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RESEARCH ARTICLE

Multimodal sensorimotor system in unicellular zoospores of a fungus

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ABSTRACT

Complex sensory systems often underlie critical behaviors, including avoiding predators and locating prey, mates and shelter. Multisensory systems that control motor behavior even appear in unicellular eukaryotes, such as *Chlamydomonas*, which are important laboratory models for sensory biology. However, we know of no unicellular opisthokonts that control motor behavior using a multimodal sensory system. Therefore, existing single-celled models for multimodal sensorimotor integration are very distantly related to animals. Here, we describe a multisensory system that controls the motor function of unicellular fungal zoospores. We found that zoospores of *Allomyces arbusculus* exhibit both phototaxis and chemotaxis. Furthermore, we report that closely related *Allomyces* species respond to either the chemical or the light stimuli presented in this study, not both, and likely do not share this multisensory system. This diversity of sensory systems within *Allomyces* provides a rare example of a comparative framework that can be used to examine the evolution of sensory systems following the gain/loss of available sensory modalities. The tractability of *Allomyces* and related fungi as laboratory organisms will facilitate detailed mechanistic investigations into the genetic underpinnings of novel photosensory systems, and how multisensory systems may have functioned in early opisthokonts before multicellularity allowed for the evolution of specialized cell types.

KEY WORDS: Multisensory, Behavior, Phototaxis, Chemotaxis, Allomyces, Opisthokont

INTRODUCTION

All organisms rely on sensory systems to gather information about their surroundings using external stimuli. The integration of individual sensory modalities into multisensory systems greatly increases the amount of information an organism can use to form responses and behaviors. Although multimodal sensory systems are common in multicellular, motile organisms, there are significantly fewer multisensory systems known from unicellular eukaryotes (Govorunova and Sineshchekov, 2005). The relative rarity of studies characterizing these systems in unicellular, laboratory-tractable organisms has resulted in a significant taxonomic gap between current model systems and animals.

To address this deficit, we focused on fungi characterized in part by motile, zoosporic life stages. Zoosporic fungi collectively form a clade within the 'early-diverging lineages' of fungi, outside the better known Ascomycota and Basidiomycota. They are largely found in freshwater ecosystems with a global distribution (James et al., 2014). Zoosporic fungi are typically characterized as saprobes, such as *Allomyces*, although parasitic life strategies on both plant and animal hosts also do exist (Longcore et al., 1999). Similar to all fungi, colonies of *Allomyces* use mycelia to absorb nutrients and ultimately grow reproductive structures. Unlike most fungi, *Allomyces* produce zoosporangia, terminations of mycelial branches that make, store and ultimately release a multitude of single-celled, flagellated propagules, termed zoospores (Olson, 1984). When the appropriate environmental cues are present, zoospores are produced *en masse*, eventually bursting from zoosporangia (James et al., 2014). Once in the water column, the zoospores rely on a single, posterior flagellum to propel themselves away from the parent colony and towards suitable substrates or hosts (Olson, 1984).

During dispersal of the zoosporic life stage, interpretation of environmental cues is critical for the survival and success of the future colony (Dukes and Apple, 1961). *Allomyces* zoospores have a finite amount of endogenous energy reserves, and no zoospore is known to metabolize energy from external sources (Suberkropp and Cantino, 1973). This energetic constraint places significant pressure on the zoospore to efficiently locate a favorable environment for settlement and growth. The evolution and maintenance of a sensory system within the unicellular zoospore allows it to evaluate external conditions, move towards suitable habitats and avoid hazards (James et al., 2014). Previous studies across zoosporic fungi led to the discovery of a number of sensory modalities that guide zoospore dispersal including chemotaxis, phototaxis and electrotaxis (Machlis, 1969; Morris et al., 1992; Robertson, 1972). However, these studies have neither tested a single species for multiple sensory modalities nor posited the possibility that these single senses may only be a portion of a more complex sensorimotor system guiding zoospore dispersal and settlement.

