Dispatches

Cell Biology: Scaling and the Emergence of Evolutionary Cell Biology

A new study investigating the origins of diversity in the structure of the mitotic spindle in nematode embryos, at timescales spanning a few generations to hundreds of millions of years, finds that most features of the spindle evolve via a scaling relationship generated by natural selection acting directly upon embryo size.

**Patrick C. Phillips*
and Bruce Bowerman**

Work over the last 30–40 years has provided deep insights into the molecular basis of most important biological processes, including signaling cascades, regulation of the cell cycle, and the dynamics of cell-fate patterning and morphological development. This new knowledge in turn has enabled novel approaches for studying the evolution of these complex systems, with evolutionary developmental biology ('evo-devo') being perhaps the best example [1]. In contrast, melding an understanding of evolutionary processes with variation in intracellular structure has been much less common [2]. While biodiversity is particularly striking at the level of whole organisms, perhaps a tendency to focus on conserved features of eukaryotic cells has obscured the fact that there also is a great deal of diversity at the cellular level, including the structure of the nucleus [3] and the Golgi apparatus [4]. This is a shame because the rigorous functional approach that is typical of cell biology has a great deal to contribute to our understanding of how molecular function evolves. Bringing cell biology to an equal footing with molecular evolution and evo-devo requires that we turn our increasingly sophisticated toolset of microscopy and single-cell analysis toward precise measurements of variation in cellular processes within and between species. A new study published in this issue of *Current Biology* by Farhadifar *et al.* [5] examines the evolutionary forces responsible for structuring natural variation and evolutionary divergence in the mitotic spindle and is an exemplar of exactly how this research program can be carried out. More importantly, use of a rigorous comparative framework has allowed these authors to suggest that a very simple scaling relationship with cell size may explain a great deal of variation in subcellular structure among species, at least for nematodes.

Farhadifar *et al.* [5] focus on one of the fundamental events in the life of every cell: the proper segregation of chromosomes into daughter cells during cell division, a process that is directly facilitated by the construction of the spindle apparatus (Figure 1). During normal mitosis, recently replicated chromosomes are aligned along the central equator of the cell via the spindle apparatus through the action of microtubules and an array of associated proteins. The geometric structure generated during this process allows several features of spindle construction — including size, orientation, and speed through anaphase — to be characterized. To this end, Farhadifar *et al.* [5] developed a novel computerized 3D imaging system that captures thousands of spindle formation events during the first cell division in eggs of the nematode *Caenorhabditis elegans* and many of its relatives.

Proper formation of the spindle is important for accurate segregation of the chromosomes into daughter cells as mitosis progresses. Failure at this point can lead to chromosomal non-disjunction, with one daughter cell receiving an extra chromosome and the other daughter cell lacking a chromosome. An error of this kind

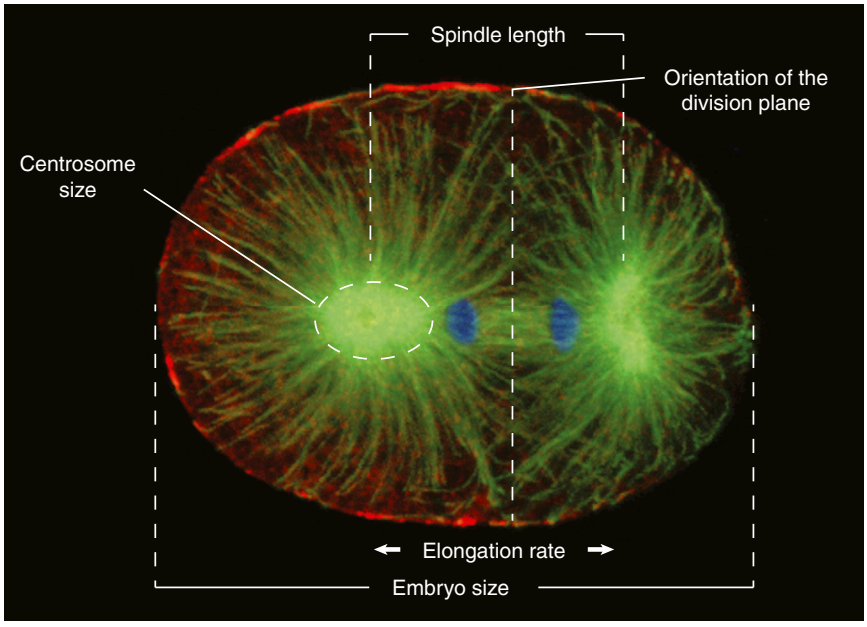

Figure 1. Automated characterization of the dynamics of spindle growth.

