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The evolution of genomic imprinting

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Summary

We explore three possible pathways for the evolution of genomic imprinting. (1) *Imprinting may be advantageous in itself* when imprinted and unimprinted alleles of a locus confer different phenotypes. If a segment of DNA is imprinted in the gametes of one sex but not in those of the other, it might lead to effects correlated with sexual dimorphism. More fundamentally, in certain organisms, sex determination might have evolved because of imprinting. When imprinting leads to chromosome elimination or inactivation and occurs in some embryos but not in others, two classes of embryos, differing in the number of functional gene copies, would result. A model for sex determination based on inequality in the actual or effective copy-number of particular noncoding, regulatory sequences of DNA has been proposed (Chandra, *Proc. natn. Acad. Sci. U.S.A.* 82. 1165–1169 and 6947–6949, 1985). Maternal control of offspring sex is another possible consequence of imprinting; this would indicate a potential role for imprinting in sex ratio evolution. (2) *Genes responsible for imprinting may have pleiotropic effects* and they may have been selected for reasons other than their imprinting ability. Lack of evidence precludes further consideration of this possibility. (3) *Imprinting could have co-evolved with other traits*. For instance, gamete-specific imprinting could lead to a lowered fitness of androgenetic or gynogenetic diploids relative to the fitness of 'normal' diploids. This in turn would reinforce the evolution of anisogamy. The reversibility of imprinting raises the possibility of occassional incomplete or improper erasure. If the site of imprinting is the egg – as appears to be the case with the human X (Chandra and Brown, *Nature* 253. 165–168, 1975) – either improper imprinting or improper erasure could lead to unusual patterns of inheritance (as in the fragile-X syndrome) or fitness effects skipping generations.

Key words: X inactivation, mealybugs, evolution of sex, anisogamy.

Introduction

By imprinting we mean that process which leads to a reversible marking of one of two homologous loci, homologous chromosomes or chromosome sets in germ cells, or more generally, during development. Such marking may result in a functional non-equivalence of genes or chromosomes; this in fact was the basis for the recognition of the phenomenon (Metz, 1938; Hughes-Schrader, 1948). The term 'imprinting' was first used in a cytogenetic context by Helen Crouse in her study of chromosome elimination in *Sciara* (Crouse, 1960, 1966; Crouse *et al.* 1971). In some organisms, a consequence of imprinting is often the (reversible) loss of activity of one of the two homologous sequences of DNA. In genetic terms, imprinting can be equivalent to hemizygosity at a locus or group of loci. Examples of imprinting include elimination of paternal chromosomes in *Sciara coprophila* (Metz, 1938) and diaspidid coccids (Brown and Bennett, 1957), heterochromatization and inactivation of paternal chromosomes in lecanoid coccids (Hughes-Schrader, 1948; Brown and Nelson-Rees, 1961; Nur, 1963), heterochromatization and inactivation of the X chromosome in mammals (Lyon, 1961; Brown and Chandra, 1973), parental chromosome strand-dependent switching of mating type in yeast (Klar, 1987), differential methylation of a transgene (which may include adjacent sequences at the site of insertion) in the mouse (Reik *et al.* 1987; Sapienza *et al.* 1987) and possibly allelic exclusion in immunoglobulin genes (Pernis *et al.* 1965; see also Chandra and Brown, 1975).

Imprinting is generally sex-specific; as will be discussed below, this does not mean that it necessarily occurs in the germ line. A consequence of imprinting is that the same locus or chromosome or chromosome set functions differently depending on its parental origin. This raises the question of why a segment of DNA in a cell lineage of a diploid individual should be active or inactive, or more generally, behave differently, based on whether it is derived from the father or the mother. In this paper we wish to consider possible evolutionary answers to this question.

Imprinting and sex

At least two distinct sets of genetic elements need to be involved in imprinting: the DNA sequences that are themselves imprinted, and the genetic and epigenetic machinery responsible for imprinting. To the extent that imprinting is sex-specific, the second set would constitute a subset of the genetic loci involved in sexual dimorphism in the broadest sense. The emphasis on sex-specificity is important, because in its absence the term 'imprinting' acquires a wider connotation. For instance, 'imprinting' can be subsumed under the phenomenon of dominance modification, in which case the evolution of imprinting could be associated with the evolution of recessiveness in a newly arisen mutation (Sapienza, 1989). On the other hand, when – as in some of the examples cited in the Introduction – imprinting *is* sex-specific, its evolutionary history must necessarily be tied up with the evolution of sexually dimorphic traits if not with the evolution of sex itself.

