

What processes drive speciation at whole-genome scales: Synergistic interactions of intra- and interchromosomal coupling produce genome-wide barriers to gene flow in a rattlesnake hybrid zone

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Abstract

A fundamental goal of biology is to understand the mechanisms that promote reproductive isolation and drive the generation and maintenance of species. Theory predicts that progress towards speciation involves the aggregate effects of direct and indirect selection across a multitude of loci that collectively produce a strong genome-wide barrier¹. Genomic coupling is the result of the interaction of selection to keep conspecific alleles together and recombination, which inherently disrupt linkage². Through coupling, genome-wide genetic associations are predicted to be the underlying mechanism by which reproductive isolation builds up within and across chromosomes and can eventually lead to genome congealing and completely prevent any gene flow between species². Here, we leveraged a rattlesnake hybrid zone and developed an approach to assess genome-wide coupling and investigate the synergistic interactions of intra- and interchromosomal linkage disequilibrium. We show evidence of a complex multi-locus barrier strengthened by coupling, involving incompatibilities related to venom resistance, which illustrate the mechanism by which coupling is predicted to lead to speciation.

Approach

- We sampled 118 rattlesnakes from a hybrid zone in New Mexico between *Crotalus viridis* and *Crotalus scutulatus*.
- We generated re-sequenced whole genomes for all sampled individuals.
- Those genomes were used to develop an approach to investigate genomic coupling
 - We used *bgc-hm*³ to measure cline variance.
 - Genomic coupling can be defined as the ration of selection for conspecific alleles and recombination (s/r)⁴.
 - Using a simulation-derived linear formula, we estimated the coupling coefficient for 100kb windows through the genome based on cline variance.
- We investigated the relation between coupling and other genome-wide inferences related to selection and linkage such as
 - Recombination rate
 - Genomic cline slope (indicative of selection^{1,4})
 - Interchromosomal linkage disequilibrium (LD) biased towards *C. scutulatus* (i.e., LD between *C. scutulatus* alleles that show resistance to introgression within the hybrid zone)
- Finally, we tested genes within regions in LD for functional enrichment

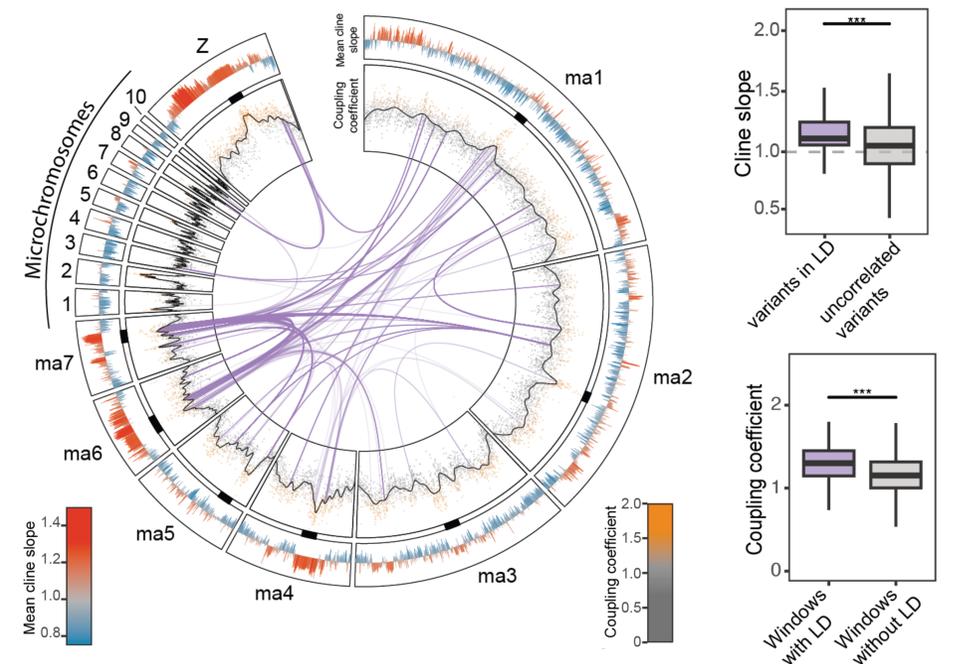


Fig 2. Genome-wide buildup of *Crotalus scutulatus* associations. The links in the center of the circle plot are showing interchromosomal associations (Linkage Disequilibrium) for SNPs biased towards *C. scutulatus*. The outer and inner tracks respectively display the average cline slope and the coupling coefficient for 100kb windows. The boxplots on the right show the distribution of cline slope and coupling coefficient for regions in LD versus uncorrelated regions.

