Mosquito heat-seeking is driven by an ancestral cooling receptor

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Introduction

Mosquitoes spread diseases that sicken >600 million people and kill >500,000 people annually. To identify hosts on which to blood-feed, female mosquitoes use a multi-sensory approach that includes the detection of odor, carbon dioxide and body heat. Discovery of the mechanisms that underlie odor and carbon dioxide detection in mosquitoes have relied on prior knowledge of the identify of receptors for these cues in *Drosophila melanogaster* [1,2]. However, the mechanisms behind heat-seeking have remained elusive.

Here, we find that heat-seeking in the malaria mosquito *Anopheles gambiae* is driven by an ancestral cooling receptor conserved between flies and mosquitoes, the ionotropic receptor (IR) IR21a. We previously showed that *Ir21a* is required for cooling detection and warm avoidance in *Drosophila melanogaster* [3]. Here we find that *Ir21a* also mediates cooling detection in *An. gambiae*. However, at a behavioral level, *Ir21a* mediates heat-seeking, rather than avoidance in the mosquito.

These results suggest that Ir21a has been functionally repurposed to support blood-feeding behavior in the mosquito. It also suggests that mosquito "heat-seeking" is driven by cooling avoidance. The discovery of a heat-seeking receptor is of potential utility in combatting against mosquito-borne diseases.

Creating *AgIr21a* mutants

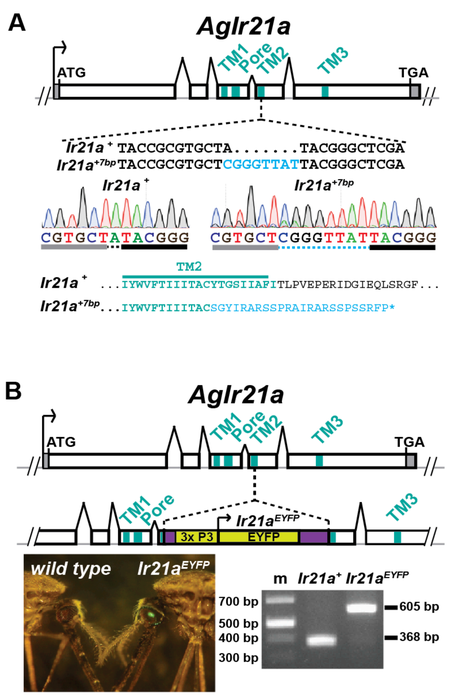

Figure 1. CRISPR/Cas9-based genetic disruption of *AgIr21a*. A) NHEJ disruption of *AgIr21a*. **B)** HDR targeted knock-in into *AgIr21a*.

AgIR21a expression at antennal tip

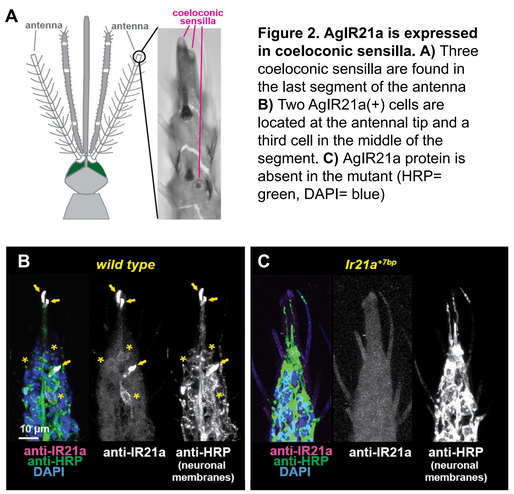

Figure 2. AgIR21a is expressed in coeloconic sensilla. A) Three coeloconic sensilla are found in the last segment of the antenna **B)** Two AgIR21a(+) cells are located at the antennal tip and a third cell in the middle of the segment. **C)** AgIR21a protein is absent in the mutant (HRP= green, DAPI= blue)

Three AgIr21a positive cells are located in the last segment of the antenna. Two have dendrites that extend into the antennal tip, and one cell located medially. These cells are located in coeloconic sensilla, known to house thermo-sensitive cells [4].

