Epigenetic Inheritance: A Contributor to Species Differentiation?

Dario Boffelli and David I.K. Martin

Multiple epigenetic states can be associated with the same genome, and transmitted through the germline for generations, to create the phenomenon of epigenetic inheritance. This form of inheritance is mediated by complex and highly diverse components of the chromosome that associate with DNA, control its transcription, and are inherited alongside it. But, how extensive, and how stable, is the information carried in the germline by the epigenome? Several known examples of epigenetic inheritance demonstrate that it has the ability to create selectable traits, and thus to mediate Darwinian evolution. Here we discuss the possibility that epigenetic inheritance is responsible for some stable characteristics of species, focusing on a recent comparison of the human and chimpanzee methylomes which reveals that somatic methylation states are related to methylation states in the germline. Interpretation of this finding highlights the potential significance of germline epigenetic states, as well as the challenge of investigating a form of inheritance with complex and unfamiliar rules.

Inheritance: DNA Only or Chromosomal?

Heritable information is carried by chromosomes (Crow and Crow, 2002). The discovery of DNA in chromosomes led to the paradigm that heredity and evolution stem from the stable transmission of DNA sequence, with occasional sequence changes that are also stably transmitted. This pattern of transmission of DNA is extremely consistent with inheritance patterns that characterize Mendelian genetics. Chromosomes, however, contain much material that is distinct from DNA yet transmitted with it, and thus has the potential to carry heritable information alongside DNA. This material, commonly known as the epigenome, is a complex assortment of proteins and chemical modifications that are associated with DNA and control its transcription (Bernstein *et al.*, 2007; Pelizzola and Ecker, 2011). The epigenome provides a means by which a single genome can generate many distinct and mitotically heritable states of cell differentiation. The question we discuss here is whether such states are entirely encoded by the genome or whether purely epigenetic states (i.e., independent of genome sequence) maintained in the germline can constrain somatic epigenetic states. These germline epigenetic states could provide an additional source of heritable variation, with the potential to contribute to differences between species (Richards *et al.*, 2010; Martin *et al.*, 2011).

There is no basis *a priori* for exclusion of a system of inheritance based on the epigenome, which requires only that alternative states of the epigenome can be transmitted between generations independently of genetic information. This requires that an epigenetic state, based on methylation or other epigenetic modifications, is maintained continu-

ously in the germline over multiple generations; this in contrast to a phenomenon, such as parental imprinting, where the epigenetic state is reset in every generation. Plants have provided multiple clear examples of this transgenerational epigenetic inheritance (Richards, 2006), beginning with the work of Brink, Coe and others on paramutation (see below) (Coe, 1959; Brink *et al.*, 1968). Plants do not segregate a germline, but the segregation of a germline in animals means that any heritable information must arise and be maintained in these cells. A small number of examples indicate that epigenetic inheritance can occur in animals. These include the behavior of the Fab-7 element (Cavalli and Paro, 1998) and heterochromatin alterations (Sollars *et al.*, 2003; Seong *et al.*, 2011) in *Drosophila*, heritable transgene silencing in mice (Hadchouel *et al.*, 1987; Sutherland *et al.*, 2000), the *A^{vy}* (Morgan *et al.*, 1999) and *ax-in(Fu)* alleles in the mouse (Belyaev *et al.*, 1981; Rakyan *et al.*, 2003), *MLH1* in human (Suter *et al.*, 2004; Hitchins *et al.*, 2007), heritable chromatin effects driven by RNAi in *Caenorhabditis elegans* (Gu *et al.*, 2012), and several others (Anway *et al.*, 2005; Greer *et al.*, 2011). Possibly the most significant examples involve centromere repositioning: studies of neocentromeres have demonstrated that centromeres are epigenetic structures—there is no specific DNA sequence requirement for placement of the protein structures of a new centromere (Marshall *et al.*, 2008). The neocentromeres can be stably inherited through meiosis (Amor *et al.*, 2004). During primate evolution, centromeres have repeatedly moved to new loci, demonstrating that an epigenetic event can be maintained and participate in a critical mechanism of speciation (Ventura *et al.*, 2004).

