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To explore the potential evolutionary relevance of heritable epigenetic variation, the National Evolutionary Synthesis Center recently hosted a catalysis meeting that brought together molecular epigeneticists, experimental evolutionary ecologists, and theoretical population and quantitative geneticists working across a wide variety of systems. The group discussed the methods available to investigate epigenetic variation and epigenetic inheritance, and how to evaluate their importance for phenotypic evolution. We found that understanding the relevance of epigenetic effects in phenotypic evolution will require clearly delineating epigenetics within existing terminology and expanding research efforts into ecologically relevant circumstances across model and nonmodel organisms. In addition, a critical component of understanding epigenetics will be the development of new and current statistical approaches and expansion of quantitative and population genetic theory. Although the importance of heritable epigenetic effects on evolution is still under discussion, investigating them in the context of a multidisciplinary approach could transform the field.

Keywords: epigenetics, inheritance, maternal effects, National Evolutionary Synthesis Center, phenotypic plasticity

Our understanding of an organism's ability to respond to its environment has advanced dramatically during the last few decades, in large part through studies controlling for genotypic variation and manipulating environmental factors (Pigliucci 2001, van Kleunen and Fischer 2005, Valladares et al. 2007). These studies typically confirm not only that genotype and environment contribute to phenotypic variation but also that these two factors interact; that is, different genotypes often respond differently to environmental variation. For example, studies have shown that some species of plants from open habitats are more responsive to shading than plants from shaded habitat. The light quality triggers an elongation response and allows for competitive growth in plants from open habitats, whereas plants from shaded environments do not respond as strongly, since they will never grow taller than the canopy plants (Dudley and Schmitt 1995, Donohue et al. 2000, Weinig 2000). Similarly, tadpoles from habitats with a wide variety of predators have been shown to be more plastic in morphological response to predators than tadpoles that experience a more constant predator environment (Van Buskirk 2002).

Yet another consequential source of phenotypic variation in ecologically relevant traits is emerging from the molecular and developmental sciences, which are revealing the mechanisms of heritable epigenetic effects. These epigenetic effects include DNA methylation, histone modification, microRNA, small interfering RNA, spatial location of DNA, and chromatin matrix or scaffold attachment regions, as well as the three-dimensional templating mechanisms and self-sustaining loops found in microorganisms. Many of these are involved in the differentiation (i.e., mitotic inheritance) of cell lines through development, but all of these mechanisms have also been shown to trigger changes in gene expression and ultimately phenotype in the next generation, without variation in genotype (Finnegan 2002, Jablonka and Raz 2009). Studies ranging across biological systems from yeast (Levy and Siegel 2008) to plants (Fieldes et al. 2005, Salmon et al. 2005, 2008, Keyte et al. 2006, Molinier et al. 2006), animal development (Ruden 2005) and behavior (Crews et al. 2007, Crews 2008), and humans (Whitelaw and Whitelaw 2006) have found that epigenetic changes induced by hybridization or environmental stress may be inherited by future generations, and therefore could contribute to explaining adaptation to novel environments. Several recent reviews of the literature (a) explore how the concept of epigenetics contributes to the areas of ecology and evolution, and (b) emphasize the lack of studies on both natural epigenetic variation and on epigenetic effects in natural environments (Finnegan 2002, Rapp and Wendel 2005, Richards 2006, 2008, Bossdorf et al. 2008, Johannes et al. 2008, Jablonka and Raz 2009).

Ultimately, we wish to understand the role of epigenetic variation and epigenetic inheritance in the natural world. Specifically, we want to achieve a more detailed mechanistic

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understanding of these processes, and we want to know how important they are, relative to DNA sequence variation and inheritance, for the ecology and evolution of natural populations. To answer this question, however, it is necessary to study epigenetic processes from an integrative perspective, and to develop theoretical tools and statistical models to tease apart epigenetic and DNA sequence–based effects on phenotypic evolution. These goals can be accomplished only if three groups of researchers join forces: (1) molecular epigeneticists, (2) experimental evolutionary ecologists, and (3) theoretical population and quantitative geneticists. To this end, the National Evolutionary Synthesis Center (NESCent) in Durham, North Carolina, recently hosted a catalysis meeting titled "What role, if any, does heritable epigenetic variation play in phenotypic evolution?" The catalysis meeting brought several experts from these different groups together, allowing them to explore the phenomenon of inherited epigenetic variation from diverse angles, and to discuss options for (and also potential difficulties in) collaborative research to develop a broad agenda for ecological and evolutionary epigenetic research.

