Can Molecular Imprinting Explain Heterozygote Deficiency and Hybrid Vigor?

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Manuscript received January 16, 1989
Accepted for publication March 29, 1989

ABSTRACT

Molecular imprinting, the phenomenon of differential expressions of a gene based on whether it is paternally or maternally derived, has been noted in mice, humans, and other nonmammalian organisms. Effects of differential imprinting are important not only in the study of the manifestation of deleterious genes; they have important evolutionary implications as well. It is shown here that molecular imprinting may mimic observations that are often construed to be due to hybrid vigor and/or inbreeding depression. Furthermore, if a locus undergoes differential imprinting, it also yields observed genotypic proportions which mimic heterozygote deficiency in the population without the aid of natural selection.

THE complementary roles played by maternal and paternal genes inherited from egg and sperm, respectively, throughout the development and life of an individual is now demonstrated in several experiments (see *e.g.*, SOLTER 1988). All of these experiments suggest that at the time of fertilization the haploid complements from maternal and paternal genes create the diploid nucleus of an individual and thereafter genes from both parents cooperate as one informational unit. Nevertheless, while contributions of both parental genomes are essential for normal embryonic development (SURANI *et al.* 1984, 1987; MCGRATH and SOLTER 1984), a memory of the gametic origin of each complement of genetic information persists, residing in some form of differential imprinting imposed on the genetic materials during gametogenesis (MONK 1987). Developmental biologic techniques of nuclear transfer, as well as genetic analyses of meiotic nondisjunction in mice indicate that some genes are differentially expressed when contributed by the maternal or the paternal gamete (SOLTER 1987, 1988). This phenomenon of molecular imprinting is apparently widespread in mice autosomal genome (SEARLE and BEECHEY 1978, 1985; CATTANACH and KIRK 1985; CATTANACH 1986; see also SOLTER 1987, 1988). It is implicated for explaining non-Mendelian transmission of several human disorders that are supposed to have classical Mendelian mode of inheritance (*e.g.*, HARPER 1975; HARDING 1981; WARRAM *et al.* 1984; MYERS *et al.* 1985; ERICKSON 1985; VADHEIM *et al.* 1986; DIAMOND 1987; FOLSTEIN *et al.* 1987; SPENCE *et al.* 1988). Although the bulk of the experimental data comes from mice, the occurrence of imprinting at molecular level is suggested in mammalian organisms ranging from human to kangaroo (SHARMAN 1971), and is suspected to occur in nonmammalian organisms such as fish (WHITT, PHILLIP and CHILDERS 1977); mealy bug (SAPIENZA *et al.* 1987); axolotl (SIGNORET *et al.* 1981; SIGNORET and DAVID 1986); sweet vernal grass, *Anthoxanthum odoratum* (KELLEY, ANTONOVICS and SCHMITT 1988); water fern, *Marsilea vestita* (TOURTE, KULIGOWSKI-ANDRES and BARBIER-RAMOND 1980); and yeast (KLAR 1987).

In spite of the growing body of literature of the widespread nature of genomic differential imprinting, the exact underpinning of the molecular mechanism through which it occurs is not yet firmly established. There are some suggestions that DNA methylation may at least partially be responsible for differential imprinting (REIK *et al.* 1987; SAPIENZA *et al.* 1987; SWAIN, STEWART and LEDER 1987). Although it is usually studied in terms of the expression of deleterious genes, or relatively uncommon events of meiotic non-disjunction in natural populations, this heralding molecular discovery may be important for explaining various evolutionary observations. SOLTER (1988), in his recent review on this topic, considered the evolutionary implications of differential imprinting, relating imprinting in mammals to the lack of parthenogenetic mammals, and the role of *X* chromosome inactivation in sex determination in mammals. Apart from this suggestion, no other evolutionary consequences of molecular imprinting have been examined thus far.

