

1 **Progress in the use of genetic methods to study insect behavior outside *Drosophila***

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13 Abstract

14 In the span of a decade we have seen a rapid progress in the application of genetic tools and genome editing
15 approaches in “non-model” insects. It is now possible to target sensory receptor genes and neurons, explore
16 their functional roles and manipulate behavioral responses in these insects. In this review, we focus on the latest
17 examples from Diptera, Lepidoptera and Hymenoptera of how applications of genetic tools advanced our
18 understanding of diverse behavioral phenomena. We further discuss genetic methods that could be applied to
19 study insect behavior in the future.

20 Introduction

21 Insects are the most numerous and diverse animal taxa on the planet. They have evolved different adaptations
22 in sensory function and neural circuitry towards performing basic behavioral tasks such as finding mates, food
23 and oviposition sites [1]. The availability of advanced genetic tools in *Drosophila melanogaster* has allowed us
24 to perform sophisticated genetic experiments to investigate everything from gene expression to brain and
25 behavior. The vinegar fly is a wonderful model for investigating odor- and light-directed locomotion and
26 courtship, but offers little insight into pollination, phyto- or haemotophagy and eusociality. From the perspective
27 of meeting the global challenges of the 21st century *D. melanogaster* fails as it is neither a crop pest nor a
28 disease vector and we are only beginning to understand its natural behaviors [2]. Paradoxically, only limited
29 genetic tools are employed for insects with better studied behavior in their ecological context [3]. The
30 application of genetic techniques in non-drosophilids is often directed by pioneering *D. melanogaster* studies
31 that have uncovered phenotypes for candidate homologous gene targets. However, while a genetic tool can
32 often be successfully ported between species (e.g. fluorescent markers [4], gene editing [5,6] or transgene
33 binary expression systems [7–9]), the behavioral outcome of targeting homologous genes is less predictable
34 and needs to be studied on species-by-species basis [e.g. 10–13].

35 A number of recent studies have employed gene silencing or editing in Diptera, Lepidoptera, Hymenoptera,
36 Orthoptera, Hemiptera, Coleoptera and Blattodea (**Table 1, Box 1**) in order to understand their behaviors. The
37 majority of these studies focused on olfaction, reflecting its importance for insect fitness and survival, and the
38 multi-sensory nature of many natural behaviors. These advances herald exciting times in studying the genetic
39 basis of insect behaviors, with increased focus on the organism itself and reduced focus on its use as a genetic
40 model. Here we review the latest progress in the use of genetic tools in behavioral studies, taking a closer look
41 at insect-plant interaction, social behaviors, human host-seeking and oviposition. We also highlight studies that
42 can potentially help decipher the neuronal basis of behavior.

43 **Box 1. Overview of genetic methods**

44 **RNA-interference (RNAi)**

45 The technique of suppressing gene transcription through the application of RNA-interference (RNAi) was first
46 described in 1998 in the nematode *Caenorhabditis elegans* [14]. Since its discovery, this technique has been
47 widely used in insects [15–20] to silence genes of interest by exogenous or endogenous delivery of double-
48 stranded RNA (dsRNA) or small interfering RNA (siRNA). In target cells, dsRNA is cleaved to siRNA by the Dicer
49 enzyme and is incorporated into the RNA-induced silencing complex (RISC) to direct degradation of
50 complementary endogenous mRNA of the targeted gene. The flexibility of this technique is partially attributed
51 to the fact that only the sequence of the gene, and not that of its chromosomal location or regulatory untranslated
52 regions, is required to design dsRNAs. However, the technique is susceptible to variable or no results depending
53 on insect species, gene, tissue, and method of delivery [16,21,22]. Yet, the ability to reduce (knock-down) and
54 not completely ablate (knock-out) the function of a targeted gene at different stages of development permits
55 the analysis of early regulators of sensory behaviors that are also essential for overall survival. Multiple methods
56 of delivery, such as injections at any developmental stage, feeding, transgenic expression and soaking (**Fig. 1**),
57 enable manipulation of insect behaviors in laboratory and the field for research or pest control purposes.
58 Interestingly, pre-blastoderm embryo injections of mRNA has been successfully applied as a forward genetics
59 approach to upregulate gene expression to study sex determination in mosquitoes [23].

60 **Directed mutagenesis by ZFNs, TALENs and CRISPR/Cas9**

61 Reverse genetics is central to associating genes with a biological function. Classic methods of altering genomic
62 DNA using X-rays to induce chromosomal breakage *in situ* revolutionized our ability to associate a particular
63 genomic locus with a behavior [24]. Later, genome sequencing helped map these loci to specific genes and,
64 most recently, gene function was explored through targeted mutagenesis. Targeting a particular gene of interest
65 became feasible for non-model organisms with the help of Zinc-finger nucleases (ZFNs) [25,26] and Transcription
66 activator-like effector nucleases (TALENs) [27]. However, costs and time associated with engineering these
67 proteins prevented a quick adoption of these methods. The discovery of CRISPR/Cas9 system, the part of the
68 bacterial adaptive immune system [5,6,28], permits a fairly quick and inexpensive mechanism for the targeted
69 modification of DNA with the ability to generate deletions from a single base pair to hundreds of kilobase pairs
70 [29]. It is currently the fastest and most effective method of genome editing in diverse organisms from bacteria
71 to human [30]. The application of the technique requires a source of Cas9 protein and the custom-designed
72 guide-RNAs (sgRNAs) that are complimentary to the gene of interest. The sgRNAs bind the Cas9 and deliver it
73 to the desired location in the genome. Cas9 induces a double-stranded break that is naturally repaired either
74 through non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanism. The latter mechanism
75 allows researchers to design specific DNA homology templates surrounding the repair site, adding elements
76 such as transgenes to be incorporated into the target site. In most cases, components of the CRISPR/Cas9 system
77 (Cas9, sgRNAs and a DNA homology template) are injected into a pre-blastoderm embryo. To overcome high
78 costs and workload, and embryo lethality associated with injection, new methods of delivery directly into a
79 gravid female are now being developed [31,32].

80 Since its introduction as a gene editing tool in 2012, CRISPR/Cas9 system has advanced research in many insect
81 species, including flies [33–38], sandflies [39], mosquitoes [40–43], moths [13,44–47], butterflies [48,49],
82 crickets [50], locusts [51], planthoppers [52], honeybees [53,54], wasps [55], ants [11,12], beetles [56], aphids
83 [57] and psyllid bugs [31].

84 **Transposon Mutagenesis**

85 The workhorse of *Drosophila melanogaster* genetics is the P-element [58]. The P-element is a sequence of
86 nucleotides recognized by a transposase found in wild *Drosophila* and applicable for insertion based
87 mutagenesis in lab strains of *D. melanogaster*. These transposable elements allow researchers to insert genes
88 and gene reporters into the germ line of the vinegar fly driving research in reverse and forward genetics.
89 However, the p-element is narrowly applicable to other insect species. The piggyBac transposable element
90 was discovered in the cabbage looper moth, *Trichoplusia ni* [59,60] and has been applied broadly to
91 generate random insertions of transgenes in non-model insects [61].

92

93 Insect-plant interaction

94
95 Insects and plants have co-evolved for approximately 400 million years and many insects rely on the sensory
96 perception of plant cues to elicit quick and adaptive behaviors [62]. Plants are also not passive in these
97 interactions, exemplified by the diversity of flower colors or plant odors driven by the selectivity of their
98 pollinators or voraciousness of their pest.

99 The crepuscular hawkmoth *Manduca sexta* uses both visual and olfactory cues to locate its host plant, the Western
100 Jimsonweed, *Datura wrightii* [3,13], which produces a relatively large, white upright trumpet flower with a strong
101 odor bouquet. Mediating the detection of this floral bouquet are a subset of diversely evolved insect
102 chemosensory receptors, one group of which is encoded by the odorant receptor (OR) genes. The ORs form
103 ligand-gated cation channels with a highly-conserved insect co-receptor ORCO [10,63], and determine the
104 channel's odorant-binding specificity. The ORCO gene is thus necessary for proper function of most olfactory
105 sensory neurons in an insect. Mutating ORCO provides a means of shutting down a large portion of the olfactory
106 system and evaluating its importance in behaviors in insects (**Table 1**). Recently, CRISPR/Cas9 was used to
107 generate an ORCO knock-out (KO) in *M. sexta* [13]. Wind tunnel experiments on ORCO mutants demonstrated
108 that while the nectar-filled and fragrant flower provides a strong visual cue, ORCO-dependent olfaction is
109 needed to complete the sensory behavior of hovering, unfurling the proboscis, and feeding [13]. Interestingly,
110 ORCO-independent sensory processes, such as vision, perception of humidity, and CO₂ do not compensate for
111 the innate behavior involved in seeking out the *Datura* flower (**Fig 2A**). This study also investigated the role of
112 ORCO in hawkmoth plant-seeking for oviposition. The hawkmoth caterpillar is an herbivore and *Datura* is a
113 preferred food source, often to the detriment of the plant. A gravid female hawkmoth evaluates a suitable host
114 plant via olfactory cues from plant leaves [64], and this host-seeking behavior is significantly disrupted in ORCO
115 mutants. However, a number of gravid ORCO-mutant *M. sexta* were still able to locate their host [13], implying
116 that other ORCO-independent olfactory cues may direct this host plant seeking behavior. Thus, the hierarchy of
117 sensory cues and the mode of their integration may vary in multi-sensory behaviors and can only be understood
118 by testing a reverse genetic phenotype in a semi-natural environment. Further implementation of genetic
119 methods, e.g. live imaging of neuronal responses as done in mosquitoes [65,66], could help us understand the
120 gene-specific representation of sensory cues in an insect's brain.

