Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis

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SUMMARY

If a mother sometimes has offspring by more than one father and if genes in the offspring are active in acquiring resources from maternal tissues, theory predicts that alleles at some loci in the offspring will evolve different patterns of gene expression depending on the gene's parent of origin (genomic imprinting). The criteria for the evolution of imprinting are satisfied in many seed plants, and imprinting has been reported from the endosperm of angiosperm seeds. This paper's purpose is to show that imprinting phenomena in endosperm can provide a coherent explanation of some failures of experimental crosses, and of the prevalence of pseudogamy among apomictic angiosperms.

As a consequence of imprinting, seed development comes to depend on a particular ratio of maternal and paternal genomes in endosperm. This ratio is normally two maternal genomes to each paternal genome. Imprinting probably accounts for the failure of crosses between diploids and their autotetraploids, because the 2m:1p ratio is disturbed in such crosses. Imprinting may also account for the breakdown of endosperm in crosses between related species, if the expression of maternal and paternal genomes in endosperm is out of balance.

When a cross fails because of such an imbalance, the reciprocal cross will have the opposite imbalance and a complementary phenotype would be expected. The embryological evidence is consistent with this prediction. For example, many incompatible crosses show delayed wall formation in one direction of the cross, but precocious wall formation in the other direction. Typically, seed development can be classified as showing 'paternal excess' or 'maternal excess'. Paternal excess is associated with unusually vigorous early growth of the endosperm, and maternal excess with the opposite. This pattern is consistent with natural selection on paternal gene expression favouring larger seeds. Genetic evidence from maize confirms an association between paternal gene expression and larger kernel size, and maternal gene expression and smaller kernel size.

Genomic imprinting creates a requirement for both maternal and paternal genomes in imprinted tissues. In mammals, imprinting is expressed in derivatives of the zygote. The requirement for a paternal genome has constituted a block to the evolution of parthenogenesis, because all the genes in a parthenogenetic embryo are maternal. In angiosperms, imprinting is primarily expressed in the endosperm rather than the embryo. If the effects of imprinting in the embryo are small, an asexually

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produced embryo can develop, provided that it is associated with a viable endosperm. Many apomicts are pseudogamous. That is, the endosperm is fertilized and contains maternal and paternal genes, but the embryo is asexual and contains maternal genes only. Thus, the division of labour between the embryo and the endosperm during development of the seed can be seen as a preadaptation for apomixis.

Some apomicts are autonomous. That is, the embryo and the endosperm both develop without fertilization. Genomic imprinting in endosperm would seem to constitute a barrier to the evolution of autonomous apomixis. Thus, there is a problem, not previously appreciated, in understanding how autonomous apomixis is possible.

1. INTRODUCTION

Genomic imprinting is the process by which a gene comes to be expressed differently in an individual, depending on whether the gene is derived from the individual's mother or father. Recently, the mechanisms and consequences of imprinting have become active areas of experimental research in the developmental and cell biology of mammals (Barton *et al.* 1984; McGrath & Solter 1984; Cattanach & Kirk 1985; Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987; Solter 1988; papers in Monk & Surani 1990; Barlow *et al.* 1991; DeChiara *et al.* 1991). Other researchers have independently uncovered evidence for imprinting in the endosperms of flowering plants. Kermicle (1970) and Lin (1982, 1984) provided direct evidence for imprinting in endosperms, but there had been indirect evidence from plant breeders for considerably longer. Plant breeders, however, usually sought other explanations for this evidence because they took it for granted that a genome's behaviour could be affected only by the alleles it contained, not by whether a particular allele was derived from an individual's mother or father. Here, and elsewhere in this paper, 'genomic imprinting' and 'imprinting' will be used interchangeably.

In the present paper, we show how imprinting in endosperm can provide a coherent explanation of known phenomena in two areas: (i) outcomes of experimental crosses, including the failure of crosses between diploids and their autotetraploids, and consistent patterns of compatibility and incompatibility in crosses between related species; and (ii) the prevalence of pseudogamy in apomictic angiosperms. (An apomict produces seeds in which the embryo develops without fertilization.) We also review evidence about the genetic basis of imprinting in endosperm, and provide historical summaries of attempts to explain the phenomena that can now be explained in terms of imprinting.

2. WHY SHOULD GENOMIC IMPRINTING BE SIMILAR IN ANGIOSPERMS AND MAMMALS?

Harper *et al.* (1970) began their important review on the shapes and sizes of seeds with the rhetorical statement that 'the development of the placental habit is one of the most remarkable examples of parallel evolution in the plant and animal kingdoms'. Whether or not one accepts this statement, we believe that the 'placental habit' is responsible for the similar effects of genomic imprinting in mammalian and angiosperm development (Haig & Westoby 1989).

Most of a seed's food reserves are transferred directly from maternal to offspring tissues after fertilization. These food reserves have a major influence on seedling fitness. In general, seeds with larger food reserves produce more vigorous seedlings. However, natural selection does not result in an indefinite increase in seed size because larger seeds are a greater metabolic cost to the mother (seed parent). This cost is experienced either as fewer resources available for other seeds in the current flowering or as fewer resources available for growth and maintenance, and thus (indirectly) as fewer resources available for future seeds. If genes expressed in the mother determine seed size, they will be selected to produce some optimal size that balances the benefits to individual seedlings of larger food reserves against the reproductive costs of fewer seeds (Smith & Fretwell 1974).

A pollen parent (father) benefits directly from an increase in the size of his offspring, but does not experience a direct metabolic cost. Rather, he experiences an indirect cost, if larger seeds mean that there are fewer ovules to fertilize. The additional seeds, that would have been produced had seeds been smaller, can be considered to be a reproductive cost to both parents. However, the cost to a pollen parent is discounted by the probability that other fathers would have fertilized these seeds. In other words, a mother experiences all of the reproductive cost if a seed grows larger but the seed's father experiences only part of the cost. In the special case of a seed parent that produces all of her seeds by the same father, both parents experience the same cost.

Given the observation that imprinting can and does exist, genes that are expressed in offspring (and that influence seed size) will be selected to promote greater growth when they enter the seed paternally than when they enter the seed maternally. Thus, genomic imprinting has evolved in mammals and angiosperms because in these organisms the offspring genome is active at the stage when the mother provisions her offspring (Haig & Westoby 1989; Haig & Graham 1991; Moore & Haig 1991).