The purpose of this note is to demonstrate two population genetic consequences of imprinting. It is shown that imprinting at a locus can cause apparent

TABLE 1

Genotypic probabilities and genotypic values under parental imprinting effect at a bi-allelic autosomal locus

Expressed genotype	Scored genotype	Genotypic value	Actual genotype of an individual			
			A_fA_m (p^2)	A_fa_m (pq)	a_fA_m (pq)	a_fa_m (q^2)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Both genes expressed						
A_fA_m	AA	2α	$1-\theta$			
A_fa_m	Aa	2α		$1-\theta$		
a_fA_m	Aa	2α			$1-\theta$	
a_fa_m	aa	2α				$1-\theta$
Maternal gene unexpressed						
A_f-	AA	α	θ			
A_f-	AA	α		θ		
a_f-	aa	α			θ	
a_f-	aa	α				θ

Note: The expressions under columns (4)–(7) are the probabilities with which the events for the different rows may occur. For example, the genotype a_fA_m will be expressed as a_f- (and scored as aa in the traditional sense) when the maternal allele (A_m) is unexpressed, which occurs with probability θ, and this will be expressed as a_fA_m (and scored as Aa) with probability $1-\theta$.

With the assumption that both alleles have equal allelic effect (α), and the allelic effects are additive, the genotypic values are shown in column (3), which are 2α for all genotypes when both parental genes are expressed, and they are all reduced to α, when the maternal gene is unexpressed.

heterozygote deficiency and at the same time may mimic hybrid vigor (heterozygote superiority) at the same locus without any fitness relevance of the alleles segregating at the locus.

POPULATION GENETIC CONSEQUENCES OF IMPRINTING

Imprinting can cause apparent heterozygote deficiency: Consider an autosomal locus having two segregating alleles (A and a) with frequencies p and q ($=1-p$) in a panmictic population. To distinguish the parental origin of these alleles in an individual, I use the allelic designations, A_f and a_f, when the alleles are derived from the father, and A_m and a_m, when they are derived from the mother of the individual. The four possible genotypes of an individual then become: A_fA_m,A_fa_m, a_fA_m, a_fa_m. With no selection differentials of alleles, their respective frequencies in a randomly mating population are p^2, pq, qp and q^2, respectively. Although differential parental imprinting may relate to either parental gamete, let us first assume that when the gene descends from the mother, with probability θ, it does not express itself in the individual (the same θ applies to both alleles). The alleles of paternal origin are assumed to be expressed always with probability one.

Table 1 shows the scenario of the effect of such differential imprinting on the genotypes of individuals in a population. If we simply collate the pooled frequencies of the three genotypes, AA, Aa, and aa (disregarding the parental origin of the alleles), the expected genotypic proportions are

$$\text{Pr. } (AA) = p^2 + \theta pq, \tag{1a}$$

$$\text{Pr. } (Aa) = 2pq(1-\theta), \tag{1b}$$

and

$$\text{Pr. } (aa) = q^2 + \theta pq, \tag{1c}$$

which represent the classic deviation from the Hardy-Weinberg equilibrium (HWE). Note that these equations are identical in form to WRIGHT's (1969) equations for inbreeding depression. Although such departure from HWE can be ascribed to nonrandom mating (inbreeding), selection, and/or the presence of subdivisions within the population, genetic imprinting as a cause of deviation from HWE has not been discussed before. This simple demonstration shows that since $0 \leq \theta \leq 1$, differential imprinting will always cause heterozygote deficiency (in comparison with HWE expectations) in the population.

Equations 1a–1c further show that the proportional heterozygote deficiency in the population, $(H_O - H_E)/H_E$, takes the form

$$(H_O - H_E)/H_E = -\theta, \tag{2}$$

where H_O is the observed heterozygosity at the locus, when the genes exhibit a differential parental imprinting effect (expression 1b), and H_E, the expected heterozygosity under HWE ($H_E = 2pq$). In other words, the absolute proportional heterozygote deficiency is simply equivalent to the extent to which a gene from one parent does *not* express itself.

