J. evol. biol. 11 (1998) 159–183
1010–061X/98/020159–25 $ 1.50 + 0.20/0

Journal of Evolutionary Biology

Target review

Epigenetic inheritance in evolution

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Key words: Epialleles; multicellularity; chromosome organization; post-mating isolation; acquired characters.

Abstract

We discuss the role of cell memory in heredity and evolution. We describe the properties of the epigenetic inheritance systems (EISs) that underlie cell memory and enable environmentally and developmentally induced cell phenotypes to be transmitted in cell lineages, and argue that transgenerational epigenetic inheritance is an important and neglected part of heredity. By looking at the part EISs have played in the evolution of multicellularity, ontogeny, chromosome organization, and the origin of some post-mating isolating mechanisms, we show how considering the role of epigenetic inheritance can sometimes shed light on major evolutionary processes.

Most biologists, including ourselves, accept that the theory of evolution developed by Darwin is basically correct: adaptive changes occur through the selection of heritable differences between individuals. What is not generally accepted is Darwin's idea that some of the heritable differences on which selection acts are generated by environmental changes. After all, geneticists have shown how ample new variation can be provided by rare mutations and the shuffling of genes during sexual processes. The ultimate source of variation is the random changes in the sequences of DNA bases that constitute the genes. Since, according to orthodox views, genes pass from generation to generation unaffected by external factors, there is little room in modern evolutionary theory for the idea that the environment can induce heritable changes. Such 'Lamarckian' beliefs are wrong, it is argued, because we know that 'acquired characters' are not inherited. The role of the environment is in the selection, not the generation, of heritable variation.

Until recently, the limit of tolerance for the notion that the environment can influence the generation of variation was the admission that it can affect the rate of mutation. However, since mutation was thought to be random and the mutation rate low, it was assumed that for most purposes small environment-dependent differences in mutation rate could safely be ignored. But things are beginning to change. First, studies of bacteria and unicellular eukaryotes have shown that some genetic changes may not be random: in some stressful conditions, the mutations that occur are adaptive to the environment inducing them. The molecular mechanisms that produce this effect are still being argued about (Sniegowski and Lenski, 1995), but it has been acknowledged that it would not be surprising to find that, through natural selection, systems have evolved which preferentially produce or stabilize those DNA changes that are adaptive in the conditions that produced them (Brenner, 1992). Second, the genome is no longer regarded as something static: it is recognized that the antics of jumping genes and different types of repair and recombination mechanism can lead to rapid expansion and contraction of various parts of the genome. As more and more regions of eukaryote genomes have been sequenced, it has become clear that the spread of repeated sequences has had profound effects on the organization and functioning of genes and chromosome regions (Zeyl and Bell, 1996).

A third recent change in the attitude to the nature and source of inherited variation is the recognition that there is more to heredity than DNA. Information can be transmitted from one generation to the next in ways other than through the base sequence of DNA. It can be transmitted through cultural and behavioural means in higher animals, and by epigenetic means in cell lineages (Holliday, 1987; Jablonka and Lamb, 1989). All of these transmission systems allow the inheritance of environmentally-induced variation. We want to concentrate on the less well-known systems, the epigenetic inheritance systems, or EISs, as they have been dubbed.

Epigenetic Inheritance Systems (EISs)

EISs are best known through their role in perpetuating the determined and differentiated states of cell lineages. They are the memory systems that enable somatic cells of different phenotypes but identical genotypes to transmit their phenotypes to their descendants, even when the stimuli that originally induced these phenotypes are no longer present. Even in culture, fibroblasts divide to give fibroblasts, keratinocytes divide to give keratinocytes, and epithelial cells divide to give epithelial cells. One of the best known and best understood examples of cell memory is the transmission of the inactive *X* chromosome in female mammals. *X*-chromosome inactivation is the dosage compensation mechanism that makes female mammals, with their two *X* chromosomes, functionally equivalent to males, with their single *X*. Early in development, one of the two female *X* chromosomes is inactivated. In some cells it is the paternal *X*, in others it is the maternal *X*, but once inactivation has occurred, all of the clonal descendants of the cell have the same *X* inactive.

Three types of EIS that may play a role in cell memory have been recognized (Jablonka et al., 1992):

(i) *Steady-state systems.* Some metabolic patterns are self-perpetuating. A simple example is a gene that regulates its own transcription by positive feedback: once turned on, a direct or indirect product of the gene's own transcriptional activity binds to a control region and maintains the activity of the gene. If the concentration of the regulatory product is not too low, and cell division is more or less equal, the pattern of activity will be inherited by daughter cells with quite high fidelity. Moreover, if the regulatory product is able to diffuse to other cells, these too could be switched to the same heritable activity pattern. Many examples of such self-maintaining regulatory loops acting at the transcriptional and post-transcriptional levels are known in *Drosophila* and other organisms (Blau, 1992).

(ii) *Structural inheritance systems.* Pre-existing cell structures can be used as templates for similar new structures. The best examples of this type of inheritance system comes from ciliates such as *Tetrahymena* and *Paramecium.* In these protozoa, genetically identical cells show heritable differences in the patterns of ciliary rows on their cell surface. Even experimentally altered patterns can be transmitted to daughter cells. Existing structures act as templates for new structures. Although little is known about the underlying mechanisms, there are good reasons for thinking that multicellular organisms also assemble new cell structures in association with existing structures (Grimes and Aufderheide, 1991).

(iii) *Chromatin-marking systems.* These are the EISs about which most is known. They depend on the way DNA is organized in chromosomes. Information is carried from one cell generation to the next because it rides with DNA. It is contained in what we have called *chromatin marks* (Jablonka and Lamb, 1989), which are binding proteins or additional chemical groups that are attached to DNA and influence its activity. When DNA is replicated, so are the chromatin marks. One type of mark is the methylation pattern a gene carries. In many eukaryotes, some of the cytosines in DNA are methylated. The addition of a methyl group does not affect the coding properties of the base, but the number and pattern of methylated cytosines is related to the functional state of the gene: usually low levels of methylation are associated with potential activity, high levels with inactivity. Changes in methylation patterns may occur: some are random, but specific changes are induced in response to particular environmental or developmental stimuli. The same DNA sequence can therefore have several different methylation patterns, each reflecting a different functional state. As Figure 1 shows, these alternative patterns, or *epialleles*, can be stably inherited through many cell divisions, because the cytosines that can be methylated occur in CG doublets or CNG triplets (where C is cytosine, G is guanine, an N can be any base). The complementary base pairing of the two DNA strands means that CG and CNG are partnered by the same sequence in the opposite direction. Following DNA replication, the parental DNA strand is methylated, but the daughter strand is not. Maintenance methylases recognise the hemimethylated sites and methylate the cytosines of the new strand. (For a comprehensive review of the role of DNA methylation in cellular inheritance, see Holliday, 1990).

Although less is known about them, chromatin marks involving DNA-associated proteins that affect the maintenance of gene activity can also be transmitted in cell lineages (Moehrle and Paro, 1994). The way these protein marks are replicated is not understood, but plausible models for the duplication of nucleoprotein complexes that would ensure that daughter cells inherit the appropriate state of activity have been developed (Wolffe, 1994).

