Network topology enables efficient response to environment in *Physarum polycephalum*

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The network-shaped body plan distinguishes the unicellular slime mould *Physarum polycephalum* in body architecture from other unicellular organisms. Yet, network-shaped body plans dominate branches of multi-cellular life such as in fungi. Which evolutionary advantage does a network structure provide when facing a dynamic environment with adverse conditions? Here, we probe how network topology impacts *P. polycephalum*'s avoidance response to adverse blue light. We stimulate either an elongated, I-shaped amoeboid or a Y-shaped networked specimen and quantify the evacuation process of the light-exposed body part. The result shows that Y-shaped specimen complete the avoidance retraction even slightly faster than I-shaped organisms, yet, at a lower almost negligible increase in migration velocity. Contraction amplitude driving mass motion is further only locally increased in Y-shaped specimen compared to I-shaped - providing further evidence that Y-shaped's avoidance reaction is energetically more efficient than in I-shaped amoeboid organisms. The difference in the retraction behaviour suggests that the complexity of network topology provides a key advantage when encountering adverse environments. Our findings could lead to a better understanding of the evolutionary transition from unicellular to multicellularity.

acellular slime mold | transport network | network topology | behavior

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Introduction

Both mycelial fungi and plasmodial slime moulds such as *P. polycephalum* shape their body into extensive networks that are highly adaptive to their environment (1). With the interlaced network-shape *P. polycephalum* is at stark difference to the single cell amoeba state of its closest relative *Dictyostelium discoideum*. *D. discoideum* never transforms from the amoeba state but itself bridges into multicellular developmental stages by forming amoeba aggregates (2, 3). Instead *P. polycephalum* can transform its amoeba by fusion into a complex and dynamic multinucleate tubular network covering tens of centimeters (4). As a network-shaped multinucleate body plan evolved twice in mycelial fungi and plasmodial slime moulds we would expect an evolutionary advantage of network over amoeboid shape.

P. polycephalum networks are highly dynamic, reorganizing when migrating and searching for food (5–7), responding to environmental conditions such as chemicals (8–10), humidity (11, 12), stiffness (13, 14), light conditions (11, 15–17) and temperature (18, 19), thereby distinguishing between attractive conditions such as nutrient-rich or adverse conditions such as blue light. When partly exposed to blue light the network rapidly reorganizes to evacuate from blue light (20, 21), as it harms the organism by inhibiting metabolism (22). By remodelling its network body in response to its environment *P. polycephalum* performs sophisticated, often termed 'intelligent' behaviours (20, 23, 24) like forming efficient adaptive and fault-tolerant networks connecting multiple food sources in a way comparable to optimized man-made transport networks (25, 26), or finding the shortest path through light (20). Being of network-shaped therefore allows for complex behaviours, but how do such advantages emerge when a tiny plasmodia grows beyond the amoeboid shape?

Amoeboid-shaped plasmodia have served as a desired model to investigate how migration arises from the coordination of the peristaltic contractions that enable mass flow (27–31). Due to the reduced size, patterns of the acto-myosin driven cross-sectional contractions rhythmically constricting the single tube and thereby pumping the enclosed fluid volume forth and back can be quantified (29–31). Yet, how dynamics differ if the topology of elongated amoeboid, i.e. I-shaped plasmodium, becomes more complex by forming a first network node as in a Y-shaped network is unknown.

Here, we investigate the advantage of network topology by comparing the response of the simplest network state of a Y-shaped *P. polycephalum* plasmodium to the elongated I-shaped amoeboid plasmodia, in adverse conditions provided by localized blue light. Quantifying in detail the dynamics of organism mass, motion and contraction dynamics of both Y-shaped and I-shaped side-by-side we discover that the higher complexity in topology of Y-shaped specimen allows them to evacuate from blue light more efficiently i.e. specimen move organism mass out of harmful blue light in a slightly shorter time period at less motion and lower overall contraction amplitude. Our finding therefore suggest that network topology may have an evolutionary advantage compared to amoeboid shape.

