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Highly efficient DNA-free gene disruption in the agricultural pest *Ceratitis capitata* by CRISPR-Cas9 ribonucleoprotein complexes

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The Mediterranean fruitfly *Ceratitis capitata* (medfly) is an invasive agricultural pest of high economic impact and has become an emerging model for developing new genetic control strategies as an alternative to insecticides. Here, we report the successful adaptation of CRISPR-Cas9-based gene disruption in the medfly by injecting *in vitro* pre-assembled, solubilized Cas9 ribonucleoprotein complexes (RNPs) loaded with gene-specific single guide RNAs (sgRNA) into early embryos. When targeting the eye pigmentation gene *white eye* (*we*), a high rate of somatic mosaicism in surviving G0 adults was observed. Germline transmission rate of mutated *we* alleles by G0 animals was on average above 52%, with individual cases achieving nearly 100%. We further recovered large deletions in the *we* gene when two sites were simultaneously targeted by two sgRNAs. CRISPR-Cas9 targeting of the *Ceratitis* ortholog of the *Drosophila* segmentation *paired* gene (*Ccprd*) caused segmental malformations in late embryos and in hatched larvae. Mutant phenotypes correlate with repair by non-homologous end-joining (NHEJ) lesions in the two targeted genes. This simple and highly effective Cas9 RNP-based gene editing to introduce mutations in *C. capitata* will significantly advance the design and development of new effective strategies for pest control management.

The Mediterranean fruitfly *Ceratitis capitata* (medfly) is an economically relevant agricultural pest infesting more than 260 crop species including fruits, vegetables, and nuts[1]. Wild populations can be contained by the Sterile Insect Technique (SIT), an eradication strategy based on the repeated release of large numbers of factory-grown sterile males into infested areas[2,3]. *C. capitata* was the first non-Drosophilidae insect species in which transposon-mediated germline transformation was established[4,5]. Various transgenic strains have been developed to improve SIT and other pest control strategies[6–14]. Furthermore, embryonic RNA interference was successfully applied to study *in vivo* functions of key *Ceratitis* genes controlling sex determination[15,16].

Nonetheless, a more comprehensive study of gene functions in *Ceratitis* will be needed to further improve existing control strategies. To generate long-lasting and heritable changes in gene function, the novel CRISPR-Cas9 gene editing system with its modular and simple components provides a promising tool for reverse genetics also in insects and to implement scalable and reproducible pest control strategies[17,18]. In short, Cas9 endonuclease recognizes a specific genomic region based on sequence complementary of a preassembled chimeric single guide RNA (sgRNA), and induces double-strand DNA breaks (DSBs) at the targeted site. DSBs

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