These epigenetic inheritance systems have properties that make them very different from the genetic system. First, although some epigenetic changes arise unpredictably through mistakes in the maintenance or copying systems, many variations are directed and predictable outcomes of environmental changes. Second, unlike most mutations, epigenetic variants are frequently, although not necessarily, adaptive. Third, the frequency with which variants arise and their rate of reversion varies widely, from 100% to almost zero: for example, during development a change in the heritable state of activity of certain genes in some cell types is a virtual

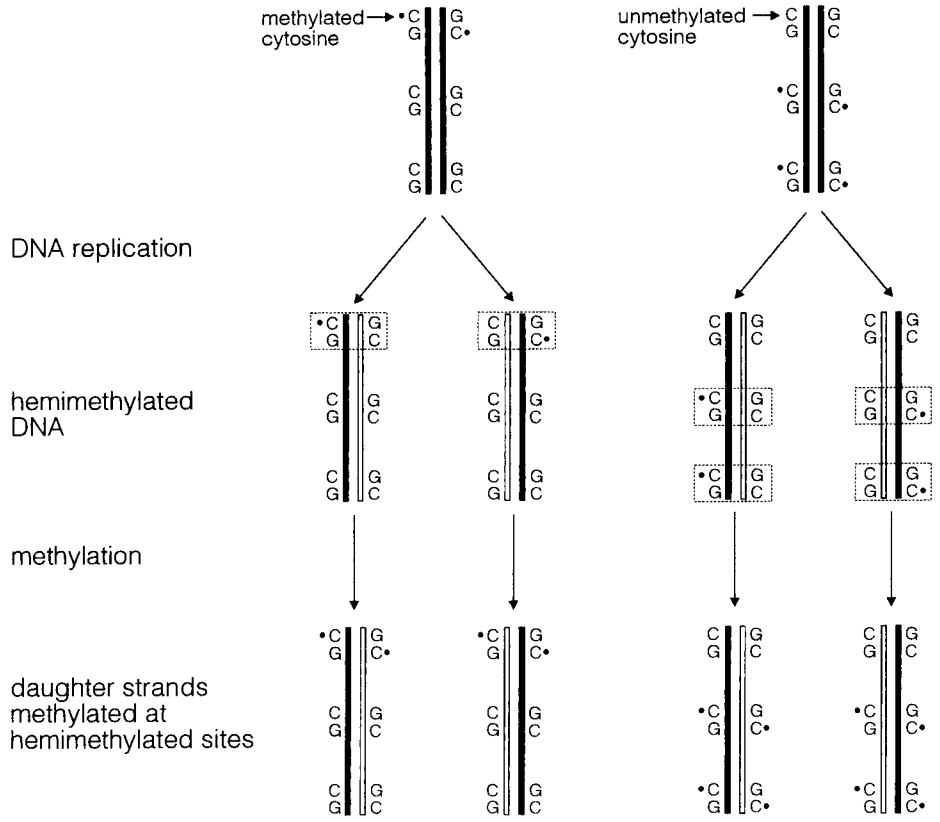

Fig. 1. The inheritance of methylation patterns on two identical DNA sequences with different methylation marks (i.e. two epialleles). After DNA replication, maintenance methylases recognize hemimethylated sites (boxed) and methylate the new DNA strand.

certainty, whereas once genes on the mammalian *X* chromosome have been inactivated, the chances of spontaneous reactivation in somatic cells is very low indeed. A fourth, and important, difference between the epigenetic and genetic systems is that epigenetic variations induced by environmental changes may be produced coordinately at several loci. This is what happens during normal development. Finally, in epigenetic systems the same changes can be induced in more than one cell within an organism, and in more than one organism. The epigenetic systems may therefore produce rapid, reversible, co-ordinated, heritable changes. However, EISs can also underlie non-induced changes, changes that are induced but non-adaptive, and changes that are very stable.

Evidence for the importance of epigenetic inheritance in the development of whole organisms has come from studies of genomic imprinting. In several organisms, mainly insects and mammals, the transmission and expression of some chromosomes, chromosomal regions, or genes depend on the sex of the parent from which they were inherited (reviewed by Solter, 1988; Barlow, 1995). Studies of imprinting have shown several things about epigenetic systems that are important for evolutionary biology. First, they have shown that epigenetic marks that originate in the parents (probably during gametogenesis) can be transmitted and affect gene activity in progeny. Second, they have shown that most epigenetic marks, such as those on the mouse autosomes, would be totally undetectable in the absence of sophisticated experimental manipulations. Hall (1990) and Ruvinsky (1988) are among those whose analyses have led them to suggest that the incomplete penetrance and variable expressivity of mutant alleles are probably often associated with differences in their epigenetic marks. Third, studies of imprinting have shown that even if a zygote inherits two complete and compatible sets of genes, if the epigenetic marks are inappropriate the zygote will not develop normally. By using genetic tricks, embryos with two maternal or two paternal chromosomes can be produced; often they fail to develop, or are abnormal, with the phenotype of those with two paternal chromosomes being different from that of those with two maternal chromosomes (Cattanach, 1986). A similar type of result is obtained in nuclear transplantation experiments in which zygotes having two maternal or two paternal genomes are constructed: the embryos fail to complete development, and gynogenetic and androgenetic embryos are abnormal in different ways (McGrath and Solter, 1984). For normal development, embryos must have the right epigenetic information, as well as a complete set of genetic information.

Transgenerational epigenetic inheritance

The role of EISs in development is clear. However, with the exception of imprinting, it is usually assumed that epigenetic inheritance can have no direct role in evolution. The arguments against a role for EISs in evolution can be divided into three categories: (i) transgenerational epigenetic inheritance is rare, pathological, and therefore unimportant; (ii) even if transgenerational epigenetic inheritance is common, it is so unstable that it has no evolutionary significance; (iii) epigenetic

inheritance can be of importance only in unicellular asexual organisms, or in multicellular organisms that reproduce by fragmentation, because if development has to start from a single cell, the resetting of epigenetic information during gamete production makes its inheritance unlikely. We need to examine the validity of each of these arguments before looking at the evolutionary effects and implications of EISs.

How common is transgenerational epigenetic inheritance?

Hereditary variations that are unstable or do not obey Mendelian laws have often been found, but rarely studied. When they have been studied, they have frequently yielded evidence suggesting epigenetic inheritance. We have previously summarized some of this evidence (Jablonka and Lamb, 1995, Tab. 6.1), and many other examples could be added. For example, Giesel (1988) found that paternal as well as maternal photoperiod influences the development time of *Drosophila melanogaster*, and attributed it to an inherited and adaptive change in genome expression; carry-over effects, the lingering of induced phenotypes despite a change in the environmental conditions that first induced them, have been found in a number of plant species (for references see Jablonka et al., 1995).