Without laboring the point, it may be noted that this holds irrespective of which parental gene is unexpressed (θ may relate to gametes from the male parent), or if both parental genes are unexpressed with certain probabilities (*i.e.*, when $\theta_f \neq \theta_m$ are used instead of a single θ). Therefore, an apparent heterozygote deficiency will result for any general type of differential expressivity of alleles of either parental origin. Multiallelic extension of this logic is also obvious, suggesting that differential parental imprinting may cause heterozygote deficiency in a multiallelic locus as well.

Imprinting also mimics hybrid vigor at a locus: Concurrent to this effect, we can also study the genotypic values of the different apparent genotypes under genetic imprinting. For simplicity again, let us assume that the allelic effects of A and a (irrespective of their parental origin) are identical (α), and additive. Therefore, the locus is assumed to be selectively neutral with no adaptive significance attached. Under this model, when the maternal allele is unexpressed in an individual, the genotypic effect will be reduced by half (pseudo-haplotypic effect), shown in Table 1 (third column). Again, collating the terms from Table 1, it can be shown that the mean genotypic values (μ_{AA}, μ_{Aa}, and μ_{aa}) of the three genotypes satisfy the equations

$$\begin{aligned}[p^2 + \theta pq]\cdot\mu_{AA} &= p^2[2\alpha(1-\theta) + \alpha\theta] + pq\alpha\theta \\ &= \alpha[p^2 + \theta pq] + \alpha p^2(1-\theta),\end{aligned} \tag{3a}$$

$$\begin{aligned}[2pq(1-\theta)]\cdot\mu_{Aa} &= pq[2\alpha(1-\theta) + 2\alpha(1-\theta)] \\ &= 2\alpha[2pq(1-\theta)],\end{aligned} \tag{3b}$$

and

$$\begin{aligned}[q^2 + \theta pq]\cdot\mu_{aa} &= q^2[2\alpha(1-\theta) + \alpha\theta] + pq\alpha\theta \\ &= \alpha[q^2 + \theta pq] + \alpha q^2(1-\theta).\end{aligned} \tag{3c}$$

In other words, the mean genotypic values are

$$\mu_{AA} = \alpha + \alpha(1-\theta)\cdot\left[\frac{p^2}{p^2 + \theta pq}\right], \tag{4a}$$

$$\mu_{Aa} = 2\alpha, \tag{4b}$$

$$\mu_{aa} = \alpha + \alpha(1-\theta)\cdot\left[\frac{q^2}{q^2 + \theta pq}\right]. \tag{4c}$$

Since the last factor in (4a) or (4c) is less than or equal to one (because $0 \le \theta \le 1$), it is clear that both μ_{AA} and μ_{aa} can never exceed $2\alpha = \mu_{Aa}$, the heterozygote mean genotypic value.

Combining both homozygotes together, the mean genotypic value of the homozygotes, μ_{Hom}, becomes

$$\mu_{\text{Hom}} = \alpha + \alpha(1-\theta)\cdot\left[\frac{p^2 + q^2}{p^2 + q^2 + 2\theta pq}\right], \tag{5a}$$

whereas the mean heterozygote genotypic value, $\mu_{\text{Het}} = \mu_{Aa}$, is

$$\mu_{\text{Het}} = 2\alpha. \tag{5b}$$

Again, $0 \le \theta \le 1$ implies that $\mu_{\text{Hom}} \le \mu_{\text{Het}}$, showing apparent overdominance effects of the scored alleles at this locus. This proves that differential parental imprinting will mimic hybrid vigor of genotypic values. The qualitative implications of (5a) and (5b) remain unaltered when the paternal gene instead of the maternal one is not expressed, and when multiple alleles are involved at a locus under an imprinting effect. The formal proof of these assertions is obvious from the above derivations.

