Promoting Convergence: The Phi Spiral in Abduction of Mouse Corneal Behaviors

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Why do mouse corneal epithelial cells display spiraling patterns? We want to provide an explanation for this curious phenomenon by applying an idealized problem solving process. Specifically, we applied complementary line-fitting methods to measure transgenic epithelial reporter expression arrangements displayed on three mature, live enucleated globes to clarify the problem. Two prominent logarithmic curves were discovered, one of which displayed the \( \phi \) ratio, an indicator of an optimal configuration in phyllotactic systems. We then utilized two different computational approaches to expose our current understanding of the behavior. In one procedure, which involved an isotropic mechanics-based finite element method, we successfully produced logarithmic spiral curves of maximum shear strain based pathlines but computed dimensions displayed pitch angles of 35\(^\circ\) (\( \phi \) spiral is ~17\(^\circ\)), which was altered when we fitted the model with published measurements of coarse collagen orientations. We then used model-based reasoning in context of Peircean abduction to select a working hypothesis. Our work serves as a concise example of applying a scientific habit of mind and illustrates nuances of executing a common method to doing integrative science.

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INTRODUCTION

Convergence science [1] is a directed effort to take advantage of collective modern expertise to solve hard problems of organized complexity [2]. The goals and missions are expanded from a new biology [3], which stressed education and the coordinated application of problem-solving methods, used by complements of experts with both deep and wide knowledge, to control and preserve the nested hierarchies that characterize healthy natural living systems. Despite the promise of
merging disciplines [4], it is recognized that an optimal level of interaction has not yet been achieved [5–7].

The scientific habit of mind is a normative attitude toward learning and acting shared by both experts and novices [8–11] (CP 1.44). The associated property of simplification serves as antidote [12,13] (CP 7.61–73) for the growing sea of information that obstructs integration across disciplines. Despite potential benefits to society, effective dissemination and acceptance of the things that need to be communicated about the scientific method [2,11] in a perspicuous manner has proved extremely difficult [14,15] (CP 7.49). One goal of this report is to exemplify [16] an idealized approach [17] through application of modern techniques to solve an unexplained biological phenomenon. Extensive discussion is intended to emphasize particular themes, such as clarifying joint problem spaces [7,18,19] that promote effective collaboration. Stated differently, we want to explain why mouse corneal epithelial cells are arranged as spirals through application of a scientific habit of mind.

Why-questions about natural phenomena are in part searches for explanations, and providing accounts is a fundamental aim of science [20]. Sufficient explanations are three-term relations between a topic, a contrast-class, and a relevance relation, which “specifies what sort of thing is being requested as answer” for a given context [21]. Explanations to why-questions are never disinterested and can be structured into a logical form [22] using the Peircean abduction (PA), an adaptive syllogistic argument that leads to selection of the working hypothesis under uncertainty [23]. The PA differs from other statements of abduction (cf., [24]) in that it draws special attention to the fundamental aim of science [20]. Sufficient explanations are searches for explanations, and providing accounts is a fundamental aim of science [20].

The surprising fact, C, is observed.

But if A were true, C would be a matter of course.

Hence, there is reason to suspect that A is true.

Therefore, the extent to which a mechanism (A) qualifies as hypothesis for explaining observation (C) depends on judgments about how much deductions of (A) suffices expectations for (C).

Spirals can be used to distinguish different forms of nature and at different growth stages [26–28]. Natural planar spirals are typically classified as either Archimedean or logarithmic depending on rate of growth of radiating arms. The \( \phi \) spiral is a special type of a logarithmic spiral in which the radius grows proportionally to the \( \phi \) ratio (1.618...) and can be described by its pitch angle of \( \sim 17^\circ \). A pitch angle is defined as the angle formed at the point where the tangent of the spiral arm meets a circle (inset Figure 3(a)). \( \phi \) is an irrational number approached by the quotient of the greater number to its previous term at the limit of the Fibonacci series. This number can also be derived by dividing a line/circle the only way allowable, such that taking the ratio of the larger to the smaller equals the proportion of the whole to the larger of the two pieces [29].

Spirals have been decomposed and computed in natural systems across vast spatial scales [30–33], which provide opportunities for synthesizing sound relations across disciplines [34] through structural alignment [35]. Therefore, the practice of explaining why spirals occur in mouse corneas may be exapted for different purposes, such as improving biomaterials design [36,37], maintaining health [38–41], or clarifying general constraints among locally planar, globally spherical systems [42,43].

