GENE/GENOME EDITING

How it's relevant to comparative biologists

Kate Montana

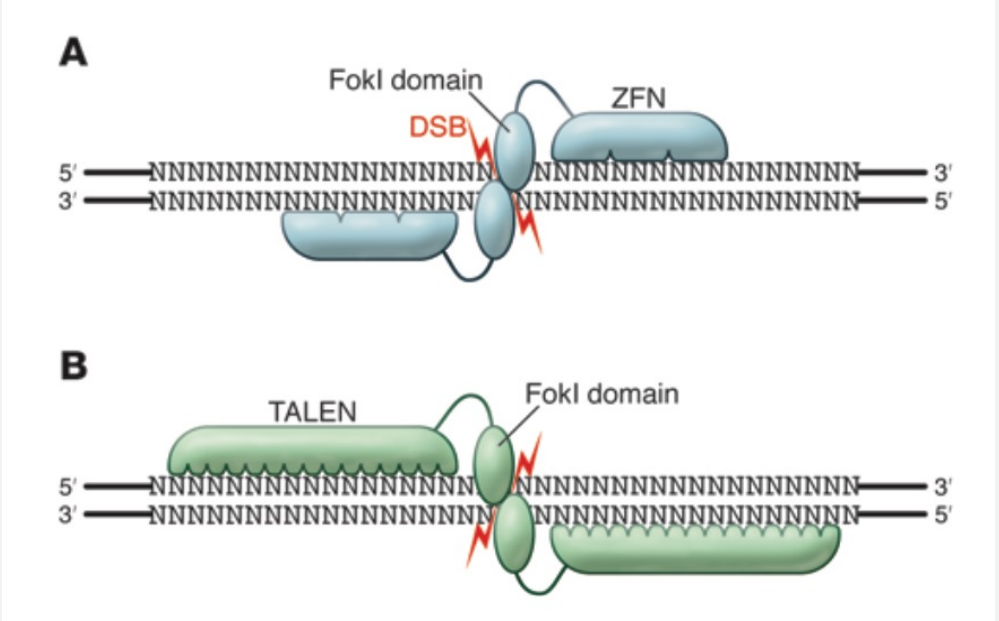
Comparative Genomics 2

MY QUESTIONS

- How did genome editing start?
- What is CRISPR-Cas9?
- How can these techniques be relevant to us as evolutionary biologists?

EARLY APPROACHES

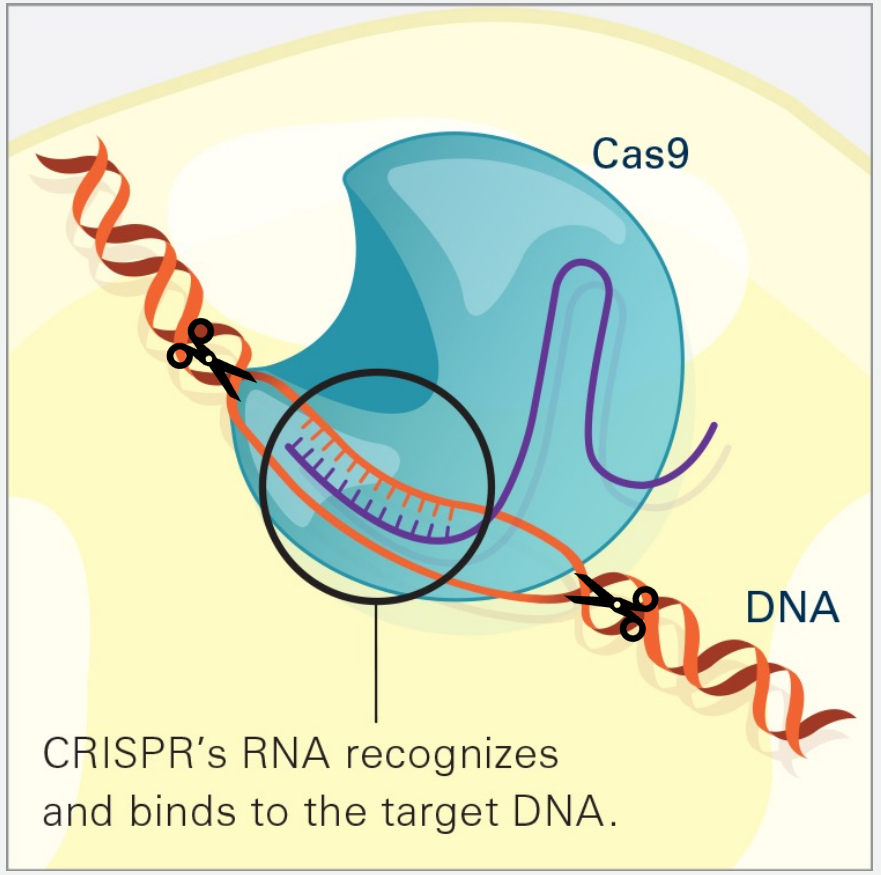
- Facilitated homologous recombination
- 1st gen zinc finger nucleases (ZFNs)
- 2nd gen transcription activator-like effector nucleases (TALENs)
- Both have danger of off-target effects



