Transposable elements and small RNAs: Genomic fuel for species diversity

Federico G Hoffmann[1,2], Liam P McGuire[3], Brian A Counterman[4], and David A Ray[3,*]

[1]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology; Mississippi State University; Mississippi State, MS USA; [2]Institute for Genomics, Biocomputing, and Biotechnology; Mississippi State University; Mississippi State, MS USA; [3]Department of Biological Sciences; Texas Tech University; Lubbock, TX USA; [4]Department of Biological Sciences; Mississippi State University; Mississippi State, MS USA

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.

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.

References

1. Hoekstra HE, Coyne JA. The locus of evolution: evo devo and the genetics of adaptation. Evolution 2007; 61:995-1016; PMID:17492956; http://dx.doi.org/10.1111/j.1558-5646.2007.00105.x
2. King MC, Wilson AC. Evolution at two levels in humans and chimpanzees. Science 1975; 188:107-16; PMID:1090005; http://dx.doi.org/10.1126/science.1090005
3. Wolf JBW, Lindell J, Backstrom N. Speciation genetics: current status and evolving approaches. Philos T R Soc B 2010; 365:1717-33; http://dx.doi.org/10.1098/rstb.2010.0023
4. Babushok DV, Ostertag EM, Kazazian HH, Jr. Current topics in genome evolution: molecular mechanisms of new gene formation. Cell Mol Life Sci 2007; 64:542-54; PMID:17192808; http://dx.doi.org/10.1007/s00018-006-6453-4
5. Hasler J, Samuelsson T, Strub K. Useful 'junk': Alu RNAs in the human transcriptome. Cell Mol Life Sci 2007; 64:1793-800; http://dx.doi.org/10.1007/s00018-007-7084-0
6. Jurka J. Origin and evolution of *Alu* repetitive elements. In: Maraia RJ, ed. Impact of short interspersed elements (SINEs) on the host genome. Austin, TX: Landes Company, 1995:25-41
7. Kazazian HH, Jr. Mobile elements: drivers of genome evolution. Science 2004; 303:1626-32; PMID:15016989; http://dx.doi.org/10.1126/science.1089670
8. Matlik K, Redik K, Speek M. L1 antisense promoter drives tissue-specific transcription of human genes. J Biomed Biotechnol 2006; 2006:71753; PMID:16877819; http://dx.doi.org/10.1155/JBB/2006/71753
9. Nigumann P, Redik K, Matlik K, Speek M. Many human genes are transcribed from the antisense promoter of L1 retrotransposon. Genomics 2002; 79:628-34; PMID:11991712; http://dx.doi.org/10.1006/geno.2002.6758
10. Peaston AE, Evsikov AV, Graber JH, de Vries WN, Holbrook AE, Solter D, Knowles BB. Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. Dev Cell 2004; 7:597-606; PMID:15469847; http://dx.doi.org/10.1016/j.devcel.2004.09.004
11. Speek M. Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. Mol Cell Biol 2001; 21:1973-85; PMID:11238933; http://dx.doi.org/10.1128/MCB.21.6.1973-1985.2001
12. Batzer MA, Deininger PL. *Alu* repeats and human genomic diversity. Nat Rev Genet 2002; 3:370-9; PMID:11988762; http://dx.doi.org/10.1038/nrg798
13. Eichler EE, Sankoff D. Structural dynamics of eukaryotic chromosome evolution. Science 2003; 301:793-7; PMID:12907789; http://dx.doi.org/10.1126/science.1086132
14. Lonnig WE, Saedler H. Chromosome rearrangements and transposable elements. Annu Rev Genet 2002; 36:389-410; PMID:12429698; http://dx.doi.org/10.1146/annurev.genet.36.040202.092802
15. Caceres M, Ranz JM, Barbadilla A, Long M, Ruiz A. Generation of a widespread drosophila inversion by a transposable element. Science 1999; 285:415-8; PMID:10411506; http://dx.doi.org/10.1126/science.285.5426.415
16. Gray YH. It takes two transposons to tango: transposable-element-mediated chromosomal rearrangements. Trends Genet 2000; 16:461-8; PMID:11050333; http://dx.doi.org/10.1016/S0168-9525(00)02104-1
17. Lim JK, Simmons MJ. Gross chromosome rearrangements mediated by transposable elements in Drosophila melanogaster. Bioessays 1994; 16:269-75; PMID:8031304; http://dx.doi.org/10.1002/bies.950160410
18. Mathiopoulos KD, della Torre A, Predazzi V, Petrarca V, Coluzzi M. Cloning of inversion breakpoints in the Anopheles gambiae complex traces a transposable element at the inversion junction. Proc Natl Acad Sci U S A 1998; 95:12444-9; PMID:9770505; http://dx.doi.org/10.1073/pnas.95.21.12444
19. Weil CF, Wessler SR. Molecular evidence that chromosome breakage by Ds elements is caused by aberrant transposition. Plant Cell 1993; 5:515-22; PMID:8390878; http://dx.doi.org/10.1105/tpc.5.5.515
20. Zhang J, Peterson T. Transposition of reversed Ac element ends generates chromosome rearrangements in maize. Genetics 2004; 167:1929-37; PMID:15342530; http://dx.doi.org/10.1534/genetics.103.026229
21. Jurka J, Bao W, Kojima KK. Families of transposable elements, population structure and the origin of species. Biol Direct 2011; 6:44; PMID:21929767; http://dx.doi.org/10.1186/1745-6150-6-44
22. Zeh DW, Zeh JA, Ishida Y. Transposable elements and an epigenetic basis for punctuated equilibria. Bioessays 2009; PMID:19472370