Allomyces zoospores are known to use either chemotaxis or phototaxis, depending on the species, to guide dispersal and settlement (Pommerville and Olson, 1987; Robertson, 1972). Chemotaxis towards the source of amino acid gradients allows zoospores to congregate at the site of an injury or on decaying material in the water column (Machlis, 1969). *Allomyces macrogynus* zoospores possess refined chemosensation, settling on substrates at varied rates in response to different amino acids (Machlis, 1969). In contrast, the zoospores of *Allomyces reticulatus* display positive phototaxis, potentially leading spores to swim towards the air–water interface (Robertson, 1972). In animals, positive phototaxis and subsequent 'rafting' on floating debris work to considerably increase the dispersal range of planktonic larvae (Epifanio et al., 1989). Similarly, spores attracted to the water surface may encounter floating debris, algal hosts or currents that aid their dispersal. Though closely related, studies to date have not tested for the presence of chemotaxis in *A. reticulatus* or phototaxis in *A. macrogynus*.

While little is known about the molecular mechanisms of chemotaxis in fungal zoospores, the underpinnings of their

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photosensitivity are beginning to come to light. The protein responsible for light detection in zoospores of *Blastocladiella emersonii*, a close relative of *Allomyces*, is a bacteriorhodopsin gene called *CyclOps* (*Beme-Cycl*) (Avelar et al., 2014). Unlike many bacteriorhodopsins that regulate ion channels, *Beme-Cycl* acts through regulating intracellular cGMP (Avelar et al., 2015). A *CyclOps* gene is present in the genome of *A. macrogynus* (*Amag-Cycl*), a species that has been anecdotally described as having phototactic zoospores (Olson, 1984). A recent study by Gao et al. (2015), however, contradicts claims of phototaxis in *A. macrogynus* by revealing the proteins encoded by *Amag-Cycl* are orders of magnitude less sensitive to dark/light transitions than *Beme-Cycl* proteins (Gao et al., 2015). This raises questions about the sensitivity of *CyclOps* proteins required for phototaxis, and the mutations that have occurred to precipitate the loss of function in *Amag-Cycl* proteins.

The uncertainty surrounding the sensory systems of *Allomyces* zoospores, created by these conflicting reports, demands experimental evidence to clarify the number and type of modalities used during zoospore dispersal and settlement. Addressing the current deficits in our understanding of fungal sensory systems is also motivated by the potential to discover a multimodal sensorimotor system that will further our knowledge of multisensory evolution and function in early opisthokonts. Here, we investigated the responses to chemical and light gradients in three species of *Allomyces*, revealing previously unknown variation in fungal sensory systems and uncovering a novel multisensory system in a zoosporic fungus.

MATERIALS AND METHODS

Culture conditions

We used *Allomyces arbusculus* (strain ATCC 10983), *Allomyces reticulatus* (strain California 70 from ATCC, cat. no. 42465) and *Allomyces macrogynus* (Roberson lab, Arizona State University). We kept cultures of *A. macrogynus* and *A. arbusculus* in both solid and liquid media. For solid media, we used Machlis (1953) Emerson YSS (HiMedia M773) at half strength. Colonies was transferred aseptically in a laminar flow hood every 4 weeks by moving a chunk of mycelia from the leading edge of the colony onto a new plate. For liquid media, we followed the protocol for Machlis' medium B (1953). We inoculated liquid cultures via sterile transfer of sporangia and mycelia into a 125 ml Erlenmeyer flask containing 50 ml of liquid media and antibiotics (Machlis, 1953) for the first generation. For all subsequent generations kept in liquid culture, we used dilute salts solution to initiate sporulation of the previous generation's colonies. We then added 1 ml of this zoospore–dilute salts solution (referred to as 'sporulation product' from here on) into new liquid media. Both liquid and solid cultures were grown on an orbital shaker at 140 rpm and kept at room temperature (~24°C). Cultures in liquid media were kept for a maximum of 5 days, and were considered ready for sporulation after 72 h. Because *A. reticulatus* did not grow well in liquid media, we cultured *A. reticulatus* on full-strength Emerson YSS media for no more than 6 weeks. Propagation of *A. reticulatus* cultures in solid media was performed identically to propagation of the other species.

Sporulation conditions

Liquid cultures of *A. macrogynus* and *A. arbusculus* were considered for sporulation after 72 h. We visually inspected colonies under a microscope to confirm the absence of gametangia. Using a stainless steel sterile mesh, we strained the colonies out of growth media and rinsed them 5 times with dilute salts solution to remove the growth media. We then placed the rinsed colonies and strainer in a pyrex dish with 10 ml of dilute salts solution and allowed them to sporulate for no more than 90 min. Once either sufficient spore density had been reached [5×10^5 spores ml^{-1} for chemotaxis (Machlis, 1969), 1×10^6 spores ml^{-1} for phototaxis (Avelar et al., 2014)] or 90 min had elapsed, the mesh and colonies were lifted out of the dish (Machlis, 1969).

