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Cover Photograph: The cover is a collage of photos with the following caption: "Equinodermos de Venezuela: Parte superior *Tropiometra carinata* (Lamarck, 1816) *Linckia guildingi* Gray, 1840 *Ophioderma rubicunda* Lütken, 1856 Parte Media *Mellita quinquiesperforata latiambulacra* H. L. Clark, 1940 *Holothuria mexicana* Ludwig, 1875 Parte Inferior *Lytechinus variegatus* (Lamarck, 1816) Diadema antillarum (Philippi & Agassiz, 1863)"

Of particular note is the bottom left picture, which is captioned as follows: "A Variegated Sea Urchin at St. Lucie County Marine Center in Fort Pierce, St. Lucie County, Florida, U.S.A. © Hans Hillewaert - CCSA 4.0"

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Editorial Comment:
On behalf of the Alabama Academy of Science, I would like to express my gratitude and appreciation to the reviewers for their valuable contributions in reviewing the manuscripts of this issue.

Thanks!
Brian Toone
Editor: Alabama Academy of Science Journal

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Parental Dietary β -Carotene Intake in *Lytechinus variegatus* Affects Early Development of Offspring Exposed to UV Radiation

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ABSTRACT

Planktonic embryos of *Lytechinus variegatus* utilize photo-protective mechanisms to minimize the deleterious effects of solar ultraviolet radiation (UVR) exposure. It was hypothesized that embryos from parents who received supplemental β-carotene in their diets would be more resistant to the effects of UVR than embryos from parents who received no supplement. Adult *Lytechinus variegatus* broodstock with gonads in the growing phase were fed diets either with or without supplemental β-carotene for 5 months and subsequently induced to spawn. Fertilized eggs were collected from each feed treatment and exposed to differing intensities of UVA (0-4 J/m^2) or UVB (0-100 mJ/cm^2) radiation. Larval mortality counts and developmental status were recorded at 34 and 55 hours post-fertilization and compared between feed treatments. Embryos derived from sea urchins consuming supplemental β-carotene developed at a slower rate than those whose parents did not consume supplemental β-carotene. Increasing intensities of UVA and UVB radiation were positively correlated with larval mortality in both dietary treatments. UVB induced higher mortality than UVA. Larval mortality in the β-carotene supplemented treatment was significantly higher than the non-supplemented treatment. These data suggest that dietary supplements of carotenoid do not provide photo-protection and may enhance, through unknown mechanisms, the deleterious effects of UV exposure.

Lytechinus variegatus are broadcast spawners, releasing gametes directly into the water column. In shallow water, eggs and developing embryos can be subject to the detrimental effects of ultraviolet radiation (UVR), which in many embryos can negatively impact survivorship, delay development, and promote genetic degradation (Lesser and Barry, 2003; Campanale et al., 2011). UVR can penetrate several meters into coastal waters (Booth and Morrow, 1997). The most biologically deleterious portion of the ultraviolet wavelength is classified as ultraviolet-B (UVB) radiation, which spans the electromagnetic spectra between 280 and 320 nanometers. UVB radiation exposure delays development and produces abnormalities in sea urchin embryos (Adams and Schick, 2001), thought to be the result of DNA damage. Additionally, UV induced protein damage interferes with normal cellular processes and lipid damage disrupts cell membranes

(Bancroft et al. 2007). While many organisms can repair UV induced cellular damage, the efficiency of these processes differ among species (Blaustein et al.. 1994). Ultraviolet-A radiation (UVA), spanning between 320 and 400 nm, can form reactive oxidant species (ROS) in irradiated cellular constituents *in vitro* (McCormick et. al., 1976, Czochralska et al., 1984; Vile and Tyrrell, 1994). Oxygen free radicals generated from UVB or UVA exposure can lead to point mutations and deletions in DNA (de Gruijl. 2002). Embryos of the sea urchin *Strongylocentrotus droebachiensis* have displayed sensitivity to UVA and UVB radiation (Lesser et al. 2006). UVR has been shown to impact covering behavior and larval settlement in *Lytechinus variegatus* (Sigg et al. 2007, Tauchman and Pomory 2011).

