An epigenetic resolution of the lek paradox

Melvin M. Bonilla[1)2)], Jeanne A. Zeh[1)] and David W. Zeh[1)]*

Female choice for traits signaling male genetic quality is expected to erode heritable variation in fitness, undermining the benefits of choice. Known as the lek paradox, this contradiction has motivated extensive population genetic theory, yet remains unresolved. Recent modeling by Bonduriansky and Day concludes that costly female preference is best maintained when male condition is determined by environmentally induced factors transmitted across single generations. Here, we reformulate their model in explicitly epigenetic terms, and review evidence that environmentally induced paternal effects are mediated through epigenetic changes in sperm. Noncoding RNA expression, DNA methylation and histone modifications are highly sensitive to diet, stress, toxicants and stochastic events. Epigenetic variation renews each generation and cannot be exhausted by selection. By choosing well-endowed males that produce gametes in epigenetically good states, females can increase their fitness directly through increased fertilization success or indirectly through epigenetic effects on the fitness of offspring and potentially subsequent generations. Also watch the video abstract.

Keywords:
condition-dependence; epigenetically good genes hypothesis; epigenetics; female choice; lek paradox

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Introduction

Female choice of attractive males as a mechanism responsible for the evolution and maintenance of exaggerated male traits remains a contentious topic in evolutionary biology [1, 2]. When males provide females with direct benefits that increase female longevity and/or fecundity [1, 3], such as nuptial gifts or paternal care [4–6], the benefits of choice are uncontroversial. However, when males provide only ejaculates, female choice presents a conundrum. Choosiness is likely to be costly [7], and, in the absence of compensating benefits, female choice should be eliminated by selection. It has therefore been proposed that females derive indirect, i.e. genetic benefits for offspring by choosing attractive males [8].

The "good genes hypothesis" proposes that females select mates based on secondary sexual characters that provide honest signals of male genetic quality [9]. According to this viability indicator model, only high-quality males can fully express costly traits, and, by mating with ornamented males, females secure good genes that increase the viability and/or reproductive value of their offspring [9, 10]. However, over time, the increased reproductive success of males bearing exaggerated traits should exhaust genetic variation for viability, and drive the traits to fixation [11, 12]. Female choice, in combination with natural selection acting on both sexes, depletes additive genetic variation for fitness, yet genetic benefits are the explanation for choice [11, 13]. Known as the lek paradox, this apparent contradiction has generated a vast literature on possible mechanisms maintaining variation in male fitness (Supporting Information, Table S1). Most hypotheses posit that variation upon which females choose is replenished by mutations each

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[1)] Department of Biology and Program in Ecology, Evolution and Conservation Biology, University of Nevada, Reno, NV, USA
[2)] Department of Environmental Health, T.H. Chan School of Public Health, Harvard University, Boston, MA, USA

***Corresponding author**:
Jeanne A. Zeh
E-mail: jaz@unr.edu

Abbreviations:
lncRNA, long noncoding RNA; **miRNA,** microRNA; **ncRNA,** noncoding RNA; **P1,** protamine 1; **P2,** protamine 2; **piRNA,** piwi-interacting RNA; **TE,** transposable element.

generation [11, 13]. However, it is unclear whether mutational-based input is sufficient to account for the persistence of female choice [14].

Recent theory by Bonduriansky and Day [12] suggests that environmentally induced variation in male condition is much more effective in maintaining costly female preferences than when condition is determined genetically or by a highly mutable but non-inducible epigenetic factor. Here, we reformulate their model in explicitly epigenetic terms, provide an overview of relevant empirical studies, identify the limitations of the model and its supporting evidence, and outline a research program for testing the hypothesis.

Environmentally induced variation in male condition maintains adaptive female choice: Theory

In the first mathematical analysis of the effect of transmission of paternal condition via sperm-borne, non-DNA sequence-based factors, Bonduriansky and Day [12] compared three models of male condition in terms of ability to support the evolution and maintenance of costly female preferences. In all three models, female preference is determined by a single locus with two alleles (A/a, female preference/non-preference). Male condition (C/c, high/low) is either: (i) genetically determined (two alleles, low spontaneous mutation rate); (ii) epigenetically controlled (two epigenetically determined states, high spontaneous "mutation" rate); or (iii) environmentally induced (two environmentally induced states). In the epigenetic-control model, changes in epigenetic states are random with respect to environmental quality, and may persist across multiple generations. With environmental induction, male condition is influenced by environmental quality and transmitted across a single generation. The terminology describing the latter two models does not imply that epigenetic mutations are not environmentally induced. In fact, environmental induction is responsible for a large fraction of epigenetic variation [15].

Comparison of the three models [12] indicated that costly female preference most likely evolves and persists when male condition is an environmentally induced factor transmitted over a single generation. Preference can also be supported when condition depends on non-inducible, highly mutable epigenetic states, but the necessary conditions are more restrictive. In this theoretical framework, in which condition was controlled by a single locus or factor, the genetic model was unable to support the evolution of costly female preference. Although based on a simplified view of the genetic control of male sexually selected traits contradicted by multilocus models of male condition (Table S1), Bonduriansky's and Day's analysis [12] nonetheless establishes the plausibility and strength of environmentally induced, condition-dependent processes in the evolution of costly female preferences. As discussed below, the environment experienced by a male directly affects epigenetic states in both his somatic and germline tissues. Environmentally mediated modifications to the sperm epigenome thus provide the most plausible and best-supported mechanism through which environmentally induced male condition can be transmitted to offspring.

