JULS

Perspectives

Population effects of chiral snail shell development relate handedness to health and disease

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Abstract

The spiral patterns of snail shells exhibit chirality, or "handedness." These patterns often heavily favor the dextral (right-handed, clockwise) over the sinistral (left-handed, counter-clockwise) phenotype. While the developmental pathways resulting in each enantiomorph (non-superimposable mirror image form) have been studied extensively, there has been limited investigation into how the emphasis on one spiral direction over the other may confer survival benefit. This perspective essay proposes that developmental events determining cell cleavage robustness, mating compatibility, and predator evasion can influence the distribution of dextral and sinistral snails. The connection between chirality and survivability has broader implications for exploring the role of handedness in diseases and their treatments.

Introduction

Just as a human can be right- or left-handed, the shell chirality of a snail such as Lymnaea (L.) stagnalis can be either dextral (coiling clockwise) or sinistral (coiling counter-clockwise).[1] The determining genetic factor of L. stagnalis shell chirality has been isolated to a single maternal gene, Lsdia1, which controls the expression of the actin-related Lsdia1 protein.[2] L. stagnalis populations are generally 98% dextral with the dominant allele, compared to 2% sinistral with the recessive allele.[2] However, this distribution of enantiomorphs does not follow the principles of basic Mendelian inheritance.[3]

The genetic lineage of a snail shell's chirality is difficult to trace. This is partly because they are hermaphrodites, meaning they have both male and

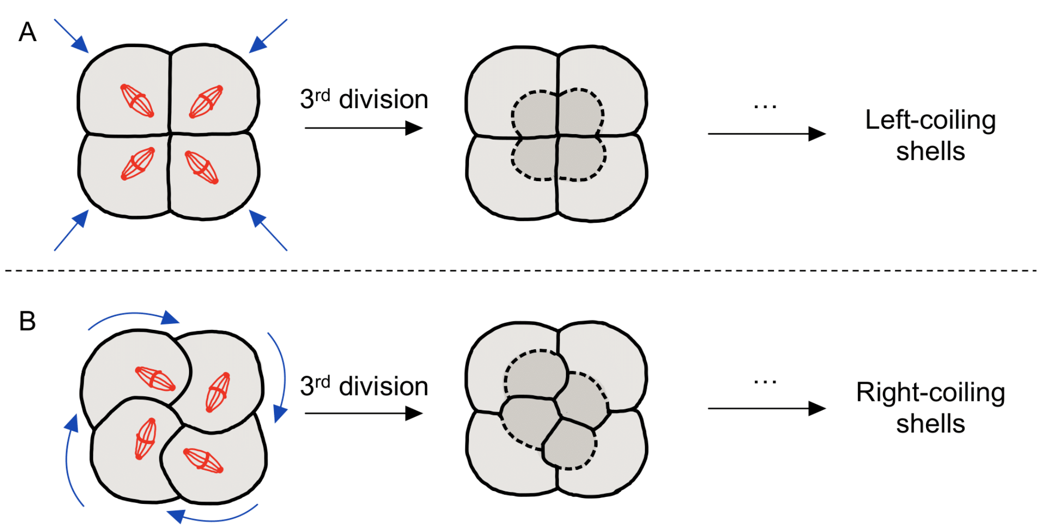

Figure 1. The third cell division. Cleavage from four to eight cells is distinctly different in sinistral and dextral embryos. (A) In sinistral embryos, the blastomeres remain neutral, limiting shell development. (B) In dextral embryos, chirality is determined by the rotation of the macromeres to form the spiral pattern. Blue arrows indicate the direction of micromere budding.

female reproductive organs and individuals can sometimes reproduce on their own.[2] Furthermore, the chiral phenotype is determined solely by the mother's genotype.[1,2] This means a homozygous recessive mother will have sinistral offspring with a homozygous dominant father. The sinistral offspring will have the heterozygous genotype and will have purely dextral offspring of their own.[2] These factors make it challenging to predict population variation.

However, the chiral distribution in different snail species varies greatly, with some species being 100% dextral or even 100% sinistral with many different distributions in between.[4] Additionally, the distributions of the same species in different geographic locations can be different. A 1917 sampling of L. stagnalis shells in Luga, Russia found a small population of snails with predominantly sinistral shells.[5] This suggests that there are additional factors that may be species-specific or environment-related that determine the distribution of dextral and sinistral snails, such as in the case of L. stagnalis.