Possible levels of selection

Assuming that a positive component of fitness is associated with imprinting, there are essentially three ways in which imprinting of a segment of DNA could have evolved: (a) the genes responsible for imprinting may have pleiotropic effects and may be selected for reasons unrelated to their ability to imprint other loci, (b) imprinting of the segment of DNA may be selectively advantageous in itself, or (c) the machinery for imprinting may have co-evolved with some other selectively advantageous trait. We discuss possibilities (b) and (c) later. The reason for mentioning (a) at all is that sex-of-parent dependent methylation has been observed to occur in the case of a number of transgenes (Reik *et al.* 1987; Sapienza *et al.* 1987), and it seems hardly likely that in each case the transgene contained sequences that were the natural substrates of host-specific methylating enzymes. This argument holds good even when the primary imprinting signal does not involve DNA methylation.

In the context of normal development, one set of circumstances consistent with (a) would be the following. Consider a mechanism for sequence-specific modification (say methylation) of DNA in individuals of one sex but not the other. The DNA so modified could include genetic loci involved in sexual dimorphism. Suppose another segment of DNA carries target sequences similar to those in the sexually dimorphic genes. If the latter segment becomes accessible to the modifying mechanism, it could be modified in the germ line and, as long as there is no reduction in fitness, could continue to carry the modification into the next generation. Lack of evidence one way or the other makes further speculation along these lines fruitless, and in what follows we therefore confine our attention to possibilities (b) and (c).

Selection in favour of imprinting *per se*

As noted earlier, if a segment of DNA is imprinted in the gametes of one sex but not in those of the other, the genes responsible for imprinting could have sexually dimorphic functions. In other words, such genes must function either at the level of, or subsequent to, the primary signal determining sex. A gene whose state of activity becomes important after primary sex has been determined can exert its effect in several ways. For example, it could cause the appearance of a zygotic phenotype that depends on the genotype of only one parent, most plausibly the mother. Some of the known genes with maternal effects could fall into this class. Thus 'maternal effect' may not always have to do with the storage of a maternally synthesised gene product. Indeed, recent work of Bander *et al.* (1989) provides evidence of this, albeit for a 'paternal' effect. In crosses between C57BL/6By and BALB/cBy strains of mice, significant differences were observed between the eggs of F_1 females from reciprocal matings with respect to sensitivity to pronase and hyaluronidase. Eggs from females born of C57BL fathers showed the increased susceptibility characteristic of C57BL while eggs derived from females born of BALB fathers were, like the BALB strain generally, relatively resistant to both enzymes. A similar patroclinous segregation was also evident in the four kinds of F_2 females. These findings are consistent with an interpretation based on a pattern of differential imprinting resulting in differential egg susceptibility to enzyme action inherited through the males of the two strains (Bander *et al.* 1989). This suggests that genetic variation exists in the imprinting machinery. If differences of this nature (see references in Bander *et al.* 1989 for other examples) have consequences in terms of the fitness of the zygote, a selective role for gamete-specific imprinting would be indicated.

Any relationship of imprinting to sex determination itself is obviously more interesting. There are at least three systems in which cytogenetic methods have indicated such a relationship: (1) *Sciara coprophila*; (2) Coccid systems with chromosome elimination; (3) Coccid systems with chromosome inactivation.

Sciara coprophila

In *Sciara* imprinting is seen as chromosome elimination. It occurs in some embryos but not in others, resulting in two classes of embryos differing in the actual or effective copy number of particular chromosomes. This inequality in copy number is the basis of sex determination. *Sciara coprophila* zygotes contain two sets of autosomes (A) and three X chromosomes. Two of these X chromosomes are paternal in origin and the chromosome constitution of the zygote can therefore be represented as $A^mA^pX^mX^pX^p$. During early embryogenesis, one X chromosome of paternal origin is eliminated from the germ line of both sexes and from the female soma. In the male soma both X chromosomes of paternal origin are eliminated. The end result is that the female and male germ lines and the female soma are X^mX^p. The male soma on the other hand is X^mO. Thus while the germ line of both sexes is AAXX, the chromosome constitutions of the soma are differ-

ent: AAXX (female) and AAXO (male). A point of particular interest, is that differential elimination of chromosomes in the soma seems to be based on maternally controlled imprinting (Crouse, 1960, 1966). The imprinted site on the X chromosome, which is responsible for the subsequent elimination of the X, is located close to the centromere. In X-autosome translocations only those translocated chromosomes which carry the X centromere are subject to imprinting and subsequent elimination (Crouse, 1960, 1966).