The Concept of the Epigenome

It is humbling to consider that the first, and still possibly the clearest description of the epigenome was derived by Brink in the 1950's from classical genetic studies without any direct analysis of its molecular components (Brink, 1960). Brink's pioneering work on paramutation at the *R* locus (Brink, 1956) led him to conceive of a system of heredity that was associated with, yet distinct from, the DNA-based system responsible for Mendelian inheritance (Brink, 1960). His view of the epigenome provided an answer to the long-standing question of how disparate phenotypes could arise from a single genome.

Brink proposed that a chromosome is composed of two general types of chromatin, orthochromatin and parachromatin, which today we would call the genome and epigenome. Orthochromatin he described as "...the genetic substance proper...whose composition and architecture are disclosed by conventional Mendelian analysis." Brink described parachromatin as "...self-replicating, and co-extensive in distribution with the orthochromatin...condition(s) the intrachromosomal processes...in response to the changing cellular environments of the developing organism...altered by accretion, diminution, and in other ways...takes on a succession of potentially reversible, but mitotically transmissible states...the nexus between chromosomal heredity and the development of the organism...capable of replicating in phase with orthochromatin and then functions as quasi-genetic material...its ultimate role is epigenetic...the meeting ground of the genetic and epigenetic functions of the chromosome" (Brink, 1960). This description sounds just like the epigenome, minus the information on its molecular composition that was not available in Brink's time, but for all that clearly laying out the functional properties that could be inferred from experimental data.

Brink's integrated view of the epigenome stands in contrast to its modern molecular description. This has revealed that histone modifications, cytosine methylation, and the accretion of a variety of proteins, such as Polycomb, vary over the length of a chromosome and are associated with particular states of gene expression (Bernstein *et al.*, 2007; Schwartz and Pirrotta, 2008; Lister *et al.*, 2009; Hawkins *et al.*, 2010; Smith and Shilatifard, 2010; Negre *et al.*, 2011). The complexity of these modifications illustrates why epigenetic inheritance is so different from Mendelian inheritance: one is based on the faithful replication of a stable molecule, while the other is based on structures, not yet fully understood but containing an assortment of molecules of varying stability. Furthermore, the epigenome has different compositions at different sites, and so each example of epigenetic inheritance displays a different pattern of inheritance, presumably because each is based on a different assortment of molecules. Another distinction between Mendelian and epigenetic inheritance is that a locus in a single individual can be described by one or two genetic alleles, but by an undetermined number of epialleles created by combinations of molecular accretions to the genome.

What Is Epigenetic Variation?

The contrast between the genome (stable, faithfully replicated) and the epigenome (less stable, error-prone replication by reassembly of a complex structure) extends to variation in both of them. The definition of an epigenetic variant is not

obvious. Because the epigenome is a complex of proteins that interact and presumably stabilize each other, it might be perturbed more readily than the genome (Li *et al.*, 2011; Richards, 2011). From a molecular perspective, an epigenetic variant can be defined as a change in the molecular composition of the epigenome at some site (e.g., methylation of a CpG dinucleotide, or gain or loss of a histone modification). Many such molecular variants may be functionally trivial. From a functional perspective, an epigenetic variant is a stable change in a transcriptional regulatory element, which changes the expression of a gene without any change in DNA sequence or in the intracellular environment. This definition excludes random or stochastic changes in gene expression that quickly revert to the normal state. Under constant environmental conditions, the reference epigenetic state at a locus is likely to be more stable than a variant state; however, a change in environmental conditions could perturb the stability profile of the epigenome at a locus, changing the probability of variant states arising, and even making a variant state more stable than the previous reference state (Skinner, 2011). For example, recent analysis of the effects of continuous methyl donor supplementation on methylation variation in mice indicates that this environmental factor increases variation, and that some of this variation is heritable (Li *et al.*, 2011).

