

A piggyBac route to transgenic honeybees

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The honeybee *Apis mellifera* is one of the most recognized and revered insect species throughout human history. The economic impact of the honeybee as an essential pollinator of key crops is estimated to be in the range of billions of dollars per year. The honeybee also serves as a powerful animal model for studies of the evolution and regulation of social behaviors at the endocrine, cellular, genomic, and molecular levels (1). However, until recently, studies of molecular processes that underlie behavior in the honeybee have mainly relied on correlative genomic studies of brain gene expression patterns (2). Nevertheless,

establishing a causal relationship between specific genetic loci and phenotypes has been hindered by the lack of robust and reliable methods to manipulate honeybee gene expression, the generation of transgenes, and genome editing. In PNAS, Schulte et al. (3) provide, to my knowledge, the first evidence that heritable germ-line manipulations of the honeybee genome are feasible by using engineered transposable elements. These welcome technical advances are likely to transform the fields of sociobiology and apiculture (commercial beekeeping) by helping investigators to establish

causal relationships between genes and behavioral, developmental, and disease-related phenotypes.

The development of transgenic insects is not new to biology. In fact, one of the first successful transgenic germ-line transformations of any animal genome was published in a series of groundbreaking studies in the fruit fly *Drosophila melanogaster* by Rubin and Spradling in the early 1980s (4). These studies were the first to show that engineered natural transposable elements, viral-like DNA sequences called “P-elements,” can be used as efficient molecular shuttles for the stable introduction of foreign DNA fragments into the fly genome. Consequently, the P-element became the workhorse of fly molecular genetics and genomics by allowing the rapid identification of affected genes in forward genetic screens of *Drosophila* lines that harbor P-elements inserted in protein-coding genes throughout the fly genome (5).

The main discovery that allowed the adoption of natural transposons for use in molecular biology and genetics was that active transposons require just two elements to enable them to “jump” across chromosomal loci. First, the DNA sequences of most transposons harbor terminal inverted repeat (IR) sequences, which define their chromosomal boundaries. Second, all active transposons code for a transposase, an enzymes that catalyzes the cutting and pasting of the transposon from one location to another. Thus, in the presence of a transposase, any DNA sequences between the two IRs will be cut out of the present location and pasted to a new random location in the genome.

New Developments in Insect Transgenesis

Despite the efficiency and robustness of engineered P-elements, they are limited in several ways. First, it is now thought that P-elements have evolved specifically in *D. melanogaster* and spread globally over the last 60 y throughout wild strains, most likely after a single horizontal transfer from a *Drosophila willistoni* strain (6). Additionally, for reasons that are not completely understood,