In a study comparing UVR sensitivity of the embryos of three urchin species Lesser et al. (2006) reported that embryos with low concentrations of UV absorbing compounds were more sensitive to UVR and endured more frequent occurrences of cyclopyrimidine dimers. There is evidence suggesting that maternally derived UV-protective compounds are obtained in the diet and sequestered in developing ova (Carroll and Shick, 1996). Examples of these compounds include microsporine-like amino acids, (MAA's) and carotenoids. MAA's have been shown to increase UV protection and prevent UV induced developmental abnormalities in the eggs of the sea urchin *Strongylocentrotus droebachiensis* (Adams and Shick, 1996; Adams and Shick, 2001). We hypothesize that these compounds function similarly in *Lytechinus variegatus*. Carotenoids and MAA's are secondary metabolites that function as molecular sunscreens in some marine organisms that live in environments with high intensities of sunlight. In addition, it is suggested that carotenoids within the embryo may function as antioxidants or photo-protectants (Lamare and Hoffman, 2004). Bottom feeders such as sea urchins are presumably shielded from the majority of harmful UVR, however their gametes and embryos are subject to upwelling currents which may increase their exposure to UVR near the water's surface. Sea urchins presumably obtain some carotenoids through the diet; however, the carotenoid echinenone is suggested to be synthesized within the urchin gut wall as well as the ovary (Tsushima et al., 1993). Nutrients from the maternal diet are sequestered in developing ova, which facilitate embryonic development post-fertilization (Herrera, 1995). This nutritive compliment includes carotenoids (Griffiths, 1966). Consequently, sea urchin embryos must solely rely on maternally derived stores of nutrition until they reach the feeding pluteus stage.

This study compares the effects of parental dietary carotenoid intake on the development and survival of early embryos exposed to either indoor fluorescent lighting (control) or to differing levels of indoor UVA or UVB radiation. Fertilized eggs from two diet treatments were exposed to environmentally relevant levels of UVA or UVB radiation.

MATERIALS AND METHODS

Sample Collection

Lytechinus variegatus (ca. 8 to 32g) were collected from the subtidal zone of St. Joseph's Bay (30°N, 85.5°W), FL and transported in 75-liter aerated coolers to the University of Alabama at Birmingham. Sea urchins were placed into multiple 75-liter aquaria plumbed into an artificial saltwater recirculating system with biological filtration and starved for 1 month to standardize their nutritional status (Spirlet et al. 2000). Water conditions were maintained as follows: 32 ± 0.5 ppt

salinity, 24 ± 1°C, D.O. 7 ± 2 ppm, ammonia <0.2 ppm, nitrite <0.2 ppm, nitrate <80 ppm, and pH 8.2.

Raceway design and water quality

The experiment was performed in a large raceway (235 cm x 53 cm x 31 cm, L x W x H, as described by Taylor 2006). A 160 x 23 cm (L x H) center baffle in the center of the raceway allowed for recirculating water flow by an in-line utility pump (Supreme® Mag Drive Utility Pump, 700 gallons of water/hour). The utility pump removed saltwater from the raceway on one side of the baffle. Water was then passed through a mechanical and biological filter and returned to the raceway on the opposite side of the baffle. The flow rate of the resulting current was approximately 9.7 – 12.6 cm s^{-1}. Water was continually pumped through a Life Guard Aquatics Ultraviolet Sterilizer Model QL-15. Water conditions were maintained as follows: 32 ± 0.5 ppt salinity, 22 ± 2°C, D.O. 7 ± 2 ppm, ammonia <0.2 ppm, nitrite <0.2 ppm, nitrate <80 ppm, and pH 8.2. Nutrients were measured using the API Saltwater Master Liquid Test Kit. A 12-hour dark, 12-hour light photoperiod was maintained. Feces were siphoned 3 times per week.

