

Running head: Organized growth of chiton eyes

**Continuous and regular expansion of a distributed visual system in the eyed
chiton *Tonicia lebruni* Rochebrune, 1884**

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Abstract

Chitons have a distinctive armature of eight articulating dorsal shells. In all living species, the shell valves are covered by a dense array of sensory pores called aesthetes, but in some taxa, a subset of these are elaborated into lensed eyes, which are capable of spatial vision. We collected a complete ontogenetic series of the eyed chiton *Tonicia lebruni* to examine the growth of this visual network, and found that it expands continuously as eyes are added at the margin during shell growth. Our dataset ranged from a 2.58 mm juvenile with only 16 eyes to adults of 25-31 mm with up to 557 eyes each. This allowed us to investigate the organization and potential constraints therein of these sensory structures and their development. Chiton eyes are constrained to a narrowly defined region of the shell, and data from *T. lebruni* indicate they are arranged roughly bilaterally symmetrically. We found deviations from symmetry of up to 10%, similar to irregularity reported in some other animals with multiplied eyes. Distances separating successive eyes indicate that, while shell growth slows during the life of an individual chiton, eyes are generated at regular time intervals. Although we could not identify a specific eye-producing tissue or organ, we propose that the generation of new eyes is controlled by a clock-like mechanism with a stable periodicity. The apparent regularity and organization of the chiton visual system is far greater than previously appreciated. This does not imply the integration of shell eyes to form composite images, but symmetry and regular organization could be equally beneficial to a highly duplicated system by ensuring even and comprehensive sampling of the total field of view.

Introduction

Vision has evolved independently many times across a wide range of animal lineages, in diverse clades such as vertebrates, molluscs, arthropods, and cnidarians that collectively contain around 96% of living species (Land and Fernald 1992). Although many of these lineages share a familiar pattern of paired, cephalic eyes, some of the more unusual visual systems can be found distributed more widely across part or all of the body surface. One such distributed system is found in polyplacophoran molluscs, chitons, which can have shell eyes numbering in the hundreds and entirely contained within the hard dorsal shells. All chitons possess an extensive system of innervated canals within their eight-part shell armature that open to the dorsal surface, but members of some families have elaborated parts of this network to form discrete light-sensing organs embedded within the shell (Schwabe, 2010).

Distributed visual systems present a variety of intriguing challenges for neuroethologists, particularly concerning the arrangement, integration and potential redundancy of many duplicated inputs. In some cases the number and organization of distributed visual structures is determinate and constrained; examples include the rhopalia of cubozoan jellyfish (Nilsson et al., 2005), the lateral eyes of arachnids (Miether et al., 2016), and the optic cushions of sea stars (Petie et al., 2016). Such systems are presumably controlled by stable and determinate developmental processes that produce a 'final' adult morphology and underlying neural architecture during ontogeny. However, in other systems this problem is compounded by the continual

63 growth and addition of eyes to an existing adult network. Such systems tend to involve
64 higher numbers of eyes with less regular arrangements, as seen in scallops (Butcher,
65 1930; Whoriskey et al., 2014), some fan worms (Del Pasqua et al., 2017), and eyed
66 chitons. Understanding how these continually expanding visual systems grow will shed
67 some much-needed light on their function, origins and developmental control. The
68 chiton visual system is additionally constrained by the rigid structure of the valves,
69 meaning that growth can only occur at the shell margins. Chitons thus present a unique
70 opportunity to study continual visual system expansion by providing a clear record of
71 past growth.

72
73 There are two broad types of pigmented photoreceptor structures that can occur in
74 chiton shells, but the terminology is often somewhat confused. All chitons have
75 aesthetes, a network of innervated pores that cover the entire surface of the valve. Shell
76 eyes and other pigmented aesthetes are only ever a small subset of this larger network
77 in any individual. Chiton “shell eyes” comprise a large chamber within the shell surface
78 covered by a convex, solid aragonite lens (Speiser et al., 2011: fig.4). These are image-
79 forming structures, and at least one species with shell eyes is capable of differentiating
80 dark shapes from uniform changes in brightness (Speiser et al., 2011). Shell eyes can
81 also be referred to as extrapigmented or extrapigmental aesthetes, but pigment occurs
82 both in the shell matrix surrounding the eye chamber, and within the aesthete tissue of
83 eye itself (Speiser et al., 2014). Shell eyes occur in only a few genera. By contrast,
84 intrapigmental aesthetes are less elaborated structures that have pigment only within
85 the aesthete body and no lens; these are found in several unrelated families (e.g.

