

# Commentary: Random variability of quantitative characteristics, an intangible epigenomic product, supporting adaptation

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The paper I wrote over 20 years ago, reprinted in this issue of the *IJE*,<sup>1</sup> confirmed observations indicating a third causal component for the random variability of quantitative biological characteristics beside genotype and environment. This ‘third’ component had previously been described<sup>2</sup> and named ‘intangible variance’. My paper revealed that intangible variance results from physiological processes, prior to the third cell division, that stipulate differential further development of any zygote. The nature of those processes remains unclear.

In the following commentary, I discuss investigations subsequent to my original paper. These have served: (i) to clarify the hypothesis that the origin of intangible variance is at the early embryonic stage; and (ii) further to characterize this intangible variance by means of studies on the individual differences between isogenic inbred rats and mice. These individual differences concern the normal distribution of many quantitative characteristics; their significance for habitual abortion; and individual differences in behavioural characteristics, stress–response characteristics and the course of infections among adults. Subsequent investigations have also considered: (iii) if intangible variance supports evolutionary advantage; and (iv) if it arises by modifications of the epigenome during early zygote stages.

(i) Findings in cloned cattle confirm the early embryonic origin of intangible variance: The proposed existence of a third variance component and its early embryonic origin<sup>3</sup> met with criticism,<sup>4</sup> because it challenged existing understanding that the uniformity of natural monozygotic twins (human and other species) is caused solely by their genetic identity. It was thought that undiscovered residual heterogeneity in the inbred strains used for these experiments might be the origin of the differences observed between natural siblings and this resulted, in what later turned out to be erroneous, in the hypothesis that there are

processes during early embryogenesis that cause intangible variance.

Assessments of cloned siblings<sup>5</sup> served to clarify the situation. During cloning, the genome is doubled prior to the start of development of each individual. This occurs when the nucleus of one isolated embryonic cell is transferred from a morula into an enucleated recipient egg cell. The remaining cytoplasmic milieu induces modification of the transplanted nucleus and its development into an individual. First and further cell divisions follow the transfer of the nucleus. The zygote produced is transferred at the blastocyst stage into the uterus of a foster mother. Here it matures into an embryo, which is carried to full term and birth. Cloned siblings originate from nucleus transfers derived from the same donor morula and prepared in the same way.

This sophisticated technique was mastered successfully 20 years ago in cattle.<sup>6</sup> Measurements of various body sizes and weights were taken from cloned calf siblings and also from monozygotic calf twins.<sup>5</sup> The data were compared using analysis of variance: The cloned siblings differed considerably in body size and body weight and did not show the remarkable uniformity of the monozygotic twins. These discrepancies also provided evidence of early individualization processes in the zygotes up to about the eighth cell stage. As this is before such zygotes split in monozygotic twins, both twin-partners are determined identically by the same earlier individualization processes and therefore develop uniformly.

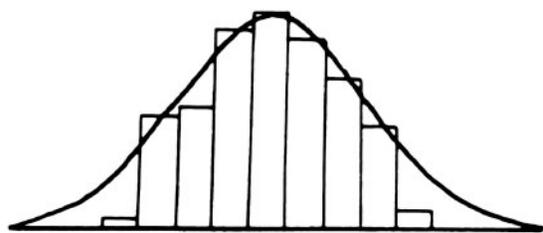
Observed differences in uniformity between monozygotic calf twins and cloned calves eliminate the crucial assumption that the intangible variance observed among isogenetic inbred mouse siblings might be caused by a residual heterogeneity, which had resisted inbreeding over more than 100 generations and was impossible to discover using the various tests for isogeny performed with inbred strains. Unaware of

the intangible variance observed in inbred mice and rats, cattle breeders assumed that these unexpected differences between the cloned calves were due to manipulation artefacts.