The verbal model presented above is a direct extension of models of parent–offspring conflict, in which offspring are selected to demand more food from parents than parents are selected to give (Trivers 1974). Formal genetic models of this conflict have not considered the possibility that a gene can have different effects depending on its parental origin. However, in these models offspring demand more resources from mothers when mothers have offspring by multiple

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fathers (see, for example, Parker & Macnair 1978; Macnair & Parker 1978, 1979). The increased conflict that results from multiple paternity must be due to selection on the paternal component, because the distribution of maternal alleles among a mother's offspring is independent of the mating system.

3. ESSENTIALS OF SEED DEVELOPMENT IN ANGIOSPERMS

An angiosperm ovule contains a haploid female gametophyte surrounded by diploid maternal tissues. At fertilization, a male gametophyte (pollen tube) releases two sperm into the female gametophyte. One sperm nucleus unites with the egg nucleus to form a zygote. The zygote develops into a diploid embryo. The other sperm nucleus fuses with two polar nuclei to form the first nucleus of a triploid endosperm. The two sperm nuclei are genetically identical, as are the polar nuclei and the egg nucleus. Therefore, the nuclear genomes of the embryo and endosperm are identical, except that the endosperm has two doses of maternal genes for every dose of paternal genes. The diploid embryo usually consists of the embryo proper and a suspensor. It is the embryo proper which gives rise to the tissues of the future seedling. The embryo proper is usually embedded within the endosperm and joined at one end to the suspensor. At its other end, the suspensor often makes contact with maternal tissues. Exceptions exist to all of the generalizations in this paragraph. Nevertheless, the generalizations apply to most species discussed below, and exceptions will be noted.

Endosperm functions as an intermediary in the transfer of nutrients from maternal tissues to embryonic tissues, and is traditionally described as a 'nurse tissue' in textbooks. In some species, the endosperm is the principal storage tissue of the mature seed, whereas in other species the endosperm is ephemeral and its nutrients are absorbed by the developing embryo before seed maturity (Brink & Cooper 1947; Maheshwari 1950; Vijayaraghavan & Prabhakar 1984). In at least some species, endosperm does not appear to be an important source of nutrients during the earliest stages of embryo development. Instead, the early embryo appears to be nourished directly from maternal tissues via the suspensor (Vijayaraghavan & Prabhakar 1984; Raghavan 1986). Nutritional relationships within the seed are complex, poorly understood, and undoubtedly variable among species.

The theory presented in the previous section predicts that imprinting should occur in all offspring tissues that influence the cost of a seed to its mother. Evidence presented in the next section suggests that, in at least some species, the effects of imprinting in the embryo (including the suspensor) are minor relative to the effects in endosperm. We infer that in these species the embryo's genome does not have a major influence on seed size. In fact, we do not know of any compelling evidence for imprinting in angiosperm embryos, but expect that such evidence will be found, particularly in those species in which the embryo has a direct role in acquiring nutrients from maternal tissues. Gymnosperm seeds do not contain endosperm, but theoretical arguments predict that imprinting should occur in gymnosperm embryos (Haig 1992). We do not have any evidence to test this prediction.

4. GENOMIC IMPRINTING IN ENDOSPERM

Mammals require the participation of both a maternal and paternal genome during embryonic development. Embryos in which all genes are maternally derived (2m:0p; this shorthand notation refers to the number of maternal and paternal genomes or gene copies in a tissue), or in which all genes are paternally derived (0m:2p), do not complete development (Surani *et al.* 1984; Barton *et al.* 1984; McGrath & Solter 1984). This is because functions that are specific to the maternal and paternal genomes are both required for normal development.

A similar absolute requirement for maternal and paternal genomes does not exist in the embryos of all angiosperms, though it may exist in some species. Maternal haploid embryos (1m:0p) and paternal haploid embryos (0m:1p) can produce viable seedlings in at least some angiosperms (Kimber & Riley 1963; Sarkar & Coe 1966; Chase 1969; Kermicle 1969), as can the maternal diploid embryos (2m:0p) of apomictic angiosperms (Lakshmanan & Ambegaokar 1984; Nogler 1984). However, such embryos usually will not complete development unless they are associated with an endosperm that contains both maternal and paternal genomes. These observations taken together suggest that imprinting does not play a crucial role in the embryo of these angiosperms, but does play an important role in their endosperm.

The recognition that genomic imprinting occurs in endosperm helps to clarify the failure of crosses between different ploidy levels of the same species, and of some crosses between closely related species. It has long been appreciated that some sort of 'genic balance' is disturbed in crosses that fail. It has only recently been generally accepted that the relevant 'genic balance' resides in the ratio of maternal to paternal genomes in endosperm. The model of Haig & Westoby (1989) can explain why natural selection has produced differential gene expression of maternal and paternal genomes in endosperm, and can predict the sorts of deviations from normal development that should be observed in unbalanced endosperms. Below, we outline the predictions of this model with respect to the outcomes of crosses, and then discuss evidence which is relevant to these expectations. This evidence is organized as a historical summary of attempts to explain the outcomes of crosses. These attempts usually assumed that imprinting was not possible.

(*a*) *Expectations*

Natural selection should act on gene expression in endosperm (and other tissues which acquire resources from the mother) in such a way that, under normal conditions, the expression of paternally derived alleles would tend to promote the production of heavier seeds, whereas the expression of maternally derived alleles

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would tend to promote the production of lighter seeds. The mass of the mature seed (and the size of its food reserves) would be the outcome of the balance between these two tendencies.

One might expect higher levels of expression in paternally than in maternally derived alleles at some loci, whereas the converse could arise at other loci. For example, preferential paternal expression would be expected for genes that encode the digestive enzymes released by an endosperm haustorium. Differential selection could also act on the relative timing of developmental processes that determine endosperm size. In cereals, an endosperm's strength as a nutrient sink during grain-filling is primarily determined by the number of endosperm cells, and this number is stabilized before filling begins (Brocklehurst 1977; Chojecki *et al.* 1986; Ouattar *et al.* 1987). Therefore, paternally derived genes might act to increase the final number of endosperm cells, and maternally derived genes might counteract this tendency. Other genes could have little direct effect on the vigour with which resources are acquired, but increases or decreases in their level of expression could nevertheless affect activity of genes that affect resource acquisition directly, if resources are limited for overall gene expression (see Haig & Westoby 1989).