Sturrock and Baxter, 1995: fig.1). These are described in vision literature as “eyespot” and it is unclear whether they facilitate spatial vision (Kingston et al., 2018). Terminology in systematic and morphological literature on chitons, however, has historically referred to these eyespots as “shell eyes”, and to shell eyes (extrapigmental aesthetes) as “ocelli” (Schwabe, 2010). Here, we use “shell eyes” to refer only to the more elaborated structures with aragonite lenses, and “eyespot” to describe intrapigmental aesthetes. This also prevents confusion with the trochophore “ocelli”, pigmented but non-homologous and probably non-image-forming structures that are prominent in chiton larvae (Fischer, 1980; Sumner-Rooney and Sigwart, 2015).

In all chiton species that have eyes or eyespots, these structures are limited to a constrained distribution on the valves (Fernandez et al., 2007; Schwabe, 2010). They do not cover the whole dorsal surface, but are restricted to the shell diagonals on intermediate valves (II-VII, Figure 1), sometimes extending distally on the lateral areas, and they form rays on the head and posterior (postmucronal) area of the tail valve that may be irregular or coalescing. There may be one or several rays of eyes on each valve, but their distribution lies within a constrained area of the shell. Eyespots (intrapigmental aesthetes) may be more widespread than eyes, but they also follow the same distinctive distribution on the lateral areas and terminal valves (Schwabe, 2010). The constrained distribution and continual addition of eyes is relevant to understanding how they function as a visual system but has not been addressed in literature on animal vision.

The aims of the present study were to use a chiton with shell eyes, *Tonicia lebruni* de Rochebrune, 1884, to investigate the growth and allometry of this unique visual system. *Tonicia lebruni* represents an exceptional model organism for this study, because it broods its young until they have fully formed shells and are competent to settle and live independently (Figure 1A; Sirenko, 2006; Sirenko, 2015; Ituarte and Arellano, 2016). The opportunity to examine late stage brooding embryos, and the known timing of release of competent juveniles, enabled us to determine the size and stage at which eyes first appear during growth. *Tonicia lebruni* also has a relatively narrowly constrained distribution of shell eyes, with a single row on each side of the intermediate valves, providing an opportunity to comprehensively examine the spatial distribution of eyes on the shell armature (Figure 1B). We used a complete ontogenetic series of post-settlement individuals to determine whether all eyes are retained for the lifetime of individuals, whether rates of addition vary through growth, and whether the visual system is bilaterally symmetrical. Differing rates of eye addition during growth could produce three measurable outcomes; where eyes are added at regular intervals, with increasing frequency over time, or with decreasing frequency over time. With constant shell growth, this would result in regular, decreasing, or increasing spacing between consecutive eyes, respectively. All this information will help us better understand the evolution, integration, and functions of distributed vision.

Methods

Specimens of *Tonicia lebruni* were collected intertidally in the Falkland Islands in September 2016 under research license agreement granted to JDS by the Falkland Islands Government. Material was collected at three sites: Cape Pembroke (51° 40' 50" S, 57° 43' 23" W) and Hooker's Point (51° 41' 57" S, 57° 46' 41" W) near Stanley, and Bleaker Island (52° 12' 54.6" S, 58° 51' 16.662" W). Specimens were held flat during fixation and preserved in 70-90% ethanol. This included brooding females with embryos that had partially to fully developed shell fields. All specimens are deposited in the California Academy of Sciences (CASIZ) collections. Additional specimens were collected from near Hooker's Point in June 2019 and fixed for histological sectioning, but were not included in allometric analysis.