23. Oliver KR, Greene WK. Mobile DNA and the TE-Thrust hypothesis: supporting evidence from the primates. Mobile DNA 2011; 2:8; PMID:21627776; http://dx.doi.org/10.1186/1759-8753-2-8

24. Oliver KR, Greene WK. Transposable elements and viruses as factors in adaptation and evolution: an expansion and strengthening of the TE-Thrust hypothesis. Ecol Evol 2012; 2:2912-33; PMID:23170223; http://dx.doi.org/10.1002/ece3.400

25. Furano AV, Hayward BE, Chevret P, Catzeflis F, Usdin K. Amplification of the ancient murine Lx family of long interspersed repeated DNA occurred during the murine radiation. J Mol Evol 1994; 38:18-27; PMID:8151711; http://dx.doi.org/10.1007/BF00175491

26. Kazazian HH, Jr., Goodier JL. LINE drive. retrotransposition and genome instability. Cell 2002; 110:277-80; PMID:12176313; http://dx.doi.org/10.1016/S0092-8674(02)00868-1

27. Feschotte C. Transposable elements and the evolution of regulatory networks. Nat Rev Genet 2008; 9:397-405; PMID:18368054; http://dx.doi.org/10.1038/nrg2337

28. Platt RN, Vandewege MW, Kern C, Schmidt CJ, Hoffmann FG, Ray DA. Large numbers of novel miRNAs originate from DNA transposons and are coincident with a large species radiation in bats. Mol Biol Evol 2014; 31:1536-45; PMID:24692655; http://dx.doi.org/10.1093/molbev/msu112

29. Borchert GM, Holdon NW, Williams JD, Heman WL, Bishop IP, Dembosky JA, Elste JE, Gregoire NS, Kim JA, Koehler WW, et al. Comprehensive analysis of microRNA genomic loci identifies pervasive repetitive-element origins. Mobile Genet Elements 2011; 1:8-17; PMID:22016841; http://dx.doi.org/10.4161/mge.1.1.15766

30. Roberts JT, Cardin SE, Borchert GM. Burgeoning evidence indicates that microRNAs were initially formed from transposable element sequences. Mobile Genet Elements 2014; 4:e29255; PMID:25054081; http://dx.doi.org/10.4161/mge.29255

31. Roberts JT, Cooper EA, Favreau CJ, Howell JS, Lane LG, Mills JE, Newman DC, Perry TJ, Russell ME, Wallace BM, et al. Continuing analysis of microRNA origins: Formation from transposable element insertions and noncoding RNA mutations. Mobile Genet Elements 2013; 3:e27755; PMID:24475369; http://dx.doi.org/10.4161/mge.27755

32. Pritham EJ, Feschotte C. Massive amplification of rolling-circle transposons in the lineage of the bat *Myotis lucifugus*. Proc Natl Acad Sci U S A 2007; 17:422-32