For development to proceed successfully, genes at different loci must be expressed in the correct ratio at the appropriate times, within some allowable range of variation. The significance of imprinting is that successful development proceeds in the context of gene expression that is sometimes antagonistic between maternal and paternal genomes. Moreover, the different genomes are sometimes responsible for different functions. Despite these complications, overall gene expression is subject to the evolutionary constraint that development produces a viable seed. Developmental processes would, therefore, be sensitive to changes in the relative number of maternal and paternal genomes in endosperm, or to combining maternal and paternal genomes from evolutionarily divergent populations.

Consider a simple model of gene expression in endosperm. Each gene can be regarded as consisting of a coding sequence, plus associated control sequences that determine where, when and at what level the gene is expressed. The control sequences remain unchanged, but can adopt different settings. At some loci, the control sequences are set independently of parental origin, but at other 'imprinted' loci the settings change, depending on whether the gene is transmitted to endosperm by a sperm or a polar nucleus. The relative expression of any two arbitrarily chosen loci will be determined by the setting of the control sequences. If one or both loci are imprinted, the ratio of maternal to paternal genomes in the endosperm (normally 2m:1p) will also affect relative expression of the loci.

Now, for members of an interbreeding population, the control sequences are set such that satisfactory development occurs in a 2m:1p endosperm. The compatibility of settings at different loci is maintained by natural selection and gene exchange. However, the settings in genetically isolated populations are free to diverge, subject to the constraint that crosses within a population produce balanced gene expression. If the balance between loci is maintained by 'imprinted' settings, and these settings diverge sufficiently among populations, then crosses between populations will be unbalanced and development abnormal.

Crosses between diploids and autotetraploids should fail, if imprinting is important, because balanced gene expression is contingent on a 2m:1p endosperm. This ratio is disturbed in crosses between ploidy levels. If the diploid is used as the pollen parent, endosperm will be 4m:1p, whereas if the autotetraploid is used as the pollen parent, endosperm will be 2m:2p. Seed development would be expected to differ in the reciprocal crosses. A diploid and its autotetraploid will have the same set of alleles, in the same relative proportions. Reciprocal differences would be due solely to imprinting and the imbalance in maternal and paternal gene dosage.

Crosses between related species, at the same or different ploidy levels, could fail if the gene expression of the maternal and paternal contributions to endosperm is unbalanced. This could arise from various causes. A cause that does not depend on imprinting would be that qualitative differences in the coding sequences between the alleles from different species are responsible for the incompatibility. Such an explanation could apply whether or not imprinting occurs, and might result in differences between reciprocal crosses (though it need not do so). Other causes would depend on imprinting. Failures of crosses between species at different ploidy levels could be explained by departures from 2m:1p. Failures of crosses between species at the same ploidy level could be explained if the parental imprints in the crossing partners had diverged sufficiently. These explanations, based on imprinting, would predict differences in seed development between reciprocal crosses.

Our theory predicts that reciprocal differences should occur in incompatible crosses, and also the nature of the differences. Reciprocal crosses should, in some sense, show complementary departures from normal development. Crosses between a diploid and its autotetraploid produce 2m:2p endosperms when the autotetraploid is the pollen parent. These should show evidence of abnormal vigour in resource acquisition. Such endosperms could be described as having a 'paternal excess'. On the other hand, the 4m:1p endosperms from the reciprocal cross should show evidence of reduced vigour, and could be described as having a 'maternal excess'.

In crosses between related species of the same ploidy, it is possible that an endosperm could show 'maternal excess' in the relations between expression of genes at some loci, and 'paternal excess' in the interactions between other loci. The complementary pattern would be predicted in the reciprocal cross. An interesting feature of the experimental evidence (discussed in subsequent sections) is that patterns of development are remarkably consistent when incompatible crosses between species and incompatible crosses between ploidy levels within a species are compared.

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(*b*) *Diploid × autotetraploid crosses*

Attempts to explain the failure of diploid by autotetraploid crosses have been entangled from the beginning with attempts to explain the failure of crosses between related species. This was because similar patterns of reciprocal differences in both sets of crosses indicated that similar processes were responsible for the failure of seed development (see, for example, Kihara & Nishiyama 1932; Wakakuwa 1934). Thompson (1930*a*, *b*) attempted to explain failures in terms of a genomic imbalance within endosperm. For example, if an allotetraploid (genotype AABB) was crossed to one of its diploid progenitors (genotype AA), the endosperm would be AAABB if the diploid was the pollen parent, but AAAB if the diploid was the seed parent. Thompson ascribed endosperm failure to the imbalance between the triple dose of the A genome and the double or single dose of the B genome.

However, similar failures are observed in diploid × autotetraploid crosses, yet there is little or no allelic differentiation between parental genomes in such crosses. Therefore, Thompson's interpretation can not account for the failure of such crosses. By extension, it need not account for failures in crosses between parents with genomes containing different alleles. We discuss diploid by autotetraploid crosses separately, before we discuss between-species crosses, because the evidence for genomic imprinting is easier to see. Interspecific crosses are discussed in the next section, though this creates an artificial division in the historical development of ideas.

Most crosses between a diploid and its autotetraploid are unsuccessful. Development is normal for the triploid endosperm from $2x \times 2x$ crosses and the hexaploid endosperm from $4x \times 4x$ crosses. However, development is abnormal for the tetraploid endosperm from $2x \times 4x$ crosses and the pentaploid endosperm from $4x \times 2x$ crosses (by convention, the seed parent appears before the pollen parent in descriptions of crosses) (Randolph 1935; Howard 1939; Cooper & Brink 1945; Cooper 1951; Håkansson 1952, 1953, 1956; Håkansson & Ellerström 1950; Woodell & Valentine 1961; Milbocker & Sink 1969). All these crosses, whether successful or unsuccessful, contain qualitatively the same genes in the same relative proportions. Moreover, the problem can not be attributed to the ploidy level of the endosperm itself, because the dysfunctional endosperms are intermediate in ploidy between functional endosperms. Thus, incompatibility appears to be a direct consequence of a difference in ploidy level between parents.