Body length measurements were taken from flat, preserved specimens. All specimens were examined under an Olympus SZX12 stereo microscope fitted with a trinocular camera attachment, and photographed with an Olympus E-M5 Mark II digital camera. Eyes were counted on 51 specimens to determine the number of eyes to the left and right of the animal midline, on each valve. Eyes were easily identified from the presence of both pigmentation and the characteristic lens (*Tonicia lebruni* and closely related species do not have eyespots, which lack the lens). To examine the allometry of eye addition during growth, we measured the radial distance from the posterior apex (the 'beak') of the valve to the centre of each eye on the right side of valve III. Valve III was chosen for measurement as valves I and VIII are the terminal head and tail valves, valve II is elongated to protect the radula, and valves III-VII are generally considered equivalent iterated units (Connors et al., 2012, Ibáñez et al., 2018). Our data on

allometry were limited to smaller specimens, with fewer than 20 eyes per side, per valve, to avoid measurement confounds from the curvature of the valve. The distance to the first (most proximal, oldest) eyes over the ontogenetic series was used to investigate whether eyes are retained during continuous growth. Depending on specimen size, measurements were either recorded directly with Vernier callipers or, for most specimens, measured from photographs using FIJI (Schindelin et al., 2012). Instances where two adjacent eyes formed a fused double-eye were counted as two eyes for total counts, where they could be identified, but were considered one point in measuring radial distances. Double-eyes represent potentially two points in a visual field, but all available evidence suggests they represent a single growth event. Relationships of morphometric parameters were examined using ordinary least squares regressions on raw or transformed data to calculate best fit and confidence intervals, implemented in R (R core team, 2019; Wickham, 2016).

One specimen was fixed in 4% paraformaldehyde for histological sectioning, and subsequently transferred to phosphate-buffered saline. A section of tissue was excised including a shell fragment with multiple complete eyes. Decalcification, resin embedding, sectioning, staining and image capture followed standard protocols (Ruthensteiner, 2008; Sigwart et al., 2014). The specimen was sectioned at 1.5 μm and stained with high contrast monochromatic Richardson's stain. Slides were studied using an Olympus BX41 compound microscope fitted with a trinocular camera attachment, and photographed with an Olympus E-M5 Mark II digital camera. The pigmented region

around the eye structure was identified in sections using color thresholding in Adobe Photoshop (release 21.0.1, 2019).

Results

Our ontogenetic series of post-settlement individuals represented the full range of post-settlement sizes for this species (2-31 mm). Embryos and early stage juveniles held in the pallial cavity of the mother and at the point of release had no shell eyes (Fig 1A). The smallest individual with shell eyes was a juvenile (2.48 mm body length) that had 16 eyes in total (Fig 1B). The largest specimen included in this analysis (31.32 mm length) had 399 eyes in total, the largest number of eyes was 557 eyes in a specimen 26.33 mm long. The eyes are added continually but in restricted zones of the shell valves (Fig 1C,D).

Tonicia lebruni is highly color polymorphic (Ibáñez et al., 2019), with individuals ranging in color from brown to pink to green and with variable patterns of stripes; however, we did not informally observe any correlation between variation in color and the distribution or number of eyes.

Symmetry

Shell eyes are distributed roughly equally on the left and right sides of the body; although there is some deviation, this is maintained throughout growth (Fig 2A). The

data showing percentage deviation exclude the smallest specimen, which had 6 eyes on the left side and 10 eyes on the right side or a deviation of 25%. Deviations from symmetry in eye number are consistent across the different valves (I-VIII) of the chiton (Fig 2B).

In considering symmetry, we only refer to the total number of eyes on the left and right sides of a valve or the total body, not their fine-scale distribution or arrangement. Individual chitons that showed asymmetry in one valve may exhibit more regularity in the adjacent valve (e.g. Fig1B). The median number of eyes on left and right is close to symmetric in all valves (Fig 2B), but there are more frequent deviations in the terminal valves, especially the head valve, most likely because this also contains the largest number of eyes in larger animals (Fig 1).

The distribution of eyes is generally confined to the shell diagonal in lateral valves, and to the ventral rays associated with insertion slits in terminal valves. The shell slit formula for *T. lebruni* is 8–9/1/9–12 meaning that there may be 8 or 9 slits, and therefore 8-9 main rays of eyes, in the head valve, and 9-12 in the tail valve, but generally only one ray on each side of the intermediate valves. The rays are usually difficult to distinguish in terminal valves, and this variability is unlikely to have a significant influence on overall symmetry of eyes counted on either side of the body midline. Although eyes were largely confined to single diagonal rays, deviations were observed where they spread out to occupy a laterally-expanding wedge of an intermediate valve (e.g. Fig. 3A, left

hand side). Individual eyes were found outside the constrained distribution in only 2 valves among 51 specimens (Fig. 3A, B).