Twenty-four sea urchins were randomly divided among six plastic mesh cages (ca. 23 cm x 23 cm x 14 cm high, with 5 mm open mesh sides and bottom) within the raceway (n = 2 – 5 urchins per cage). The floor of each cage was elevated above the bottom of the raceway with PVC spacers (ca. 4 cm) to allow unimpeded water circulation. Cages were rotated within the raceway each week to prevent bias due to cage position. Urchins in three cages were fed a formulated diet (C-) without supplemental carotenoids, and urchins in the other three were fed the same formulated diet supplemented with carotenoids (C+). A fluorescent light fixture was mounted above the center of the raceway, and it was fitted with 2 bulbs emitting blue actinic light (450-500 nm peak spectral output). In a previous study, urchins exposed primarily to blue actinic light for over 12 weeks developed gonads that were redder in color than urchins that were not (Taylor et al., 2014). We hypothesized that urchins exposed to actinic light would sequester higher levels of carotenoids into their gonads and transfer them to developing ova. The light fixture was provided by Nova Extreme 91.44 cm, 2 * 39 Watt T5 systems. Actinic bulbs were supplied by Giesemann 39 watt Power Chrome Actinic + fluorescent bulbs. According to Geiseman, these bulbs may emit a small amount of UVA radiation; however, the peak emission for these bulbs is between 450 and 500 nm. Each parental treatment was exposed to the same levels of actinic and possible UVA spectra throughout the feeding period. The raceway was fitted with an enclosure made from opaque plastic sheeting and suspended from supports 70 cm high around the top of the raceway to obstruct outside light from entering. The enclosure was vented at the top to allow heat dissipation. Lux was measured daily with a Milwaukee MW700 waterproof probe in the raceway from 3 equally spaced locations along a central line beneath the lighting system. A lux reading of 2400 +/- 200 lux at the center measuring site was maintained by raising or lowering the light ballasts. Lux levels were maintained to insure that any possible carotenoid metabolism or deposition in the gonad due to light intensity or quality was kept consistent in the parents (Taylor et al., 2014).

Feed and feed preparation

Two formulated feeds were produced using both purified and semi-purified ingredients. One feed was supplemented with a commercial carotenoid preparation to a final weight per volume of 1.7%

(MP Biomedicals) containing β-carotene and xanthophylls (C+), and the other was not supplemented with carotenoids (C-, replaced with diatomaceous earth). The carotenoid concentration selected for this study had been shown to optimize gonad color between yellow and orange in previous experiments (Taylor et al., 2014). Previous experiments show no negative growth outcomes due to this carotenoid inclusion in previous experiments with adults. All other nutrients remained constant between diets including any ingredients that may have contained microsporine-like amino acids (not tested). Dry ingredients were mixed with a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 minutes. Liquid ingredients were added, and the mixture was blended for an additional 10 minutes to a mash-like consistency. The feed was extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays for 48 hours. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4°C until used. Feed proffered to urchins was kept in opaque and air tight containers in order to prevent degradation of β-carotene within the urchin diet.

Within the raceway, 12 sea urchins were proffered a daily *ad libitum* ration of the diet supplemented with carotenoids (C+). The remaining 12 were proffered an *ad libitum* ration of the diet that lacked supplemental carotenoids (C-).

Spawning and fertilization

After 5 months (May – October 2012) of feeding, sea urchins from each feed treatment were induced to spawn via injection of 1 mL 0.1 M acetylcholine through the peristomial membrane. Gametes from each feed treatment were collected and diluted with synthetic saltwater (ASW, Crystal Sea salt). Sperm from 2 males of each treatment was diluted by adding 2-3 drops of sperm into 1 L of ASW, inducing activation, and individuals were then combined. This protocol has been shown previously to optimize fertilization success in previous studies (Moseley, 2009). Eggs from 2 females from each treatment were mixed within each treatment and diluted with 1 L ASW. Eggs were fertilized with 3 mL dilute sperm solution from 2 males in the same treatment. One mL of media containing fertilized eggs from each treatment was transferred to 30 mL glass Petri plates and labeled according to UVR exposure or as control (exposed to fluorescent room light only). Successful fertilization was determined by visualization of a raised fertilization envelope within 5 min. Control fertilization was always >95%.

UVR exposure and data acquisition

Two replicates of fertilized eggs from each treatment were exposed to one of 6 levels of UVB radiation (10, 20, 40, 60, 80 or 100 mJ/cm^2) or 3 levels of UVA radiation (1, 2, or 4 J/m^2) using a Daavlin, UVA/UVB Research Irradiation Unit (Bryan, OH). This device was programmed to irradiate samples at predefined levels of UVA or UVB. Three replicates of controls for each broodstock feed received no UVR exposure. Exposures were conducted in glass Petri dishes with the tops removed during the exposure period. Embryos were incubated at 24-26^o C.