The richest source of examples of heritable epigenetic marks with phenotypic effects is plants. In the 1960s Brink initiated studies of paramutation, a process which leads to the heritable alteration of the expression of one allele by another allele of the same gene (reviewed by Brink, 1973; Patterson and Chandler, 1995). The maize *R* locus, which affects pigment intensity, has paramutagenic alleles that, when heterozygous with a sensitive *R* allele, alter the sensitive allele so that in subsequent generations it produces less pigment. The change in the *R* allele is somatic in origin, but is heritable through meiosis, although it can revert. If the *R* allele is passed through more and more generations of heterozygotes, the paramutagenic effect is progressive, and the phenotype gets more extreme. The *R* locus also shows imprinting, and the strength of the paramutagenic effect depends on environmental conditions during a particular stage in the development of the heterozygous plant (Mikula, 1995). Paramutation of the *R* locus is associated with methylation changes.

McClintock's pioneering studies have led to a wealth of information about germline-transmitted epigenetic variants of maize transposable elements. Heritable shifts between states of activity and inactivity are correlated with changes in methylation patterns, and depend on the element's parental origin, position in the plant, and the presence of other transposable elements (reviewed in Fedoroff, 1989). Plant transgenes also show germline transmitted differences in their states of activity. For example, Meyer et al. (1992) found that environmentally-induced changes in the heritable activity of a maize pigment transgene in petunia flowers was associated with changes in its methylation status.

Das and Messing (1994) found increased methylation associated with phenotypic effects in a study of two new variants of the *P* pigment locus in maize. The

methylation level and phenotype segregated regularly for the four and six generations they were studied. The new epialleles arose spontaneously and were not associated with DNA sequence changes. In a phenotypic revertant the methylation level was reduced, although not to the normal level, and this new level of methylation was maintained for the two further generations it was tested. Cocciolone and Cone (1993) studied a similar heritable variant of the maize *Pl* pigment gene, and they too concluded that the altered phenotype was the result of a changed methylation pattern rather than a changed DNA sequence.

It can be objected that most of the plant systems just described have involved unusual interacting alleles, or transposable elements or transgenes that would disturb chromatin structure, so they are not particularly relevant for 'normal' genes. This is not a very powerful argument, however, since transposable elements are not rare, and many kinds of epigenetic interactions between homologous 'normal' genes are known (Matzke and Matzke, 1995). It seems to us that the reason why plant systems have yielded some of the best evidence for the inheritance of spontaneous and induced epigenetic variants is fourfold: (i) long before molecular analysis was possible, people like Brink and McClintock recognized and studied non-Mendelian inheritance in plants, so it has been an active (albeit not always fashionable) area of research for many years; (ii) the germline and soma of plants do not separate until late in development, so there is more opportunity for induced changes to be transmitted to the next generation; (iii) the commercial importance of genetically engineered plants, in which inserted 'useful' foreign genes are commonly found to become heritably inactive, has focused research on transgenes and transposons; (iv) the subtle changes in gene activity that are likely to result from epiallelic variants can be monitored fairly readily as changes in the intensity or distribution of pigment in plant seeds or flowers, and do not affect survival.

Examples of well-characterized heritable epigenetic variants in metazoa are certainly less common than in plants. The most likely reason for this is the type of metazoan used for genetic and developmental studies. All have a segregated germline, and as Bolker (1995) has stressed, the 'model systems' used in developmental studies (*Drosophila*, *Caenorhabditis*, species of sea urchin, *Xenopus*, mice) are so strongly canalized they can tell us little about the role of the environment, and probably give us an overly deterministic view of development.

One example of epigenetic inheritance in metazoa has come from studies of a *Drosophila* mutant system, position effect variegation (PEV), in which part of the phenotype is no longer highly canalized (reviewed by Weiler and Wakimoto, 1995). As with many of the plant examples, the system showing epigenetic inheritance is one in which small changes in the activity of a pigment gene can be detected. If a chromosome rearrangement brings a normally euchromatic eye-colour gene near to heterochromatin, it often results in a variegated eye. In some cells the eye colour gene becomes heterochromatic and inactive, and the inactive state is clonally inherited during development. The degree of mottling depends on environmental temperature, on whether the rearranged chromosome was transmitted through the sperm or the egg (i.e. there is an imprinting effect), on the amount of heterochromatic *Y* chromosome present, and on various suppressor and enhancer genes. Some

of the latter have been cloned, and their nature is consistent with them coding for proteins that affect the assembly of chromatin. Dorn et al. (1993) studied an autosomal gene that is a strong enhancer of PEV: male offspring of fathers who carry the gene show an enhanced phenotype, even though they themselves do not carry the enhancer. Analysis of crosses showed that the *Y* chromosome is responsible for this effect: the product of the enhancer gene changes the *Y* chromatin in a way that affects the degree of variegation. What is remarkable is that this changed chromatin structure can be inherited for many generations in the absence of the enhancer that induced it.

There is clearly a lot of evidence suggesting that epigenetic inheritance is not rare and, if the system is appropriate, it can be detected. But is epigenetic inheritance, as some have suggested, 'pathological'? Certainly some is not, since normal coding genes are involved, but what about transposons, PEV, etc.? One feature that many of the sites of inherited epigenetic variants have in common is that they are associated with duplicated or repeated DNA sequences. Repeated sequences seem to be good carriers of epigenetic marks (Lohe and Hilliker, 1995). Therefore it is not the 'peculiarity' of the particular system that allows transgenerational epigenetic inheritance, it is the way DNA is organized in that system. Repeated sequences, transposable elements, and heterochromatic regions can no longer be thought of as being 'peculiar', 'atypical' parts of the genome. If they were, 'normal' coding sequences would be small islands in a large sea of peculiarity!

Many of the examples of non-Mendelian inheritance that we have discussed (those involving transgenes, paramutations, transposons, and imprinting) are known to be associated with inherited chromatin marks. Direct tests of the hypothesis that epigenetic variations underlie other cases of non-Mendelian inheritance are feasible: current methods easily and reliably detect differences in methylation patterns, and immunological methods may sometimes be appropriate for identifying alternative protein marks.

How stable are heritable epigenetic variations?

From what we know about the biochemical basis of heritable epigenetic variations, we would expect them often to be sensitive to environmental conditions, and to have a range of stabilities from fairly unstable, lasting only a few generations, to very stable and therefore behaving in the same way as classical mutations, lasting for hundreds of generations. This is indeed what has been found. Work on mammalian cell lines has shown that some epigenetic variants are so stable that for a long time they were mistaken for classical mutations (Holliday, 1987; Harris, 1989). Other cells in culture show frequent changes in epigenetic state: for example, tobacco leaf cells in culture changed phenotype with a frequency of 10^{-2}–10^{-3} (Meins, 1989). In maize plants the spontaneous rate of epigenetic change (epimutation rate) in a coding gene was as low as 10^{-6} (Das and Messing, 1994). In contrast, in many clonal plants, carry-over effects persist for only a limited number of clonal generations (Jablonka et al., 1995). For the marks on transgenes and

transposable elements, the rate of change is very variable and dependent on environmental and developmental conditions (Mittelsten Scheid, 1995; Jorgensen, 1995). In some cases it has been found that once a transgene or a repeated gene has become repressed by heavy methylation, it is very difficult to re-activate it (Hadchouel et al., 1987; Allen et al., 1990; Rhounim et al., 1992). Often the extent of trans-generational stability of epigenetic marks is unknown, because it has been followed for only a limited number of generations: the induced dwarfism and lowered level of methylation found by Sano et al. (1990) in rice was stable for the three generations it was studied, and the induced changes in the phenotype and level of methylation in Triticale were stable for three generations (Heslop-Harrison, 1990); the changed *Y* chromosome that affected variegation in *Drosophila* was examined and was stable for 11 generations (Dorn et al., 1993). The general picture emerging from studies of epigenetic variation in plants and mammals suggests a wide range of stabilities, between 10^{-1} to 10^{-6}, and for any particular system both the genetic background and environmental factors are important in determining stability.