33. Ray DA, Feschotte C, Pagan HJ, Smith JD, Pritham EJ, Arensburger P, Atkinson PW, Craig NL. Multiple waves of recent DNA transposon activity in the bat, *Myotis lucifugus*. Genome Res 2008; 18:717-28; PMID:18340040; http://dx.doi.org/10.1101/gr.071886.107

34. Ray DA, Pagan HJT, Thompson ML, Stevens RD. Bats with hATs: evidence for recent DNA transposon activity in genus myotis. Mol Biol Evol 2007; 24:632-9; PMID:17150974; http://dx.doi.org/10.1093/molbev/msl192

35. Thomas J, Sorourian M, Ray D, Baker RJ, Pritham EJ. The limited distribution of Helitrons to vesper bats supports horizontal transfer. Gene 2011; 474:52-8; PMID:21193022; http://dx.doi.org/10.1016/j.gene.2010.12.007

36. Thomas J, Phillips CD, Baker RJ, Pritham EJ. Rolling-circle transposons catalyze genomic innovation in a mammalian lineage. Genome Biol Evol 2014; 6:2595-

37. 610; PMID:25223768; http://dx.doi.org/10.1093/gbe/evu204

37. Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PDN. Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey. Mol Ecol 2009; 18:2532-42; PMID:19457192; http://dx.doi.org/10.1111/j.1365-294X.2009.04184.x

38. Clare EL, Barber BR, Sweeney BW, Hebert PDN, Fenton MB. Eating local: influences of habitat on the diet of little brown bats (Myotis lucifugus). Mol Ecol 2011; 20:1772-80; PMID:21366747; http://dx.doi.org/10.1111/j.1365-294X.2011.05040.x

39. Blood BR, Clark MK. *Myotis vivesi*. Mammalian Species 1998; 588:1-5; http://dx.doi.org/10.2307/3504455

40. Dietz C, von Helversen O, Nill D. Bats of Britain, Europe, and Nortwest Africa. London: A&C Black Publishers Ltd., 2009

41. Ma J, Jones G, Zhang SY, Shen JX, Metzner W, Zhang LB, Liang B. Dietary analysis confirms that Rickett's big-footed bat (Myotis ricketti) is a piscivore. J Zool 2003; 261:245-8; http://dx.doi.org/10.1017/S095283690300414X

42. Robson SK. Myotis adversus (Chiroptera: Vespertilionidae): Australia's fish-eating bat. Aust Mammal 1984; 7:51-2

43. Ibanez C, Juste J, Garcia-Mudarra JL, Agirre-Mendi PT. Bat predation on nocturnally migrating birds. Proc Natl Acad Sci U S A 2001; 98:9700-2; PMID:11493689; http://dx.doi.org/10.1073/pnas.171140598

44. Fukui D, Dewa H, Katsuta S, Sato A. Bird predation by the birdlike noctule in Japan. J Mammal 2013; 94:657-61; http://dx.doi.org/10.1644/12-MAMM-A-172.1

45. Thabah A, Li G, Wang YN, Liang B, Hu KL, Zhang SY, Jones G. Diet, echolocation calls, and phylogenetic affinities of the great evening bat (Ia io; Vespertilionidae): another carnivorous bat. J Mammal 2007; 88:728-35; http://dx.doi.org/10.1644/06-MAMM-A-167R1.1

46. Frick WF, Heady PA, Hayes JP. Facultative Nectar-feeding behavior in a gleaning insectivorous bat (Antrozous Pallidus). J Mammal 2009; 90:1157-64; http://dx.doi.org/10.1644/09-MAMM-A-001.1

47. Fullard JH, Dawson JW. The echolocation calls of the spotted bat Euderma maculatum are relatively inaudible to moths. J Exp Biol 1997; 200:129-37; PMID:9317482

48. Goerlitz HR, Ter Hofstede HM, Zeale MRK, Jones G, Holderied MW. An aerial-hawking bat uses stealth echolocation to counter moth hearing. Curr Biol 2010; 20:1568-72; http://dx.doi.org/10.1016/j.cub.2010.08.057

49. Agosta SJ, Morton D, Kuhn KM. Feeding ecology of the bat Eptesicus fuscus: 'preferred' prey abundance as one factor influencing prey selection and diet breadth. J Zool 2003; 260:169-77; http://dx.doi.org/10.1017/S0952836903003601