Environmentally induced, intergenerational epigenetic effects can resolve the lek paradox

Three premises underlie the lek paradox, namely that female choice occurs, is costly, and provides no direct (material) benefits [11]. These premises generate two contradictory predictions that constitute the paradox: (i) females gain genetic benefits from choice; and (ii) female choice depletes genetic variance, thus precluding choice-based genetic benefits. The most compelling of previously proposed resolutions of the paradox have questioned either the no direct benefits premise or the prediction that additive genetic variance in male condition becomes exhausted. For example, the phenotype-linked fertility hypothesis posits direct benefits, in which costly ornament expression provides a reliable signal of male fertilization efficiency [16, 17]. Alternatively, the genic capture hypothesis [11, 18] proposes that the cumulative effects of mutations across the genome are sufficient to replenish additive genetic variation in male sexually selected traits as rapidly as it is eroded by female choice and natural selection.

Building on previous theory [12, 19–21], we propose that the key to resolving the lek paradox lies in the emerging field of molecular epigenetics. The epigenome consists of a complex regulatory system, involving somatically, intergenerationally, and, in some cases, transgenerationally heritable epigenetic marks (DNA methylation and histone modifications), as well as diverse classes of noncoding RNA (ncRNA), that determines whether, where and when genes are expressed [22, 23]. Epigenetic marks and ncRNAs are much more sensitive than DNA sequence to environmental factors and stochastic events experienced during an individual's lifetime [24–27]. We propose that these biotic and abiotic environmental effects provide an inexhaustible source of epigenetic variation that affects male sexually selected traits signaling phenotypic condition. Males in good phenotypic condition are likely to produce sperm with epigenetic profiles that contribute to high offspring fitness.

Our female choice for genes in an epigenetically good state hypothesis (henceforth the epigenetically good genes hypothesis) proposes that, by choosing well-endowed males who produce gametes in epigenetically good states, females increase their fitness either directly through increased fertilization success or indirectly through epigenetic effects on fitness of offspring and potentially subsequent generations. Like the phenotype-linked fertility hypothesis and genic capture models, the epigenetically good genes hypothesis proposes that male sexually selected trait expression is condition-dependent and reflects an individual's capacity to acquire and assimilate environmental resources. Where it differs is in the recognition that the physical and biotic environments experienced by a male, and, in some cases, by his recent ancestors, can induce epigenetic changes that influence his capacity to produce epigenetically good sperm. Stress, diet, exposure to toxicants, infection by parasites and gut microbiota diversity all endow sperm with an epigenetic legacy that influences their ability to achieve fertilization and successfully modulate development, growth and reproduction in offspring [26–28]. The lek paradox is resolved because

epigenetic variation is renewed every generation independent of selection acting on male condition (Fig. 1).

Three pillars of epigenetics: DNA methylation, histone modifications and noncoding RNAs

A fundamental tenet of modern biology is that the characteristics inherited by organisms are ultimately controlled by the DNA sequences of their parents. However, it is now apparent that there is more to the phenotype than nucleotide sequence alone. Associated with the DNA scaffold is a system of somatically, intergenerationally, and potentially transgenerationally heritable epigenetic marks. In conjunction with ncRNAs, DNA methylation and chemical modifications to core histone proteins affect how tightly DNA is packaged in chromatin (Fig. 2; Box 1). By providing differential access to underlying genetic information in a reversible, dynamic and inducible manner, epigenetic marks mediate the developmental pattern, tissue specificity and environmental context of gene expression [63]. Increasing evidence identifies epigenetic states as determinants of health, with environmentally induced, epigenetic dysfunction implicated in the pathogenesis of complex diseases, such as atherosclerosis [64], cancer [65], diabetes [66], and neurological disorders [67], as well as fertility disruption in both sexes [23, 68]. The various components of epigenetic gene regulation interact to establish cellular identity and respond to internal threats posed by genomic parasites and physiological challenges emanating from the external environment [63].

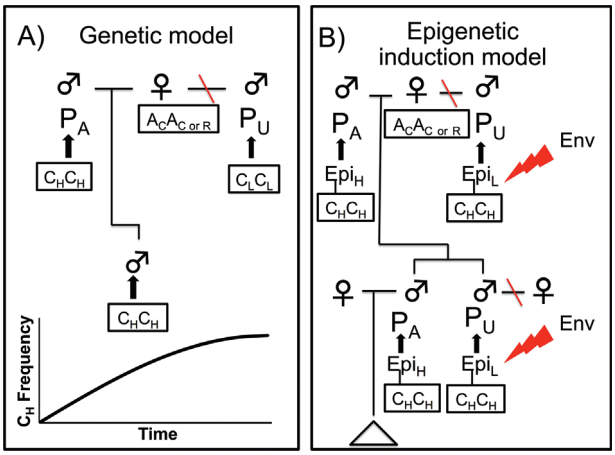

Figure 1. Comparison of genetic and epigenetic-induction models for the maintenance of costly female preference. In both conceptual frameworks, female choice is controlled by a single locus with two alleles: A_C encodes preference for phenotypically attractive (P_A) males in high condition, whereas A_R encodes random mating. **A**: In genetic models, male condition is genetically determined by one or more loci with two alleles (C_H/C_L, high/low expression of sexually attractive trait). Because unattractive males (P_U) achieve low mating success, C_H is driven rapidly to fixation (bottom left). At equilibrium, phenotypic variation in male attractiveness stems from non-additive genetic variation or non-heritable environmental variation. **B**: In the epigenetic induction model, variation in male condition is epigenetically based (Epi_H/Epi_L, high/low quality epigenetic states) and is induced by the set of environmental conditions and chance events (Env) experienced by the male during development and adulthood. Because environmental quality directly impacts epigenetic states in male somatic and germline tissues, acquired epigenetic states are transmitted intergenerationally from males to offspring.

