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Intraspecific phenotypic variation in deer: the role of genetic and epigenetic processes

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Abstract. Intraspecific phenotypic variation (PV) in deer is common, at times impressively diverse, and involves morphology, development, physiology, and behaviour. Until recently considered a nuisance in evolutionary and taxonomic studies, PV has become the primary target to study fossil and extant species. Phenotypes are traditionally interpreted to express primarily interactions of inherited genetic variants. PV certainly originates from different genotypes, but additional PV, referred to as phenotypic plasticity (PP), results from gene expression responsive to environmental conditions and other epigenetic factors. Usage of 'epigenetics' for PP has increased exponentially with 20 316 published papers (Web-of-Science 1990 – May 2010), yet it does not include a single paper on cervids (1900 to the present). During the 'genomic era', the focus was on the primary DNA sequences and variability therein. Recently however, several higher order architectural genomic features were detected which all affect PV.

(1) *Genes*: poli-genic traits; pleiotropic genes; poli-allelic genes; gene dosage (copy number variants, CNV); single nucleotide variance in coding and gene regulatory regions; mtDNA recombinations and paternal mtDNA inheritance.

(2) *Gene products*: pleiotropic gene products; multiple protein structures through alternative splicing; variable gene product reactions due to gene dosage.

(3) *Gene expression*: (i) epigenetic regulation at the DNA, nucleosomal and chromosomal levels; (ii) large-scale genomic structural variation (i.e. CNV imbalance); (iii) transcription factor proteins (TF), each regulating up to 500 target genes, with TF activity varying 7.5–25% among individual humans (exceeding variation in coding DNA by 300–1000×); (iv) non-protein-coding RNA (98.5% of genome) constituting maybe hundreds of thousands RNA signals; (v) gene expression responsive to external and internal environmental variation; (vi) transgenerational epigenetic inheritance (e.g. from ubiquitous non-gametic interactions, genomic imprinting, epistasis, transgenerational gene–diet interactions); (vii) epigenetic stochasticity resulting in random PP. A unique example of labile traits in mammals is the yearly regrowth of a complete appendage, the antler in cervids.

Highly complex assortments of genotypes lead to a spectrum of phenotypes, yet the same spectrum can result if a single genotype generates highly complex assortments of epigenotypes. Although DNA is the template for the DNA–RNA–protein paradigm of heredity, it is the coordination and regulation of gene expression that results in wide complexity and diversity seen among individual deer, and per-generation variety of phenotypes available for selection are greater than available genotypes. In conclusion, epigenetic processes have fundamental influences on the great intraspecific PV found in deer, which is reflected in broad ranges of environmental conditions under which they can persist. Deer management and conservation of endangered cervids will benefit from appreciating the large inherent PV among individuals and the immense contribution of epigenetics in all aspects of deer biology and ecology.

Additional keywords: adaptation, cervids, evolution, gene expression.

Introduction

Intraspecific phenotypic variation (PV) in deer is common, and at times impressively diverse. Such variations include: (i) *physiology*: living in temperature ranges exceeding 100°C, age at maturity varying by several years, threshold weight for ovulation varying several fold, compensatory extension of the gestation length being almost twice the oestrous cycle length, varying the gut length and gastrointestinal anatomy, or adjusting body size by up to 7.6-fold; (ii) *proportional morphometry*: metatarsus proportionally up to 70% longer and ratio of hind foot length to body length up to 3.1-fold larger between different herds; and (iii) *behaviour*: variably sedentary or migratory, highly variable social or spatial segregation between the sexes, wide range of group sizes with large compositional turnover, highly flexible mating strategy, which can include mating territories, clustered territories, leks, classic harem, mixed-sex herds with dominance system, and opportunistic wanderers (see the review by Putman and Flueck[1]). Whereas PV also affects morphology

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(size, shape, colour), development, life history traits,[2] and susceptibility to diseases,[3] it also includes variations (e.g. reaction rates) that may not result in gross phenotypic expressions. These variations, of both genetic and non-genetic origin, are of interest in terms of contributing to adaptability of individuals and species, whether they are heritable, and their role as major forces of micro- and macroevolution.[4,5]

Interestingly, small morphological differences were commonly employed by palaeontologists to differentiate supposedly distinct species. However, only recently, and after over a century of poor taxonomy, is PV becoming accepted as an important feature having acted also in the past (e.g. Mihlbachler[6]). Although PV has been considered as a nuisance in evolutionary studies of extant species, it has now become the primary target of investigations, as much for fossils as for extant species.[7–9]