The Characteristics of Epigenetic Inheritance Make It Difficult to Observe

Epigenetic inheritance is so different from Mendelian inheritance, in its mechanisms and consequently the diversity of its patterns and predictability, that it can be very difficult to study. Known patterns of epigenetic inheritance (see examples of epigenetic inheritance described above) do not follow established population genetics models; accurate description of epigenetic inheritance will require new models informed by sufficient experimental data describing the phenomenon (Slatkin, 2009; Tal *et al.*, 2010; Johannes and Colome-Tatche, 2011). This discussion deals only with transgenerational epigenetic inheritance, which we define as the transmission of epigenetic variants that are maintained in germ cells over generations and independently of the genome sequence—in other words, more than one epigenetic state can be inherited in association with the same genome. It is important to distinguish this pure epigenetic inheritance from examples in which the epigenetic state of a locus is fully or partially dependent on the genome sequence (Richards, 2006). Because genome sequence, acting either in *cis* or in *trans*, can control or influence epigenetic state (e.g., during development and cell differentiation), it can be difficult to distinguish genetic inheritance of epigenetic states from pure epigenetic inheritance. In practice this distinction can be made only by studying isogenic populations, where genetic variation can be neglected.

What is inherited? DNA has provided this answer for Mendelian inheritance, but there is no such simple answer for epigenetic inheritance. As mentioned at the beginning, chromosomes are inherited, but the particular complement of proteins and chemical modifications at a particular locus will vary, and influence the heritability of an epigenetic state. In essence, it is the epigenetic state that is inherited, and there may be no single molecule or structure responsible for that state.

Another point for consideration is the evidence that animal germlines undergo extensive resetting of their epigenomes (Feng *et al.*, 2010; Hajkova, 2011). This has traditionally been considered as creating an epigenetic blank slate onto which a genetically encoded developmental program writes somatic epigenetic states. The several extant examples of epigenetic inheritance (see first section above) demonstrate that this resetting is not universal (Feng *et al.*, 2010). Furthermore, we have little information on the real extent of resetting: the complexity of the epigenome, and the difficulty in accessing the variety of cell types that make up the germline, leaves open the possibility that substantial epigenetic information is retained in the germline.

Can Epigenetic Inheritance Be So Stable as to Underlie Species Characteristics?

Epigenetic inheritance—defined as above as transgenerational passage of a purely epigenetic state—is by now an established phenomenon, but its biological significance is still very much an open question. The instability of many inherited epigenetic states has led to the view that it can be a mechanism of phenotypic response to environmental factors, one that can readily be reversed when such factors change (Jirtle and Skinner, 2007; Petronis, 2010; Richards, 2011; Skinner, 2011; Cropley *et al.*, 2012). But, is it more than that? Could epigenetic inheritance be in some cases so stable that it creates essentially permanent changes in a species? Epigenetic inheritance is compatible with Darwinian evolution if epigenetic states that specify traits can remain stable in the germline through generations; in other words epigenetic states in the parents must predict states in succeeding generations (Jablonka and Lamb, 1989; Haig, 2006; Slatkin, 2009; Richards, 2011). However, because its molecular basis is very unlike the basis of Mendelian inheritance, extremely stable epigenetic inheritance could be very difficult to identify. The first difficulty lies in the identification of epigenetic changes that could be a source of heritable phenotypic variation: as we discussed above, there is no single definition of epigenetic variation. The second is the necessity of ensuring that any identified epigenetic variant is not encoded in DNA sequence. Consequently, an estimate of the contribution of epigenetic inheritance to the establishment of phenotypic differences between species has so far been elusive.

We considered this issue in regard to humans and their closest relatives, the chimpanzees. Genetic approaches have implicated a limited number of genome sequence differences in human evolution, but it is not clear how many of the phenotypic differences between human and chimp are explained by these genetic differences. Our comparative analysis of the human and chimp methylomes illustrates the insights that can be gained from, but also the limitations of, epigenomic comparisons (Martin *et al.*, 2011).

