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Commentary: Gärtner's 'third component': still an open question

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In this paper, published in 1990, Klaus Gärtner[1] provides evidence that there is more to phenotype than simply genotype and environment. He presents data, collected over many years, that suggest that genotype and environment, alone, cannot explain the random variability seen in quantitative traits in mammals and suggests the existence of something else, which he calls a 'third component'. He compares this to Falconer's 'intangible variation', a term coined much earlier to explain much the same phenomenon.[2] This manuscript is rarely discussed and has been cited less than 100 times, despite its potential significance. Over the 20 years since publication, our understanding of genetics has progressed considerably. Do Gärtner's ideas survive the test of time?

Gärtner had, for many years, been puzzled that despite inbreeding, many laboratory animals displayed considerable variability in most measurable traits and he reasoned that a reduction in variability would make research easier; smaller numbers of animals would be required to detect differences between control and experimental groups. He spent much of his scientific career trying to standardize the phenotypes of laboratory animals. In this particular paper, he presents two main findings. First, he shows that standardization of the environment, such as standardizing the food, cage conditions, group size, etc. has little effect on the range of kidney weights in inbred rats. In most inbred strains this ranged from 80 to 120% of the mean. He also found that the range did not change significantly even when the rats were housed in 'wild' conditions, where temperature changes were extreme. Similar findings were made using other phenotypic measures, and he concludes that the postnatal physical environment has no major role in random variability.

Secondly, he carried out twinning experiments in which he divided eight-cell embryos in half and transferred each half separately to one foster mother and found that the phenotypic variation, in this case body weight, was reduced. In these experiments, he used inbred mice and some of this data had appeared in a previous publication.[3] He argues that if the random variability of body weight is

environmentally caused, then the variation within these twins should be the same as that between natural born littermates. He concludes that the variability cannot be caused by environmental factors acting after the eight-cell stage. He postulates the influence of factors in the egg or zygote. Quantitative geneticists use the expression 'environment' for all non-genetic variation that influences an individual after fertilization. Gärtner suggests that this is made up of two components; one is 'inborn' and may originate from differences in the cytoplasm of the egg or from 'different modulations of the isogenic genome'. Today, we might call this epigenetics. The second component, the real environment, has only a small effect on the creation of variability. More recently, support for these ideas has come from studies in invertebrates.[4] Batchmates of clonal crayfish, shown to be 'isogenic' by analysis of nuclear microsatellite loci, exhibited surprisingly broad ranges of variation in colouration, growth, lifespan and other phenotypic traits, even when reared under identical conditions.[4]

Gärtner's studies have been cited, by the authors and others, as evidence for the importance of epigenetics in the determination of phenotypic variation.[5,6] However, Gärtner's main conclusion, i.e. there is more contributing to phenotype than genotype and environment alone, is only valid if inbred mice are truly isogenic and there is growing evidence that this may not be the case. Individuals within inbred strains do differ genetically in some regions of the genome. For example, inbred mice show unique patterns of telomere lengths and these unique patterns appear to be established either in the germ cells of the parents or in the zygote.[7–9] It has been assumed that these regions are transcriptionally silent but this may not be the case. Genes have been identified in subtelomeric regions[10,11] and the silencing effects of chromatin can spread from repeats into adjacent 'genic' regions.[12,13] So changes in the length of a telomere could, through this mechanism, produce variable phenotypes. Furthermore, some non-telomeric regions of the genome have been found to vary among inbred C57Bl/6 littermates.[14,15] Repeat regions of the genome remain largely unstudied in this regard but the advent of deep sequencing technologies provides us with an opportunity to investigate this in much more detail. The recent discovery of extensive somatic retrotransposition in human brain attests to the power of this technology.[16]

If, indeed, inbred mice are not isogenic, then the finding that the two halves of one eight-cell embryo are phenotypically more similar to each other than to other littermates is unsurprising.

Similar comments could be made about the crayfish; the fact that batch-mates are genetically identical and homozygous at a small number of microsatellite markers, does not rule out the possibility of genetic variation elsewhere. Admittedly, they are parthenogenetically-derived but genetic rearrangements, such as those seen at telomeres in the mouse, could occur in the first stages after gametic activation. At this stage, we know little about the genome of the crayfish.

The issues raised by Gärtner continue to fascinate those of us with an interest in phenotypic diversity. His studies, in particular the twinning of preimplantation mouse embryos, suggest a novel mechanism for the generation of phenotypic diversity. Whether this process turns out to involve DNA sequence changes or epigenetic gene silencing, remains to be established.

Conflict of interest: None declared.

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