Permissive chromatin states allow pervasive transcription during spermatogenesis

Animal spermatogenesis is generally viewed as a three-stage process encompassing: (i) a diploid mitotic phase, involving increases in cell size and number; (ii) a diploid meiotic phase (Meiosis I) characterized by transcriptional activity and structural changes; and (iii) a post-meiotic phase of sperm maturation (spermiogenesis), in which the haploid genome is unexpressed. Haploid genome silencing is an oversimplification, as it is now known that hundreds of genes are expressed post-meiotically in sperm [69–71]. Recent RNA sequencing of multiple tissue types across vertebrates indicates that complexity of the testes transcriptome exceeds that of other tissues, due to extensive meiotic and post-meiotic expression in spermatocytes and spermatids, respectively [71]. Nearly all protein-coding genes are abundantly expressed, as are long noncoding RNAs (lncRNAs), pseudogenes and transposable elements (TEs). Pervasive transcription is associated with permissive chromatin states, including DNA hypomethylation of gene, lncRNA and TE promoters, as well as high H3K4me2, a histone modification indicative of open chromatin configuration [71].

In the sections below, we focus on modifications to the sperm epigenome that affect female fitness indirectly through offspring. However, environmentally induced epigenetic effects also impact ejaculate quality and fertilization efficiency, and thus influence direct benefits to female choice. It is important to note that, even though post-meiotic haploid gene expression occurs in sperm, is not required for epigenetically mediated direct benefits to female choice. Epigenetic disruption to DNA methylation and/or small ncRNA pathways during meiosis can unleash TE expression (Box 1), resulting in spermatogenesis arrest and reduced viable sperm production. In addition, epigenetic disruption in male accessory glands can affect the quality of seminal fluid. Finally, exercise and diet can induce epigenetic changes in somatic tissues and sperm, and these epigenetic effects have been linked to ejaculate characteristics affecting fertilization efficiency, such as sperm number and semen volume (see below).

Environmentally induced, intergenerational epigenetic effects mediated through sperm: Empirical evidence

Molecular epigenetics challenges the supremacy of DNA sequence as the basis for phenotypic variation and has yet to enter the mainstream of evolutionary biology [72]. Consequently, support for the epigenetically good genes hypothesis comes largely from biomedical research, in which epigenetic effects on health and disease are now widely

Box 1

Overview of epigenetic mechanisms: DNA methylation, noncoding RNAs and histone modifications

DNA methylation occurs pervasively in eukaryotes, although lineages differ markedly in levels and genomic distribution of methylation [29]. In animals, methylation primarily occurs symmetrically (on both strands) on cytosine residues in CpG dinucleotides. Cytosine methylation functions to silence transposable elements (TEs), regulate transcription or establish tissue-specific patterns of gene expression [30]. In vertebrate genomes, which are highly methylated, most genes contain CpG-rich regions (CpG islands) at or near transcription start sites. While methylation at these gene promoters results in repression, methylation and expression may be positively correlated within gene bodies [31]. Gene body methylation functions in alternative splicing and tissue-dependent gene expression [32]. TE sequences, which constitute ~40% of mammalian genomes, often contain promoter sequences that are hypermethylated and constitutively silenced [31]. In invertebrates, DNA methylation patterns are highly variable, and range from near absence in *Drosophila* to genome-wide methylation levels approaching those of vertebrates [33]. Invertebrate DNA methylation is uncommon in TEs and other intergenic regions but enriched in transcriptionally active gene bodies [34]. Among insects, methylation is abundant in social Hymenoptera, where it regulates reproductive caste determination, longevity and behavioral plasticity [35, 36]. DNA methylation may also underlie phenotypic plasticity in other invertebrates, such as locusts [37] and *Daphnia* [38].

Diverse classes of ncRNA, including microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), and long noncoding RNAs (lncRNAs), have emerged as key players in the regulation of gene expression, genome stability, and defense against parasitic sequences [39]. Among small ncRNAs, miRNAs are conserved sequences that regulate gene expression at the post-transcriptional stage through mRNA degradation and/or translational repression [40]. miRNAs associate with Argonaute proteins and guide the RNA-induced silencing complexes (RISCs) to target mRNAs by base pairing complementary [41]. Because binding is restricted to a short "seed" region of the miRNAs [40], a single miRNA may bind to the mRNAs of numerous genes, and a single gene may be regulated by several miRNAs, resulting in regulatory networks that function in almost all developmental, physiological and disease-related processes [42, 43]. piRNAs are highly expressed in gonads where they function as a defense against TE activity [44]. In *Drosophila*, PIWI proteins are essential for male and female fertility [45–47], and, in mouse, deficiency in PIWI proteins results in germline activation of TEs and complete male sterility [48–50]. Like miRNAs, piRNAs function via homology-dependent post-transcriptional gene silencing, in which piRNAs guide TE transcripts to RISCs for targeted destruction. piRNAs also target genes involved in early development and sex determination, as well as de novo methylation of imprinted genes, and establishment of memory in neurons [51–54]. The piRNA pathway also modifies chromatin and targets gene expression via RNA interference pathways [39]. Long non-coding RNAs constitute a heterogeneous class of RNA sequences >200 nucleotides in length, and possess little or no protein coding potential. An important epigenetic regulatory role for lncRNAs was first recognized with the discovery of X inactive specific transcript (XIST), which coats and silences virtually the entire inactive X chromosome in female mammals. More recent evidence indicates that lncRNAs associate with chromatin to recruit chromatin-modifying complexes that detect and silence aberrant transcription events, and establish a memory of these events, using self-reinforcing epigenetic loops [39]. Gene targeting and knockdown experiments have established an essential role for lncRNAs in development and organogenesis [55].