Because *A. reticulatus* was only grown on solid media, we took a surface scraping to lift sporangia from the agar and placed it into a pyrex dish with 10 ml dilute salts solution (Saranak and Foster, 1997). If no zoospores were present, we replaced the dilute salts solution every 20 min for the first hour. Sporulation typically occurred within 8 h, after which the colonies were strained from the dilute salts solution.

Phototaxis trials

We conducted phototaxis trials in a custom-made 1×3×5 cm (W×H×L) Plexiglas chamber. We added 10 ml of sporulation product, diluted to 1×10^6 spores ml^{-1} (Avelar et al., 2014), to the test chamber and left the solution for 15 min in total darkness to dark adapt and randomize spore distribution. Preliminary trials showed that an adjustment period of 15 min, rather than 30 min (Avelar et al., 2014), was sufficient for zoospore randomization and dark adaptation. After the adjustment period, spores were exposed to a white light (USHIO halogen bulb) through a 5 mm diameter fiber optic cable positioned 5 cm from the leading edge of the test chamber. To calibrate the light intensity, we used a JAZ Oceanoptics light sensor with Spectrasuite v2.0.162. We adjusted the intensity of the light to $1.8–1.0\times10^{13}$ mol of photons cm^{-2} on the edge closest to the light source. We measured temperature with a thermometer placed in the center of the test chamber with the light source on for 15 min. The chamber was designed with thick plastic to limit heat transfer to the zoospore suspension; no appreciable thermal turbulence or change in temperature was observed after 15 min of light exposure, at which point we divided the test chamber into four sequential sub-chambers (1×3×1.25 cm) using sterile glass slides. This resulted in four sub-chambers (1–4) arranged linearly so that sub-chamber 1 was closest to the light source, while sub-chamber 4 was the farthest away (Fig. S1A). We gently agitated the liquid in each sub-chamber to homogenize swimming spore distribution and counted swimming spore density in four, 10 µl samples from each sub-chamber using a hemocytometer. To account for the variation in spore counts between each trial in both control and light treatments, we quantified the number of swimming spores in each chamber as a proportion of the total number of spores counted in that trial. This resulted in less inter-trial variation. A total of 10 control treatments (no light exposure) and 18 experimental treatments were conducted for each species.

Chemotaxis trials

Chemotaxis trials followed the protocol established by Machlis (1969). The amino acids and combinations thereof we tested were lysine (K), leucine (L), proline (P), L+K, L+P, K+P, L+K+P and buffer (5×10^{-3} mol l^{-1} KH_2PO_4) solution (referred to as 'treatment solutions' from here on). All amino acid concentrations were 5×10^{-4} mol l^{-1} for each amino acid in all treatments. We created a chemical dispersal apparatus by drilling a hole through the lid of a 60×15 mm Petri dish and inserting a 5 mm inner diameter glass pipette. We secured dialysis membrane (3500 MWCO) to the tip of the pipette and positioned it 3 mm above the bottom of the Petri dish (Fig. S1B). This creates a gradient in the Petri dish of whatever solution is placed behind the dialysis membrane, allowing

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zoospores to navigate to the membrane, where they settle and can later be counted. The dialysis membrane was soaked and rinsed with deionized water for 24 h to remove potential contaminants and bubbles that would affect results (Carlile and Machlis, 1965). Turbulence in the Petri dish would greatly affect the strength of the gradient. To avoid this, we placed the modified Petri dishes in a larger, sealed container to avoid drafts and the treatment solution was added last. This resulted in no movement of any container between the addition of treatment solution and the end of the trial.

To test chemotactic and settlement responses to these treatments, we added 10 ml of sporulation product (diluted to 5×10^5 spores ml^{-1}) to the Petri dish and 300 µl of treatment solution into the pipette. As a control, we used 300 µl of buffer alone. We allowed the spores to react to the gradient in total darkness for 90 min. At the end of the trial time, we removed the pipette and dialysis membrane from the dish and gently shook it to remove excess liquid (Machlis, 1969). We counted spores settled on the membrane under an Olympus szx7 microscope at 400× or greater magnification.