Spirals have been reported on the anterior surface of human eyes under both normal and aberrant conditions. Vortex keratopathies are recognized by metabolic or drug depositions on the anterior surface while hurricane keratopathies are classified as diseased or natural forms associated with epithelial cell behaviors [44]. The stability of some corneal spirals are illustrated by longitudinal analyses, which showed that subtle changes to neural plexus arrangements can be detected over weeks-long timescales [45]. An in vitro study of corneal limbal explants exposed to a specific magnetic field produced spirals that maintained up to \( \sim 1 \) week before unfurling [46].

In the rodent, spirals are prominently displayed as transgenic epithelial cell segregation arrangements [47,48] or as neural extensions [49–51] migrating through the subbasal nerve plexus [52]. Mosaic studies [47,53] showed that spirals of reporter expression arrangements begin to emerge \( \sim 3 \) weeks after birth \( (\sim 1 \) week after eye opening) and resolve to robust patterns after \( \sim 10 \) weeks, a time that marks the later part of sigmoidal growth [51,54]. These observations of spiral patterning in distinct layers of the cornea is suggestive of a global systemic force that regulates formation and maintenance [55]. Despite proposal of a contrast class of mechanisms (e.g., electrical, magnetic, mechanical, cellular [44,56–59]), there is currently no consensus on a best explanation.

In this report, we surveyed live, enucleated globes containing transgenic mouse corneas between 4 and 7 months using confocal laser scanning microscopy. We applied complementary line-fitting measurements to demonstrate that 17° and 21° pitch angles are predominantly represented. We constructed two different computable schemas to simulate observed behaviors. Subsequently, we combined abduction and model-based reasoning to make inferences about deduced solutions, which reflect our current understanding of the phenomenon. We close with a discussion of justifications for choosing the working hypothesis (CP 7.163).
RESULTS

Mouse Cornea Anatomy

The cornea is the superficial, transparent, avascular tissue at the anterior-most position of the eye (Figure 1). It may be considered a special type of skin [60–62] with balanced optical and mechanical properties that may be exploited for development and application of therapeutic technologies [63–65]. Structurally, it is a three-layered system [Figure 1(d,l–l)] with the stratified corneal epithelial cells further subdivided into superficial cells [white arrows, Figure 1(d,e)], intermediate wing cells and basal epithelial cells [red arrows, Figure 1(d,f,j)]. The different cell types are marked by layer-specific expression of different proteins, such as the tight junction marker, ZO-1 [Figure 1(e)] only in superficial cells, while basal epithelial cells predominantly express the gap junction protein, Cx43 [Figure 1(f) and inset] and Integrin β4, a mechanical/signaling integrator, but with local concentration differences [Figure 1(f)] [66]. The middle layer of the cornea, the stroma [Figure 1(d,k)], is comprised mostly of a collagen-rich material secreted by keratocytes [67] [green arrows, Figure 1(d,j,k)], the path-dependent arrangements and interactions of which provide the majority of mechanical support. The deep corneal endothelial cells [purple arrows, Figure 1(d,l)], arranged in shapes reminiscent of the Benard phenomenon [68], provide important fluid regulation required to maintain transparency and general nutritive balance [69,70].

The limbus [light blue arrows, Figure 1(a–c,g–i)], a morphologically distinct region that also serves as a specialized niche for stem cells [71], marks the corneal periphery. The stroma is split immediately posterior to the limbus, with the superficial portion providing basal support to the conjunctiva, a goblet cell-rich layer contiguous with the cornea that provides mucus and protection against foreign substances in combination with the lacrimal apparatus. The sclera constitutes the exterior portion of the posterior eye.

Qualitative Determination of Spiral Progression Through Comparisons

Previously, we used a landmarking method to demonstrate that logarithmic spiral patterns are conserved in chimeric rat corneas [48]. In the current report, we used a line-fitting method to measure variegated arrangements displayed by mTmG alleles [72], that is, from individuals of a single genotype. The mTmG transgenic line expresses a single-copy, membrane-targeted version of a fusion reporter gene homologously recombined into the ROSA26 locus, supplemented by a strong artificial promoter to enhance activation. Although expression was expected in every cell, only mosaic patterns were observed in adult mouse corneal epithelia. We observed different types of patch patterns using the same artificial promoter expressing a different downstream reporter integrated randomly into the genome [73] (data not shown). Variegation in Krt12-Cre/+; ZEG reporter mice have also been reported [74]. In rats, transgenic lines produced by lentiviral injection of a reporter construct driven by a human polyubiquitin-C promoter that express a more complete pattern has been reported [48]. The reasons for corneal-specific variegated patterns is a subject of future investigations but is expected to involve combinations of epigenetic [75,76], physiological [77], and/or dynamic protein turnover mechanisms. Therefore, whether spiral patterns represent clonal cell migration paths or transient reporter effects are not known, although the former has generally been assumed in the absence of complete information.

