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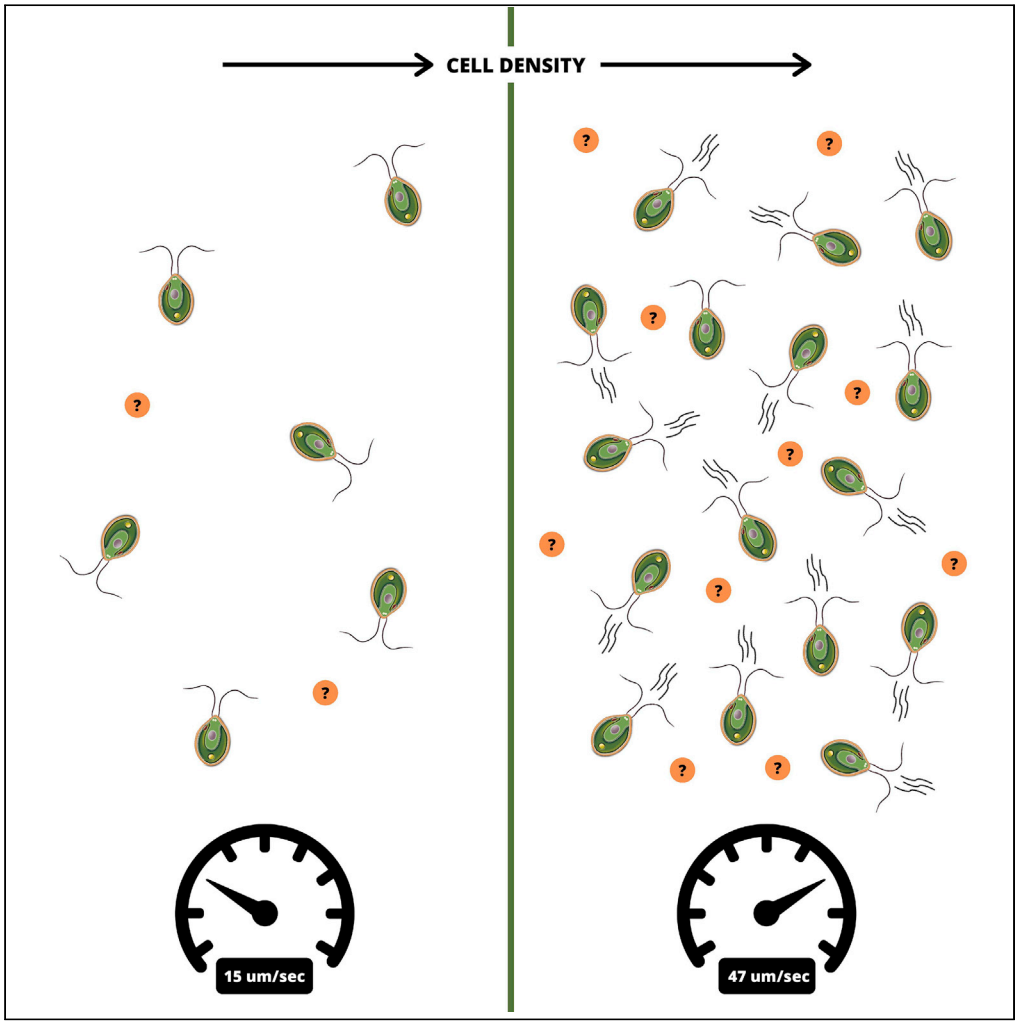

Alexandra M. Folcik, Kirstin Cutshaw, Timothy Haire, ..., Faizan Zaidi, Brianna Richardson, Andrew Palmer

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HIGHLIGHTS

Swimming speed in *Chlamydomonas reinhardtii* increases as with population density

Increased swimming speed depends on a low-molecular-weight organic compound

This response is conserved and interchangeable between *C. reinhardtii* and *C. moewusii*

This indicates expansion of quorum sensing to a new genus of unicellular eukaryotes

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Quorum Sensing Behavior in the Model Unicellular Eukaryote *Chlamydomonas reinhardtii*

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SUMMARY

Microbial communities display behavioral changes in response to variable environmental conditions. In some bacteria, motility increases as a function of cell density, allowing for population dispersal before the onset of nutrient scarcity. Utilizing automated particle tracking, we now report on a population-dependent increase in the swimming speeds of the photosynthetic unicellular eukaryotes *Chlamydomonas reinhardtii* and *C. moewussi*. Our findings confirm that this acceleration in swimming speed arises as a function of culture density, rather than with age and/or nutrient availability. Furthermore, this phenomenon depends on the synthesis and detection of a low-molecular-weight compound which can be transferred between cultures and stimulates comparable effects across both species, supporting the existence of a conserved phenomenon, not unlike bacterial quorum sensing, among members of this genus. The potential expansion of density-dependent phenomena to a new group of unicellular eukaryotes provides important insight into how microbial populations evolve and regulate "social" behaviors.