121 Contact chemoreception mediated by gustatory receptors (GRs) is important for oviposition in many insects,
122 especially in Lepidoptera [67]. Female swallowtail butterflies *Papilio xuthus* evaluate the suitability of a
123 substrate while drumming their front legs against the leaves of their Rutacea (citrus) plant host. Synephrine, a
124 citrus plant alkaloid, induces a physiological response in the female tarsi [68]. Gustatory receptor *PxutGR01*
125 was found to be expressed only in females and respond to synephrine when heterologously expressed in an
126 insect cell line [67]. An injection of dsRNA in the pupae downregulated the *PxutGR01* transcript and
127 physiological response to synephrine was reduced in the tarsi of adults. While there was no change in the
128 drumming activity, the oviposition behavior in response to synephrine was reduced in the knock-down individuals,
129 demonstrating that this GR is responsible for the evaluation of synephrine. Laying eggs on the right plant is
130 important since caterpillars need to overcome the plant's defense mechanisms and feed the moment they hatch,
131 and not all leaves provide adequate nutrients to support growth and development. The peripheral sensory
132 system mediating this choice has been studied further in the monophagous silkworm, *Bombyx mori*. This moth is
133 cultivated for its silk cocoons and has been a Lepidopteran model for the development of genetic tools [69].
134 Additionally, many behaviorally abnormal strains of *B. mori* have been cultivated and their genetic loci
135 characterized. The silkworm feeds exclusively on the leaves of the common mulberry plant and a specific
136 cultivated strain was found to have an abnormal food preference. A putative bitter sensing gustatory receptor
137 *BmorGr66* was identified within the mapped genetic loci of the abnormal strain [70]. The application of
138 CRISPR/Cas9 to mutate the *BmorGr66* led to the silkworm accepting foods like fruits and grains in addition to
139 mulberry leaves [70]. Electrophysiological analyses of the mutants did not reveal any general sweet or bitter
140 contact chemoreception deficit; the ligand for mulberry leaf preference remains to be identified. CRISPR/Cas9
141 mutation of ORCO in *B.mori* silkworms also determined that OR-related olfaction was important for feeding
142 behaviors; the ORCO-mutants had trouble localizing the mulberry leaves in a test arena [71]. These studies
143 highlight the importance of single sensory receptors for complex phenomena like foraging preference.
144 Identification of genes like *BmorGr66* may further instruct genetic pest control strategies.

145

146 Social interactions

147

148 Eusocial insects live in complex societies and interact with each other in fascinating ways to maintain social
149 integrity [72,73]. However, understanding the genetic basis of these sensory behaviors has been hindered by
150 the lack of genetic tools, which are particularly difficult to establish when only very few female individuals
151 reproduce sexually, and generation times often span many months. Moreover, these females often have to be
152 isolated to start new colonies. Genetic crossing and outcrossing routines are thus difficult to achieve in eusocial
153 insects in a laboratory setting.

154 Pheromones play a crucial role in regulating social behaviors in eusocial Hymenoptera like ant communities.
155 Antennal olfactory neurons respond to conspecific cuticular hydrocarbons in *Camponotus floridanus* ants [74],
156 and ORCO-dependent receptors of the ponerine ant *Harpegnathos saltator* respond to its cuticular
157 hydrocarbons and pheromones when ectopically expressed in *Drosophila* [75]. Recent studies have taken the
158 next step in genetic characterization of the role of olfaction for intraspecific communications of two species of
159 ants, using CRISPR/Cas9 for the first time in eusocial insects to mutate the ORCO gene [11,12]. Workers of
160 *Harpegnathos saltator* present a unique advantage that facilitates a genetic modification – all workers of this
161 species normally mate, and can take over the queen's place after the queen dies or is removed from the colony
162 [11]. The unmated workers may thus lay haploid eggs that develop into males, or the workers may be allowed
163 to mate and lay diploid eggs that produce females. Another ant species, the clonal raider ant *Ooceraea biroi*
164 was selected for these experiments because it reproduces asexually via parthenogenesis, thus overcoming the
165 obstacles related to difficult genetic crosses [12]. In addition, these ants are blind, which simplifies analysis of
166 their behaviors in response to multisensory cues.

167 ORCO mutant individuals of two species showed deficiencies in olfactory response to pheromones and other
168 volatiles, and abnormal social behaviors (**Fig 2B**). For instance, the ORCO-mutant *O. biroi* could not detect and
169 follow the pheromone trail to their nest, spending a significant amount of time wandering. Additionally, a
170 permanent Sharpie marker line drawn on a surface often deters wild type ants from approaching the line,
171 however, the ORCO-mutants were not repelled and often crossed these lines [12]. Both studies also reported a
172 surprising defect in olfactory neurodevelopment. The ORCO-mutants exhibited a dramatic reduction of olfactory
173 receptor neurons in the antennae [12] and the number of glomeruli in their antennal lobe. Interestingly, ORCO
174 mutation leads to no visible changes in the brain of *Drosophila* [10], and only minor reduction in the relative
175 volume of pheromone-specific glomeruli in *M. sexta* [13]. These results reveal that olfactory neurodevelopment
176 in the ant is largely dependent on the presence of functional ORCO, and raise intriguing questions about the
177 role of ORCO and other olfactory genes in neurodevelopment of other insects.

178 Within the ant colony necrophoric behavior, the removal of dead individuals from the nest by workers, is an
179 important innate behavior that is triggered by olfactory cues from dead individuals [76]. A study on the red
180 fire ant *Solenopsis invicta* showed that a chemosensory protein gene *Si-CSP1*, which is highly expressed in the
181 antenna of workers, is involved in detecting volatile oleic and linoleic acids from dead nestmates and in
182 regulating the necrophoric behavior of *S. invicta* workers. The behavior was suppressed by RNAi through
183 feeding with siRNA mixed into sugar water [77], demonstrating that siRNA feeding is a feasible method of
184 genetic intervention in red fire ants, and could even be a means of population control.

185 Attempts are currently underway to introduce genetic tools into another eusocial insect, the honeybee.
186 CRISPR/Cas9-mediated genetic editing of the *major royal jelly protein 1 (MRJP1)* gene and a mushroom-body-
187 specific protein *mKast* were successful (as verified by genotyping) in honeybees but did not affect the normal
188 development of drones [53,54]. These studies pave way for generating genome-edited honeybee workers for
189 investigating their neurodevelopment, innate and learnt behaviors. This work is especially exciting given the
190 economic importance of honeybees as pollinators and the long history of learning and memory studies on
191 honeybees.

192

193 Human host-seeking

194

195 Insects are vectors of malaria, Zika, Dengue, yellow fever, Chagas and other lethal diseases. Female mosquitoes
196 (**Fig 2C**) and triatomine bugs [78] target their human and animal hosts in order to obtain a blood meal and
197 develop their eggs. Zinc-finger nucleases (ZFNs) were used to mutagenize the ORCO [25] and CO₂ co-receptor

198 GR3 [26] of *Aedes aegypti* mosquitoes. The mutations impaired the mosquitoes' ability to detect components of
199 human body odor and CO₂, but still left them able to find humans. Mosquitoes with a CRISPR/Cas9-generated
200 mutation for ionotropic olfactory co-receptor, *IR8a*, were impaired in their ability to respond to acidic odorants
201 that are components of human sweat [79]. This mutation also significantly reduced attraction of female
202 mosquitoes to a human arm. The attraction was reduced further, but not abolished, in mosquitoes carrying two
203 mutations, *IR8a+ORCO* or *IR8a+GR3* indicating that other cues outside chemosensation mediate attraction to
204 humans. One of these cues is human body heat. For example, mutation in *Aedes TRPA1* gene affects the
205 mosquitoes' preference for human body temperature (40 °C) and avoidance of warmer objects (50-55 °C)
206 [80]. These studies have highlighted the multisensory and additive nature of sensory cues mosquitoes employ in
207 finding humans (summarized in [81]), drawing certain parallels with plant host-seeking in hawkmoths.

208 Interestingly, female *Aedes aegypti* mosquitoes discontinue host-seeking for four days after a blood meal, and
209 resume after the eggs have been laid. A recent study has discovered that human neuro-peptide Y (*NPY*) Y2
210 receptor agonists efficiently target the *Aedes* *NPY*-like receptor 7 (*NPYLR7*), suppressing mosquito attraction to
211 humans [82]. *NPY* antagonists had the opposite effect, leading to increased host-seeking. Mosquitoes that
212 carried a CRISPR/Cas9-induced mutation in *NPYLR7* resumed host-seeking only one day after the blood meal,
213 in contrast to four days in wild-type mosquitoes. A drug screen, conducted on wild-type and *NPYLR7*-mutant
214 mosquitoes, identified six *NPYLR7*-specific agonists that suppress mosquito attraction to humans. These findings
215 suggest an exciting new pathway for behavioral analysis of mosquitoes and the potential for vector disease
216 control by deploying mosquito drug feeders.

217 The neuronal circuits that underlie mosquito host-seeking are currently unknown. Thus the next step is to
218 investigate how multimodal sensory stimuli and systemic signals are processed in the mosquito brain. First steps
219 in this direction have been taken in *Anopheles gambiae* [83,84] and *Aedes aegypti* [85] by employing the
220 fluorescent calcium indicator GCaMP to image live neuronal responses from the peripheral organs and the brain
221 of mosquitoes. The same approach has been taken to study *Aedes* oviposition choices [65,66].