Methods

Culturing of *P. polycephalum*. To ensure comparability of individual experiments in particular regarding the nutritional state, one day old microplasmodia grown in a liquid culture using the medium by Daniel and Rusch (32) with hematin ($5\,\mathrm{mg/mL}$) instead of chicken embryo extract were used. The method to prepare plates with small I- and Y-shaped *P. polycephalum* plasmodia was adopted from the preparation of plasmodial networks as reported in (33). First, 1.5% agar plates were left to be air dried within the sterile envi-

ronment with petri dish lids open for 5 min, before $1\,\mathrm{mL}$ of microplasmodia solution was added to the plates. The plates were then carefully tilted in all directions to ensure an even dispersion of plasmodia, this is to reduce the possibility of them fusing into one single plasmodium with complex network structure. After dispersion, the plates were left with lids opened to be air dried for another 15 min before being sealed with parafilm and incubated overnight under dark conditions at T = 25 °C. This second drying step is again crucial in preventing *P. polycephalum* from fusing together, it also ensures there are no visible traces of liquid medium during imaging which will affect the image quality. After plating, plates were used for imaging within 16-20 hours.

Microscope setup and data acquisition. Images were recorded using a Zeiss Axio Zoom V16 with a Zeiss PlanNeofluar 1x/0.25 objective and a Hamamatsu ORCA-Flash 4.0 digital camera. The microscope setup is shielded from ambient light by a canopy, and a Lee 740 Aurora Borealis green filter was added in between the bright field light source and specimen to minimise the environmental stress on *P. polycephalum* (15).
After the petri dish was taken out from the incubator, it was sealed with parafilm and placed upside down onto the imaging stage to reduce the effect of agar drying out and visible condensation droplets during the experiment. The specimen was left under microscope light for 40 min before the start of the experiment to accommodate the change of *P. polycephalum* behaviour towards the change of the environment. The first 20 min of the experiment were carried out without blue light to capture a 'baseline' dynamics. Then, a blue light, created by a Zeiss HXP 200C light source at maximum intensity, and a Zeiss HE38 470/40 nm filter, was introduced at a predefined position. The intensity of the blue light used varies depending on the optical zoom, in the following experiments it was between 150 and $250\,\mathrm{W\,m^{-2}}$ as was measured by a Thorlabs PM16-122 Power Meter at the sample stage. The Zeiss Zen 2 (Blue Edition) software was used for imaging. An image was acquired every 6 s.
The position of the blue light need to be predefined in Zen software before the start of the experiments, meanwhile the *P. polycephalum* could have migrated away from the blue light region during the first hour before the blue light was triggered. In those cases, the experiment was aborted, and a new petri dish with a sample of the right topology was taken from the incubator, to ensure all samples were in a similar state when meeting the blue light.

Image analysis. The resulting experimental images were first passed through a custom-written MATLAB code following the steps described in (33) to extract a measure of organism volume and its dynamics in terms of the intensities of transmitted light at every pixel along the backbone of the specimen. Briefly, during this process, the background of each image was first removed with the rolling-ball method before a threshold was applied to separate the network from the background. Small holes were filled and single-pixel edges were smoothed before the biggest structure was extracted as a binary 'mask'. In the next step, the mask was etched to extract a 'skeleton'. A local diameter, which was calculated as the largest fitting disk radius around the point within the mask, was associated with each point on the skeleton. Within this disk, the average intensity was computed and saved with the skeleton data. Because the diameter has a lower resolution than the intensity, from here on only the intensity data was used.
As *P. polycephalum* performs avoidance reaction and migrates out of the blue light region, a reference skeleton image that captures all organism location data throughout the experiment was created in order to achieve a kymograph-like file where the time evolution of the network pixel intensity along the tube can be recorded and tracked. This reference image was achieved by first overlay and then etch all skeleton data. Next, for each time frame, each pixel on the instantaneous skeleton data was mapped on to a corresponding pixel in the reference image using the shortest distance criteria. Lastly, the corresponding intensity data at each time frame for each pixel can be loaded onto the reference position, and the kymograph was generated.