INTRODUCTION

Once thought to operate as completely independent entities, microorganisms are now understood to engage in behaviors as complex and diverse as any of the large, multicellular organisms, with which they frequently associate. These interactions are crucial to allowing the community to respond to changing environmental conditions such as a decline in nutrients, changes in temperature, the introduction of invasive species, etc (Bowers and Parke, 1993; Hibbing et al., 2010; Tans-Kersten et al., 2001; Xie and Wu, 2014). The phenomenon known as quorum sensing (QS) allows microorganisms to couple phenotypic switching to cell density, ensuring the emergence of specific behaviors when they will be most productive. For example, at low cell densities, the production of exopolysaccharides by bacteria is unlikely to produce a beneficial biofilm. However, at high cell densities, the excretion of these extracellular matrices results in biofilms that provide significant advantages to resource sharing and persistence (Stewart and Franklin, 2008). Other QS-regulated phenotypes include motility, conjugation, antibody production, bioluminescence, virulence factor production, and more (for recent reviews, see (Papenfort and Bassler, 2016; Welsh and Blackwell, 2016)). QS has provided new targets for the regulation of specific microbial behaviors and revolutionized the field of synthetic biology (Palmer et al., 2011a; Tamsir et al., 2011).

QS is regulated by the diffusion of a broad assortment of low-molecular-weight organic compounds, generically referred to as autoinducers (AIs). At low cell densities, these signals are synthesized at a constitutive level and then diffuse or are actively transported out of the cell into the surrounding environment. AIs are perceived by dedicated receptors either at the cell surface or intracellularly once a sufficient concentration has been reached. Formation of the AI:receptor complex directs changes in the gene expression either directly by receptor binding to specific promoter sites on DNA or indirectly through a phosphorylation cascade. Regardless of the exact mechanism, these relatively simple biological circuits allow AI concentration to serve as a proxy for cell density.

While originally discovered in bacteria, QS has also been observed in the other two major domains (superkingdoms) of life: archaea and eukarya. For example, *Methanothrix harundinacea*, a methanogenic

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archaeon, forms filaments due to the accumulation of carboxylated *N*-acyl-L-homoserine lactones, a class of AIs commonly used by gsram-negative bacteria (Montgomery et al., 2013). Similarly, *Candida albicans*, a fungus found primarily in human microbiota, exhibits a filamentous phenotype at low cell densities but grows instead as budding yeast at high cell densities. In this example, the isoprenoid farnesol serves as the AI which regulates QS (Albuquerque and Casadevall, 2012). Additional studies have established the presence of other QS-regulated phenotypes among fungi, typically regulated by a variety of aromatic amino acid alcohols which serve as the AIs for these systems (Hornby et al., 2004; Roca et al., 2005). The above examples support QS as a convergent evolutionary strategy employed by unicellular microorganisms across all three domains of life to coordinate behaviors and increase fitness.

While prevalent in fungi, there are no examples of this phenomenon among other eukaryotes. Yet, most eukaryotic microorganisms face many of the same challenges faced by prokaryotes, suggesting QS would provide an equally effective strategy for success. The obvious challenge to the discovery process is the identification of the phenotypic switch, *i.e.* what behavior manifests in response to a quorum being met.

Motility is one commonly regulated phenotype in prokaryotic QS, as in *Staphylococcus aureus*, which uses the phenomenon to detect high cell densities prior to reaching the carrying capacity of their environment. Strategies for dealing with nutrient availability have proven a 'good bet' over evolutionary time across multiple kingdoms. Indeed, simple population dispersion to explore new environments may have been one of the earliest pressures for QS.

One obvious limiting factor to the study of the behavioral responses of the individuals within these communities is their small average size (<10 μm). We recently developed a simple particle tracking method utilizing recorded video to observe the distribution of swimming speeds in cultures of microorganisms (Folcik et al., 2020). These videos are processed using an optimized analysis pipeline including publicly available software Fiji (ImageJ), the TrackMate plug-in for this package, and R statistical computing software. Using this automated small particle tracking method, we are able to gather significant amounts of data (>3000 tracks) on culture speed, directionality of movement, and cell size to investigate population dynamics in real time.

In this study, we have utilized this tracking technique to investigate the effects of culture age on the swimming speed of the model unicellular eukaryote *Chlamydomonas reinhardtii*. This model organism has been used extensively to study flagellar motility as well as both phototaxis and chemotaxis (Fujiu et al., 2011; Huang et al., 1982; W. F. Marshall, 2009; Stavis and Hirschberg, 1973; Wakabayashi et al., 2011). Our findings reveal a previously unreported correlation between swimming speed and cell density. This response is due to the accumulation of one or more low-molecular-weight organic molecules which can be isolated from these cultures and used to stimulate increased swimming speeds at lower density cultures. This phenomenon is conserved in the closely related *Chlamydomonas moewusii*, and the signals in both species are equally active in the other. We note the similarities in this phenomenon to prokaryotic QS circuits. Finally, we discuss the potential impact of this newly observed instance of QS on nutrient acquisition and utilization in this well-represented and ecologically important genus of photoautotrophs, as well as the broader implications to our understanding of microbial ecology.