A number of authors recognized that normal endosperms always had a ratio of two maternal genomes to every paternal genome, and that this ratio was disturbed in endosperms with abnormal development (see, for example, von Wangenheim 1957; Valentine & Woodell 1963). However, the ratio of maternal to paternal genomes was discounted as a cause of endosperm failure, because, once a sperm had united with the polar nuclei, maternal and paternal genomes were presumed to be indistinguishable, and it was 'difficult to think of the failure or success of the endosperm as being caused merely by an interaction between identical nuclear genomes' (Valentine & Woodell 1963). A number of theories attempted to account for the failure of diploid by autotetraploid crosses while continuing to assume that maternal and paternal genomes behave identically in endosperm.

Müntzing (1930, 1933) proposed that incompatibility could be explained by disturbances to the ratio of ploidies in different seed tissues. Thus, the ploidy ratio of maternal tissues to endosperm to embryo was 2:3:2 in $2x \times 2x$ crosses and 4:6:4 in $4x \times 4x$ crosses, which gave normal development, but was 2:4:3 in $2x \times 4x$ crosses and 4:5:3 in $4x \times 2x$ crosses, which gave abnormal development. This theory could also explain the differences between reciprocal crosses, because reciprocal crosses had opposite deviations from a 2:3:2 ratio.

Watkins (1932) argued that normal development depended on the correct ratio of endosperm and embryo ploidies but was independent of maternal ploidy. He based his argument on the observation that seed development was normal for a 2:6:4 ratio, produced when diploid sperm fertilized occasional unreduced female gametophytes in $2x \times 4x$ crosses. Thus, he proposed that seed development was normal for $-$:3:2. Valentine (1954) preferred to explain endosperm failure as the result of a departure from the normal ratio of maternal ploidy to endosperm ploidy (2:3:$-$), because viable seeds were occasionally produced with haploid embryos (presumably 2:3:1).

von Wangenheim (1957, 1961) argued that the causes of endosperm failure must lie within the endosperm itself, rather than in the relation between endosperm ploidy and ploidy of other tissues. In interploidy crosses of *Solanum*, normal development did not depend on a 2:3 ratio of maternal to endosperm ploidy, because unreduced female gametophytes on diploid mothers produced normal endosperms when fertilized by diploid sperm (von Wangenheim 1957). Thus, a 2:6:4 ratio of seed tissues was compatible with normal development. Similarly, endosperm development did not depend on the ratio of endosperm to embryo ploidy because hexaploid endosperms developed normally in seeds without embryos and in seeds with diploid or tetraploid embryos (von Wangenheim *et al.* 1960).

Endosperm failure in interploidy crosses of *Solanum* could not be a consequence of abnormally high chromosome numbers in aberrant endosperms, because hexaploid endosperms from $4x \times 4x$ crosses were normal whereas tetraploid and pentaploid endosperms from interploidy crosses were abnormal. Therefore, von Wangenheim (1957) reasoned that nuclear genes alone could not be responsible for endosperm failure, and that the critical relation was the balance between nuclear and cytoplasmic genes within the endosperm.

von Wangenheim (1962, 1967) extended his research to study endosperm development in reciprocal crosses between diploid and autotetraploid *Oenothera hookeri*. *Oenothera* is unusual because its endosperm is normally diploid, produced by the fertilization of a single polar nucleus. Therefore, endosperm was triploid in both $4x \times 2x$ and $2x \times 4x$ crosses, yet reciprocal differences

were still observed (von Wangenheim 1962). For von Wangenheim, these differences resulted from the relation between endosperm ploidy and the quantity of extrachromosomal factors in the endosperm. In apparent agreement with this theory, the volume of cytoplasm per endosperm nucleus differed in the two directions of the cross (von Wangenheim 1967). However, the reciprocal differences could also be explained by genomic imprinting because the endosperm is 2m:1p in the $4x \times 2x$ cross, but 1m:2p in the reciprocal.

Sarkar & Coe (1971) rejected any hypothesis that attempted to explain endosperm success or failure in terms of the ratio of maternal to endosperm ploidy or of endosperm to embryo ploidy, because they observed normal development of maize endosperm for 2:3:0, 2:3:1, 2:6:4 and 4:6:2. They proposed instead that proper seed development required an endosperm that was $3n$ or a multiple of $3n$, but did not offer any explanation for this requirement.

All these attempts to find a model which could account for the evidence of interploidy crosses were hampered by the implicit or explicit assumption that maternal and paternal genomes had to behave identically in endosperm. Direct evidence for genomic imprinting in plants finally came from genetic studies in maize (Kermicle 1970; Lin 1982, 1984). This led to the recognition that normal endosperm required a 2:1 ratio of maternal to paternal genomes in endosperm, and previous hypotheses became redundant. The genetic evidence for imprinting will be discussed in a subsequent section.

(*c*) *Incompatibility in crosses between species*

Some matings between species at the same ploidy level show consistent differences between reciprocal crosses (see, for example, Valentine 1947, 1952, 1955; Reusch 1959*b*; Dhaliwal 1977; Nishiyama & Yabuno 1978). The differences are often similar to those observed in reciprocal crosses between a diploid and its autotetraploid (see, for example, Woodell & Valentine 1961). Conversely, some polyploids behave in crosses as if they were diploids. Certain crosses between a diploid from one species and a tetraploid from another give viable seeds in both directions (Howard 1942; Stephens 1942; Nishiyama & Inomata 1966). An incompatible cross between diploids sometimes becomes compatible if one of the species is replaced in the cross by its autotetraploid (Gill & Waines 1978; Johnston & Hanneman 1982).