Eye structure and pigmentation

Eyes are of consistent diameter within and among individual chitons; they do not increase in size with body growth. Eyes were occasionally seen to be fused (Fig. 3B) and in this case were counted as two eyes for symmetry and count data, although they may share a single subsurface chamber (Sigwart, in prep.). The eye is formed of a large asymmetrical chamber covered by an ovate transparent lens that is contiguous with the dorsal layer of the shell, in line with previous studies (e.g. Speiser et al. 2011: fig. 1, Plate 1897: fig. 121). A large pigment halo surrounds the eye within the shell matrix (Fig. 1, 3B, 4). The chamber is approximately 45 μm in diameter, measured from direct visual inspection of slides with an ocular micrometer and calibrated digital images, while the pigmented area extends laterally around the chamber, in a region of 65-70 μm in diameter based on persistence through serial sections. The pigment halo is not symmetrically distributed but is predominantly on the proximal side of the eye chamber, i.e. this pigment field would be added to the growing shell before the eye chamber itself (Fig 4).

Rates of eye addition

The total number of eyes on an individual chiton increases approximately linearly in proportion to body size (Fig 5A; $R^2 = 0.718$, $p \ll 0.005$). The distance separating the valve posterior apex and the first (earliest) eye increases slightly with body size; however a linear increase is a poor approximation of this correlation (Fig 5B; $R^2 = 0.239$, $p = 0.0002$). We also measured the interspace between the first and second eye on a subset including the smallest and largest specimens, and found no correlation between valve width and the interspace distance ($R^2 = 0.261$, $p = 0.254$). The distance between the apex and the first eye increases very slightly over ontogeny, with shell material being added slowly to the posterior margin, but the distance between the first and second eye does not increase across specimens of increasing sizes. In a geometric morphometric sense, it should be noted that the shell apex is clearly a Type II landmark, with a clearly defined position that is homologous across specimens based on geometry, but that nonetheless shifts over ontogeny. The homology of any given eye on one specimen cannot be assumed.

We considered three potential hypotheses that might be appropriate models for the addition of shell eyes during growth: in a model where the shell expands outward at a constant rate, eyes might be added at regular intervals, with increasing frequency over time, or with decreasing frequency over time. Each of these three scenarios would produce a different pattern of the distances separating eyes along the diagonal of intermediate valves or the rays on terminal valves (Fig 6A). Accelerating eye production relative to shell growth would result in eyes being increasingly closer together, and a power relationship would describe the sequence of radial distances from posterior apex

to each successive eye. Conversely, declining rate of eye production relative to shell growth would cause eyes to be increasingly further separated as the shell expands and hence radial distances to each eye would increase in approximately exponential fashion. In *T. lebruni*, radial distances of sequential eyes combined from 34 individuals were equally well described by a linear ($R^2 = 0.802$) or power relationship with an exponent of approximately 0.647 ($R^2 = 0.797$) (Fig 6B). Based on the coefficient of determination (R^2) in ordinary least-squares regressions for each separate specimen, most individuals had a stronger fit to the accelerating (power) model (n=20) but a large number had a stronger or equivocal fit to the regular (linear) model (n=14). These models assume sustained regular shell growth; if eyes were produced at regular intervals when shell growth is slowed, that would also produce the pattern where the rate of eye production is increasing relative to shell growth.

Discussion

The distinctive distribution patterns of shell eyes along the valve diagonals in chitons were noted from the very first time the structures were reported (Moseley, 1884). These structures are distinct from the larger network of aesthetes (also visible in Fig. 4), which were recognized earlier (Marshall 1869). It is remarkable that the recognition of shell eyes came so late in the long history of conchology, especially given that detailed shell features were used as the main basis for chiton systematics. The original description speculated that shell eyes had been overlooked because: they do not occur in any European species, the eyes are not very distinct in dried shell specimens, and “a

molluscan shell is, moreover, almost the last place in which the naturalist would expect to find eyes” (Moseley, 1885: p. 38).