Late Rotation in Sinistral Cleavage Hinders Development

The chiral phenotyping seen in adult gastropods is driven by the Nodal pathway, whereby the Nodal signaling molecule, a ligand in the TGF-β superfamily, acts on the Pitx1 transcription factor to elucidate left-right asymmetry.[6] The handedness generated by the Nodal pathway depends on the localization of Nodal and Pitx expression in the embryo. Grande and Patel (2009) showed, using whole-mount in situ hybridization for trochophore-stage embryos, that dextral species had Nodal and Pitx mRNA localization on the right side of the embryo, while this characteristic was flipped in sinistral species.[7]

These differential mRNA localizations for right- and left-handed strains of L. stagnalis are determined by their respective spiral cleavage patterns, beginning upstream of the Nodal pathway at the third cell division.[8] Spiral cleavage involves the budding of new micromeres (smaller blastomeres) off of the animal poles of the larger macromeres. In dextral snails, rotation of the micromeres to form a spiral pattern occurs simultaneously to cleavage—blastomeres undergo spiral deformation and mitotic spindle inclination in the clockwise direction.[9] In sinistral snails, blastomeres remain neutral and spindles remain radially directed, with rotation only occurring very late at the furrow ingression stage of cleavage.[9] This difference is shown in Figure 1.

The difference in cleavage patterns provides a first explanation for the strong tendency toward dextral chirality in L. stagnalis. Kuroda et al. (2014) showed that sinistral micromeres were less likely than their dextral counterparts to complete the full 45° rotation (54% of sinistral embryos, 100% of dextral embryos).[10] The incomplete micromere rotation, characterized by partial-counterclockwise, neutral, or partial-clockwise movement, resulted in a 53% survival rate, while 90% of the dextral embryos developed into healthy juveniles.[10] The late determination of cleavage direction in the sinistral blastula during the third cell division makes further development less robust, and more susceptible to adverse environmental effects. This "incomplete" morphology may also decrease survival to adulthood by influencing the mechanics and rate of success of egg hatching.[11]

Sinistral Morphology is Caused by a Rare Frameshift Mutation

The weakness of sinistral spiral cleavage as seemingly a loss-of-function phenotype so early in embryonic development suggests that genetic factors may be involved as well. Indeed, a homolog of the gene encoding diaphanous formin, Lsdia1, has been identified in L. stagnalis as the single, maternally determined gene dictating chirality.[12] In fact, Davison et al. (2016) inhibited formin activity in dextral L. stagnalis using the SMIFH2 drug and found that micromeres emerged neutrally during the third cleavage, characteristic of sinistral embryos.[13] Lsdia1 catalyzes formation of actin filaments and stabilizes microtubules. Actin plays an integral role in blastomere deformation and cytokinesis, and microtubules are responsible for the spindle inclination that occurs in dextral embryos. Thus, Lsdia1 is essential for the development of dextral morphology.

Considerable empirical evidence supports the hypothesis that the stark sinistral minority can be explained by a genetic mutation. Bacterial artificial chromosome cloning and sequencing was used to show that a single nucleotide deletion in exon 3 of Lsdia1 for sinistral (and not dextral) snails leads to a premature stop codon in its mRNA, causing a loss of the aforementioned formin functionality.[12] Quantitative polymerase chain reaction and western blotting showed that while Lsdia1 was present in dextral zygotes and slowly decreased throughout cleavage, it was never detectable in sinistral zygotes.[12] The results of CRISPR-Cas9 gene-knockout targeting Lsdia1 corroborated these findings. Regardless of their phenotype, L. stagnalis mothers that underwent biallelic frameshift mutations produced sinistral offspring, while mothers with mutations where at least one allele was either non-mutated or had a non-frameshift mutation produced dextral offspring.[14]

The results of these experiments with Lsdia1, which characterize sinistral snails as having rare mutations in an otherwise completely dextral genome, further explain the scarcity of sinistral L. stagnalis in the wild. However, the molecular mechanism by which LsDia1 drives spiral deformation and spindle inclination to