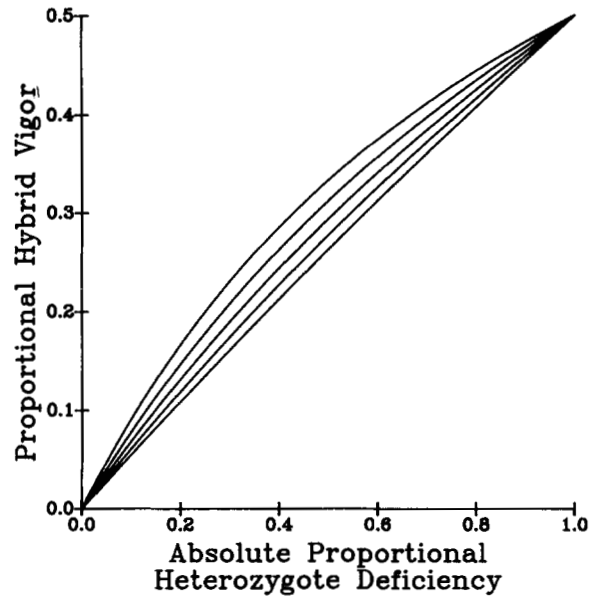

FIGURE 1.—Relationship between absolute proportional heterozygote deficiency and proportional hybrid vigor of genotypic value at a two-allele autosomal locus under the model of molecular imprinting. See text for the definitions of these proportional measures. The graphs from bottom to top are for average expected heterozygosity (under Hardy-Weinberg equilibrium) in the population, $H = 0.10, 0.20, 0.30, 0.40$ and 0.50.

Equations 5a and 5b can be used to study the relationship between absolute proportional deficiency of heterozygotes ($=\theta$, shown in Eq. 2) at a locus and proportional heterozygote advantage, measured by

$$\omega = (\mu_{\text{Het}} - \mu_{\text{Hom}})/\mu_{\text{Het}}.$$

It is easy to show that

$$\omega = \frac{\theta}{2[1 - H(1-\theta)]}, \tag{6}$$

where $H = 2pq$, represents the expected heterozygosity under HWE at a two-allelic codominant locus. Figure 1 graphically illustrates the relationship (6) for different values of H. It clearly establishes that under differential parental imprinting, a positive relationship exists between heterozygote deficiency and hybrid vigor of genotype values. Furthermore, such a relationship appears to be stronger for populations (or loci) that are more heterozygous. For $\theta = 0$, there is no heterozygote deficiency (Eq. 2) and no hybrid vigor (Eq. 6), and this relationship is locus-specific; *i.e.*, if there is no differential imprinting at a locus, it will not produce a deviation from HWE of genotype frequencies, nor will it contribute to a positive correlation between heterozygosity and genotype values. Since a fitness differential does not appear in this derivation, we have the result that heterozygote deficiency and a concurrent hybrid vigor may be generated in a population by molecular imprinting alone.

DISCUSSION

The above results indicate that a departure from HWE as well as a mimicry of hybrid vigor can result from the phenomenon of molecular imprinting. These population genetic consequences of imprinting effects are analogous to that of the presence of null alleles, chromosomal deletion, and incomplete penetrance. Thus, at a population level, the consequences of imprinting cannot be differentiated from these alternative mechanisms. However, since the effect of imprinting is transient and specific to the parental origin of the alleles, whereas the above alternatives cause more permanent effect which are symmetric with regard to the parental source of alleles, family data extending over two or more generations may be used to distinguish the imprinting hypothesis from the others. Nonrandom segregation specific to only one parent's genotypes are expected under the imprinting hypothesis, and it should not repeat over generations, since the imprinting starts over afresh in the next generation, irrespective of the grandparental origin of alleles.

Furthermore, as shown above, no selection is required to explain the imprinting effects, whereas maintenance of null alleles and chromosomal deletions involving specific loci must have to be examined in the light of their selective implications for which evidence is difficult to obtain from population data alone.

The two implications of molecular imprinting, discussed above, are also important in view of the availability of molecular tools for studying imprinting at the organismic, cellular, chromosomal, and DNA level. This is so, because using this theory it is possible to identify the candidate genes where this might occur, and to find organisms in which such events may apply. Since imprinting effects are locus-specific, a validation of this hypothesis is possible when the parental genotype-specific nonrandom segregation can be studied in relation to the molecular make-up of the alleles, showing that at the molecular level the structures of the expressed and unexpressed alleles are identical, as done in SWAIN, STEWART and LEDER (1987).