These observations of *T. lebruni* confirm that individuals retain all their eyes during their adult life, and that the eye network is continually expanded over post-larval growth. The shell valves of chitons expand continuously, on multiple fronts, as all valves expand laterally and anteriorly as the animal grows. The posterior apex also expands, but at a lower rate than the main lateral extension. The dorsal shell layer, the tegmentum, folds over the apex to create a ventral callus or mixoperipheral fold which expands throughout ontogeny (Sigwart & Sutton, 2007; Kingston et al., 2019).

The overall distribution of eyes is constrained to the valve diagonals (and additional radiating lines in the head and tail valves), and animals and their valves are roughly bilaterally symmetrical in terms of the numbers of eyes. The arrangement of shell eyes is described as “random” in taxonomic descriptions of some species, where the general constraint on distribution is already well understood by specialists (e.g. Ibáñez et al., 2019). Even in *T. lebruni*, which typically has a single row per side on each valve, there are minor irregularities that seem to be sporadic, frequent, and inconsistent among specimens and even among valves on a single individual. This pattern has been illustrated in other species (e.g. in shell eyes in *Onithochiton neglectus* de Rochebrune, 1881; Boyle 1969: fig 2). Double eyes were found relatively frequently; we did not quantify their occurrence, as they are too subtle to be visible without significant magnification, yet there are at least two double-eyes on the specimen selected to

312 illustrate the regular nature of the eye network (Fig. 1C, left side of valves VI and VII).
313 Despite these irregularities in the individual components, patterns emerge of a
314 bilaterally symmetric, regularly arranged sensory network.

315

316 We found the strongest statistical support for a model where eyes are closer together
317 toward the distal edge of the shell, a pattern that could be explained by two hypotheses.
318 These are that (1) shell growth is constant, but the rate of production of eyes increases
319 over time or (2) the rate of shell growth decreases with total size, but the production of
320 eyes occurs at regular intervals in time, as if regulated by a constant clock. While
321 chitons, and many marine invertebrates, have continuous growth in terms of volume,
322 their linear dimensions tend to fit asymptotic growth models (Lord and Shank 2012).
323 Long-term growth studies in chitons are relatively sparse; however, existing work on
324 multiple species (not including *Tonicia*) indicate that growth rates, and specifically the
325 rate of valve expansion, decrease with age (Booolootian, 1964; Baxter and Jones, 1978;
326 Baxter, 1982; Jones and Crisp, 1985; Lord, 2012). This also explains why our results
327 are equivocal between eye production appearing constant, or increasing relative to shell
328 growth. Our dataset was limited to smaller individuals under 20 mm length, compared to
329 the maximum body size of < 40 mm for the species (Ibáñez et al., 2019), but
330 nonetheless included animals that would have experienced the flattening of asymptotic
331 growth. Shell layers are regular in earlier parts of the valve but become more dense
332 toward the distal margin of older animals (Lord, 2012). This suggests that the production
333 of eyes may be constant, even if the lateral expansion of the valve slows over time.

Given that chiton shell growth is constant or slightly slowing, the generation of new eyes is apparently controlled by a clock-like mechanism with a stable periodicity.

We examined the secretory tissues at the terminus of the shell diagonal through histological sections and did not find anything that morphologically differentiates mantle tissue in this eye-producing region from other parts of the shell field. The ability to generate the components of a chiton shell eye apparently does not require any anatomically specialized tissue or organ. Recent work on molluscan genomics has highlighted the ability of molluscs to co-opt conserved genes to create novel structures (Sun et al., 2020), and the shell eye may be an excellent example of this. The eye itself is an enlarged aesthete chamber, and no discrete tissue or organ has been identified as the site of production of non-eye aesthetes, either. Aesthetes occur over the whole shell surface and grow on the full perimeter of the lateral and anterior shell margins. The lens, a key distinguishing feature of the eye, is structurally identical to the surrounding aragonite valve (Speiser et al., 2011). Thus, many of the necessary components and pathways to build shell eyes are likely to be available throughout the shell field. In the apparent absence of a morphologically distinct eye-generating region, the potential ability to produce eyes may also be widespread in the shell field, but only enacted in a restricted region around the valve diagonal. This reduced specificity would be concordant with the slight irregularity and asymmetry that characterizes the fine details of the distribution of eyes on an individual chiton. However, the control mechanism remains unknown.