Phenotypic variants are traditionally interpreted to result primarily from interactions of inherited genetic variants.[10–12] Certainly, phenotypes are the expressed physical traits, which in part originate from different genotypes. However, additional PV referred to as phenotypic plasticity (PP) results from unique gene expression in response to environmental conditions and other epigenetic factors.[13] PP includes 'non-labile' traits which are expressed once, and 'labile' traits which are expressed repeatedly and reversibly during an individual's lifetime. Labile traits (also been called life-cycle stages, or phenotypic flexibility) recorded in wild populations include those related to reproduction (e.g. timing of reproduction and the number or size of offspring produced), or morphological characters that are regularly regrown.[14]

Variability in phenotypes unrelated to DNA sequences are epigenetic phenomena. The increased use of 'epigenetics' is due to recent shifts in life science research from genome sequencing to the understanding of mechanisms regulating genomic expression.[15] Searching Web-of-Science (Thomson Reuters) for 'epigen*', 1990–99, we found 2450 papers, versus 17 866 papers for the period from the year 2000 to 5 December 2010. Searching epigen* with common domestic animals revealed 488 hits. However, searching for 'epigen*' and 'cervid*' (or 'deer') for the year 1900 to the present resulted, astonishingly, in no results.

In this paper we review factors, particularly in relation to epigenetics, which play a role in the variable intraspecific phenotypic expressions observed in deer (for definitions of terms, see Table 1).

Processes generating phenotypic variation: an overview

During the 'genomic era', the focus was on hard wiring, the primary DNA sequences and variability therein. Recently however, several higher order architectural features of the genome have been detected.[9,12,15] The physical expression of a genome is determined not only by the genes actually present, but also by the various factors acting on gene expression, and by other epigenetic factors (Fig. 1).

Currently known factors and mechanisms (some overlapping with others) resulting in enormous complexity of phenotypic expression involve genes, gene products and gene expression (Table 2).

Coding genes

To assess the role of genetic inheritance in PV, several concepts are reviewed. Traditionally, PV is explained by interactions of multiple genes modulated by environmental factors. However, where population PV results from different genotypes with traits unresponsive to environmental variation, additional variation stems from a given genotype having traits which are responsive to non-genetic factors that contribute to PP, like the classic genotype–environment interactions (precipitation, temperature, diseases, population density, etc.). However, additional epigenetic factors add to PP, such as genomic imprinting, epistasis, epigenetic inheritance and DNA modification (Table 2), with two effects. First, PP includes non-labile (fixed) traits (expressed once during a life time), which present reaction norms (if continuous) or switch points (if discontinuous). However, these cannot be studied in an individual having traits expressed only once, instead studies of natural and artificial clones provide valid models. Second, labile traits (expressed repeatedly) also represent reaction norms or switch points for that genotype in relation to environmental gradients.[14] Empirical research to date, however, has largely ignored variation in reaction norms at the individual level, yet non-genetic sources of variation in labile trait plasticity are common in nature.[13]

Genes

Apart from the inadequacy of Mendelian one-gene inheritance models, the definition of what comprises a gene has moved from early simplistic descriptions to a rather undetermined one at present. Hopkin summarised the current view of a gene as follows: the unit of heredity – made of DNA or RNA, that encodes a coherent set of potentially overlapping functional product molecules, either protein or RNA – that influences phenotype in ways we may or may not be able to measure.[16] About 3.5% of our genome is evolutionarily conserved, and up to 1% even highly conserved, yet with still unknown functions as it does not code for protein.[17] For instance, intense studies of human chromosome 21 revealed that 65% of highly conserved blocks have unknown functions, but most were neither protein-coding nor RNA genes.[18] Whereas ~1.5% of genomes encode for proteins, another 98.5% produce non-protein-coding RNA fractions which until recently were considered junk.[19,20] Although several hundred functional RNA fractions are known already, there may in fact be tens or even hundreds of thousands of RNA signals which constitute a hitherto hidden control network that regulates gene expression during mammalian ontogeny and which may underpin the development and much of the PV in mammals.[10,11,19] Additionally, signatures for adaptive evolution of such non-coding sequences were identified, although reasons for it are still unclear.[21]

Moreover, recently discovered, a transcription factor protein (TF) typically regulates several dozen to some 500 target genes and thus exhibit an enormous scale of pleiotropism.[9] Further, variation of binding of a given TF (and thus gene expression) between individual humans was 7.5–25%, which exceeds estimates for sequence variation in coding DNA by 300–1000-fold.[22] Because some 10% of all DNA codes for TF, it makes this the single largest family of human proteins. Not only do TF