Molecular methods

PCR, cDNA synthesis and sequencing

Because genomic data existed for *A. macrogynus* but expression data did not, we used PCR to attempt to identify whether CyclOps genes are expressed in *A. macrogynus* zoospores. mRNA was extracted from zoospores using a NucleoSpin RNA XS kit. We synthesized cDNA using the Clontech cDNA synthesis kit. Primers were designed from putative rhodopsin/guanalyl cyclase fusion proteins identified from the BROAD institute's *Allomyces macrogynus* genome, using the CyclOps protein from *Blastocladiella emersonii* as the bait sequence (Avelar et al., 2014). Sets of primers were designed in IDT PrimerQuest. All PCR products were visualized using a 1% agarose gel with 100 bp ladder. PCR for CyclOps was only done on *A. macrogynus* as transcriptomes for *A. reticulatus* and *A. arbusculus* would yield expression data.

RNA was isolated from zoospores of *A. reticulatus* and *A. arbusculus* using a Nucleospin xsRNA kit, and cDNA was synthesized using the NEB Next RNA First and Second Strand Synthesis modules. cDNA was sequenced using a multiplexed Illumina HiSeq lane at approximately 50× coverage.

Bioinformatics and statistics

We trimmed Illumina data using Trimmomatic (Bolger et al., 2014), assembled using Trinity 2.0, and analyzed on the UCSB Osiris bioinformatics platform (Giardine et al., 2005; Oakley et al., 2014). Putative CyclOps proteins were identified using *Beme-Cycl* as a bait sequence in BLASTn searches against the *A. macrogynus* genome (NCBI bioproject 20563) and the new *A. reticulatus* and *A. arbusculus* zoospore transcriptomes. Any sequence with an e-score lower than 1e−40 was considered as a candidate. We then used the 'get orf' feature from Trinity to produce predicted proteins from candidate genes, selecting the longest open reading frames (ORFs) with the highest similarity to *Beme-Cycl* protein when reciprocally BLASTed using blastp. We identified the putative *CyclOps* gene in both *A. reticulatus* and *A. arbusculus* (*Aarb-Cycl*). Reads from the *A. arbusculus* transcriptome were mapped back to the putative *Aarb-Cycl* using Bowtie2 (Langmead and Salzberg, 2012) and visualized using IGV viewer (Thorvaldsdóttir et al., 2013). Because the bacteriorhodopsin and guanylate cyclase domains appeared in different ORFs on the same strand, each base was manually examined for uncertainty and low support. A guanine at site 378 was manually removed because of low coverage and low support in the reads, implying that the addition of guanine at position 378 was most likely an assembly artifact. The manually edited *Aarb-Cycl* gene produced a single predicted ORF with the appropriate bacteriorhodopsin–guanylyl cyclase domains (Fig. S2).

Candidate proteins were aligned using MAFFT under the L-INS-i strategy. Outgroups were selected based on a previous analysis (Avelar et al., 2014) (Fig. S2). The alignment was used to create a phylogeny of candidate genes with RAxML 8 (Stamatakis, 2014) and 100 bootstrap replicates using the GTR+Γ model. Trees were visualized in Evolview (He et al., 2016) and annotations were added in Adobe Illustrator.

Comparisons of zoospore phototaxis behavior were analyzed using JMP v13.0. Average spore counts per sub-chamber per trial were analyzed using a one-way ANOVA. Although sample size was low, each sample represents an average of four replicates for each treatment. Zoospore chemotaxis was analyzed using Wilcoxon each pair test because of the non-parametric distribution of results and small sample size (n=10).

RESULTS

Allomyces reticulatus relies on phototaxis

Zoospores of *A. reticulatus* showed no significant deviation from the control when exposed to any amino acid treatment (P>0.05 for all treatments) (Fig. 1A). In accordance with existing literature, *A. reticulatus* showed a significant response to a directional light source (Robertson, 1972). The number of zoospores swimming in the sub-chamber closest to the light source (Fig. 2A) was significantly higher than that when no light source was present (P=0.0001).

Allomyces macrogynus relies on chemotaxis

As seen in previous experiments, *A. macrogynus* zoospores displayed a significant response to all amino acid treatments compared with the control (Machlis, 1969) (P, P=0.0294; L, P=0.0275; K, P=0.0294; and any combination of two or three amino acids when compared to a control P<0.001) (Fig. 1C). The zoospore response to increasing treatment complexity was non-linear though roughly equal for all unique combinations of equal complexity. *Allomyces macrogynus* zoospores showed no response when exposed to a directional light source (Fig. 2C). The number of zoospores in all sub-chambers was the same for both light and dark trials (P=0.8801).