AgIr21a mediates cooling detection

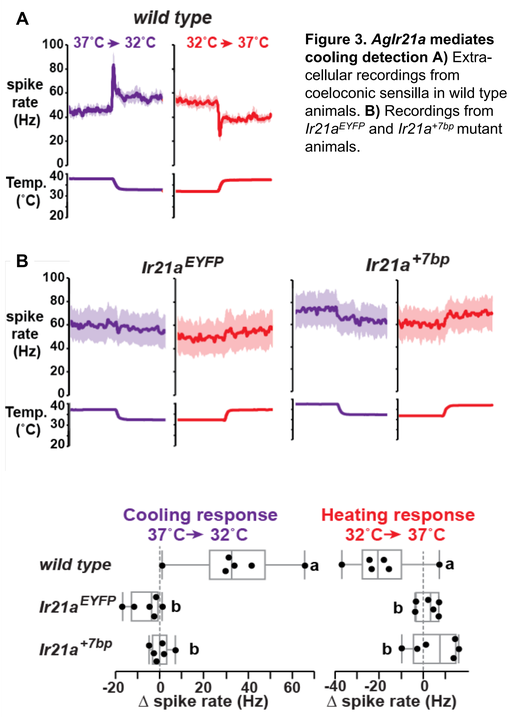

Figure 3. *AgIr21a* mediates cooling detection A) Extracellular recordings from coeloconic sensilla in wild type animals. **B)** Recordings from $Ir21a^{EYFP}$ and $Ir21a^{+7bp}$ mutant animals.

AgIr21a mutants show reduced heat seeking

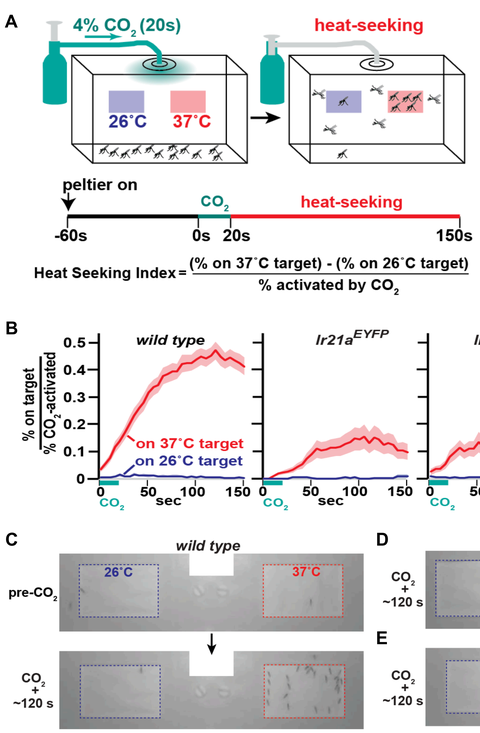

Figure 4. *AgIr21a* mediates heat-seeking. A) Female mosquitoes are placed in a Plexiglass box with two Peltier-heated targets. Peltiers are turned on for 1 minute, then a 20 sec 4% CO_2 pulse is provided and mosquitoes are given the opportunity to land on either target. **B)** Wild type, $Ir21a^{EYFP}$ and $Ir21a^{+7bp}$ mosquito landings on 37ºC and 26ºC target. % landing on each target is normalized to % mosquitoes that take flight in response to CO_2 pulse. **C)** Representative images of 26°C (blue) and 37°C (red) targets before (upper) and ~120 s after (lower) CO2 pulse initiation in wild type. **D-E)** ~120 s after CO2 pulse initiation in $Ir21a^{EYFP}$ (D) and $Ir21a^{+7bp}$ (E). Wild type n=27 assays, $Ir21a^{EYFP}$ n=12, $Ir21a^{+7bp}$ n=13.