Gupta & Musunuru (2014)

CRISPR-CAS9

- Cas9 is developed from *Streptococcus pyogenes* adaptive immune defense
- Developed for mammals
- Clustered regularly interspersed tandem repeats
- System consists of Cas9 protein which holds
  - Guide RNA
  - PAMs
- NHEJ or HDR synthesize new DNA at the site



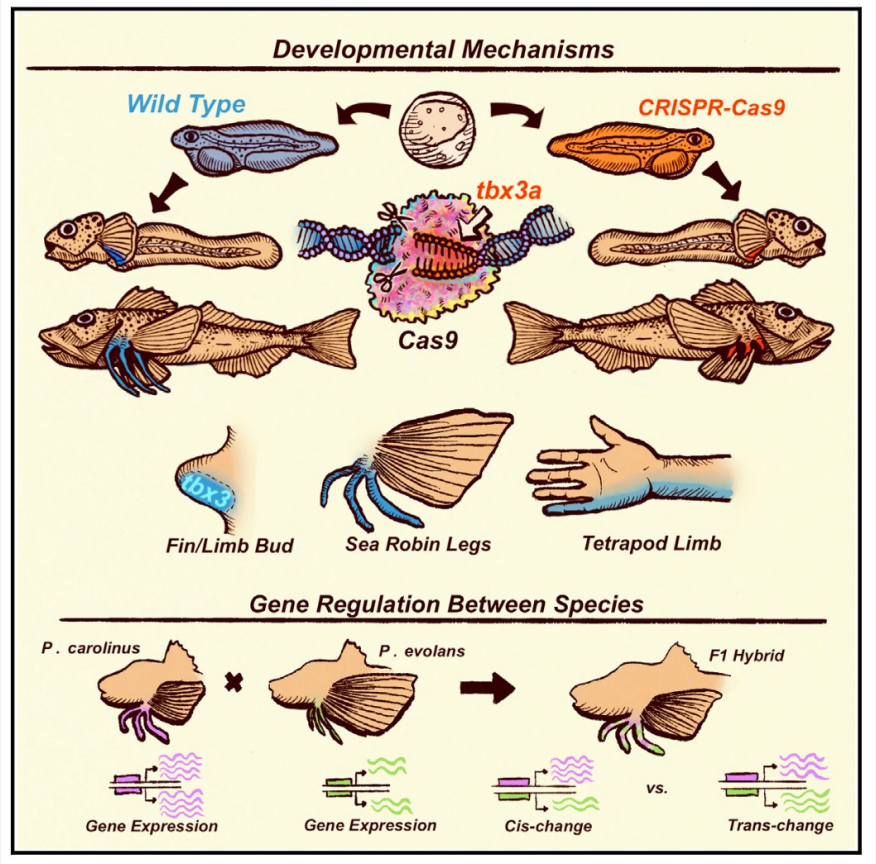
NIGMS/NIH, edited

APPLICATIONS AND CONSIDERATIONS

- Cheaper and more efficient than other genome editing techniques
- CRISPR changes are heritable
- New CRISPR technologies like Cpf1 and C2c2 are becoming available
- Can apply to gene therapy, pathogen disruption, agriculture, and many other fields
    - Lots of ethical considerations

