THE EPIGENOME IN EVOLUTION: BEYOND THE MODERN SYNTHESIS

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Evolutionary biology is at a turning point. During the last ten years, biologists' views about heredity have been changing, and these changes are inevitably beginning to modify established views about evolution. In the West, the established view is known as the «Modern Synthesis», which is a framework for evolutionary thinking that was forged during the 1940s and 1950s, mainly in the USA and England (Mayr, Provine, 1980). This framework has dominated evolutionary thinking for nearly sixty years.

Ernst Mayr, one of the architects and ardent promoters of the Modern Synthesis, described its development in his book, the *Growth of Biological Thought* (Mayr, 1982). He showed how, in order for it to emerge, naturalists and geneticists in the West had to find common ground:

«It was in these years (1936–1947) that biologists of the most diverse subdivisions of evolutionary biology and from various countries accepted two major conclusions: (1) that evolution is gradual, being explicatory in terms of small genetic changes and recombination and in terms of the ordering of this genetic variation by natural selection; and (2) that by introducing the population concept, by considering species as reproductively isolated aggregates of populations, and by analyzing the effect of ecological factors (niche occupation, competition, adaptive radiation) on diversity and on the origin of higher taxa, one can explain all evolutionary phenomena in a manner that is consistent both with the known genetic mechanisms [Mendelian genetics and the chromosomal theory] and with the observational evidence of the naturalists. Julian Huxley (1942) designated the achievement of consensus on these points as *the evolutionary synthesis*. It required that the naturalists abandon their belief in soft inheritance and that the experimentalists give up typological thinking and be willing to incorporate the origin of diversity in their research programs. It led to a decline in the concept of "mutation pressure," and its replacement by a heightened confidence in the powers of natural selection, combined with a new realization of the immensity of genetic variation in natural populations» (Mayr, 1982, P. 567).

This view was accepted, with minor modifications, until quite recently. To a large extent, the Synthesis defined itself negatively – by what it excluded. The major, positive and negative, assumptions relating to heredity and variation in the molecular version of the Modern Synthesis can be summarized as follows (Jablonka, Lamb, 2005):

- Heredity is through the transmission of germline genes, which are discrete units located on chromosomes. Genes are DNA sequences and hereditary variation is equated with variation in DNA. There are no inherited non-DNA variations that cannot be reduced to genetic inheritance.
- Hereditary variation is the consequence of the many random allelic combinations generated by sexual processes, and each allele usually has only a small phenotypic effect. New variations in genes – mutations – are the result of accidental changes; hereditary variation is not affected by the developmental history of the individual. There is no «soft inheritance», in which heritable variations are the result of environmental effects, use and disuse, or other factors.
- Selection occurs among individuals that are, at all times, well-defined entities. Gradually, through

the selection of individuals with phenotypes that make them more adapted to their environment than others are, some alleles become more numerous in the population. Mutation pressure (including genome-wide changes) is of secondary, marginal importance.

- Evolution occurs through modifications from a common ancestor, and is based on vertical descent. Horizontal gene transfer (HGT) is of minor significance – it does not alter the basic tree structure of biological evolution.
- Macroevolution is continuous with microevolution, and does not require any extra selective processes or molecular mechanisms beyond those operating during microevolution.

This accepted view is now beginning to be challenged in the West. Biologists are arguing that:

1. Not all heritable variation stems from DNA differences

2. Not all heritable variation is random in origin

3. Not all evolutionary change is gradual

4. Not all patterns of evolutionary divergence are tree-like.

In the former USSR, the first three of these challenges were accommodated by the broader view of heredity that existed there. Although Trofim Lysenko abused hereditary research by delegitimizing Mendelian genetics, there were Russian scientists, like Dmitry Belyaev and his colleagues in Novosibirsk, who acknowledged and studied Mendelian heredity, but also explored hereditary phenomena that were ignored in the West. They observed and carried out research into rapid evolutionary changes that included patterns of inheritance that did not conform to what was considered to be the «normal» behaviour for nuclear or cytoplasmic genes. Their studies led them to suggest that evolutionary change can be saltational, and that genes can be transmitted in «dormant» and «non-dormant» states; they reasoned that transitions between the two states depends on the physiological status of the organism, which is affected by environmental conditions such as ecological and hormonal stresses. Their work on the domestication of silver foxes, and later the domestication of other mammalian species, was, and remains, one of the most important long-term evolutionary experiments, and it suggested a role for stress-induced inherited variations in evolution (Belyaev *et al*., 1981a, b; Belyaev, Borodin, 1982; Ruvinsky *et al*., 1983a, b, 1986; Trut *et al*., 2004; Popova, 2006). Today their studies can be interpreted within the developing framework of epigenetic inheritance, particularly the aspect that sees a role for epigenetic control in macroevolution under conditions of stress.