We determined methylation state differences in the epigenomes of neutrophils purified from human and chimpanzee. Neutrophils were chosen because they are homogeneous and accessible cells with a deeply conserved function of engulfing and destroying microorganisms. We derived accurate genome-wide methylation maps from multiple individuals by deep sequencing of ends generated by digestion with a methylation-sensitive restriction enzyme, and inference of methylation states with a computational

pipeline designed to correct biases inherent in the data (Singer *et al.*, 2010). Using orangutan as the outgroup, methylation differences were not distributed randomly among the individuals we analyzed, but recapitulated the known phylogenetic relationships of the three species in a pattern consistent with their stable inheritance (Martin *et al.*, 2011).

This study provided the first comprehensive dataset indicating that epigenetic states are maintained and predictably transmitted within species. The ability to reconstruct phylogenies is not unique to CG methylation states: many phenotypic characters can be used for this purpose. However, these characters reflect genomic sequences that encode the characters; that is, the character merely acts as a surrogate for information encoded in the genome. CG methylation differs from these characters because of its potential for independent heritability: it is a covalent modification of DNA itself, and is a component of the set of interacting epigenetic modifications that form the epigenome (Klose and Bird, 2006). However, this data does not permit the inference that methylation states are independent of DNA sequence: our data are consistent with a full spectrum of epigenetic states that are obligate (determined by underlying DNA sequence), facilitated (influenced but not determined by sequence), or pure (sequence-independent) (Richards, 2006).

If methylation states are heritable and participate in evolution, they must be present in the germline. Methylated CGs undergo mutation to TG much more frequently than unmethylated CGs (Coulondre *et al.*, 1978); CG decay takes place in all cell types, but only CG decay that occurs in the germline results in heritable sequence changes that can become fixed within a species. (Sved and Bird, 1990). This phenomenon, known as CG decay, is responsible for the underrepresentation of CG dinucleotides in vertebrate genomes. We combined phylogenomic and somatic methylation data to infer germline methylation states. CG decay rates were higher in CG islands that are methylated in neutrophils of human, chimp, and orang, relative to CG islands that are not methylated in these species. The rate of CG decay that we infer for the germline methylated CG islands is consistent with estimates obtained in other studies (Nachman and Crowell, 2000; Arndt *et al.*, 2003; Kondrashov, 2003; Hwang and Green, 2004; Zhang *et al.*, 2007); however, in this case the availability of somatic methylation data allowed us to distinguish CG islands that are methylated in the germline from those that are not (Martin *et al.*, 2011). We also found evidence that CG islands whose somatic methylation differs in human and chimp are differentially methylated in the germline, supporting the idea that epigenetic events have contributed to the divergence of the two species, as discussed in this review. Using a purely computational approach, Tanay and colleagues found evidence of germline methylation in CG-rich regions of the human genome (Cohen *et al.*, 2011), although the regions they identified are in general very short and do not overlap with our set of CG islands.

The combination of phylogenomic and somatic methylation data provides only an indirect assessment of germline methylation, but direct characterization of germline methylation is difficult. The germline is not a single cell type: it is the lineage that gives rise to gametes, the gametes themselves, and the pluripotent cells of the early embryo. Most of these cell types are rare and inaccessible in primates. Sperm

is the only germline cell type that is readily accessible, but it is a highly differentiated cell with a peculiar chromatin structure, unlikely to be representative of the germline as a whole. We found little correlation between our data and a recent genome-wide analysis of sperm methylation in human and chimp (Molaro *et al.*, 2011), as well as with an older sperm dataset from Beck's group (Eckhardt *et al.*, 2006). However, we find good correlation between our data and genome-wide ES cell methylation (Lister *et al.*, 2009). It is important to note that the presence of methylation in sperm cannot be used to conclude that it is present in other cell types of the germline, nor can the absence of methylation in sperm be used to conclude the absence of methylation in the germline: the evidence of CG decay stands by itself, and where it conflicts with sperm methylation states it serves to emphasize that the germline is not a single cell type.