222

223 Oviposition

224

225 *Drosophila* neuroscience heavily relies on transgenic lines, and in particular on three orthogonal binary
226 expression systems (reviewed in [86]). Reporter transgenes are especially useful for labelling neurons,
227 monitoring or manipulating their functional responses. Generating transgenic lines in other insects has, until
228 recently, been hampered by the need to identify and clone out the native enhancer and promoter region for
229 the gene of interest (although see [83,85,87,88]). The advance of CRISPR/Cas9 has removed this requirement,
230 and now allows us to introduce a transgene, with a T2A or a similar linker, immediately into or after the coding
231 sequence of a gene [89]. By using live Ca²⁺ imaging of genetically encoded activity indicators, we can now
232 investigate the neuronal basis of the observed behavioral phenotypes. This method has been elegantly used to
233 study oviposition choices in *Aedes aegypti* [65,66] (**Fig 2D**). Gravid mosquito females lay their eggs in or near
234 water sources, because their larvae and pupae are aquatic. Female *Aedes*, mutant for the *pickpocket* cation
235 channel subunit gene *ppk30*, lay fewer eggs and fail to avoid water with high salinity that is harmful for their
236 larvae [66]. Live Ca²⁺ imaging of ventral nerve cord that is innervated by *ppk30* expressing neurons from the
237 mosquito legs, has shown that these neurons responds both to water and to NaCl, implying that there must be a
238 parallel neuronal pathway that prevents oviposition in salty water in wild-type *Aedes*. Live Ca²⁺ imaging from
239 the *Aedes* antennal lobe has been used to observe sparse neuronal responses to geosmin, an oviposition
240 attractant [65]. The preference for geosmin has been abolished in *ORCO* mutant mosquitoes, indicating that, as
241 in *Drosophila*, geosmin binds an *ORCO*-dependent receptor. *Drosophila*, however, find geosmin repulsive [90]
242 and avoid it in oviposition and other assays.

243

244 In summary, these latest studies have shed light on general principles that guide insect behavior. Not surprisingly,
245 complex behaviors such as host-seeking and oviposition in moth and mosquitoes are controlled by multisensory
246 cues. The relative importance of these cues is different for different behaviors, and depends on the internal
247 state of the animal (fed, hungry, host-seeking, etc). Mutations of the highly conserved *ORCO* gene in different
248 species lead to strikingly different developmental and behavioral consequences, highlighting the necessity of

249 an era of comparative genetic studies. These are also instrumental for the development of pest and disease
250 vector control strategies.

251

252 Outlook

253 CRISPR/Cas9 provides a unique opportunity to use gene editing to study the molecular and neuronal basis of
254 insect behavior, ranging from sensory perception to memory formation and retrieval [50,91]. Either mutating a
255 gene of interest or simultaneously introducing transgenes into precisely defined locations with CRISPR/Cas9
256 would permit the functional re-programming of neurons. The successful use of transgenes to monitor neuronal
257 responses in mosquitoes will be undoubtedly followed by similar studies in other non-model insect organisms.
258 Work on *Drosophila* has developed multiple methods for activating or silencing neurons by ectopic expression
259 of sensory receptors or ion channels [86,92]. These techniques are now being adapted to other insects [e.g. 93],
260 promising us greater understanding of the neural basis of insect behaviors.

261 Gene knockouts deliver a unique opportunity for observing the comparative evolution of gene function. For
262 example, the ORCO gene knock out has been generated in 8 species (**Table 1**). ORCO is a highly conserved
263 gene with putative chaperone function and forms functional co-receptors with the highly diverse ORs. OR gene
264 numbers range from 10 to > 300 across species and are tuned to diverse natural ligands [1,94]. The Orco KO
265 has consistently demonstrated disrupted neurophysiological responses to a range of odorants and pheromones.
266 However, insect OR-mediated behaviors distinctively integrate with other sensory modalities (**Fig 2**). For
267 instance, copulation behaviors continue to occur in ORCO KO *D. melanogaster*, presumably through the flexible
268 multi-sensory nature of their mating cues. On the other hand, the strict OR-mediated perception of pheromones
269 is critical for copulation behaviors in some Lepidoptera [13,47]. A more striking example involves the role of
270 ORCO in neurodevelopment, where the loss of ORCO leads to dramatic reduction and loss of olfactory glomeruli
271 and olfactory sensory projections from the antennae, indicating a developmental role for ORCO via an unknown
272 mechanism in ants [11,12]. The application of genetic techniques to other genes and their respective homologues
273 will no doubt advance our understanding of many novel biological phenomena based on expanding
274 comparative observations.

275 However, care should be taken in understanding certain unforeseen effects of current gene editing techniques.
276 CRISPR-Cas9 may introduce unintended mutations beyond the targeted genes and *in silico* methods of off-
277 target detection are often unverified in current non-model organisms. These off-target effects may provide
278 misleading information for behavioral phenotypes or disrupt other factors involved in fitness or fecundity.
279 Especially when a genetic rescue lines are not feasible, techniques in testing off-target effects *in vivo* should be
280 considered. Research is quickly advancing in the development of rapid and accurate techniques applying
281 methods in next generation sequencing to identify sites that go through the natural cellular nucleotide repair
282 mechanism after CRISPR application, providing a reliable and un-biased method of off-target detection in any
283 organism [95,96].

284 Advances in genetic techniques in other insect species will also have practical implications in pest management
285 of major crop and disease vectors. For example, new methods of RNAi delivery now allow applications of it in
286 field conditions for crop protection [17,19]. Additionally, a combination of CRISPR/Cas9 and RNAi can lead to
287 the generation of more insects susceptible to RNAi transcript downregulation. Releases of sterile [97] or
288 bacteria-carrying [98] mosquitoes have been adopted as methods to limit mosquito population. Gene-drive
289 technology now allows us to propagate various genetic modifications and transgenes throughout an insect
290 population [41]. These modifications do not need to eliminate an insect population, but may also rely on
291 manipulating insect behavior, e.g. to divert them from economically important crops or from ourselves. Ultimately
292 the application of these techniques and the observations gained from different insects may provide the
293 conceptual framework to better address these challenges.

294

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300 limitations or our ignorance.

301 **Figures**

302 **Figure 1. Ways to deliver RNAi in insects.**

303 **A. Soaking.** Immersion in dsRNA solution against a detoxification enzyme gene has been successfully used in
304 adult fleas [99]. The fleas were incubated at 4°C, which excludes active ingestion of dsRNAs.

305 **B. Feeding.** RNAi feeding has been applied in e.g. larval mosquitoes [100–103] and *Tribolium* [104], triatomine
306 bug nymphs [105], caterpillars [106], ants [76] and aphids [104,107]. dsRNA may be mixed directly into food
307 [76,104], or presented in the form of nanoparticles [100,101] that slow down the degradation of dsRNA.
308 Bacteria [102] and yeast [103] have been genetically engineered to produce siRNA. Finally, plants may be
309 genetically modified to produce siRNA or sprayed with dsRNA against insect genes [107].

310 **C. Injections.** Injection of dsRNA or siRNA is the most common laboratory delivery method. Injections may be
311 given at any of an insect's life stages (e.g. embryos [23,108], larvae [109–111], pupae [91,112–114], adults
312 [115,116]). While labor-intensive, this method normally provides the highest efficiency of gene silencing, with
313 the caveat that giving the injection may impair an animal's survival.

314 **D. Transgenes.** Transgenic expression of dsRNA is most commonly used in *Drosophila*, where thousands of UAS-
315 RNAi lines have been established, and may now be used by simple genetic crosses with a driver line of interest.
316 The same transgenic approach is feasible in other insects, but the need to create a stable transgenic line has so
317 far prevented its implementation.

318

319 **Figure 2. Insect behaviors, studied with genetic tools.**

320 **A. Insect-plant interactions.** Flowers provide visual and olfactory cues (odor bouquet, relative humidity and
321 CO₂), while the leaves of the plant also provide olfactory and gustatory cues for adult female butterflies and
322 moths, and their caterpillars. ORCO-mutant *Manduca sexta* moths are impaired in their foraging behaviors [13]
323 (left). Caterpillars choose their food based on its taste. *Bombyx mori* caterpillars with mutated GR66 receptor
324 expanded their food preference from mulberry leaves to fruit and grains [70] (right).

325 **B. Social behaviors.** Social behaviors of ants heavily rely on olfactory perception of pheromones. Recent studies
326 have shown that ORCO-mutant ants are seriously impaired in their social interactions, indicating that ORCO-
327 dependent olfactory receptor neurons are necessary for pheromone perception [11,12].

328 **C. Human host-seeking.** Female mosquitoes, as moths, integrate multiple sensory cues to find their human host.
329 Mutations in ORCO [25], GR3 [26] and IR8a [79] receptors that detect human body odors, CO₂ and acidic
330 components of human sweat respectively, have significantly reduced the ability of *Aedes aegypti* to find humans.

331 **D. Oviposition.** Female mosquitoes lay their eggs in or near water, and their larvae and pupae develop in
332 water. Thus, oviposition sites need to be carefully selected by the females. Two recent studies have found that
333 *Aedes aegypti* mosquitoes prefer to lay their eggs in geosmin-scented water [65], and tend to avoid salty water
334 [66]. Neurons that respond to salt and water were found in the mosquitoes' legs, and geosmin-sensing neurons
335 – in the antennae.