Epigenetics, epigenetic inheritance, and epigenetic inheritance systems

Epigenetics is concerned with those aspects of development that lead to flexibility and adjustment when the environment or the genome changes. The complementary nature of developmental stability and developmental plasticity, and their ecological and evolutionary significance, were recognized long ago, particularly by C. Waddington (1957) in Great Britain and I. Schmalhausen (1949) in the USSR. Epigenetics, a term coined by Waddington, explores the interactions between genes, their products, and the environment, and highlights the processes that decouple genetic and phenotypic variation. Epigenetic studies explore the regulatory mechanisms that can lead to long-term, persistent, developmental effects: to the establishment of variant cellular states that are transmitted across cell divisions, or that are dynamically maintained for a long time in non-dividing cells. These mechanisms are referred to as epigenetic control mechanisms or epigenetic control systems. Usually changes in DNA sequence are not involved, but in some cases, for example in the mammalian immune system and in ciliate development, epigenetic control mechanisms do generate regulated alterations in DNA.

Epigenetic inheritance is a component of epigenetics, and an important aspect of development. Epigenetic inheritance is seen when environmentally-induced and developmentally-regulated variations, or variations that are the result of developmental noise, are transmitted to subsequent generations of cells or organisms (Jablonka, Lamb, 2005). Today the term epigenetic inheritance is used in two overlapping ways (Jablonka, Lamb, 2007a):

(i) *Epigenetic inheritance in the broad sense* is the inheritance of developmental variations that do not stem from differences in DNA sequence or from persistent inducing signals in the environment. It includes cell heredity in unicellular and multicellular

organisms, and soma-to-soma information-transfer that is based on interactions between groups of cells, between systems, and between individuals. Soma-to-soma transmission *by-passes* the germ-line; it takes place through developmental interactions between mother and embryo (e.g. Weaver *et al*., 2004), through social learning (Avital, Jablonka, 2000), and through symbolic communication (Jablonka, Lamb, 2005).

(ii) *Cellular epigenetic inheritance* is the transmission from mother cell to daughter cell of variations that are not the result of DNA differences or persistent inducing signals in the cell's environment. It occurs during cell division in prokaryotes, during mitotic cell division in the soma of eukaryotes, and sometimes also during the meiotic divisions in the germ-line that give rise to sperm or eggs. In the latter case, offspring may inherit epigenetic variations. In both the soma and germ-line, transmission can be through chromatin marks (the non-DNA parts of chromosomes, which includes binding proteins and DNA modifications that do not affect the sequence or code), through RNAs, through self-reconstructing three-dimensional structures, and through self-sustaining metabolic loops (Jablonka *et al*., 1992; Jablonka, Lamb, 1995, 2005, 2007a). Following Holliday (reviewed in Holliday, 2002, 2006), many biologists tend to restrict cellular epigenetic inheritance to the inheritance of chromatin marks and RNA-mediated cellular inheritance (e.g., see: Wu, Morris, 2001). However, yeast geneticists use the term epigenetic inheritance for the inheritance of protein conformations, such as prions (e.g. Uptain, Lindquist, 2002), and the term is also used by those studying self-sustaining loops (e.g. Laurent *et al*., 2005) and chromatin inheritance (e.g.Grandjean *et al*., 1998) in bacteria. Chromatin and RNA-mediated cellular inheritance (through DNA methylation, histone and other DNA binding protein, and the RNA-mediated system) are at present the major focus of the study of epigenetics, and they seem to play an important role in cellular inheritance through the germ-line of both females and males.

The mechanisms that lead to cellular epigenetic inheritance also underlie *cell memory* – the persistence of functional and structural cellular states in long-lived, non-dividing cells. For example, epigenetic mechanisms, including DNA methylation and histone modification, are involved in stable gene-expression patterns in neurons (reviewed by Levenson, Sweatt, 2005). In rats, early maternal behaviour has long-term behavioural effects on the young , and these are associated with chromatin marks in a key gene in brain cells (Weaver *et al*., 2004); changes in DNA methylation are also associated with fear conditioning (Miller, Sweatt, 2007). Learning impairment in chicks caused by stress imposed on their parents also seems to involve epigenetic modifications (Lindqvist *et al*., 2007).