These types of evidence suggest that the genetic differences responsible for endosperm failure in interspecific crosses may sometimes be quantitative rather than qualitative. Several authors have recognized that the outcomes of crosses between related species can often be predicted, if each species is assigned a genetic 'value' that is determined by its behaviour in crosses with an arbitrary reference species. A cross is predicted to produce normal, or nearly normal, seeds if the parents' genomes have similar genetic values. Such a model is more parsimonious than developing separate rules for every cross, and suggests that outcomes are controlled by some general process rather than by the specifics of qualitative gene combinations produced in each cross. This approach also provides a common conceptual framework for describing the results of interspecific crosses and interploidy crosses within species, because autotetraploids behave as if their genetic value is double that of their diploid progenitors. The remainder of this section reviews some of the attempts to understand crossing behaviour in terms of genetic value or a similar index. In all the unsuccessful crosses discussed in this section, incompatibility is due to developmental failure after fertilization.

Stephens (1942) found that diploid *Gossypium arboreum* did not produce viable seed when crossed to diploid congeners, but did produce viable seed when crossed as an autotetraploid to the same diploid species. Stephens accepted Watkins's (1932) hypothesis that a 3:2 ratio of endosperm to embryo genomes was necessary for normal seed development in intraspecific crosses. He recognized that the same hypothesis could be extended to the results of interspecific crosses in *Gossypium* if each haploid genome of *G. arboreum* was assigned half the genetic 'strength' of a haploid genome from the other species. Thus, Stephens proposed that compatible crosses required a 3:2 ratio of genetic strengths in endosperm and embryo, rather than a 3:2 ratio of chromosome numbers as proposed by Watkins. Howard (1947) similarly assigned genetic strengths to different species of *Nasturtium* and used the endosperm:embryo ratio of genetic strengths to explain the results of interspecific crosses in that genus.

Valentine (1954) adapted Stephens's (1942) hypothesis to explain the outcomes of reciprocal crosses among three diploid species of *Primula*. Each species was assigned a 'genetic value', and the ratio of endosperm genetic value to maternal genetic value was found to be a good predictor of the length, germinability and appearance of hybrid seeds. Valentine preferred the endosperm:maternal ratio to the endosperm:embryo ratio because endosperm development appeared to be independent of embryo ploidy. Woodell & Valentine (1961) and Valentine & Woodell (1963) extended this analysis to explain reciprocal differences in crosses between diploid and autotetraploid primulas.

Kihara & Nishiyama (1932) adopted a different approach to explain reciprocal differences in crosses between *Avena* species. They emphasized the ratio of maternal to paternal genomes within the embryo or endosperm, rather than the ratio of genetic values in different seed tissues. They observed that the larger the deviation from the normal 1m:1p ratio in the embryo and 2m:1p ratio in the endosperm, the less the percentage of germinated seed. Kihara & Nishiyama (1932) were only concerned with reciprocal differences in interploidy crosses, and their hypothesis implicitly assumed that haploid genomes from all species had the same value. Müntzing (1933) considered their hypothesis 'gratuitous', and 'an unproved assumption which covers the empirical results but which can scarcely be accepted as an explanation'.

Nishiyama & Yabuno (1978) presented a modified version of Kihara & Nishiyama's (1932) hypothesis, in

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which haploid genomes could have different genetic values. Their model assumed that the actions of maternal and paternal genomes were essentially antagonistic. Each polar nucleus was associated with a response value (RV) that acted in opposition to the activating value (AV) of the sperm nucleus. The ratio AV:2RV was called the activation index. Seed development was more or less normal for activation indices between 30% and 80%. Crosses with activation indices greater than 80% produced large, shrivelled kernels that did not germinate, whereas activation indices less than 30% resulted in small kernels that were fully or partly filled. If the activation index fell below 20%, these kernels did not germinate.

An essentially similar hypothesis was developed by Johnston *et al.* (1980) to explain crossing behaviour in *Solanum*. These authors were aware of Lin's (then unpublished) work showing that a 2:1 ratio of maternal to paternal genomes was necessary for normal endosperm development in maize (see below). Among *Solanum* species, all intraspecific interploidy crosses conformed to the 2m:1p hypothesis. However, some crosses between species of the same ploidy were incompatible, whereas other crosses between species of different ploidies were compatible. Johnston *et al.* (1980) proposed that the genome of each species be assigned a specific value in endosperm, called its endosperm balance number (EBN). Each species' EBN described its effective ploidy in crosses with an arbitrarily chosen species. By this hypothesis, the ratio of the summed EBNs of the polar nuclei to the EBN of the sperm nucleus should be 2:1, if endosperm development were to be normal.

It is interesting that much crossing behaviour can apparently be described in terms of variation in a single dimension (activation index in *Avena*, endosperm balance number in *Solanum*) and that the imbalance between maternal and paternal genomes in hybrid endosperms can sometimes be corrected by a change in the ploidy of one of the parents. The simplest way to account for these observations would be if incompatibility is primarily the result of an imbalance in a single pair of factors. If several pairs of factors were out of balance, the imbalances would all have to be in the same 'direction'. Otherwise, a change in parental ploidy would not restore the correct balance for all factors at once. On the other hand, genetic evidence suggests that multiple loci are responsible for incompatibility between species (see below). The two viewpoints, of multiple loci and of a single pair of factors, could be compatible if the multiple loci contribute to variation in a single pair of quantitative characters.

The relevance of imprinting to understanding crossability can be summarized as follows. Maternal and paternal genomes have different expression in endosperm, and both sets of functions are required for normal development. The correct balance between maternal and paternal genomes is disturbed in crosses between different ploidy levels of the same species, and in some interspecific crosses. The next section considers the embryological basis of reciprocal differences in incompatible crosses.

(*d*) *Embryological basis of reciprocal differences*

Reciprocal differences are expected in incompatible crosses because the balance of maternal and paternal factors in endosperm is disturbed by a maternal excess in one direction of the cross, but by a paternal excess in the other direction. Seeds with a paternal excess should be associated with enhanced nutrient-acquiring activity of the endosperm, whereas seeds with a maternal excess should be associated with reduced endosperm activity (Haig & Westoby 1989). There is strong evidence for this pattern.