Measurements of the spindle apparatus taken by Farhadifar *et al.* [5] of the first cell division in the nematode *C. elegans* using automated image capture. Microtubules are shown in green, chromosomes in blue, and the cell background in red. The actual measurements were taken from a series of black and white video images. (Image of dividing *C. elegans* embryo by Aaron Severson, reproduced with permission from [14].)

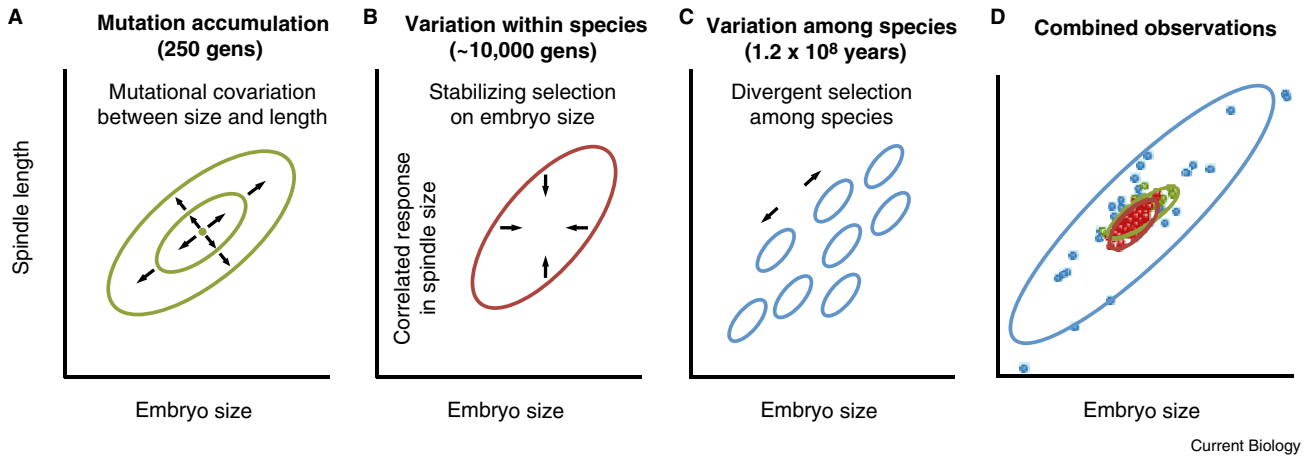

Figure 2. Micro- and macro-evolution of cell size and spindle structure.

(A) The relationship between the size of an embryo and the characteristics of the spindle formed at the first cell division depends on a balance between mutations generated each generation and the strength of selection operating for or against those mutations. Farhadifar *et al.* [5] use a mutation accumulation approach to find that, on average, mutations that increase embryo size also tend to increase spindle length. (B) An analysis of variation among natural populations (which have probably been diverging from one another for about 10,000 generations (gens)) shows, however, that most of the variation generated each generation by mutation must be eliminated by natural selection. The model that fits the data the best is that selection actually occurs directly on embryo size, with spindle length evolving as a correlated response to this selection, with the correlation being generated by the mutational covariance between the two traits. (C) Assuming that a similar pattern of mutation and selection is operating between species, then the total variation in embryo size and spindle morphology within this family of nematodes is structured by a shifting pattern of natural selection that favors a slightly different sized embryo within each species. (D) Combining these factors together gives the overall pattern of variation within and between species that was observed by Farhadifar *et al.* [5] for the influence of mutation (green), variation within species (red), and variation among species (blue).

means that whole sets of genes will be missing from or disproportionately abundant within the cell — decidedly bad things — which suggests that proper spindle formation should be under strong purifying natural selection for the maintenance of proper function. Yet just because an essential endpoint (such as proper chromosomal segregation) is under selection, it does not necessarily follow that every single feature of a cellular process and/or structure is also under strong selection [6].

To test this hypothesis quantitatively, Farhadifar *et al.* [5] used *C. elegans* lines that had been maintained for 250 generations by restricting the population size of each line to one self-reproducing individual each generation. Such a small population size maximizes the effects of genetic drift and minimizes the chance that natural selection will be able to eliminate new mutations before they become fixed within the line. In this near absence of natural selection, Farhadifar *et al.* [5] found that all aspects of spindle morphology rapidly accumulated variation from new mutations (Figure 2). Indeed, 250 generations of mutation accumulation was sufficient to recapitulate nearly all of the variation in spindle structure observed among a worldwide collection of 97 different natural isolates of *C. elegans* (Figure 2).