Jablonka and Lamb used the collective term epigenetic inheritance systems (EISs) for the mechanisms that underlie cellular epigenetic inheritance. They characterized four broad types of EIS that are based on epigenetic control (Jablonka, Lamb, 1995, 2005, 2007a; Jablonka *et el*., 1992):

(i) *Self-sustaining metabolic loops.* The cellular patterns of activity of genes and their products can be maintained by the regulatory organization of the metabolic circuit. For example, through positive feedback, an inducible gene product can act as a transcriptional activator for its own transcription. The transmission of the components of the circuit (proteins, RNAs, and metabolites) can lead to the same patterns of gene activity being reconstructed in daughter cells after cell division. Such positive feedback may lead to alternative and heritable cell phenotypes, and is commonly found in fungi (Malagnac, Silar, 2003) as well as in bacteria and probably other microorganisms (Smits *et al*., 2006). It also plays an important role in the development of multicellular organisms (Ferrell, 2002).

(ii) *Structural inheritance.* Mechanisms based on spatial templating, in which pre-existing cellular structures act as templates for the production of similar structures, which then become components of daughter cells. This type of templating covers a wide spectrum of mechanisms, including prion-based inheritance in fungi (Shorter, Lindquist, 2005), the inheritance of cortical structures in ciliates (Grimes, Aufderheide, 1991), and the reconstruction of what Cavalier-Smith (2004) calls «genetic membranes».

(iii) *Chromatin marking.* Chromatin marks are the variant, modifiable, histone and non-histone proteins that are non-covalently bound to DNA, as well as small chemical groups (such as methyls) that are covalently bound directly to

DNA. Chromatin marks influence gene activity and may segregate (semi-conservatively or conservatively) with the DNA strands during replication, nucleating the reconstruction of similar marks in daughter cells (Henikoff *et al*., 2004). The ways in which chromatin marks are transmitted between generations of cells, especially through gametes, are only partially understood.

(iv) *RNA-mediated variation in gene expression.* Silent transcriptional states are actively maintained through repressive interactions between small RNA molecules and the mRNAs or DNA to which they are complementary (Meister, Tuschl, 2004; Bernstein, Allis, 2005; Matzke, Birchler, 2005). These repressive interactions can be transmitted between cell and organism generations through an RNA-replication system, and/or *via* the interaction of small RNAs with chromatin, which leads to heritable modifications of chromatin marks. RNA-DNA and RNA-RNA pairing interactions may lead not only to silencing, but also to targeted gene deletions and amplifications (Mochizuki, Gorovsky, 2004). Small RNAs also seem to be involved in processes of paramutation (Rassoulzadegan *et al*., 2006).

Transgenerational epigenetic inheritance seems to be ubiquitous. Jablonka and Raz (forthcoming) have collated data on inherited epigenetic variations in bacteria, protists, fungi, plants and animals. Their list includes nearly a hundred cases, and this number is increasing almost daily. Here we give only few examples to illustrate the scope and range of epigenetic inheritance.

In microorganisms and fungi, switches between alternative heritable forms that are underlain by self-sustaining loops are common. For example, in Candida albicans, an epigenetic switch underlies the transition between white and opaque cells, two states that are heritable for many generations. Wor1 is the key regulator protein that is necessary for the initiation and maintenance of the opaque state, and it positively regulates its own transcription, forming a stable self-sustaining feedback loop (Zordan *et al*., 2006). In fungi there are also several examples of the inheritance of alternative protein conformations (Malagnac, Silar, 2006). For example, in Saccharomyces cerevisiae there are several well-characterized cases of prion inheritance in which variations are reproduced through self-templating mechanisms (Benkemoun, Saupe, 2006).

There are many cases of epigenetic inheritance in plants. A famous case is the inheritance of a variant of *Linaria vulgaris*. This variant, which was described over 250 years ago by Carl Linnaeus, has a floral structure that is very different from that of the normal toadflax. Linnaeus named the new variant «Peloria», the Greek word for «monster». Enrico Coen and his colleagues looked at *Lcyc*, a gene that is known to control dorso-ventral asymmetry, and lead to the peloric variant in other plant species (Cubas *et al*., 1999). They found that in *Linaria* the DNA sequences of the normal and peloric forms were the same, but the pattern of methylation differed: in the peloric variant the gene was heavily methylated and transcriptionally silent. Peloric strains are not totally stable, and occasionally branches with partially or even fully wild-type flowers develop on a peloric plant, but the epigenetic marks are transmitted to progeny for at least 2 generations (Parker, personal communication).