336

337 **References**

338 * of special interest

339 ** of outstanding interest

- 340 1. Hansson BS, Stensmyr MC: **Evolution of insect olfaction.** *Neuron* 2011, **72**:698–711.
- 341 2. Mansourian S, Enjin A, Jirle E V., Ramesh V, Rehermann G, Becher PG, Pool JE, Stensmyr MC: **Wild**
342 **African *Drosophila melanogaster* Are Seasonal Specialists on Marula Fruit.** *Curr Biol* 2018,
343 **28**:3960-3968.e3.
- 344 3. Stöckl AL, Kelber A: **Fuelling on the wing: sensory ecology of hawkmoth foraging.** *J Comp Physiol A*
345 2019, **0**:1–15.
- 346 4. Berghammer AJ, Klingler M, A. Wimmer E: **A universal marker for transgenic insects.** *Nature* 1999,
347 **402**:370–371.
- 348 5. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E: **A Programmable Dual-RNA-**
349 **Guided DNA Endonuclease in Adaptive Bacterial Immunity.** *Science* (80-) 2012,
- 350 6. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, et al.:
351 **Multiplex genome engineering using CRISPR/Cas systems.** *Science* 2013, **339**:819–23.
- 352 7. Potter CJ, Tasic B, Russler E V., Liang L, Luo L: **The Q system: A repressible binary system for**
353 **transgene expression, lineage tracing, and mosaic analysis.** *Cell* 2010, **141**:536–548.
- 354 8. Riabinina O, Luginbuhl D, Marr E, Liu S, Wu MN, Luo L, Potter CJ: **Improved and expanded Q-system**
355 **reagents for genetic manipulations.** *Nat Methods* 2015, **12**.
- 356 9. Brand AH, Perrimon N: **Targeted gene expression as a means of altering cell fates and generating**
357 **dominant phenotypes.** *Development* 1993, **118**:401–15.
- 358 10. Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB: **Or83b Encodes a Broadly**
359 **Expressed Odorant Receptor Essential for *Drosophila* Olfaction.** *Neuron* 2004, **43**:703–714.
- 360 11**. Yan H, Opachaloemphan C, Mancini G, Yang H, Gallitto M, Mlejnek J, Leibholz A, Haight K, Ghaninia
361 M, Huo L, et al.: **An Engineered *orco* Mutation Produces Aberrant Social Behavior and Defective**
362 **Neural Development in Ants.** *Cell* 2017, **170**:736-747.e9.
- 363 First genetic editing with CRISPR/Cas9 in an eusocial insect to investigate the role of olfactory co-receptor
364 ORCO.
- 365 12**. Tribble W, Olivos-Cisneros L, McKenzie SK, Saragosti J, Chang N-C, Matthews BJ, Oxley PR, Kronauer
366 DJC: ***orco* Mutagenesis Causes Loss of Antennal Lobe Glomeruli and Impaired Social Behavior in**
367 **Ants.** *Cell* 2017, **170**:727-735.e10.
- 368 First genetic editing with CRISPR/Cas9 in an eusocial insect to investigate the role of olfactory co-receptor
369 ORCO.
- 370 13**. Fandino RA, Haverkamp A, Bisch-Knaden S, Zhang J, Bucks S, Nguyen TAT, Schröder K, Werckenthin A,
371 Rybak J, Stengl M, et al.: **Mutagenesis of odorant coreceptor *Orco* fully disrupts foraging but not**
372 **oviposition behaviors in the hawkmoth *Manduca sexta*.** *Proc Natl Acad Sci* 2019,
373 doi:10.1073/PNAS.1902089116.
- 374 CRISPR/Cas9 application in *Manduca sexta* to study ecologically-relevant insect-plant interactions.
- 375 14. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC: **Potent and specific genetic**
376 **interference by double-stranded RNA in *Caenorhabditis elegans*.** *Nature* 1998, **391**:806–811.
- 377 15. Whitten MM: **Novel RNAi delivery systems in the control of medical and veterinary pests.** *Curr*
378 *Opin Insect Sci* 2019, **34**:1–6.
- 379 16. Vélez AM, Fishilevich E: **The mysteries of insect RNAi: A focus on dsRNA uptake and transport.**
380 *Pestic Biochem Physiol* 2018, **151**:25–31.
- 381 17. Zhang J, Khan SA, Heckel DG, Bock R: **Next-Generation Insect-Resistant Plants: RNAi-Mediated Crop**

- 382 **Protection.** *Trends Biotechnol* 2017, **35**:871–882.
- 383 18. Terenius O, Papanicolaou A, Garbutt JS, Eleftherianos I, Huvenne H, Kanginakudru S, Albrechtsen M,
384 An C, Aymeric J-L, Barthel A, et al.: **RNA interference in Lepidoptera: An overview of successful and**
385 **unsuccessful studies and implications for experimental design.** *J Insect Physiol* 2011, **57**:231–245.
- 386 19. Joga MR, Zotti MJ, Smaghe G, Christiaens O: **RNAi Efficiency, Systemic Properties, and Novel**
387 **Delivery Methods for Pest Insect Control: What We Know So Far.** *Front Physiol* 2016, **7**:553.
- 388 20. Wang K, Peng Y, Pu J, Fu W, Wang J, Han Z: **Variation in RNAi efficacy among insect species is**
389 **attributable to dsRNA degradation in vivo.** *Insect Biochem Mol Biol* 2016, **77**:1–9.
- 390 21. Shukla JN, Kalsi M, Sethi A, Narva KE, Fishilevich E, Singh S, Mogilicherla K, Palli SR: **Reduced stability**
391 **and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects.**
392 *RNA Biol* 2016, **13**:656–669.
- 393 22. Bellés X: **Beyond Drosophila: RNAi In Vivo and Functional Genomics in Insects.** *Annu Rev Entomol*
394 2010, **55**:111–128.
- 395 23*. Krzywinska E, Dennison NJ, Lycett GJ, Krzywinski J: **A maleness gene in the malaria mosquito**
396 **Anopheles gambiae.** *Science* 2016, **353**:67–9.
- 397 Applications of RNAi and mRNA injections to downregulate and upregulate respectively sex determination
398 genes in mosquitoes
- 399 24. Fujii T, Fujii T, Namiki S, Abe H, Sakurai T, Ohnuma A, Kanzaki R, Katsuma S, Ishikawa Y, Shimada T:
400 **Sex-linked transcription factor involved in a shift of sex-pheromone preference in the silkworm**
401 **Bombyx mori.** *Proc Natl Acad Sci* 2011, **108**:18038–18043.
- 402 25*. DeGennaro M, McBride CS, Seeholzer L, Nakagawa T, Dennis EJ, Goldman C, Jasinskiene N, James
403 AA, Vosshall LB: **orco mutant mosquitoes lose strong preference for humans and are not repelled**
404 **by volatile DEET.** *Nature* 2013, **498**:487–491.
- 405 ZFN application to mutate olfactory co-receptor ORCO in *Aedes aegypti* mosquitoes, and explore its role for
406 human host-seeking.
- 407 26. McMeniman CJ, Corfas RA, Matthews BJ, Ritchie SA, Vosshall LB: **Multimodal Integration of Carbon**
408 **Dioxide and Other Sensory Cues Drives Mosquito Attraction to Humans.** *Cell* 2014, **156**:1060–
409 1071.
- 410 27. Hatakeyama M, Yatomi J, Sumitani M, Takasu Y, Sekiné K, Niimi T, Sezutsu H: **Knockout of a**
411 **transgene by transcription activator-like effector nucleases (TALENs) in the sawfly, *A thalia rosae***
412 **(Hymenoptera) and the ladybird beetle, *Harmonia axyridis* (Coleoptera).** *Insect Mol Biol* 2016, **25**:24–
413 31.
- 414 28. Doudna JA, Charpentier E: **The new frontier of genome engineering with CRISPR-Cas9.** *Science (80-*
415 *)* 2014, **346**.
- 416 29. Gantz VM, Akbari OS: **Gene editing technologies and applications for insects.** *Curr Opin Insect Sci*
417 2018, **28**:66–72.
- 418 30. Sun D, Guo Z, Liu Y, Zhang Y: **Progress and Prospects of CRISPR/Cas Systems in Insects and Other**
419 **Arthropods.** *Front Physiol* 2017, **8**:608.
- 420 31*. Hunter WB, Gonzalez MT, Tomich J: **BAPC-assisted CRISPR/Cas9 System: Targeted Delivery into**
421 **Adult Ovaries for Heritable Germline Gene Editing (Arthropoda: Hemiptera).** *bioRxiv* 2018,
422 doi:10.1101/478743.
- 423 Alternative methods of generating heritable mutations, with reduced costs and increased success rates.
- 424 32*. Chaverra-Rodriguez D, Macias VM, Hughes GL, Pujhari S, Suzuki Y, Peterson DR, Kim D, McKeand S,
425 Rasgon JL: **Targeted delivery of CRISPR-Cas9 ribonucleoprotein into arthropod ovaries for heritable**
426 **germline gene editing.** *Nat Commun* 2018, **9**:3008.
- 427 Alternative methods of generating heritable mutations, with reduced costs and increased success rates.
- 428 33. Aumann RA, Schetelig MF, Häcker I: **Highly efficient genome editing by homology-directed repair**