Rather than simply stopping at a description of variation per se, Farhadifar *et al.* [5] used equations drawn from multivariate evolutionary quantitative genetics theory that allow the pattern of selection operating within natural populations to be inferred from the contrast between standing genetic variation and the amount of variation expected under neutral mutation accumulation [7]. One of their important observations here is that most mutations do not appear to have independent effects on different aspects of spindle structure. Instead, mutations that affect egg size also affect spindle length, elongation rate, centrosome size and the orientation of the division plane (Figure 2A). So, while it is clear that stabilizing selection must in general be keeping mutational variation in check, a simple model in which selection is operating only on egg size, with all other aspects of spindle structure resulting from a correlated response to selection on size, fits the data extremely well (Figure 2B). To support this idea, Farhadifar *et al.* [5] measured offspring production in a subset of the natural isolates and found that lifetime production of offspring tends to be maximized at intermediate egg sizes — a strong signal of stabilizing selection.

Having established an evolutionary linkage between spindle size and egg size within a single population, Farhadifar *et al.* [5] then applied this model to variation in spindle structure among 40 additional species of nematodes. Strikingly, they found that the pattern of covariation between egg and spindle size observed within mutation accumulation lines and within natural isolates of *C. elegans* is echoed at the level of an entire order of nematodes, albeit with higher levels of total variation, as would be expected for an additional 100+ million years of evolution (Figure 2C). In fact, the model that best fits the overall pattern is that each species has been selected for a slightly different optimal egg size, with changes in most other features of the spindle tagging along with that simple change.

It is important to note that the accumulation of mutations could easily span the total range of variation among species within a few thousand generations. Thus, as is often the case [8], long-term evolutionary change in the early nematode embryo

is characterized by very slow conservative change among species dominated by strong stabilizing selection within species.

Overall, the most interesting suggestion that emerges from this study is that essential features of the cell, such as the structure and function of the spindle, might arise from very simple scaling rules with cell size per se, which makes sense since the spindle itself is stretched during the course of cell division. This relationship also appears to be true within an individual because centrosome and spindle size also scale with cell size as embryogenesis in both *C. elegans* and the amphibian *Xenopus laevis* proceeds to produce smaller and smaller cells [9–11]. Strong relationships between overall size and global organismal features, such as body proportion and metabolic rate, have been a central feature of comparative biology for more than one hundred years [12]. It will be interesting to see whether the scaling relationship observed here is simply an interesting hypothesis that appears to fit data from a particular group of nematodes or whether it is indeed a general rule that explains the structure of the mitotic spindle across all animals. Application of similar methods will also open up other areas of cell biology to similar questions about patterns of organelle variation and evolution, such as the distribution and abundance of mitochondria, Golgi and endoplasmic reticulum. Advances in automated subcellular microscopy pioneered by Farhadifar *et al.* [5], in addition to work from a number of other groups [13], now make it possible to conduct the high-precision, high-throughput analysis needed to examine a large number of specimens from many different species. Their approach of combining an extensive collection of cell structure features with a rigorous evolutionary analytical framework points toward a new unified approach for addressing many long-standing questions in cell biology. In this way, this work is a harbinger of what is sure to be an exciting new era marking the emergence of evolutionary cell biology as a proper field of study [2].

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Institute of Ecology and Evolution, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA.
*E-mail: pphil@uoregon.edu

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Life History Evolution: What Does a Menopausal Killer Whale Do?

Menopause evolved in humans and whales, presumably because older females can help their kin. But how do they help? New research shows that post-menopausal female killer whales lead foraging groups. This leadership is most significant when food is scarce.

Hal Whitehead

Menopause, in which human females routinely live a substantial part of their lives after last giving birth, even following their ability to give birth, is an evolutionary puzzle [1]. If the currency of evolution, fitness, is the number of genes passed to subsequent generations, menopause appears to be a "Darwinian abdication" [2]. Even though reproduction may become increasingly problematic with age, as it does for most female mammals, surely they should try? The evolutionary conundrum is avoided if females rarely lived beyond about age 45 during most of human evolution: there would be no selective pressure for reproduction at older ages [3]. However, this "menopause as an epiphenomenon" hypothesis is countered by the presence of numbers of elderly women in societies without the survival benefits of modern societies, such as plumbing, democracy and health care [4]. Even more damning is the presence of menopause in non-human animals, such as killer whales and long-finned pilot whales. Like human women, female killer and sort-finned pilot whales stop reproducing in their early forties, but may live into their eighties [5,6]. So, menopause is a true evolutionary puzzle, and a broader one than had originally been thought. In recent years explanations for menopause have focussed on what have been called the 'mother hypothesis' [1] and the 'grandmother hypothesis' [7] — that older females help their children and grandchildren, respectively, and that this help outweighs the potential for reproduction when older. But how do they help? In this issue of