In plants, many of the cases of epimutation have appeared under conditions of genomic or chemical stress (Jablonka and Raz, forthcoming), and it seems that changes in ploidy are always accompanied by heritable epigenetic changes. As we argue later, the epigenetic mechanisms that bring these changes about are also involved in the widespread (epigenetic and genetic) re-patterning of the genome.

Animals, too, provide many good examples of epigenetic inheritance. Feeding the nematode worm *Caenorhabditis elegans* with bacteria that express double-stranded RNA that targets specific *C. elegans* genes resulted in many different morphological and physiological variations, and they were transmitted for at least 10 generations (Vastenhouw et al., 2006; N. Vastenhouw, personal communication). Epimutations have also been studied in an isogenic strain of *Drosophila melanogaster* that carried a mutant allele of the *Krüppel* gene, which affects eye morphology (Sollars *et al.*, 2003). Adding geldanamycin, a drug that inhibits the activity of the heat shock protein Hsp90, to the food of larvae enhanced the development of the abnormal eye phenotype. Addition of the drug to the food for only one generation, followed by six generations of selective breeding for the eye anomaly, increased the proportion of flies showing it from just over 1 % to more than 60 %. Since the strains used were isogenic, the selectable variation probably stemmed

from new heritable epiallelic differences, not from differences in gene sequences.

Most examples of epigenetic inheritance in mammals come from studies of mice and rats. In the mouse, 'Fused' is a dominant trait, with carriers of the gene manifesting a kinked tail phenotype. The expression of the gene is very variable, with some individuals showing an extremely kinked tail, others only a slight kink, and some having a completely normal tail. More than 20 years ago, D. Belyaev and his group concluded that the patterns of inheritance observed with «Fused» are the manifestations of epigenetic, rather than purely genetic, phenomena (Belyaev *et al*., 1981a, 1983). Rakyan *et al*. (2003) subsequently confirmed that the degree of expression of Fused is epigenetically inherited through both male and female parents. They found that the phenotypic expression of the Fused gene (now known as $Axin^{Fu}$) is correlated with the degree of methylation of a transposon-derived sequence in one of the introns of the *Axin* gene. Heavy methylation leads to the development of a normal tail, whereas a demethylated transposon element leads to abnormal RNA transcripts and a kinked tail. How exactly methylation patterns are reconstructed is unknown, but the phenotype seems to be inherited via the chromatin marking system.

Another mammalian example of epigenetic inheritance is that of the variations induced by vinclozolin, an anti-androgenic endocrine disruptor, in rats. Anway and his colleagues (2005, 2006a, b) injected pregnant females with vinclozolin during a sensitive period 8–15 days post coitum, and showed that the abnormalities induced in male offspring were inherited through the male line for at least four generations. They found 15 different DNA sequences that had altered methylation patterns in the F1 males, and these were transmitted from the F1 to the F3 generation.

These examples are just a small sample of the reported cases of epigenetic inheritance. The data reviewed by Jablonka and Raz suggest that epigenetic inheritance has been found in every taxon in which it has been sought, and that it can affect every type of locus in the genome (although some regions are more prone to heritable epigenetic modifications than others). The conditions for the induction of cellular epigenetic variants and the stability of their inheritance depend on the type of epigenetic system and the type of organism. However, despite this context sensitivity, and although some epigenetic variations may be the consequence of developmental noise, a feature that emerges from many studies is that extreme environmental conditions (stresses) often induce heritable epigenetic variations.

Epigenetic inheritance in conditions of stress: guiding genetic selection, generating local mutational biases, and causing systemic mutations

I. Schmalhausen (1949) and C. Waddington (1957, 1968, 1975) suggested that development has a guiding role in evolution. Developmental adjustments to the changes experienced by organisms, especially under conditions of stress, reveal previously hidden genetic differences between individuals in their ability to adjust, and this variation can be selected. The genetic variants that contribute most to the adaptive responses therefore increase in frequency. In this way, selection can lead to a change from a stimulus-dependent to stimulus independent (or less dependent) phenotype, a process that was called «stabilizing selection» by Schmalhausen (1949) and «genetic assimilation» by Waddington (Waddington, 1957; Pigliucci *et al*., 2006).