- 429 **using Cas9 protein in *Ceratitis capitata***. *Insect Biochem Mol Biol* 2018, **101**:85–93.
- 430 34. Heinze SD, Kohlbrenner T, Ippolito D, Meccariello A, Burger A, Mosimann C, Saccone G, Bopp D:
431 **CRISPR-Cas9 targeted disruption of the yellow ortholog in the housefly identifies the brown body**
432 **locus**. *Sci Rep* 2017, **7**:4582.
- 433 35. Choo A, Crisp P, Saint R, O’Keefe L V., Baxter SW: **CRISPR/Cas9-mediated mutagenesis of the white**
434 **gene in the tephritid pest *Bactrocera tryoni***. *J Appl Entomol* 2018, **142**:52–58.
- 435 36. Sim SB, Kauwe AN, Ruano REY, Rendon P, Geib SM: **The ABCs of CRISPR in Tephritidae: developing**
436 **methods for inducing heritable mutations in the genera *Anastrepha*, *Bactrocera* and *Ceratitis***. *Insect*
437 *Mol Biol* 2019, doi:10.1111/imb.12550.
- 438 37. Rajaratnam G, Supeinthiran A, Meier R, Su KFY: **CRISPR/Cas9 deletions in a conserved exon of**
439 **Distal-less generates gains and losses in a recently acquired morphological novelty in flies**.
440 *iScience* 2018, **10**:222–233.
- 441 38. Meccariello A, Monti SM, Romanelli A, Colonna R, Primo P, Inghilterra MG, Del Corsano G, Ramaglia
442 A, Iazzetti G, Chiarore A, et al.: **Highly efficient DNA-free gene disruption in the agricultural pest**
443 ***Ceratitis capitata* by CRISPR-Cas9 ribonucleoprotein complexes**. *Sci Rep* 2017, **7**:10061.
- 444 39. Martin-Martin I, Aryan A, Meneses C, Adelman ZN, Calvo E: **Optimization of sand fly embryo**
445 **microinjection for gene editing by CRISPR/Cas9**. *PLoS Negl Trop Dis* 2018, **12**:e0006769.
- 446 40*. Kistler KE, Vosshall LB, Matthews BJ: **Genome Engineering with CRISPR-Cas9 in the Mosquito *Aedes***
447 ***aegypti***. *Cell Rep* 2015, **11**:51–60.
- 448 First application of CRISPR/Cas9 in *Aedes* mosquitoes.
- 449 41**. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E,
450 Russell S, et al.: **A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria**
451 **mosquito vector *Anopheles gambiae***. *Nat Biotechnol* 2016, **34**:78–83.
- 452 Gene-drive application in mosquitoes for high inheritance rate of transgenes or mutated genes
- 453 42. Li M, Li T, Liu N, Raban R, Wang X, Akbari O: **Methods for the generation of heritable germline**
454 **mutations in the disease vector *Culex quinquefasciatus* using CRISPR/Cas9**. *bioRxiv* 2019,
455 doi:10.1101/609206.
- 456 43. Dong Y, Simões ML, Marois E, Dimopoulos G: **CRISPR/Cas9 -mediated gene knockout of *Anopheles***
457 ***gambiae* FREP1 suppresses malaria parasite infection**. *PLOS Pathog* 2018, **14**:e1006898.
- 458 44. Ma S, Chang J, Wang X, Liu Y, Zhang J, Lu W, Gao J, Shi R, Zhao P, Xia Q: **CRISPR/Cas9 mediated**
459 **multiplex genome editing and heritable mutagenesis of BmKu70 in *Bombyx mori***. *Sci Rep* 2015,
460 **4**:4489.
- 461 45. Huang Y, Chen Y, Zeng B, Wang Y, James AA, Gurr GM, Yang G, Lin X, Huang Y, You M:
462 **CRISPR/Cas9 mediated knockout of the abdominal-A homeotic gene in the global pest,**
463 **diamondback moth (*Plutella xylostella*)**. *Insect Biochem Mol Biol* 2016, **75**:98–106.
- 464 46. Garczynski SF, Martin JA, Griset M, Willett LS, Cooper WR, Swisher KD, Unruh TR: **CRISPR/Cas9**
465 **Editing of the Codling Moth (*Lepidoptera: Tortricidae*) CpomOR1 Gene Affects Egg Production and**
466 **Viability**. *J Econ Entomol* 2017, **110**:1847–1855.
- 467 47*. Koutroumpa FA, Monsempes C, François M-C, de Cian A, Royer C, Concordet J-P, Jacquin-Joly E:
468 **Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth**. *Sci Rep* 2016,
469 **6**:29620.
- 470 CRISPR/Cas9-induced mutation in olfactory co-receptor ORCO leads to abnormal responses to host plant
471 volatiles and pheromones in a moth.
- 472 48*. Li X, Fan D, Zhang W, Liu G, Zhang L, Zhao L, Fang X, Chen L, Dong Y, Chen Y, et al.: **Outbred**
473 **genome sequencing and CRISPR/Cas9 gene editing in butterflies**. *Nat Commun* 2015, **6**:8212.
- 474 First successful application of CRISPR/Cas9 in butterflies.
- 475

- 476 49. Markert MJ, Zhang Y, Enuameh MS, Reppert SM, Wolfe SA, Merlin C: **Genomic Access to Monarch**
477 **Migration Using TALEN and CRISPR/Cas9-Mediated Targeted Mutagenesis.** *G3 (Bethesda)* 2016,
478 6:905–15.
- 479 50*. Awata H, Watanabe T, Hamanaka Y, Mito T, Noji S, Mizunami M: **Knockout crickets for the study of**
480 **learning and memory: Dopamine receptor Dop1 mediates aversive but not appetitive**
481 **reinforcement in crickets.** *Sci Rep* 2015, 5:15885.
- 482 First successful application of CRISPR/Cas9 in crickets to target dopamine receptor that is essential for learning
483 and memory.
- 484 51*. Li Y, Zhang J, Chen D, Yang P, Jiang F, Wang X, Kang L: **CRISPR/Cas9 in locusts: Successful**
485 **establishment of an olfactory deficiency line by targeting the mutagenesis of an odorant receptor**
486 **co-receptor (Orco).** *Insect Biochem Mol Biol* 2016, 79:27–35.
- 487 First successful application of CRISPR/Cas9 in locusts to mutate olfactory co-receptor ORCO.
- 488 52. Xue W-H, Xu N, Yuan X-B, Chen H-H, Zhang J-L, Fu S-J, Zhang C-X, Xu H-J: **CRISPR/Cas9-mediated**
489 **knockout of two eye pigmentation genes in the brown planthopper, Nilaparvata lugens**
490 **(Hemiptera: Delphacidae).** *Insect Biochem Mol Biol* 2018, 93:19–26.
- 491 53*. Kohno H, Suenami S, Takeuchi H, Sasaki T, Kubo T: **Production of Knockout Mutants by CRISPR/Cas9**
492 **in the European Honeybee, Apis mellifera L.** *Zoolog Sci* 2016, 33:505–512.
- 493 First report of successful gene editing in honeybees.
- 494 54. Kohno H, Kubo T: **mKast is dispensable for normal development and sexual maturation of the male**
495 **European honeybee.** *Sci Rep* 2018, 8:11877.
- 496 55*. Li M, Au LYC, Douglah D, Chong A, White BJ, Ferree PM, Akbari OS: **Generation of heritable**
497 **germline mutations in the jewel wasp Nasonia vitripennis using CRISPR/Cas9.** *Sci Rep* 2017,
498 7:901.
- 499 First successful application of CRISPR/Cas9 in *Nasonia*.
- 500 56*. Gilles AF, Schinko JB, Averof M: **Efficient CRISPR-mediated gene targeting and transgene**
501 **replacement in the beetle Tribolium castaneum.** *Development* 2015, 142:2832–9.
- 502 First successful application of CRISPR/Cas9 in *Tribolium*.
- 503 57. Le Trionnaire G, Tanguy S, Hudaverdian S, Gleonnec F, Richard G, Cayrol B, Monsion B, Pichon E,
504 Deshoux M, Webster C, et al.: **An integrated protocol for targeted mutagenesis with CRISPR-Cas9**
505 **system in the pea aphid.** *Insect Biochem Mol Biol* 2019, doi:10.1016/J.IBMB.2019.04.016.
- 506 58. Rubin GM, Spradling AC: **Genetic transformation of Drosophila with transposable element vectors.**
507 *Science* 1982, 218:348–53.
- 508 59. Cary LC, Goebel M, Corsaro BG, Wang H-G, Rosen E, Fraser MJ: **Transposon mutagenesis of**
509 **baculoviruses: Analysis of Trichoplusia ni transposon IFP2 insertions within the FP-locus of**
510 **nuclear polyhedrosis viruses.** *Virology* 1989, 172:156–169.
- 511 60. Fraser MJ, Brusca JS, Smith GE, Summers MD: **Transposon-mediated mutagenesis of a baculovirus.**
512 *Virology* 1985, 145:356–361.
- 513 61. Handler AM: **Use of the piggyBac transposon for germ-line transformation of insects.** *Insect Biochem*
514 *Mol Biol* 2002, 32:1211–1220.
- 515 62. Bruce TJA: **Interplay between insects and plants: dynamic and complex interactions that have**
516 **coevolved over millions of years but act in milliseconds.** *J Exp Bot* 2015, 66:455–465.
- 517 63. Butterwick JA, del Mármol J, Kim KH, Kahlson MA, Rogow JA, Walz T, Ruta V: **Cryo-EM structure of**
518 **the insect olfactory receptor Orco.** *Nature* 2018, 560:447–452.
- 519 64. Allmann S, Späthe A, Bisch-Knaden S, Kallenbach M, Reinecke A, Sachse S, Baldwin IT, Hansson BS:
520 **Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition.** *Elife* 2013, 2:1–
521 23.