The constellation of DNA, RNA, and protein constituents that comprise chromatin exists in various conformational and biochemical states at multiple scales of organization. At the most fundamental level, genomic DNA is wrapped around octamers of histone core proteins, forming repeated units called nucleosomes. The amino termini (tails) of these histones protrude from nucleosomes and can be reversibly modified in more than a dozen ways, generating tremendous combinatorial complexity, sometimes called the histone code [56]. Acetylation and methylation are the most common and best understood of these histone modifications. The transfer of an acetyl group to lysine neutralizes the positive charge of the histone tail, promoting a more relaxed chromatin structure and facilitating recruitment of transcription factors and other components of the transcriptional machinery [57]. Among the many potential sites for lysine acetylation, lysine 16 of histone 4 (H4K16) is particularly critical in regulating chromatin folding and in the switching from silent heterochromatin to an active, euchromatin configuration [58]. Histone methylation, which occurs on arginine, lysine, and histidine residues, may be either repressive or activating, depending on site and level of methylation. Lysine residues, can exist in mono-, di-, or tri-methylated forms. Whereas H3K4me3 is a distinctive feature of transcriptionally active genes, H3K27me3 is associated with repressed chromatin [59]. Histone modifications do not operate in isolation but interact with DNA methylation and ncRNAs to establish cellular identity, and to respond to internal threats posed by genomic parasites and physiological challenges emanating from the external environment [60]. For example, in mammals and *Drosophila*, piRNAs not only inactivate TEs through post-transcriptional mRNA degradation but also target TE sequences for DNA methylation and/or histone modifications that result in a repressive chromatin environment and transcriptional silencing [39]. This crosstalk between post-transcriptional and transcriptional gene silencing mechanisms may underlie the ability of sperm-borne ncRNAs to transmit altered epigenetic states to subsequent generations [61–62].

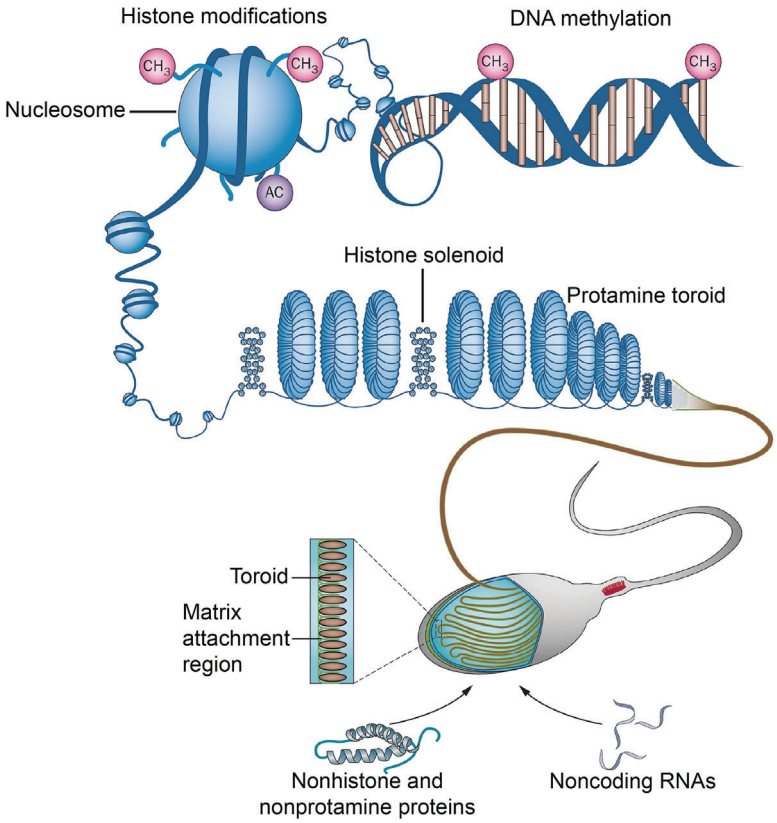

Figure 2. Sperm epigenetics. During spermiogenesis, a high percentage of histone proteins are replaced with smaller protamines, enabling an order of magnitude greater compaction than nucleosome-bound chromatin. The remaining histone solenoids exhibit modifications to histone tails, including methylation (CH_3) and acetylation (AC). Covalent modifications to DNA most commonly occur as methylation of cytosine. Sperm also contain nonhistone and nonprotamine proteins, as well as noncoding RNAs, including microRNAs and piwi-interacting RNAs. Permission from Elsevier Ltd. Carrell, DT. 2012 *Fertil. Steril*. 97: 267–274.

appreciated [15, 23–24]. The hypothesis yields a number of testable predictions regarding the relationships between epigenetic states in males and sperm, male fertility, offspring fitness and male condition. Specifically, it predicts that: (i) epigenetic disruption should be a key factor in male infertility; (ii) environmentally induced epigenetic changes in males should affect epigenetic states in sperm that influence offspring phenotypes and disease susceptibility; (iii) sperm should have the capacity to mediate intergenerational and transgenerational epigenetic effects; (iv) the contribution of environmentally induced epigenetic variation to phenotypic variation and disease phenotypes should be substantial; and (v) epigenetic states should influence male traits that serve as indicators of condition.

Epigenetic disruption as a key factor in male infertility

Whereas genetic causes, such as karyotypic abnormalities and Y chromosome microdeletions, account for a minority of male infertility cases in humans [73], sperm epigenetic defects are increasingly linked to fertility disorders and aberrant embryogenesis [74]. Because of the small size and motility of sperm, the sperm epigenome is highly specialized and vulnerable to disruption [26, 27]. During spermiogenesis, ~85% of histones are replaced with protamines, small, nuclear proteins unique to sperm that enable compaction up to 10 times greater than nucleosome-bound chromatin (Fig. 2) [75]. Fertility disorders correlate with a range of aberrant epigenetic states in sperm, involving not only the stepwise transition from histone- to protamine-bound DNA (protamination) but also chemical modifications to histones that escape protamine replacement, and the concentration and diversity of ncRNAs. Protamination involves the establishment of two protamine isomers, P1 and P2, at a tightly regulated ratio of ~1:1. Distorted P1/P2 ratios are common among infertile and subfertile males, and are linked to low sperm concentration, poor motility, and abnormal morphology [76], as well as decreased fertilization capacity and poor embryogenesis in assisted reproductive technology [77].