EVO DEVO

- Example of application relevant to us
- Compare fish with extra limbs vs. CPRISPR-removed limbs
- ID'd *tbx3a* as top differentially expressed gene when compared to pectoral fins
- Homology between walking fish fins and tetrapod limbs



Herbert et al. (2024)

Progress and Prospects of CRISPR/Cas Systems in Insects and Other Arthropods

Dan Sun[1,2†], Zhaojiang Guo[2†], Yong Liu[1] and Youjun Zhang[2]*

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- Cas9 might not be best option for arthropods
  - Smaller, more fragile eggs

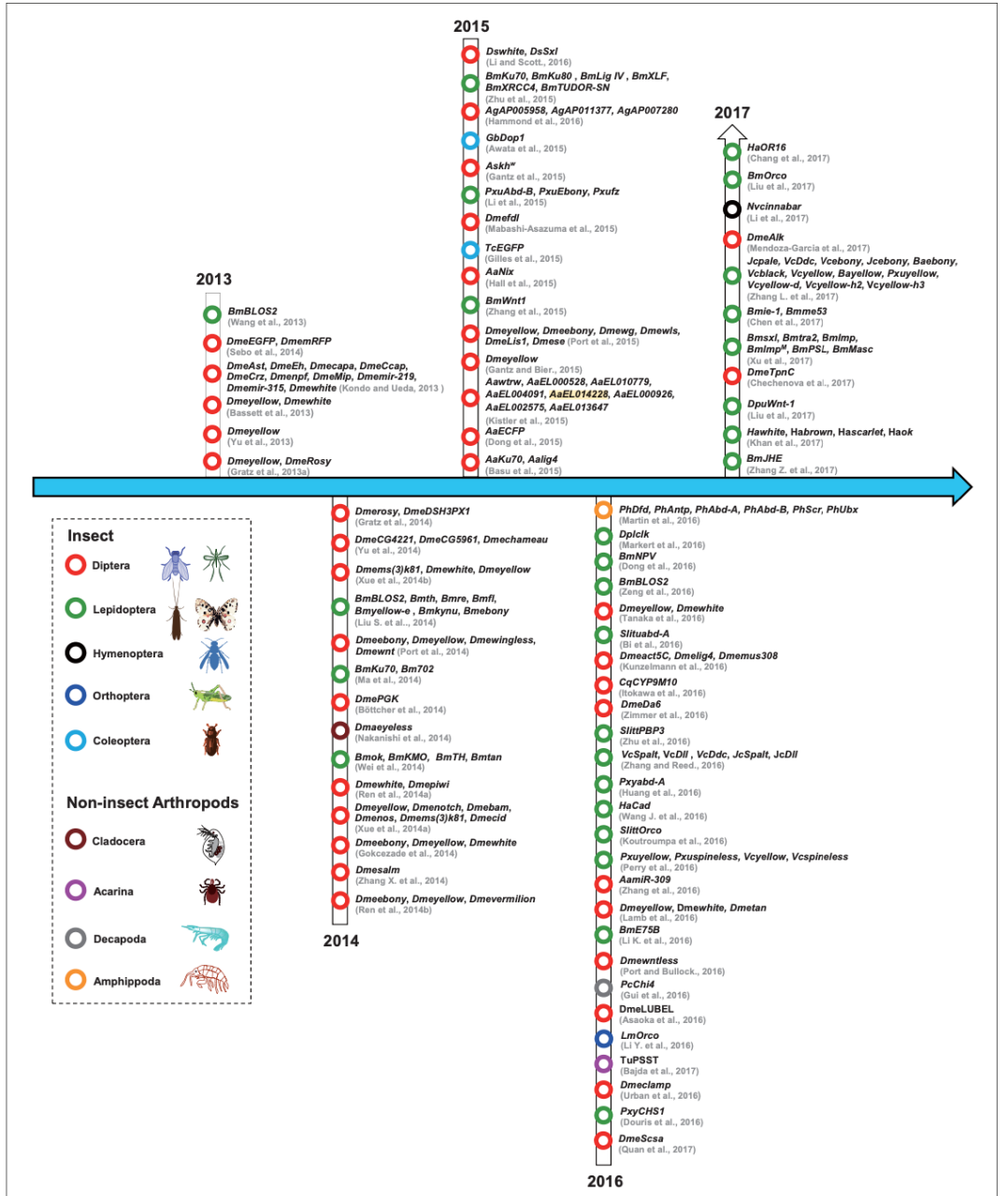

FIGURE 2 | The history of the use of CRISPR/Cas9 technology in arthropods. The dotted lines indicate different arthropod classifications. Red circles represent