The group was characterized by people with experience in a wide variety of systems and approaches, a condition essential to a profitable discussion about (a) what methods are available to investigate epigenetic variation and inheritance, (b) how we can assess the relevance of epigenetic processes in natural populations, and (c) how we can separate DNA sequence–based effects from epigenetic effects and evaluate their relative importance for phenotypic evolution. Here we briefly summarize the emergent themes from the meeting.

Definitions of epigenetics and epigenetic inheritance

One issue that inevitably arose repeatedly during the NESCent meeting was how to define epigenetics and epigenetic inheritance, as there is still no widely accepted definition of the term "epigenetics." Its meaning in the last decades has shifted from a very broad definition of "the study of how the genotype translates into the phenotype" (developmental biology) to the "study of mitotically or meiotically heritable changes in gene function that cannot be explained by changes in DNA-sequence" (Jablonka and Lamb 2002, Richards 2006). However, while the latter definition is now accepted by large parts of the (molecular biological) scientific community, other uses of the term epigenetics, including the broad definition, are still common (see, e.g., Jablonka and Raz 2009).

A similar situation exists for the term "epigenetic inheritance," from the broadest possible definition, which includes all processes that are not specifically DNA sequence–based (including behavioral and cultural inheritance, as well as niche construction), to narrower conceptions that restrict epigenetic inheritance to cellular processes (including soma-to-soma transmission and a number of other biochemical processes that are not related to chromatin modifications; Jablonka and Raz 2009). Another important question is whether inheritance across cell lineages should also be referred to as epigenetic inheritance, or whether this term should be reserved for transgenerational phenomena only (Bossdorf et al. 2008, Jablonka and Raz 2009).

It remains to be seen whether the scientific community will eventually converge on a unique definition, or whether pluralistic concepts of epigenetics and epigenetic inheritance—analogous to, for instance, the idea of a pluralistic species concept (Mishler and Donoghue 1982)—will be more helpful. Defining epigenetics will certainly continue to be an area of debate, and it is obvious that as long as the terms epigenetics and epigenetic inheritance are used in different ways, one cannot use them without making clear which definition he or she employs. However, even with pluralistic concepts of epigenetics and epigenetic inheritance, we suggest that for multicellular organisms, the term "inheritance" should be used for transgenerational phenomena only, whereas "somatic transmission" can be used to describe cell-to-cell lineages; that is, mitotic inheritance that occurs during ontogeny. Of course, this dichotomy does not apply to unicellular organisms, where transgenerational inheritance is identical to cell-to-cell transmission. Indeed, there are possible areas of investigation where being able to go from lineage-to-lineage to transgenerational inheritance will be helpful—most obviously in the study of the evolutionary transition between uni- and multicellularity (Maynard Smith and Szathmáry 1995).

How is epigenetics related to phenotypic plasticity and maternal effects?

An important issue that came up during our meeting, and one that stirs discussion whenever ecologists and evolutionary biologists are introduced to the field of epigenetics, is how epigenetics and epigenetic inheritance are related to concepts such as phenotypic plasticity and maternal environmental effects—concepts that also deal with environmentally induced phenotypic variation or inheritance. In a general sense, both phenotypic plasticity and maternal effects may result from underlying epigenetic mechanisms that cause persistent phenotypic effects, either ontogenetic or transgenerational. How to tease apart the finer details of the relationships between these concepts depends on which definitions of epigenetics and epigenetic inheritance one uses.