To determine regularity of mTmG variegated patterns, we examined a developmental cross section of enucleated live mouse corneas from 4 to 7 months, taking great care not to perturb the natural shape of the globe. Comparisons of globes from different individuals arranged in series suggested that spirals evolve over time but ordering does not behave monotonically [Figure 2(a–c)]. When intraindividual corneas were compared, similar or topologically nonequivalent behaviors were displayed on surfaces of paired eyes. For example, in one 6-months old female, clockwise (CW) spirals were displayed on each cornea [Figure 2(d,e)], whereas a different individual displayed a counter CW (CCW) pattern on one eye but two counter-rotating curves separated by an equator on the other [Figure 2(f,g)]. In mice, CW spiral patterns dominate ~5% over CCW patterns but these patterns only represent a subset of possible forms [47]. Probability distributions of main pattern types are characterized in [53] based on examinations of 135 globes. Combined, these observations demonstrate that both genetic and environmental effects influence mature patterns, which may depend on unequal spontaneous symmetry breaking processes operating at earlier sensitive periods [78,79].

Visual Search for Spirals

To demonstrate the existence of logarithmic spirals on mouse corneas and to determine their precise pitch angle, we matched preselected curves to features. Due to incomplete information about features, we used reasonable grouping principles [80] (viz., proximity, similarity, continuity, smooth continuation, symmetry, familiarity, and common fate) for categorization. Only corneas from age-matched animals with qualitatively similar shapes were selected for measurement to focus our problem to a discrete growth stage.

We produced a range of curves for direct comparisons. The range was selected based on resemblance to natural forms; the 12.44° pitch angle representing the silver ratio displayed by nautilus [81], the φ ratio displayed in Phyllotaxis [82], the 21.1° pitch displayed by the M51 Galaxy
Mouse cornea anatomy. a) Lateral and b) centric views of live, enucleated cornea. The red color is epifluorescence from the mTmG allele. c) Sagittal section of limbus infused immunostained using an Intβ1 primary antibody to label some endothelial compartments. d) High magnification of region marked by the yellow arrow in inset. Basement membranes adjacent to Bowman’s and Descemet’s membranes are indirectly labeled with a Pan-Laminin antibody. e) Superficial cells (white arrows) are labeled by a tight junction marker, αZO-1. f) Intβ4 and Cx43 antibodies mark puncta in basal epithelial cells, some of which are clustered in series. Live imaging of limbus using confocal (g,h) or polarization microscopy (i). Note loss of birefringence in solera, indicating poor collagen alignment. Centric views of an optical plane through Bowman’s membrane (j), stroma (k) or Descemet’s membrane (l). Light blue arrows mark the same area of the limbus, white, red, green, and purple arrows mark superficial cells, basal epithelial cells, keratocytes, and corneal endothelial cells, respectively. SHG = second harmonic generation. All colored images are of fixed tissues, while grayscale images are of live tissue.
Corneal patterns are sensitive to both genetic and environmental effects. Inter (a−c) and intra (d−g) individual comparisons to demonstrate sample range of possible behaviors. Although the series (a−c) is placed according to age, the most symmetric form is (b). Therefore, a linear description requires increased time resolution. Both similar (d,e) and dissimilar (f,g) forms are possible within individuals. All scale bars are 100 μm in this report. Images were captured only under two magnifications: size of “low-magnification” field is 1240 μm wide (a,b,d,e), while “high-magnification” is 500 μm wide (c,f,g).

[83], as well as extreme forms, which are together represented in Figure 3.