357 Although the general structure of individual shell eyes has been known for over 100
358 years, detailed studies for most species are still lacking. The ‘halo’ of pigment
359 surrounding the shell eyes is typically visible through the transparent outermost layer of
360 solid shell tegmentum (Schwabe, 2010). Previous work on *Acanthopleura granulata*
361 (Gmelin, 1791) identified the pigment in the aesthete tissue and shell matrix around the
362 chamber of shell eyes as pheomelanin, which is presumably used as a visual screening
363 pigment (Speiser et al., 2014). Whether the same pigment is used in *T. lebruni* remains
364 unconfirmed, but the eye chamber anatomy is strongly similar; the pigment, and new
365 results here on growth, likely apply equally to Acanthopleurinae and Toniciinae. In *T.*
366 *lebruni* at least, this surrounding extracellular pigment was not evenly distributed and is
367 predominantly found on the proximal side of the eye chamber, preceding the growth
368 front (Fig. 4). This implies that the production of new eyes in the mantle tissue must
369 begin by laying down pigment in the appropriate position of the aragonite shell matrix,
370 followed by the initiation of the eye chamber and lens, providing another potential clue
371 to eye production. This pigment cannot have come from within the eye, because it is
372 consistently distributed in eyes across the shell (effectively a time series of eye
373 production) and the “cloud” of pigment in the shell matrix around the eye chamber is not
374 distributed in a way that aligns with remaining intracellular pigment within the aesthete
375 tissue (Fig. 4). From the dorsal side, the raised lens is surrounded by an apparently
376 symmetrical and complete ring of pigment (Fig. 3B), but from sections this layer is
377 apparently of variable depth and mainly on the proximal side, while the ventral
378 extension of the aesthete canal is on the distal side of the eye chamber. In specimens
379 with pale valves, it is also possible to see dark streaks emanating from the individual

eyes on the distal side (e.g. Fig.1C, 3A). This may be coincidental shell pigment unrelated to the screening pigment, but more likely it reflects additional screening pigment that occurs in the aesthete tissue that extends below the eye chamber toward the shell margin. Such pigmented extensions in the direction of the shell margin can be seen clearly in decalcified valves (illustrated in Speiser et al., 2014: fig. 1A).

Further comparisons between *T. lebruni* and existing work, largely concerning members of the subfamily Acanthopleurinae (Speiser et al. 2011; Currie, 1989), should account for the distinct differences in their visual environments. *Acanthopleura* and related species are largely of tropical and subtropical distribution, where high light intensity and relatively clear waters likely provide an abundance of visual information and facilitate, for example, the detection of overhead shadows. By contrast, *T. lebruni* (in the sister subfamily Toniciinae) inhabits choppy sub-Antarctic to temperate waters with poor visibility (Sirenko ,2006; Ibanez et al., 2019). Despite its presence in the intertidal and shallow waters, the availability of visual cues is likely to be starkly different from species at lower latitudes. Such differences can drive substantial divergence between visual systems, but the impacts of changing light environments also depend on a range of ecological, physiological and life-history traits (e.g. Sumner-Rooney 2018). It appears that the individual eyes of both subfamilies are similar, but there are organizational-level differences among acanthopleurine species in the aesthete networks around the eyes (Currie, 1989). There may be also be significant differences in their visual networks that we are yet to discover.

403 The constant expansion of the chiton shell eye array presents a potential challenge for
404 integrating and interpreting visual signals. Eyes are confined to well-defined regions,
405 increase steadily in number and are produced at a regular rate; the level of organization
406 of the chiton visual system is greater than has been previously appreciated. This
407 potentially has important implications for the function of the shell eyes and the feasibility
408 of signal integration between visual units across a valve or even a whole animal.