The best embryological evidence comes from species with nuclear endosperm. In these species, the primary endosperm nucleus undergoes several cycles of mitotic division before the resulting nuclei are segregated into separate cells (Maheshwari 1950). When seeds have a maternal excess, nuclear division is frequently slower, and cell formation usually earlier, than occurs in controls with balanced endosperms. Seeds with a maternal excess are often plump and well formed, but smaller than normal. Such seeds frequently give good germination. The reciprocal crosses yield seeds with a paternal excess. Such seeds are often normal-sized but shrivelled. Germination is usually poor or non-existent, except when the paternal excess is small. The rate of nuclear division in the endosperm is often enhanced, but cell formation is usually delayed or suppressed and the endosperm frequently degenerates without accumulating food reserves. Some of the evidence for these generalizations is discussed below.

Interspecific, interploidy crosses of *Avena* and *Triticum* yield similar results (Kihara & Nishiyama 1932; Wakakuwa 1934). Maternal excess is associated with plump, sometimes small seeds and good germination, whereas paternal excess is associated with large, shrivelled seeds and poor or non-existent germination. The differences between reciprocal crosses become more accentuated as the maternal:paternal imbalance increases. In *Avena*, a maternal excess is accompanied by slower initial growth of the endosperm, and early formation of cell walls. In reciprocal crosses, endosperm growth is initially rapid, but cell walls are formed belatedly or not at all (Kihara & Nishiyama 1932; Håkansson & Ellerström 1950). Delayed wall formation is also associated with paternal excess in *Triticum* (Johnson & Dhaliwal 1976). Among other grasses, similar patterns have been observed in diploid × autotetraploid crosses of *Zea mays* (Cooper 1951) and *Hordeum vulgare* (Håkansson 1953), and in reciprocal crosses of *Lolium perenne* with *Festuca pratensis* (Reusch 1959*a*, *b*).

Kihara & Nishiyama (1932) observed that seed set was poor in interploidy crosses of *Avena* when the species with the higher chromosome number was used as the seed parent, but the resulting seeds tended to be small but viable (maternal excess). By contrast, seed set was good when the species with the higher chromosome number was used as the pollen parent, even though the resulting seeds were empty and inviable (paternal excess). Wakakuwa (1934) made similar observations in *Triticum*. Poor seed set does not appear to have been caused by a failure of fertilization, because seed set was

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good in the reciprocal crosses, as well as when either parent was selfed. A possible explanation is that the low vigour of endosperms (or embryos) with a maternal excess resulted in many seeds being aborted before they reached a sufficient size to be counted as seeds. Thus, a distinction must be made between early and late seed abortion. Seeds with a paternal excess have low levels of early abortion because of their enhanced vigour during the earliest stages of development, but these seeds abort after they have attained large size because of genomic imbalance.

Among dicotyledons with nuclear endosperm, maternal excess is also associated with early wall formation and paternal excess with delayed wall formation. In *Brassica*, nuclear divisions are accelerated in the endosperm from $2x \times 4x$ crosses and formation of cell walls is completely inhibited. On the other hand, in $4x \times 2x$ crosses, nuclear divisions are retarded in the endosperm, cell walls are formed precociously, and the final amount of endosperm is reduced, compared with $2x \times 2x$ and $4x \times 4x$ controls. Viable seeds are not produced in either direction of the cross (Håkansson 1956; Nishiyama & Inomata 1966). In *Citrus*, paternal excess is associated with delayed wall formation and maternal excess with smaller than normal endosperms (Esen & Soost 1973).

Valentine (1955) performed reciprocal crosses among diploid species of *Primula* and found that, in any pair of crosses, seed development departed from normality in two characteristic ways. Type A seeds were subnormal in size but generally well filled, whereas type B seeds were nearly normal in size but empty at maturity and inviable. He noted that the Type A seeds 'may be thought of as resulting from a general inhibition of slowing down of all processes which lead to seed formation . The nature of the Type B seeds on the other hand suggests that here some of the processes leading to seed formation are stimulated or speeded up, and that breakdown occurs because different parts of the mechanism get out of phase' (Valentine 1956). When the paternal excess was small, type B seeds were larger than normal, a fact that Valentine considered was consistent with some form of stimulation.

Type A and type B seeds contain endosperms with a maternal excess and paternal excess respectively. Thus, diploid × autotetraploid crosses in *Primula* yield type A seed when the tetraploid is the maternal parent, and type B seeds in the reciprocal cross (Woodell & Valentine 1961; Valentine & Woodell 1963). Woodell (1960*a*, *b*) studied seed development in interspecific crosses of *Primula*. The endosperm of type A seeds was characterized by precocious cell formation and the early accumulation of food reserves, whereas cell formation was delayed in type B seeds. This is the same pattern as found in other groups with Nuclear endosperm.

In *Oenothera hookeri*, cell walls were first formed after the eighth or ninth division cycle in $2x \times 2x$ and $4x \times 4x$ crosses, after the sixth or seventh division cycle in $4x \times 2x$ crosses, and after the tenth or eleventh division cycle in $2x \times 4x$ crosses. Cell divisions continued after initial cell formation, and the final number of cells was about 5500 in a normal 1m:1p endosperm ($2x \times 2x$), about 1800 in a 2m:1p endosperm ($4x \times 2x$), and 13000 in a 1m:2p endosperm ($4x \times 4x$) (von Wangenheim 1962). Normal endosperm in this family is diploid (1m:1p) rather than triploid (2m:1p).

Much less is known, embryologically, about the causes of endosperm failure in incompatible crosses that involve species with cellular endosperm. Endosperm is described as cellular if the earliest divisions of the primary endosperm nucleus are accompanied by the formation of cell walls (Maheshwari 1950). Reciprocal crosses between diploid and autotetraploid *Datura stramonium*, and between diploid and autotetraploid *Lycopersicon pimpinellifolium*, showed abnormally slow development of the endosperm in both directions of the cross, but without obvious reciprocal differences (Sansome *et al.* 1942; Cooper & Brink 1945). On the other hand, Håkansson (1952) observed marked differences between reciprocal crosses of diploid and autotetraploid *Galeopsis pubescens*. When the diploid was used as seed parent (paternal excess), the endosperm's early growth was vigorous, with a strongly developed micropylar haustorium which penetrated the integument and entered the ovarian cavity. However, rapid growth was not maintained, and the endosperm soon degenerated. In the reciprocal cross (maternal excess), endosperm development was slower than normal and came to a premature end. Moreover, the micropylar haustorium was weakly developed and did not penetrate the integument.