(ii.a) Intangible variance results in Gaussian distributions of quantitative characteristics that lack extreme categories: Geneticists consider the Gaussian frequency distribution of quantitative characteristics in natural plant or animal populations to reflect the genetic variability of these natural populations. However, in inbred strains genetic variability is not present, yet quantitative characteristics are still distributed in the same manner. More than 70% of the total variance in the body weight, for example, of inbred strains is caused by intangible variance.<sup>7</sup> This poses the question whether the distribution produced by intangible variance among the individuals of an inbred strain really resembles a Gaussian distribution.

The shape of the frequency distribution of body weights in 150 groups of growing and adult inbred laboratory rats and mice was investigated.<sup>8</sup> Each group consisted of between 80 and 100 animals of the same strain, age and sex, reared under optimal standardized conditions. Skewness and kurtosis of the distributions, as well as mean values and standard deviations, were assessed in each group at different ages. All the distributions bore strong resemblances to Gaussian distributions. However, they very frequently showed small but significant negative deviations of kurtosis ( $-0.12$  to  $-0.76$ ) but no general tendency to skewness ( $+0.30$  to  $-0.43$ ). All the coefficients of variation of the body weights were similar.

We assume that the processes prior to the third cell division would tend to produce a Gaussian distribution of quantitative characteristics among individuals. We further assume that the regularly observed negative deviations of kurtosis of these distributions are due to the low number of individuals observed in the extreme categories of those distributions, or their absence from these categories. Figure 1 demonstrates this discrepancy between an ideal Gaussian distribution (black line) and the distributions of body weights observed in all groups of adult rats (columns). Computer simulation<sup>7</sup> shows that cutting off both the tails of a normal distribution results in negative deviation of values for kurtosis. For example, elimination of 10% and more of the smallest and



**Figure 1** Ideal Gaussian distribution (black line) and the actual weight distribution of adult rats (columns)

largest values out of a normal distribution results in kurtosis of  $-0.12$  to  $-0.76$ . The following section discusses the hypothesis that these individuals may have been lost due to habitual abortion.

(ii.b) Habitual abortion, the outcome of early embryonic individual determination by intangible variance: The causes of habitual abortion remain unclear. Lethal and sublethal genetic dispositions are assumed to be the causes. However, following the elimination of lethal and sublethal genetic dispositions by means of inbreeding, the rate of prenatal loss in inbred mice and rat strains remains remarkably high. The rate of habitual abortions in inbred strains is 14–57%.<sup>9–11</sup> This is comparable with that in outbred mice strains (12–57%) and corresponds to that in other animal species and human beings.<sup>10,12</sup> These findings mean that non-genetic causes must be taken into account. Maternal influences play a significant role in individual prenatal survival ability, since the prenatal rate of loss of reciprocal F1-hybrids from inbred strains<sup>9</sup> has been observed to differ and show similarities to the maternal strains. The transplant of such F1-embryos into foster mothers of the paternal strain revealed that individual survival ability is only minimally dependent on the uterine milieu of the foster mother. Individual survival ability of an embryo is mainly determined by the cytoplasm of the eggs from which the embryo originates.<sup>10,13</sup> We assume that processes which take place prior to the third cell division determine later individual development and produce a Gaussian distribution of quantitative characteristics between adults. However, these processes also determine the extreme variants that are unable to survive and are removed by habitual abortion.

(ii.c) Diversity of behavioural or stress-response characteristics and infection resistance among adult isogenetic inbred animals are also caused by mechanisms producing intangible variance: Behavioural and endocrine stress-response characteristics in highly standardized inbred rats show a considerable degree of variability.<sup>14</sup> They are not normally distributed, rather distributed with two or three peaks, and inbred strains and outbred stocks show similarities in the shape of the distribution of these characteristics. This means that these discrepancies do not result primarily from genetic differences between the animals.

These remarkable diversities between genetically identical siblings are unexpected. They appear incompatible with the amazing uniformity of behavioural traits observed in monozygotic twins and often described in human beings. For this reason, we assume that the obvious differences in behavioural traits among isogenetic rats or mice raised under standardized conditions are not only the result of the manifold stochastic environmental noise to which the animals are subjected during their lifetime. While this might create random differences of

a dimension similar to that shown among monozygotic twins, the particular shape of the distribution in inbred sisters would indicate that it is based on processes which take place before third cell division and are similar to those which create intangible variance.