Conclusions

Stable phenotypic differences mediated by the epigenome have been considered a purely somatic phenomenon in animals, with epigenetic modifications reset to a base state during germ cell development (Feng *et al.*, 2010; Hajkova, 2011). But what is the base state? We have found evidence that some methylation states are sufficiently stable in the germline to be predictably transmitted within a species, and to distinguish human and chimpanzee. This satisfies one key requirement for epigenetics as a component of human evolution: the presence and maintenance of epigenetic states in the germline.

The finding of germline methylation is tantalizing; it raises the possibility that a substantial portion of the epigenome is heritable, but it does not present a clear path to clarify the relative importance of genomically encoded states versus purely epigenetic states. If one considers inheritance as a chromosomal phenomenon, it is possible that purely epigenetic variants associated with DNA, but not strictly dependent on its sequence, carry heritable information. However, while the presence of epigenetic states in the germline is a requirement for their inheritance, it does not demonstrate that they are inherited. The mechanisms by which epigenetic states are maintained in the germline are not clear: their molecular complexity makes it possible that some components are removed, but re-established based on information that is retained; elucidation of these processes will be necessary if we are to understand the contribution of pure epigenetic inheritance to species evolution.

Our CG decay analysis indicates that somatic methylation reflects the presence of methylation at some stage in the germline, supporting the conclusion that differences observed in somatic cells arise in the germline. This finding implies that the somatic potential of transcriptional regulatory elements might be constrained by epigenetic states that are present in the germline, in contrast to the view that somatic states are established by the execution of a genetic program during development. King and Wilson hypothesized that divergence of human and chimp is based on sequence changes in gene regulatory elements rather than protein-coding regions (King and Wilson, 1975). We suggest a twist on this idea: the genome contains a reservoir of sequences whose gene regulatory function is already established, but restricted to a limited number of somatic cell

types by germline methylation. Changes in the germline epigenetic state can alter somatic function of an element without any change in its DNA sequence (Fig. 1).

We have discussed a novel source of heritable variation, which could be genetically encoded, or purely epigenetic, or some combination of these. Genetic variation that is present in natural populations will always confound analysis and separation of the two sources of variation. At the least, our study provides an upper bound to the number of purely epigenetic variants in human and chimp, because it is likely that at least some of the variants that we have found are genetically encoded. An unbiased assessment of the extent of heritable variation will require working with isogenic populations, in which any genetic contribution to variation can be neglected. Efforts along this line have begun in plant systems (Johannes *et al.*, 2008, 2009; Becker *et al.*, 2011; Eichten *et al.*, 2011; Johannes and Colome-Tatche, 2011; Richards, 2011; Schmitz *et al.*, 2011), where evidence for an epigenetic component to variation and inheritance has always been more prominent than in animals, perhaps due to the lack of a segregated germline and the greater tolerance of plants for morphological variants. In metazoans, the problem is complicated by the great variety of highly differentiated cell types, with their distinct epigenomes, and the relative inaccessibility of the germline. The study of heredity has been dominated by Mendelian genetics, which is based on

FIG. 1. Model for the gain of an active state in somatic tissues following a change of epigenetic state in the germline. We assume that the germline state of certain transcriptional regulatory elements constrains the range of somatic cell types in which those elements will be active (the open elements, representing the ancestral state of an element, are active in a restricted set of cell types). A change in the germline epigenetic state of such an element (e.g., loss of methylation) will result in a broader range of somatic activity, so that the element is now active in cell types where it was silent in the ancestral species (the closed elements are active in more cell types). If germline epigenetic states can be faithfully transmitted between generations, such a change may create a heritable phenotypic difference. This model predicts that CpG islands whose methylation state has been found to deviate from the ancestral state will have activity in a broader range of somatic cell types than in the ancestral state, but this prediction has not yet been tested.

the faithful replication and transmission of DNA sequence. Epigenetic inheritance has a very different, and far more complex, molecular basis. The challenges of studying this form of heredity are only beginning to be recognized.