Results

I- and Y-shapes require similar time to evacuate from a blue light region. To study how network topology impacts the response of *P. polycephalum* to blue light stimuli, we collected time series of bright-field images of the organism's evacuation response over approximately $2\,\mathrm{h}$, during which a single, localised blue light stimulus was applied on a part of the organism (Fig. 1A). For I-shaped specimen, individual experiments differ in the fraction of the elongated organism that was exposed to light. For Y-shaped specimen, only one out of the three 'arms' connected into the Y was stimulated with blue light. During the $20\,\mathrm{min}$ before the application of blue light, where the organism is freely living on the agar plate (the 'baseline'), the body mass, approximated by the sum of recorded bright-field intensity values related to the organisms mass by Lambert-Beer law, is conserved (Fig. 1B).
For each experiment, the instantaneous body mass was compared to the average of the baseline value. To obtain generalised I-shaped and Y-shaped behaviour, we then averaged the data points for I- and Y-shapes, respectively. Whole *P. polycephalum* mass decreases under the exposure to blue light, albeit slightly stronger for I-shaped than for Y-shaped specimen (Fig. 1B). To quantify the evacuation response we followed the *P. polycephalum* mass exposed to blue light over time (Fig. 1C). On average I-shaped took $71\,\mathrm{min}$ (N = 12, SD = 17) and Y-shaped took $65\,\mathrm{min}$ (N = 6, SD = 18) to evacuate. While for extreme cases, the slowest Y-shape took $79\,\mathrm{min}$ to complete the evacuation, the slowest I-shape took $108\,\mathrm{min}$ to complete evacuation. The evacuation from the region of blue light is accompanied by the specimen's part outside of blue light increasing over time (Fig. 1D).
Despite only subtle differences in the overall mass shrinkage and evacuation statistics, the process of how evacuation is achieved differs strongly between I- and Y-shaped specimen. For I-shaped, the part inside the blue light undergoes both