Although the presence of imprinting impinges on the basic premises of Mendelian segregation ratios, the loci which are affected by imprinting are not necessarily unsuitable for population genetic studies, since allele sharing in parents and offspring, or among sibs is not mitigated by imprinting. It may be noted that apparent nonrandom segregation of alleles, which is one consequence of differential imprinting, has been noted in humans and other organisms at several immunological and isozyme loci (WARRAM *et al.* 1984; VADHEIM *et al.* 1986; WILKINS 1976; BEAUMONT, BEVERIDGE and BUDD 1983; HVILSOM and THEISEN 1984; GAFFNEY and SCOTT 1984; FOLTZ 1986), yet such loci are well known for being useful for population genetic analyses. Finally, this theory does not deny the existence of overdominance as a cause of hybrid vigor, it suggests an alternative explanation of apparent hybrid vigor when no demonstrable selection differential exists at a locus, even in the presence of a departure from the Hardy-Weinberg expectations of genotype frequencies and heterozygote superiority of genotype values.

This study is supported by U.S. Public Health Service Research grants from the National Institutes of Health and National Science Foundation. Discussions with W. J. SCHULL, M. NEI, D. GRAUR, C. L. HANIS and E. BOERWINKLE had been helpful in developing this theory.

LITERATURE CITED

BEAUMONT, A. R., C. M. BEVERIDGE and M. D. BUDD, 1983 Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). Mar. Biol. Lett. **4:** 151–161.

CATTANACH, B. M., 1986 Parental origin effects in mice. J. Embryol. Exp. Morphol. **97** (Suppl.): 137–150.

CATTANACH, B. M., and M. KIRK, 1985 Differential activity of maternally and paternally derived chromosome regions in mice. Nature **315:** 496–498.

DIAMOND, J. M., 1987 Causes of death before birth. Nature **329:** 487–488.

ERICKSON, R. P., 1985 Chromosomal imprinting and the parent transmission specific variation in expressivity of Huntington disease. Am. J. Hum. Genet. **37:** 827–829.

FOLSTEIN, S. E., G. A. CHASE, W. E. WAHL, A. M. MCDONNEL and M. F. FOLSTEIN, 1987 Huntington disease in Maryland: clinical aspects of racial variation. Am. J. Hum. Genet. **41:** 168–179.

FOLTZ, D. W., 1986 Segregation and linkage studies of allozyme loci in pair crosses of the oyster *Crassostrea virginica.* Biochem. Genet. **24:** 941–956.

GAFFNEY, P. M., and T. M. SCOTT, 1984 Genetic heterozygosity and production traits in natural and hatchery populations of bivalves. Aquaculture **42:** 289–302.

HARDING, A. E., 1981 Genetic basis of autosomal dominant late onset cerebellar ataxia. J. Med. Genet. **18:** 436–441.

HARPER, P. S., 1975 Congenital myotonic dystrophy in Britain. II. Genetic basis. Arch. Dis. Child. **50:** 514–521.

HVILSOM, M. M., and B. F. THEISEN, 1984 Inheritance of allozyme variations through crossing experiments with the blue mussel *Mytilus edulis* (L.). Hereditas **101:** 1–7.

KELLEY, S. E., J. ANTONOVICS and J. SCHMITT, 1988 A test of the short-term advantage of sexual reproduction. Nature **331:** 714–716.

KLAR, A. J. S., 1987 Differential parental DNA strands confer developmental asymmetry on daughter cells in fission yeast. Nature **326:** 466–470.

MCGRATH, J., and D. SOLTER, 1984 Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell **37:** 179–183.

MONK, M., 1987 Memories of mother and father. Nature **328:** 203–204.

MYERS, R. H., L. A. CUPPLES, M. SCHOENFELD, R. B. D'AGOSTINO, N. C. TERRIN, N. GOLDMAKHER and P. A. WOLF, 1985 Maternal factors in onset of Huntington disease. Am. J. Hum. Genet. **37:** 511–523.