Despite conversion of most histones to protamines, modifications to remaining histones play a significant role in sperm function, male fertility, and the transgenerational inheritance of acquired epigenetic states [78]. Histone retention and location within the sperm epigenome occurs in association with promoters of developmentally important genes, imprinted genes, transcription factors, and microRNA genes [79]. Genome-wide analyses have identified marked abnormalities in histone retention patterns at these developmentally important loci in sperm of infertile men [78].

Numerous studies have tied abnormal DNA methylation to fertility disruption and defective embryological development in humans and animal models [79]. In a pioneering study, Benchaib et al. [80], detected a correlation between global sperm DNA hypomethylation and poor pregnancy outcomes among IVF patients, a finding substantiated in subsequent studies conducted using locus-specific methylation detection methods [81, 82]. Hypomethylation of genomically imprinted genes and *Alu* TEs was particularly significant and associated with poor semen parameters, male infertility, and high rate of spontaneous abortion [81]. In the X-linked *Rhox* gene cluster, the association between methylation and infertility is reversed, with *RHOX* genes more likely to be hypermethylated in infertile patients than in fertile men [83]. Moreover, level of hypermethylation was significantly correlated with severity of ejaculate abnormalities [83]. Because *RHOX* genes encode homeobox transcription factors that are highly expressed in testes and regulate several genes controlling spermatogenesis, this association is likely to be causal [83]. The importance of DNA methylation in spermatogenesis has also been experimentally confirmed in mice. *Dnmt3L* is a DNA methyltransferase regulator that

is expressed during spermatogenesis and promotes DNA methylation at paternally imprinted regions, heterochromatic sequences, and interspersed repeats [84]. *Dnmt3L*-knockout mice exhibited increased retrotransposition of an LTR-ERV1 TE, abnormalities in heterochromatin, spermatogenesis arrest, and spermatocyte loss due to apoptosis [84].

Sperm deliver a complex ensemble of noncoding RNAs to zygote

Despite possessing a compact nucleus with minimal cytoplasm, sperm deliver a complex mix of RNAs to the oocyte, including mRNAs, microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), transfer RNA-derived small RNAs (tsRNAs), ribosomal RNAs, and TE transcripts [85, 86]. Because of their abundance, short length, resistance to fragmentation, and capacity to orchestrate DNA methylation and histone modifications, small ncRNAs, including miRNAs and piRNAs, are likely to be particularly important for male fertility and embryological development [87]. Oligoasthenozoospermia (low sperm count and poor sperm motility) is the leading cause of reduced fertility/infertility in human males. In a recent study of sperm of oligoasthenozoospermic men, 50 miRNAs were found to be significantly up-regulated and 27 significantly down-regulated, compared to sperm from normal males [88]. Experimental studies suggest a causative relationship between differentially expressed small ncRNAs and male fertility, with deletion of the miRNA loci, miR34b/c and miR-449, impairing both meiosis and the final stages of spermatozoan maturation, and resulting in oligoasthenoteratozoospermia [89]. Analysis of pachytene spermatocytes revealed a set of deregulated genes enriched as targets for silencing by the miR-34 family of miRNAs [89].

Environmentally induced epigenetic changes in males affect sperm epigenetic states that influence offspring phenotypes and disease susceptibility

Epigenetics provides a molecular basis for the developmental origins of health and disease hypothesis, according to which environmental challenges experienced during critical stages of pre- and post-natal mammalian development induce lifelong physiological changes that modulate risk for chronic diseases, including metabolic syndrome, obesity, cardiovascular disease, psychoses, osteoporosis, and asthma [90]. This hypothesis incorporates two hallmarks of epigenetics, namely, susceptibility to dysregulation during critical developmental phases, and metastability, involving self-propagating biochemical signatures that provide memories of previously experienced stimuli [91]. While epigenetically based maternal effects are now widely acknowledged in gestating mammals [23], only recently has it become apparent that paternal effects may have equally important consequences for offspring health and reproduction [26, 27, 92]. With both maternal and paternal effects, alterations to parental epigenomes occurring at critical life history stages may be propagated in offspring and subsequent generations.

Two recent studies illustrate the relevance of maternal effects on male offspring and paternal effects on sperm epigenomes for the epigenetically good genes hypothesis. Clear evidence for epigenetically based maternal effects on male offspring and sperm is provided by a study in which female mice were nutritionally restricted during gestation [93]. In utero undernourishment resulted in F_1 offspring with low birth weight and multiple metabolic defects. The timing of nutritional restriction coincided with reestablishment of methylation in F_1 male primordial germ cells, resulting in more than 100 regions that were hypomethylated in F_1 males' sperm relative to controls. When mated to control females, F_1 males sired F_2 offspring with metabolic phenotypes similar to their own, including low birth weight and glucose intolerance. Although sperm whole-genome methylation analysis in the F_2 revealed a loss of differential methylation, tissue-specific differences persisted in expression of metabolic genes neighboring previously differentially methylated regions.