Coccid systems involving elimination

In the armoured scale insects, which are among the most highly specialized coccids, paternal chromosomes are eliminated during the cleavage divisions in some embryos; these develop into males. In other embryos, no such elimination occurs, and these develop into females (Brown and Bennett, 1957). Thus, by definition, paternal chromosomes are imprinted in some embryos but not in others. Hence, although fertilization is necessary for embryonic development to begin, the imprinting of paternal chromosomes leading to their elimination in male embryos leads to a haplo-diploid system of sex determination similar in effect to that in wasps and honeybees.

Coccid systems involving chromosome inactivation

In certain sexually reproducing coccids, the paternal set of chromosomes becomes heterochromatic and genetically inactive in embryos that develop into males. Such inactivation occurs during early embryogenesis and the chromosomes remain in a heterochromatic state in most tissues during subsequent development (Fig. 1). In female embryos no such distinction between paternal and maternal chromosomes is apparent and genetic tests reveal that both sets of chromosomes are functional. There is no recombination in males. During meiosis the paternal, heterochromatic set of chromosomes is eliminated and all sperm contain only the maternal chromosomes. This system is referred to as the Lecanoid system after the group in which it was discovered (Hughes-Schrader, 1948; Brown and Nelson-Rees, 1961).

In the *Comstockiella* system, which is an extraordinary variant of the above, paternal chromosomes turn heterochromatic in male embryos and the system is indistinguishable from the Lecanoid system except in the premeiotic and meiotic stages. There is selective destruction of one or more paternal chromosomes during premeiotic prophase. Those chromosomes not so destroyed are eliminated subsequently, but before spermiogenesis. Here also, only the maternal chromosomes form sperm (Brown, 1963; Kitchin, 1970).

In certain coccids which usually reproduce parthenogenetically, rare males can arise in the absence of fertilization. The mechanism by which such male embryos originate provided compelling evidence that imprinting occurs in the egg (Nur, 1963). In *Pulvinaria hydrangeae*, the haploid egg pronucleus divides once and the two haploid nuclei that result fuse to form a zygote substitute. Such diploid embryos normally develop into females which continue facultative parthenogenesis. However, in a small number of such parthenogenetic embryos one of the two haploid sets of chromosomes turns heterochromatic early in development; such embryos become males. Since all chromosomes are maternal in origin, the two sets of haploid chromosomes, being mitotic products of the haploid egg pronucleus, would be expected to be genetically identical. Thus imprinting must have occurred *in* the egg, presumably during the brief period when the two division products of the egg pronucleus were spatially separate. These results, by extrapolation, suggest that in sexually reproducing coccids also,