Development of each embryonic treatment was assessed at 6.5 and 34 hours post fertilization (HPF). Embryos were sorted into developmental categories which were chosen after an initial survey of the population but before counting. Survival was recorded at 34 and 55 HPF. The

counter was blinded to each treatment assessed for development and survival. Evaluations were recorded after removing a 1 mL subsample containing embryos from each replicate Petri dish and transferring it to a clean Petri dish.. The mean n value for 6.5 hrs was 167 (range: 24-286). The mean n value for 34 hrs was 97 (range: 63-101) and the mean n for 55 hrs was 100 (range: 94-102). Counts at each diet treatment and exposure level were combined among replicate Petri dishes and compared for each of 3 assessment periods. Samples from each Petri dish were preserved in 50% Davidson's solution (40% formalin, 220 ml; glacial acetic acid, 115 ml; 95% ethanol, 330 ml; distilled water, 335 ml) at each counting period and were photographed for developmental stage identification. Developmental stage categories were compared between diet treatments within a UVR exposure level.

Statistics

Data from each replicate were pooled in this study. Differences in survival or development within and between diet treatments were calculated using a 2x2 contingency table with a Yates corrected Chi-Sq. 2-tailed p value. Significance was determined at $p<0.05$.

RESULTS

Development at 6.5 HPF

Since embryos were not moving at this time period, survival could not be accurately determined. Exposure to as little as 1 J/m^2 UVA (Fig. 1) and 20 mJ/cm^2 UVB (Fig. 2) altered development within both diet treatments. For treatments that received no UVR, C- (no carotenoid supplementation) produced higher numbers of early blastula and mature blastula than C+(supplemented with carotenoids) ($p<0.001$, 0.001 respectively). At all levels of UVA exposure (1, 2, and 4 J/m^2), C- produced higher numbers of blastula than C+ ($p<0.001$, 0.001, 0.001, respectively) (Fig. 1). No significant differences in development were found between diet treatments at 10 mJ/cm^2 UVB exposure. Significantly higher numbers of normal blastula were recorded in C+ at UVB exposures of 20 and 40 mJ/cm^2 ($p< 0.001$, 0.001, respectively). A significant majority of embryos in C+ at 60 mJ/cm^2 were pre-blastula (Fig. 2). A majority of embryos from both diet treatments at exposure levels of 80 and 100 mJ/cm^2 were abnormally developed at the preblastula stage and were not counted at this stage (Fig. 2).

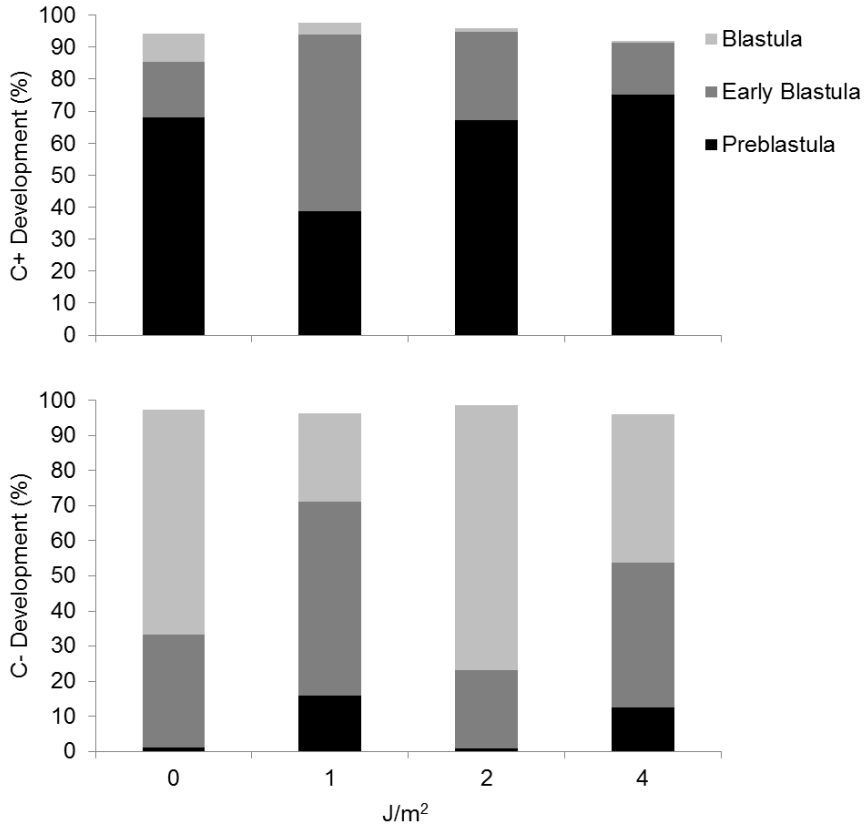

Figure 1. Percent of *Lytechinus variegatus* embryos (6.5 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to one of 3 intensities of UVA radiation at 1 HPF. Exposure to as little as 1 J/m^2 UVA resulted in significant alteration in development within both diet treatments. At all levels of UVA exposure (1, 2, and 4 J/m2) there were significantly higher numbers of blastula in C-.