- 522 65*. Melo N, Wolff GH, Costa-da-silva AL, Arribas R, Triana MF, Gugger M, Riffell JA, Degennaro M,
523 Stensmyr MC: **Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites.** *bioRxiv* 2019,
524 doi:10.1101/598698.
- 525 Application of live imaging with genetically-encoded indicators in *Aedes* mosquitoes to study responses to an
526 oviposition attractant
- 527 66**. Matthews BJ, Younger MA, Vosshall LB: **The ion channel ppk301 controls freshwater egg-laying in
528 the mosquito *Aedes aegypti*.** *Elife* 2019, **8**.
- 529 Application of the Q-system to create a CRISPR/Cas9 knock-in driver line for a specific class chemosensory
530 neurons, and anatomical and functional characterization of these neurons and corresponding behavior.
- 531 67. Briscoe AD, Macias-Muñoz A, Kozak KM, Walters JR, Yuan F, Jamie GA, Martin SH, Dasmahapatra
532 KK, Ferguson LC, Mallet J, et al.: **Female Behaviour Drives Expression and Evolution of Gustatory
533 Receptors in Butterflies.** *PLoS Genet* 2013, **9**.
- 534 68. Ozaki K, Ryuda M, Yamada A, Utoguchi A, Ishimoto H, Calas D, Marion-Poll F, Tanimura T, Yoshikawa
535 H: **A gustatory receptor involved in host plant recognition for oviposition of a swallowtail
536 butterfly.** *Nat Commun* 2011, **2**:542.
- 537 69. Reid W, O'Brochta DA: **Applications of genome editing in insects.** *Curr Opin insect Sci* 2016, **13**:43–
538 54.
- 539 70**. Zhang Z-J, ZhangID S-S, Niu B-L, Ji D-F, Liu X-J, LiID M-W, Bai H, Reddy Palli S, Wang C-Z, TanID A-J,
540 et al.: **A determining factor for insect feeding preference in the silkworm, *Bombyx mori*.** *PLoS Biol*
541 2019, doi:10.1371/journal.pbio.3000162.
- 542 Application of CRISPR/Cas9 to mutate a gustatory receptor GR66 that is essential for feeding preference in
543 caterpillars.
- 544 71. Liu Q, Liu W, Zeng B, Wang G, Hao D, Huang Y: **Deletion of the *Bombyx mori* odorant receptor co-
545 receptor (BmOrco) impairs olfactory sensitivity in silkworms.** *Insect Biochem Mol Biol* 2017, **86**:58–
546 67.
- 547 72. Favreau E, Martínez-Ruiz C, Rodrigues Santiago L, Hammond RL, Wurm Y: **Genes and genomic
548 processes underpinning the social lives of ants.** *Curr Opin Insect Sci* 2018, **25**:83–90.
- 549 73. LeBoeuf AC, Benton R, Keller L: **The molecular basis of social behavior: models, methods and
550 advances.** *Curr Opin Neurobiol* 2013, **23**:3–10.
- 551 74. Sharma KR, Enzmann BL, Schmidt Y, Moore D, Jones GR, Parker J, Berger SL, Reinberg D, Zwiebel LJ,
552 Breit B, et al.: **Cuticular Hydrocarbon Pheromones for Social Behavior and Their Coding in the Ant
553 Antenna.** *Cell Rep* 2015, **12**:1261–1271.
- 554 75. Pask GM, Slone JD, Millar JG, Das P, Moreira JA, Zhou X, Bello J, Berger SL, Bonasio R, Desplan C, et
555 al.: **Specialized odorant receptors in social insects that detect cuticular hydrocarbon cues and
556 candidate pheromones.** *Nat Commun* 2017, **8**:297.
- 557 76. Diez L, Lejeune P, Detrain C: **Keep the nest clean: Survival advantages of corpse removal in ants.**
558 *Biol Lett* 2014, doi:10.1098/rsbl.2014.0306.
- 559 77*. Qiu H-L, Cheng D-F: **A Chemosensory Protein Gene Si-CSP1 Associated With Necrophoric Behavior
560 in Red Imported Fire Ants (Hymenoptera: Formicidae).** *J Econ Entomol* 2017, **110**:1284–1290.
- 561 Application of RNAi feeding in ants to study their chemosensory behaviour.
- 562 78. Franco TA, Oliveira DS, Moreira MF, Leal WS, Melo ACA: **Silencing the odorant receptor co-receptor
563 RproOrco affects the physiology and behavior of the Chagas disease vector *Rhodnius prolixus*.**
564 *Insect Biochem Mol Biol* 2016, **69**:82–90.
- 565 79. Raji JI, Melo N, Castillo J, Gonzalez S, Saldana V, Stensmyr M, DeGennaro M: ***Aedes Aegypti*
566 Mosquitoes Detect Acidic Volatiles in Human Odor Using the IR8a Pathway.** *Curr Biol* 2019,
567 **29**:1253-1262.e7.
- 568 80. Corfas RA, Vosshall LB: **The cation channel TRPA1 tunes mosquito thermotaxis to host**

- 569 **temperatures.** *Elife* 2015, doi:10.7554/eLife.11750.001.
- 570 81. Potter CJ: **Olfaction: Mosquitoes Love Your Acid Odors.** *Curr Biol* 2019, **29**:R282–R284.
- 571 82**. Duvall LB, Ramos-Espiritu L, Barsoum KE, Glickman JF, Vosshall LB: **Small-Molecule Agonists of Ae.**
572 **aegypti Neuropeptide Y Receptor Block Mosquito Biting.** *Cell* 2019, **176**:687–701.e5.
- 573 Application of CRISPR/Cas9 to mutate an NPY-like receptor that regulated mosquito blood-seeking and may
574 be targeted by common drugs.
- 575 83. Riabinina O, Task D, Marr E, Lin C-C, Alford R, O’Brochta DA, Potter CJ: **Organization of olfactory**
576 **centres in the malaria mosquito *Anopheles gambiae*.** *Nat Commun* 2016, **7**.
- 577 84*. Afify A, Betz JF, Riabinina O, Potter CJ: **Commonly used insect repellents hide human odors from**
578 ***Anopheles* mosquitoes.** *bioRxiv* 2019, doi:10.1101/530964.
- 579 First implementation of genetically-encoded indicators of neuronal activity to study activity of ORCO-expressing
580 neurons in *Anopheles* mosquitoes.
- 581 85*. Bui M, Shyong J, Lutz EK, Yang T, Li M, Truong K, Arvidson R, Buchman A, Riffell JA, Akbari OS: **Live**
582 **calcium imaging of *Aedes aegypti* neuronal tissues reveals differential importance of**
583 **chemosensory systems for life-history-specific foraging strategies.** *BMC Neuro* 2019, **20**, 27
584 doi:10.1186/s12868-019-0511-y.
- 585 First implementation of genetically-encoded Ca²⁺ indicators in *Aedes* mosquitoes.
- 586 86. Gohl DM, Morante J, Venken KJT: **The Current State of the Neuroanatomy Toolkit in the Fruit Fly**
587 ***Drosophila melanogaster*.** In *Decoding Neural Circuit Structure and Function*. . Springer International
588 Publishing; 2017:3–39.
- 589 87. Mysore K, Li P, Duman-Scheel M: **Identification of *Aedes aegypti* cis-regulatory elements that**
590 **promote gene expression in olfactory receptor neurons of distantly related dipteran insects.** *Parasit*
591 *Vectors* 2018, **11**:406.
- 592 88. Bernardini F, Haghghat-Khah RE, Galizi R, Hammond AM, Nolan T, Crisanti A: **Molecular tools and**
593 **genetic markers for the generation of transgenic sexing strains in Anopheline mosquitoes.** *Parasit*
594 *Vectors* 2018, **11**:660.
- 595 89. Li-Kroeger D, Kanca O, Lee P-T, Cowan S, Lee MT, Jaiswal M, Salazar JL, He Y, Zuo Z, Bellen HJ: **An**
596 **expanded toolkit for gene tagging based on MiMIC and scarless CRISPR tagging in *Drosophila*.**
597 *Elife* 2018, **7**.
- 598 90. Stensmyr MC, Dweck HKM, Farhan A, Ibba I, Strutz A, Mukunda L, Linz J, Grabe V, Steck K, Lavista-
599 Llanos S, et al.: **A conserved dedicated olfactory circuit for detecting harmful microbes in**
600 ***drosophila*.** *Cell* 2012, **151**:1345–1357.
- 601 91. Vinauger C, Lahondère C, Wolff GH, Locke LT, Liaw JE, Parrish JZ, Akbari OS, Dickinson MH, Riffell
602 JA: **Modulation of Host Learning in *Aedes aegypti* Mosquitoes.** *Curr Biol* 2018, **28**:333–344.e8.
- 603 92. Simpson JH: *Chapter 3 Mapping and Manipulating Neural Circuits in the Fly Brain.* Elsevier Inc.; 2009.
- 604 93*. Wang H, Dewell RB, Ehrenguber MU, Segev E, Reimer J, Roukes ML, Gabbiani F: **Optogenetic**
605 **manipulation of medullary neurons in the locust optic lobe.** *J Neurophysiol* 2018, **120**:2049–2058.
- 606 First application of optogenetics in a locust to study visual processing.
- 607 94. Brand P, Robertson HM, Lin W, Pothula R, Klingeman WE, Jurat-Fuentes JL, Johnson BR: **The origin of**
608 **the odorant receptor gene family in insects.** *Elife* 2018, **7**:1–13.
- 609 95. Wienert B, Wyman SK, Richardson CD, Yeh CD, Akcakaya P, Porritt MJ, Morlock M, Vu JT, Kazane KR,
610 Watry HL, et al.: **Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq.** *Science*
611 2019, **364**:286–289.
- 612 96. Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar V V., Thapar V, Wyvekens N, Khayter C, Iafrate AJ,
613 Le LP, et al.: **GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas**
614 **nucleases.** *Nat Biotechnol* 2015, **33**:187–198.