(*e*) *Genetic evidence of imprinting*

In this section, we review evidence from maize about the genetic basis of imprinting. The first convincing evidence for imprinting came from studies of the *r* locus in maize. The *r* allele causes colourless kernels when homozygous. If *rr* is crossed to *RR*, the resulting kernels are solidly coloured when *RR* is the female parent but mottled when *RR* is the male parent. Kermicle (1970) showed that the difference between reciprocal crosses was due to preferential expression of the *R* allele when maternally derived. Endosperm was solidly coloured whenever it possessed a maternal copy of *R*, but mottled whenever it possessed paternal copies of *R* without a maternal copy. Extra paternal copies of the allele could not substitute for a maternal copy. The imprinting effect was allele-specific, because the expression of another allele at the same locus was independent of parental origin.

Further evidence of imprinting came from studies of the 'small-kernel' effect in maize. Reciprocal translocations between autosomes and B-chromosomes can be used to generate endosperms that are deficient for a paternal copy of the segment translocated on to the B-chromosome. For some chromosomal regions (but not others), paternal deficiency caused the development of small kernels (Beckett 1978). Initially, this was interpreted as a simple dosage effect: small kernels had two rather than three copies of the chromosomal region in their endosperm. Parent-specific effects were first demonstrated for the long-arm of chromosome 10. Lin (1982) showed that extra maternal copies of *10L* could

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not compensate for the absence of a paternal copy. Maternal dosage had little or no effect on kernel size, and this was interpreted as evidence for preferential paternal expression at some loci on *10L*. This contrasted with Kermicle's (1970) evidence for preferential maternal expression at the *r* locus (also on *10L*).

The small-kernel effect has been shown to be a consequence of a paternal deficiency *per se* rather than a simple dosage effect, for translocations involving three particular chromosome arms. These are *1L*, *1S* and *10L* (Lin 1982; Birchler & Hart 1987). Whether imprinting also explains the small-kernel effect observed for other translocations awaits experimental test. Lin (1982) did not report any phenotypic effects of extra maternal copies of *10L*. On the other hand, extra maternal copies of *1S* enhance, rather than compensate for, the small-kernel effect caused by a paternal deficiency for *1S*. The same is true for extra maternal copies of *1L* (Birchler & Hart 1987).

This is not the only evidence that extra maternal copies of some chromosomal regions may contribute to the formation of small kernels. Paternal deficiency for *5L* does not cause a small-kernel effect (Beckett 1978), but an extra maternal dose of this region in each polar nucleus causes an 11% reduction in seed size (Beckett 1983). Similarly, extra maternal copies of *1S*, *1L*, *3S*, *4S*, *7L* and *10L* enhance the small-kernel effect caused by paternal deficiency for some other chromosomal regions (Birchler & Hart 1987). The underlying mechanisms are unclear, but the basic pattern of paternal deficiency and maternal excess both causing reductions in kernel size is predicted by Haig & Westoby's (1989) model.

Crosses that generate endosperms with a paternal deficiency also produce occasional endosperms that are mosaics for sectors with and without the deficiency. Birchler (1980) found no evidence for a small-kernel effect in sectors with a paternal deficiency, even though the same genotype would cause small kernels in non-mosaic grains. Birchler concluded that paternal genes in non-deficient sectors were able to supply deficient sectors with sufficient gene products to prevent the small-kernel effect. The simplest explanation is that the gene products which prevent the small-kernel phenotype are produced before cell walls are formed, at a stage when deficient and non-deficient nuclei are located in a common cytoplasm.

Segments of several chromosomes cause small-kernel effects in maize, suggesting that a substantial number of loci may be subject to imprinting. Moreover, the chromosome segments that are known to cause small-kernel effects are large enough to contain many loci, more than one of which may be imprinted. Lin (1982) showed that four smaller regions of *10L* each contributed to the reduction in endosperm size caused by paternal deficiency for this chromosome arm. Multiple imprinted loci are also implicated as causes of endosperm failure in interspecific crosses of *Primula* (Valentine 1956) and *Solanum* (Ehlenfeldt & Hanneman 1988).

Lin (1984) conclusively demonstrated that a ratio of two maternal genomes to one paternal genome was necessary for normal endosperm development in maize. Lin studied endosperm development in kernels produced on mothers homozygous for the *indeterminate gametophyte* allele. This allele causes variation in the number of polar nuclei that contribute maternal genomes to the endosperm. Lin independently varied the number of paternal genomes by using haploid and diploid pollen. By these means, Lin (1984) was able to generate endosperms with one to eight maternal genomes and one or two paternal genomes. All endosperms were produced on a diploid mother. Endosperms of the same ploidy developed differently, depending on the relative numbers of maternal and paternal genomes. Thus, hexaploid endosperms with four maternal genomes and two paternal genomes developed normally, but hexaploid endosperms with five maternal genomes and one paternal genome were abortive. The only endosperms which developed normally were triploid with two maternal genomes and one paternal genome, and hexaploid with four maternal genomes and two paternal genomes. The number of maternal genomes per paternal genome could not explain all observations: 3m:1p endosperms produced small kernels (consistent with maternal excess), but 6m:2p endosperms were abortive.

In summary, the available genetic evidence indicates that several loci, perhaps many, are imprinted. However, the embryological evidence indicates that most consequences of imprinting can be satisfactorily summarized as effects along a single dimension, from paternal excess, through normal development, to maternal excess. This outcome can perhaps be understood in the following terms. Selection for genomic imprinting arises through the impact of one offspring's development on the fitness of its half-sibs. While the effects of different loci on development may be complex and far from additive, the effects which matter to fitness of half-sibs may reduce simply to acquiring more resources faster (with negative effects on half-sibs, hence stronger selection for imprinting) versus acquiring fewer resources or acquiring them more slowly (with converse effects). Thus variation between lineages with respect to imprinting might be expected to reduce to a single dimension, even though many loci are involved and even though the developmental consequences of mutants at different loci do not, as a general principle, simply have additive effects.