Allomyces arbusculus uses both chemotaxis and phototaxis in a multisensory system

As expected from existing literature, *A. arbusculus* zoospores responded similarly to the zoospores of *A. macrogynus* when exposed to amino acid treatments. The number of spores settled increased in a non-linear fashion as the complexity of the treatment increased, though at a lower average number of settled spores when compared with *A. macrogynus* (K+P, P=0.0014; K+L, P=0.0014; L+P, P=0.0008; K+L+P, P=0.0004) (Fig. 1B). As opposed to *A. macrogynus*, *A. arbusculus* did not respond to, or could not detect, gradients of single amino acids (P>0.05 for all single amino acid treatments) with the possible exception of proline. We found that no zoospores settled when *A. arbusculus* was exposed to trials of proline alone, potentially indicating negative chemotaxis or inhibition of settlement in response to gradients of proline by itself. However, we cannot definitively resolve this reaction with our sample size (P versus control: P=0.072, N=10). When exposed to a directional light source, *A. arbusculus* displayed positive phototaxis (Fig. 2B). The number of zoospores in the sub-chamber closest to the light was significantly higher (P<0.0001) in light versus

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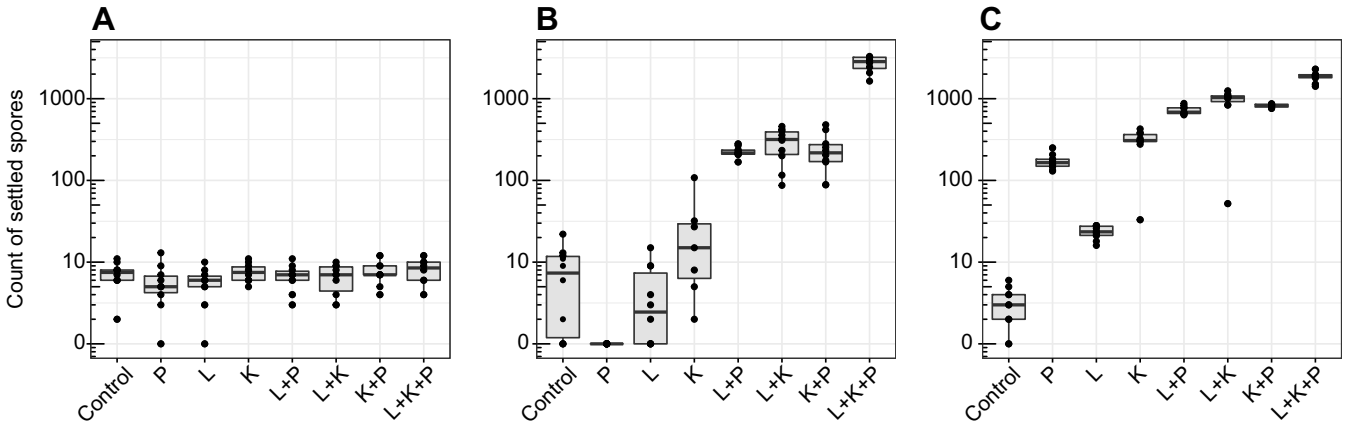

Fig. 1. The number of zoospores settled on the dialysis membrane in response to varying amino acid treatments. (A) *Allomyces reticulatus*, (B) *Allomyces arbusculus* and (C) *Allomyces macrogynus*. Each column represents the number of zoospores settled on 2 mm^2 dialysis membrane after 90 min. Data are for control, proline (P), leucine (L), lysine (K) and combinations thereof. *N*=10 for all treatments. Note the log scale for the *y*-axis.

dark trials and was comparable to the response of *A. reticulatus* zoospores.

CyclOps is present in all species, may not be expressed in *A. macrogynus* zoospores

Phylogenetic analysis of putative *CyclOps* genes revealed the presence of *CyclOps* in all three samples (*A. arbusculus* and *A. reticulatus*: transcriptome data; *A. macrogynus*: previously available genome data). The single copy of *CyclOps* recovered from the *A. arbusculus* transcriptome showed a possible truncation of the guanylyl cyclase domain (Fig. 3). *Aarb-Cycl* and *Amag-Cycl* share a mutation at the putative functional residue F313 to I313. Despite the success of positive controls indicating successful PCR amplification, no primers successfully amplified *Amag-Cycl* from *A. macrogynus* zoospores.