In a study of environmentally induced paternal effects in isogenic lines of *D. melanogaster*, 48-hr exposure of males to a high sugar diet resulted in metabolic reprogramming and obesity in offspring [94]. In a process involving histone methylation marks, H3K9me3 and H3K27me3, paternal sugar acted to decondense chromatin domains in both mature sperm and embryonic offspring. The researchers found that similar chromatin signatures also predict obesity susceptibility in mouse lines and human obesity cohorts, suggesting a general mechanism through which acute dietary effects may underlie sperm-borne, intergenerational metabolic reprogramming.

Sperm epigenomes mediate intergenerational and transgenerational epigenetic effects

The sperm epigenome: A messenger of ancestral exposures [27: 80]

Parental exposure to environmental stimuli that modify epigenetic states can directly affect both somatic and germline tissues, resulting in intergenerational epigenetic effects [95]. In gestating mammals, direct environmental induction impacts three generations simultaneously: maternal somatic tissue in the F_0 generation, fetal somatic tissue in the F_1 generation and fetal primordial germ cells that will contribute to the F_2 generation [96]. In males, direct exposure is restricted to two generations, paternal somatic tissue and sperm. The more stringent phenomenon of transgenerational epigenetic effects refers to perpetuation of altered epigenetic states in the absence of direct exposure, which requires demonstration of F_2 involvement for paternal transmission and F_3 involvement for gestating females. While intergenerational epigenetic effects are now well documented, transgenerational epigenetic effects remain controversial [96], although examples are steadily accumulating [68]. A relatively high percentage of cases result from paternal transmission, and most commonly involve alterations to DNA methylation and ncRNAs [97].

Altered epigenetic states in sperm and transgenerational inheritance of disease can be induced by ancestral exposure to fungicides, hydrocarbons, pesticides, and poor nutrition [68]. A recent study of paternal inheritance of odor fear conditioning provides a striking example of transgenerational epigenetic effects [98]. When male mice were conditioned to fear the odor of acetophenone, their offspring and the F_2 generation were born with an increased sensitivity to that odor. The olfactory receptor gene involved in the fear response was differentially demethylated in the sperm of conditioned males, and this epigenetic change was transferred to F_1 and F_2 generations, indicating that epigenetic alterations escaped postfertilization and primordial germ cell erasure of DNA methylation. In a second study of mice [99], early life traumatic stress significantly altered miRNA and piRNA expression in sperm, and behavioral and metabolic phenotypes of F_1 and F_2 progeny. A causal link between sperm ncRNA disruption and altered behavioral phenotypes was demonstrated by microinjecting RNAs purified from traumatized males' sperm into fertilized oocytes of wild-type females. In F_3 progeny, small ncRNA profiles returned to normal but behavioral and metabolic trauma symptoms persisted, suggesting that initial disruption in ncRNAs was transferred to other epigenetic mechanisms.

Controversy over the role of epigenetics in evolution often centers on the supposition that epigenetic effects must be transgenerational to be evolutionarily significant [96]. Although transgenerational epigenetic inheritance can occur, only intergenerational epigenetic effects encompassing males and F_1 offspring are required for the environmental induction model of female choice [12].

Epigenetics contributes to phenotypic variation and disease phenotypes

Our hypothesis is based on the premise that environmentally induced epigenetic variation contributes significantly to variation in male condition that, in turn, affects male ability to produce sperm of high epigenetic quality. The relative importance of epigenetically based variation should be inversely related to the amount of variation and the proportion of heritability in fitness-related traits that can be explained by sequence variation. Genome-wide association studies (GWAS) of complex phenotypes, including complex disease phenotypes, therefore set bounds on the potential contribution of intergenerational epigenetic effects. While the proliferation of GWAS has revealed numerous sequence variants contributing to variation in morphological traits, metabolic diseases and behavioral and neurological disorders [100], identified genetic variants often account for a small fraction of heritability [101]. For example, human height is 80–90% heritable [100], yet a GWAS meta-analysis, involving ~250,000 individuals, could account for only 20% of the heritability [102]. Missing heritability is equally problematic for metabolic phenotypes, such as type 2 diabetes and resting glucose levels, for which association with single nucleotide polymorphisms explains only ~10% and ~5% of heritability, respectively [101]. For major depressive disorder, a condition that affects ~13% of the US population and exhibits a heritability of 0.31–0.42 [103], the proportion of variation explained by GWA is <1% [104]. Although improvements in sequence coverage, sampling design and statistical analysis should improve the predictive power of GWAS, solving the missing heritability problem requires a more inclusive theory of inheritance that incorporates epigenetic sources of variation [95]. The impact of epigenetics on disease phenotypes is particularly evident from studies of monozygotic twins who frequently exhibit high discordance for diseases, such as autism, schizophrenia, osteoarthritis, biliary cirrhosis, congenital heart disease, and Beckwith–Wiedemann syndrome [105–107].

Epigenetic states influence male traits that serve as indicators of condition and sperm epigenetic quality

Signals of health and vigor are universal features of sexual attractiveness [1], and are closely tied to male condition or ability to acquire and convert nutritional resources into secondary sexual characteristics in the face of environmental challenges [108]. Environmentally induced epigenetic effects likely impact male condition via nutrition and metabolic processes, because essentially all epigenetic modifications depend on substrates from intermediary metabolism, such as nicotinamide adenine dinucleotide, acetyl CoA, and S-adenosyl methionine [109]. Diet and exercise studies in humans and rodents provide striking evidence that environmentally induced epigenetic states influence male traits that serve as indicators of male condition and sperm epigenetic quality. In western societies, human females exhibit a preference for masculine facial features [110], and waist-to-chest ratios and body mass indices indicative of an athletic body type [111]. In obese human males, weight loss from diet and exercise interventions improves glucose control and insulin function, alters DNA methylation and miRNAs in leucocytes, and enhances sexual function, hormone profiles and ejaculate quality, as measured by sperm count and semen volume [112–114]. These studies have been replicated in mice and extended to demonstrate that short-term diet and exercise regimens in obese founder males normalized miRNA abundance in testes and sperm, dramatically enhanced male mating success, increased embryonic survival and pregnancy establishment, and reestablished normal adiposity and insulin sensitivity in offspring [115–117]. Assuming these findings hold for other species, they corroborate the full set of assumptions required for an epigenetic resolution of the lek paradox.