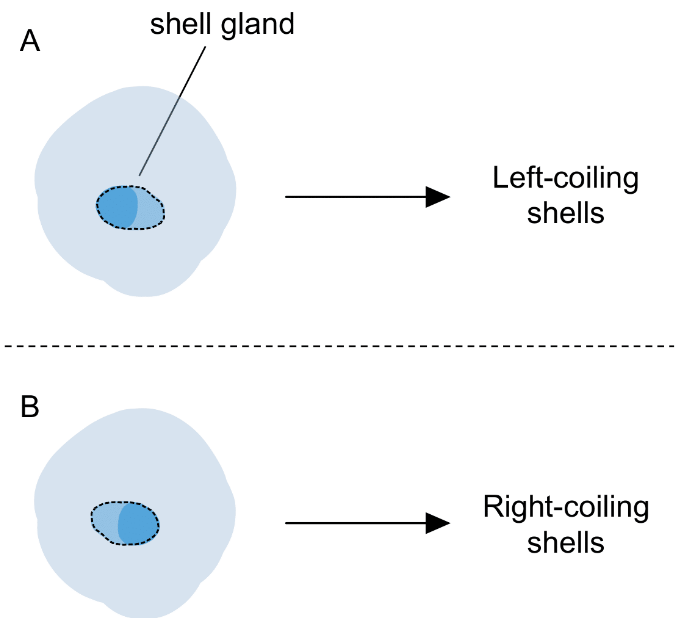

Figure 2. Expression patterns of Lsdpp in the late trochophore stage. (A) Expression of Lsdpp in the shell gland of the sinistral embryo is localized to the left, and (B) expression of Lsdpp in the shell gland of the dextral embryo is localized to the right. Diagrams adapted from the in situ hybridization experiments in Shimizu et al.[4]

cause dextrality is still unknown.[12] Nevertheless, the findings from the CRISPR-Cas9 application confirm the dominant-recessive relationship and the maternal effect, providing a baseline understanding for why dextral L. stagnalis are so pervasive.

Shell Chirality Influences Mating Compatibility and Success

Chirality has a considerable effect on mating behavior in L. stagnalis and gastropods in general. The handedness, position, and size of a snail's shell make mating with a snail of the opposite chirality extremely difficult.[15] This means that the rare sinistral snails are largely limited to mating with other sinistral snails in the wild, which could be a significant factor in explaining the dextral-favoring population distribution of L. stagnalis. Understanding the morphogenetic mechanisms behind chiral shell formation provides a better understanding of why inter-chiral copulation is so uncommon.

Shell development begins early in gastrulation, with a first invagination occurring at just 27 hours post first cleavage.[16] This causes the ectodermal and endodermal layers to come into contact, and subsequently begin differentiation into the shell gland that will secrete the shell matrix proteins.[16] In the late trochophore stage of development, the shell gland constructs the mantle—a thin layer of tissue that forms the basis for shell growth into the veliger stage and beyond.[17] Mantle expansion and further shell development are directed by Lsdpp, a homolog of the Drosophila (D.) melanogaster wing-patterning morphogen gene dpp.[18] Shimuzu et al. (2013) found using in situ hybridization that Lsdpp expression is localized to the shell gland and that this expression forms a left-right asymmetric Lsdpp gradient dependent on the chirality of the embryo (see Figure 2).[17] Lsdpp not only promotes the secretion of shell matrix proteins to grow the mantle, but also "translates" the chirality formed during spiral cleavage into the chirality of the shell. This dual function was confirmed by observing that embryos exposed to dorsomorphin, a bone morphogenetic pathway inhibitor, developed into juveniles with either immature or non-coiling shells.[19]

For L. stagnalis to mate with an opposite-chirality partner, it must change the positioning with which it mounts to its partner's shell.[20] This behavioral alteration is highly unfavorable. In a study of Partula suturalis, a dimorphic snail species with shell morphology similar to that of L. stagnalis, only 3 of the 26 interchiral pairs observed successfully copulated.[21] It is clear that shell chirality, which is determined by a highly-specialized gastrulation and morphogenesis pathway, has an immense influence on L. stagnalis mating, and thus the population distribution of dextral and sinistral strains.