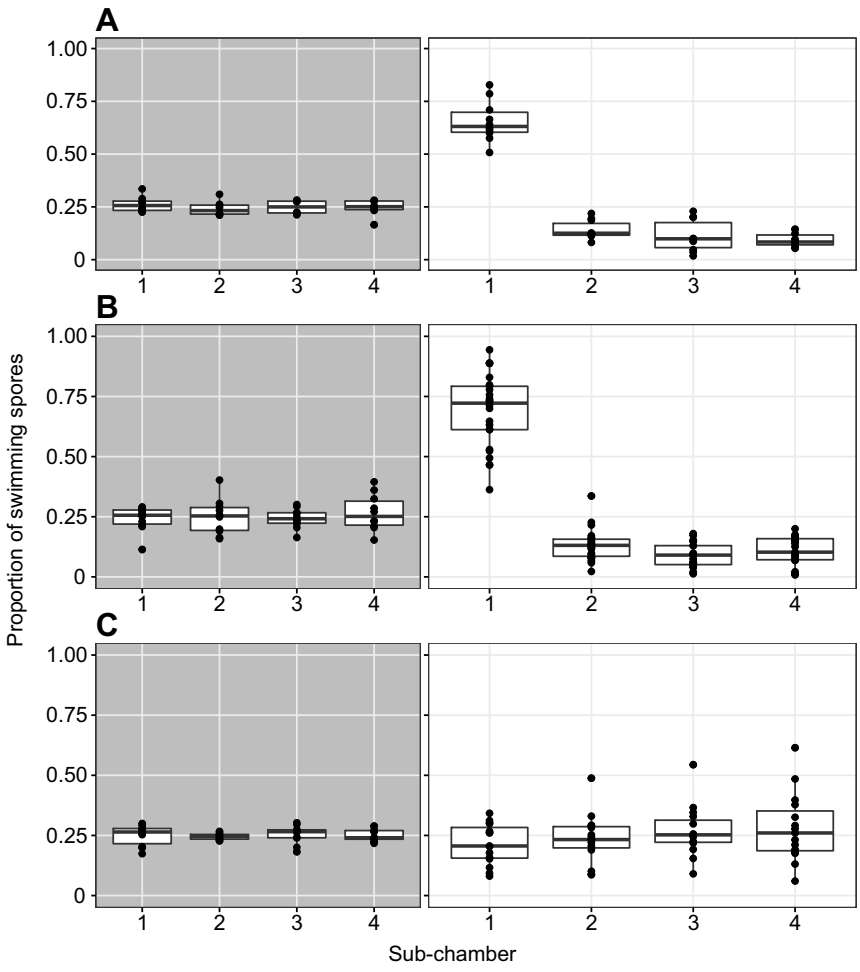

Fig. 2. The proportion of swimming zoospores found in each sub-chamber in phototaxis trials. Zoospore distribution of (A) *Allomyces reticulatus n*=10, (B) *A. arbusculus n*=26 or (C) *A. macrogynus n*=16 after 15 min of darkness followed by 15 min exposure to directional light (white background) or 30 min of darkness (gray background, *n*=10 for all species). The directional light source was positioned so light intensity was strongest in sub-chamber 1 and lowest in sub-chamber 4. Data points in each sub-chamber represent the proportion of swimming zoospores counted in that sub-chamber relative to the total number of swimming zoospores counted in that trial. This method reduces the effect of variable intertrial numbers of swimming zoospores on the overall trend.

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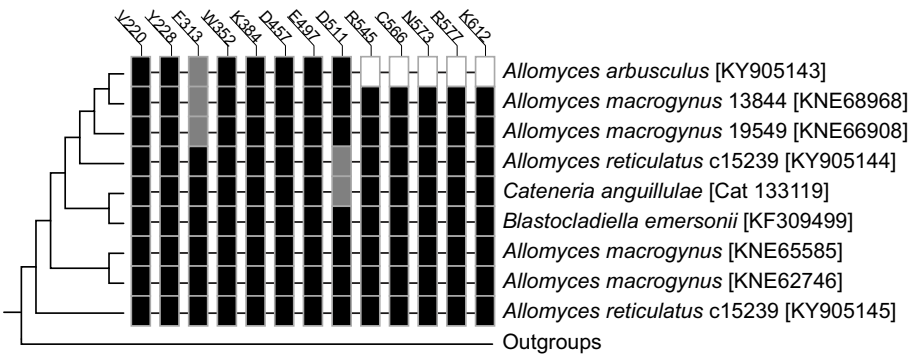