West-Eberhard (2003) has recently developed and extended the idea that developmental plasticity plays a key role in evolution. In the general framework for evolutionary thinking that she constructed, environmentally-induced changes in development are followed by genetic changes, which are selected because they simulate or stabilize the induced developmental changes, or ameliorate their adverse effects. She called this developmental guiding process, which includes but is not limited to genetic assimilation, «genetic accommodation». Jablonka and Lamb (1995, 2005) argued that processes of genetic assimilation and accommodation would be enhanced if the induced developmental effects can be inherited between generations, and this possibility has been modelled by Pál (1998). During conditions of stress, epigenetic inheritance is likely to be particularly important because of this accelerating effect (Badyaev, 2005; Siegal, Bergman, 2006).

Epigenetic inheritance and the mechanisms underlying it may have a role not only in guiding

the selection of genetic variations, they may also have direct effects on the generation of genetic variants. Heritable variations in chromatin can bias changes in DNA sequence: they can affect genetic variation by influencing rates of mutation, transposition, and recombination (Belyaev, Borodin, 1982; reviewed in Jablonka and Lamb 1995, chapter 7). For example, whereas highly methylated transposable elements in plants rarely move, when the same elements are demethylated they are usually mobile. When transposable elements move to new locations, they introduce changes in coding or regulatory sequences, and they are regarded as a major source of mutations, so their epigenetic state (e.g. the extent to which they are methylated) affects the rate at which mutations are generated. Since the movement of some transposable elements is known to be markedly influenced by various types of internal (cellular/genomic) and external (environmental) stress, new genetic variants may be more common in circumstances in which the survival of existing forms is threatened (Kidwell, Lisch, 1997).

There is a close relationship between genetic and epigenetic variation in repeated sequences, and this is evolutionarily significant. Sequence studies have shown that, during plant and animal phylogeny, developmental genes have been duplicated and re-used (Gu et al., 2004). S. Rodin and his colleagues (2005) have suggested that the position-effects resulting from altered patterns of epigenetic marks following gene duplication and repositioning can play an important role in the re-use of the duplicated genes.

Duplications, movements of transposable elements, increases in the rates of recombination and of mutation, all occur under conditions of stress. «Stress» is an intuitively clear term but it is theoretically tricky, and Hans Selye, who pioneered the study of physiological stress, focused on the characteristic physiological systemic *response* to it. He defined stress as a state «manifested by a specific syndrome which consists of all the non-specifically induced changes within a biological system» (Hans Selye, 1956, quoted in Hoffmann and Parsons 1991). Using a similar approach, but with respect to genomic changes affecting evolutionary trajectories, R. Goldschmidt (1940) suggested that stress often initiates systemic changes in the genome, which lead to macro-evolutionary changes. This idea was not in line with the then crystallizing evolutionary synthesis, and it used to be derided, but recent data from many biological fronts is changing attitudes (see Shapiro, 1999, and Bateman and DiMichele 2002 for a re-evaluation of Goldschmidt's position). In plants, ecological stresses such as nutritional changes during a sensitive period of growth can induce significant variations in repeated sequences, probably through DNA methylation and RNAi. Ongoing hormonal stress in animals can also lead to systemic changes. During their work with silver foxes, D. Belyaev and his colleagues (1974) observed that in the lines selected for tameness and aggression, the changes in physiology and behaviour that resulted from the stress imposed by domestication were accompanied by an increase in the frequency of micro-chromosomes.

Genomic stresses may have even more dramatic and more predictable consequences. Stresses such as those imposed by auto- and allo-polyploidization seem to induce genome-wide changes in both epigenetic and genetic organization. Recent studies have shown that in many naturally occurring and experimentally induced polyploids and hybrids, DNA methylation patterns are dramatically altered, and genes in some of the duplicated chromosomes are heritably silenced (e.g. Levy, Feldman, 2004). It seems that following auto- and allo-polyploidization, there is a burst of selectable variation that opens up opportunities for adaptation, very much in line with the suggestions made by McClintock (McClintock, 1984; Jorgensen, 2004; Fontdevila, 2005; Rapp, Wendel, 2005). Work with other organisms suggests that during conditions of genomic and ecological stress, developmentally-induced variations in DNA are often (if not invariably) mediated by chromatin marking or RNA-mediated EISs. Representative examples of different types of stress on epigenetic variations or on epigenetically guided genetic re-patterning mechanisms are shown in the Table.