50. Arlettaz R. Feeding behaviour and foraging strategy of free-living mouse-eared bats, Myotis myotis and Myotis blythii. Anim Behav 1996; 51:1-11; http://dx.doi.org/10.1006/anbe.1996.0001

51. Merzendorfer H. Insect chitin synthases: a review. J Comp Physiol B 2006; 176:1-15; PMID:16075270; http://dx.doi.org/10.1007/s00360-005-0005-3

52. Bell GP. Birds and mammals on an insect diet: a primer on diet composition analysis in relation to ecological energetics. Studies in Avian Biology 1990; 13:416-22

53. Whitaker JO Jr., Dannelly HK, Prentice DA. Chitinase activity in insectivorous bats. J Mammal 2004; 85:15-8; http://dx.doi.org/10.1644/1545-1542(2004)085%3c0015:CIIB%3e2.0.CO;2

54. Strobel S, Roswag A, Becker NI, Trenczek TE, Encarnacao JA. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. PloS one 2013; 8:e72770; PMID:24019876

55. Cauchie HM. Chitin production by arthropods in the hydrosphere. Hydrobiologia 2002; 470:63-96; http://dx.doi.org/10.1023/A:1015615819301

56. Finke MD. Estimate of chitin in raw whole insects. Zoo Biol 2007; 26:105-15; PMID:19360565; http://dx.doi.org/10.1002/zoo.20123

57. Alfoldi J, Di Palma F, Grabherr M, Williams C, Kong L, Mauceli E, Russell P, Lowe CB, Glor RE, Jaffe JD, et al. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature 2011; 477:587-91; PMID:21881562; http://dx.doi.org/10.1038/nature10390

58. Novick P, Smith J, Ray D, Boissinot S. Independent and parallel lateral transfer of DNA transposons in tetrapod genomes. Gene 2010; 449:85-94; PMID:19747963; http://dx.doi.org/10.1016/j.gene.2009.08.017

59. Novick PA, Basta H, Floumanhaft M, McClure MA, Boissinot S. The evolutionary dynamics of autonomous non-LTR retrotransposons in the lizard Anolis carolinensis shows more similarity to fish than mammals. Mol Biol Evol 2009; 26:1811-22; PMID:19420048; http://dx.doi.org/10.1093/molbev/msp090

60. Novick PA, Smith JD, Floumanhaft M, Ray DA, Boissinot S. The evolution and diversity of DNA transposons in the genome of the Lizard Anolis carolinensis. Genome Biol Evol 2011; 3:1-14; PMID:21127169; http://dx.doi.org/10.1093/gbe/evq080

61. Campbell T. The Brown Anole (Anolis sagrei Dumeril and Bibron 1837). The Institute for Biological Invasions: The Invader of the Month 2002

62. Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ, Losos JB. Rapid evolution of a native species following invasion by a congener. Science 2014; 346:463-6; PMID:25342801; http://dx.doi.org/10.1126/science.1257008

63. Coates BS, Hellmich RL, Grant DM, Abel CA. Mobilizing the genome of lepidoptera through novel sequence gains and end creation by non-autonomous Lep1 helitrons. DNA Res 2012; 19:11-21; PMID:22086996; http://dx.doi.org/10.1093/dnares/dsr038

64. Lavoie CA, Platt RN, Novick PA, Counterman BA, Ray DA. Transposable element evolution in Heliconius suggests genome diversity within Lepidoptera. Mobile DNA 2013; 4; PMID:24088337; http://dx.doi.org/10.1186/1759-8753-4-21

65. Supple M, Papa R, Counterman BA, McMillan WO. The genomics of an adaptive radiation – insights across the *Heliconius* speciation continuum. In: Landry C, Aubin-Horth N, eds. Ecological Genomics. New York: Springer, 2014

66. Chuma S, Nakano T. piRNA and spermatogenesis in mice. Philos T R Soc B 2013; 368:20110338; PMID:23166399

67. Kiuchi T, Koga H, Kawamoto M, Shoji K, Sakai H, Arai Y, Ishihara G, Kawaoka S, Sugano S, Shimada T, et al. A single female-specific piRNA is the primary determiner of sex in the silkworm. Nature 2014; 509:633-6; PMID:24828047; http://dx.doi.org/10.1038/nature13315

68. Coyne JA, Orr HA. Two rules of speciation. In: Otte D, Endler JA, eds. Speciation and its consequences. Sunderland, MA: Sinauer, 1989:180-207