Phenotypic plasticity is usually defined as the ability of a genotype to express different phenotypes in different environments (Pigliucci 2001). If the concept is restricted to reversible phenotypic changes that do not act across generations, then phenotypic plasticity is related to epigenetics only if the latter is defined in the broadest possible way; that is, if cell-to-cell inheritance is considered to be part of epigenetic inheritance. Of course there are developmental processes underlying phenotypic plasticity, and some of these will involve epigenetically controlled differentiation processes. Sometimes the concept of phenotypic plasticity is expanded to also include transgenerational phenotypic effects (e.g., Galloway and Etterson 2007), and the term "transgenerational plasticity" is often used interchangeably with that of maternal effects (e.g., Donohue and Schmitt 1998, Fox and Mousseau 1998).

Maternal environmental effects are phenomena where parents influence their offspring's phenotype in ways other than just passing on their nuclear genes. In the broadest sense, this includes influences of cytoplasmic genes, the endosperm, and a variety of maternal phenotypic effects based on structural, physiological or behavioral processes (Roach and Wulff 1987, Rossiter 1996, Crews et al. 2007, Crews 2008). As maternal effects are transgenerational, they can be related to epigenetics only if the latter is concerned with transgenerational effects. Obviously, there is a large potential overlap between maternal effects and epigenetic inheritance. For instance, if epigenetic inheritance is defined in the broadest possible way as all processes of inheritance that are not DNA sequence based, then all maternal phenotypic effects are also at the same time cases of epigenetic inheritance. If epigenetic inheritance is defined narrowly as the inheritance of chromatin variation, then the two concepts are related because part of the observed maternal phenotypic effects will be due to underlying mechanisms of epigenetic inheritance.

How to study the impact of epigenetic inheritance

Isolating the contribution of epigenetic effects to phenotypic variation can be approached in a wide variety of systems by using classic ecological genetics experimental designs conducted under controlled conditions, such as in the greenhouse or through reciprocal transplant studies in the field (Bossdorf et al. 2008). This approach traditionally allows for quantification of the contributions of genotype and environment, both of which must be controlled to additionally isolate the epigenetic component (Bossdorf et al. 2008). The potential importance of epigenetic effects can be explored by either manipulating the level of epigenetic effects (e.g., through the use of a demethylating agent such as 5-azacytidine [5-azaC] or endocrine-disrupting chemicals) or by exposing organisms to extreme environments that may trigger epigenetic changes that alter the phenotype of individuals with the same genotype. By growing the progeny of genetically identical individuals that have been exposed to different treatments in a common environment, a study can identify the contribution of heritable sources of phenotypic variation that are not based on DNA sequences. For example, methylation patterns and associated changes in early versus late flowering that resulted from 5-aza-C treatments of *Linum usitatissimum* persisted not only throughout the lifetime of the individual but also in lines that were five to nine generations beyond the treatment generation (Fieldes et al. 2005). Crews and colleagues (2007) showed that rat females exposed to endocrine-disrupting chemicals preferred unexposed males up to three generations after exposure. External temperature has been shown to change methylation patterns, which induce early flowering time in *Triticum* (Sherman and Talbert 2002) and *Arabidopsis* (Burn et al. 1993). Offspring of individuals exposed to these kinds of different treatments can then be grown in common environments to identify whether some of these environmentally induced differences are heritable and stable (Bossdorf et al. 2008, Johannes et al. 2008).

Some molecular tools have been developed for nonmodel systems that have no sequence data yet available. These include methylation-sensitive amplified fragment length polymorphisms (AFLP), which is similar to standard AFLP but measures methylation variation instead of DNA sequence variation at random markers in populations (Salmon et al. 2005, 2008, Keyte et al. 2006). Another approach is the use of high-performance liquid chromatography for detection of methyl-C to get a gross measure of genomewide methylation (Fieldes et al. 2005). For nonmodel systems for which there are some sequence data available, researchers can use methylation-sensitive gene probes combined with quantitative polymerase chain reaction to determine whether specific genes are methylated (as identified by homologues of model organisms). Next-generation sequencing of complementary DNA libraries could allow researchers to expand research on nonmodel systems beyond what is currently feasible (Vera et al. 2008). Using this technology, individuals grown under different conditions can be screened for differential expression to identify which genes are affected by the environment. Again, offspring of differently treated individuals that are grown in a common environment can be screened to identify differential gene expression that is inherited and which could result from an epigenetic effect (Bossdorf et al. 2008).