409

410 Whether chiton shell eyes act as independent visual units or form part of a network
411 somehow integrated across valves or whole animals remains unclear. Recent work
412 found higher than anticipated cephalization and nervous system diversity in chitons
413 (Sumner-Rooney and Sigwart 2018); however, it seems unlikely that chitons are
414 capable of the required computation to combine hundreds of visual inputs to form a
415 single or series of composite images. In *Acanthopleura granulata*, the eyes mediate
416 defensive behavior in response to overhead shadows (Speiser et al., 2011). The lenses
417 of the shell eyes appear to be individually capable of image formation, and therefore
418 they may act as independent but highly duplicated organs. Similar systems are thought
419 to facilitate shadow responses in sabellid and serpulid fanworms and ark clams
420 (Nilsson, 1994; Bok et al., 2016; Bok et al., 2017), which also appear to lack the large
421 associated neural centers we might expect to see if hundreds of images were being
422 combined. Although such high levels of duplication may superficially seem excessive in
423 the absence of sophisticated integration, there are many potential advantages to having
424 large numbers of eyes acting independently, including redundancy, coverage of a larger
425 field of view, and increased sampling of the visual environment. Regular and constant

expansion of the visual network in species with continuous growth could contribute to all three of these potential benefits.

In visual systems with such high levels of duplication, an element of redundancy is widely expected. Where eyes are vulnerable to loss via damage or occlusion, particularly where they facilitate crucial behaviors such as predator avoidance, replication mitigates the potential impacts. In chitons, the continual addition of shell eyes presumably reduces the impacts of epifaunal growth, damage and erosion of the valve surface. The specimens used here had no visible signs of erosion nor large epibionts, but the valve apex of chitons is frequently worn or broken, and in species such as *Acanthopleura* spp. this natural damage can sometimes remove the whole tegmentum layer, including eliminating eyes. Previous work has speculated on the potential secretory function of aesthetes, and a potential role in shell repair (Baxter et al., 1987), but there is no evidence to date that chitons are able to regenerate lost eyes.

The regular and symmetrical distribution of eyes may also be advantageous, even if chitons are not forming composite images. Firstly, the continual addition of eyes at regular intervals may be an efficient way to ensure comprehensive coverage of the global field of view as the animal grows. The presence of additional eye rows on the very curved head and tail valves also presumably improves coverage. Secondly, regularly spaced eyes may ensure even sampling within the total field of view. Speiser and colleagues (2011) estimated that the eyes of *Acanthopleura granulata* covered a 75° field of view, assuming the nodal point of the lens was at its centre. By a similar

estimate, the field of view of individual eyes of *T. lebruni* would be around 85°. Given that a single animal can have hundreds of eyes, it is likely that these fields of view, at least between adjacent eyes, overlap to some extent. As well as providing redundancy in the case of damage to individual eyes, this could also facilitate the detection of moving objects or the improvement of signal-to-noise ratios via relatively simple signal comparison or summation between neighboring eyes. This might be advantageous in visually noisy environments, where particulate matter detected by one eye could initiate an erroneous defensive response, for example. If instead, detection by two adjacent eyes was required to trigger a response, such false positives would decrease in frequency (Nilsson et al., 1994). If such local comparisons were to take place, regular spacing between eyes would ensure consistent levels of resampling of the total visual field.

Broad bilateral symmetry is seen in almost all visual systems, even including some distributed visual systems such as sabellid fanworms. Symmetry likely facilitates simpler signal comparison between left and right sides. The slight departures from complete symmetry we find in *T. lebruni*, however, are not unique and wouldn't necessarily be prohibitive to broad signal comparison between left and right sides of the body. Minor asymmetry in visual systems is not uncommon, particularly in those comprising many duplicated units. Scallops, scorpions and fanworms can all exhibit left-right asymmetry in eye number (Loria and Prendini, 2014; Whoriskey et al., 2014; Bok et al., 2016), for example. Although symmetry would benefit a complex integrated visual system, its presence is not evidence of this. It is unlikely that scallops and sabellid worms use eyes

to reconstruct composite images. Overall left-right signal comparisons between the lateral nerve cords innervating the aesthetes, eyespots and shell eyes could mediate orientation to visual stimuli, as observed in *Chiton tuberculatus* (Kingston et al., 2018). In scorpions, which exhibit complex visual behaviors such as navigation, one study found that left-right asymmetry in eye number affected 11% of examined individuals (Loria and Prendini, 2014), and that the maximum percentage deviation from symmetry was 20% (two ocelli on one side, and three on the other), similar to that observed in *T. lebruni*.