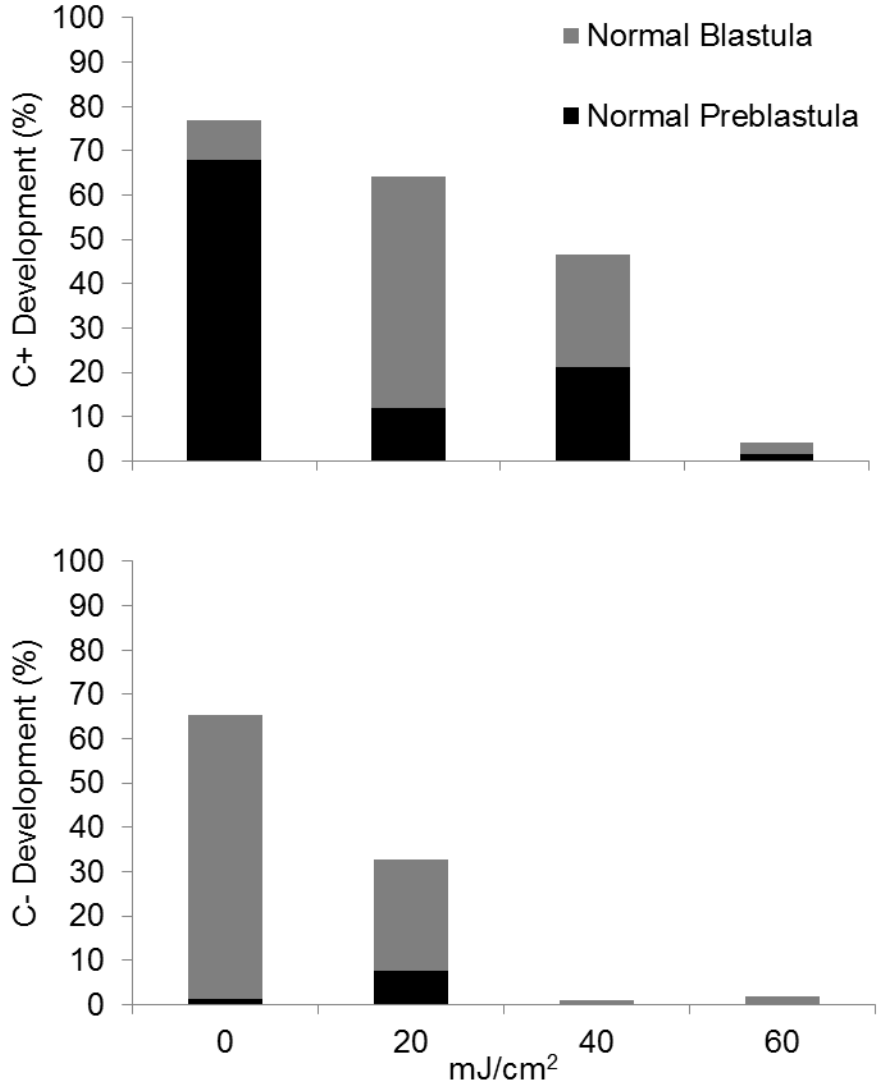

Figure 2. Percent of *Lytechinus variegatus* embryos (6.5 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to one of 3 intensities of UVB radiation at 1 HPF. Exposure to as little as 20 mJ/cm^2 UVB resulted in significant alterations in development within both diet treatments. Significantly higher numbers of normal blastula were recorded in C+ at UVB exposures of 20 and 40 mJ/cm^2 as compared to C-.

Survival at 34 HPF

Exposure to as little as 2 J/m^2 UVA (Fig. 3) and 40 mJ/cm^2 UVB (Fig. 4) resulted in a significant decrease in survival within both diet treatments. For embryos that received no UVR, there was no significant difference in survival between diet treatments ($p= 0.086$). Significantly lower survival was found in C+ at UVB exposures of 60, 80 and 100 mJ/cm^2 ($p= 0.0439$, <0.001, <0.001, and

<0.001, respectively)(Fig. 4). Significantly lower levels of survival were recorded in C+ at UVA levels of 2 and 4 J/m^2 (p=0.0341, 0.0001, respectively) (Fig. 3).