The low stability of some epigenetic variants does not mean that they are of no evolutionary importance. Selection can maintain and increase the frequency of even unstable variants. As with the genetic system, the maintenance of epialleles in a population depends on both the coefficient of selection and the rate of epimutation. Theoretical models have shown that induced and non-induced semi-stable epialleles are advantageous in some types of fluctuating environments (Jablonka et al., 1995; Lachmann and Jablonka, 1996). The evolutionary effects of semi-stable epigenetic variants depend on the strength of selection and the nature of the environment.

Is transgenerational epigenetic inheritance possible in organisms that begin life as a single cell?

Many people are prepared to accept that epigenetic variations can have an evolutionary role in unicellular organisms, or in simple organisms that reproduce by fragmentation. However, most people find it harder to accept that in complex multicellular organisms epigenetic variations in the germline may be developmentally stable, or 'stubborn', and behave like variations in DNA sequence, passing from one generation to the next. After all, it is argued, the need to erase epigenetic information in the germline is a developmental necessity: totipotency has to be restored. However, totipotency is not to be identified with a 'clean slate', and being totipotent is not the same as being invariant. For example, small differences in conventional genetic factors can influence timing in development, or spatial differentiation, yet all zygotes with these genetic variations remain totipotent. If we accept that there is variability that does not involve loss of totipotency when discussing the influence of DNA variations on development, then we must also accept that transmitted epigenetic variations can influence development without loss of totipotency. The need to preserve totipotency is a selective constraint on both DNA variations and epigenetic variations, but the ground state can be variable,

and different in different organisms. The question of whether or not epigenetic variations are transmitted through gametes is empirical; it does not pose any theoretical problems.

What is the empirical evidence for the transmission of epigenetic marks from one sexual generation to the next? Molecular studies have shown that there are extensive changes in methylation patterns and DNA-bound proteins during gametogenesis and early embryogenesis. For example, there is widespread demethylation during the pre-implantation stage of the mouse. However, this does not mean that all marks are erased leaving all DNA sequences 'unmarked'. Evidence of this comes from studies of imprinted genes: for example, Tremblay et al. (1995) have shown that methylation differences between maternally and paternally inherited alleles of the mouse H19 gene are preserved during the demethylation phase.

As Maynard Smith and Szathmáry (1995) have pointed out, for epigenetic inheritance to be important in the evolution of multicellular organisms, epigenetic marks on identical DNA sequences have to be transmitted to the next generation and have consistent, reproducible effects on ontogenesis. The only question is how frequently this occurs. The evidence from the systems that we have described in this section suggests that it is probably quite common.

The role of EISs in evolution

The ubiquity and importance of epigenetic inheritance in plants is recognized by most plant geneticists (e.g. see Jorgensen, 1993; Phillips et al., 1995), and the importance of epigenetic inheritance in plant evolution is slowly beginning to be accepted (Matzke and Matzke, 1995). But, in general, study of the evolutionary implications of epigenetic inheritance is in its infancy. One exception is the attention that has recently been given to the significance of genomic imprinting. It has been suggested that in mammals and flowering plants, parental imprinting is associated with conflicts between parental genes over the rate of growth of the offspring (reviewed by Haig, 1992). Other evolutionary explanations of imprinting have been put forward: in some groups it plays a role in sex determination and dosage compensation, and it may facilitate the control of gene activity (reviewed by Jablonka and Lamb, 1995). Since the evolutionary significance of imprinting has been widely acknowledged and discussed, and we wish to focus on the less well known long-term inheritance of epigenetic marks, we shall not discuss imprinting further.

If long-term epigenetic inheritance is to be fully integrated into evolutionary theory, it will be necessary to study and estimate the extent of heritable epigenetic variation in populations. With existing molecular techniques, it should be possible to study the rate at which new methylation variants are generated, and the fidelity of their transmission. Pure lines of different genotypes could be exposed to new environmental conditions for a number of generations before transferring them back to the original environment; changes in the methylation patterns of selected DNA segments (such as heterochromatic regions and imprinted genes) are ex-

pected, and the extent to which they are carried over from one generation to the next could be monitored. Such molecular studies would provide an insight into the origin and stability of new variants, but they would not enable us to estimate the prevalence of epigenetic variations. For this what is needed is a concept comparable to the classical concept of heritability, and a model similar to those used for measuring the effects of cultural inheritance on human behaviour in populations (Cavalli-Sforza and Feldman, 1981). Such a concept is *epigenetic heritability*, which has been defined as the relative contribution of heritable epigenetic variability to the total phenotypic variance in a population. By using the classical quantitative genetic models and adding some reasonable assumptions about random or environmentally-induced changes in epigenetic states, epigenetic heritability can be calculated from covariances between relatives, and distinguished from customary genetic heritability (Kisdi and Jablonka, pers. comm.).

In what follows we shall argue that recognizing a role for epigenetic inheritance adds a complementary interpretation to conventional gene-based explanations of evolutionary events and leads to specific testable predictions. The areas on which we shall focus are the evolution of ontogeny, the evolution of genome organization, and speciation.

The origin of EISs and their importance in the evolution of ontogeny

EISs are probably very ancient. Steady-state EISs are an inevitable result of the properties of most self-maintaining gene-controlled metabolic circuits, and have been found in all living organisms in which genetic regulatory systems have been studied. Although much less is known about the structural inheritance system, the three-dimensional templating of complex organic structures probably played an important role in the transmission of information in primordial cells (Jablonka, 1994). Bestor (1990) argued that the chromatin-marking system that is based on DNA methylation probably evolved from the prokaryote restriction-modification defence system against viral parasites. Bacterial DNA is methylated in a sequence-specific way that protects the cells against their own restriction enzymes; these enzymes recognise and destroy inappropriately methylated foreign DNA. Bestor suggested that this defence system was adapted in primitive eukaryotes to become a defence system against intra-genomic parasites: extensive DNA methylation was used to suppress the activity of selfish repetitive elements such as transposons and genomically-integrated viral sequences. This role is still retained today in many organisms, including fungi, plants, and mammals, which selectively methylate newly integrated DNA sequences that are present in multiple copies (Doerfler, 1991; Matzke and Matzke, 1995); in some groups methylated sequences are subsequently targets for point mutations, excisions and rearrangements, a process known as 'ripping' (*r*epeat *i*nduced *p*oint mutation) (Selker, 1990). It has been argued that the role of methylation in regulating endogenous genes evolved in proto-vertebrates from this defence role (Bestor, 1990; Bird, 1995). However, since DNA methylation is involved in gene regulation in bacteria and many non-vertebrate eukaryotes

(Jablonka and Regev, 1995), it seems more likely that the methylation EIS was present in ancient unicellular organisms and preceded the origin of multicellularity.