What are the mechanisms underlying genomic stress responses? We are only just beginning to understand how epigenetic control systems are involved in the generation of systemic mutations, but it is plausible that processes such as those seen in ciliates, where epigenetic control systems cause targeted deletions and amplifications of genes in the developing macronucleus (Mochizuki, Gorovsky,

Table

Epigenetically-mediated genomic alterations under stress

Type of stress	Taxon	Extent and severity of stress	Nature of heritable epigenetic variations	Extent of epigenetic and genetic change	Stability	References
1	2	3	4	5	6	7
Genomic: hybrid formation and allopolyploidization; autopolyploidization	Many plant taxa	Evolutionarily recurrent; potentially catastrophic	Chromatin (DNA methylation, histone modification) and RNA-mediated	Genome-wide change; depends on taxon. In case of hybrids, depends also on divergence between hybridizing partners and direction of cross	Varies: some very stable, some metastable	Biol J. Linn. Soc. V. 82(4) 2004; Pikaard 2000, 2001; Comai *et al.*, 2000; Mittelsten Scheid *et a*l., 2003; Rapp, Wendel, 2005
Genomic: change in mode of reproduction to agamospermy	Sugar beet; probably other plants	Significant, probably recurring during evolution	Unknown	Many loci	Varies, some stable	Levites, 2000; Maletskii, 1999
Genomic: introduction of foreign genome or RNA through infection or introduction of transgenes	Plants, fungi, mammals	Evolutionarily recurrent; potentially catastrophic	Chromatin and RNA-mediated?	Introduced genes and related endogenous genes silenced	Varies: some very stable, some metastable	Hadchouel *et a*l., 1987; Matzke *et al.*, 1989; Martienssen, Colot, 2001; Kovalchuk *et a*l., 2003
Genomic: meiotic mis-pairing	Fungi, insects, mammals; (all sexually reproducing animals?)	Common, recurrent problem	Chromatin; RNAi	Potentially every locus	Leads to epigenetic changes in offspring	Shiu *et a*l., 2001; Bean *et al.*, 2004;Turner *et al.*, 2005, 2006
Physical: heat	Brassica	Evolutionarily recurrent; usually non-catastrophic	Presumably chromatin	r-DNA; many sequences	Varies: some very stable, some metastable	Waters, Schaal, 1996

to be continued

Continued

1	2	3	4	5	6	7
hydrostatic pressure	Rice	Novel	Chromatin	Not specified	Not known	Long *et al.*, 2006
irradiation	Animals, plants	Recurrent	Chromatin	Many sequences	3 or more generations	Dubrova, 2003; Molinier *et al.*, 2006
Physiological: nutritional	Flax	Evolutionarily recurrent?	Chromatin and RNA-mediated?	r-DNA genes and repetitive sequences	Varies: some very stable	Cullis, 2005
	Aphids	Usually non-catastrophic	Not known	Not specified	Stable	Shaposhnikov, 1965
Physiological: (aging: Lansing effects)	Many: animals and protists	Cumulative, chronic	Chromatin, possibly others EISs	Probably many sequences	Varies: metastable, reversible	Lamb, 1994; Jablonka, Lamb, 1995
Toxins and mutagens: (e.g. 5-azacytidine, nicotinic acid)	Plants	Novel, usually catastrophic	Chromatin and RNA-mediated?	Many sequences	Varies: some very stable, some metastable	For example: Flavell, O'Dell 1990; Janousek *et al*., 1996; Jacobsen, Meyerowitz, 1997; Bogdanova, 2003; Akimoto *et al*., 2007
Behavioural: (mediated by hormones)	Silver foxes	Evolutionarily recurrent; usually non-catastrophic	Probably DNA methylation, histone modification and RNA-mediated?	Heterochromatin? B chromosomes more common in selected lines	Varies: metastable	Belyaev *et al*., 1974; Trut *et al*., 2004
	Rats	Androgen suppressors	Chromatin; DNA methylation	15 sequences identified	At least 4	Anway *et a*l., 2005; 2006a,b; Chang *et al*., 2006; Crews *et al*., 2007
	Mouse	Hydrocortisone	Probably chromatin	Not known	At least one	Belyaev *et al*., 1983
	Plants	Cytokinins	Probably DNA methylation	Locus specific?	Stable	Meins 1989a, b; Meins, Thomas, 2003