Model systems still provide the most power for assessing the importance of epigenetic effects in response to ecologically relevant scenarios. Model organisms can be used in the already-mentioned classic experimental designs in the field or greenhouse, but in addition, several genomics tools allow for a more mechanistic dissection of the epigenetic component of the response. For example, with a full genome sequence, researchers can use tiling arrays to identify which parts of the genome are methylated and how that varies across different genotypes (Vaughn et al. 2007, Zhang et al. 2008), or can develop recombinant inbred lines that are genetically identical but vary in which parts of the genome are methylated (Johannes et al. 2008, 2009, Reinders et al. 2009). These organisms and tools can be brought into an evolutionary ecological context and used to monitor changes that occur in response to different environments by replicating genotypes under the conditions of interest. Growing the offspring in a common environment will then reveal which of those epigenetic changes are stable and contribute to persistent phenotypic differences (Bossdorf et al. 2008). An important next step in understanding the evolutionary impacts of epigenetic effects will involve the development of quantitative genetic models and statistics that apply across taxa and incorporate epigenetic inheritance (Johannes et al. 2008, Richards 2008, Jablonka and Raz 2009).

Consequences of epigenetics for evolutionary theory

The obvious question for evolutionary biologists when contemplating new research on epigenetic inheritance is: So what? What exactly are the consequences of epigenetics for the way we see evolution? The participants in the NESCent workshop tackled this question from a variety

of perspectives, with an eye toward sketching practical empirical research programs for the near future.

An interesting feature that seems to be common (though not necessarily universal) in epigenetic heritable systems is that they are more labile (i.e., less stable) than their DNA sequence counterpart (Kalisz and Purugganan 2004, Vaughn et al. 2007, Johannes et al. 2008, 2009, Reinders et al. 2009), which implies higher mutation rates (Finnegan 2002, Kalisz and Purugganan 2004, Richards 2008, Zhang et al. 2008). These higher rates suggest the possibility of a two-track system of inheritance underlying phenotypic variation: A fast but unstable (epigenetic) track and a slow but stable (DNA sequence) one (Bossdorf et al. 2008). This may open up an obvious solution to a classic problem in population genetics: It is well known (Hartl and Clark 2007) that new advantageous mutations appearing in a population face an immediate evolutionary hurdle, in that they start at a very low frequency (depending on the population size) and can easily be lost by genetic drift. If, however, the new heritable variant is causally dependent on high-frequency (possibly environmentally induced) epigenetic variation, the novel phenotype may appear at a nonnegligible frequency from the onset, which would facilitate the role of natural selection in overcoming stochastic loss.

The scenario sketched above is consistent with West-Eberhard's (2003) suggestion that sometimes genes are "followers" rather than initiators of evolutionary change, meaning that they stabilize phenotypic changes that are started by epigenetic or developmental processes. Indeed, epigenetic inheritance systems could provide a reasonable mechanistic link between West-Eberhard's interesting but rather speculative suggestions about the role of developmental plasticity in evolution on one hand, and standard population genetic models of evolutionary change on the other.

The NESCent group also considered how population genetic theory can accommodate findings about epigenetic inheritance. The consensus was that the clear way to do this is through the addition of parameters formally equivalent to mutation and back-mutation rates, but ones characterized by much higher values (several orders of magnitude) than the standard rates (for a recent first attempt, see Slatkin 2009). Indeed, in the case of epigenetic inheritance, the high frequency of back mutation becomes a crucial engine of epigenetic dynamics and cannot be ignored in first approximation, as is often done with standard back-mutation frequencies (Hartl and Clark 2007). A more problematic aspect of incorporating epigenetics into population genetic theory needs to be considered when we wish to model the coevolution between the two tracks. From a mechanistic perspective, epigenetic phenomena such as methylation depend on the genes, since gene-coded enzymes are necessary for epigenetic processes to take place. By the same token, however, gene action—for instance transcription rate—is in turn affected by epigenetic processes, making the causality between the two systems bidirectional (Johannes et al. 2008). Current population genetic models do not incorporate anything like this sort of dynamic, and would need to be modified in novel ways to be able to do so.

