Experimental evidence needed to demonstrate inter- and trans-generational effects of ancestral experiences in mammals

Brian G. Dias\(^1\)\(^2\)* and Kerry J. Ressler\(^1\)\(^2\)\(^3\)

Environmental factors routinely influence an organism’s biology. The inheritance or transmission of such influences to descendant generations would be an efficient mode of information transfer across generations. The developmental stage at which a specific environment is encountered by the ancestral generation, and the number of generations over which information about that environment is registered, determines an inter- vs. trans-generational effect of ancestral influence. This commentary will outline the distinction between these influences. While seductive in principle, inter- and trans-generational inheritance in mammals is a hotly debated area of research inquiry. We present constructive criticism of such inheritance, and suggest potential experimental avenues for reconciliation. Finally, epigenetic mechanisms present an avenue for gene regulation that is dynamic. We briefly discuss how such malleability affords the potential for a reversal of any detrimental environmental influences that might have adversely impacted ancestral or descendant generations.

Keywords: epigenetics; inheritance; non-coding RNA; olfaction

Adapting to changing environments is often critical for survival. Events such as malnourishment, childhood maltreatment, terrorist attacks, and war violence have been shown to profoundly affect the exposed generation. Examples of the influence of ancestral environmental perturbations on descendant biology abound and are accumulating. For example, exposure to famine during the Dutch Hunger Winter of 1944 profoundly affected the F1 generation gestating in utero at the time that the F0 generation was subjected to famine conditions. Notably, that next, F1, generation went on to have a higher propensity for developing diabetes and obesity. What is striking is that this F1 generation, despite now living in non-impoverished conditions, gave birth to an F2 generation that also suffered from obesity and diabetes [1]. In another example, the more recent 9/11 terrorist attacks in New York saw the detection of lower cortisol levels in the 1-year old offspring that were gestating in utero at the time that their mothers witnessed the attacks [2]. Lower cortisol levels are predictive of PTSD-like symptoms, across generations, as has been shown in the case of adult offspring of Holocaust survivors [3]. We now possess the tools to begin to ask how such ancestral experiences could influence subsequent generations [4, 5].

When discussing the influence of ancestral environments on descendant generations, distinctions between inter- and trans-generational influences must be made (Fig. 1). Inheritance implies information transfer via the germ-line (sperm and eggs); a phenomenon that cannot be disentangled if the germ cells of the descendant generations are themselves affected by the ancestral environment. The perturbation in the ancestral environment of pregnant females was also experienced by the descendant generation, in utero, and as such presents an example of inter-generational inheritance of ancestral exposures in the F1 and F2 generations. This is because of the in utero nature of the ancestral perturbation affecting not only the somatic and germ cells of the developing F1 fetus, but also the germ cells of the F2 generation. Effects must also be observed in the F3 generation to

---

1) Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA
2) Yerkes National Primate Research Center, Atlanta, GA, USA
3) Howard Hughes Medical Institute, Chevy Chase, MD, USA

*Corresponding author: Brian G. Dias
E-mail: bdias@emory.edu

DOI 10.1002/bies.201400105
be considered trans-generational. In contrast, if the paternal environment were altered, any consequent effects on the F1 generation would be considered inter-generational, while persistence into the F2 generation would be considered true trans-generational inheritance. The persistence of any effects in the F2 generation would be considered true trans-generational inheritance. In the case of the maternal environment being altered during gestation, or early peri-natal environments like maternal care being manipulated, a trans-generational effect should survive into the F3 generation.

Figure 1. Inter- and trans-generational influences of ancestral environments in descendant populations are often investigated by manipulating the environment of A: the paternal ancestor, B: a pregnant maternal ancestor, or C: a peri-natal population. Exposing the paternal ancestor to an environmental perturbation affects not only the ancestor but also his sperm and as a consequence the F1 generation in an inter-generational manner. The persistence of any effects in the F2 generation would be considered true trans-generational inheritance. In the case of the maternal environment being altered during gestation, or early peri-natal environments like maternal care being manipulated, a trans-generational effect should survive into the F3 generation.

Having talked about the observations of inter- and trans-generational effects of ancestral experiences, the distinction between those two effects and a broad scientific discipline that is thought to underlie such observations, how might we leverage animal studies to model and investigate such effects? To do so, animal researchers have typically subjected the ancestral population (F0) to a manipulation and then asked how the descendant generations are affected.

An eloquent example of the impact of maternal care quality on descendant biology has been highlighted by the work of Szyf, Meaney and colleagues [11, 12]. Taking advantage of naturally occurring variation in the maternal care exhibited by female rats post-parturition, these researchers have shown that higher levels of good maternal care at post-natal time-points causes the female rat pups to show the same high quality care toward their own offspring when they care for them. A cross-fostering strategy shows that this phe-

Figure 1. Inter- and trans-generational influences of ancestral environments in descendant populations are often investigated by manipulating the environment of A: the paternal ancestor, B: a pregnant maternal ancestor, or C: a peri-natal population. Exposing the paternal ancestor to an environmental perturbation affects not only the ancestor but also his sperm and as a consequence the F1 generation in an inter-generational manner. The persistence of any effects in the F2 generation would be considered true trans-generational inheritance. In the case of the maternal environment being altered during gestation, or early peri-natal environments like maternal care being manipulated, a trans-generational effect should survive into the F3 generation.

