On Chirality of Slime Mould

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Abstract

Left-right patterning and lateralized behaviour is an ubiquitous aspect of plants and animals. The mechanisms linking cellular chirality to the large-scale asymmetry of multicellular structures are incompletely understood, and it has been suggested that the chirality of living cells is hardwired in their cytoskeleton. We examined the question of biased asymmetry in a unique organism: the slime mould *Physarum polycephalum*, which is unicellular yet possesses macroscopic, complex structure and behaviour. In laboratory experiment using a T-shape, we found that *Physarum* turns right in more than 74% of trials.The results are in agreement with previously published studies on asymmetric movement of muscle cells, neutrophils, liver cells and growing neural filaments, and for the first time reveal the presence of consistently-biased laterality in the fungi kingdom. Exact mechanisms of the slime moulds direction preference remain unknown.

1   Introduction

Above the quantum level, the universe does not distinguish left from right. Yet, left-right (LR) asymmetry or chirality is prevalent in the biosphere [39]. Many animal body plans appear to be bilaterally symmetrical; however this overt symmetry belies asymmetry of the morphology and location of various internal organs [40]. LR Asymmetry is defined here as a consistent bias of the left-right axis with respect to the other two orthogonal axes (as opposed to randomly-oriented asymmetries, or fluctuating asymmetries due to developmental noise). LR asymmetries encompass not only the placement and morphology of single organs such as the heart, spleen and stomach, but also organs such as the lungs, which have distinct

morphologies on the left and right sides [63, 50]. Establishing LR asymmetry is an important aspect of normal embryonic development, and disorders of laterality are a serious birth defect that affects approximately 1 in 8000 births [21, 19, 27, 47, 49]. Understanding how and when embryos reliably distinguish their left from its right is an important question for developmental and cell biology, evolution, and the biomedicine of birth defects. In addition to morphological asymmetry, most animals also show behavioural lateralization, which derives in part from asymmetries of the brain and nervous system. Consistently-biased asymmetry is prevalent throughout the tree of life [46]. Behavioural lateralization has been extensively observed in invertebrates [25], and morphological asymmetry has been studied in the growth of plants [30, 41] and chirality of single-celled organisms [11, 24]. Even individual cells from Metazoan bodies exhibit consistent chiral behaviours in culture in the direction of neurite outgrowth, cell-substrate interactions, rotation, and docking with neighbouring cells [24, 29, 65, 66, 44, 20, 68, 69]. A fundamental question in this field is the evolutionary conservation of LR-generating and amplifying mechanisms [64, 63, 7, 55, 56], and the relationship between single cell chiral properties and asymmetries of a large-scale body structure [59, 54, 8]. Recent data suggest deep conservation of cytoskeletal components as the ancient origin of asymmetry [38], although chromatic segregation [52, 33, 6] and ciliary activity [10] may also play important roles. Moreover, the relationship between behavioural and morphological asymmetry is still open [28]. We report a novel asymmetry in the growth properties of *Physarum*, which preferentially turns right when grown in a T-shape. The slime mould *Physarum polycephalum* is a large single cell capable to nearly optimally adapt shape of its body in overlapping gradients of attractants and repellents. This behaviour has been fruitfully interpreted in terms of computation [43], and a wide range of distributed sensing, concurrent information processing, parallel computation and decentralised actuation devices made of the living slime mould has been produced [2, 3]. *Physarum* is a fascinating model blurring many of the categories in the LR patterning field: it is a "unicellular" yet large-scale organism, and its growth pattern simultaneously encompasses elements of both, morphological patterning (developmental growth) and functional exploration of the environment (behavior). These data are, to our knowledge, the first demonstration that consistent left-right asymmetry extends even to the Fungi Kingdom, and is not essentially tied to traditional cellular structures, cell-cell communication pathways, or multicellular architectures.