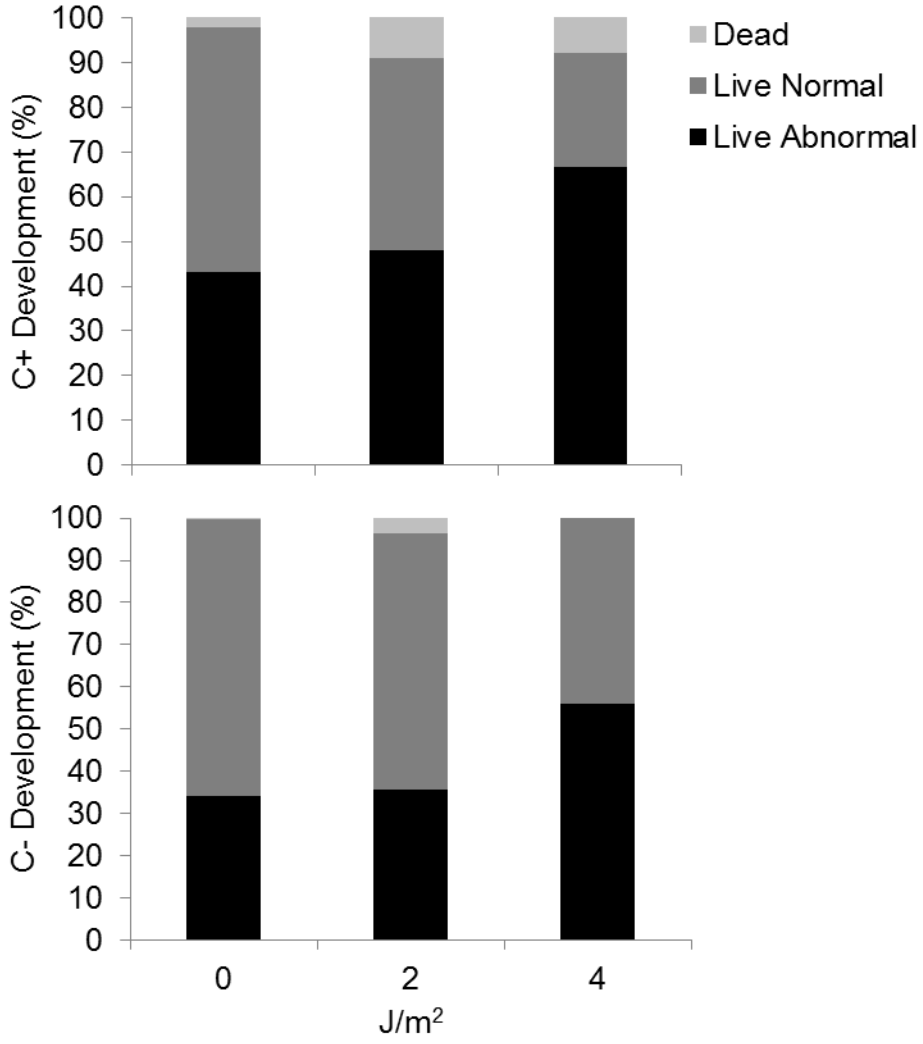

Figure 3. Percent of *Lytechinus variegatus* embryos (34 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to 2 or 4 J/m^2 UVA radiation at 1 HPF. Exposure to as little as 2 J/m^2 UVA resulted in a significant decrease in survival within both diet treatments. Exposure to as little as 2 J/m^2 in C+ and 4 J/m^2 in C- resulted in a significant decrease in the numbers of normally-developing embryos. Significantly lower survival was recorded in C+ at UVA levels of 2 and 4 J/m^2. Between treatments, higher percentages of normally-developing embryos were observed in C-.