Fig. 3. Cladogram showing the relationships and conserved amino acid residues in CyclOps proteins. Boxes indicate amino acid residues critical for binding (Avelar et al., 2014), with residue numbers based on position in unaligned Beme-Cycl (AIC07007.1). Black boxes indicate an amino acid matching at that residue when compared with Beme-Cycl; gray boxes indicate a mutation; and white boxes indicate a gap.

DISCUSSION

How sensory modalities evolve and integrate with other behavioral circuits remains an open question in both neurobiology and evolutionary biology. The *Allomyces* genus, with new variation in sensory systems first discovered in this study, will aid in answering these questions. Previous studies showed *Allomyces* spores use either chemotaxis or phototaxis to guide dispersal. Here, we reveal that the sensorimotor system in *A. arbusculus* is multimodal – able to process both chemical and light cues. Additionally, our results reveal the previously unknown complexity and variation of sensorimotor systems in *Allomyces*.

Variation in sensory modalities across *Allomyces*

We report previously unknown variation in the distribution of sensory modalities across the genus. This variation manifests in two ways: the types of sensory modalities used by each species of *Allomyces* and the responses of *A. arbusculus* and *A. macrogynus* zoospores to the same amino acids. The lack of phototaxis, coupled with the inability to amplify *CyclOps* from zoospore mRNA in *A. macrogynus* is quite interesting because of conflicting reports between previous studies. These studies state either that *A. macrogynus* zoospores are phototactic (through anecdotal evidence) (Olson, 1984) or that the photosensitive protein, Amag-Cycl, does not respond effectively to light (Gao et al., 2015). Our findings support the hypothesis that *CyclOps* in *A. macrogynus* no longer effectively differentiates between light and dark, and suggest that *CyclOps* expression has been lost in *A. macrogynus* zoospores. The shared distribution of *CyclOps* expression and photosensitivity in *A. reticulatus*, *A. arbusculus* and *Blastocladiella* make it likely that phototaxis was an ancestral character state in *Allomyces* (Fig. S3). It is currently unclear whether this loss occurred in a natural ecosystem or as a byproduct of being kept in culture. Anecdotal evidence exists for the loss/suppression of phototaxis in lab-cultivated fungus (Olson, 1984), and particular long-term algal cultures show degraded eyespot organization (Moldrup et al., 2013). Unfortunately, because all evidence of phototaxis in *A. macrogynus* is anecdotal, there is no way to know the state of the original culture without re-isolation of a wild strain. If phototaxis was lost in a natural setting, the dispersal and settlement patterns of *A. macrogynus* zoospores would likely deviate greatly from those of both *A. reticulatus* and *A. arbusculus* in the wild. These differences would present a mechanism for potential speciation driven by mutation in sensorimotor systems. However, if the relatively recent cultivation of *A. macrogynus* led to the degradation of photosensitivity in zoospores, it would present an excellent opportunity to examine how multisensory systems in simple organisms remodel after the loss of an input.

The variation in *Allomyces* sensory systems discovered in this study coupled with the convergent function of *CyclOps* and animal opsins make it critical to our understanding of the evolutionary history of light sensing. Animal photoreception, mediated through Type II opsins, operates through intracellular regulation of cyclic nucleotides. Non-animal photoreception, mediated through Type I opsins, operates through channel and sensory rhodopsins (SRII). However, unlike Type II opsins, SRIIs do not regulate cyclic nucleotide concentrations. Instead, SRIIs often indirectly regulate CheY, a protein that controls flagellar motion (Klare et al., 2004). Type I and II opsins are considered a spectacular example of convergent evolution (Larusso et al., 2008). *CyclOps* represents a functional evolutionary convergence, where the protein may modulate intracellular cGMP levels like some Type II phototransduction cascades, yet the sequence of *CyclOps* indicates its origin as a Type I opsin (Avelar et al., 2015). Studying the evolution of *CyclOps* sequence function through the variation in *Allomyces* will yield further insight into the evolution of this novel photosensory mechanism.