REIK, W., A. COLLICK, M. L. NORRIS, S. C. BARTON and M. A. H. SURANI, 1987 Genomic imprinting determines methylation

of parental alleles in transgenic mice. Nature **328:** 248–251.

SAPIENZA, C., A. C. PETERSON, J. ROSSANT and R. BALLING, 1987 Degree of methylation of transgenes is dependent on gamete of origin. Nature **328:** 251–254.

SEARLE, A. G., and C. V. BEECHEY, 1978 Complementation studies with mouse translocations. Cytogenet. Cell. Genet. **20:** 282–303.

SEARLE, A. C., and C. V. BEECHEY, 1985 Noncomplementation phenomena and their bearing on nondisjunction effects, pp. 363–376 in *Aneuploidy: Etiology and Mechanisms*, edited by V. L. DELLARCO, P. E. VOYTEK and A. HOLLAENDER. Plenum Press, New York.

SIGNORET, J., and J. C. DAVID, 1986 DNA-ligase activity in axolotl early development: Evidence for a multilevel regulation of gene expression. J. Embryol. Exp. Morphol. **97** (Suppl.): 85–95.

SIGNORET, J., J. LEFRESNE, D. VINSON and J. C. DAVID, 1981 Enzymes involved in DNA replication in axolotl. II. Control of DNA ligase activity during very early development. Dev. Biol. **87:** 126–132.

SHARMAN, G. B., 1971 Late DNA replication in the paternally derived X chromosome of female kangaroos. Nature **230:** 231–232.

SOLTER, D., 1987 Inertia of the embryonic genome in mammals. Trends Genet. **3:** 23–27.

SOLTER, D., 1988 Differential imprinting and expression of maternal and paternal genomes. Annu. Rev. Genet. **22:** 127–146.

SPENCE, J. E., R. G. PERCIACCANTE, G. M. GREIG, H. F. WILLARD, D. H. LEDBETTER, J. F. HEJTMANCIK, M. S. POLLACK, W. E. O'BRIEN and A. L. BEAUDET, 1988 Uniparental disomy as a mechanism for human genetic disease. Am. J. Hum. Genet. **42:** 217–226.

SURANI, M. A. H., S. C. BARTON and M. L. NORRIS, 1984 Development of reconstituted mouse eggs suggesting imprinting of the genome during gametogenesis. Nature **308:** 548–550.

SURANI, M. A. H., S. C. BARTON and M. L. NORRIS, 1987 Experimental reconstruction of mouse eggs and embryos: an analysis of mammalian development. Biol. Reprod. **36:** 1–16.

SWAIN, J. L., T. A. STEWART and P. LEDER, 1987 Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. Cell **50:** 719–727.

TOURTE, Y., J. KULIGOWSKI-ANDRES and C. BARBIER-RAMOND, 1980 Comportement différential des chromatines paternelles et maternelles au cours de l'embryogenèse d'une fourgère: le Marsilea. Eur. J. Cell. Biol. **21:** 28–36.

VADHEIM, C. M., J. I. ROTTER, N. K. MACLAREN, W. J. RILEY and C. E. ANDERSON, 1986 Preferential transmission of diabetic alleles within the HLA complex. N. Engl. J. Med. **315:** 1314–1318.

WARRAM, J. H., A. S. KROLEWSKI, M. S. GOTTLIEB and C. R. KAHN, 1984 Difference in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. N. Engl. J. Med. **311:** 149–152.

WHITT, G. S., D. P. PHILLIP and W. F. CHILDERS, 1977 Aberrant gene expression during the development of hybrid sunfishes (Preciformes, Teleostei). Differentiation **9:** 97–109.

WILKINS, N. P., 1976 Genetic variability in marine Bivalvia: implications and applications in molluscan mariculture, pp. 549–563 in *Proceedings of the Tenth European Symposium on Marine Biology*, edited by G. PERSOONE and E. JASPERS. Universa Press, Wetteren, Belgium.

WRIGHT, S., 1969 *Evolution and the Genetics of Population*, Vol. 2. University of Chicago Press, Chicago.

Communicating editor: J. R. POWELL