RESULTS

Swimming Speed Increases Over Time in cc124 Cultures

We began by comparing *C. reinhardtii* wildtype (strain cc124) motility between 24-, 48-, and 72-h cultures to observe any baseline differences which might emerge as a function of culture age (videos are available on Dryad, see Data Availability Statement for information). As shown in Figure 1, swimming speed in cultures of *C. reinhardtii* appeared to be positively correlated with culture age. Average swimming speeds of this wildtype culture at 24, 48, and 72 h were 15, 28, and 47 μm/sec, respectively (standard error of the mean [SEM] = +/− 6–12 μm/sec). While the average at 72 h is slower than what has been reported in some of the literature (Marshall, 2009), it is consistent with our previous studies as well as others (Chen et al., 2017; Folcik et al., 2020). In addition to average speed, our method allows us to observe speed distributions within the population, as shown by the histograms provided in Figure 1. Over the 48 h of this assay, there was a significant reduction in low or no motility ($\lesssim$20 μm/sec) cells.

Over the duration of this study, cultures increased in density from $\approx 10^5$ cells/ml at 24 h to $\approx 10^7$ cells/ml at 72 h. This 100-fold increase in cell density would almost certainly reduce the amount of nutrients available,

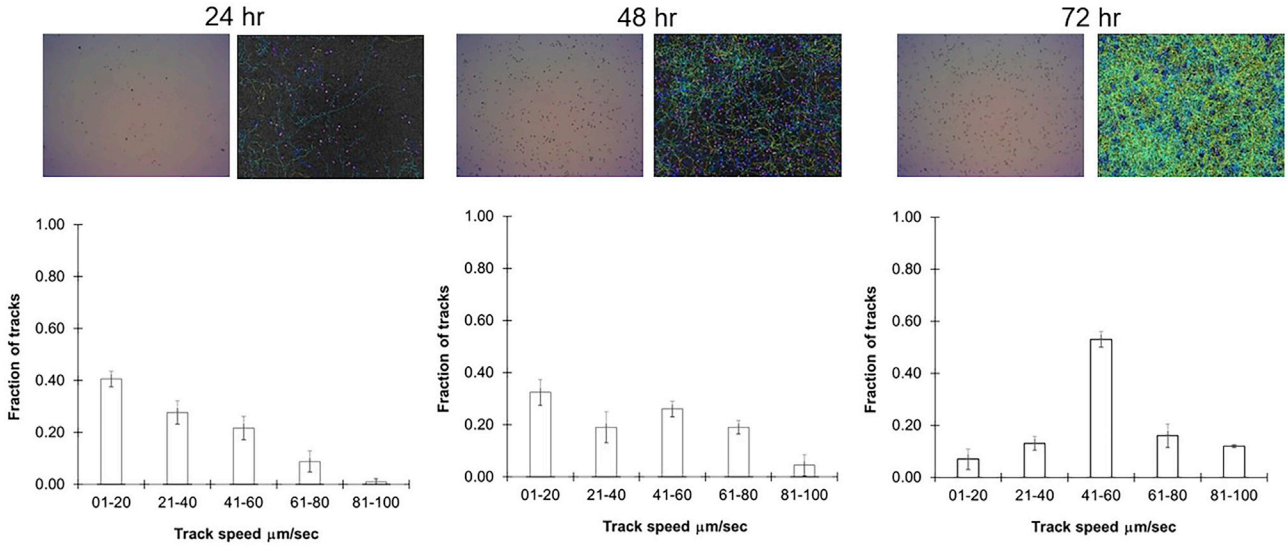

Figure 1. Cultures of *Chlamydomonas reinhardtii* Increase Swimming Speed as a Function of Culture Age

Cultures were grown for the indicated time, then a fraction was isolated, and analyzed for motility. (Top) For each time point, the top left image is a single frame from a 30 s video, while the top right is an analyzed image highlighting the total number of tracks. (Bottom) Histogram of *C. reinhardtii* swimming speeds as a function of culture age. Results are the average of three videos with error bars indicating +/− SEM.

potentially driving the search for new nutrient sources. Prior observations that *C. reinhardtii* is capable of chemotaxis toward nitrogen sources when this nutrient is scarce support this "direct" model based on nutrient deficiency (Byrne et al., 1992; Ermilova et al., 2007). Alternatively, increased cell-cell contact, as density increases over culture age, could trigger increased swimming. Prior studies have confirmed that *C. reinhardtii* can respond to physical stressors, supporting the viability of this "mechanical" model (Herron et al., 2019). Finally, as cultures age, a low-molecular-weight compound that increases swimming speed could be released (signal/cue model).

Cell-Cell Contact Does Not Increase Swimming Speeds

We hypothesized that a simple media swap experiment would allow us to distinguish simple mechanosensitivity (the response to physical contact) from the "direct" and "signal/cue" models described above. As seen in Figure 2, 6 hours after their exposure to filter-sterilized (0.2 μm cutoff) 72-h-old media, 24-h cultures showed a significant increase in the average speed of the population (51 ± 12 μm/sec SEM). This increase was statistically indistinguishable from that for 72-h cultures (47 ± 11 μm/sec SEM, p value <0.01). Seventy-two-hour cultures displayed a moderate reduction in their swimming speeds following exposure to 24-h culture media (42 ± 6 μm/sec SEM). These results support the "direct" or "signal/cue" models for increasing swimming speed, while arguing against the "mechanical" model, as cell density does not increase in these studies.