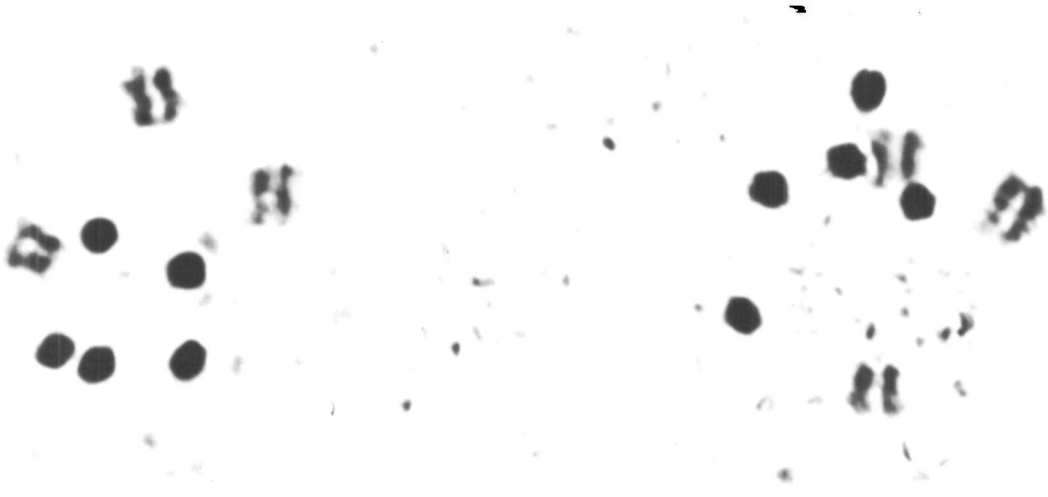

Fig. 1. Imprinting and inactivation of paternal chromosomes in a mealybug, *Planococcus citri* (Risso) [Lecanoidea; Homoptera]. Two cells with chromosomes in mitotic prophase, from an embryo which was derived from a cross between a triploid female (3n=15) and a normal diploid male (2n=10). The three euchromatic chromosomes in each cell are maternal, and the five heterochromatic chromosomes paternal, in origin. The mother, being triploid, had contributed only three chromosomes to this embryo. The euchromatic chromosomes are undergoing endomitotic replication and therefore appear split (Chandra, 1963).

imprinting occurs in the egg at the time of fertilization (Nur, 1963; Chandra, 1963; Brown and Chandra, 1977).

Thus in both *Sciara* and coccids, there is evidence of *maternal* control of imprinting (Chandra and Brown, 1975). Since primary sex determination in these insects appears to be a consequence of imprinting, it follows that the mother can determine the sex of her offspring. In addition to providing a mechanism for the maternal control of sex, this also provides a means for the maternal control of progeny sex ratio, a central issue in the area of sex ratio evolution (Charnov, 1982). Therefore, at least in these insects, imprinting seems to be a means exercised by the mother to control the sex of her offspring. This opens up a possible evolutionary route in that imprinting could be a device utilised by the mother to arrive at the sex ratio appropriate to a particular ecological setting.

In mammals a one-to-one sex ratio is normally controlled by Mendelian segregation of X and Y chromosomes. The evidence from *Sciara* and mealybugs raises the intriguing question whether even among mammals sex determination, and so the sex ratio, may in part be under maternal control. The evidence in favour of maternal control of imprinting in mammals comes from human ovarian teratomas. These are maternally derived diploid parthenogenetic growths, and genetic evidence suggests that they are postmeiotic in origin. (Linder, 1969). They result either from an abnormal proliferation of polar body I or from a fusion product of polar body II and the egg pronucleus. Since one of the two Xs in such teratomas is inactive, it suggests that the machinery to imprint and inactivate the X chromosome can be entirely maternal (Chandra and Brown, 1975). However, the relation of mammalian X-inactivation to sex determination remains hypothetical (Chandra, 1985).

Co-evolution of imprinting

Arguments for the evolutionary origin of sex involve selective advantages associated with recombination and segregation, especially in spatially or temporally varying environments (Bell, 1982). Could imprinting, that is, gamete-specific marking, also be advantageous for the *origin* of sex (as opposed to its maintenance)? For this to be so, one would need to postulate a positive component of fitness associated with the union of two haploid genomes, one marked and the other unmarked. This does not seem to be a promising line of reasoning to follow. Current theories for the origin of sex tend to be based on an initial variation at a single locus between otherwise identical genomes (Bull, 1983). If neither the genomes nor the gametes of the two sexes were significantly different to begin with, why would there be a fitness differential associated with marking as such? Unless marking itself conferred an advantage, or was associated with an advantage, it is not obvious that the union of two similarly marked (or two unmarked) haploid genomes would have been any less fit than that of one marked and one unmarked genome. Therefore, it is unlikely that imprinting *per se* conferred a specific advantage to sexual as opposed to asexual reproduction.