Table 1. Description of major concepts relevant in genetics and epigenetics

Concept	Description
Phenotype	Includes all variations of an organism other than those caused by the genotype, from enzyme products, learned behaviours, to disease resistance. It is the individual's adjustment to environments to survive and prosper based on their genetic potential – to thwart natural selection and defeat evolution.[75,76] This occurs because phenotypic variation renders selection 'myopic' because it cannot see the entire potential of a genotype's plastic responses, but only the phenotype that happens to be produced in a particular environment.
Phenotypic plasticity	The ability of a single genotype to produce more than one alternative form of morphology, physiological state, behaviour, etc. in response to environmental conditions.[75] The 'environment' includes both external surroundings of an organism and internal conditions affecting gene expression, resulting in an enormous diversity of responses.
Pleiotropism	A single gene controls several distinct and seemingly unrelated phenotypic outcomes. The underlying mechanism includes that a gene codes for a product used by various distinct cells, or has a signalling function on various targets. Pleiotropism also occurs with gene products (enzymes, transcription factors, etc.) and non-coding RNA affecting several distinct systems, implying that genetic regulatory networks exhibit an enormous scale of complexity.
Epistasis	Multiple genes involved in a phenotypic trait; effects of one gene are modified by one or several other genes, including suppression at the genomic level; or genes which in combination produce an entirely new trait.
Qualitative traits	Traits producing distinct categories of phenotypes. The pattern of inheritance is typically monogenetic, i.e. the trait is only influenced by a single gene. Inherited diseases caused by single mutations, or fur colour, are examples of qualitative traits. The environment has very little influence on phenotypes of such traits.
Quantitative traits	Traits producing phenotypes showing a gradient of continued variation, due to the trait being the sum of effects from numerous genes. Animal metabolism, i.e. milk yield or growth rate, are examples of quantitative traits. If several small gene effects are present, the phenotype values for a population will typically have a normal distribution. However, in some cases the phenotype values are not distributed normally, even though the trait has a polygenetic inheritance. Quantitative traits, which only express a few classes, are called threshold traits.
Non-coding DNA or RNA	DNA or RNA sequences that do not encode for proteins.
Epigenetics	Changes in gene expressions caused by mechanisms other than changes in the underlying DNA sequence. These changes play a role in short-term adaptation of individuals, and include reversibility. It can be transmitted somatically or inherited through modification of DNA regions and allows organisms, on a multigenerational scale, to switch between phenotypes. However, epigenetic modifications also play a crucial role in silencing or expressing of non-coding sequences and thus regulatory networks, and by guaranteeing genomic stability via silencing of centromeres, telomeres, and transposable elements.[59]
Transposable elements	About 50% of the mammalian genome are pieces of genetic information that manage to multiply themselves and move around in the genome.[65,77] A substantial fraction, numbering about 0.5 of the number of genes, has been subjected to strong selective constraint.[78] Transposable elements, frequently polymorphic, carry transcription regulating signals and have an important potential to contribute to pre-transcriptional, transcriptional and post-transcriptional gene regulation. Transposable elements have been a profuse source of new regulatory sequences throughout mammalian evolution.

regulate and control the amount of gene products (RNA and proteins) available to cells, but TF themselves are regulated (often by other TF). Thus, variation in TF networking, which regulates gene expression, is much greater than variation in coding genes.

Genetic variation influencing the expression of non-coding RNA would add even more complexity, and biomolecular interactions depending on multiple genetic variations would make it impossible to explain PV simply by adding together independent genetic effects.[23]

These new developments and insights from epigenetics (see below) suggest that conceptions of gene regulation and approaches to molecular genetic analysis will have to be revised, as the genome might encompass an RNA-based information suite that is far more sophisticated than expected.[24,25]

Inheritable gene-encoded traits and phenotypic variation

The understanding of genetics and inheritance has taken new directions with important implications regarding PV. The notion that various alleles of one gene result in the observed variation of an inheritable character (Mendelian) is too simplistic and deterministic, and the least relevant conceptual model for PV and for biology in general.[26] For one, most characters, especially those with continuous features and life history characters, are polygenic. Even in many cases of discrete phenotypes, these may have a polygenic pattern of inheritance due to underlying continuously distributed traits, but with corresponding thresholds of expression, resulting in discrete phenotypes.[27] Simple features like wing dimension were shown to have at least 8–11 genes involved,[28] disease resistance involved 20 or more genes,[29] which amounts to many variant responses given the multiple alleles per given gene. For instance, challenging the plant *Arabidopsis* with pathogen stimuli, up- or down-regulation was detected in 705 mRNA, with >100 genes involved. Stimuli from a fungus increase the abundance of 168 mRNA more than 2.5-fold, whereas that of 39 mRNA was reduced, and Schenk *et al*.[30] concluded that a substantial network of regulatory interactions and coordination occurred during defence induced from pathogens. Another example is the human blood group system consisting of 38 genes with 643 alleles.[31] Moreover, irreducibly complex biochemical systems are those with many interacting parts, each contributing so critically to the basic function that deletion or modification of any one of the parts