Extensive investigations have acknowledged these individual differences in behaviour patterns, vasomotoric and endocrine stress-response characteristics in isogenetic inbred rats and mice. In spite of the high standardization in genotype, age and environmental conditions, for instance, male rats within groups of four can be divided into at least two or three categories for each behavioural activity pattern.<sup>7,14</sup> Such differences enable certain animals to perform particular tasks more successfully than other members of a group. In one instance, out of a group of four, one or two rats proved to be keen learners. They discovered quickly where the food had been hidden. However, when living together with handicapped learners, the latter find the location of the food just as quickly by social learning. In another case, various female rats on heat selected only the same one or two out of four highly standardized inbred adult male rats as fathers of the next generation. Within cage groups, one male performed much more than 50% of the total number of ejaculations counted among all four cage mates. One or two were sexually passive and the other male showed moderate sexual activity.

Different endocrine and vasomotoric stress-response characteristics and systolic blood pressure were studied before and during social isolation for 10 days. For this adult male rats were socially isolated by keeping each alone in a metabolic cage, which enables the measurement of the daily renal output of corticosterone, adrenaline, noradrenaline, dopamine, creatinine, sodium and potassium and of the blood pressure. Using these results, adult, male, inbred rats, highly standardized in genotype, age and environment could be classified into different types for vasomotoric and endocrine responses to social isolation stress. Multiple correlations and cluster analyses between these characteristics and the behavioural traits showed dependencies between stress-response, sexual activity and curiosity traits.<sup>15-19</sup>

Despite all efforts at standardization, the animals within groups of male inbred rats vary and can be compared with a well-established team of specialists, each qualified to solve a particular task most effectively. Together, they form a team that most effectively masters the art of survival and negotiating natural selection. The individual talents they bring to the team are inborn and it seems they may be established by processes creating intangible variance.

In this connection, resistance of the individual against infection was investigated. The level of resistance also displays a high degree of variability within groups of highly standardized inbred rats or mice.

It does not follow a normal distribution, rather a distribution with more than one peak. Extensive investigations have documented these individual differences by comparing the clinical course of model infections with *Mycoplasma pulmonis* or *Mycoplasma arthritidis* between highly standardized male adult inbred rats over a period of 120 days.<sup>20-23</sup> These investigations showed the severity of the illness to vary greatly between the animals; some were severely ill, whereas others only suffered mild forms of the disease. Severity of the illness in adult male isogenetic rats correlated with their Darwinian fitness, estimated by their copulation behaviour before infection. Therefore, the individual resistance against infection of adult inbred rats might be influenced by psychoimmune components, which differ considerably among these highly standardized animals.<sup>23</sup> We assume that processes producing intangible variance might also be the origin of these differences.

(iii) Does intangible variance support the evolutionary search for adaptation? Intangible variance decisively determines the differences between isogenic individuals of the same generation. While many of their quantitative characteristics are normally distributed, the distributions of others have several peaks. This enables the differentiation of specialists among isogenetic individuals who then become leaders when that population has to cope with particular environmental challenges.

Normal distributions produced by intangible variance, surprisingly, show qualities essential to adaptation processes by means of trial-and-error<sup>24,25</sup>: Gaussian distributions as shown by fitness-relevant characteristics; size of variation fixed for each trait; and variation prospectively determined, i.e. prior to the first confrontation with the environment of grown individuals. However, intangible variance is produced independently from genetic diversity. Such incidental variation appears to be counterproductive for trial-and-error processes based on genetic variation. The question arises, does intangible variance still have any evolutionary significance or is it only the outcome of 'developmental noise'? Knowledge of population genetics and of sciences concerned with algorithmic search processes shed some light on this question.

For breeding selective strains, breeders choose as parents for the next generation not only the individuals which best fit the aim, but also those which effectively pass the genetic variability of the parental generation to the filial generation.<sup>26</sup> Possibly one of the evolutionary objectives of intangible variance is to resist the inbreeding effects caused by the selection of the fittest.