2   Methods

Two series of tests have been developed to probe intrinsic bias in *Physarum* growth behaviour: one with an agar substrate and one with a filter paper substrate. For

the first series, 2 mm thick agar plates were prepared with 1.5% non-nutrient agar (Sigma Aldrich). T-shapes were cut out of agar plate. The vertical channel was 5 mm wide and 20 mm long. The horizontal channel was 5 mm wide and 30 mm long. For the second series, 0.5 mm thick filter paper was used. T-shapes were cut out of the paper. The vertical channel was 2 mm wide and 20 mm long, while the horizontal channel was 2 mm wide and 30 mm long. The T-shapes were placed in Petri dishes with lids closed. The Petri dishes were placed in a metal enclosure. Vertical channels of all T-shapes were oriented from South to North, and care was taken to avoid external cues. Indeed, there were no equipment in the room, which might emit EM waves etc. Dishes were in the darkness into a metal cupboard to avoid any causes of maze/medium. Moreover, the agar was homogeneous, and flat, as the paper, except for its implicit roughness. Samples were on a horizontal plane, with the upper part of the T-shape oriented to North during the whole experiment. We kept the dishes in a horizontal position without tilting them, even during the checking phase. A piece of *Physarum* (5 μl in case of agar and 2μl in case of filter paper substrate) was placed the end bottom end of the vertical channel. Each experiment is assumed finished when *Physarum* reached end of one of the shoulders of the horizontal channel. 120 experiments were conducted, 60 with agar gel substrate and 60 with filter paper respectively.

3   Results

We found statistical significance of *Physarum polycephalum* tendency in turning right is not influenced by substrate. In the first experimental session, we undertook 60 experiments of *Physarum* growing on a T-shaped non-nutrient agar substrate. Samples were kept in a dark and humid metal box and periodically checked.

Figure 1 shows a photograph of one of the samples with agar and Fig. 2 the slime mould growing on the filter paper. Figure 3 presents three photographs of the slime mould turning right. *Physarum* grows in different ways with a variable number of small capillaries near the edge, but regardless the type of growth, the chirality remains. Geometry of the thickest protoplasmic tube shows that the slime mould can 'understeer' (Fig 3a), 'oversteer' (Fig 3b) or even make a right turn at the last moment (Fig 3c). In all three cases the slime mould 'probes' edge of the shape with a fine branches and only then implements the turn.

The second experimental set-up has been developed in order to avoid the possible effect that can be attributed to the networks created by *Physarum polycephalum* during its growth. Indeed, Physarum is self repellent, thus many lamellopodia release a mould, repelling between neighbouring lamellopodia and external effects of cyto-skeleton chirality might be lost. Therefore, knowing that the degree of branching is inversely proportional to *comfort*, i.e. a concentration of nutrients,
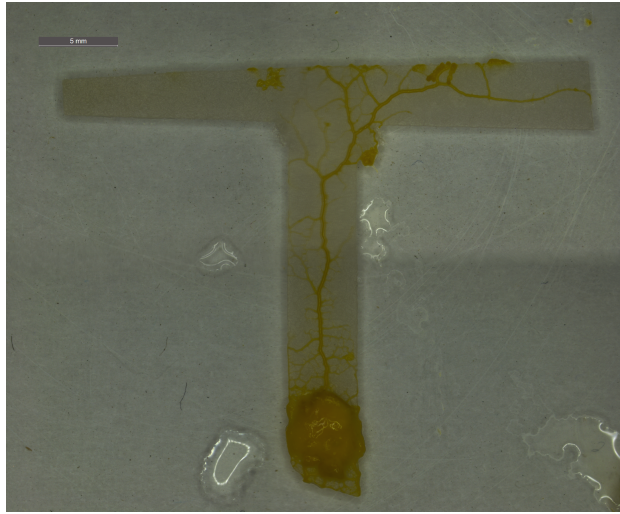
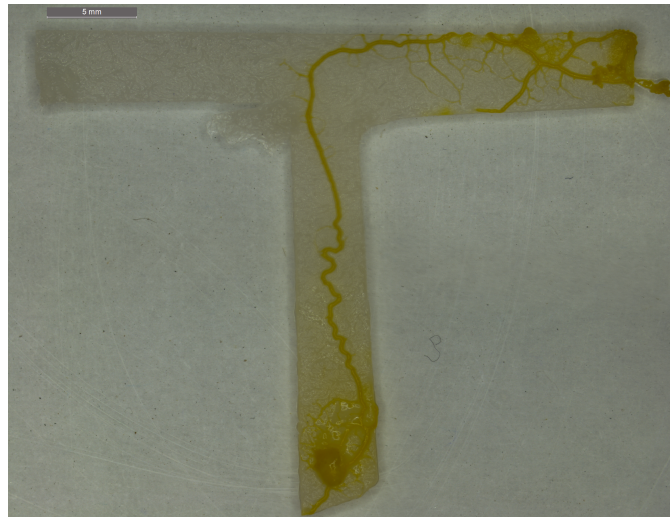Figure 1: Photograph of the slime mould on a T-shaped agar plate.

