**Doudna JA, Charpentier E, The new frontier of genome engineering with CRISPR-Cas9, Science 346: 1258096, 2014.**

This review paper outlines that major innovations in CRISPR-Cas9 gene editing technology, in the two years since its publication in 2012. CRISPR-Cas9 is an example of site-specific editing, which means you can change a very specific place in the genome. Earlier examples of geneome-editing techniques included zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), proteins that, when combined with another enzyme called FokI, could function as a site-specific nucleases, thereby generating DNA at specific points that the researcher wants to target. These were difficult to produce, however, so CRISPR-Cas9 has taken off.

CRISPRs are genetic loci with plasmid and viral origins: clustered regularly interspaced palindromic repeats. The cas part of the name indicates genes that are CRISPR-associated and code for helicase or nuclease domains. They are naturally occurring parts of genomes associated with immune response. CRISPR-Cas9, specifically, is a gene that codes for a large protein that is thought to be associated with resistance to viral invasion via cleaving existing DNA. In tandem, single guide RNA (sgRNA), which is a human-synthesized RNA based on combining two naturally occurring RNAs, guides the protein to the specific DNA sequence that the researcher wants to cleave. Protospacer adjacent motifs (PAMs) are short sequences that are critical for Cas9 binding to existing DNA.

CRISPR-Cas9 has incredible implications for editing human genomes for medicinal purposes, but it also can be used for “systematic analysis of gene functions.” Knocking out particular genes is a sure way to see how the loss of that gene affects a biological process in an organism. Engineering directed evolution of cell lines is also possible with CRISPR-Cas9.

**Gupta RM, Musunuru K, Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9, The Journal of Clinical Investigation 124: 4154, 2014.**

Similarly to Doudna & Charpentier (2014), this is a review paper that outlines the history of genome editing and the major technologies. Facilitated homologous recombination is the earliest example of gene editing. ZFNs are outlined as fusion proteins that recognize a 3- to 4-base pair DNA sequence with tandem FokI domains that cleave the DNA. TALENs come from plant pathogens and are tandem array genes that fuse to FokI domains to cleave DNA similarly to ZFNs. In both cases, the damaged DNA is repaired via nonhomologous end joining (NHEJ), or homology-directed repair (HDR). CRISPR-Cas9 technology is also outlined similarly to above.

**Herbert AL, Allard CAH, McCoy MJ, Wucherpfennig JI, Krueger SP, Chen HI, Gourlay AN, Jackson KD, Abbo LA, Bennett SH, Sears JD, Rhyne AL, Bellono NW, Kingsley DM, Ancient developmental genes underlie evolutionary novelties in walking fish, Current Biology 34: 4339-4348.e6, 2024.**

This paper is a terrific example of using genome editing technology (CRISPR-Cas9) to ask questions about organism development in an evolutionary context (EvoDevo). Researchers sought to identify the genetic mechanisms related to limb development in a curious fish species, the northern sea robin *Priotonus carolinus*, that has limbs for swimming, walking, and digging. Via knock-out of gene *tbx3a*, the study identifies this gene as important for limb development and differentiation between limbs and pectoral fins. Mutations in tetrapod tbx3a also have an effect on limb development.

**Martin A, Wolcott NS, O’Connell LA, Bringing immersive science to undergraduate laboratory courses using CRISPR gene knockouts in frogs and butterflies (MH Dickinson, LB Vosshall, and JAT Dow, Eds.), Journal of Experimental Biology 223: jeb208793, 2020.**

This paper gives an overview of a course-based undergraduate research experience (CURE) designed to provide practical experience of current biotechnical innovations, CRISPR-Cas9 in this case. Students were guided through all stages of asking research questions about gene function in a frog and butterfly genome. They got to design guide RNAs and do the injections to knock out known genes related to functions like eye development and wing patterning. Thus, the students got direct, visually striking results of their research. Bioethics were also discussed in the course. This paper gives practical guidance for others planning similar courses.

**Sun D, Guo Z, Liu Y, Zhang Y, Progress and Prospects of CRISPR/Cas Systems in Insects and Other Arthropods, Frontiers in Physiology 8, 2017.**

This review paper summarizes applications of CRISPR-Cas9-related technology to studies of arthropods, my taxa of interest. The paper covers many of the technical explanations of CRISPR-based genome editing, as in Doudna & Charpentier (2014), but also outlines two other technologies CRISPR-Cpf1 and CRISPR-C2c2, which are more efficient than even Cas9. The review provides a comprehensive timeline of major applications of CRISPR-based genome editing in nine arthropod orders. This review offers a good reference for researchers who want examples of previous work to refer to when crafting their own gene-function analyses in other arthropods.