Having talked about the observations of inter- and trans-generational effects of ancestral experiences, the distinction between those two effects and a broad scientific discipline that is thought to underlie such observations, how might we leverage animal studies to model and investigate such effects? To do so, animal researchers have typically subjected the ancestral population (F0) to a manipulation and then asked how the descendant generations are affected.

An eloquent example of the impact of maternal care quality on descendant biology has been highlighted by the work of Szyf, Meaney and colleagues [11, 12]. Taking advantage of naturally occurring variation in the maternal care exhibited by female rats post-parturition, these researchers have shown that higher levels of good maternal care at post-natal time-points causes the female rat pups to show the same high quality care toward their own offspring when they care for them. A cross-fostering strategy shows that this phe-

Figure 1. Inter- and trans-generational influences of ancestral environments in descendant populations are often investigated by manipulating the environment of A: the paternal ancestor, B: a pregnant maternal ancestor, or C: a peri-natal population. Exposing the paternal ancestor to an environmental perturbation affects not only the ancestor but also his sperm and as a consequence the F1 generation in an inter-generational manner. The persistence of any effects in the F2 generation would be considered true trans-generational inheritance. In the case of the maternal environment being altered during gestation, or early peri-natal environments like maternal care being manipulated, a trans-generational effect should survive into the F3 generation.
social transmission of behavioral traits across generations. But note that it is distinctively different from inheritance. This distinction notwithstanding, follow-up studies using this model have provided insight into how alterations of DNA methylation around the glucocorticoid receptor gene in the hippocampus and the estrogen receptor in diverse brain regions profoundly affect behavior and physiology [12].

A model first generated by Michael Skinner’s group exemplifies the transgenerational influence of ancestral experience on descendant behavior. In this model, the exposure of pregnant female rats (F0 generation) to the fungicide, Vincozolin, has been shown to affect male fertility, and mate preference of subsequent generations [13, 14]. Among more recent works that document an inter- or trans-generational inheritance of ancestral experience, rodent F0 generations have been subjected to maltreatment during rearing, social defeat, and stress during adulthood [15–17]. All these studies have demonstrated that the ancestral environment affects anxiety- and depressive-like states in descendant generations. These examples utilize broad perturbations to the ancestral generation and query the inter- and trans-generational effects in the descendants.

Asking how manipulations of specific features of the ancestral environment affect the descendant generations is a useful way to focus effort on where and how in the (epi)genome might the effects of ancestral experience reside. An ecological example of this comes from the work in which the diet of a pregnant mouse female was supplemented with “cherry” or “mint” odors. This resulted in the descendant F1 generation showing a preference for those odors. Accompanying this behavioral preference was increased volumes those odors. Accompanying this behavioral preference was increased volumes

There are also the recent data indicating the barrier that exists between the germ cells and circulation. Weismann’s theory of the germplasm talks about the barrier that exists between the germ cells and circulation. This suggests that there exists an immunization of the germ cells from any environmental information that the somatic cells might have been privy to. However, the discovery of small RNA species such as microRNA, piRNA, and tRNA-derived RNA fragments (trFs) at high levels in sperm [22–25], and that of cargo-containing exosomes traveling through circulation [26] present potential conduits between the environment and the germ-cells. In keeping with this idea, miRNA have been shown to be involved in the trans-generational inheritance of the Kit phenotype [10]. There are also the recent data indicating that an increase in non-coding RNA in
sperm of a manipulated F0 generation results in an inter-generational effect in the descendants [27]. The question of how any marked loci escape epigenetic reprogramming that occurs soon after fertilization and then again in the primordial germ cells of the developing fetus is a trickier one to answer. Parent-of-origin-allele-specific resistance to reprogramming provides evidence for escape of genetic loci from epigenetic reprogramming [28, 29]. As more epi- and molecular genetic mechanisms of reprogramming come to light, more molecular candidates would come to light as avenues by which this epigenetic reprogramming might be escaped at genetic loci that have been tagged to be salient imprints of ancestral experience.

While one should certainly question this field of research and revise our impression of it as more light is shone on its mechanisms, one should also appreciate the phenomenology of inter- and trans-generational inheritance and the potential dynamic nature of epigenetic modifications that might underlie it. Just as one environment is laying down epigenetic marks at a specific genetic locus, so could another environment strip those marks or lay down an antagonistic set of marks at that same locus. Such malleability in the control of gene expression could well be harnessed to ameliorate the effects of detrimental influences of the ancestral environment, and examination of such processes will be an important practical trajectory of future research.

That an ancestral environment can influence descendant generations is slowly being accepted and debated in light of the accumulating evidence [30]. The mechanisms by which this occurs, and any potential avenues by which descendant generations could be buffered from such influences will undoubtedly see a plethora of continued scientific debate and inquiry.

Acknowledgments
Funding for [20] was provided by the Howard Hughes Medical Institute and the Burroughs Wellcome Fund to K. J. R. In addition, this project was partially funded by the National Center for Research Resources P51RR000165 and is currently supported by the Office of Research Infrastructure Programs/OD P51OD011132 to Yerkes National Primate Research Center.

The authors have declared no conflict of interest.

References