We first performed a blinded study to mitigate subjective bias. Protocol for matching involves feature selection/visual recognition of areas of contrast [arrows, Figure 3(a,b)] followed by masking spiral arms through translation and/or rotation to generate the best fit. After a visually apparent feature is selected [purple arrow, Figure 3(a)], a relationship to a second feature, such as the major curve (blue arrow) or distance to the pole (green dot) can be scanned and compared with each other. That is, the investigator is able to resolve distance to the center [84] to micrometer resolution by taking advantage of subordination of coiling forms [85], namely, the relationship between the pole and radiating arms [26]. Selection of two features also constrains rotational freedom, which drastically reduces the space to be examined for correlations (purple and orange arrows). That is, the protocol makes the difficult problem of describing the full extent of the complex pattern displayed on mouse corneas tractable by reducing the problem to measurements of localized, selected regions. Comparison to a counterexample demonstrates that the visually apparent argument for a better match is self-evidencing [Figure 3(b,c)].

Best fit was determined through direct contrast against overlain curves of similar angles [Figure 3(d,e)]. An external viewer is then able to verify the investigator’s intention through assessment of the precise relationship between crossing point and selected feature at the bound site [purple arrow, Figure 3(e)]. Intuitive preliminary matching at the intended site [purple arrow, Figure 3(d)] yielded imprecise crossings, which can be refined through deliberate recursion onto a selected representation [Figure 3(e), [13,86]].

In the absence of explicit knowledge about underlying mechanisms of cell variegation, judgment [87] was used to determine best fit. For example, if the conspicuous major arm was deemed most important, the 21.1°/C14 spiral was the best match [Figure 3(g,h)]. However, this came at a cost of losing match with the edge marked by the purple arrow [Figure 3(g,h)] that extended to a bending feature at a distal site [yellow arrow, Figure 3(i)]. If all features are commensurable, then the φ spiral was the best match because it touched the greatest number of features along the greatest distance from the pole.

Once selected, targeted search [88] for periodic effects can be implemented by rotating the selected curve at desired increments [fivefold rotation, green arrow, Figure 3(f)], which suggests discrete parsing of territories. Rotating
FIGURE 3

Generating best fit through line fitting. (a–c) Rules of matching. Features (colored arrows) are selected and logarithmic forms with specific pitch angle are masked over features. Best match is produced through direct comparison after rotation and translation upon selection of two features. For example, closeness to a bright cluster of cells (orange arrow in b) is determined after selecting the pole (green dot) and edge (purple arrow) to root features. Flipping the symmetry demonstrates reasonable agreement that purple curves are matched better onto features in (b) than in (c). Curves in (a–c) represent a blinded trial. Once selected, purple curves (17°) were colored gold in (d–i), which contains a series ranging from 8° (pink) to 35° (red). To perform targeted search, the golden spiral is rotated in 72° increments in (f). (i) is a lower-magnification view of (a–h). Orientation is not exactly preserved in (i) compared to (a–h).

measuring sticks 180° after matching to the major arm always produced overlap with features of the minor arm in this sample [range: 8° to 35°, orange arrows, Figure 3(g,h)]. These measurements suggest the existence of opposite symmetrical logarithmic spiral arms in this cornea. In summary, placement of each curve onto a scene produces a hypothesis about nonrandom effects being responsible for generation of that correlation.

**Verification for Existence of \( \psi \) and M51 Spirals**

To support the validity of our method and to minimize influence of illusory effects, we transformed the images from Cartesian to log-polar coordinates (Figure 4). Each pixel is then referenced by the logarithm of its distance to the pole and the angle between that line and the \( x \)-axis. With this method, any logarithmic curve is converted into an oblique line, the slope of which is related to the pitch angle. For some of the data, we performed log transformations using different poles [Figure 4(b–e)] and visually inspected the data for presence of straight lines. Display of degree angle is sensitive to pole selection and subsequent confirmation through visual perception is affected by feature transformation.

We primarily chose visually apparent features, such as clusters of bright cells lying in sequence [yellow arrows,
Figure 4(b) or edges to determine linearity. The reader can then assess correspondence of matched features or verify existence of parallel lines in adjacent regions [orange arrows Figure 4].

We emphasize that while the \( \phi \) spiral was noticed, it was not the most conspicuous form displayed in the three samples using this method. Rather, the 21.1° \( /C_14 \) angle was. Moreover, while the entire set of possible logarithmic curves is not currently known, the range was found to be discontinuous. For example, although the 21° \( /C_14 \) curve was dominant [white lines, Figure 4(f–j)], more extreme pitch angles [green arrows, Figure 4(c,d)] were also represented.