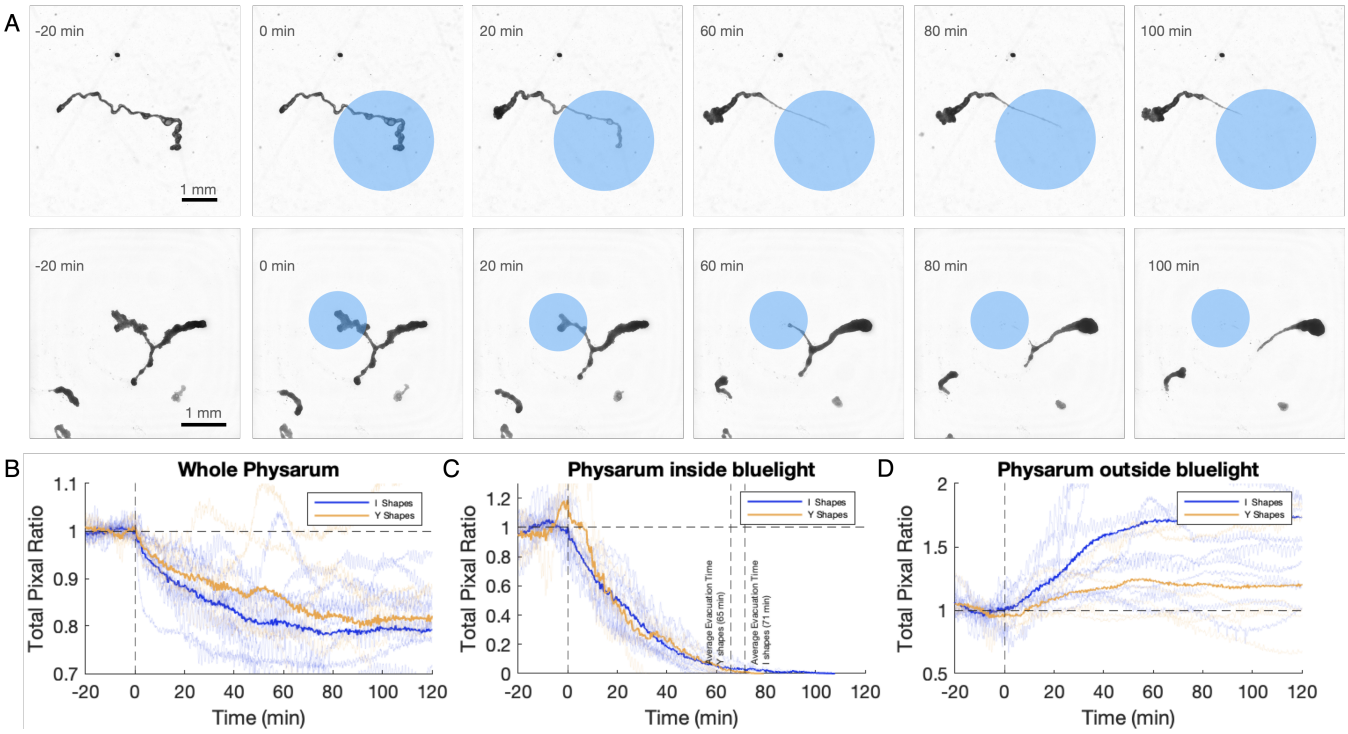

Fig. 1. Y-shaped *P. polycephalum* evades blue light region slightly faster than I-shaped, while I-shaped suffer a slightly more significant loss in overall mass. (A) Sequential bright-field images of a previously stable *P. polycephalum* evading the localized blue light stimulus (blue circle) applied at $0\,\mathrm{min}$. (B) Averaged change of *P. polycephalum* total mass for the whole organism shows a statistically stronger decrease in mass for I-shaped specimen. (C) Averaged change of the *P. polycephalum* mass for the specimen part exposed to blue light indicates a statistically slightly faster evacuation for Y-shaped specimen. (D) Evacuation response is compensated by averaged change of *P. polycephalum* specimen mass outside of blue light stimulus. The y-axis presents the ratio of the instantaneous mass (total pixel value) over the baseline value (mean pixel value before blue light was applied), respectively. Bold yellow line indicates averaged I-shape data (N = 12) and bold blue line averaged Y-shape data (N = 6). Light blue and yellow lines in the background are the change in the total mass for individual experiments, see individual traces in supplementary information.

thinning and retracting process (see i.e. Fig. 1A at 60 min and Supplemental Videos), while Y-shaped only smoothly retract the light exposed part.

I-shapes exhibit higher velocity during evacuating from the blue light region. To measure the differences in the evacuation process we next quantify the changes in motion velocity of the whole organisms as well as the individual parts exposed or not exposed to blue light. Here, the instantaneous velocity is calculated from the position change of the weighted centroids of the *P. polycephalum* between frames. Again as a baseline velocity we calculated the average velocity during the $20\,\mathrm{min}$ before blue light was applied to individual *P. polycephalum*, and the instantaneous velocities were compared to this baseline velocity. As blue light exposure has been implicated to change the mechanical properties of both *P. polycephalum* tube wall and cytoplasm (21), we suspect these mechanical changes can have a direct implication on the velocity. Hence, the velocity data of individual specimen was binned according to the ratio of specimen's body mass exposed to blue light compared to that of the whole *P. polycephalum* body. Different specimen have different proportions of their body initially exposed to blue light, with particularly Y-shaped being exposed less than 60%, but as evacuation continues this proportion always shrinks to zero. Thus, the x-axis in Fig. 2 can be read as inverse time. Both I- and Y-shaped *P. polycephalum* increase their velocity as the evacuation process continues (Fig. 2A), yet the velocity of Y-shaped is always lower than that of I-shaped. After being exposed to blue light I-shaped first continue the overall moving at their baseline velocity, only as the evacuation process continues the velocity increases. Velocity dynamics reach a plateau as the organism is halfway out of the blue light, maxing out at 1.7 times the baseline velocity. For Y-shaped, instead of continuing with the baseline velocity, it lowers its locomotion velocity upon the onset of blue light. Over time as it evacuates, it slowly recovers to the baseline. It only uses a higher overall velocity at the last step of the evacuation process when there is less than 10% of the mass remaining in the blue light.