Engineers and informatic scientists look for optimal technical setups or optimal economic and even social constellations using algorithmic search processes and have proposed theoretical reflections about evolutionary search processes. Based on 'evolution

strategy,<sup>24</sup> they have discussed a 'gene-saving hypothesis' as the possible biological significance of intangible variance.<sup>27</sup> Based on theoretical reflections, they reveal that the number of genes involved correlates with the number of generations that is needed to reach the objective of the search. When the number of participating genes rises above a certain limit, the number of generations needed to complete the search increases rapidly.

Fitness-relevant, polygenetic characteristics in animals may require a great number of genes to achieve a Gaussian distribution, thereby the number of generations would rise. Intangible variance avoids that disadvantage by providing a Gaussian distribution independently. Optimal 'gene-saving' occurs if genetic variation accounts only for ~42% of the total variance of a characteristic and intangible variation for the rest.<sup>27</sup> This proportion is of interest since in natural populations many polygenetic and fitness-relevant characteristics have values for heritability ( $h^2$ ) of ~0.5. Intangible variance forms the largest part of their non-genetic variability.

(iv) Does modification of the epigenome in fertilized eggs cause intangible variance? As mentioned previously, intangible variance results from early individualization processes in zygotes, up to the eighth cell stage, that determine their further development differently. The nature of these processes remains obscure. Maternal involvement has been reported. Reciprocal F1-hybrids were bred repeatedly from two inbred mouse strains that differed considerably in the coefficients of variation of their body weights. The resultant reciprocal hybrid strains showed a difference in the coefficient of variation of their body weights, which resembled those of their mother's strain.<sup>28,29</sup> Reciprocal F1-hybrids are genetically identical but differ in the cytoplasmic milieu of the oocytes. The cytoplasmic milieu influences the individualization processes that result in intangible variance in body weight. Duration of the first cell cycle of the fertilized eggs was also compared between the same reciprocal F1-hybrids. It differed in the G1, G2 and the S-phase.<sup>30</sup> The complete duration of the first cycle resembled that of the mother's strain.<sup>30</sup> The different cytoplasmic milieu of fertilized oocytes influences their early developmental progress just when intangible variance is determined.

Our recent knowledge of the early development of mammalian embryos and the changes that take place in their epigenetic profiles enable us to speculate on processes that might determine the different individual development of genetically identical siblings prior to the third cell division. In effect, these changes should act like random generators because they result in the random distribution of quantitative characteristics among isogenic siblings. Such processes have been described recently.<sup>11,31-33</sup>

Passive demethylation of the epigenome during the first three cell divisions is of particular interest.<sup>33</sup>

Already during gametogenesis germ cells undergo extensive demethylation and the formation of new epigenetic profiles.<sup>34</sup> The sperm DNA is fully demethylated after passing through the oocyte wall and prior to commencement of the male pronucleus. In contrast, the oocyte DNA remains highly methylated. At the monocellular stage, only the maternal DNA strand has epigenetic methylations, except for a few genes with paternal imprinting provisions. During the first three cell divisions, the DNA doubles without methylation. As the DNA methyltransferases are not active during these stages, the DNA demethylates passively. This means that the existing epigenetic methylations of the maternal DNA strand are passed onto one strand only of the filial DNA. Its other strand remains demethylated. Following the next division, both filial strands are demethylated. Thus, eighth cell zygotes, identical in genotype, differ randomly in the pattern of their DNA methylation among the eight cells. Following the third cell division, passive demethylation ceases and *de novo* methylation of DNA starts. Monozygotic twin partners derive from one zygote that splits after the third cell division. Both partners have identical patterns of DNA methylation distribution at their eighth cell stages.

While the above paragraphs outline the progress that has been made over the past 20 years, clearly further investigations are required to clarify the processes responsible for the formation of intangible variance.

**Conflict of interest:** None declared.

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## Commentary: The presence of bifurcations as a 'third component of individual differences': implications for quantitative (behaviour) genetics

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