Gaps in evidence: Is expression of sexually attractive traits epigenetically controlled?

Although epigenetic contributions to variation in male sexually attractive traits have yet to be systematically investigated, human and model organism studies indicate

that characters tied to fitness, including chronic disease risk and a range of complex morphological, metabolic and behavioral traits, are influenced by environmentally induced epigenetic effects. In addition, experimental manipulations of laboratory and natural populations increasingly reveal large non-genetic and possibly epigenetic contributions to variation in sexually attractive traits. Male traits significantly affected by diet quantity and/or quality include body size and cuticular hydrocarbons in flies [118, 119] and pheromone attractants in beetles [120]. In a study of pre- and post-copulatory sexual traits in guppies, diet manipulation exerted significant effects on courtship display intensity, body size, color ornamentation and sperm traits, including number, motility, viability and length [121].

The insulin/insulin-like signaling (ILS) pathway represents a promising avenue for investigating the extent to which variation in sexual traits is epigenetically determined. ILS is an evolutionarily conserved, intracellular pathway that regulates cell proliferation in response to nutritional status, and functions in metabolism, growth, reproduction and aging [122]. ILS is implicated in controlling expression of male sexually dimorphic traits, ranging from antlers in deer to elaborate horns in rhinoceros beetles [122]. Emlen et al. [123] argue that such exaggerated male traits exhibit extreme variability because of their acute sensitivity to ILS. According to this hypothesis, well-nourished, unstressed males exhibit high levels of ILS components compared to poorly nourished, diseased, and/or stressed individuals. Heightened sensitivity to ILS signaling magnifies between-male differences in condition, enabling females and rival males to reliably discern variation in male quality.

Although additional research is needed, particularly on invertebrates, recent studies suggest a critical role for epigenetic regulation of ILS. In mammals, expression of the IGF-1 gene, a main component of vertebrate ILS, is essential for late gestational and postnatal growth, with IGF-1 knockout mice exhibiting ~70% reduction in adult body mass [124]. Intrauterine growth restriction (IUGF), a common complication of human pregnancy, increases risk for early onset metabolic syndrome, and is associated with persistent reduction in circulating IGF-1 protein. IUGF studies in mouse models have revealed complex epigenetic regulation of IGF-1 expression, involving promoter DNA methylation, histone modifications, nucleosome depletion and posttranscriptional modulation via miRNAs. IUGF increases IGF-1 promoter methylation and modifies chromatin configuration, resulting in intergenerational epigenetic effects on IGF-1 expression and metabolism [125]. Epigenetic disruption is more persistent in male offspring [126] but can be ameliorated in both sexes by nutrient supplementation [125]. Interestingly, in mammals, somatic ILS signaling, involving receptors for insulin and IGF-1, is required for male gonad development and sexual differentiation [127]. ILS effects on male reproduction are not limited to mammals, as demonstrated by a recent study of flour beetles, in which ILS and nutrition were shown to regulate male accessory gland growth and maturation [128]. Taken together, these findings suggest that ILS is an epigenetically regulated pathway that modulates not only condition-dependent expression of sexually selected traits but also ejaculate and sperm quality.

Evidence for epigenetically good genes hypothesis: Caveats and limitations

Even in species in which males and females associate only to mate, paternal effects may extend beyond direct contribution of the sperm's haploid genome and epigenome [12, 23, 92]. Paternal transfer of microbiota and seminal fluid can potentially influence maternal behavior and physiology, and females may adjust resource allocation to offspring based on assessment of male quality [92]. Distinguishing these cryptic paternal effects from sperm epigenetic effects may be challenging, but can be achieved through manipulative experiments, e.g. involving microinjection of ncRNAs. Moreover, microbial and seminal fluid effects may be mediated epigenetically [23, 28]. In hamsters, excision of male accessory glands altered offspring postnatal growth, elevated anxiety [129] and was associated with disrupted acetylation in male pronuclei and retarded de-and re-methylation kinetics in cleavage-stage embryos [130].

There are three potential criticisms of the evidence presented here. First, it could be argued that cited studies are not relevant to sexual selection in nature due to limited genetic variation in laboratory populations. Experimental manipulations might also involve unnatural challenges that generate aberrant epigenetic effects that could be further confounded by genetic mutations. While one or more of these criticisms might apply in specific cases, they cannot be used to reject the body of evidence. The criticism that epigenetic explanations for phenotypic, intergenerational, and transgenerational effects ignore genetic effects does not apply to several cited experimental studies, such as microinjection experiments, involving ncRNAs that result in intergenerational or transgenerational effects (e.g. [131]). Similarly, this argument does not apply to environmental effects, such as improved diet and exercise, that result in elevated male mating success, production of gametes with epigenetically altered states, and the transmission of these acquired epigenetic states to offspring [115–117]. In addition, the criticism is not applicable to non-experimental lines of evidence, including: (i) failure of GWAS to account for the high heritability of complex phenotypic traits; and (ii) numerous studies demonstrating strong condition dependence of sexually selected traits. The criticism that epigenetic effects are only important when animals are subjected to artificially extreme perturbations is not consistent with: (i) laboratory and field studies showing strong effects of moderate manipulation of diet and other environmental factors on male phenotypic traits and gamete characteristics; and (ii) human studies revealing aberrant epigenetic profiles in the sperm of males exhibiting poor fertility. More generally, the epigenetically good genes hypothesis does not preclude genetic contributions to variation in male condition and gamete quality, and both genetic and induced epigenetic effects likely contribute to the maintenance of costly female preferences.