Measuring Size of Core

If radiating arms are measurable, then so should centers of curves. Briefly, three spiral corneal samples were normalized by drawing a line across a linear feature (white lines, Figure 5), rotating five times, then matching to generate the outlines of a pentagram [Figure 5(a,d)]. Samples were oriented by subjectively rotating the image with respect to the top horizontal line [Figure 5(a,c), purple arrows]. Adjusting samples proved useful for partitioning the space for easier comparison and representation [Figure 5(a–d)]. That is, it fixed searchable space to a smaller region by restricting free rotation that facilitated representation of pole distributions.

Using this method, we identified poles for each image based on matching distal-arm features [Figure 5(d)]. Curve-fitted poles from each rotated sample were then compiled onto single images [colored dots, Figure 5(b,c)]. We defined the size of the core as the maximum distance of the two most separated poles. The results from images captured with a 10× lens were 50, 145, and 210 \( \mu \)m, respectively [Figure 5(a–c)]. Projecting 3D curved samples onto a 2D plane is expected to increase measurement error in samples collected at lower magnification. These data argue that curves are not always tiled on a plane. That is, although the poles in the first sample pivoted about a single point when examined at higher magnification [Figure 3(f)], the arms of samples with larger cores crossed each other near the center.

Deducing Solutions from One Model

One model of corneal epithelial patterning proposes that spirals result from preferential placement of stem cells at the limbus followed by migration of differentiating cells toward the pole [53,54]. To illustrate the chemotactic mechanism as a potential explanation with respect to the three-term relation, we produced a multiagent model using Netlogo software. The multiagent modeling platform allows constructions of appropriate problem representations by facilitating conversion of mental models [25] into computer simulations [89]. For our purposes, we integrated rules for firefly flashing and Dictyostelium aggregation behaviors.

Briefly, stem cells [large dark blue discs, Figure 6(a)] placed at the limbus (light blue annulus) produce...
epithelial cells (agents, maple leafs). Agents move in response to two different parameters. The first value is assigned to patches (static positions represented as yellow surface), that is, highest at the pole. The second value is dynamic; a diffusing chemical secreted by agents, to which neighbors respond with an assigned probability. Chemical concentrations can be assessed through visual inspection of signal intensity [bright green, Figure 6(b)]. The rules allow up to 10 cells to occupy the same discrete space to produce a dynamically stable number of cells. Global arrangement can be approximated using a box-counting algorithm, that is, exported to calculate fractal dimension $D$ [Figure 6(c)]. These rules are sufficient to satisfy the conditions of the stem-cell based model proposed to explain epithelial spiraling, although stem cell positions are fixed [53].

Simulations allow views of time slices during the ontogenetic process. Export views at $t = 0$ or $t = 400$ iterations are presented to give the reader an idea of the effects of parameter selection on final solutions [Figure 6(d–g)]. Export views from a single simulation with Sniff angle 45 are presented in series in Figure 6(h). “Sniff angle” is a simulation parameter that allows agents to select detection range in direction of heading that affects direction of the next movement. $D$ is plotted for increments up to 800 iterations under different conditions ($\sim 80$ days, based on rate of spiral emergence, and speed of migration, measured at $\sim 26$ $\mu$m/day [54]). Based on both direct visualization and plot, dynamic equilibrium was achieved after $\sim 400$ iterations. Comparisons of different initial conditions demonstrated that the most constrained global shape resulted from sniff-angle 68° and addition of pacemakers. Parameters can be adjusted to yield
different forms, including a spiral [Figure 6(j)], target [Figure 6(k)] or saddles [Figure 6(l)].

**Induction Through Comparison Against Target Behavior**

Comparison of the hypothesized global representation of observed behavior against computed solutions quickly exposed the ambiguous meaning of the term “spiral” [Figure 6(j)]. For example, juxtaposed breaks that contribute to the sense of spirality are positioned at the periphery, whereas in natural corneas, peripheral patterns are radial and break into spirals ~300 μm from the pole [Figure 3(i)]. To our knowledge, epithelial assortment patterns that resemble targets have never been observed on natural corneas, which suggest that real conditions do not allow for
its formation and maintenance. Although counter-rotating pairs have been observed in simulations, its shape is different and positioning is outside the domain in which it is normally found (field size = 3 mm, each parallel segment in this image is ~70 μm, central domain radius = 3 segments, closest saddle point is 4 segments from pole).