Acknowledgments

We thank Cath Suter and Jen Cropley for helpful comments and discussions. This work was supported by the NIH grants HL084474 (DB), ES016581 (DM), and CA115768 (DM).

Disclosure Statement

No competing financial interests exist.

References

- Amor, D.J., Bentley, K., Ryan, J., Perry, J., Wong, L., Slater, H., *et al.* (2004). Human centromere repositioning "in progress". *Proc Natl Acad Sci U S A* **101**, 6542–6547.
- Anway, M.D., Cupp, A.S., Uzumcu, M., and Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469.
- Arndt, P.F., Petrov, D.A., and Hwa, T. (2003). Distinct changes of genomic biases in nucleotide substitution at the time of Mammalian radiation. *Mol Biol Evol* **20**, 1887–1896.
- Becker, C., Hagmann, J., Muller, J., Koenig, D., Stegle, O., Borgwardt, K., *et al.* (2011). Spontaneous epigenetic variation in the Arabidopsis thaliana methylome. *Nature* **480**, 245–249.
- Belyaev, D.K., Ruvinsky, A.O., and Borodin, P.M. (1981). Inheritance of alternative states of the fused gene in mice. *J Hered* **72**, 107–112.
- Bernstein, B.E., Meissner, A., and Lander, E.S. (2007). The mammalian epigenome. *Cell* **128**, 669–681.
- Brink, R.A. (1956). A genetic change associated with the R locus in maize which is directed and potentially reversible. *Genetics* **41**, 872–889.
- Brink, R.A. (1960). Paramutation and chromosome organization. *Q Rev Biol* **35**, 120–137.
- Brink, R.A., Styles, E.D., and Axtell, J.D. (1968). Paramutation: directed genetic change. Paramutation occurs in somatic cells and heritably alters the functional state of a locus. *Science* **159**, 161–170.
- Cavalli, G., and Paro, R. (1998). The Drosophila Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* **93**, 505–518.
- Coe, E.H. (1959). A Regular and continuing conversion-type phenomenon at the B locus in maize. *Proc Natl Acad Sci U S A* **45**, 828–832.
- Cohen, N.M., Kenigsberg, E., and Tanay, A. (2011). Primate CpG islands are maintained by heterogeneous evolutionary regimes involving minimal selection. *Cell* **145**, 773–786.
- Coulondre, C., Miller, J.H., Farabaugh, P.J., and Gilbert, W. (1978). Molecular basis of base substitution hotspots in *Escherichia coli*. *Nature* **274**, 775–780.
- Cropley, J.E., Dang, T.H.Y., Martin, D.I.K., and Suter, C.M. (2012). The penetrance of an epigenetic trait is progressively yet reversibly increased by selection and environment. *Proc R Soc Lond B Biol Sci* **279**, 2347–2353.
- Crow, E.W., and Crow, J.F. (2002). 100 years ago: Walter Sutton and the chromosome theory of heredity. *Genetics* **160**, 1–4.
- Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V.K., Attwood, J., Burger, M., *et al.* (2006). DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* **38**, 1378–1385.
- Eichten, S.R., Swanson-Wagner, R.A., Schnable, J.C., Waters, A.J., Hermanson, P.J., Liu, S., *et al.* (2011). Heritable epigenetic variation among maize inbreds. *PLoS Genet* **7**, e1002372.
- Feng, S., Jacobsen, S.E., and Reik, W. (2010). Epigenetic reprogramming in plant and animal development. *Science* **330**, 622–627.