A Low-Molecular-Weight Compound Triggers the Increase in Swimming Speed

The previous media swap experiment is unable to distinguish between the remaining two models proposed for increased culture swimming speeds: the "direct" detection of reduced nutrients or signal/cue-induced changes in swimming speeds. We therefore envisioned an experiment that would allow us to extract organic compounds from the culture media and apply them to cultures of different ages and nutrient content. To accomplish this, we performed an organic extraction of cell-free 24- and 72-h *C. reinhardtii* culture media with ethyl acetate.

The addition of the 72-h extracts to 24-h-old cultures showed an increase in cell swimming speed upon treatment (44 ± 7 μm/sec SEM) (Figure 3). Twenty-four-hour extracts had no significant effect on the swimming speeds of either 24- or 72-h cultures of *C. reinhardtii* (data not shown). Dimethyl sulfoxide (DMSO) loading controls also had no effect on motility in either 24- or 72-h cultures.

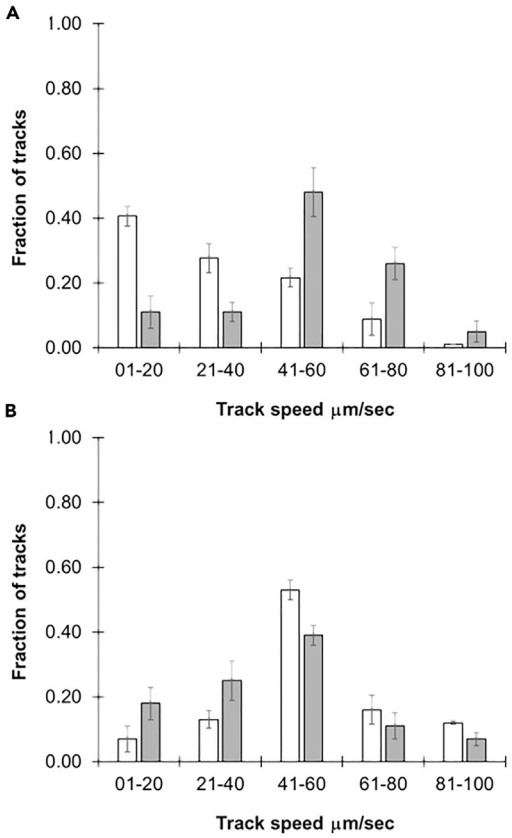

Figure 2. Older *Chlamydomonas reinhardtii* Media Induces Higher Swimming Speeds

Cultures were grown for (A) 24 or (B) 72 h and then either evaluated for motility directly (white bars) or after media swap (gray bars). For the media swap, pelleted cultures of *C. reinhardtii* were re-suspended in the other media (24-h cells in 72-h media in A and 72-h cultured cells in 24-h culture media in B). The effects on swimming speed were determined by image analysis after 6 h. Results are expressed as the mean of 3 videos with error bars indicating +/− SEM.

As 72-h extracts were able to increase swimming speeds in the more nutrient-rich 24-h cultures, it argues against a "direct" nutrient deficiency model to explain the increase in swimming speed. These findings are consistent with our proposed "signal/cue" model of a diffusible low-molecular-weight compound being responsible for this effect. We refer to this unknown signal(s) or cue(s) as the Chlamydomonas swimming speed factor (CSSF) for the remainder of the text.

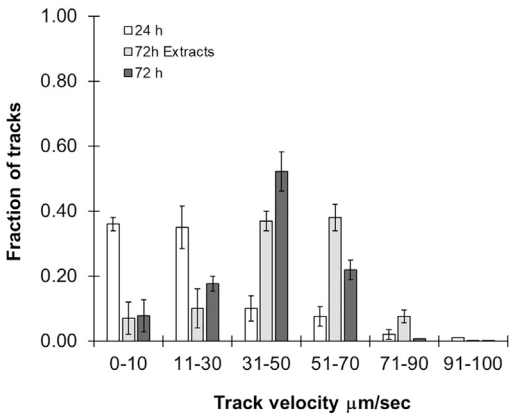

Figure 3. Motility in *Chlamydomonas reinhardtii* is Modulated by a Low-Molecular-Weight Signal

Extracts of 24-h (white) and 72-h (dark gray) cultures of *C. reinhardtii* were dissolved in DMSO and added directly to 24-h (light gray) cultures of *Chlamydomonas reinhardtii* in Tris-acetate-phosphate (TAP). Samples were incubated for 2 h and then evaluated for changes in swimming speed. Results are expressed as the mean of 3 videos with error bars indicating +/− SEM.