Although our agent-based model can be criticized for many deficiencies, such as individuated migration rather than contact-mediated coordination, failure to cover the expanse of the migrating field, large migration step sizes, and so forth, it is nevertheless useful for exposing unstated assumptions that require explanation, namely, its location at the center of the cornea and the specific pitch angle. Moreover, it is not clear that even a perfectly executed chemotactic model would produce the desired logarithmic spiral solution as primary forms in Dictyostelium models resemble Archimedean spirals. That is, computed solutions did not reduce the number of possibilities, as no clear relevance relation was recognized.

**Deducing and Comparing Solutions from a Different Model Versus Target Behavior**

To produce a contrast class, we constructed a bulk material model with either isotropic stiffness or one that adds anisotropy through collagen alignments matched to reported experimental measurements [90] (Figure 7(a)). Under stated constraints, displacement is greatest at the center when a pressure of 13 mm Hg is applied to the interior surface [Figure 7(b)]. From this solution, we mapped a vector field of maximum shear strains in the plane of the surface of the stroma [Figure 7(c)]. To simplify visualization, we devised an algorithm that traced pathlines from the perimeter based on sampling local maximum shear strains using an unstructured mesh under isotropic conditions [black curves, Figure 7(d)], center region magnified below and collated as black curves in Figure 7(f)]. When a radial mesh was applied to isotropic conditions, pathlines resolved more closely to logarithmic maximal in-plane spiral shear strains. Our results did not reduce the number of possibilities, as no clear relevance relation was recognized.

To test the hypothesis that simulated collagen arrangements can affect pathline behaviors, we repeated procedures under anisotropic conditions using the radial mesh [blue curves, Figure 7(e,f)]. We directly compared pathlines initialized from five independent positions under the three conditions, that is, black, green, and blue curves. When anisotropic collagen conditions were applied, shapes of curves were qualitatively different at the center [Figure 7(f)]. Curves that were both greater [purple arrow, Figure 7(e)] and lesser [orange arrow, Figure 7(e)] than a logarithmic maximal in-plane spiral shear pathlines were produced under isotropic conditions that differed from the ϕ spiral by 18°. Adding collagen anisotropies affected shape of solutions maximally near the center. These data support the argument that local anisotropies of collagen arrangements can affect pathline behavior. Moreover, we hypothesize that if collagen arrangements are major effectors of patterns displayed on mouse corneal epithelial cells, then actual arrangements, which are known to be more complex than the coarse forms that we used to fit our model, can lead to ϕ spiral shear strains. Our approach offers a complementary method by which to solve mesoscopic collagen arrangements [91].

**DISCUSSION**

Being clear about the relation [21] (CP 7.218) between behaviors to be explained [92,93] and mechanisms of formation [37,94–96] is an underappreciated requirement for productively advancing transdisciplinary investigations of scientific explanation, which ultimately will bear on practical judgment making [87]. We applied complementary line-fitting methods to verify existence of two prominent logarithmic spiral forms. Despite resolvability of the measuring method, we were not able to agree on a single value to describe the global state of the system. Specifically, the nondiscursive nature of the matching method [Figure 3(d)] and the lack of awareness of underlying biology both affected feature appraisal, which affected consensus building. However, if future investigations [7,87] (CP 7.114-115) reveal grounds for assigning higher value to the conspicious feature (blue arrows, Figure 3), then the best description would be 21° [Figure 3(g)]. If all features are commensurable, then the ϕ spiral is the best representation because a single curve touches the most number of landmarks along the greatest distance from the pole [yellow arrow, Figure 3(i)]. Irrespective of such considerations, the resolution of the measuring method was resolvable at least to within 4°, and makes description of the end flexible but not ambiguous [87].

To avoid vacillation [97], we hypothesized that the ϕ spiral is the global attractor [34,82], or a type of regulating line that saves the phenomenon. It was preferred over the 21° spiral based on its utility as a computable descriptor for minimal energy/maximum entropy/perfect growth [28,32,98] states.

Qualitative matching of the ϕ spiral against solutions to Netlogo simulations quickly exposed unstated expectations of the problem, namely, explaining its location at the center of the cornea and whether the phenomenon can even be said to be present. However, consonance between the Netlogo model and the observation is not produced the way the mechanism is currently posed.
To generate a computable contrast-class, we constructed a Finite Element Method (FEM) model that produced logarithmic spiral shear strains under isotropic conditions. On simulation of intraocular pressure, curves displayed 35° pitch angles. Adding anisotropic collagen arrangements to the model impacted in-plane shear strain behaviors maximally near the center. Therefore, admissibility of the FEM model as a potential explanation depends on the likelihood that generation of a logarithmic curve is a veridical statement that the phenomenon is present and whether further modifications to the model reflecting finer measurements can affect tightening of
correlation to $17^\circ$. That is, we choose the working hypothesis ($\phi$, FEM model), in contrast to rest of contrast-class X (i.e., Netlogo model), because $A$ (deduction of logarithmic spiral, relevance relation) [21]. Adopting the hypothesis that the $\phi$ spiral describes the global state of the system proved valuable for structuring the abduction, which then allowed simple performance evaluation via resemblance during induction (CP 7.218), sc., inference to the best explanation [99].

