Sex-linked dosage-sensitive modifiers as imprinting genes

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Summary

It is proposed that differential genome imprinting is the result of dosage-sensitive modifier genes located on the sex chromosomes. Parallels between variegating position-effects in *Drosophila*, the phenotype elicited by transgenes in the mouse and data from several pediatric tumors indicate that the net result of the activity of such modifier genes is often cellular mosaicism in the expression of affected alleles. The mechanism by which inactivation of affected alleles is achieved is proposed to be through the formation of heterochromatic domains.

Because the relevant sex-linked modifying loci are dosage sensitive in their activity, differential imprinting will occur even within homogeneous genetic backgrounds. The presence of allelic variants at these loci in non-inbred populations will give rise to variation in the observed expressivity and mode of inheritance of affected traits.

Key words: sex-linked modifying loci, dosage-sensitive modifiers, *Drosophila* variegation, Huntington's disease.

Introduction

Genome imprinting may be operationally defined as the process that results in the gamete-of-origin-dependent modification of phenotypes (Crouse, 1960; Monk, 1988). The involvement of genome imprinting in the modification of any particular phenotype has most often been recognized through a negative effect on the expression of affected alleles (Sapienza, 1989). Whether the inactivation of alleles at particular loci is the primary purpose of genome imprinting, or whether this phenomenon is simply a reflection of the primary purpose is unknown. Nevertheless, the existence of the process has important consequences for the observed mode of inheritance, penetrance and expressivity of affected traits.

I have argued (Sapienza, 1989) that the phenotypes affected by genome imprinting represent special cases of dominance modification (Fisher, 1928). As such, the phenomena are most easily treated within the confines of models that invoke the activity of modifier genes (imprinting genes) on particular loci (imprinted or modified genes). While these models, as initially proposed (Sapienza, 1989), have been useful in explaining differences in the phenotype observed for imprinted loci between individuals in outbred populations or between inbred strains, they do not address the fundamental problem of gamete-of-origin-dependent modification: genome imprinting persists, even within inbred populations (Spofford, 1959; McGrath and Solter, 1986). Males imprint their genomes differently than do females, even though each may be genetically identical.

This observation would seem to lend support to the notion that differential imprinting may reflect the fact that the physiology and biochemistry of gamete formation is different in males and females. Within such a model, genetics need play no part other than the role of genes in determining sex. However, the genotype-dependent differences we (Sapienza *et al.* 1989; McGowan *et al.* 1989) and others (Surani *et al.* 1990; Allen *et al.* 1990; Reik, personal communication) observe in the phenotypes yielded at some imprinted loci indicates that this cannot be completely true.

It is the purpose of this paper to present a hypothesis that accommodates both of these observations, and provide a predictive genetic model for genome imprinting. A formal statement of the hypothesis is that

'Genome imprinting is a result of the activity of dosage-sensitive modifier genes located on the sex chromosomes.'

For the purposes of the following models, I will assume that the inactivation of imprinted alleles by modifier loci is accomplished through the formation of heterochromatic domains. I will further assume that the process generally results in variegation of expression of the affected allele. This latter assumption may not be demonstrable for all phenotypes observed to be affected by imprinting, but if not, then these phenotypes are expected to be secondary to an underlying variegated phenotype. It should be pointed out that, within these models, DNA methylation need not play a primary role in imprinting. The strong correlation observed between the gamete-of-origin of an affected locus and the methylation phenotype of that locus (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987; Hadchouel *et al.* 1987) is predicted to be a consequence

of a role for DNA methylation in the maintenance of heterochromatic domains rather than a primary role in their establishment (Deobagkar *et al.* 1990; Selker, 1990).

Genome imprinting often gives rise to variegated phenotypes

Allelic differences between individuals at loci responsible for generating genome imprints are predicted to give rise to variability in the expression of imprinted loci that are identical by descent. (Fig. 1 and Sapienza, 1989). In the case of transgene loci introduced into the mouse genome by microinjection, we and others have gathered both indirect and direct evidence that such variability has a genetic component and an epigenetic component (Sapienza *et al.* 1989; McGowan *et al.* 1989; Surani *et al.* 1990; Allen *et al.* 1990) and sometimes reflects different degrees of cellular mosaicism in the expression of the introduced sequences (McGowan *et al.* 1989). Conceptually similar models, including somatic mosaicism, account for the observed preferential retention of paternal tumor suppressor alleles in sporadic cases of several pediatric tumors (Reik and Surani, 1989; Scrable *et al.* 1989; Sapienza, 1990), as well as genetic linkage data (Scrable *et al.* 1989) in familial cases of several others (Grundy *et al.* 1988; Huff *et al.* 1988).