Sinistral Coiling Produces an Advantageous Shell Morphology for Predator Aversion

Sinistral L. stagnalis are disadvantaged in early development and mating, so why does the 2% sinistral population remain at all? It may be that predators have specialized to better hunt the more common dextral population.[22] In laboratory experiments of snake attacks in Satsuma and B. similaris snails, sinistral Satsuma snails had an 87% survival rate compared to a 0% survival rate in dextral snails.[23] Sinistral B. similaris snails had a survival rate 5 times higher than their dextral counterparts. It was found that snakes that predominantly hunt snails attack in specific orientations and have even developed asymmetrical mandibular tooth numbers to favor attacking dextral snails.[22] Analysis of worldwide snail populations have found a strong coexistence of sinistral flat snails and snail-eating snakes.[22]

In L. stagnalis, sinistral snails may benefit from similar predator speciation favoring dextral snails. While L. stagnalis has a variety of predators, its shell morphology suggests significantly better protection from attacks on one flank.[23,24] Furthermore, the sinistral L. stagnalis shell is not just a mirrored copy of the dextral one. Dextral shell whorls, or coils, are translated further along the coiling axis while sinistral shell whorls translate and expand the last whorl.[1] This leads to a shorter, stubbier shell in sinistral shells by up to 10%. A shorter shell with an expanded final whorl means a wider shell opening. As is evident in the Satsuma and B. similaris snails, a predator cannot kill a sinistral snail unless the shell aperture (or opening) is small enough for the mandible to enclose it. This relative advantage of sinistrality may be greater in species with larger shells, such as L. stagnalis.[23,25]

While shell chirality is determined at the third cleavage, dextral and sinistral embryos are not mirrored images at this stage (Figure 1). The sinistral embryos lag significantly behind their dextral counterparts in rotation development. Continuous rotation during development forms the shell whorls and increasing the number of whorls grows the snail's shell at the aperture.[26] The speed of dextral development leads to faster whorl development down the coiling axis, resulting in the longer translation.[1,10] The lag of the sinistral rotation leads to the shorter and stubbier physiology.[10] Although slower development causes lower embryonic survival rates, the different shell physiology may improve a sinistral snail's rate of survival from predation.[23]

Conclusions and Connections

Understanding why the dextral strain of L. stagnalis is so prevalent in the wild is a puzzle that can be pieced together by considering the contributions of chirality in both genetic and epigenetic factors. For example, the relative robustness of spiral deformation and spindle inclination in dextral spiral cleavage explains why more dextral than sinistral embryos survive to adulthood. The identification of a single frameshift in a single gene as the cause of sinistrality demonstrates that left-handedness in snails is a rare mutation. Chirality-dependent factors that improve the survival of the species and the individual, such as compatibility in mating and specialized predator evasion, also affect the population distribution.

This investigation has greater significance in developmental and evolutionary biology because most animals, including humans, develop asymmetrically. As such, the hypothesis that the chirality in embryonic development leads to preferential survival of certain population subsets need not be limited to snails as a model organism. For example, while D. melanogaster embryos do not undergo spiral cleavage, chirality plays a crucial role in male genital orientation, and thus mating and reproduction. Drosophila male genitalia undergo a 360° clockwise (dextral) rotation to ensure that the copulatory organ is oriented to facilitate the male-above mating position.[27] Mutants for the Myo31DF gene exhibit counterclockwise (sinistral) rotation.[27] While a full 360° sinistral rotation would likely be harmless, the rotation of the genitalia in Myo31DF mutant flies can be prematurely stopped, causing angle deviations that make them incompatible with the female organ.[27] This greatly reduces, and in some cases completely eliminates, reproductive success.[27] Chirality is also relevant to the understanding of human health and disease, as the locations of the visceral organs are highly asymmetric. Accounting for "chiromorphogenesis" as coined by R. Kuroda (2014) may shed light on enigmatic disorders such as situs inversus, where the locations of a patient's visceral organs are mirrored as compared to those in healthy people, and could help investigate the highly-debated relationships between human asymmetry, developmental homeostasis, and adult health outcomes.[28]

There are likely many other factors contributing to chiral distribution such as food sources, geographical factors, and environmental factors. Accounting for the external factors that contribute to asymmetry transforms the study of chirality from a niche domain into one that contributes more broadly to understanding health and disease.

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COMPETING INTERESTS

No competing interests declared.

Received: April 3, 2022
Accepted: November 30, 2022
Published online: December 26, 2022

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