EISs may be very important for unicellular organisms that live in unpredictable or fluctuating environments. For example, if the environment fluctuates regularly between two different conditions, each lasting longer than the generation time of the organism but not long enough to allow classical mutations to become fixed (i.e. each environmental state lasts 2–100 generations), even non-inducible heritable epigenetic variations are an advantage (Lachmann and Jablonka, 1996). In this type of fluctuating environment, the optimal rate of change in phenotype is approximately $1/n$, where n is the number of generations each environmental state lasts. This means, for example, that when each type of environment lasts 25 generations, the rate of change must be nearly 4% per generation. This is much higher than the rate of classical mutation, but well within the range found for EISs. The model predicts that altering the periodicity of environmental fluctuations will lead to alterations in the rate of epimutation. Experimental tests of this prediction may be possible using microorganisms that have epigenetic phase variations, i.e. routinely switch between two functional states of the same gene.

If heritable variations are induced by the environment rather than being random, then according to theoretical models there are several environmental regimes in which inheriting the phenotype induced in ancestors is advantageous (Jablonka et al., 1995). Rather surprisingly, the models show that in a randomly changing environment the inheritance of induced epigenetic variations is even more advantageous than individual plasticity. In such environmental conditions, not only would selection preserve EISs, it would also lead to their refinement. Jablonka et al. explained the repeatability seen in many ecological successions as consequences of past selection for inducible epigenetic variations.

The EISs that evolved in early unicellular organisms probably played an important role in the transition to multicellularity (Jablonka, 1994). For a transition to a new level of organization to occur, selection at the higher level (in this case, the multicellular individual) must be stronger than selection at the level of the units (the component cells) that make up the new individual. In other words, during transitions, group selection must overwhelm individual selection (Maynard Smith and Szathmáry, 1995). One of the consequences of epigenetic inheritance is that the selectable variation within a group of cells originating from a single cell is small. Even if the cells in a group come from different lineages, diffusion of trans-acting factors and the subsequent stabilization of the induced state by steady-state or chromatin-marking EISs could lead to phenotypic uniformity within the group. Since the same phenotype is inherited by every individual cell, the phenotypic variance between groups of cells can be larger than the variance within the groups, and group adaptations leading to an increased interdependence and division of labour can evolve. Looking at the evolution and maintenance of multicellular organisms from this epigenetic point of view makes it easier to see why some multicellular units were able to maintain their integrity in spite of mutation. For a mutant cell to destroy the coherence of a group, it must forget its epigenetic heritage and defy the inductive influences of other members of the group, as well as

proliferate more rapidly than its neighbours. The validity of these ideas about the importance of EISs in the evolution of multicellularity can be tested by modelling competition between groups of mutating cells with and without epigenetic inheritance.

The evolution of the ontogenies that produce complex multicellular organisms required the evolution and sophistication of EISs. EISs are essential because they enable the structure and function of organs to persist despite the turnover of their component cells. Yet the very same EISs that enable the stable cellular inheritance that is necessary for complex development also threaten the integrity of the multicellular organism. EISs allow competition within organisms: cells with new heritable epialleles may compete with existing cells. This can lead to cancer and somatic death, and if such selfish cells achieve germline status, they can jeopardize the development of descendants. Many basic features of the development of multicellular organisms can be interpreted as evolutionary responses to the potential dangers of transmitting selfish epialleles as well as selfish mutations to the next generation (Buss, 1987; Jablonka and Lamb, 1995). For example, beginning development from a single cell avoids competition from rogue cells with different genotypes or different epialleles. Maternal control of development and the early segregation of the germline are effective ways of preventing rogue cells from forming germ cells. Irreversible differentiation and loss of totipotency, and the strong dependence of individual cell survival on inter-cellular interactions (Raff, 1992) prevent rogue cells from assuming the epigenetic state necessary for germ cell differentiation. Finally, the extensive chromatin restructuring that occurs during gametogenesis restores marks to a ground state that allows a fresh (although variable!) epigenetic start.

The constraints that EISs impose on development can thus explain the evolution of many basic features of ontogeny. However, transgenerational inheritance of epialleles also has more direct adaptive evolutionary consequences, especially in organisms without a segregated germline. The presence of several induced or randomly produced epiallelic variants at the same locus increases selectable variation. Moreover, the formation of new variation at a limited number of loci in response to an environmental change is less costly than a general increase in the overall rate of variation, because it imposes a relatively low load on the population. Since similar epiallelic variants may be produced in many individuals in the same population, the opportunities for establishing and fixing new advantageous variants are increased. The targeting of variation to particular loci, and the presence of similar variants in more than one individual, can lead to rapid evolutionary change.

The view of differentiation and development we have outlined is rather different from that presented by Kauffman (1993). Kauffman suggested that the determined or differentiated state of a cell reflects stable, self-maintaining circuits of gene activities based on regulatory interactions among gene products. Although Kauffman's model may apply to genetic systems in primitive cells and in relatively simple organisms such as some bacteria, we believe it is insufficient to account for the maintenance of the determined and differentiated states in more complex organisms. For the perpetuation of the many functional states, eukaryotic cells use

specialized cell memory systems, such as the chromatin-marking methylation EIS, that can stabilize any pattern of gene activity, even one that would otherwise be stable for only a few cell generations. Like the system responsible for DNA replication, chromatin-marking EISs are unlimited inheritance systems, which can perpetuate and maintain any number of variant patterns (Jablonka and Szathmáry, 1995). Kauffman points to the correlation between gene number and the number of differentiated cell types in different groups to support his hypothesis that a cell type is the result of a stable, spontaneously ordered, network of gene activities. However, this general correlation probably reflects not only the cybernetic constraints of gene networks, but also the different developmental strategies that are related to differentiation in different phyla. For example, organisms that use the methylation EIS, which is a very efficient cell memory mechanism, can 'afford' to have more cell types as well as a higher cell turnover than organisms that do not use this EIS. In addition, the organization of the chromosome into distinct bands (see next section) may have a substantial effect on the efficiency of gene regulation and the number of cell types in vertebrates (Holmquist, 1989). Our view is that differentiation in eukaryotes involves the operation and interactions of all three EISs; the evolution of differentiation is associated with the evolutionary refinement of these EISs and chromosomal organization, and the evolution of general developmental strategies.

The hypothesis that EISs have influenced the evolution of development and life histories would be supported if correlations are found between modes of development and the type of EIS used. For example, organisms with high cell turnover are expected to have a reliable cell memory system, such as comes from having the methylation EIS, whereas small, short-lived organisms may make less use of this type of EIS. A preliminary survey of existing data supports this expectation (Jablonka and Lamb, 1995). The hypothesis relating EISs to life histories also suggests that long-term epigenetic inheritance may be particularly common in obligatory self-fertilizing organisms or ameiotic parthenogens, since having stubborn marks that produce carry-over effects would be a way of compensating for lack of genetic variability.