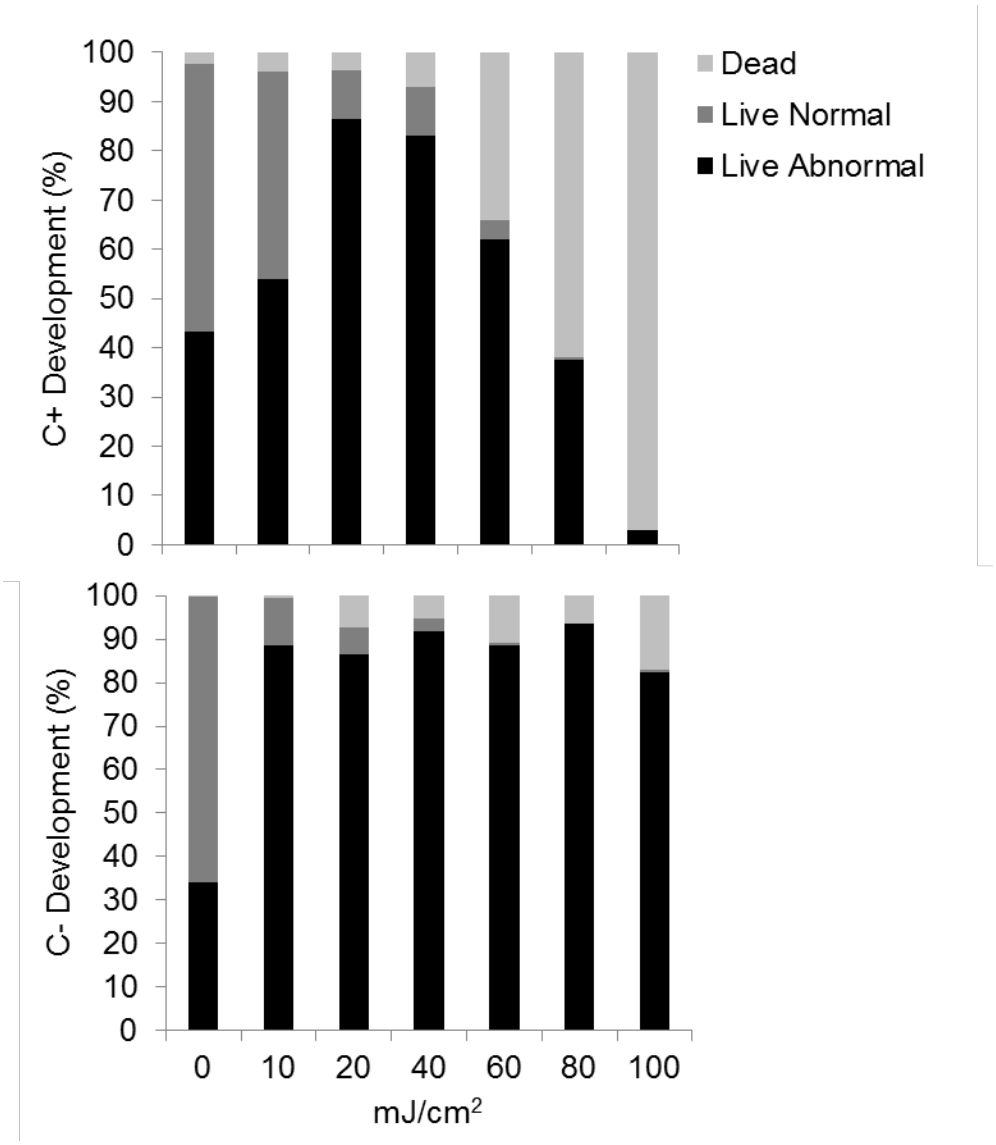

Figure 4. Percent of *Lytechinus variegatus* embryos (34 HPF) at each developmental stage in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to one of six intensities of UVB radiation at 1 HPF. Exposure to as little as 40 mJ/cm^2 UVB resulted in a significant decrease in survival within both diet treatments. Significantly lower survival was found in C+ at UVB exposures of 10, 60, 80 and 100 mJ/cm2.

Development at 34 HPF

Exposure to as little as 2 J/m^2 in C+ (Fig. 3) and 4 J/m^2 in C- (Fig. 4) resulted in a significant decrease in developmental rate in normally-developing embryos. In treatments that received no UVR or in UVA exposures of 2 or 4 J/m^2, a significantly higher number of normally developed living embryoswere recorded in C- as compared to C+($p=0.007$, <0.001, <0.001, respectively) (Fig. 3). Higher numbers of normally developed embryoswere recorded at UVB levels of 10, 40, and 60 mJ/cm^2 in C+ as compared to C-($p= <0.001$, 0.011, 0.043, respectively) (Fig. 4).