It should be noted that epigenetic responses to stressful environments can be adaptive, as in predator-induced phenotypic plasticity, and can accelerate adaptive evolution [25]. However, in the context of our hypothesis, low-quality, stressful environments will generally prevent males from attaining the

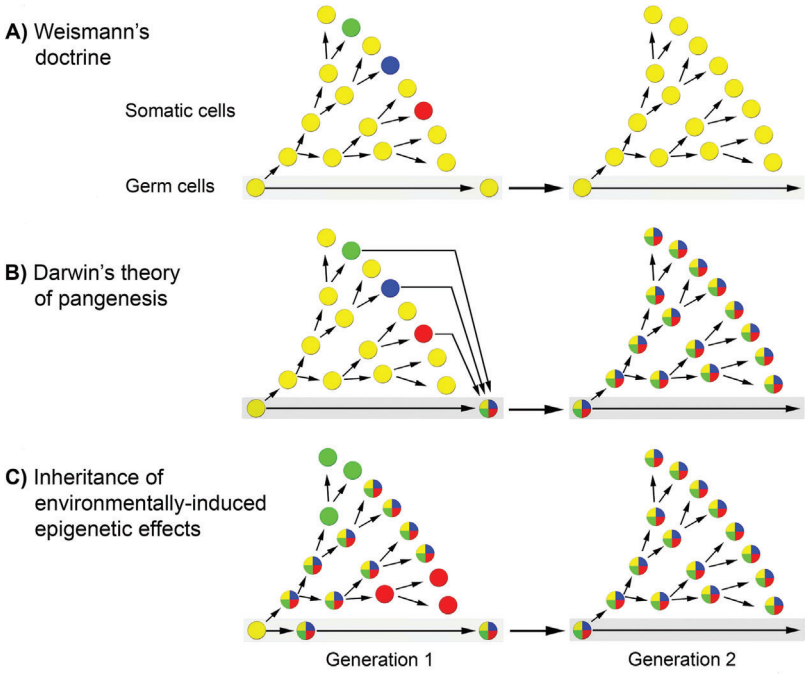

Figure 3. Inheritance according to Weismann's doctrine, Darwin's theory of pangenesis and the inheritance of environmentally induced epigenetic effects. **A**: According to Weismann's doctrine of the continuity of the germline [134], there is a molecular distinction between the soma and the germline: the germline is resistant to environmental influences, and mutations in somatic cells cannot be transmitted to offspring. **B**: In pangenesis, somatic tissues undergo environmentally induced changes that are then transmitted to the germline via gemmules. **C**: In the case of epigenetics, the germline and soma are distinct, but epigenetic states in gonadal tissues are sensitive to the environment, and epigenetic alterations can be transmitted to offspring. For simplicity, no mutations or epigenetic changes are shown in generation 2. The epigenetic model is more complicated than shown here because male physiological condition can influence the epigenetic states of sperm throughout the reproductive lifespan of the male.

preferred male phenotype, thus compromising his ability to produce sperm of high epigenetic quality.

Epigenetics can resolve the lek paradox just as particulate inheritance resolved the paradox of variation maintained under selection

Ever since Darwin, breakthroughs in understanding heredity have played a pivotal role in advancing evolutionary theory. In the context of the then prevailing view of blending inheritance, evolution by natural selection required an untenably high mutation rate to maintain the trait variation required for selection [132]. To address this criticism, Darwin [133] proposed pangenesis, a complex theory for the inheritance of acquired characteristics, in which environmentally induced changes in somatic cells could be transmitted to offspring via hypothetical particles called gemmules (Fig. 3). By identifying hereditary factors that could maintain their integrity across generations, Mendel's theory of particulate inheritance rescued the principle of natural selection from the homogenizing effect of blending inheritance. Similarly, we suggest that paternal transmission of acquired epigenetic states via the sperm epigenome rescues condition-dependent indicator (good genes) models as an explanation for the perpetuation of costly female choice.

Conclusions and outlook

Evidence is accumulating that males bestow a paternal environmental legacy on offspring by transmitting acquired epigenetic states through sperm [26, 27]. Paternal transmission of environmentally induced epigenetic effects provides an inexhaustible source of variation in male quality that resolves the lek paradox, and challenges the view of female preference as a dichotomy between choice for direct, material benefits and choice for indirect and therefore genetic benefits. The rapidity with which epigenetic states respond to diet, exercise and stress also renders male condition and its heritability more labile than traditionally assumed. Chance events and environmental heterogeneity may substantially alter both male and sperm epigenomes.

These findings have two important implications for sexual selection research. First, because male quality is a complex random variable that fluctuates through time, testing the epigenetically good genes hypothesis requires that male phenotype, sexual attractiveness, reproductive success, and epigenetic states in soma and sperm be measured as concurrently as possible. Second, in assessing epigenetic states of sperm, females are likely to utilize multifaceted assessments of attractiveness that reflect both the male's developmental trajectory and his current physiological status. Systematic studies of natural populations should therefore be carried out to test the predictions that: (i) the correlation between male attractiveness and sperm epigenetic quality should increase with the number of informative variables measured; (ii) in addition to standard measures of ejaculate quality, sperm epigenetic profiles should differ systematically between attractive and unattractive males; and (iii) manipulations of environmental factors affecting health and vigor should simultaneously influence male epigenetic quality, sperm epigenetic quality, male attractiveness and offspring fitness. From a theoretical perspective, more complex multilocus models are needed that incorporate both genetically based and epigenetically based components of male condition, as well as epigenetic effects that attenuate across generations.

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