In the case of quantitative genetic (i.e., more statistically based) models, epigenetic effects could be treated as additional parameters in an analysis of variance–type approach, alongside standard parameters such as effects due to genotype, environment, and genotype-by-environment interactions. The complication here is that a main epigenetic factor will then of course also bring to the model a slew of interaction factors (i.e., epigenotype-by-genotype, epigenotype-by-environment, and so on). While this does not represent a problem in terms of mathematical formalism, it would require formidably complex experimental designs to actually be able to reliably estimate such additional parameters (e.g., Wu et al. 2004, O'Hara et al. 2008).

Another major research question concerning the consequences of epigenetic inheritance systems on evolution is related to their connection, if any, with the issue of evolvability (Pigliucci 2008). The latter is often understood as a property of biological systems that facilitates the exploration of phenotypic space, and hence the evolutionary process itself. Epigenetic modifications have been linked with examples of "evolutionary capacitors" such as the Hsp90 stress-response system (Sollars et al. 2003) and prions (Brookfield 2001), themselves often invoked as candidate mechanisms for increased evolvability. More generally, however, the higher mutation levels of epigenetic markers can be seen—despite their lower long-term stability—as a factor accelerating the exploration of phenotypic space and augmenting the searching capability of natural selection, perhaps in a manner directly analogous to a similar role hypothesized for phenotypic plasticity, the feasibility of which has been confirmed by mathematical models (Borenstein et al. 2006).

There are several other intriguing potential consequences of epigenetic inheritance systems for our understanding of evolution, including a possible role in speciation (think of genetic incompatibility through imprinting; Jablonka and Lamb 1991) and an apparent causal link with the evolution of transposons (Finnegan 2002, Vaughn et al. 2007, Richards 2008), themselves likely involved both in speciation and the evolution of evolvability. Clearly, several intellectual horizons are vastly open here, and much empirical as well as theoretical work is awaiting the epigenetically inclined evolutionary biologist.

Conclusions

At this time in the science of epigenetics, the relevance of heritable epigenetic effects for the ecology and evolution of most organisms is still highly speculative. We assembled a unique group of scientists that was equipped with experience from a wide variety of systems and approaches to begin the discussion about what methods are available to investigate epigenetic variation and epigenetic inheritance in a broad array of organisms, and how we can evaluate their relative importance for phenotypic evolution.

We found that because of the many different uses of the terms epigenetics and epigenetic inheritance, it is extremely important to always make clear what definition one uses, and to avoid confusion among evolutionary biologists, we suggest the term "inheritance" should be reserved (in multicellular organisms) for transgenerational epigenetic phenomena. We also found that, depending on the definitions employed, there is a large conceptual overlap between the fields of epigenetics and maternal environmental effects, and, to a lesser extent, epigenetics and phenotypic plasticity.

Classic approaches to understanding sources of phenotypic variance can be used to quantify the importance of epigenetic effects, even in nonmodel organisms, but will typically require the rearing and phenotyping of offspring in a common environment. The rapidly advancing next-generation molecular technology promises to allow for investigating the mechanistic bases of epigenetic effects in a broader array of nonmodel organisms. We expect that this will transform the field in terms of available techniques though the development and refinement of new and current statistical approaches will also be necessary.

Finally, our group outlined some of the challenges faced by population geneticists in particular, and by evolutionary theorists more broadly, in tackling the more complex population dynamics that result from the explicit consideration of epigenetic inheritance systems in evolution. Basic population genetic theory, the mathematical backbone of evolutionary theory, can be expanded in novel directions, and relatively new concepts such as evolvability and the idea of genes as "followers" in the evolutionary process will become more viable candidates for an extension of the current paradigm in evolutionary studies.

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