5. GENOMIC IMPRINTING AND APOMIXIS

As a side effect of imprinting, successful development comes to depend on maternal and paternal genomes being present in the correct relative doses. If imprinting is important during an embryo's development, a parthenogenetically derived embryo would have unbalanced gene expression because all its genes are maternally derived. In fact, imprinting is thought to be the main reason why mammals are the only major group of vertebrates in which successful parthenogenesis is unknown (Surani 1987; Solter 1988).

Haig & Westoby's (1989) model predicted that imprinting should also be important during seed development, and we have shown above that it is of widespread occurrence in angiosperms. At first sight,

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imprinting ought to present an obstacle to the evolution of asexual reproduction by seeds (apomixis) in plants, as it has presented an obstacle to the evolution of parthenogenesis in mammals. Consistent with this expectation, apomixis is unknown among gymnosperms (Richards 1986). However, apomixis has evolved many times in angiosperms (Nygren 1967), and we argue below that this has been possible because of the division of labour between endosperm and embryo during seed development. Specifically, the endosperm, rather than the embryo, has been the tissue primarily subject to natural selection for imprinting and, as a consequence, apomictic embryos can develop successfully if they are associated with an endosperm that contains both maternal and paternal genomes in the proper ratio.

There are two fundamentally different types of apomixis, called adventitious embryony and gametophytic apomixis (Nogler 1984). In adventitious embryony, apomictic embryos are derived directly from an unreduced somatic cell, whereas the endosperm is derived from a meiotically produced female gametophyte (Lakshmanan & Ambegaokar 1984). In gametophytic apomixis, the embryo and endosperm both develop from an unreduced female gametophyte (Nogler 1984). Richards (1986) compiled data from Nygren's (1967) comprehensive review of the apomixis literature. In Richards' compilation, apomixis was reported from 34 families, but 75 % of apomictic taxa belonged to only three families, the Asteraceae, Poaceae and Rosaceae. Gametophytic apomixis was the only reported mode of apomixis in these three families, whereas adventitious embryony was the dominant mode of apomixis in the remaining families. These data suggested that some families were predisposed to the evolution of apomixis, and that different families were predisposed to evolving different forms of apomixis (Richards 1986).

Apomicts can also be classified by whether they have pseudogamous or autonomous development of endosperm. In pseudogamous apomixis, endosperm only develops after the polar nuclei are fertilized. That is, asexually derived embryos can only complete their development if they are associated with a sexually produced endosperm. In autonomous apomixis, the embryo and endosperm both develop without fertilization. Adventitious embryony and gametophytic apomixis each include pseudogamous and autonomous taxa (Nygren 1967; Nogler 1984). There are clear phylogenetic patterns in the distribution of pseudogamous and autonomous apomixis. Most apomictic members of the Asteraceae have autonomous development of endosperm, but autonomous apomixis has only a sporadic occurrence in other families. Most apomictic members of the Rosaceae and Poaceae are pseudogamous (Nogler 1984).

Apomicts must have evolved from sexual ancestors. Seed development in these ancestors is expected to have depended on the correct ratio of maternal to paternal genomes in endosperm. Therefore, seed development in their apomictic descendants would also be expected to depend on the same balance between paternal and maternal gene expression in endosperm. This may clarify why so many apomicts should be pseudogamous, but at the same time it raises difficulties (not previously appreciated) in understanding how autonomous apomixis has evolved, because the endosperm of autonomous apomicts develops in the absence of a paternal genome. One cannot circumvent this problem by proposing that autonomous apomicts evolved from sexual ancestors in which the embryo, rather than the endosperm, took the primary role in nutrient acquisition, because in that case natural selection would be expected to favour a paternal requirement in the embryo. The problem of genomic balance in the endosperm of autonomous apomicts remains unresolved.

(*a*) *The endosperm of pseudogamous apomicts*

The endosperm of pseudogamous apomicts contains both maternal and paternal genomes. It therefore poses less of a problem to the theory of genomic balance than does the endosperm of autonomous apomicts. However, normal development in sexual species usually depends on the correct maternal to paternal ratio, and this ratio might be disturbed in pseudogamous apomicts. In this section, we discuss the formation of endosperm in pseudogamous apomicts, and consider whether their endosperms conform to a 2m:1p ratio.

Most species with adventitious embryony are pseudogamous (Richards 1986), though there are exceptions (Kapil 1961; Nygren 1967). Typically, pollination is followed by double fertilization of a reduced female gametophyte and the initiation of a sexual embryo and endosperm. The sexually produced endosperm is then commandeered by an apomictic embryo derived from a somatic cell (Richards 1986). The endosperm is 2m:1p and genomically balanced.

The pollen of pseudogamous, gametophytic apomicts usually has the reduced number of chromosomes, whereas the polar nuclei have the unreduced number (Nogler 1984). Thus, the fusion of a male gamete with two polar nuclei would produce an unbalanced 4m:1p endosperm. Such an endosperm has been reported from *Tripsacum dactyloides* (Brown & Emery 1958). However, in most gametophytic apomicts that have been studied, fertilization is modified to produce endosperms with a 2:1 ratio of maternal to paternal genomes. Many apomictic grasses produce unreduced female gametophytes that are 4-nucleate, with one rather than two polar nuclei (Warmke 1954; Brown & Emery 1958). Fertilization of the single polar nucleus produces a 2 m:1p endosperm. In *Dichanthium annulatum*, the unreduced, 8-nucleate female gametophyte has two unfused polar nuclei. Either a reduced male gamete fuses with one polar nucleus, and the other polar nucleus degenerates, or each polar nucleus is fertilized by a reduced male gamete (Reddy & d'Cruz 1969). Other species produce 4m:2p endosperms. In *Paspalum secans*, an unreduced male gamete fertilizes two unreduced polar nuclei (Snyder 1957). In *Ranunculus auricomus*, two fused unreduced polar nuclei are fertilized by two reduced male gametes (Rutishauser 1954).

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From our reading of the literature, we cannot tell whether 2m:1p and 4m:2p endosperms are exceptional among pseudogamous gametophytic apomicts or whether they are the usual condition. However, we predict that such endosperms should be typical, and 4m:1p endosperms exceptional, because the sexual ancestors of most apomicts would have had balanced 2m:1p endosperms.

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