These observations bear striking resemblance to the
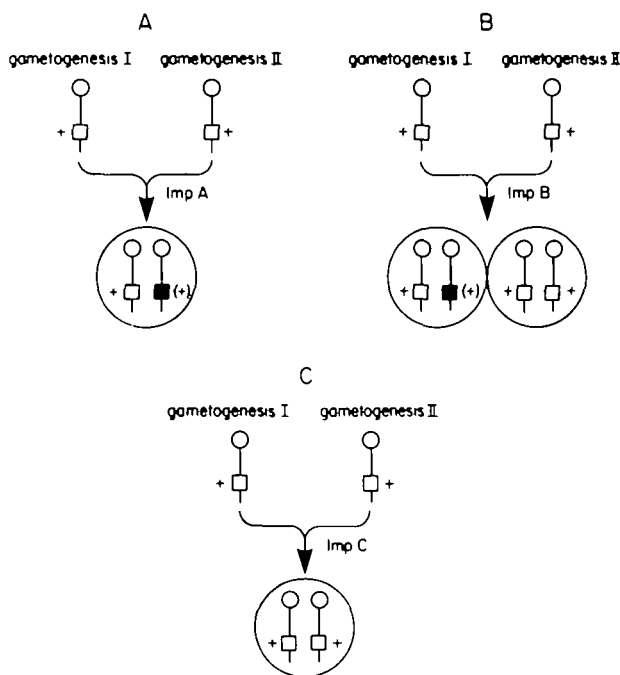
Fig. 1. Imp A, B and C represent alleles at a gamete-of-origin-dependent modifier locus. Boxes represent alleles at a modified locus. The activity of an allele at the Imp locus results in inactivation of (filled boxes) or failure to inactivate (open boxes) alleles at the modified locus, resulting in different degrees of mosaicism of expression of the affected allele, ranging from completely enhanced (Imp A) to completely suppressed (Imp C). See text.

behavior elicited by certain rearranged chromosomes in *Drosophila*. Rearrangements that result in the juxtaposition of normally euchromatic regions adjacent to normally heterochromatic regions often give rise to mosaic expression of loci within the normally euchromatic domains. This phenomenon is referred to as position-effect variegation (reviewed in Spofford, 1976). There are several parallels between the *Drosophila* and mouse systems that bear mention. (1) Both phenomena may exhibit gamete-of-origin effects (reviewed in Spofford, 1976 and Solter, 1988). (2) The expression, or lack of expression, of affected loci is cell autonomous. In *Drosophila*, variegation is usually observed as a mosaic of wholly wild-type and wholly mutant patches (Spofford, 1976). In the mouse, none of the 15–50 copies of some transgene constructs are expressed in some cells (Katsuki *et al.* 1988; Sweetser *et al.* 1988; McGowan *et al.* 1989). (3) Additionally, because such introduced transgene loci may contain>100 kilobases of repeats of the same sequence, in those cells in which no copy is expressed, the epigenetic effect must affect a domain of at least 100 kilobases, analogous to the 'spreading' of variegation to adjacent loci in *Drosophila* (Spofford, 1976; Locke *et al.* 1988). (4) Both phenomena are responsive to the activity of unlinked modifier genes (Spofford, 1976; Sapienza, 1989).

Many modifiers of variegating position-effects (Locke *et al.* 1988), as well as modifiers of other phenotypes in *Drosophila* (Rabinow and Birchler, 1989), are responsive to gene dosage. Locke *et al.* (1988) have described a number of modifying genes that affect white-mottled and yellow variegation. Their extensive genetic analyses define two classes of modifiers. Those in class I are deficiency-dependent suppressors and duplication-dependent enhancers of variegation. (A *suppressor* of variegation is defined as a modifying gene that gives rise to more wild-type tissue, *i.e.* fewer cells that display the mutant phenotype; an *enhancer* of variegation gives rise to less wild-type tissue, *i.e.* more cells that display the mutant phenotype). Class II modifiers behave in a reciprocal fashion and are deficiency-dependent enhancers and duplication-dependent suppressors of variegation.