- 615 97. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo MN, Lacroix R, Naish N,
616 Morrison NI, et al.: **Successful suppression of a field mosquito population by sustained release of**
617 **engineered male mosquitoes.** *Nat Biotechnol* 2012 309 2012,
- 618 98. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y,
619 Axford J, Kriesner P, et al.: **The wMel Wolbachia strain blocks dengue and invades caged Aedes**
620 **aegypti populations.** *Nature* 2011, **476**:450–453.
- 621 99*. Edwards CH, Baird J, Zinser E, Woods DJ, Shaw S, Campbell EM, Bowman AS: **RNA interference in**
622 **the cat flea, Ctenocephalides felis: Approaches for sustained gene knockdown and evidence of**
623 **involvement of Dicer-2 and Argonaute2.** *Int J Parasitol* 2018, **48**:993–1002.
- 624 Administration of RNAi by soaking in fleas
- 625 100. Mysore K, Flannery E, Leming MT, Tomchaney M, Shi L, Sun L, O'Tousa JE, Severson DW, Duman-Scheel
626 M: **Role of semaphorin-1a in the developing visual system of the disease vector mosquito Aedes**
627 **aegypti.** *Dev Dyn* 2014, **243**:1457–1469.
- 628 101. Mysore K, Flannery EM, Tomchaney M, Severson DW, Duman-Scheel M: **Disruption of Aedes aegypti**
629 **Olfactory System Development through Chitosan/siRNA Nanoparticle Targeting of semaphorin-**
630 **1a.** *PLoS Negl Trop Dis* 2013, **7**:e2215.
- 631 102*. Taracena ML, Hunt CM, Benedict MQ, Pennington PM, Dotson EM: **Downregulation of female**
632 **doublesex expression by oral-mediated RNA interference reduces number and fitness of**
633 **Anopheles gambiae adult females.** *Parasit Vectors* 2019, **12**:170.
- 634 Use of genetically-modified bacteria to produce dsRNA that is fed to mosquito larvae.
- 635 103*. Mysore K, Hapairai LK, Sun L, Harper EI, Chen Y, Eggleston KK, Realey JS, Scheel ND, Severson DW,
636 Wei N, et al.: **Yeast interfering RNA larvicides targeting neural genes induce high rates of**
637 **Anopheles larval mortality.** *Malar J* 2017, **16**:461.
- 638 Use of yeast to produce dsRNA that is fed to mosquito larvae.
- 639 104. Cao M, Gatehouse JA, Fitches EC: **A Systematic Study of RNAi Effects and dsRNA Stability in**
640 **Tribolium castaneum and Acyrthosiphon pisum, Following Injection and Ingestion of Analogous**
641 **dsRNAs.** *Int J Mol Sci* 2018, **19**.
- 642 105. Araujo RN, Santos A, Pinto FS, Gontijo NF, Lehane MJ, Pereira MH: **RNA interference of the salivary**
643 **gland nitrophorin 2 in the triatomine bug Rhodnius prolixus (Hemiptera: Reduviidae) by dsRNA**
644 **ingestion or injection.** *Insect Biochem Mol Biol* 2006, **36**:683–693.
- 645 106. Choi M-Y, Vander Meer RK: **Phenotypic Effects of PBAN RNAi Using Oral Delivery of dsRNA to**
646 **Corn Earworm (Lepidoptera: Noctuidae) and Tobacco Budworm Larvae.** *J Econ Entomol* 2019,
647 **112**:434–439.
- 648 107. Pitino M, Coleman AD, Maffei ME, Ridout CJ, Hogenhout SA: **Silencing of Aphid Genes by dsRNA**
649 **Feeding from Plants.** *PLoS One* 2011, **6**:e25709.
- 650 108. Yoshiyama N, Tojo K, Hatakeyama M: **A survey of the effectiveness of non-cell autonomous RNAi**
651 **throughout development in the sawfly, Athalia rosae (Hymenoptera).** *J Insect Physiol* 2013,
652 **59**:400–407.
- 653 109. Liu C, Pitts RJ, Bohbot JD, Jones PL, Wang G, Zwiebel LJ: **Distinct Olfactory Signaling Mechanisms in**
654 **the Malaria Vector Mosquito Anopheles gambiae.** *PLoS Biol* 2010, **8**:e1000467.
- 655 110. Liu C, Zwiebel LJ: **Molecular Characterization of Larval Peripheral Thermosensory Responses of the**
656 **Malaria Vector Mosquito Anopheles gambiae.** *PLoS One* 2013, **8**:e72595.
- 657 111. Werren JH, Loehlin DW, Giebel JD: **Larval RNAi in Nasonia (parasitoid wasp).** *Cold Spring Harb*
658 *Protoc* 2009, **2009**:pdb.prot5311.
- 659 112. Schultheis D, Weißkopf M, Schaub C, Ansari S, Dao V-A, Grossmann D, Majumdar U, Hakeemi MS,
660 Troelenberg N, Richter T, et al.: **A large-scale systemic RNAi screen in the red flour beetle Tribolium**
661 **castaneum identifies novel genes involved in insect muscle development.** *bioRxiv* 2019,
662 doi:10.1101/397117.

- 663 113. Zhu F, Xu P, Barbosa RMR, Choo Y-M, Leal WS: **RNAi-based demonstration of direct link between**
664 **specific odorant receptors and mosquito oviposition behavior.** *Insect Biochem Mol Biol* 2013,
665 **43:916–923.**
- 666 114. Colinet D, Kremmer L, Lemauf S, Rebuf C, Gatti J-L, Poirié M: **Development of RNAi in a Drosophila**
667 **endoparasitoid wasp and demonstration of its efficiency in impairing venom protein production.** *J*
668 *Insect Physiol* 2014, **63:56–61.**
- 669 115. Meyering-Vos M, Müller A: **RNA interference suggests sulfakinins as satiety effectors in the cricket**
670 **Gryllus bimaculatus.** *J Insect Physiol* 2007, **53:840–848.**
- 671 116. Gatehouse HS, Gatehouse LN, Malone LA, Hodges S, Tregidga E, Todd J: **Amylase activity in honey**
672 **bee hypopharyngeal glands reduced by RNA interference.** *J Apic Res* 2004, **43:9–13.**
- 673 117*. Vinauger C, Breugel F Van, Locke L, Tobin K, Dickinson M, Fairhall A, Akbari O, Riffell J: **Visual-**
674 **olfactory integration in the human disease vector mosquito, Aedes aegypti.** *Curr Biol* 2019, doi:
675 10.1016/j.cub.2019.06.043.
- 676 Application of genetically-encoded neuronal activity indicators to uncover multisensory integration in Aedes
677 mosquitoes
- 678 118. Liu Y-J, Yan S, Shen Z-J, Li Z, Zhang X-F, Liu X-M, Zhang Q-W, Liu X-X: **The expression of three opsin**
679 **genes and phototactic behavior of Spodoptera exigua (Lepidoptera: Noctuidae): Evidence for**
680 **visual function of opsin in phototaxis.** *Insect Biochem Mol Biol* 2018, **96:27–35.**
- 681 119. Yang B, Fujii T, Ishikawa Y, Matsuo T: **Targeted mutagenesis of an odorant receptor co-receptor using**
682 **TALEN in Ostrinia furnacalis.** *Insect Biochem Mol Biol* 2016, **70:53–59.**
- 683 120. Hanin O, Azrielli A, Applebaum SW, Rafaeli A: **Functional impact of silencing the Helicoverpa**
684 **armigera sex-peptide receptor on female reproductive behaviour.** *Insect Mol Biol* 2012, **21:161–**
685 **167.**
- 686 121. Merlin C, Beaver LE, Taylor OR, Wolfe SA, Reppert SM: **Efficient targeted mutagenesis in the**
687 **monarch butterfly using zinc-finger nucleases.** *Genome Res* 2013, **23:159–168.**
- 688 122. Merrill RM, Rastas P, Martin SH, Melo MC, Barker S, Davey J, McMillan WO, Jiggins CD: **Genetic**
689 **dissection of assortative mating behavior.** *PLOS Biol* 2019, **17:e2005902.**
- 690 123. Niehuis O, Buellesbach J, Gibson JD, Pothmann D, Hanner C, Mutti NS, Judson AK, Gadau J, Ruther J,
691 Schmitt T: **Behavioural and genetic analyses of Nasonia shed light on the evolution of sex**
692 **pheromones.** *Nature* 2013, **494:345–348.**
- 693 124. Desjardins CA, Perfectti F, Bartos JD, Enders LS, Werren JH: **The genetic basis of interspecies host**
694 **preference differences in the model parasitoid Nasonia.** *Heredity (Edinb)* 2010, **104:270–277.**
- 695 125. Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, Kang L: **CSP and Takeout Genes Modulate the Switch**
696 **between Attraction and Repulsion during Behavioral Phase Change in the Migratory Locust.** *PLoS*
697 *Genet* 2011, **7:e1001291.**
- 698 126. Awata H, Wakuda R, Ishimaru Y, Matsuoka Y, Terao K, Katata S, Matsumoto Y, Hamanaka Y, Noji S,
699 Mito T, et al.: **Roles of OA1 octopamine receptor and Dop1 dopamine receptor in mediating**
700 **appetitive and aversive reinforcement revealed by RNAi studies.** *Sci Rep* 2016, **6:29696.**
- 701 127. Kim HG, Margolies DC, Park Y: **Roles of transient receptor potential channels in eclosion and**
702 **movement in the red flour beetle Tribolium castaneum.** *Physiol Entomol* 2018, **43:79–85.**
- 703 128. Xu L, Jiang H-B, Chen X-F, Xiong Y, Lu X-P, Pei Y-X, Smagghe G, Wang J-J, Xu L, Jiang H-B, et al.:
704 **How Tyramine β -Hydroxylase Controls the Production of Octopamine, Modulating the Mobility of**
705 **Beetles.** *Int J Mol Sci* 2018, **19:846.**
- 706 129. Kim HG, Margolies D, Park Y: **The roles of thermal transient receptor potential channels in**
707 **thermotactic behavior and in thermal acclimation in the red flour beetle, Tribolium castaneum.** *J*
708 *Insect Physiol* 2015, **76:47–55.**
- 709 130. Liu X-M, Zhang B-X, Li S-G, Rao X-J, Wang D-M, Hu X-X, Liu S: **Knockdown of the olfactory co-**
710 **receptor Orco impairs mate recognition in Tenebrio molitor (Coleoptera: Tenebrionidae).** *J Asia Pac*