Tantalizingly, we can imagine what the benefits of regular distribution might be if chitons were indeed compiling visual information from across their valves; although we consider this an unlikely scenario, there is no functional evidence to disprove it. Regular or near-regular spacing of shell eyes, if they were to form ommatidium-like units of a larger network, would provide regular sampling across the field of view and thus simplify the reconstruction of one or more global images. In analogy to an insect compound eye, this would be equivalent to having regular facet diameters, or at least a regular pattern to facet diameters, and thus regular pixel size and spacing in the composite image.

The relative stability of the chiton visual system, owing to its integration into the resilient shell valves, make them an ideal model to study distributed vision and visual allometry in a continuously growing animal. From a comprehensive growth series of *Tonicia lebruni* we find that eyes are added throughout adult life, likely at regular time intervals, symmetrically and within a restricted region in all valves. A clock-like mechanism in a

local region of the shell field may be responsible for the regular production of shell eyes. The first stage of eye production is extracellular pigment laid in the shell matrix. While the lateral spread of the eye region expands over ontogeny, the distances between adjacent eyes in a series becomes smaller as the rate of shell growth slows in larger animals. This represents a greater level of organization than is commonly attributed to the chiton visual system, and could confer multiple benefits in terms of visual function, whether eyes act independently or as integrated units of a larger system.

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Figure captions

Figure 1. *Tonicia lebruni* is a polymorphic species with many shell colors from pink to brown to green. A. Late stage embryos brooded by a female (preserved specimen in Universidad Andres Bello, Santiago, Chile). B. Juvenile, length 2.48 mm, collected at Cape Pembroke, Falkland Islands. The right eyes on the intermediate valves are indicated with arrowheads. C. The rows of dark shell eyes are clearly visible on the pale areas of the valves in this adult specimen collected at Hooker's Point, Falkland Islands, length 17.07 mm. D. Schematic showing the arrangement of shell eyes on the valve diagonals of specimen shown in C. Shaded areas indicate the generalised region where shell eyes occur. This restricted arrangement is consistent in all chitons that possess shell eyes or pigmented aesthetes.

Figure 2. A. Symmetry of eye distribution in *T. lebruni* does not change with increasing body size; the deviation from symmetry is the difference in number of eyes on the right vs left side of the body, shown as a percentage of total eyes per individual for 51 specimens. B. The symmetry of eyes on each valve within the eight-part armature, shown as the numerical count of eyes that differ on the left or right side. In both panels, positive deviations indicate more eyes to the right, negative deviations are more eyes to the left.

Figure 3. A. Valves IV and III of a specimen from Cape Pembroke, length 22.62 mm, photograph and schematic showing the placement of eyes. Scale bar = 2 mm. This

includes one eye in valve IV that is outside the normal constrained distribution. B.

Photograph of 6 eyes from a larger row, including one fused double-eye. The additional regular aesthete pores can be seen over the whole shell surface between and around the eyes. Scale bar = 200 μm .

Figure 4. Section of a shell eye in *Tonicia lebruni*, from a 1.5 μm section. The lens is removed from the section due to decalcification of the shell matrix. The schematic to the right, of the same section, indicates the former position of the lens (dashed line) and shaded areas indicating pigmented areas. Scale bar applies to both A and B.

Figure 5. A. Correlation of body length and the total number of eyes per individual in *Tonicia lebruni*, B. Correlation of body length and the distance separating the shell posterior apex to the first (earliest) shell eye, as shown in inset schematic. Measurements were taken from the right side of valve III. Shading indicates the 95% confidence interval of the line of best fit.

Figure 6. A. Three potential hypotheses for the expected patterns of the radial distances from shell apex to the series of eyes on a single valve in *Tonicia lebruni*, if shell growth is constant and eyes are produced at an accelerating, constant, or slowing rate compared to the pace of shell growth. B. Radial distance from the posterior apex to consecutive eyes on the right hand side of valve III. Eye number 1 is the closest to the apex (oldest eye). Shading indicates the 95% confidence interval of the line of best fit.