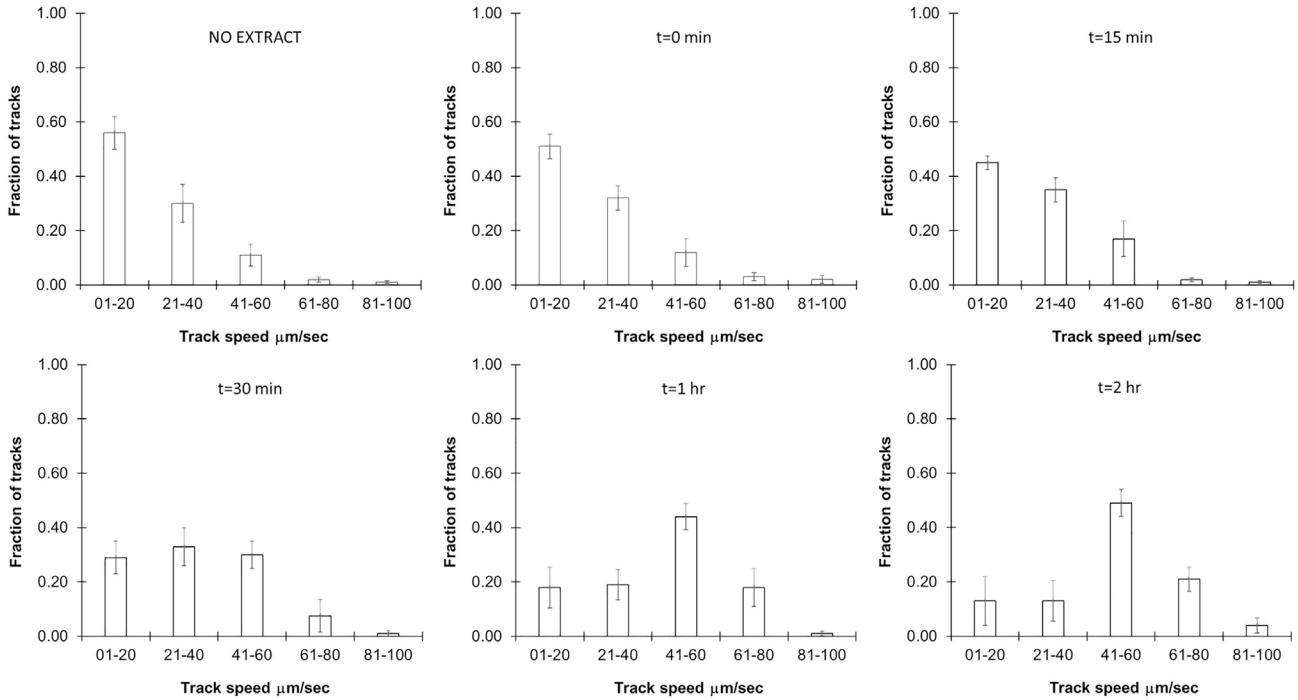

Figure 4. Extract Time Dependence in *C. reinhardtii*

Twenty-four-hour cultures of *C. reinhardii* cc124 were treated with 72-h extracts and then evaluated for changes in swimming speed at the indicated time points. Results are expressed as the mean of 3 videos with error bars indicating +/− SEM.

CSSF Increases Swimming Speed in a Time-Dependent Manner

Next, we evaluated the time dependence for the observed increase in swimming speeds due to CSSF exposure. Seventy-two-hour extracts were applied to 24-h *C. reinhardtii* cultures and then monitored for changes in swimming speed over a two-hour period. As shown in Figure 4, a population-wide shift in the swimming speed is observable after 30 min of CSSF exposure (from <20 μm/sec to 31 μm/sec). One hour after CSSF exposure, swimming speeds in 24-h cultures were indistinguishable from two-hour CSSF exposures (average: 44 vs 47 $\pm$ 7–9 μm/sec SEM). This similarity was also reflected in the distribution of swimming speeds across the population.

CSSF Activity Requires Changes in Gene Expression/Protein Production

CSSF-induced increases in swimming speeds in *C. reinhardtii* may be a purely physiological response or coupled to a change in gene/protein expression. We hypothesized that cycloheximide, a fungal metabolite capable of inhibiting mRNA translation in microorganisms, could help resolve these two models. As seen in Figure 5, 24-hour cultures of *C. reinhardtii* co-incubated with 72-h extracts and 1 μM cycloheximide showed no increase in swimming speed and were indistinguishable from extract-free controls (p value = 0.55). Seventy-two-hour *C. reinhardtii* cultures incubated with an equal concentration of cycloheximide for 2 h showed no significant reduction in swimming speed (data not shown). These findings are consistent with the production of new molecular machinery playing a significant role in the switch to increased swimming speeds rather than a simple physiological switch.

Culture Density, Not Age, Determines Swimming Speed

Our results thus far are consistent with the density, rather than age, dependent accumulation of a chemical signal or cue responsible for the observed increase in swimming speeds. If this is indeed the case, then artificially increasing cell density should mimic the effect of an older culture, *i.e.* by increasing swimming speeds. To evaluate this possibility, we artificially increased the cell density of 24-h cultures from $\approx 5.00 \times 10^5$ to 2.00×10^7 cells/ml. As shown in Figure 6, concentrated cultures showed a statistically significant increase in swimming speed over 30 min relative to untreated controls (p value <0.001). The

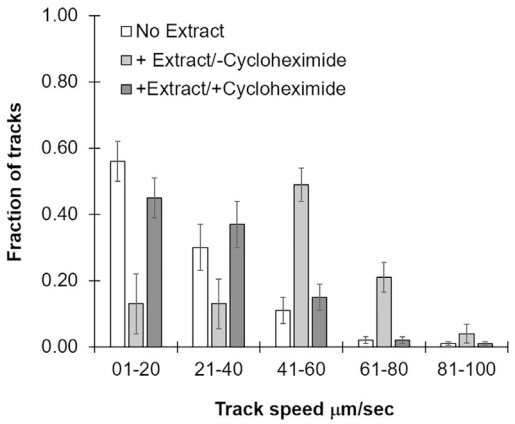

Figure 5. *C. reinhardtii* Response to Extract Signals Is Transcription Dependent