- 711 *Entomol* 2016, **19**:503–508.
- 712 131. Waris MI, Younas A, ul Qamar MT, Hao L, Ameen A, Ali S, Abdelnabby HE, Zeng F-FF, Wang M-QQ:
713 **Silencing of chemosensory protein gene *NlugCSP8* by RNAi induces declining behavioral**
714 **responses of *Nilaparvata lugens***. *Front Physiol* 2018, **9**:1–17.
- 715 132. Li S, Zhou C, Zhou Y: **Olfactory co-receptor *Orco* stimulated by Rice stripe virus is essential for host**
716 **seeking behavior in small brown planthopper**. *Pest Manag Sci* 2019, **75**:187–194.
- 717 133*. Bazalova O, Kvicalova M, Valkova T, Slaby P, Bartos P, Netusil R, Tomanova K, Braeunig P, Lee H-J,
718 Sauman I, et al.: **Cryptochrome 2 mediates directional magnetoreception in cockroaches**. *Proc Natl*
719 *Acad Sci U S A* 2016, **113**:1660–5.
- 720 dsRNA injections to study magnetoreception in cockroaches
- 721 134. French AS, Meisner S, Liu H, Weckström M, Torkkeli PH: **Transcriptome analysis and RNA**
722 **interference of cockroach phototransduction indicate three opsins and suggest a major role for**
723 **TRPL channels**. *Front Physiol* 2015, **6**:207.
- 724

Table 1. Applications of genetic methods to study behavior (non-exhaustive list)

Order	Species	Method	Target	Phenotype	Reference
Diptera	<i>Aedes aegypti</i>	chitosan-siRNA nanoparticle feeding	<i>SEMA1α</i>	Impaired larval light avoidance due to improper targeting of photoreceptor neurons	[100]
	<i>Aedes aegypti</i>	chitosan-siRNA nanoparticle feeding	<i>SEMA1α</i>	Impaired larval yeast attraction due to improper targeting of olfactory neurons	[101]
	<i>Aedes aegypti</i>	CRISPR/Cas9; RNAi injection	<i>DOP1</i>	Impaired olfactory learning	[91]
	<i>Aedes aegypti</i>	ZFN	<i>ORCO</i>	Loss of OR-mediated olfaction. Disrupted host localization	[25]
	<i>Aedes aegypti</i>	ZFN	<i>GR3</i>	Impaired CO ₂ detection and host localization	[26]
	<i>Aedes aegypti</i>	ZFN	<i>TRPA1</i>	Impaired avoidance of high temperatures	[80]
	<i>Aedes aegypti</i>	CRISPR/Cas9	<i>PPK301</i> (also 304, 216, 306)	Impaired oviposition decisions in response to salty water	[66]
	<i>Aedes aegypti</i>	CRISPR/Cas9	<i>NPYLR7</i>	Abnormal host-seeking after a recent blood meal	[82]
	<i>Aedes aegypti</i>	CRISPR/Cas9	<i>IR8a</i>	Impaired detection of lactic acid and host localization	[79]
	<i>Aedes aegypti</i>	GCaMP imaging	<i>ORCO, Ubi-GCamp6s</i>	Olfactory responses to geosmin observed <i>in vivo</i> . Demonstration that geosmin is oviposition attractant.	[65]
	<i>Aedes aegypti</i>	GCaMP imaging	<i>Ubi-GCamp6s</i>	<i>In vivo</i> recordings from antennal and optic lobes, evidence of visual - olfactory integration.	[117]
	<i>Anopheles gambiae</i>	RNAi injection	<i>OR7, OR40, IR76b</i>	Impaired larval olfactory behavior	[109]
	<i>Anopheles gambiae</i>	RNAi injection	<i>TRPA1</i>	Impaired larval thermotaxis	[110]
	<i>Culex quinquefasciatus</i>	RNAi injection	<i>OR37, OR99</i>	Impaired oviposition preference for 4-ethylphenol	[113]
Lepidoptera	<i>Spodoptera littoralis</i>	CRISPR/Cas9	<i>ORCO</i>	Disrupted antennal function towards plant host and pheromone volatiles. Disrupted mating.	[47]
	<i>Spodoptera exigua</i>	RNAi injection	<i>Se-uv, Se-bl, Se-lw</i>	Phototaxis towards green light	[118]
	<i>Manduca sexta</i>	CRISPR/Cas9	<i>ORCO</i>	Disrupted plant host localization and foraging behaviors. Disrupted mating	[13]
	<i>Ostrinia furnacalis</i>	TALEN	<i>ORCO</i>	Ablated pheromone response	[119]
	<i>Bombyx mori</i>	CRISPR/Cas9	<i>GR66</i>	Feeding assay used to determine a gustatory receptor involved in the deterring generalist feeding behavior	[70]
	<i>Bombyx mori</i>	CRISPR/Cas9	<i>ORCO</i>	Pheromone detection	[71]
	<i>Papilio xuthus</i>	RNAi	<i>PxutGr01</i>	Tarsal contact chemosensation of plant host compounds	[68]

	<i>Helicoverpa armigera</i>	RNAi injection	Sex peptide receptor	Oviposition and ovary development	[120]
	<i>Danaus plexipus</i>	ZFN, TALENs, CRISPR/Cas9	CRY2, CLK	Group eclosion behavior	[49,121]
	<i>Heliconius melpomene</i> , <i>Heliconius cydno</i>	QTL analysis		Mating preference	[122]
Hymenoptera	<i>Ooceraea biroi</i>	CRISPR/Cas9	ORCO	Deficiencies in social behavior and fitness. Disrupted antennal lobe development	[12]
	<i>Harpegnathos saltator</i>	CRISPR/Cas9	ORCO	Deficiencies in social behavior and fitness. Disrupted antennal lobe development	[11]
	<i>Solenopsis invicta</i>	RNAi feeding	Si-CSP1	Chemosensory protein, involved in necrophoric behavior	[76]
	<i>Nasonia vitripennis</i>	Genetic crosses, RNAi, hybrids, QTL analysis	NV10127-29	Production and perception of male sex pheromone components	[123]
	<i>Nasonia vitripennis</i>	Hybrids, genotyping		Egg-laying preference	[124]
Orthoptera	<i>Locusta migratoria</i>	CRISPR/Cas9	ORCO	Olfactory response to conspecifics	[51]
	<i>Locusta migratoria</i>	RNAi injection	CSP3, TO1	Olfactory response to conspecifics	[125]
	<i>Gryllus bimaculatus</i>	CRISPR/Cas9	DOP1	Appetitive and aversive olfactory learning	[50]
	<i>Gryllus bimaculatus</i>	RNAi injection	OA1, DOP1, DOP2	Appetitive and aversive learning	[126]
Coleoptera	<i>Tribolium castaneum</i>	RNAi injection	TRP channels	Motor behaviors based on anatomical defects of hind leg folding; tonic immobilization	[127]
	<i>Tribolium castaneum</i>	RNAi injection	TcTBH	Mobility	[128]
	<i>Tribolium castaneum</i>	RNAi injection	TRPA1	Thermotaxis	[129]
	<i>Tenebrio molitor</i>	RNAi injection	ORCO	Impaired mate recognition	[130]
Hemiptera	<i>Rhodnius prolixus</i>	RNAi injection	ORCO	Impaired host localization, ecdysis, survival, oviposition rate and blood ingestion	[78]
	<i>Nilaparvata lugens</i>	RNAi injection	CSP8	Decreased olfactory attraction	[131]
	<i>Laodelphax striatellus</i>	RNAi feeding	ORCO	Olfactory host-seeking	[132]
Blattodea	<i>Periplaneta americana</i> , <i>Blattella germanica</i>	RNAi injection	CRY1, CRY2, TIMELESS	Responses to magnetic field	[133]
	<i>Periplaneta americana</i>	RNAi injection	Opsins Trp Channels	Electrophysiological characterization of phototransduction	[134]