Distinguishing *P. polycephalum* into a specimen's part outside of blue light and a part exposed to blue light the weighted centroids and, hence, the velocity were recalculated for each part (Fig. 2B, C). For I-shaped organism, both parts show a constant increase in the instantaneous velocity as evacuation progresses. Yet, the increase in velocity outside of blue light is much less vigorous as for the exposed part. For Y-shaped, the dynamics of the organism part outside of blue light resembles the overall velocity, recovering steadily from a decrease in motion velocity upon the impact of blue light. For the part of the organism under blue light, an increase in motion velocity similar but not as steady and fast as for I-shaped organisms is observed.

From the slightly faster evacuation of Y-shaped versus I-shaped observed in the previous section, here, finding motion velocity of I-shaped being always faster than Y-shaped

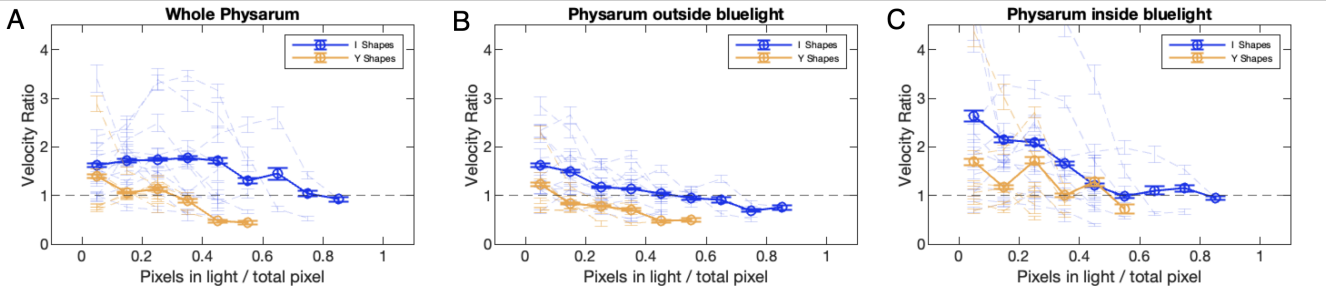

Fig. 2. Change of velocity bigger for I- than Y-shaped *P. polycephalum*. Histograms of the change in velocity (A) for the whole specimen body, (B) for the specimen part outside of blue light, and (C) for the specimen part under blue light. The histograms present data obtained from the ensemble average of the instantaneous velocity over the mean velocity before the blue light was introduced. The velocities were binned into the relative mass of *P. polycephalum* in blue light relative to the whole body. For both topologies, there is a clear increase of velocity for the region directly under blue light. Meanwhile, the response from the region outside of the blue light differs, which resulted in the overall higher evacuation velocity for the I-shapes. Each light blue and yellow line in the background represents a single data set, and bold blue and yellow lines represent the ensemble average within each bin.

is against our intuition if we assume that mass motion is driving evacuation. Thus, there needs to be a more subtle mechanism to evacuate the light-exposed organism part that gives advantage to more complex Y-shaped network topologies over I-shaped amoeboid topologies. Due to their simple topology, I-shaped *P. polycephalum* are constrained in how their body mass can be reallocated using shuttle streaming. Body mass that needs to be moved out of blue light either needs to be pumped by the light exposed part or sucked by the non-exposed part by shuttle flow into the part outside of blue light. In the Y-shaped topology, however, the two arms outside of blue light do not need to undergo a net motion as they may symmetrically inflate to take up mass. To identify the pumping dynamics we next turn to quantify the contraction dynamics.