Corneal mechanical properties are scale dependent [91]. Collagen molecules self-organize into right-handed triple helical procollagen, are secreted by the generating cell, is processed enzymatically, associate laterally and longitudinally to produce collagen fibrils that are constrained in its properties by the microenvironment. Fibrils are $\sim 30$–$35$ nm dense and $\sim 1$ mm long [100,101] in the cornea. Fibrils then associate into larger lamellar forms $\sim 2$ $\mu$m thick and $\sim 5$–$100$ $\mu$m wide that interdigitate and insert directly into the anterior limiting lamina [102,103]. Within lamella, $\sim 50$–$60$ nm fibril spacing is maintained in a quasihexagonal arrangement by osmotic and electrostatic pressure mediated by proteoglycan coating of fibrils [70]. Fibril and lamellar sizes are controlled by region-specific, overlapping [104] and independent [105] mechanisms. Cholesteric forms detected with polarized microscopy [37,102] [Figure 1(k)] implicate extracellular liquid crystalline packing as potential effectors of corneal mechanics [106].

We experienced difficulty imaging the cornea at high resolution in whole organisms due to corneal pulsing. We suspect this is due to perfusion pressure driven from retinal and uveal vessels [107]. Given the rapid mouse heartbeat [108,109], ocular pulsing may drive strain stiffening depending on the structure of interparticle interactions [110].

Despite atomistic simulations of idealized microfibrils that neatly illustrate reliance of mechanical properties on the level of description [91], the great disparity of observations reported in the large-strain regime demonstrates that mesoscale behaviors remain unexplained. Moreover, there is currently no systematic means by which to select samples that represent local corneal microenvironments appropriately enough to identify mesoscale-level regularities. If our hypothesis that corneal spiral patterns are dependent on collagen arrangements is true, then the shape of the spiral, which situates the complex interactions that control its form [111], will be useful for marking specific local microenvironments that can be developed into a reference system of reduced complexity [7]. That is, selection of a region that fits in a single optical field will help reduce experimental and computational costs of determining typicalities of mesoscopic arrangements, which can then complement studies concurrent with bottom-up methods [91]. Producing a comprehensible model that demonstrates quantitative dependence of the corneal spiral on molecular packing [112] will be an important and valuable contribution to understanding relationships between patterns and scaling phenomena [113]. Comparisons of behaviors using natural and engineered mice with defective organization [38,40,114] will support this mission to integrate diverse expertise to construct hypotheses that can also explain emergence of topologically nonequivalent forms [Figure 2(g)]

The mouse cornea shares similarity with plant phyllotaxis in that each can be considered a centric representation [32,93,115] of multilayered pressure vessels displaying visually apparent spiral arrangements. Yet, the $\phi$ ratio is expressed differently in the cornea, a transparent animal tissue in which growth and migration is towards the apex. Such properties make corneal spiraling incongruous with phyllotactic models in which the optimal process involves successive appearance of elements displaced at golden angles that grow away from the center.

To our knowledge, only an indirect phyllotactic mechanism has been applied for the cornea. Investigators have applied phyllotactic algorithms to compute tropocollagen packing into cylindrical fibrils that retain the property of placing elements on a generative spiral, which produce secondary nearest neighbor-aligned parastichies [116]. Whether these nanoscale effects address our search for an explanation across the micrometer scale relevant for cell interactions [91,103,117,118] is unclear. In summary, while both systems are marked by the $\phi$ ratio, the extent of common relational structure is ambiguous.

**CONCLUSIONS**

The surprising fact, C ($\phi$ spiral), is observed. But if A (FEM model) were true, C would be a matter of course. Hence, there is reason to suspect that A is true.