By genetic criteria, class I modifiers are the most numerous and it has been estimated that 20–30 such modifiers are present in the *Drosophila* genome. Based on their observations, Locke *et al.* (1988) propose a mass-action model to explain the behavior of suppressor/enhancer loci. Each class I modifier is thought to encode a structural protein component of heterochromatin. Class I modifiers are proposed to act on variegation through a dosage-dependent effect on the extent of heterochromatin assembly. Biochemical and genetic experiments have yielded data consistent with this interpretation for at least 3 loci (Moore *et al.* 1979; James and Elgin, 1986; Reuter *et al.* 1990).

In contrast, only 2 loci that behave as class II modifiers have been described. Because class II modifiers of variegation behave reciprocally to those in class I (the reduction or removal of a class II gene
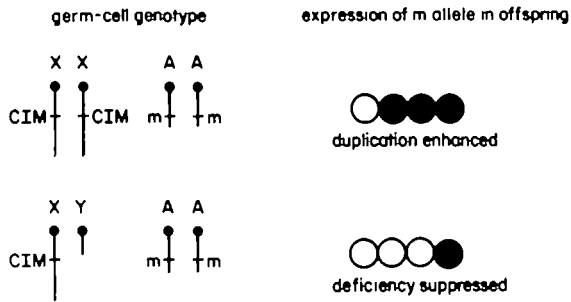
germ-cell genotype   expression of m allele m offspring

Fig. 2. Effect of an X-linked, dosage-sensitive, class I modifier (CIM) on the expression of an allele at a modified (m) locus. Open circles represent cells in which the allele is expressed. Filled circles represent cells in which the allele is not expressed. See text.

product increases the spread of a heterochromatic domain), they propose that class II products inhibit class I products directly, bind to 'termination sites' that define heterochromatic domains, or promote euchromatin formation.

In the mouse, we and others have demonstrated the existence of modifier loci that alter the phenotype produced by unlinked transgenes (Sapienza *et al.* 1989; McGowan *et al.* 1989; Surani *et al.* 1990; Allen *et al.* 1990). We further infer the existence of more than one such modifier locus that may affect different transgene loci in different ways. That such modifying loci may behave in a manner similar to that observed in the modification of variegating position-effects in *Drosophila* is illustrated by at least one recent study (see Fig. 3 in McGowan *et al.* 1989).

The demonstrated dosage sensitivity of modifier genes in *Drosophila* provides a mechanism by which parental-specific imprinting may be accomplished. With these concepts in mind, we may predict the genetic behavior of modifier genes that may lie on the sex chromosomes by their location (X or Y) and type (class I or class II).

Dosage-sensitive modifiers on the sex chromosomes

Any X-linked modifier that is dosage sensitive in its activity will necessarily give rise to gamete-of-origin-dependent modification (imprinting). In the case of class I modifiers, therefore, males (who will have a deficiency because they are hemizygous for the X-linked modifier) will suppress variegation of affected loci in their offspring. Females, on the other hand, will have a 'duplication' (two X chromosomes) and a class I modifier will enhance variegation in their offspring (Fig. 2).

The predictions for this type of modifier locus provide an explanation for what has been a puzzling set of observations on the behavior of transgene loci in the mouse. In contrast to some of the endogenous loci examined (Sanford *et al.* 1987; Monk *et al.* 1987), the methylation phenotype of imprinted transgenes has, with one exception (Sapienza *et al.* 1987), proved to be

hypomethylated when transmitted by a male and hypermethylated when transmitted by a female. If the transgene locus was affected by an X-linked class I modifier, however, the prediction is that the observed phenotype *will be suppressed by male transmission* (deficiencies give rise to a higher proportion of cells that are 'wild type'; *i.e.* the transgene will be expressed in a larger fraction of cells and/or the transgene will be hypomethylated) and enhanced by female transmission (duplications give rise to more nearly 'mutant' tissue and the transgene will be expressed in fewer cells and/or be hypermethylated).