Twenty-four-hour cultures of *C. reinhardtii* were evaluated for changes to swimming speed without modification (white), after a 2-h exposure to 72-h *C. reinhardtii* extract (light gray) or after a 2-h exposure to both 72-h extract and 1 μM cycloheximide (dark gray). Results are expressed as the mean of 3 videos with error bars indicating +/− SEM.

perturbation caused by centrifugation and resuspension of these cells does cause an observable increase in swimming speed; however, this was still significantly less than that in the concentrated samples. Taken together, these results support the hypothesis that cell density rather than age determines the switch in behavior.

CSSF and Response Is Conserved in Chlamydomonas spp

Finally, we wanted to determine if this behavior was unique to *C. reinhardtii* or could be found in other members of this genus. We ultimately selected *Chlamydomonas moewusii* (cc1419) as this strain is well characterized and readily available for further study (Watanabe and Lewis, 2017). As shown in Figure 7A, cultures of *C. moewusii* showed a similar trend of increased speed as a function of culture age/density. As with *C. reinhardtii*, this increase in swimming speed could be induced in 24-h cultures by exposure to extracts from 72-h culture media (Figure 7B). Furthermore, the signals or cues responsible for this behavior appear conserved between these species as 72-h extracts from *C. reinhardtii* and *C. moewusii* were able to increase swimming speed in the other species after 2 h treatments (Figure 7C). Taken together, these findings strongly support the conservation of a density-dependent phenomenon, not unlike QS, within this genus, although the extent of this remains to be determined.

DISCUSSION

Investigating how microbial communities change behavior in response to external stimuli, cell density, and other factors is important to our fundamental understanding of how these populations interact with the surrounding world. Many unicellular microorganisms, including yeast and bacteria, display phenotypic switches like QS to regulate crucial behaviors such as bioluminescence, antibiotic production, biofilm

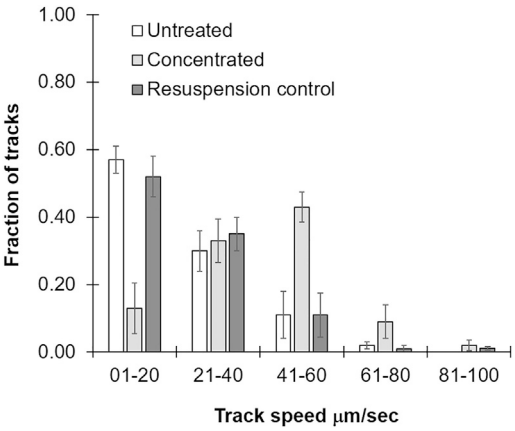

Figure 6. Population Density Determines Phenotypic Switch

Twenty-four-hour cultures of *C. reinhardtii* were pelleted by centrifugation and then re-suspended in either a reduced volume of the original TAP media to increase cell density from 10^5 cells/ml to 10^7 cells/ml (light gray) or the full volume of the original TAP media (dark gray). Samples were evaluated for changes to swimming speed relative to untreated control cultures (white) after 30 min. Results are expressed as the mean of 3 videos with error bars indicating +/− SEM.

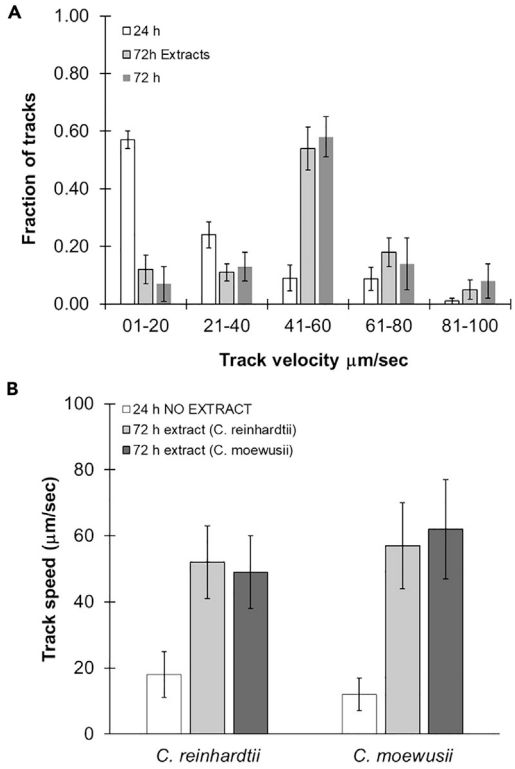

Figure 7. QS Is Conserved Among Members of the Genus Chlamydomonas

(A) Cultures of *C. moewusii* were evaluated for swimming speed at 24 (white) and 72 h (dark gray). The effects of 72-h *C moewusii* culture extracts on swimming speeds in 24-h cultures of *C moewusii* after two hours are shown in light gray. (B) Twenty-four-hour cultures of *C. reinhardtii* or *C. moewusii* were exposed to extracts of 72-hour cultures of either *C. reinhardtii* (light gray) or *C. moewusii* (dark gray) algae for 2 hours and then imaged for changes in swimming speed. Results are the average of three videos with error bars indicating +/− SEM.