We produced the above working hypothesis by stating the topic, contrast-class and relevance relation [21] explicitly. The logarithmic spiral played different roles during the procedural stages of scientific explanation: (1) it served as a single numerical description for the observation, which rooted the abduction; (2) it served as the relevance relation by which to judge the quality of deductions from among the contrast class of models, which embodies the context [25] during inference to the best explanation. Our work serves as a concise example of scientific reasoning during early stages of inquiry and illustrates dependence of induction on abduction, and the serial manner by which different forms of hypothesis partition the process (CP 7.218).

In summary, we propose that spiral angles are stable in mature mouse corneas (Figure 8). They (co) exist primarily as either $17^\circ$ or $21^\circ$ forms, the poles of which are superposed to different levels [Figure 8(d)]. We selected the combination of $\phi$ spiral and FEM model as the working hypothesis based not only on resemblance of computed solutions to the observation, but also on integrative
qualities such as contiguity, communicability and sustainability [17,120].

MATERIALS AND METHODS

Immunohistochemistry

Standard immunohistochemical protocols were followed. The major modification added to the current method was to include overnight 50 nM Deoxycholate incubation in Phosphate Buffered Saline (PBS) at 4°C prior to vibratome sectioning and incubation with primary antibody. Primary antibodies were purchased from BD Biosciences (Integrin β1, β4), ZO-1 and all secondaries from Invitrogen, Cx43 from Cell Signaling Technology and Laminin from Sigma.

Live Imaging of Enucleated Globes

Animal work was approved by the Lurie Children's Research Center IACUC protocol 2009-09. Mice were euthanized according to IRB protocol. Globes were carefully removed using small scissors and placed in Dulbecco's Modified Eagle Medium (DMEM) at room temperature. Corneas were placed in a culture dish with glass bottom and images were collected using an Olympus FV1000MPE with Spectra Physics Maitai-OL HP ultrafast IR laser and a BX61WI fixed stage upright microscope or using a Zeiss LSM 700. The Second Harmonic Generation (SHG) images were acquired at 860 nm and filtered at 420–460 nm bandpass filter in a reflected nondescanned photomultiplier tube (PMT). Images were processed with Photoshop, conforming to epistemic standards for processing [121].

Netlogo Model

Coarse-grained algorithms borrowed from a library of validated models were modified to simulate conditions of corneal patterning. Example models used were Slime.nlogo and Firefly.nlogo. The model and details are freely available at the Center for Connected Learning at Northwestern. http://ccl.northwestern.edu/netlogo/models/community/Cornea%20patch%20formation.

Finite Element Model

Interior and exterior surfaces were fit to spheres of different radii and centers to create a central thickness of 0.3 mm and a peripheral thickness of 0.1 mm. The radius of the cornea is 1.4 mm. The geometry is then meshed.
with standard finite element meshing software using eight-node hexahedral elements with a B-bar option. Both an unstructured mesh and a radial mesh with more elements near the center were used.

The material model is adapted from anisotropic, hyperelastic model for human corneas described in (104), but with a single preferred fibril orientation. We model only the stroma. We treat the stroma as an isotropic, incompressible Neo-Hookean matrix, with a set of oriented and dispersed fibers that add stiffness in the direction in which they are oriented. Individual fibers are not modeled explicitly. The list of material properties for FEM of the mouse cornea is the following:

\[
\lambda(kPa) = 5500; \mu_0(kPa) = 60; k_1(kPa) = 20; k_2 = 400
\]

We developed an approximate model for collagen orientations in the cornea based on the D28 data from Figure 5 of (65). It is worth noting that while the collagen is predominately nasal-temporal near the center in the figure, the authors report other corneas exhibited a more oblique orientation near the center. Near the limbus, 90% of fibrils are assumed to be oriented circumferentially, while 80% are oriented horizontally near the center. Random fibril orientation, which makes up the difference, is not represented in the image. A transition zone bounded by the green and red circles linearly interpolates both the fibril direction and percentage of oriented fibrils. In this model,

we use \( r_{in} \) of 0.5 mm and an \( r_{out} \) of 1.1 mm. The edge of the cornea is not allowed to displace but may rotate under pressure. An intraocular pressure of 13 mm Hg was applied to the inside face of the model.

The displacements, strains, and stresses are solved using a nonlinear finite element code. As there are two orthogonal directions of maximum shear strain, we chose the one at each point that point CCW as it moves toward the center. We then traced pathlines of the vector field along the top surface. To simplify this procedure, we first averaged the shear strains in the element. As the maximum shear strain direction is then constant within a given element, the trace of the pathline is piecewise linear. We then find the endpoint in each element, and trace the entire pathline, as described in [122].

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