Blue light triggers increase in amplitude in light exposed part but also non-exposed part for I-shaped. *P. polycephalum* uses peristaltic pumping as a transport mechanism (34), we, hence, analysed the contraction characteristics, namely the instantaneous frequency and amplitude, for I- and Y-shaped organisms. Both frequency and amplitude are extracted individually from pixel intensity time dynamics ('intensity kymograph') employing the Hilbert transform (33), subsequently averaging over pixels in respective parts of an organism. Similar to Fig. 2, frequency and amplitude change are quantified by normalizing with the baseline, the average frequency and amplitude during the 20 min prior to light onset, and subsequently binned according to the relative mass exposed to light. The changes in frequency of both I- and Y-shaped almost collapse regardless of which part of the organism is considered. Upon blue light exposure frequency initially decreases (15, 35–37) to then slowly recover to the baseline frequency as the evacuation process continues.

On the contrary, upon light exposure the amplitude increases (36) both for I-shaped and Y-shaped organisms within the organism part exposed to blue light. However, for the part outside of the blue light, the two topologies showed different responses: for Y-shaped plasmodia the amplitude was maintained at around the baseline value, while for I-shaped plasmodia an increase in amplitude was observed as the evacuation proceeded. And the differences in the dynamics in organism's part outside of blue light dominate the overall *P. polycephalum* body amplitude dynamics.

In a peristaltic wave, as frequency decreases, pumping performance decreases, yet, an increase in contraction amplitude counteracts. When the amplitude increase outperforms the frequency decrease, overall the pumping performance may increase. Here, as amplitude increase rises to more than twofold, at a less than half decrease in frequency, the exposure of blue light seems to up-regulate pumping performance in the light-exposed part. The localized up-regulation of pumping performance appears to be sufficient to efficiently evacuate the light-exposed part for Y-shaped specimen. For the simpler topology of I-shaped amoeboid organisms contraction amplitude also increases in the non-exposed body parts. This more wide-spread increase in contraction amplitude suggests that a similar effectiveness in evacuation speed for I-shaped specimen can only be achieved if also the pumping i.e. sucking performance in the non-exposed organism part is increased. However, up-regulating contraction amplitude in a larger portion of an organism is likely to be more energetically costly (38) and, thus, may account for the observation of a stronger mass consumption of I-shaped specimen during evacuation than Y-shaped (Fig. 1B). The more complex albeit still simple topology of Y-shaped organism body may therefore allow for a more efficient response to adverse conditions.

Discussion

Comparing the dynamics of the response to localized blue light exposure of elongated amoeboid I-shaped versus network-state Y-shaped *P. polycephalum*, we find that Y-shaped are slightly faster in evacuating their light exposed body part than I-shaped. All the while I-shaped body parts exhibit an overall faster motion velocity and increase contraction amplitude across the entire organism while in Y-shaped only the light exposed body part increases contraction amplitude. This increased dynamics of I-shaped go at the expense of their body mass decreasing statistically slightly stronger than Y-shaped. We therefore conclude that the network topology represented by the Y-shaped organisms requires less energy and is, thus, more efficient in evacuating body parts exposed to harmful conditions.