formation, and many other phenotypes with significance to host-microbial associations, microbial ecology, and more (Miller and Bassler, 2001). QS has emerged as a convergent strategy among various microorganisms as a way to couple phenotypic switching to cell density (Fuller et al., 2017). However, while prevalent in fungi, there are no examples of this phenomenon among other eukaryotes. Yet, most eukaryotic microorganisms face many of the same challenges as prokaryotes, suggesting QS would provide an equally effective strategy for success. The obvious challenges to the QS discovery process are (i) what behavior changes in response to a quorum being met and (ii) the identity of the signal(s) or cue(s).

Herein, we utilized an in-house particle tracking method to monitor individual, as well as population-wide, changes in the swimming behavior of *C. reinhardtii* and determined that they were positively correlated with culture age and/or population density (Figure 1). Following this observation, we sought to identify the driving factor (or model) associated with this change. Swapping the media of younger and older *C. reinhardtii* cultures ruled out increased physical contact (mechanosensory) as the driver of increased swimming speeds but could not distinguish if the shift in motility was due to a decrease in nutrient availability (direct model) or due to the accumulation of a particular molecule (Figure 2). Yet, organic extracts from 72-h cultures added directly to comparatively nutrient-rich 24-h cultures show an increase in swimming speeds (Figure 3) over a one-hour period (Figure 4).

These findings are consistent with the presence of a low-molecular-weight organic compound which accumulates over time as the driver for increased swimming speeds in this model photosynthetic eukaryote. We have named this molecule the CSSF as a placeholder while ongoing studies attempt to identify it. As with many phenomena in which an external factor elicits a response in biology, most especially QS, there is some question as to whether the CSSF should be defined as a signal or a cue (Diggle et al., 2007). A signal would imply a purposeful exchange between the cells which, presumably, provides some selective

advantage to the group. For example, the CSSF could be a mechanism for these cells to inform each other of the need to increase swimming speed, based on the amount of CSSF as a proxy for cell density, due to pending nutrient deficiency. However, a clear benefit to the coordination of these responses remains unclear, and as a result, it is best to refer to the CSSF as a cue at present.

CSSF-induced changes are likely dependent on the synthesis of new molecular machinery, specifically protein, as inhibition of the ribosome, via cycloheximide, prevented 72-h extracts from increasing the swimming speed of 24-h cultures of *C. reinhardtii* (Figure 5). We further verified that this process was indeed density dependent, by artificially concentrating a 24-h culture, mimicking the density of 72-h cultures. Results suggest that an increase in cell density, and thus accumulated CSSF, is responsible for this behavior and not culture age (Figure 6). Taken together, these results strongly support the observed phenomenon as a new example of QS among unicellular eukaryotes.

This QS or QS-like phenomenon does not appear to be unique to *C. reinhardtii* as cultures of the closely related species, *C. moewussi*, display a similar behavior (Figure 7). The use of similar QS signals (AIs) for multiple species is well documented (Palmer et al., 2011b) and appears to be the case between these two species, which are sensitive to each other's cognate CSSF. Indeed, this phenomenon is potentially broadly conserved among the chlorophycean algae given the evolutionary distance between the two species tested: *C. reinhardtii* of the Reinhardtinia phylogroup and *C. moewusii* (cc1419) of the *Moewusinia* phylogroup (clade Moewusii), as this strain is well characterized and readily available for further study (Watanabe and Lewis, 2017). This underscores the potential conservation of the molecular mechanisms at work in this new QS-like behavior.

The observation of a potential QS circuit conserved between two members of this genus suggests that the phenomenon of density-dependent phenotypic switching may be even more broadly distributed among unicellular eukaryotes than once thought. However, one hallmark of QS is that AI detection increases AI synthesis, as the name implies, thereby ensuring smooth and population-wide switches in behavior. As a specific molecule has yet to be identified for this process, it is unclear whether such autoinduction actually occurs. We are currently working to identify the molecule(s) responsible for this process, as well as the timing and the molecular mechanisms which regulate it. These findings will be the subject of a subsequent manuscript.

In conclusion, the potential that QS occurs in this crucial class of photoautotrophs across multiple ecosystems suggests this phenomenon is more broadly distributed than once thought. This discovery may provide novel insight into the formation and maintenance of microbial communities. It also suggests the potential, as with prokaryotic QS systems, to employ chemical biology approaches to create synthetic regulators of this phenomenon, capable of both characterizing as well as controlling these phenotypes (Njoroge and Sperandio, 2009; Palmer et al., 2011a; Studer et al., 2014). *C. reinhardtii* in this model system for the study of motility and photosynthesis now provides a genetically tractable and well-characterized platform for the study of this new form of eukaryotic QS.

Limitations of the Study

The cycloheximide experiment performed here shows no increase in swimming speed in response to CSSF exposure. While this can be interpreted as evidence that changes in gene expression are required for the increase in swimming speed, no additional positive control is provided that shows an increase in swimming speed which can be inhibited by cycloheximide. Furthermore, at this time, we have not established the identity of the low-molecular-weight compound(s) associated with the increase in swimming speed.

