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Transposable elements and small RNAs: Genomic fuel for species diversity

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While transposable elements (TE) have long been suspected of involvement in species diversification, identifying specific roles has been difficult. We recently found evidence of TE-derived regulatory RNAs in a species-rich family of bats. The TE-derived small RNAs are temporally associated with the burst of species diversification, suggesting that they may have been involved in the processes that led to the diversification. In this commentary, we expand on the ideas that were briefly touched upon in that manuscript. Specifically, we suggest avenues of research that may help to identify the roles that TEs may play in perturbing regulatory pathways. Such research endeavors may serve to inform evolutionary biologists of the ways that TEs have influenced the genomic and taxonomic diversity around us.

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Introduction

For an organism to expand to a new ecological niche, the tools to cope with the stresses inherent to such a change must first be present as heritable variation in the genome. Thus, on the most basic level, our understanding of evolutionary process must be informed by an understanding of the mechanisms that introduce genomic variation. A long standing goal of this research is to assess the relative impact of mutations in genes related to structure and function vs. those associated with changes to regulatory regions,[1,2] as either are capable of inducing phenotypic changes and promoting biological diversity.[3]

Transposable Elements (TEs), genomic parasites with the ability to make copies of themselves in the host genome, have been shown to contribute to function and regulatory variation via their ability to insert in coding and regulatory regions. TEs are powerful mutagens that can influence gene expression via the introduction of alternative regulatory elements, exons, and splice junctions.[4-11] However, TEs need not be actively mobilizing to have an effect on genome structure. TEs mediate genome rearrangements through non-homologous recombination[12-14] and have been implicated in chromosomal rearrangements in plants and animals. In fact, deletions, duplications, inversions, translocations and chromosome breaks have all been linked to the presence of TEs in a variety of genomes.[15-20]

With all of these realized and potential impacts, TEs have long been seen as sources of genome instability, and more specifically, TEs have been seen as potential players in the speciation process. Indeed, multiple hypotheses have been proposed over the past 5 years that directly implicate TEs in one way or another. These hypotheses range from 1) the suggestion that differences among taxa with regard to intragenomic populations of TEs are established primarily by chance as a consequence of founder effects and drift as populations subdivide[21] to 2) Zeh's[22] ideas that environmental stressors trigger TE expansions within genomes, leading to punctuated equilibrium-like patterns, to 3) the TE-Thrust Hypothesis of Oliver and Green,[23,24] which implicates TE's

more broadly in the process of diversification.

These ideas are not completely novel, as Furano,[25] Kazazian,[7,26] and Feschotte[27] all postulated that TEs might be intimately involved in mechanisms of diversification. Rather, these more recent works formalized particular hypotheses that can now be tested rigorously, taking advantage of the ever-expanding genome data available.

In particular, the potential impact of TE insertions to modify gene regulatory pathways is an area of research that is gaining substantial interest.[27] This is where the impact of our recent manuscript lies.[28] While Feschotte and others have mentioned several potential impacts of TEs on regulatory pathways, one of these was the ability of TEs to serve as sources of novel miRNAs that may act as post-transcriptional regulators.[27,29-31] Unfortunately, we still know very little of how TE-derived miRNAs can affect global gene expression patterns. Thus, the relationship between TE-derived miRNAs and species diversification needs to be examined in a wide range of organisms, to better understand the roles of TEs and miRNAs in speciation and identify examples where the functional consequences of TE-derived miRNA diversity can be examined in detail.

Part of the challenge has been the lack of tractable systems to examine the interrelationships among post-transcriptional regulation, species diversification and TEs. Fortunately, bats in the family Vespertilionidae provide such a model. These bats are exceptional among mammals in that they have experienced multiple waves of Class II TE (aka DNA transposon) activity over the past 30–40 my which are coincident with their evolutionary radiation.[32-35] In this regard, one genus in particular, *Myotis*, has been more fecund than all others in the family, generating approximately one third of the ~400 species in the family within the last ~30 my.

We asked whether there was any link between the diversification of these taxa, the observed burst of TE activity, and changes in the numbers of TE-derived miRNAs. We found large numbers of miRNAs expressed in the testes of one vesper bat, *Eptesicus fuscus*, were derived from the TE family most active during the early evolutionary history of the family. Indeed when compared to 2 other mammals, the differences were striking. Over 85% of the miRNAs that originated in the common ancestor of *Myotis* and *Eptesicus* are derived from transposable elements compared to only 25% and 17% in dog and horse, respectively. Furthermore, DNA transposons made up the majority of the TE-derived miRNAs. Strikingly, the deposition of these miRNAs was contemporaneous with the diversification of vesper bats into the plethora of clades currently observed, suggesting a causal or, at least, explanatory relationship. To confirm the causal nature of this relationship, the next step will be to determine whether the functional consequences of TE-derived miRNA diversity can be linked directly to a mechanistic understanding of their role in species diversification. Thomas et al.[36] recently made some advances in that direction, demonstrating the impact of TEs on potentially functional aspects of genome structure, but much work remains to be done.