AgIr21a mutants can still perform host approach

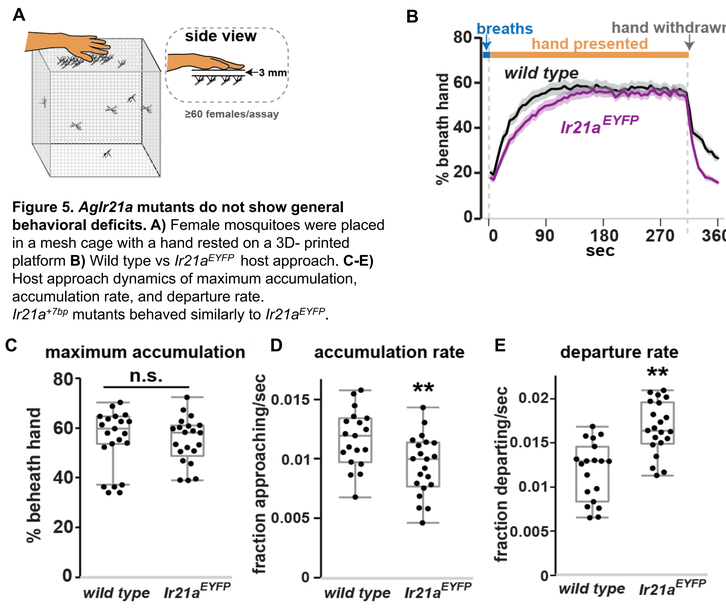

Figure 5. *AgIr21a* mutants do not show general behavioral deficits. A) Female mosquitoes were placed in a mesh cage with a hand rested on a 3D- printed platform **B)** Wild type vs $Ir21a^{EYFP}$ host approach. **C-E)** Host approach dynamics of maximum accumulation, accumulation rate, and departure rate. $Ir21a^{+7bp}$ mutants behaved similarly to $Ir21a^{EYFP}$.

AgIr21a promotes warmth-stimulated blood feeding

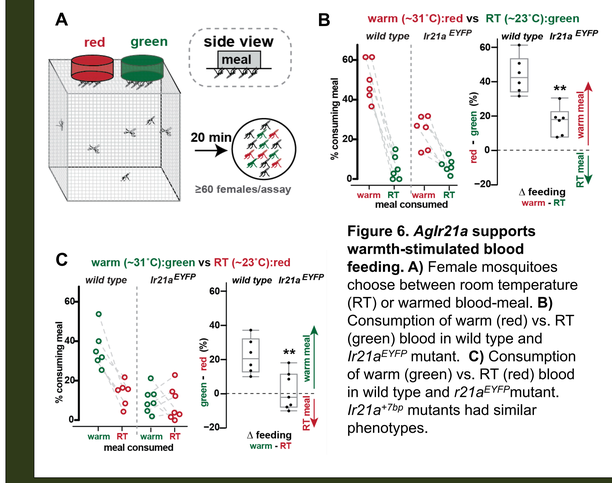

Figure 6. *AgIr21a* supports warmth-stimulated blood feeding. A) Female mosquitoes choose between room temperature (RT) or warmed blood-meal. **B)** Consumption of warm (red) vs. RT (green) blood in wild type and $Ir21a^{EYFP}$ mutant. **C)** Consumption of warm (green) vs. RT (red) blood in wild type and $r21a^{EYFP}$mutant. $Ir21a^{+7bp}$ mutants had similar phenotypes.

Conclusions and open questions

Heat-seeking in *An. gambiae* relies on an ancestral cooling receptor [5]. This suggests heat seeking behavior involves cooling avoidance [5].

In *Drosophila*, IR21a functions with two co-receptors conserved in mosquitoes, IR25a and IR93a [6,7]. Do these co-receptors have similar functions in *Anopheles*?

Is IR21a's role in heat seeking conserved in other mosquitoes?

Blood feeding emerged > 12 times during insect evolution. Has IR21a been repurposed for heat seeking in other lineages of hematophages?

How does the detection of other sensory cues like carbon dioxide alter IR21a-dependent thermosensing?

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