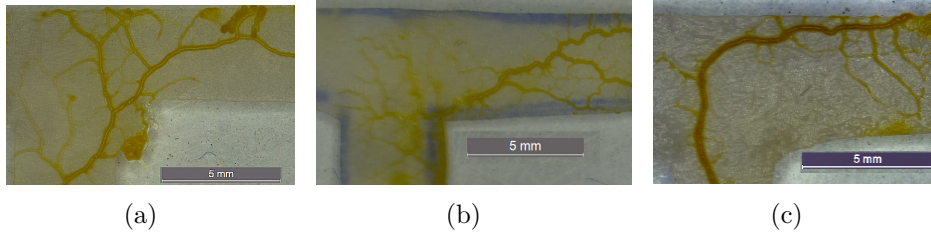Figure 2: Photograph of the slime mould on a T-shaped filter paper.

Figure 3: Photographs' zoom of the area near the right edges taken from T-shaped samples. It is visible how the small capillaries are going in both directions, showing they are not chiral, as the big protoplasmic tube. Note that the scale bar has been added later. (a) 'Understeering'. (b) 'Oversteering'. (c) 'Last moment' turn.

	Agar	Filter paper	Total
right turn, %	74	78	76
left turn, %	15	8	11.5
branching in both directions, %	11	13	12
p-value	$3.9 \cdot 10^{-4}$	$1.2 \cdot 10^{-5}$	$1.2 \cdot 10^{-8}$

Table 1: The results obtained in the experiments with the slime mould growing on T-shapes made of non-nutrient agar plates and filer paper. P-value has been calculated from the obtained results with a two tailed binomial with a 0.5 success value

surface patterning and humidity, of a substrate [57], we also undertook experiments with the slime mould growing on T-shapes made from a filter paper (Fig. 2).

The results obtained in both types of experiments are summarised in the Tab. 1. Right turn substantially dominate. We also shown the statistical significance against a 50/50 null hypothesis expectation serving a two tailed binomial (see last line of Tab. 1).

4 Discussion

These data indicate that even fungi exhibit lateralized growth and behaviour, similar to preferences for turning in a T-shape that have been observed in planaria [13], sperm [17], mealworms [12], and sheep [5]. Because the cytoskeleton was recently shown to be fundamental to asymmetry in both plants and animals [38, 62, 42, 60, 1, 61], and has an important role in regulation of *Physarum* growth [67], it is tempting to suppose that symmetry breaking is very ancient and highly conserved. While distinct mechanisms may amplify asymmetries in different ways upon diverse body plans, it may be hypothesized that the overall strategy of
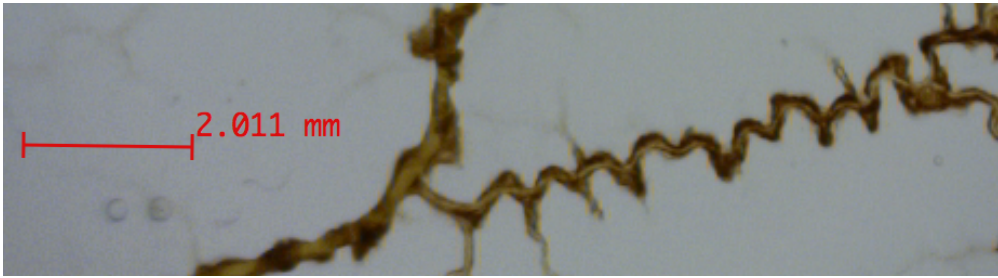
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Figure 4: Evidence of zigzag motion growth of Physarum.

deriving structural and behavioural asymmetries from subcellular chirality [16] is universal across kingdoms of life.

An evolutionary advantage of preferential right turn (left turn is also OK as long as it always left) is in decreasing exploration time of an unknown geometrically constrained environment. Right-hand (or left-hand) turn on encountering an obstacle is the key rule of the Wall Follower Algorithm of maze solving [26, 51]. The algorithm does not produce a shortest path out of the maze but finds the exit. The algorithm is not always efficient and sometimes produces wrong solutions. However, comparing to random walk approaches [4], the algorithm allows an agent, being in inside a maze, to solve the unknown maze in shorter time. The algorithm, and its many versions and enhancements, are used in robotics [23, 53, 9].