Resource Availability

Lead Contact

Further information and requests for video and/or analysis files should be directed to Andrew G. Palmer (apalmer@fit.edu, 321-674-7226).

Materials Availability

All *C. reinhardtii* lines used in this study are available through the Chlamydomonas Resource Center. However, we will provide these lines as well, upon request, with reasonable compensation by the requestor for processing and shipping.

Data and Code Availability

No specific code was developed for this work. Macros associated with ImageJ are not required but are available upon request. All data sets from this publication are available upon request. Videos are available on Dryad: https://doi.org/10.5061/dryad.w6m905qnj.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101714.

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AUTHOR CONTRIBUTIONS

A.M.F., K.C., and T.H. contributed equally to the initial discovery of the cell density phenomenon, as well as the development of the technique. A.M.F. and T.H. performed the culture swap assays. A.M.F., K.C., J.G., and B.R. did the extract studies of *C. reinhardtii*. K.C. performed the concentration study and the effects of *C. moewussi* extracts on *C. reinhardtii*. F.Z. collected the time dependence extract data. P.S. performed all studies on *C. moewussi*. A.G.P. collected and analyzed the cycloheximide data and supervised all aspects of this research.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Quorum Sensing Behavior

in the Model Unicellular Eukaryote

Chlamydomonas reinhardtii

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Quorum Sensing Behavior in the Model Unicellular Eukaryote *Chlamydomonas reinhardtii*

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Transparent Methods

Algae Growth and Media - *C. reinhardtii* wild-type (cc124) and *C. moewusii* wild-type (cc1420, formerly *C. eugametos*) were acquired from the Chlamydomonas Resource Center (http://www.chlamycollection.org/) and separately maintained on plates of Tris-acetate-phosphate (TAP) media with 1.5% agar. Liquid cultures were prepared by growing lines individually in 25 ml of TAP for 24, 48, or 72 hours under continuous light (~13,800 W/m^2) at 23°C on a shaker (150 rpm).

Video Acquisition - All motility data was collected according to the methods stated in Folcik, et al. (Folcik *et al.*, 2020). Briefly, cell density was determined using a hemocytometer. All video acquisition was completed in a darkroom to minimize background phototactic effects. Videos were taken using an Olympus CH30 binocular microscope at x100 magnification with a mounted AmScope FMA050 microscope camera. Videos were collected using ToupView software (www.touptek.com) with a frame rate of 7.5 frames/sec for approximately 30 seconds.

Video Analysis - Video analysis was also completed following Folcik, et al. methodology (Folcik *et al.*, 2020). Briefly, videos were imported into Fiji and analyzed with the TrackMate plugin (Schindelin *et al.*, 2012; Tinevez *et al.*, 2017). R statistical software using the RStudio interface was utilized for combining data files and computing statistics (www.rstudio.com; www.r-project.org). The statistical analysis package in Microsoft Excel was used to generate histograms for visualization of population speed and direction. A CSV file containing the speed of the individual tracks from each video used here is available as Supplemental File 4.

Media Swap Study - *C. reinhardtii* cc124 cultures were grown in TAP media for 24 or 72 hours. Each culture was then pelleted via centrifugation (2400 g; 10 min) and the media was removed. The media was then filtered to remove residual cells through a 0.2 μm filter. The filtered 72 hour culture media was then used to re-suspend the 24 hour cell culture pellet. Similarly, the filtered 24 hour culture media was used to re-suspend the 72 hour cell culture pellet. Samples were then vortexed for ~30 seconds to re-suspend. Media swapped cultures were then incubated for 6 hours. Following incubation, aliquots of each sample were taken, recorded, and analyzed using the above methods.

Media Extract Study - *C. reinhardtii* cc124 cultures were grown in TAP media for either 24 or 72 hours. The cultures were then pelleted via centrifugation and the media was removed. The culture medium was then subjected to organic phase liquid-liquid extraction using ethyl acetate. This process was repeated three times and the organic phase was concentrated under vacuum. Concentrated extracts were then dissolved in DMSO and kept in a (-40 ℃) freezer until needed. Extracts were allowed to come to room temperature before being added to 1.0 ml cultures via micropipette (25 µl). The samples were then incubated for 2 hours before videos were acquired to observe changes in swimming speed.

Cycloheximide Study - *C. reinhardtii* cc124 cultures were grown in 25 ml of TAP media for either 24 or 72 hours. 24 hour cultures were co-incubated for 2 hours with 72 hour extracts (25 µl/ml) and 1µM cycloheximide, or with 72 hour extract only (25 µl/ml). As a control, 72 hour cultures

were incubated for 2 hours with only 1 μM cycloheximide. Following the designated incubation period samples were imaged for changes in swimming speed.

Artificial Population Density Increase Study - C. reinhardtii cc124 cultures were grown in 25 ml of TAP media for 24 hours. 1 ml aliquots were then transferred to microcentrifuge tubes and pelleted via centrifugation (1400 rpm, 5 minutes). The pelleted cultures were re-suspended in either 250 μl of the used TAP media, to concentrate the culture, or in 1 ml of the used TAP media. The samples were imaged for changes in swimming speed after 30 minutes.

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