We wish to explore an alternative, namely that imprinting evolved in conjunction with something else. This 'something else' could have been a trait which co-evolved with sex, was selected for independently, and selection in whose favour was accelerated by the occurrence of imprinting. Since a consequence of most cases of imprinting is that a haploid genome 'knows' which sex it originated from, one is motivated to consider possible advantages that the co-evolution of imprinting may have had for gametic differentiation (that is, anisogamy). At a time subsequent to the origin of sex but preceding the differentiation of sex chromosomes, sex must have been based on a dichotomy between one or more genetic (sex) factors on homologous chromosomes which functioned differentially in the two sexes. Anisogamy, or a tendency to anisogamy, would appear thereafter. There are two basic assumptions leading to such a conclusion (Parker *et al.* 1972; Maynard Smith, 1978; Bell, 1982). One is that the number of gametes produced by an individual is inversely proportional to gamete size; and the other, that the fitness (e.g. the probability of survival to a defined stage) of the zygote formed by the union of two gametes increases disproportionately with its size, that is, with the combined size of the two gametes. Thus doubling the size of a zygote would confer on it a much greater than two-fold fitness advantage. With the appropriate relationship of zygote size to fitness, it turns out that no single gamete size – very small or very large – represents an evolutionarily stable strategy for an individual. (Parker *et al.* 1972; Maynard Smith, 1978; Bell, 1982). Basically, the point is that once a small imbalance appears, disruptive selection starts operating in favour of increasing the size difference between the gametes of the two sexes. Starting with a situation in which all individuals produce only microgametes, not only can an individual producing macrogametes successfully 'invade', but the individuals producing microgametes gain in fitness if their gametes fuse preferentially with macrogametes (Maynard Smith, 1978). This is because the fitness of a microgamete–macrogamete combination would be higher than that of a microgamete–microgamete combination.

Once there is a tendency towards anisogamy, purely physical considerations might influence the relative probabilities of union between differently sized gametes: the chances of a small motile gamete meeting a large sessile one can be higher than those of two similar gametes of either type uniting (Scudo, 1967). Alternatively, especially at a time when gamete size differences were not significant, imprinting may have played a role by lowering the fitness of zygotes formed from gametes of the same sex. We suggest that any form of gametic marking – imprinting – which tends to lower the fitness of the union of a pair of like gametes would be selectively advantageous.

Consider the gametes of an individual carrying a newly arisen mutation that caused imprinting.

Imprinted gametes would have a higher fitness than those of a randomly chosen individual because imprinting would, by assumption, lead to one of two consequences: either it would foreclose the possibility of two similar gametes fusing or, perhaps more plausibly, lower the fitness of 'like–like' unions relative to that of 'like–unlike' ones. It follows as a corollary that imprinting would also favour cross-fertilization over self-fertilization and thereby aid in the evolution of sex as well (Fig. 2).

Two pieces of circumstantial evidence are in favour of this hypothesis for the origin of imprinting. In some cases imprinting involves DNA methylation, and this is reminiscent of the means used by bacteria to modify DNA. The phenomenon of modification was discovered on the basis of its association with restriction, the degradation by host-specific enzymes of foreign (viral) DNA which had not been appropriately modified. It has been conjectured that one evolutionary function of the modification-restriction system could be to prevent the 'contamination' of the genome of one bacterial species by that of another (Luria, 1973). Among higher animals such 'contamination' is prevented by means of mating barriers, hybrid sterility and so forth; but it makes for an attractive conjecture to think that similar mechanisms may be responsible, in part, for preventing the inappropriate union of one haploid genome with another from the same species.

The second bit of information suggesting the involvement of imprinting in the evolution of sex is that, at least in the mammals, successful embryogenesis seems to require the presence of both male-derived as well as female-derived germ cells (McGrath and Solter, 1984; Barton *et al.* 1984). There is evidence that even the presence of two homologous chromosome segments, both derived from individuals of the same sex, can be lethal (Cattanach and Kirk, 1985; Cattanach, 1986). Thus there is a clear-cut negative component of fitness associated with the union of two 'like', by implication similarly imprinted, gametes. The requirement that the two homologous chromosomes in a diploid zygote be derived from individuals belonging to different sexes would lead to a refinement of the meiotic process by favouring proper chromosome segration. One does not have equally compelling evidence pertaining to the fitness of like-sex zygotes from other orders, but it should be pointed out that even an apparently negative result – the successful development of a gynogenetic or androgenetic zygote – might simply mean that the fitness differentials are very small.