Epigenetic inheritance and genome organization

The eukaryotic genome is a highly organized system. The picture of genes and chromosome as 'beads on a string' has long since changed into something much more elaborate. First, chromosomes consist of different types of DNA sequences organized into large regions, each with a characteristic base composition and patterns of repeated sequences. Second, genes are not randomly distributed along the chromosomes: in vertebrates most genes are concentrated near the telomeres and very few are found near centromeres (Bernardi, 1995). Third, there is much more to a chromosome than DNA. The chromosome is an integrated system consisting of DNA, RNAs and proteins. DNA is wrapped around a core of histone proteins to form nucleosomes, and various proteins bind directly to DNA or other proteins to form large complex structures that condense or extend the DNA, bend

it, and organize its location within the nucleus; RNA molecules bind to DNA and associate with chromatin proteins, and small chemical groups such as methyl groups are attached to some nucleotides. All these components of chromatin interact, and together form the three dimensional structure we recognize as a chromosome. In addition to specialized chromosomal regions like telomeres and centromeres, and regions of very condensed chromatin (heterochromatin), the chromosomes of higher organisms are organized into bands and domains. Chromatin domains containing several genes form a unit of regulation which is insulated from the regulatory effects of neighbouring domains by special boundary elements (Eissenberg and Elgin, 1991). These bounded domains are in turn embedded in larger regions (bands) that have a region-specific base composition and pattern of repeated sequences, which seem to affect the time of replication of the whole band and the probability of gene activity within it (Holmquist, 1989). Furthermore, chromatin and gene activity in one chromosome are affected by the chromatin structure of other chromosomes, and by physical interactions with them. The associations of regions of r-RNA genes to form nucleoli and of heterochromatic regions to form chromocentres are well known, and many other cases of ectopic pairing of long repeats have been found (Matzke and Matzke, 1995).

The behaviour of mobile genetic elements such as transposons, and processes like slippage, gene conversion and unequal crossing over, produce much of the variation on which selection affecting chromosome structure acts. However, the structure of chromosomes and the interactions between them suggest that the genome is more than the outcome of selection for the regulated transcription of genes and the curtailment of the behaviour of selfish genetic elements. The genome is a highly evolved system for the transfer of genetic and epigenetic information. We believe that the evolution of the chromosomes must have involved interactions between the genetic inheritance system and the chromatin-marking system. Inducible and heritable chromatin marks affected DNA sequence changes, and DNA base sequence changes affected the acquisition and stability of chromatin marks.

Environmental factors can have direct influences on the conformation of chromatin. External conditions like temperature, diet, or behavioural stress, as well as internal conditions like sex and age, all affect gene activity and the probability of DNA sequence changes. Whether a gene is inactive or potentially active effects the rate of mutation, recombination, and transposition. For example, the probability of crossing over and of transposition is higher in active chromatin than in inactive chromatin (for references see Jablonka and Lamb, 1995). Moreover, cells seem to have different genomic responses to different kinds of stresses, and activate specialized mutational systems such as ripping in particular stressful conditions (McClintock, 1984; Wills, 1991; Jablonka and Lamb, 1995). Since the environment affects the frequency of mutation and recombination, the usual practice of treating the generation of variation as if it is independent of selection is clearly wrong. Furthermore, if chromatin marks are inherited, an environmentally-induced active chromatin region that is a mutational or recombinational 'hot spot' may persist longer than the conditions that induced it. We have shown through a theoretical model that this can substantially increase the rate of gene substitution (Jablonka

and Lamb, 1995). We predict that higher mutation rates will be found in genes that are newly expressed in changed conditions, especially in genes that can carry variations in inherited marks.

Chromatin structure clearly affects the rate of change in DNA, but equally clearly DNA organization affects chromatin structure. CG sequences and repetitive sequences are known to be particularly important carriers of methylation and protein marks. The number and organization of these genetic elements affects the fidelity with which marks are inherited and hence the length of phenotypic memory. For example, Boyes and Bird (1992) found that the density of CG sites in the promoter region of a gene is associated with the stability of the inactive state: the more CG sites, the more stable the inactive state. In both plants and animals repeated sequences have been found to be important in regulating gene activity, either by binding regulatory proteins that alter the local conformation of chromatin, or by pairing with similar sequences in the same or other chromosomes (Vogt, 1990; Dorer and Henikoff, 1994; Matzke and Matzke, 1995). Tandem repeats and CG clusters are important in *X*-chromosome inactivation (Penny et al., 1996), in genomic imprinting in mammals (Barlow, 1995), and in gene-silencing in plants (Assaad et al., 1993; Mittelsten Scheid, 1995). Studies of PEV in *Drosophila* and position effects in yeast have shown that repeated sequences play a central role in the nucleation and perpetuation of protein complexes (Moehrle and Paro, 1994).

Clearly, DNA sequence architecture underlies the ability to transmit epigenetic marks. However, this does not mean that DNA sequences determine all variations in epigenetic marks. When more than one type of mark can be carried and transmitted by a given sequence, epigenetic inheritance that is independent of DNA variation occurs. This can be seen in genetically identical cell lines initiated from different tissues: identical genes have different heritable methylation patterns. Similarly, in two cells lines derived from a single tissue of a female mammal, the marks carried by identical *X*-linked genes are very different if different *X*-chromosomes have been inactivated. Genetic differences are not necessary for epigenetic differences.

Since blocks of heterochromatin, repeated sequences, and CG sites seem to affect the stability of gene activity, changes in their abundance and distribution may lead to changes in the ease with which genes are switched on and off, and in the fidelity with which epigenetic marks are transmitted both in cell lineages and from one generation to the next. Such changes could affect the response of cells to a spatial gradient of morphogens and the timing of gene activity during ontogeny. The vast amount of polymorphism for the number and distribution of repeated DNA sequences, and the high rate at which they change, suggest that populations have an enormous potential for ontogenetic adjustment through selection of the random or induced marks these repeated sequences allow. Systematic comparisons of the fidelity of epigenetic transmission in clones differing in the number of repeats and CG clusters are needed to verify this. According to the evidence cited earlier, populations are probably also polymorphic for heritable methylation marks, which can also lead to subtle, selectable variations. Since environmental conditions can affect chromatin marks, and chromatin marks affect the likelihood of DNA

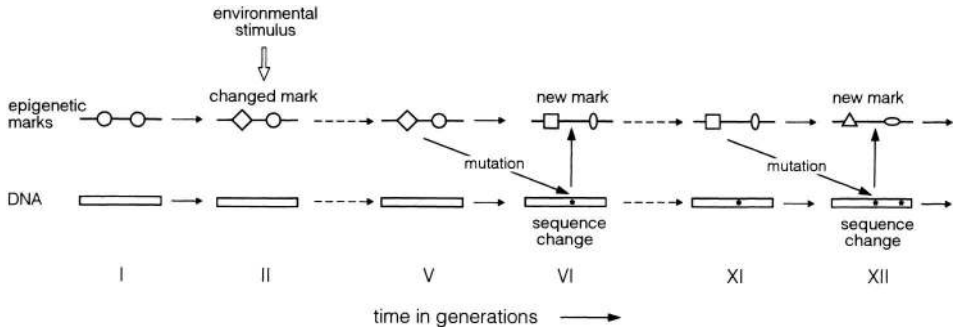

Fig. 2. Interactions between a DNA sequence and the chromatin marks it carries. In generation II an environmental stimulus alters a mark; this increases the probability of a mutational change (*) which eventually occurs in generation VI. This DNA sequence change alters the organization of chromatin, which again leads to a mutational change.

sequence change, which in turn affects chromatin marks, the interaction between the two inheritance systems is one of spiralling feedback (see Fig. 2). In addition, adaptations that were originally epigenetic, and did not involve changes in DNA sequence, can become more permanent through selection of DNA sequence changes. The process is one of genetic assimilation, in the sense described by Waddington (1957).