The failure of *A. reticulatus* to move and settle on the cellulose membrane does not necessarily indicate a complete lack of chemotaxis, but does highlight variation in *Allomyces* sensory system evolution. The amino acids tested here are frequently associated with wounded tissue and decaying organic matter, and were isolated from crushed, natural substrate in previous studies, making them appropriate for guiding the distribution and settlement of sessile saprobes (Carlile and Machlis, 1965; Machlis, 1969). While previous studies uncovered differences in the combinations of amino acids that *A. arbusculus* and *A. macrogynus* zoospores move toward (Machlis, 1969), no one tested the possible loss of this chemotactic response in closely related species. The absence of chemotaxis to these amino acids in *A. reticulatus* may reveal a turnover in sensory capabilities responsible for controlling a vital behavior across the genus. In future studies, we will use the variation in both chemotaxis and phototaxis across *Allomyces* as a platform to understand behavioral integration, multisensory evolution and sensory remodeling in an organism closely resembling ancestral opisthokonts.

The multisensory system of *A. arbsuculus*

Allomyces arbusculus zoospores present an easily culturable, laboratory tractable system for investigating multimodal sensation in unicellular systems and its underlying mechanisms. Previous studies have independently confirmed that zoospores use a diversity of senses, but fungal zoospores have only been tested for a single sense per species (Avelar et al., 2014; Machlis, 1969; Morris et al., 1992; Robertson, 1972). Our findings represent the first study of zoospore multimodal sensing, and a concrete example of

multimodal sensorimotor control in a unicellular opisthokont (Figs 1,2). Though choanoflagellates fall within Opisthokonta (Cavalier-Smith et al., 2014) and potentially exhibit both aerotaxis and pH-taxis, it remains unknown whether the sensory modalities in colonies (aerotaxis) are also used to direct the dispersal stage (pH-taxis) (Kirkegaard et al., 2016; Miño et al., 2017).

Zoospores, as unicellular, flagellated cells, might closely represent the ancestral opisthokont phenotype (Cavalier-Smith et al., 2014). Specialized cell types and functions may often evolve through subfunctionalization followed by elaboration of the ancestral cell's functions (Arendt et al., 2016). This implies that as multicellular opisthokonts evolved, the foundation for specialized sensory modalities/cells already existed in 'pluripotent', generalized pathways. Under the subfunctionalization hypothesis, multimodal systems in unicellular organisms, such as we report in *A. arbusculus*, must have evolved prior to subfunctionalization in ancestral, multicellular opisthokonts. Future studies of the multimodal sensorimotor system in *A. arbusculus* zoospores and the variation in modalities across *Allomyces* may uncover how multiple senses became integrated into behavioral responses in ancestral opisthokonts. Understanding these mechanisms in the context of the cellular subfunctionalization hypothesis will further our understanding of sensorimotor evolution, elaboration and individuation through cellular specialization.

Conclusions

We present a multimodal sensorimotor system in a unicellular life stage of a fungus. The multisensory system of *A. arbusculus* zoospores is an excellent system to study how sensory modalities integrate into existing behavioral regimes. Together with existing models of unicellular sensory mechanisms, the variation in sensory modalities in *Allomyces* and other early diverging fungi will allow us to formulate more accurate conclusions about the evolution of complex sensory systems and multisensory systems in ancestral eukaryotes. Lastly, the relatively narrow taxonomic breadth associated with the multiple transitions in sensory systems during *Allomyces* evolution will allow testing of broad questions in evolution; such as the role multimodal cell types play in the origin and evolution of specialized sensory systems, and how emergent behaviors evolve during sensory remodeling.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.J.M.S.; Methodology: A.J.M.S., T.H.O.; Software: A.J.M.S.; Validation: A.J.M.S.; Formal analysis: A.J.M.S.; Investigation: A.J.M.S.; Resources: A.J.M.S., T.H.O.; Data curation: A.J.M.S.; Writing - original draft: A.J.M.S., T.H.O.; Writing - review & editing: A.J.M.S., T.H.O.; Visualization: A.J.M.S.; Supervision: A.J.M.S., T.H.O.; Project administration: A.J.M.S.; Funding acquisition: A.J.M.S., T.H.O.

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Data availability

Alignment, newick trees and behavioral data collected for this study is available for download at https://bitbucket.org/swafford/multimodal_sensorimotor_systems_jeb_2018/overview

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.163196.supplemental

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