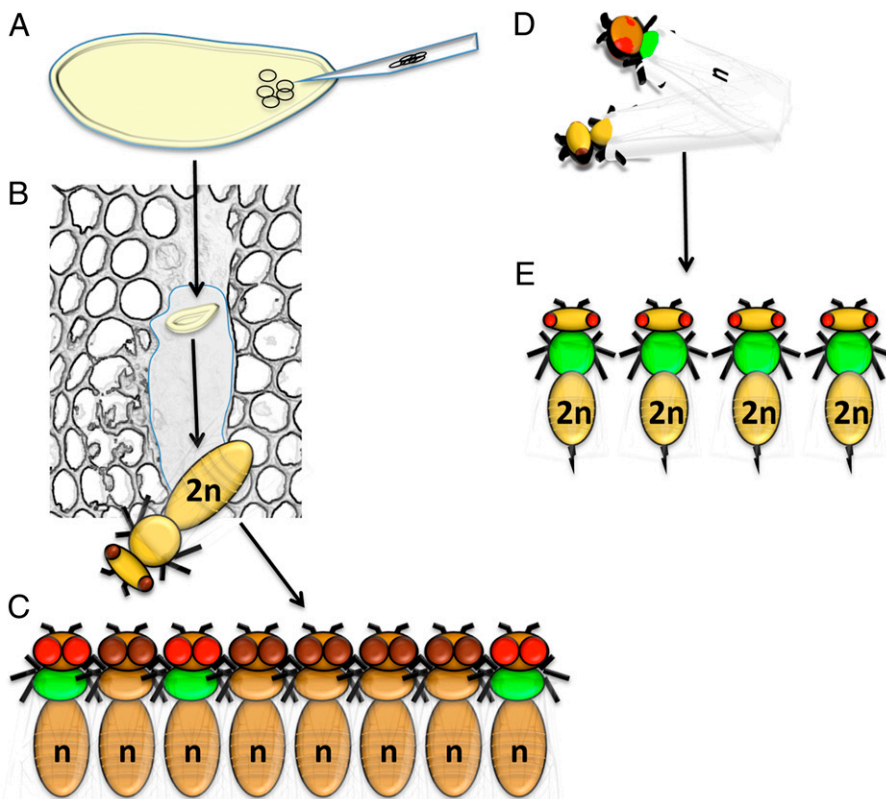


Fig. 1. Transgenesis in the haplo-diploid honey bee. (A) Engineered *piggyBac* DNA that includes red and green fluorescent expression cassettes are injected as plasmids together with transposase mRNA into fertilized diploid eggs. (B) Hatched first-instar larvae are placed in queen cells in a queenless colony, which induces workers to raise these diploid females (2n) as fertile queens rather than sterile workers. (C) Virgin injected queens are treated with CO₂ to induce the laying of unfertilized haploid eggs, which develop exclusively into male drones. (D) Transgenic drones are identified by the expression of fluorescent markers, which are then allowed to mate with WT queens naturally or via artificial insemination. (E) Queens fertilized with transgenic semen would produce 100% heterozygous transgenic female workers. Transgenic female embryos can also be manipulated to develop as heterozygous queens, which can be used to propagate specific transgenic lines.

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P-elements show very low activity levels in insects other than *D. melanogaster*, including other species members of the *Drosophila* clade. P-elements have other limits such as the tendency to integrate into genomic hotspots, often in sequences that are 5' upstream of the transcriptional start site of protein coding genes. This phenomenon made the P-element an excellent tool for trapping enhancer elements, but made it a less-than-ideal instrument for forward genetic screens, because integration events rarely generate null alleles. Nevertheless, since the original discovery of transposons by Barbara McClintock in the early 1950s (7), it has become clear that all eukaryotic genomes harbor many different transposons, some of which can serve as excellent tools for genetics and transgenesis of insects and other animals (8). Thus, a diversity of engineered natural transposons such as *hermes* (9), *hobo*, *minos*, *mosI*, and *piggybac* were developed for the purpose of transgenesis in diverse insect species (10, 11). However, due to various biological and technical issues, many of these transposons seem to work very well for the transgenesis of some insect species but fail in others.

The *piggyBac* Transposon and Transgenic Honeybees

Not without some skepticism (12), the release of the honeybee genome led many in the social insect and apicultural research communities to expect that the development of techniques for generating transgenic bees and other bee genome manipulations would follow (13). However, the low success rates of earlier attempts (14, 15), in combination with the technical difficulties of injecting honeybee embryos with DNA constructs, halted the development of universal and efficient tools for the generation of heritable and stable germ-line transformation of honeybees.

Schulte et al. (3) succeed where others have failed by revisiting the widely used Lepidopteran *piggyBac* transposon as a tool for generating transgenic honeybees. Their success in generating transgenic honeybees was driven by the optimization of the embryo injection and larval-rearing protocols, coupled with the improvement of vectors for the expression of transgenes specifically in honeybee cells. First, Schulte et al. (3) develop and optimize gene expres-

sion promoters that allow them to express fluorescent reporter genes that are easily identified in successfully transformed animals. Second, they devise an efficient protocol that takes advantage of the honeybee's social lifestyle and haplo-diploid sex

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determination system, to increase the efficiency of screening for successful transgenesis events, by injecting the *piggyBac* transgenic constructs into diploid female embryos and then inducing colonies to raise these females as virgin reproductive queens. Subsequently, they screen the haploid male progeny of the injected queens for transgenic individuals by efficiently using the identification of fluorescent markers, followed by a genomic PCR screening methodology, to identify transgenic haploid males. Although not yet tested, Schulte et al. suggest (figure 2B of their paper) that these F1 males could subsequently be used for fertilizing unrelated diploid female queens for the generation of 100% transgenic honey bee colonies (Fig. 1).