Epigenetic inheritance and speciation

So far, evolutionary genetics has been able to tell us very little about the genic basis of post-mating reproductive isolation. As Wu and Palopoli (1994, p. 284) put it, there is an "absence of an adequate description of the genetic architecture underlying post-mating reproductive isolation for even a single species pair". In view of the acknowledged failure of traditional genetic analysis to identify more than a few critical genes, it is worth considering the possibility that the factors responsible for post-mating isolation do not behave like classical Mendelian genes. We believe that in many cases epigenetic divergence may have played a significant part in the origin of post-mating isolation (Jablonka and Lamb, 1991).

One of the first effects of geographical or ecological isolation is likely to be divergence in chromatin structure resulting from environmentally-induced changes in gene activity. Consequently, even without DNA sequence changes, in new environments changes in chromatin marks in germline cells can occur, particularly in organisms with somatically derived or late germline segregation. If the new epigenetic marks persist and accumulate over several generations, they may initiate reproductive isolation between populations. How readily this occurs could be investigated in experiments similar to those that Shaposhnikov (1966) found led to reproductive isolation between aphid clones. He showed that a subpopulation of aphids allowed to reproduce parthenogenetically on a novel host rapidly became

reproductively isolated from the original population. If, in similar experiments, genetic divergence is avoided by founding new sub-populations with parthenogenetic sisters, it should be possible to follow the build-up of reproductive isolation and study changes in methylation and protein marks.

In addition to divergence of chromatin marks on identical DNA sequences, the enormous amount of intrapopulation polymorphism for the repeated sequences that can carry epigenetic marks means that when populations split, the two parts are unlikely to have the same distribution of repeats. Through the processes of molecular drive, these inherently unstable elements can rapidly diverge further (Dover, 1986). Moreover, since in many cases the events that initiate speciation, i.e. changed environmental conditions and a decrease in population size leading to inbreeding, are themselves stressful, they may elicit stress-association genetic changes, such as an increase in transposition and chromosome-breaks (McClintock, 1984). The feedback between the genetic and epigenetic inheritance systems means that changes in the two systems will tend to go together.

In organisms that have a somatically derived germline, or in which germline determination occurs late in development, variations that are tested somatically can be transferred to the germline. Buss (1988) suggested that this is why groups with no or late germline segregation are relatively species rich: genetic variants that were tested in the soma can be passed to the next generation where they may initiate new developmental programmes. Random and induced epigenetic variants are also likely to launch such ontogenetic experiments. If they do, it is not difficult to see how they could lead to very early reproductive isolation through hybrid inviability. As the nuclear transplantation experiments in mammals that were discussed earlier have shown, epigenetic compatibility is as important as genetic compatibility: although genetically adequate, embryos produced from two haploid nuclei from the same parent do not reach adulthood because their imprints are incompatible (McGrath and Solter, 1984). Therefore, if the chromatin marks of the two populations diverge, a hybrid zygote may fail to survive even though genetically there are no incompatibilities.

Some of the hybrids between species that do survive show evidence of carry-over into the hybrid of the parental sex- and species-specific marks (Jablonka and Lamb, 1995, Tab. 9.1). For example, in both animal and plant hybrids, commonly only one of the two parental nucleolar organizer regions is active. In wheat hybrids this preferential expression of r-RNA genes from one parent is related not to changes in coding sequences, but to the number and methylation of repeats in an inter-genic region (Flavell and O'Dell, 1990). The similar nucleolar dominance in *Xenopus* hybrids is also associated with repeated sequences in non-coding regions (Reeder, 1984). In both cases, preferential expression of the genes from one parent is associated with divergence of those sequences that affect the inheritance of epigenetic marks.

Interspecific hybrids are frequently somatically vigorous, but one or both sexes are sterile. This dramatic difference between normal somatic functions and the failure of gametogenic functions intrigued Spurway (1955). She suggested that the difference is due to the contrasting selection pressures acting on somatic and

gametogenic processes. For somatic functions selection is for developmental homeostasis – for survival in spite of genetically and environmentally produced 'noise'; with gametogenesis selection is for processes that detect and eliminate germ cells that are inadequate – the next generation must be started from perfect gametes. Cytogenetic evidence suggests that the pairing of homologous chromosomes is a critical stage in gamete formation: normal gametogenesis requires homologous chromosomes to pair properly during meiosis, or if they cannot pair, for unpaired regions to become heterochromatic (Miklos, 1974; Jablonka and Lamb, 1988). When the chromatin-restructuring processes that are necessary for pairing or protective heterochromatinization are impaired, for example as a consequence of chromosomal rearrangements, there are problems with DNA packaging at post-meiotic maturation stages, and gametocyte degeneration and sterility often follow. Pairing may also be impaired if homologous regions in the hybrid have species-specific marks that lead to differences in their chromatin structure at the pairing stage. The observed effects of pairing failure on gametogenesis have led to the suggestion that, as Spurway anticipated, there is a cellular 'quality control' system in gametocytes that detects and eliminates cells in which pairing is inadequate (Burgoyne and Baker, 1984; Jablonka and Lamb, 1988; Burgoyne and Mahadevaiah, 1993).

Since, in general, spermatogenesis demands greater chromosome restructuring than oogenesis, hybrid males, regardless of the sex-determining mechanism, are expected to show more sterility than hybrid females. This is expected even in groups with environmental sex-determination. When sex-determination involves heteromorphic sex chromosomes, additional effects on sterility are expected in the heterogametic sex because of the peculiar behaviour of the sex chromosomes during meiosis. We have argued that since chromatin restructuring is central to successful gametogenesis, the basis of Haldane's rule may be found in the chromatin changes of sex chromosomes (Jablonka and Lamb, 1991). Haldane's rule states that when in the offspring of an interspecific cross one sex is inviable or sterile, that sex is nearly always the heterogametic sex (Haldane, 1922). Many explanations of the genetic basis of the rule have been suggested (Wu and Davis, 1993), including X-autosome imbalance, X-Y interactions (including meiotic drive), and faster accumulation of recessive alleles on the sex chromosomes than on the autosomes. After examining these theories and the experimental evidence, Wu et al. (1996) concluded that there was no satisfactory unifying explanation of the observations on both sterility and viability in both the homogametic and heterogametic sex of species hybrids. They suggested that Haldane's 'rule' is really a composite phenomenon, and several different forces have contributed to the observed patterns of hybrid viability and sterility.