Diptera; black circles represent Hymenoptera; dark blue circles represent Orthoptera; cambridge blue circles represent Coleoptera; gray circle represents Decapoda; *(Continued)*

CRISPR AS EDUCATIONAL TOOL

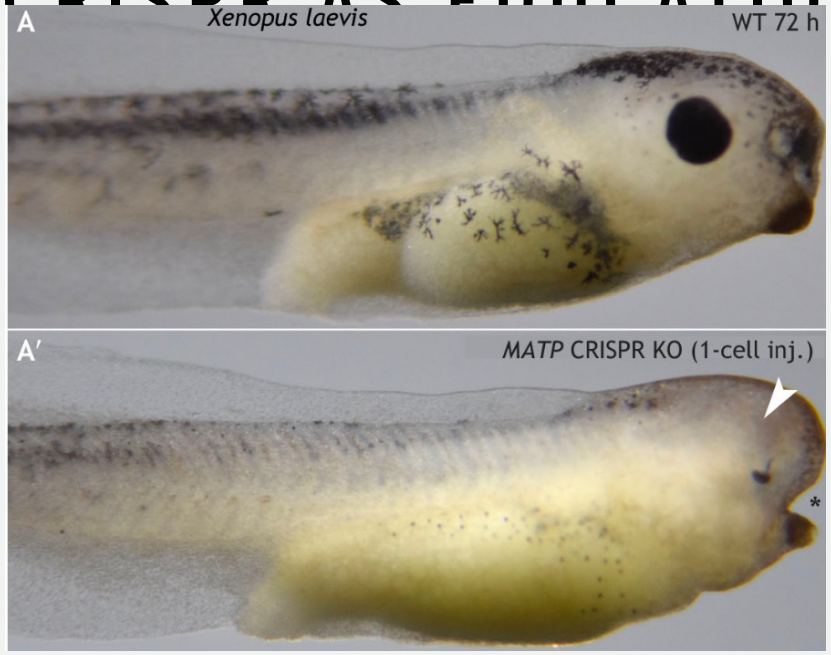

Table 2. Gene targets used for student assignment on designing sgRNAs

Protein name	Species	GenBank ID	Species for TBLASTN analysis
Abdominal-A	*Bombyx mori*	ACD10794	*V. cardui*
Distal-less	*Bicyclus anynana*	AAL69325	*V. cardui*
Membrane-associated transporter protein (SLC45A2/MATP/OCA4)	*Homo sapiens*	AIK67168	*X. laevis*
Optix	*Heliconius erato*	AEO13434.1	*V. cardui*
Tyrosinase (Tyr-a+Tyr-b)	*Homo sapiens*	NP_000363.1	*X. laevis*
WntA	*Heliconius erato*	AFC75683	*V. cardui*

Martin et al. (2020)