2004), may be involved in other organisms under conditions of genomic and ecological stress. It is very intriguing that the deletion or silencing (through heterochromatinization) of chromosomal regions that remain unpaired during meiosis (including the unpaired regions of the X and Y chromosomes in heterogametic males) are also mediated by epigenetic control systems, probably involving small RNAs that are generated from the unpaired regions (Shiu *et al*., 2001; Bean *et al*., 2004; Turner *et al*., 2005, 2006). Mechanisms based on DNA-DNA, DNA-RNA and RNA-RNA pairing interactions, coupled with chromatin or DNA enzymatic modifications, may be the genomic responses that underlie the systemic mutations that occur under conditions of stress. These genomic stress response mechanisms are evolved mechanisms, selected to deal with various hazards, including DNA damage, genomic parasites, infections, and physiological (nutritional, chemical, climatic) extremes.

The effect of various types of stress on evolutionary change is not a negligible aspect of evolution. The conditions enumerated in the table are common. The introduction of foreign genomes, especially viral genomes, through infection (sometimes leading to parasitism and rarely to symbiotic relations) is frequent, and may explain the widespread occurrence of epigenetic silencing. Similarly, extreme or chronic environmental changes that have deleterious but non-lethal effects are unexceptional occurrences for natural populations. Genomic stresses due to hybridization and polyploidization are frequent in plant phylogeny. Most flowering plants evolved through hybridization (the estimated figure is 70–90 %), and in some clades this is a recurrent process. Polyploidy is not restricted to flowering plants: whole genome duplication (polyploidy and allopolyploidy) characterizes the entire fern family Aspleniaceae, and in bryophytes, Natcheva and Cronberg (2004) suggested that polyploidization is the rule rather than the exception.

Hybridization and polyploidy are also important in animal evolution. Arnold (2007) has suggested that hybridization underlies the origin of many parthenogenetic fish taxa, and allopolyploidy occurs in some vertebrate groups such as rodents and frogs. It is also possible that during homoploid hybridization, when there is no difference in the chromosome number of the parental species and no subsequent genome duplication, epigenetic control mechanisms are activated. These may lead to changes in epigenetic states, and possibly some re-organization of the genome, the extent of which will depend on the degree of divergence of the homoploid parental species.

Conclusions

Going back to the four challenges to the Modern Synthesis with which we began this paper, it should be clear from the evidence we have outlined, first, that many heritable developmental variations are epigenetic rather than genetic. Second, that soft inheritance is common, since many new variants arise in response to environmental signals and are developmentally regulated. Such soft inheritance can affect the direction of evolution, revealing cryptic genetic variation and enhancing the generation of local genetic variations. Third, epigenetic control mechanisms affect genomic re-patterning under conditions of stress, which can lead to macro-evolutionary changes.

We have not explored here the fourth challenge to the Modern Synthesis – the challenge to the tree metaphor of phylogeny – which is beyond the scope of this paper, but we would like to outline the nature of this challenge. The tree metaphor is based on the assumption that the pattern of evolution is branching, with each branch-point starting from a single common ancestor; phylogenies do not have a web-like pattern, with branches having several common ancestors. However, if cellular stresses arising from genetic exchanges through hybridization, horizontal gene transfer, or other forms of genetic exchange are common in evolution, this assumption has to be re-evaluated. In early evolution, horizontal gene transfer may have been the rule rather than the exception, and it may still be of major importance today, especially for the evolution of microorganisms (Goldenfeld, Woese, 2007). The actual pattern of evolution is probably partly tree and partly web, with tree or web patterns dominating at different times and for different taxa.

We are living through a period of revolutionary change in the biological sciences, and we believe that that a post-Synthesis era is beginning in evolutionary biology. During the sixty years of its

reign, the Modern Synthesis has been stretched – for example, it was forced to incorporate neutral mutations and punctuational changes, which significantly extended its boundaries. Today evolutionary biology also has to incorporate soft (mostly epigenetic) inheritance, saltational changes due to systemic mutations, and various types of genetic exchange (Jablonka, Lamb, 2007b). These do more than extend the Modern Synthesis – with so much change, we now need a new evolutionary theory, one that acknowledges Darwinian, Lamarckian, and saltational processes. The era of the type of evolutionary biology that Belyaev was exploring has, at last, arrived.

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