In systems such as the vesper bats, we suggest testing specific hypotheses of how TE-derived miRNAs may drive divergence in gene expression. This will necessarily require RNA-Seq data and the identification of particular gene targets related to species-specific differences. One potential avenue to pursue would be dietary preference. Although many, and perhaps most, vespertilionid bats are considered generalist insectivores[37,38] there are species that may provide examples of dietary variation sufficient for detecting differences in gene expression. Several species of *Myotis* including *M. vivesi*,[39] *M. capaccinii*,[40] *M. ricketti*,[41] and *M. adversus*[42] include fish in their diet, although the relative dietary importance is unclear (i.e. the species may be primarily insectivorous, and only occasionally piscivorous). A better option may be species that prey on migratory birds, such as *Nyctalus lasiopterus*,[43] *Nyctalus aviator*,[44] or *Ia io*,[45] where up to 70% of individuals prey on birds during peak migration seasons.[43] Even more dramatically, as a facultative nectarivore *Antrozous pallidus* is the only known Vespertilionid to include plant material in the diet.[46] Compared to insectivorous diets, the inclusion of vertebrate prey (fish or birds) or nectar may present altered gene expression patterns. Due to differences in lipid and protein composition of vertebrate prey, enzymes related to lipid or protein metabolism would likely shift to allow for the dietary switch.

On a more subtle level, there is the potential to identify differences more broadly. For example, many species (e.g., *Euderma maculatum, Barbastella barbastellus*) are considered moth specialists with echolocation strategies adapted to avoid detection by eared moths.[47,48] Other species specialize on hard bodied arthropods, as is the case for beetle specialists such as *Eptesicus fuscus*[49] or *Myotis myotis*.[50] Comparison of these 2 broad groups of Vespertilionid bats suggests a potential gene target with species-specific expression patterns. Chitin is an important structural polymer in insects[51] that was previously thought to be indigestible.[52] More recently, chitinolytic activity has been demonstrated for insectivorous bats from both bacteria in the digestive tract[53] and chitinase produced by the bats themselves.[54] All vespertilionids tested (7 species) produced acidic mammalian chitinase (AMCase), which may provide a target gene for comparing expression between moth and beetle specialists. Insect chitin content varies among species and developmental stages (1.1–16.2% of whole dry body weight).[55,56] Thus upregulation of AMCase may be required in species with a high chitin diet.

We need not limit ourselves to mammals, however. There are several other taxa that may also serve as laboratories for this kind of study. One of the most obvious examples to us is the lizard genus *Anolis*, which, like *Myotis*, is species rich. Indeed, even more so, with ~400 species occupying a vast array of habitats in the Caribbean and southeastern North America. Also like *Myotis, Anolis* has experienced a huge diversity of TE activity in the recent past.[57-60] There are multiple examples of these lizards being introduced by humans to novel habitats,[61] which suggests numerous opportunities for adaptive change that could be investigated. In one example, a native anole, *Anolis carolinensis*, in response to invasive *Anolis sagrei*, adaptively evolved larger toepads, presumably

to cope with the pressure of moving to higher perches.[62] While the molecular mechanism of this adaptive change has not been determined, it could conceivably be due to regulatory changes, which in turn could be the result of TE activity.

Insects also provide a plethora of potential study systems. One prime example exists in the butterflies of genus *Heliconius*. The TE landscape of these mimetic insects was recently characterized and the same family of elements that contributed a majority of the novel miRNAs to the vesper bats (Helitrons) has also been exceptionally active in these butterflies.[63,64] It is conceivable that the introduction of large numbers of novel miRNAs via Helitrons or other TEs may have played a role in the remarkable adaptive radiation of wing colors and the ability of these butterflies to rapidly evolve to match their co-mimetic species.[65]

While these studies may represent a good start, there are numerous other aspects of TE-small RNA interactions that also deserve attention. An important observation is that TE-derived miRNAs often show sex and tissue specific expression patterns. For instance, in mice there are piRNAs that are expressed only in the male testes.[66] EsiRNAs, the female equivalent, are expressed only in the female ovaries and also appear to be TE-derived. These piRNA and esiRNAs repertoires are distinct and probably derive from different sets of TEs. Therefore, these small RNAs are essentially "shutting down" sex-specific TE activity. The key is that these TEs are only being shut down in the sex expressing the suppressing small RNA, but in the other sex, those same TEs are presumably still actively mobilizing. This suggests there might be sex-specific TE activity and small RNA expression shaping genomic change during species diversification.

Sex and tissue specific TE activity may be an important mechanism of lineage diversification. Perhaps the most striking example of sex-specific activity is the silk moth (*Bombyx mori*), where the key sex determination switch is a TE-derived piRNA.[67] However, these patterns of sex-specific TE activity are likely biologically widespread and probably have major impacts on the evolution of sex chromosomes and sex-specific gene expression. For example, in the female germline of XY organisms, the X chromosome has ½ the copy numbers of any autosome, and therefore TEs with female limited activity should receive only ½ the number of TE insertions as an autosome. Similarly, the Y and W chromosome would only accumulate TE insertions that occurred in the male germline, similar to that observed during the evolution of the silk moth W chromosome and sex-determining miRNA.[67] These differences in the accumulation of TEs between the sexes could contribute to the rapid accumulation of genetic incompatibilities on the sex chromosomes and the large effect of the X chromosome on speciation.[68] Incorporating sex-specific miRNA expression and TE activity into models of TE and species diversification provides a previously lacking connection to mechanisms of speciation.

Clearly, variation in TEs and small RNAs can be potential sources for lineage specific divergence in gene expression. Collectively, these studies suggest that in vertebrates and invertebrates the interplay between TE activity and small RNA evolution can be an important source of the regulatory variation underlying adaptive divergence and speciation. We encourage researchers to investigate these connections to increase our understanding of the TE-diversity link.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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