- Greer, E.L., Maures, T.J., Ucar, D., Hauswirth, A.G., Mancini, E., Lim, J.P., *et al.* (2011). Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* **479**, 365–371.
- Gu, S.G., Pak, J., Guang, S., Maniar, J.M., Kennedy, S., and Fire, A. (2012). Amplification of siRNA in *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. *Nat Genet* **44**, 157–164.
- Hadchouel, M., Farza, H., Simon, D., Tiollais, P., and Pourcel, C. (1987). Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with *de novo* methylation. *Nature* **329**, 454–456.
- Haig, D. (2006). Weismann Rules! OK? Epigenetics and the Lamarckian temptation. *Biol Philos* **22**, 415–428.
- Hajkova, P. (2011). Epigenetic reprogramming in the germline: towards the ground state of the epigenome. *Philos Trans R Soc Lond B Biol Sci* **366**, 2266–2273.
- Hawkins, R.D., Hon, G.C., Lee, L.K., Ngo, Q., Lister, R., Pelizzola, M., *et al.* (2010). Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell* **6**, 479–491.
- Hitchins, M.P., Wong, J.J., Suthers, G., Suter, C.M., Martin, D.I., Hawkins, N.J., *et al.* (2007). Inheritance of a cancer-associated MLH1 germ-line epimutation. *N Engl J Med* **356**, 697–705.
- Hwang, D.G., and Green, P. (2004). Bayesian Markov chain Monte Carlo sequence analysis reveals varying neutral substitution patterns in mammalian evolution. *Proc Natl Acad Sci U S A* **101**, 13994–14001.
- Jablonka, E., and Lamb, M.J. (1989). The inheritance of acquired epigenetic variations. *J Theor Biol* **139**, 69–83.
- Jirtle, R.L., and Skinner, M.K. (2007). Environmental epigenomics and disease susceptibility. *Nat Rev Genet* **8**, 253–262.
- Johannes, F., and Colome-Tatche, M. (2011). Quantitative epigenetics through epigenomic perturbation of isogenic lines. *Genetics* **188**, 215–227.
- Johannes, F., Colot, V., and Jansen, R.C. (2008). Epigenome dynamics: a quantitative genetics perspective. *Nat Rev Genet* **9**, 883–890.
- Johannes, F., Porcher, E., Teixeira, F.K., Saliba-Colombani, V., Simon, M., Agier, N., *et al.* (2009). Assessing the Impact of Transgenerational Epigenetic Variation on Complex Traits. *PLoS Genet* **5**, e1000530.
- King, M.C., and Wilson, A.C. (1975). Evolution at two levels in humans and chimpanzees. *Science* **188**, 107–116.
- Klose, R.J., and Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* **31**, 89–97.
- Kondrashov, A.S. (2003). Direct estimates of human per nucleotide mutation rates at 20 loci causing Mendelian diseases. *Hum Mutat* **21**, 12–27.
- Li, C.C.Y., Cropley, J.E., Cowley, M.J., Preiss, T., Martin, D.I.K., and Suter, C.M. (2011). A sustained dietary change increases epigenetic variation in isogenic mice. *PLoS Genet* **7**, e1001380.
- Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., *et al.* (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315–322.
- Marshall, O.J., Chueh, A.C., Wong, L.H., and Choo, K.H. (2008). Neocentromeres: new insights into centromere structure, disease development, and karyotype evolution. *Am J Hum Genet* **82**, 261–282.