The activity of an X-linked class II modifier will be revealed by reciprocal behavior at an affected locus (Fig. 3). Variegation of an imprinted locus will be enhanced by male transmission, i.e. the phenotype will appear more nearly mutant, and suppressed by female transmission, i.e. the phenotype will appear more nearly wild type.

Only one transgene locus has been described that appeared to be under the influence of a class II modifier (Sapienza *et al.* 1987). The methylation phenotype of this locus was hypermethylated when transmitted by a male and hypomethylated when transmitted by a female. The failure to identify more transgene loci that behaved in this manner was, *a priori*, unexpected, but if the relative abundance of identifiable class I and class II modifiers in the *Drosophila* genome is mirrored in the mouse genome, then it is not surprising that the activity of variant alleles of rare class II modifiers should be difficult to discern.

The behavior of modifiers on the Y chromosome is difficult to predict strictly on the basis of gene dosage models, because Y-linked modifiers will be either present or absent, rather than present in one dose or two doses. If a gene for a protein component of heterochromatin were present on Y, then the additive nature of these modifiers (Locke *et al.* 1988) would predict that such an activity lead to enhancement of variegation as a consequence of male gametogenesis. This prediction is identical to that made for an X-linked class II modifier. The only way to distinguish one from the other is by determining the mode of inheritance of the modifier by monitoring its activity on an affected locus in pedigrees.
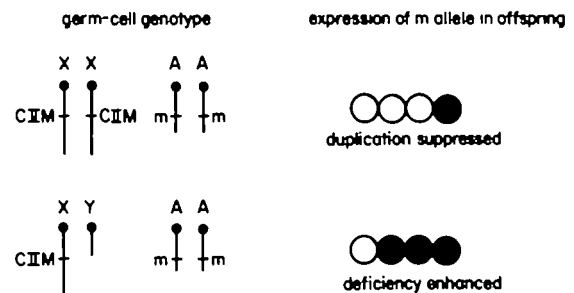
A Y-linked class II modifier yields a prediction

germ-cell genotype   expression of m allele in offspring

Fig. 3. Effect of an X-linked, dosage-sensitive, class II modifier (CIIM) on the expression of an allele at a . modified locus. Symbols as in Fig. 2.

similar to that for an X-linked class I modifier; enhancement through females and suppression through males. In *Drosophila*, the addition of a Y chromosome or part of a Y chromosome has a marked suppressive effect on variegation (Spofford, 1976). However, this is probably not due to the activity of a class II modifier like those shown in Fig. 3. Within these models, suppression results from the failure of the affected locus to be assembled into heterochromatin (Locke *et al.* 1988 and Fig. 4). This is accomplished by reducing the effective supply of any one of the class I components, either directly (class I deficiency suppression) or indirectly (class II duplication suppression). One additional mechanism by which this may be accomplished is to increase the size of the target that is subject to heterochromatin assembly (Fig. 4). It seems
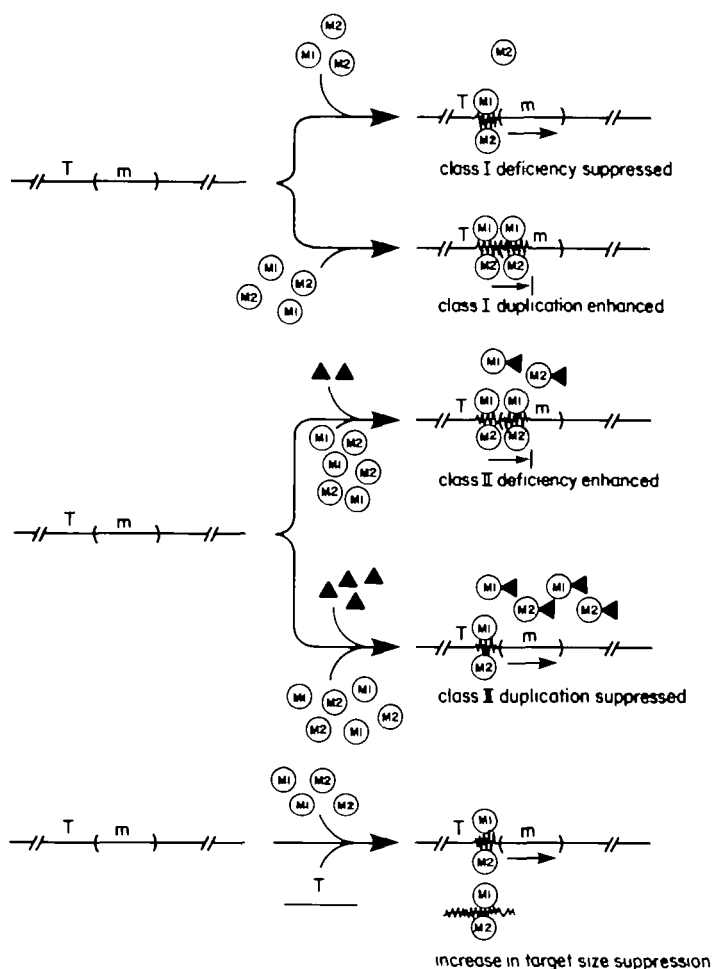
Fig. 4. Mechanism by which variegation of an affected allele may be enhanced or suppressed by the dosage of modifier gene products (Class I and Class II enhanced and suppressed, adapted from Locke *et al.* 1988). M1 and M2 represent protein components of heterchromatin (class I modifiers). Triangles represent class II modifier gene products. T denotes the target of class I modifiers and m represents an adjacent locus to which variegation may spread. In this version of the model, class II products are depicted as exerting their effect through direct interaction with class I products, however, direct interaction of class II products with the target may also occur (see text).