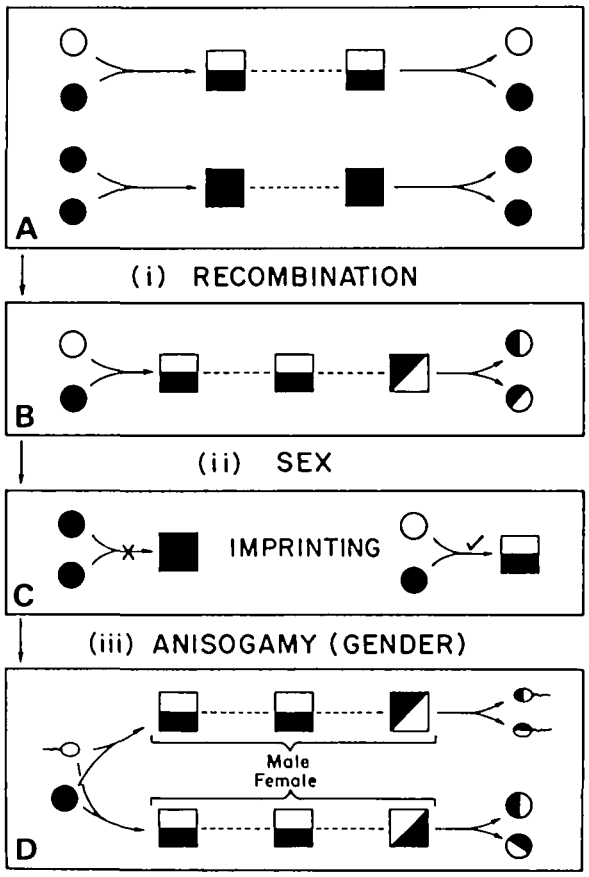

Fig. 2. Schematic representation of a possible pathway for the evolution of genomic imprinting. Circles and ellipses stand for haploid cells (or genomes), squares for diploid cells (or genomes). Shaded and unshaded areas, either between symbols or within the same symbol, stand for genomes from different individuals. The lower sequence in A and the first sequence in C both represent the union of two haploid genomes deriving from the same individual (i.e. self-fertilization). Recombination and meiosis are indicated as changes in the pattern of shading. The initial state represents a primitive situation in which diploid and haploid states alternate; transitions between the two are mediated by nuclear fusion and segregation of homologous chromosomes. The evolution of recombination, sex and gender (anisogamy) are indicated as steps (i), (ii) and (iii) respectively (see text for details). Following step (i), the diploid state undergoes recombination; haploidy is restored by meiosis. After step (ii) the union of haploid genomes from two distinct individuals is more fit than a similar union between genomes deriving from the same individual. Following step (iii) gender differentiation occurs: the two distinct individuals of step (ii) become separated into distinguishable classes depending on whether they produce very large or very small gametes. Both step (ii) and step (iii) would be accelerated if the selective differential between favoured and disfavoured unions of haploid cells were to be enhanced. Our hypothesis is that imprinting may have ensured such enhancement.

Concluding remarks

Except in the germ line, imprinting is often resistant to erasure during development. Incomplete erasure can have two consequences. It could lead to phenotypic variation of the kind ascribed to developmental 'noise'. On the other hand it can lead to unusual, apparently non-Mendelian, patterns of familial segregation – including fitness effects skipping generations – of the type associated with the fragile-X syndrome (Laird, 1987; Sapienza *et al.* 1989*a*). If chromosome-specific, gamete-of-origin-dependent, variations in the pattern of imprinting occur, groups of cells within the same

embryo might come to differ with respect to the patterns of imprinting carried in their genomes. This suggests that the pattern of imprinting might be a meaningful signature for a particular cell lineage. The preferential heterochromatization and inactivation of paternal X chromosomes in the extraembryonic membranes of the mouse (Takagi and Sasaki, 1975) provides a precedent. Sapienza *et al.* (1989*b*) have proposed that this might even be the evolutionary function of imprinting: by allowing for epigenetic mosaicism, imprinting could lead to the early establishment of different cell lineages, in particular of the germ cell lineage. Interestingly, *Caenorhabditis elegans* seems to provide a counter-example. In this nematode, even though cell lineage is an important determinant of cell fate, parental genome-specific imprinting does not appear to provide a basis for demarcating different cell lineages. This is because DNA, irrespective of whether it is derived from the egg or the sperm, is distributed in a spatially random manner during embryonic development (Ito and McGhee, 1987).

Finally, if a segment of DNA is marked cumulatively (rather than reversibly), one can imagine it as carrying a serial imprint of its lineage. The imprint might read PMPPM.... etc. with each P or M referring to the sex, in a particular generation, of the individual through whom the gene has been passaged. If the imprint affects the fitness of its bearer, one is led to consider the possibility that the selective value of a gene might lie to some extent in its history.

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