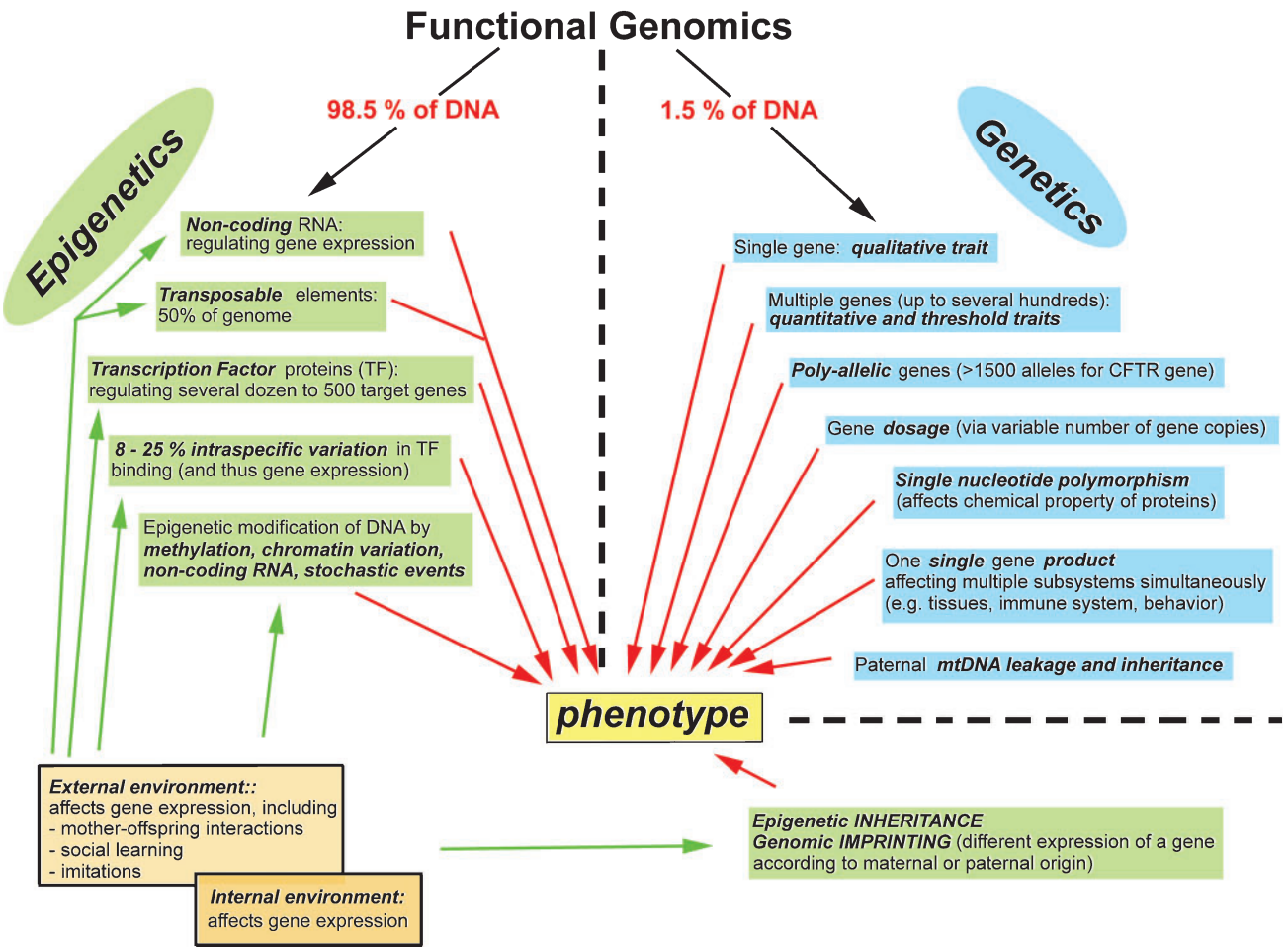

Fig. 1. Factors involved in determining the phenotype: the interplay of genetics, epigenetics and environment.

fully disrupts normal function and leaves the system with no identifiable function for itself or for its component parts. One of the better studied systems, the thermogenic system based on brown fat, uses ~300 genes, with an unknown quantity of alleles.[32]

Another layer of network complexity stems from most genes being poly-allelic. For instance, the single gene coding for the well-studied cystic fibrosis transmembrane conductance regulator protein (aberrant alleles causing an individual to have cystic fibrosis) has over 1500 known allelic variants, most causing disease.[33]