likely that the addition of large tracts of constitutive heterochromatin in the form of a Y chromosome accounts for such suppression, and that the increase in target size is effective even when supplied in *trans* (Spofford, 1976).

Within the context of this mode of suppression, it is interesting to note that one of the few human chromosomes that show a high degree of polymorphism at the level of the karyotype is the Y. Large variations in the length of the Y chromosome have been described, and the bulk of this variation is thought to be due to the absolute amount of a simple tandem repeat which is a major component of Y heterochromatin (Schmid *et al.* 1990).

Specific applications: Huntington's disease

In order to illustrate the utility of such models in the analysis of imprinting, we may take Huntington's disease as a specific example. Huntington's disease is a genetic disorder that is characterized by a variety of neurological symptoms, including chorea, dystonia and behavioral abnormalities (McKusick, 1986). The disease segregates as an autosomal dominant and is invariably fatal (McKusick, 1986).

The Huntington's disease mutation (HD) exhibits two properties of considerable interest to the geneticist. The first is the fully dominant nature of the HD allele; i.e. HD/+ individuals appear to have the same phenotype as HD/HD individuals (Wexler *et al.* 1987). Such completely dominant mutations are uncommon in the genetic literature (Fisher, 1928; Lindsley and Grell, 1968). The second property is the large degree of variability in the age of onset of the disease: HD patients range from $2\frac{1}{2}$ to 81 years of age (Boehnke *et al.* 1983; Wexler, personal communication). Those patients who are diagnosed as having the disease before age 20 are classified as 'juvenile-onset' (Ridley *et al.* 1988) (approximately 10% of cases). Within the 'juvenile-onset' class, the vast majority have inherited the HD allele from their fathers (Ridley *et al.* 1988), leading to the suggestion (Ridley *et al.* 1988; Reik, 1988) that the HD allele is subject to 'genome imprinting'.

I have previously proposed that the variability in the expressivity of HD reflected allelic differences at imprinting loci capable of modifying the HD locus (Sapienza, 1989). Laird has put forth a more specific proposal which models the genetics of Huntington's disease as a variegating position-effect (Laird, 1990) analogous to the brown-dominant mutation in *Drosophila*. Laird's model, in particular, has several interesting implications concerning the molecular nature of the HD mutation on chromosome 4, but a major feature of both models is the existence of modifier loci that affect expressivity of the trait.