Enrichment for ion channel genes

- We found 324 genes within 100kb of variants in interchromosomal *C. scutulatus* LD.
- We tested this gene set for functional enrichment in order to test if this interchromosomal interaction is driven by functional enrichment.
- Using STRING-predicted interactions, we found large network of interacting genes that includes 22 genes related to ion channel activity.
- Several of these 22 genes are a direct target of a PLA₂ neurotoxin (crotoxin) specific to *C. scutulatus*⁵.
- This suggests that there is a venom self-resistance driven incompatibility

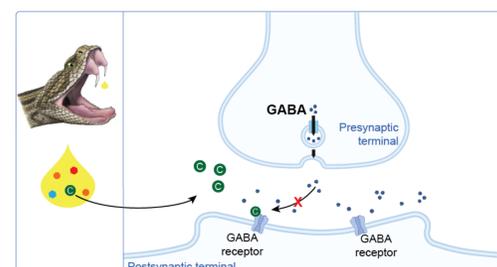


Fig 3. Mode of action of crotoxin. Crotoxin bind to GABA receptor and prevents the binding of the GABA neurotransmitter.



Fig 4. Network of genes in the *Crotalus scutulatus* LD network. STRING-predicted interaction between genes found in *C. scutulatus* LD. colored dots represent genes that are part of up to four gene sets functionally related to ion channels.

LD and coupling between ion channel genes and crotoxin

- To further demonstrate the link between ion channel genes and the *C. scutulatus* specific neurotoxin, we analyzed LD between the ion channel loci and the PLA₂ locus containing the crotoxin.
- We found that 9 out of 22 ion channel loci were located in genomic regions associated with elevated PLA₂ locus LD and intrachromosomal coupling.
- This further shows that coupling of venom toxins and their ion channel targets is likely driven by selection to avoid incompatibilities related to self-resistance to venom.
- This example provides evidence for associations related to key adaptive barrier traits being further strengthened into aggregate barrier effects through coupling, which can eventually lead to complete RI.

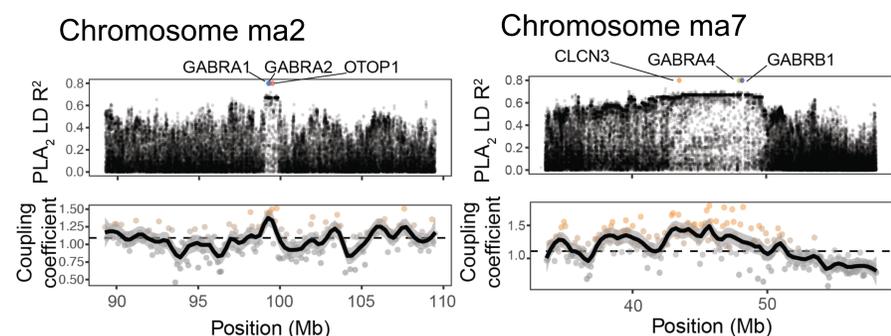


Fig 5. PLA₂ locus LD and coupling coefficient in ion channel loci. Top panels show LD between the PLA₂ locus and variants within and surrounding ion channel genes. The intrachromosomal coupling coefficient for these genomic regions is displayed in the bottom panels.

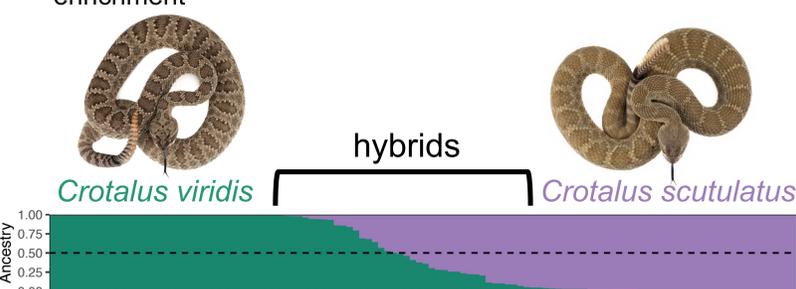


Fig 1. Parental ancestry of sampled rattlesnakes. Overall genomic ancestry of all sampled rattlesnakes within and surrounding the hybrid zone.

Buildup of associations within and across chromosomes in hybrid rattlesnakes.

- We assessed linkage disequilibrium (LD) between *C. scutulatus* biased variants in hybrid rattlesnakes.
- Variants in *C. scutulatus* LD are associated with elevated intrachromosomal coupling and cline slope.
- This indicates a synergistic interaction between intra- and interchromosomal associations across the genome.
- Overall, these interactions produce a genome-wide reproductive barrier, that manifest as associations in the genomes of hybrid individuals

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