As proposed in [60] the asymmetry may arise during self-organisation of α-actinin-enriched radial fibres, myosin-enriched transverse fibres, and longitudinal actin bundles of actin filaments. The radial fibres undergo unidirectional tilting and the longitudinal fibres — swirling motion. This might explain rotating of tips of Physarum growing zones however right turn on encountering obstacle still remains unclear. Due to a strong architectural similarity between Physarum growing zones and neural growths, we believe that, given a chance to prolate in a three-dimensional substrate with low friction, the Physarum filopodia would show the right-screw rotation similar to that of neural filopodia [58]. The explanation provided by Tamada et al [58] seem plausible: myosin pull actin filaments toward their minus end and rotate in the right-screw direction, axial rotation result in swirling motion.

Why Physarum does not move in circles then? First, this is because it propagates on a planar substrate and circling would mean self-intersection. Second, again referring to [60], we can assume that when accumulation of a torsional strain in the filament bundle exceeds certain threshold a relaxation is triggered, which lead to rotation of the filament bundle into opposite direction. Therefore a single growing tube of Physarum moves rather in zigzags (Fig. 4) similarly to alternating right/left/right movement of amoeba's pseudopodium [14].
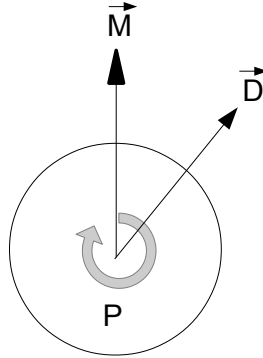
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Figure 5: Analogies between right walk of a drill bit [32] and deviation of Physarum pseudopodium. Rotation direction P of a drill bit is analogous to polymerisation of actin filament bundles. Direction of drilling M corresponds to direction of Physarum growth. Total force D of the drilling bit is analogous to deviation of the Physarum growing tip.

Some analogues can be drawn up with rock drilling. Not uncommonly, a drilling hole deviate from its preset direction due to anisotropy of mechanical properties of the rock [15] and due to unbalancing of the drill construction and materials [31]. Even more phenomenological similarities can be uncovered between a roller cone drill bit and active growing cone of *Physarum*. When drilling with the roller cone drill bit, a rotation of the drill pipe is in a clockwise direction. The roller cones rotate anti-clockwise. The drilling bit tends to 'walk' to the right [32]. This is because the bit experiences minimal left hand reactive torque, and also due to combined effects of drop, bite and fling progression [32] (Fig. 5). The right walk of the drill bit results in larger hole. *Physarum* propagates on a two-dimensional substrate, therefore it shows deviation to the right. The drilling bit does not have a compensation mechanism. *Physarum*'s cytoskeleton compensates for deviation by triggering rotation of the filament bundle into opposite direction, exact mechanism is unclear. When *Physarum* encounters an obstacle it could not compensate its right wise deviation by left moves. It moves along the obstacle therefore. Whether the bias has ecological significance (adaptive utility) for Physarum, or is a byproduct (*frozen accident*) of evolution, is as yet unknown. Further studies would be required to uncover exact mechanisms of the asymmetric motion. Continuing analogy between *Physarum*'s filopodium and neural growth cones we can propose that there are two actin sub-architectures: longitudinal long bundles resting along the ventral membrane and short branching networks between the long bundles and the dorsal membrane [36]. Ordered longitudinal bundles do contribution to rotation but what is a role of short branching networks? Mechanistic functional experi-

ments could focus on exact mechanical model of polymer networks in Physarum growing cones and exploiting their non-uniformity, e.g. a rotation component could be added to existing models [45]. Given that specific molecular-genetic and pharmacological reagents have been used to probe conserved roles of cytoskeletal proteins in asymmetry of Xenopus embryos [38, 48] and human cells in culture [68], future work targeting these pathways will address the roles of chiral intracellular structures in Physarum. The identification of biased asymmetry in Physarum is consistent with an ancient origin of chirality prior to the diversification of the Kingdoms of life. These data highlight this unique model organism as an important addition to the field of laterality research, in which the role of asymmetry in multicellularity, structure, and behavior can be addressed. It is unknown whether gravity influences the behavior of Physarum in this assay. While it is not clear how a uniform gravitational field pointing downward could induce a bias of left-right turning, especially over the small scale of our T-maze, prior data have indicated an effect of gravity on the cytoskeleton and other cellular processes [18, 22, 34, 35, 37]. Thus, the role of gravity and other geophysical parametdrs (such as geomagnetic field, hemisphere location, etc.) in this phenomenon remains to be explicitly tested in Physarum asymmetry.

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