A further layer of complexity is due to variation in copy numbers of DNA sequences (CNV), which affect 12% of the human genome.[34] CNV influence gene expression, PV and adaptation by disrupting genes and altering gene dosage, also causing disease. CNV also influence gene expression indirectly through positional effects, predisposing to deleterious genetic changes, or providing substrates for chromosomal change in evolution.[34] However, because of the repetitive and multi-copy nature, these genes are considered inaccessible by most existing genotyping and sequencing technologies. Excluding these most variable and diverse regions of genetic variation is thus a loss in the pursuit of genotype–phenotype correlations.[23]

Other complex interactions

Although many factors affect the three-dimensional structure of DNA (methylation, phosphorylation, copy numbers of DNA sequences, single nucleotide variance, etc.) resulting in significant variations in gene expression, these important sources of variability on PV have been little explored. Other sources of PV are known, like single pleiotropic gene products, proteins or hormones, which frequently have multiple, independent effects on many different aspects of development, behaviour, physiology and morphology.[9] The hormone testosterone influences a wide range of characters, from primary and secondary sex characteristics, to effects on muscle and aggression, to immunosuppression and oncogenic effects. Instructive is protein p53, discovered in 1979 and one of the most studied; it even commands its own conferences.[35] Protein p53 binds to thousands of DNA sites, some of which are thousands of base pairs away from any genes. It influences cell growth, death and structure; and DNA repair. It also binds to numerous other proteins, which can modify their activity, and these protein–protein interactions can be tuned by the addition of chemical modifiers, such as phosphates and methyl groups. Through alternative splicing, p53 can take nine different forms, each of which has its own activities and chemical

Table 2. Description of factors and mechanisms involving genes, gene products and gene expression that result in the complexity of phenotypic expression

Factor	Description
Genes	*Poly-genic traits* with up to several hundred involved genes, each likely with multiple alleles *Poly-allelic genes*: the gene for the cystic fibrosis transmembrane conductance regulator protein has >1500 alleles *Pleiotropy*: one gene affects several traits *Gene dosage*: variation in numbers of gene copies (copy number variants), affecting quantity of gene product *Single nucleotide variance* in coding and gene regulatory regions (e.g. single nucleotide polymorphisms, affecting quality of gene product like reaction rates *Mitochondrial DNA*: recombinations in animals is widespread; mtDNA leakage resulting in paternal mtDNA inheritance
Gene products	*Pleiotropic gene products* (proteins, hormones) with multiple, independent effects on many different aspects of development, behaviour, physiology and morphology. Protein p53 binds to thousands of sites on DNA strand *Multiple protein structures through alternative splicing*: p53 exhibits nine different forms, each with its own activities and chemical modifiers *Variable gene product concentrations* affecting chemical reactions: influenced by the copy number of genes and regulation of gene expression
Gene expression	(*a*) *Epigenetic regulation occurs at the DNA, nucleosomal and chromosomal levels:* – DNA methylation – Chromatin remodelling – Non-coding RNA – Histone tails modification – Evidence for epigenetic effects also from: • Combinations of small RNA • RNA-binding proteins involved in both RNA editing and microRNA access to their target mRNA • DNA methylation mediated by RNA-editing enzymes, all control translation in RNA granules that are abundant in gametes of both sexes (*b*) *Large-scale genomic structural variation* (i.e. copy number imbalance through large insertions or deletions, called copy number variants as well as balanced chromosomal rearrangements). Copy number variants affect 12% of the human genome, altering gene dosage, and influencing gene expression and phenotypic variation by altering the three-dimensional structure (*c*) *Transcription factor proteins represent 10% of all gene products* Every transcription factor regulates several dozens to some 500 target genes. Variation of transcription factor activity (and thus gene expression) was 7.5–25% between individual humans, which exceeds variation in coding DNA by 300–1000-fold. Transcription factors themselves are regulated (often by other transcription factors) (*d*) *Non-protein-coding RNA (98.5% of genome):* functions of several hundred are known, but there may be even hundreds of thousands such RNA signals. There are indications that these non-coding genomic sequences are also undergoing adaptive evolution, implying functionality (*e*) *Genes responsive to external and internal environmental variation, with labile and non-labile trait expressions:* • Maternal physiology • Parent–offspring interactions affecting the chemistry of offspring DNA • Cultural interactions like social learning, symbolic communication, and imitation • Diet resulting in inheritance of feeding behaviour, affecting morphology and disease susceptibility (*f*) *Transgenerational epigenetic inheritance:* • From non-gametic interactions which are ubiquitous • As genomic imprinting in mammals: gene expression in offspring depends on which parent provided the gene • From epistasis and genetic effects where genetic factors in one generation affect phenotypes in subsequent generation • From transgenerational gene–diet interactions (*g*) *Epigenetic stochasticity* resulting in random phenotypic plasticity