Almost all recent discussions of Haldane's rule have centred on gene interactions and ignored chromosome behaviour and chromatin restructuring. We have argued that the sterility described by Haldane's rule occurs because the structural and functional inequality of the sex chromosomes in the heterogametic sex makes them particularly sensitive to genetic and epigenetic divergence (Jablonka and Lamb, 1990, 1991). In XY males, the Y chromosome in somatic cells is largely heterochromatic, so at meiosis part of the euchromatic X chromosome has no pairing

partner; the consequences of pairing failure are avoided because this part of the *X* becomes heterochromatic. When the female is the heterogametic sex (*WZ*), the *W*, which in somatic cell is heterochromatic, becomes partially euchromatic in oogenesis, and thus provides the *Z* with a pairing partner (Jablonka and Lamb, 1988). If these processes are impaired, as they are for example with *X*-autosome translocations, the result in both *Drosophila* and the mouse is pairing failure and sterility (Miklos, 1974; Burgoyne and Baker, 1984; Rugarli et al., 1995; Palmer et al., 1995). Consequently, when chromosomes from two previously isolated populations meet in a hybrid germline, the sex chromosomes of the heterogametic sex are particularly vulnerable to any divergence that affects chromosome restructuring, e.g. *X*-autosome translocations, or changes in DNA-binding proteins, methylation marks, or the number and distribution of tandem repeats.

The Table summarizes the differences in sex-chromosome chromatin structure in somatic and germline cells in groups with male and female heterogamety. It also gives the observed number of cases of single-sex viability and sterility in different groups. On the basis of the amount of chromatin restructuring that the sex chromosomes must undergo, predictions can be made about the relative likelihood of sterility in different hybrids. Consider gametogenesis:

XY *males*: if the *X* fails to inactivate during spermatogenesis, it will lead to pairing failure and male sterility.

XX *non-mammalian females*: since *X* chromosomes behave in the same way as autosomes, they will have no special effects on fertility.

XX *mammalian females*: if the inactive *X* fails to reactivate in the germ line, reduced fertility, such as is seen in *X*0 females, is likely.

ZZ *males*: since the *Z* chromosomes behave in the same way as the autosomes, they will have no special effects on fertility.

Table

Group	Sex	Chromosomes	Sex chromosome conformation[1]		Single-sex hybrid	
			Somatic cells	Gametocytes	Inviability[2]	Sterility[2]
Drosophila	♂♂	*XY*	*X* active; *Y* inactive	*X* inactivated	14 m	199 m
	♀♀	*XX*	Both *X*s active	Both *X*s active	9 f	3 f
Mammals	♂♂	*XY*	*X* active; *Y* inactive	*X* inactivated	0 m	25 m
	♀♀	*XX*	One *X* active, one inactive	Inactive *X* reactivated	1 f	0 f
Birds	♂♂	*ZZ*	Both *Z*s active	Both *Z*s active	2 m	0 m
	♀♀	*ZW*	*Z* active, *W* inactive	*W* reactivated	21 f	30 f
Lepidoptera	♂♂	*ZZ*	Both *Z*s active	Both *Z*s active	4 m	0 m
	♀♀	*ZW*	*Z* active, *W* inactive	*W* reactivated	36 f	15 f

[1] Based on Jablonka and Lamb (1988).

[2] Taken from Wu et al. (1996); m: cases where only males are inviable/sterile; f: cases where only females are inviable/sterile.

ZW females: if the *W* fails to reactive in the germ line, it will lead to pairing failure and female sterility.

The predicted 'sterility gradient' is therefore:

$$XY \geq ZW > XX \text{ mammals} > XX \text{ non-mammals} = ZZ$$

The data in the Table are consistent with this prediction. Since no other explanation of Haldane's rule predicts that more sterility should be found in hybrid female mammals than non-mammals, additional comparative data will help to assess the validity of the chromosomal hypothesis.

Changes in chromatin structure are less directly associated with hybrid inviability, because in embryogenesis there are no pairing and segregation processes comparable to those of gametogenesis. However, if in early embryogenesis the sex chromosomes fail to activate or inactivate appropriately, it may affect gene expression and lead to imbalances between sex-linked and autosomal genes. Muller (1940) proposed that the inviability of heterogametic hybrids was the result of *X*-autosome imbalance: whereas homogametic hybrids have a complete haploid genome from each parent species, the heterogametic sex has an *X* (or *Z*) from only one parental species, but a complete set of autosomes from both parents. Consequently, in the heterogametic sex there are imbalances in gene expression which lead to inviability. According to our chromosomal hypothesis, such imbalances will also occur in *XX* females if the paternally derived *X* fails to reactivate normally. In female mammals the additional need to inactivate one *X* chromosome in early embryogenesis may enhance hybrid female inviability (Jablonka and Lamb, 1991). The limited amount of data available (Tab. 1) are consistent with these expectations.

If defects in chromosome pairing and chromatin restructuring underlie hybrid sterility, the most promising approach to the study of post-zygotic isolation may be investigating divergence in the elements responsible for chromatin structure and the condensation-decondensation cycles of chromosomes, especially the sex chromosomes. These will include not only conventional coding sequences, but also repeated sequences, CG clusters and other elements that determine chromatin conformation, and species-specific marks (a type of imprint) which are carried over from the parents. Looking at chromatin proteins, and at their binding sites on the *X* chromosome and autosomes, might be one of the most productive ways of finding out more about post-mating isolation. An integrated genetic and epigenetic outlook is needed, rather than one focused solely on classical Mendelian genes.

Conclusions

We have shown how epigenetic inheritance can play a role in evolution. The approach we have used recognises the role of the environment in inducing as well as selecting variation. This is particularly significant in plants and in invertebrates in which the germline is not segregated and development is not highly canalized. The mechanism of evolution we envisage is thoroughly Darwinian: epigenetic

inheritance simply provides an additional source of variation, much of which may be as random as mutation, but some of which will be induced in those genes that respond to changed environmental conditions. Induced epigenetic variations need not to be identical in every individual, but they will be concentrated in a limited number of loci, and hence selection will be focused on these loci. The cost of selection will be small, because the variable loci are relatively few and are relevant to the environmental conditions. The environment in such cases is an agent of variation as well as an agent of selection.

With the tools of molecular biology it is possible to distinguish between heritable genetic and epigenetic variations, and to study their effects. However, during evolution there will be constant feedback between the genetic and epigenetic systems, so that in populations that are clearly distinct, it will probably be impossible to say whether differences were initiated by genetic or epigenetic events. Only in the initial stages of divergence, such as during adaptation to a new environment, will it be possible to unravel the effects of genetic and epigenetic inheritance. Nevertheless, recognising that EISs have a role in evolution, and incorporating them into evolutionary thinking can be fruitful. We have shown that the epigenetic perspective can be useful when thinking about the evolution of development, chromosomes, and speciation, and that it leads to testable predictions. We believe that for these and other evolutionary problems, the epigenetic approach, which in some ways is a Lamarckian approach, can be more informative than a purely genetic approach.

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Received 1 May 1997.