In Laird's model, there exist at least two alleles of an X-linked modifier of variegation: a prevalent allele, en^+, and a rare allele, en^{HD}. Males who are of genotype en^+/Y, HD/+ and females who are of genotype en^+/en^+, HD/+ or en^+/en^{HD}, HD/+ will have HD
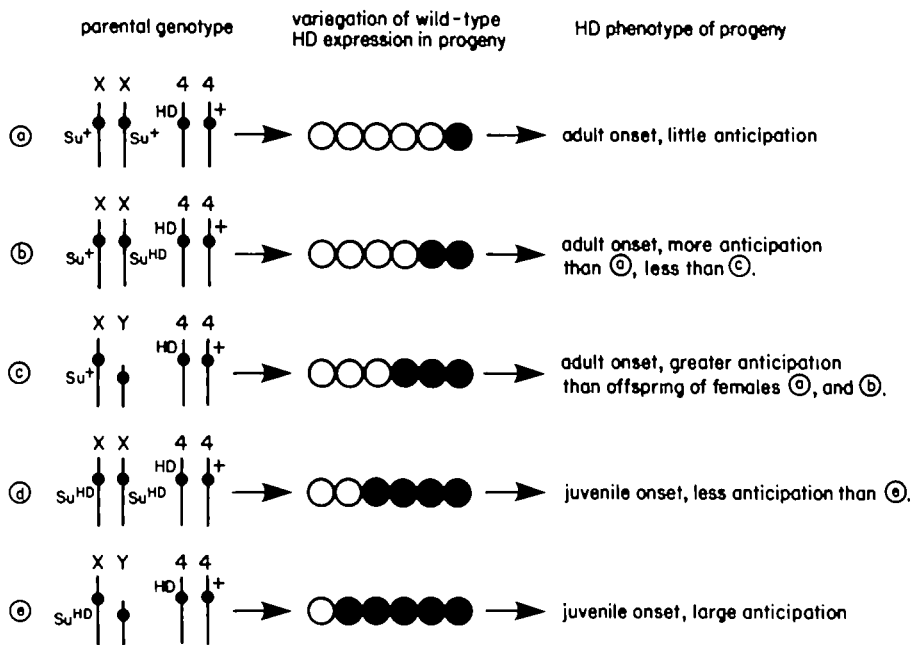
parental genotype   variegation of wild-type
                     HD expression in progeny   HD phenotype of progeny

X X   4 4
     HD   +
ⓐ   Su⁺   Su⁺   → ○○○○○● → adult onset, little anticipation

X X   4 4
     HD   +
ⓑ   Su⁺   Suᴴᴰ   → ○○○○●● → adult onset, more anticipation
                                  than ⓐ, less than ⓒ.

X Y   4 4
     HD   +
ⓒ   Su⁺   → ○○○●●● → adult onset, greater anticipation
                               than offspring of females ⓐ, and ⓑ.

X X   4 4
     HD   +
ⓓ   Suᴴᴰ   Suᴴᴰ   → ○○●●●● → juvenile onset, less anticipation than ⓒ.

X Y   4 4
     HD   +
ⓔ   Suᴴᴰ   → ○●●●●● → juvenile onset, large anticipation

Fig. 5. Effect of a dosage-sensitive modifier of variegation on HD expression. Su⁺ represents a wild-type suppressor of variegation, giving rise to fewer cells in which the HD locus has been heterochromatinized. Suᴴᴰ is a hypomorphic allele which is less effective in preventing heterochromatinization of the HD locus. Open circles: cells in which the wild-type HD gene product is expressed. Filled circles: cells in which no product is expressed.

offspring with a late age of onset. Males who are of genotype enᴴᴰ/Y, HD/+ and females of genotype enᴴᴰ/enᴴᴰ, HD/+ will have HD offspring with juvenile onset. In this model, the modifier enᴴᴰ acts as a simple X-linked recessive trait and there should be no difference between enᴴᴰ/Y fathers and enᴴᴰ/enᴴᴰ mothers. However, Ridley et al. (1988) have reported on 169 cases of juvenile onset HD. Of these, 75 show anticipation >15 years and 94 show anticipation of <15 years. If there are no differences between enᴴᴰ/Y, HD/+ fathers and enᴴᴰ/enᴴᴰ, HD/+ mothers, one might expect an equivalent proportion of large anticipation (>15 years) and small anticipation (<15 years) cases in both maternally and paternally derived groups. However, for maternally derived juvenile onset cases, only 9 of 46 (19.6%) show anticipation >15 years, while 66 of 123 (53.7%) paternally derived cases show anticipation of >15 years (X^2=43.3, P<.001). These data may be more easily explained by including a dosage-sensitive imprinting modifier in Laird's model.