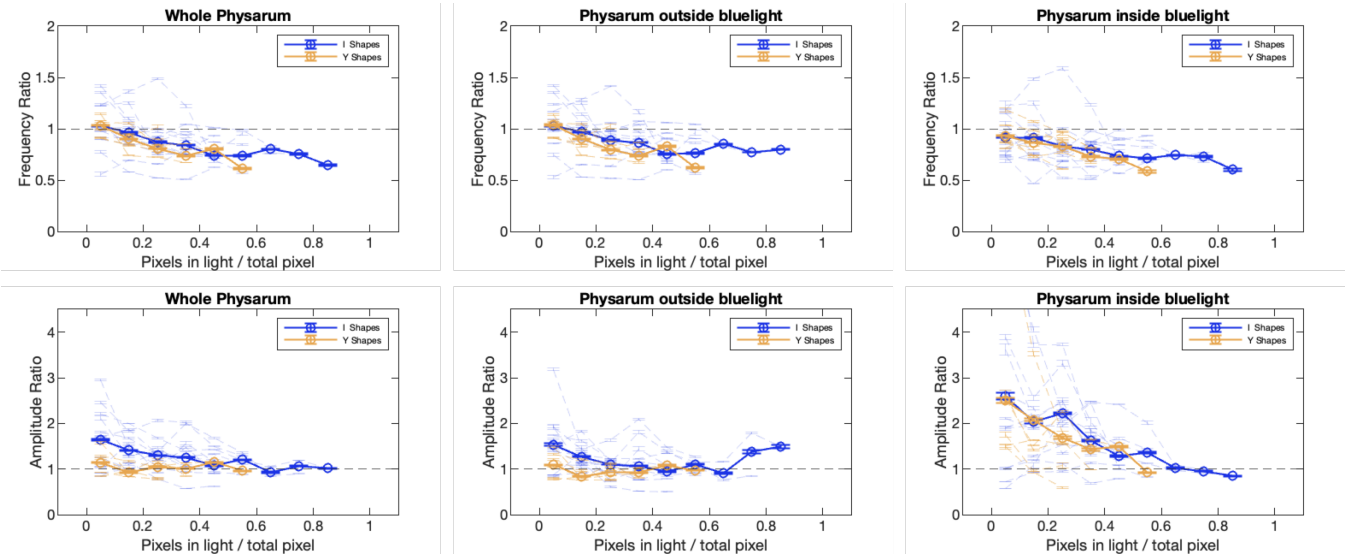

Fig. 3. While contraction frequency decreases, contraction amplitude increases and even broader for I-shaped specimen. Histograms of the change of frequency (upper row) and amplitude (lower row) for I- and Y-shaped *P. polycephalum* versus ratio of instantaneous light exposure as in Fig. 2. In both topologies there is a decrease in contraction frequency upon light exposure and recovery upon evacuation. The contraction amplitude, on the contrary, exhibits an increase in the organism part under blue light for both topologies, but I-shaped also experiencing an increase in the organism part outside of blue light.

The decisive difference in the dynamics of contractions channelling the mass movement is the across-organism increase in contraction amplitude in I-shaped versus the localized contraction amplitude increase in Y-shaped specimen. It is likely that this difference is deeply rooted in the contrasted topologies. Within amoeboid I-shaped organisms dynamics along the organism are inherently coupled and an overall increase in pumping efficiency is best to quickly increase the mass movement along the peristaltic pressure drop along the organism (39). For Y-shaped networks, however, the two unexposed arms may maintain a low pressure at the network node and therefore passively facilitate the mass motion ignited by the increase in pumping of the light exposed network arm. This could also explain the seemingly two different escaping mechanisms we are observing, namely the I-shaped needs as much body length as possible to generate the maximum amplitude, hence choose to decouple the thinning and retracting process during evacuation, while Y-shaped organisms can benefit from the coordination of the 2-arms outside the blue light and, hence, smoothly retract the light exposed part without a visible thinning step.

Network complexity is multiplexed. It ranges from the information storage opening up from the pattern of thicker and thinner tubes (40, 41), to dynamic stability arising from symmetries in networks topology (42). Here, we demonstrated, the mere existence of a network node can allow for behavioural advantage. The puzzle of how networked body forms emerged gets added a new puzzle piece that may also shine light on the evolutionary process toward multicellular organisms and the beginning of the more complicated forms of life.

ACKNOWLEDGEMENTS

We sincerely thank Jean-Daniel Julien for insightful discussions. This project has received funding from the Max Planck Society and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 947630, FlowMem) to K.A..

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