- Martin, D.I., Singer, M., Dhahbi, J., Mao, G., Zhang, L., Schroth, G.P., *et al.* (2011). Phyloepigenomic comparison of great apes reveals a correlation between somatic and germline methylation states. *Genome Res* **21**, 2049–2057.
- Molaro, A., Hodges, E., Fang, F., Song, Q., McCombie, W.R., Hannon, G.J., *et al.* (2011). Sperm methylation profiles reveal features of epigenetic inheritance and evolution in primates. *Cell* **146**, 1029–1041.
- Morgan, H.D., Sutherland, H.G., Martin, D.I., and Whitelaw, E. (1999). Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* **23**, 314–318.
- Nachman, M.W., and Crowell, S.L. (2000). Estimate of the mutation rate per nucleotide in humans. *Genetics* **156**, 297–304.
- Negre, N., Brown, C.D., Ma, L., Bristow, C.A., Miller, S.W., Wagner, U., *et al.* (2011). A cis-regulatory map of the *Drosophila* genome. *Nature* **471**, 527–531.
- Pelizzola, M., and Ecker, J.R. (2011). The DNA methylome. *FEBS Lett* **585**, 1994–2000.
- Petronis, A. (2010). Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature* **465**, 721–727.
- Rakyan, V.K., Chong, S., Champ, M.E., Cuthbert, P.C., Morgan, H.D., Luu, K.V., *et al.* (2003). Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci U S A* **100**, 2538–2543.
- Richards, E.J. (2006). Inherited epigenetic variation—revisiting soft inheritance. *Nat Rev Genet* **7**, 395–401.
- Richards, E.J. (2011). Natural epigenetic variation in plant species: a view from the field. *Curr Opin Plant Biol* **14**, 204–209.
- Richards, C.L., Bosserdof, O., Pigliucci, M. (2010). What role does heritable epigenetic variation play in phenotypic evolution? *BioScience* **60**, 232–237.
- Schmitz, R.J., Schultz, M.D., Lewsey, M.G., O'Malley, R.C., Urlich, M.A., Libiger, O., *et al.* (2011). Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**, 369–373.
- Schwartz, Y.B., and Pirrotta, V. (2008). Polycomb complexes and epigenetic states. *Curr Opin Cell Biol* **20**, 266–273.
- Seong, K.H., Li, D., Shimizu, H., Nakamura, R., and Ishii, S. (2011). Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell* **145**, 1049–1061.
- Singer, M., Boffelli, D., Dhahbi, J., Schoenhuth, A., Schroth, G.P., Martin DIK, *et al.* (2010). MetMap Enables Genome-Scale Methylotyping for Determining Methylation States in Populations. *PLoS Comput Biol* **6**, e1000888.
- Skinner, M.K. (2011). Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. *Epigenetics* **6**, 838–842.
- Slatkin, M. (2009). Epigenetic inheritance and the missing heritability problem. *Genetics* **182**, 845–850.
- Smith, E., and Shilatifard, A. (2010). The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. *Molecular Cell* **40**, 689–701.
- Sollars, V., Lu, X., Xiao, L., Wang, X., Garfinkel, M.D., and Ruden, D.M. (2003). Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nat Genet* **33**, 70–74.
- Suter, C.M., Martin, D.I., and Ward, R.L. (2004). Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* **36**, 497–501.
- Sutherland, H.G., Kearns, M., Morgan, H.D., Headley, A.P., Morris, C., Martin, D.I., *et al.* (2000). Reactivation of heritably silenced gene expression in mice. *Mamm Genome* **11**, 347–355.
- Sved, J., and Bird, A. (1990). The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model. *Proc Natl Acad Sci U S A* **87**, 4692–4696.
- Tal, O., Kisd, E., and Jablonka, E. (2010). Epigenetic contribution to covariance between relatives. *Genetics* **184**, 1037–1050.
- Ventura, M., Weigl, S., Carbone, L., Cardone, M.F., Misceo, D., Teti, M., *et al.* (2004). Recurrent sites for new centromere seeding. *Genome Res* **14**, 1696–1703.
- Zhang, W., Bouffard, G.G., Wallace, S.S., and Bond, J.P. (2007). Estimation of DNA sequence context-dependent mutation rates using primate genomic sequences. *J Mol Evol* **65**, 207–214.

Address correspondence to:

David I.K. Martin, M.D.

Children's Hospital Oakland Research Institute

5700 Martin Luther King, Jr. Way

Oakland, CA 94609

E-mail: dimartin@chori.org

Received for publication January 31, 2012; received in revised form August 3, 2012; accepted August 6, 2012.