modifiers. Only recently it became clear that p53 is also involved in processes beyond cancer, such as fertility and early embryonic development. Essentially it is not possible to understand p53 on its own, and efforts have shifted to studying the p53 network. Clearly, 'signalling pathways', in which proteins would trigger a defined set of downstream consequences, is generally unrealistic. The complexity of networks, however, presents a block as there may be no way to gather all the relevant data about each interaction included in the network: 'life is complicated'.[35] This multiplicity of action of single proteins and hormones suggests that there are tradeoffs rather than selection for an 'optimal' genotype or reaching an 'optimal' reaction norm, thereby constraining even PP.[2,9]

Mitochondrial DNA

Inheritance of mtDNA is often assumed to be through clonal maternal transmission. However, it has recently been shown that recombinations in animal mtDNA leading to heteroplasmy is widespread and would necessitate a reexamination of earlier phylogenetic and biohistorical inference.[36] Moreover, the existence of paternal mtDNA leakage with implications for

disease has unequivocally been shown.[37,38] High levels of mtDNA heteroplasmy were also found in guanaco (*Lama guanicoe*)[39] and direct evidence of paternal inheritance of mitochondria DNA was shown in sheep.[40] These new and dramatically unanticipated insights about mtDNA refer to a structure containing only 37 genes, whereas the genome may contain 25 000, mostly unknown, genes (e.g. human). Yet all genes together comprise only ~1.5% of the total genome, with the remainder presenting an even greater puzzle yet to be understood[24] (see below).

Non-coding genes and environmental factors, the realm of epigenetics

Epigenetics refers to changes in gene expression which result in different phenotypes, and are caused by mechanisms other than changes in the underlying genetics.[8,15,20,41] It occurs at DNA, nucleosomal and chromosomal levels, and through modification of histone tails that underlie developmental plasticity and canalisation and that bring about persistent developmental effects. Recognised mechanisms include DNA methylation, chromatin remodelling, and non-coding RNA.[8,20]

Epigenetic alterations in gene expression result from 'environmental' effects, both via external surroundings of an organism and via internal conditions. The latter is significant prenatally[42] and also acts through developmental interactions between mother and offspring, as the mother's behaviour can affect the chemistry of DNA in her offspring, making quality of early maternal care an epigenetic factor.[41,43] For instance, licking and grooming behaviour of maternal rats towards their pups within the first week of life presents a clear case of epigenetic maternal transmission.[44] Epigenetics also results from other important cultural interactions like social learning, symbolic communication, and imitation,[42,45–47], and through diet. Thus, epigenetic heredity of feeding and drinking behaviour during pregnancy can influence the appetite for certain foods, like alcohol, in offspring of rats.[44] Diet can also determine morphology like hair colour or body proportions, or disease susceptibility in adulthood.[20,48] Anaemia during only a few days of near-term fetal sheep results in increased heart size and cardiac output, a doubling of coronary artery conductance, all of which persist into adulthood.[49] Lastly, epigenetic stochasticity results in random PP.[10,11]

Natural clones (monozygots) are instructive models because of their shared genotype. Although twins at a very young age are epigenetically indistinguishable, the level of difference increased with age[15,41,50] and explains for instance differential disease susceptibility.[43] Artificial cloning has still limited success as most (if not all) cloned animals have epigenetic defects due to the prevailing lack of understanding of how epigenetic marks are reprogrammed.[15,20,51] Some of the anomalies encountered in cloned embryos suggest a disruption of imprinted gene expression,[52] and gross PV in pig clones was large.[53]

An informative example is provided by clonal crayfish, a highly fecund parthenogenetic animal. Clonal (isogenic) mates exhibited very broad ranges of variation in colouration, growth, life-span, reproduction, behaviour, number of sense organs, and fluctuating asymmetry, even when reared under identical conditions. Although in the same environment, clonal genotypes mapped to numerous phenotypes, thus generating variability among clone mates and individuality in a parthenogenetic species. This variation can thus introduce components of randomness into life histories, modifying individual fitness and population dynamics.[54]

One of the most unique examples of a labile trait in mammals is found among cervids: the yearly regrowth of a complete appendage, the antler.[55] Moreover, cervids provide a unique example of epigenetics, the memory of injury on live antler tissue. When a growing antler is injured on the beam, it will produce a hypertrophic growth as a response to healing. Then the antler dies and falls off; the subsequent regrowing new antler will again grow a hypertropic area, at times even more pronounced, and such responses have been documented to last up to 10 years.[56,57] This epigenetically induced memory appears to reside in a very localised tissue containing antler stem cells.