Implications and Future Directions

The protocol and tools developed by Schulte et al. (3) to allow the production of transgenic honeybees represent a technological breakthrough that has the potential to transform studies of sociobiology and apiculture and to greatly expand the use of honeybees as a model organism. Examples that come to mind include the generation of honeybee lines that express genetically encoded reporters for various intracellular signaling molecules such as Ca^{2+} , tools that will enable the selective activation of neuronal populations in vivo with *channelrhodopsin* and similar proteins (16), and the selective overexpression of any other RNA of choice. Although maintaining stable transgenic lines under laboratory conditions is still going to be difficult, the ability to freeze sperm will enable researchers to reestablish key transgenic honeybee lines as needed and will facilitate sharing of important transgenic lines between laboratories. In the long term, the work by Schulte et al. (3) paves the road for the application of state-of-the-art genome editing techniques such as the zinc finger nucleases (17), transcription activator-like effector nucleases (TALENs) (18), and the more recent clustered regularly interspaced short palindromic repeats (CRISPR/*Cas9*) system (19) to the honeybee genome.

- Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. *Nat Rev Genet* 9(10):735–748.
- Rittschof CC, Robinson GE (2014) Genomics: Moving behavioural ecology beyond the phenotypic gambit. *Anim Behav*, 10.106/j.anbehav.2014.02.028.
- Schulte C, Theilenberg E, Müller-Borg M, Gempe T, Beye M (2014) Highly efficient integration and expression of *piggyBac*-derived cassettes in the honeybee (*Apis mellifera*). *Proc Natl Acad Sci USA* 111:9003–9008.
- Rubin GM, Spradling AC (1982) Genetic transformation of *Drosophila* with transposable element vectors. *Science* 218(4570):348–353.
- Cooley L, Berg C, Spradling A (1988) Controlling P element insertional mutagenesis. *Trends Genet* 4(9):254–258.
- Daniels SB, Peterson KR, Strausbaugh LD, Kidwell MG, Chovnick A (1990) Evidence for horizontal transmission of the P transposable element between *Drosophila* species. *Genetics* 124(2):339–355.
- McClintock B (1950) The origin and behavior of mutable loci in maize. *Proc Natl Acad Sci USA* 36(6):344–355.
- Atkinson PW, Pinkerton AC, O'Brochta DA (2001) Genetic transformation systems in insects. *Annu Rev Entomol* 46:317–346.
- O'Brochta DA, Warren WD, Saville KJ, Atkinson PW (1996) *Hermes*, a functional non-*Drosophila* insect gene vector from *Musca domestica*. *Genetics* 142(3):907–914.
- Handler AM (2002) Use of the *piggyBac* transposon for germ-line transformation of insects. *Insect Biochem Mol Biol* 32(10):1211–1220.
- Fraser MJ, Jr. (2012) Insect transgenesis: Current applications and future prospects. *Annu Rev Entomol* 57(57):267–289.
- Menzel R (2012) Genetics and molecular biology: Commentary. *Honeybee Neurobiology and Behavior*, eds Galizia CG, Eisenhardt D, Giurfa M (Springer, Dordrecht, The Netherlands), pp 387–389.
- Weinstock GM, et al.; Honeybee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443(7114):931–949.
- Robinson KO, Ferguson HJ, Cobey S, Vaessin H, Smith BH (2000) Sperm-mediated transformation of the honey bee, *Apis mellifera*. *Insect Mol Biol* 9(6):625–634.
- Omholt SW, et al. (1995) Successful production of chimeric honeybee larvae. *J Exp Zool* 272(5):410–412.
- Olsen SR, Wilson RI (2008) Cracking neural circuits in a tiny brain: New approaches for understanding the neural circuitry of *Drosophila*. *Trends Neurosci* 31(10):512–520.
- Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. *Nat Rev Genet* 11(9):636–646.
- Miller JC, et al. (2011) A TALE nuclease architecture for efficient genome editing. *Nat Biotechnol* 29(2):143–148.
- Cong L, et al. (2013) Multiplex genome engineering using CRISPR/*Cas* systems. *Science* 339(6121):819–823.