In this model (Fig. 5), Su⁺ is an X-linked, dosage-sensitive class II modifier of the HD locus. Its effect is to inhibit heterochromatization of the HD allele and its wild-type homolog as described by Laird (1990). A hypomorphic allele of Su, Suᴴᴰ, also exists that is relatively inefficient at inhibiting the heterochromatization of alleles at the HD locus. The five possible genotypes of interest and the predicted dosage-dependent effect of each on variegation is shown in Fig. 5. Females who are of genotype Su⁺/Su⁺, HD/+ and males who are Su⁺/Y, HD/+ are both predicted to have adult onset progeny, but progeny of males will show greater anticipation and lower average age of onset (Ridley et al. 1988). Females who are of genotype Suᴴᴰ/Suᴴᴰ, HD/+ and males who are Suᴴᴰ/Y, HD/+ will both have juvenile onset progeny, but progeny of females will show less anticipation (Fig. 5). Progeny of females heterozygous for the hypomorphic

allele (Su⁺/Suᴴᴰ, HD/+) are predicted to be intermediate between the progeny of Su⁺/Su⁺, HD/+ females and Su⁺/Y, HD/+ males in anticipation. In this model, the HD 'maternal protective factor' identified in previous studies (Boehnke et al. 1983; Ridley et al. 1988) is simply two doses of the modifier gene. The existence of such a factor is more easily explained if Suᴴᴰ is a hypomorphic allele rather than a recessive, null allele.

This genetic model for HD makes some of the same predictions as Laird's model: (1) it should be possible to map Suᴴᴰ on the X chromosome; (2) females who are homozygous for Suᴴᴰ should be present in the population as the square of the frequency with which Suᴴᴰ hemizygous fathers are present. Additionally, in the case of juvenile onset offspring, the grandparental source of the HD allele in these cases will more often be grandmaternal because grandfathers who carry Suᴴᴰ on their X chromosome will have non-reproducing juvenile onset progeny if they also carry the HD allele. This prediction is consistent with the data obtained by Ridley et al. (1988).

Because the X-linked modifier in this model is responsive not only to gene dosage, but also to the activity of variant alleles at the modifying locus, one may postulate the existence of not only a hypomorphic Suᴴᴰ allele, but also a hypermorphic 'super-suppressor' allele at this locus. The effect of such an allele would be to push the age-of-onset of affected offspring later, accounting for rare individuals who are not diagnosed until late in life (Wexler, personal communication).

Conclusions

The variable effects of modifying loci on individual phenotype have been described many times in the genetic literature (Haldane, 1941). Although the identity and possible biochemical activity of the

modifiers is known in only a few cases (James and Elgin, 1986; Pillus and Rine, 1989; Reuter *et al.* 1990), two generalizations may be made: (1) the effect of individual modifiers is extremely sensitive to gene dosage; and (2) the effect of multiple modifying loci on individual phenotypes is additive, rather than epistatic.

These characteristics, coupled with the probability that many alleles of any individual modifier will exist between inbred strains and within outbred populations, should result in a large range of variation of phenotype for any particular modified locus. This variation will make the genetic mapping of any individual modifier in outbred populations extremely difficult unless certain criteria are met.

In those cases where extremely hypomorphic or extremely hypermorphic alleles of a modifier exist (and result in near epistasis), the effect will be most easily recognized when the modifier is sex-linked because the difference in the activity of the variant allele will be accentuated by the sex-dependent difference in gene dosage. In such cases, the trait will appear to be inherited as an autosomal dominant or incomplete dominant with a gamete-of-origin-dependent effect on the expressivity or penetrance of the phenotype. Several human diseases appear to fulfill these characteristics, including neurofibromatosis (Miller and Hall, 1978), familial glomus tumors (van der Mey *et al.* 1989), myotonic dystrophy (Glanz and Fraser, 1984), Huntington's disease (Ridley *et al.* 1988) and, perhaps, several others (reviewed in Hall, 1990). Such families will be a valuable resource in the characterization and isolation of the modifying genes involved in genome imprinting.

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