It is now recognised that intraspecific variation in behaviour is extensive, being influenced by environmental conditions from the moment the egg is fertilised until death.[42] Some of the most prominent personality traits can be categorised in terms of risk-taking behaviour. A good example is the correlation between aggressiveness towards conspecifics and boldness towards predators: individuals that risk more in intraspecific fights also risk more when confronted with a predator.[58] Variation in behaviour in social mammals like deer is often the first aspect of the phenotype to evolve in a new direction or to bring a population into a new habitat or niche.

A highly relevant component of epigenetics is transgenerational epigenetic inheritance (TEI), which is ubiquitous.[10,43,47,51,59] TEI occurs when phenotypic traits not stemming from DNA variations are transmitted to subsequent generations, with traits persisting across several to many generations.[5,60] TEI is common and usually as strong as conventional inheritance.[12,23,44] A clear example is TEI of the Agouti viable yellow allele in mice.[59] TEI also stems from transgenerational epistasis, transgenerational genetic effects where genetic factors in one generation affect phenotypes in subsequent generation without inheritance of the genetic variant in the parents, and transgenerational gene–diet interactions.[12] TEI can result from non-gametic interactions, but in sexual reproduction, heritable epigenetic variations in germ line cells can result in transmission of developmentally induced and stochastically generated phenotypes from one generation to the next through the gametes. Mammalian genomes have an additional layer of epigenetic information referred to as genomic imprints, so called because they carry a molecular memory of parental origin that is acquired in the germ line.[52] Germ line cells contain small RNA, known as Piwi-associated interfering RNA (piRNA). Mammalian spermatocytes are filled with piRNA, and similar RNA occurs in oocytes as well. Epigenetic marks that are imposed on parental chromosomes during oogenesis differ from those imposed during spermatogenesis; therefore, in the offspring, a gene's expression pattern depends on whether it was inherited from the father or from the mother.[61] For instance, the insulin growth factor 2 (IGF2) gene promotes, whereas the receptor gene (IGF2R) inhibits fetal growth. The maternal copy of IGF2 is

silenced and expression occurs only of the paternal copy. On the other hand, IGF2R is silenced paternally and expression occurs only of the maternal copy.[62] It has long been known that mules are offspring of a male donkey and female horse, and hinny are offspring of a male horse and female donkey. In mammals, it is in fact paternal imprinting which prevents parthenogenesis, ensuring that paternal contribution is obligatory for descendants. Because epigenetic reprogramming occurs during folliculogenesis and embryogenesis, any disturbance of the normal natural environment during these critical phases could cause epigenetic alterations.[59] Thus, factors like toxins, stress, or undernutrition exert a large influence over offspring phenotypes. Although many genes remain imprinted throughout the entire life of an organism, some genes are imprinted in a tissue-specific or temporal manner.[59]

The obvious molecular mechanisms for epigenetic inheritance are DNA methylation and histone modifications. However, evidence from plants, flies and mice suggests additional mechanisms: combinations of small RNA, RNA-binding proteins that are involved in both RNA-editing and microRNA access to their target mRNA, and DNA methylation mediated by RNA-editing enzymes, all control translation in RNA granules that are abundant in gametes of both sexes.[12,23] Adaptation can thus occur through the selection of heritable epialleles, without any genetic change.[12,47,51,59]

Implications of genetics and epigenetics on intraspecific phenotypic variation

Whereas highly complex assortments of genotypes lead to a spectrum of phenotypes, the same spectrum can result if a single genotype generates highly complex assortments of epigenotypes.[10] While most traits have a polygenic basis with up to hundreds of involved genes, a trait depending on only 10 genes and assuming only two alleles per gene for example, already results in 59 049 possible genotypic combinations for diploid offspring. However, as the number of genes per trait is substantially higher, and the average number of alleles per gene is perhaps 10–15, the quantity of genotypic variants is immense.

However, additional enormous layers of complexity are superimposed by epigenetic effects via genetic regulatory networks. For instance, epigenetic TF typically regulate many individual target genes (up to 500). The overall complexity, therefore, leads to the currently accepted generalisation that complex genotypic architectures are less, not more, amenable to being altered by natural selection.[63] It also underscores that PV and plasticity of a species is expected to be very profound, ranging from obvious to more cryptic variations (in terms of our perception and capacity for detection). The overall number of variants is large enough to make every individual very different from another, even if they are perfect clones, i.e. isogenic.[35]

Discussion

Although DNA is the template for the DNA-RNA-protein paradigm of heredity, it is the coordination and regulation of gene expression that results in the wide complexity and diversity seen among individual deer. In fact, per generation there will usually be a greater variety of phenotypes available for selection than available genotypes. Thus, 'soft' or epigenetic inheritance is a more pliable system for the fine tuning of the next generation to novel environments than the slow reactivity of Mendelian 'hard inheritance'.[64] Overall, the 'genetic blueprint' is conceptually inadequate and considered positively misleading to describe how PV is produced.[25]

An important aspect of DNA expression is the resulting quantity of DNA products due to the kinetics of subsequent chemical interactions. Gene expression is regulated through changes in the number and type of interactions between molecules that collectively influence transcription of DNA and translation of RNA. But it also depends on variable gene dosage (CNV), because multiple gene copies result in more products: yet even for the human genome it is still unknown how many genes exist as single copies only.[65] It is thus evident that simple changes in gene product dosage (protein) may be sufficient to trigger epigenetic responses.[12] Such epigenetic effects can get passed to the next generation, and offspring then show the trait not because they carry the responsible genetic variants, but instead because these variants were present in one to several generations before.[12]

With the bewildering amount of factors and networks determining gene expression, we would expect that two genotypes, via distinct epigenetic adjustments, could produce the same phenotype: i.e. the same outcome via different solutions as response to the same problem. Indeed, some animal models have shown just that, like transgenic mice with genes to develop specific pathologies, but when raised in semi-naturalistic enclosures from birth on, could not be differentiated by their behavioural profiles and basal levels of stress from animals without the transgene, neither at early ages, nor later in life.[42] Given current technologies for genetics, these phenomena would be indistinguishable because of intrinsic genomic redundancy (M. Pigliucci, pers. comm.).

Although the overwhelming role of epigenetics in PP is presently well recognised, applications even in livestock production are in their infancy: new technologies for sequencing are emerging to allow the determination of the epigenome, such as CNV and genomic methylation states.[66] As a result, there has been a resurgence of interest in pre-natal programming of post-natal production, embracing reproductive performance, behaviour, development of the immune system, appetite and longevity.[67] For instance, knowledge of the sensitivity of mammalian embryos to their environment, as seen in altered gene expression, is rapidly expanding. Thus, undernutrition during the peri-conception period affects cells in day-6 sheep embryos and significantly resets the growth trajectory of a majority of fetal organs and tissues; or certain subclinical trace mineral deficiencies during the first half of pregnancy reduce neonatal vigour and acquisition of passive immunity, reduce early life activity and investigatory behaviour, and result in less time spent interacting with their mothers.[67,68] Similarly, as traditional genetics explains only up to 30% of the variability in milk production of dairy cows, a substantial proportion of unexplained variation is likely due to PP, with an emerging potential to manipulate mammary function via epigenetic regulation, including the heritability of epigenetic marks to modify lactation performance of offspring.[69]

Intraspecific variation, particularly PP, has important consequences for deer management and conservation of threatened cervids. Commonly, a certain phenotype in a given population is equated to individual fitness, for instance, by using the proxy of numbers of offspring produced. However, the continued existence of polymorphism suggests that no single morph is the best fit in all situations, and individuals judged less fit in a particular study population likely become the fittest when environmental conditions change accordingly. Focusing on a mean 'optimal' phenotype diverts attention away from variation around the mean, even discarding 'outliers' as noise, to better fit the chosen model. Yet, selective pressures are working on all characteristics and their determinants in combination, and not in isolation.[70] Thus, no morph has a universally higher fitness as there are tradeoffs, with the relative fitness of two morphs being contingent upon environmental conditions. Such tradeoffs have indeed been observed in most cases studied, certainly in all cases where detailed analyses have been undertaken.[27] Such negative genetic correlations are common,[71] all of which prevent that single characters evolve unidirectionally.[72] For spatio-temporal heterogeneous environments, maintenance of intraspecific variation also is an essential ingredient for the continuous existence of a species, although evolutionary history, including genetics, certainly present constraints.[73] These are the most parsimonious explanations for the continued observed existence of apparently 'unfit'-type individuals in wild populations, and the currently available theory leads away from the idea of survival of the fittest and towards a model of survival of the barely tolerable.[58,63]

In conclusion, deer management and conservation of endangered cervids will benefit from appreciating the large inherent intraspecific PV among individuals and the immense contribution of epigenetics in all aspect of deer biology and ecology. However, promoting some cervid phenotype (necessarily of artificial choice, in the wild or *ex situ* for purpose of reintroductions), is counter natural processes. No doubt, it will give results, just as with men's selection imposed on dogs for only a few hundred years, resulting in phenotypic variance exceeding the natural known variance not only in canids, but in excess of anything known among Carnivora.[74]

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