Survival at 55 HPF

At 55 HPF, survival was significantly decreased within C+ at all levels of UVA exposure (Fig. 5). There was no significant difference in survival at any UVA exposure in C- (Fig. 5). Exposure to as little as 20 mJ/cm^2 UVB resulted in a significant decrease in survival within both diet treatments (Fig. 6). There was no significant difference in survival between diet treatments that were not exposed to UVR (p= 0.0291). There was significantly lower survival within C+ at UVB exposures of 60, 80, and 100 mJ/cm^2 (p= 0.0020, <0.001, 0.0418, respectively), but not at 10, 20 and 40 mJ/cm^2 (p=0.2984, 0.8437, 0.6094, respectively) (Fig. 6). Higher survival was recorded in C- at UVA levels of 1 and 2 J/m^2 (p= <0.001, 0.0004, respectively). There was no significant difference in survival between the diet treatments at a UVA exposure of 4 J/m^2 (p= 0.0903) (Fig. 5).

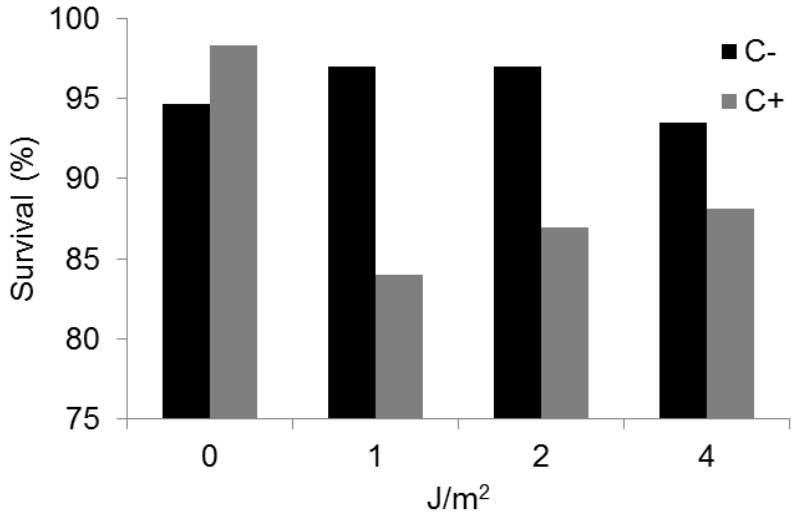

Figure 5. Percent survival at 55 HPF of *Lytechinus variegatus* embryos in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to one of 3 intensities of UVA radiation at 1 HPF. Survival was significantly decreased within C+ at all levels of UVA exposure. Between treatments, highest survival was recorded in C- at UVA levels of 1 and 2 J/m2.

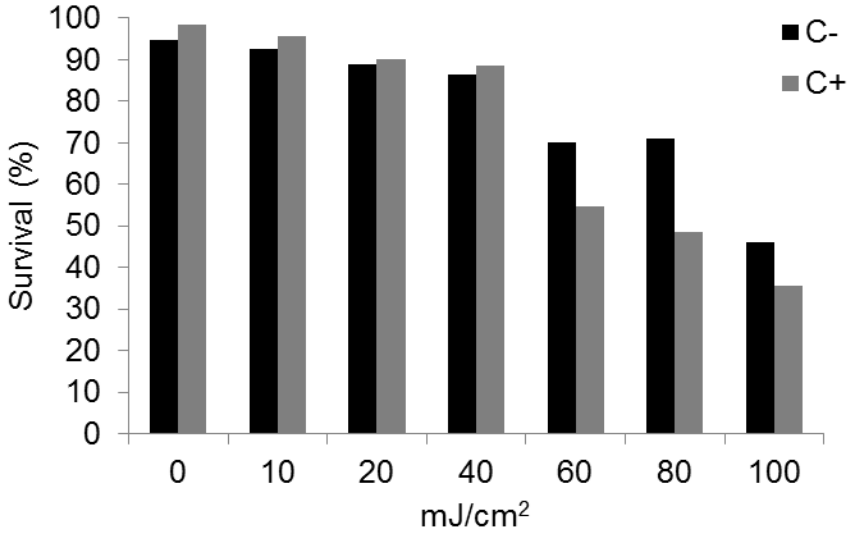

Figure 6. Percent survival at 55 HPF of *Lytechinus variegatus* embryos in which the parents were fed a diet (A) with or (B) without supplemental β-carotene. Embryos were exposed to one of six intensities of UVB radiation exposure at 1 HPF. Exposure to as little as 20 mJ/cm2 UVB resulted in a significant decrease in survival in both diet treatments. There was significantly lower survival within C+ at UVB exposures of 60, 80, and 100 mJ/cm2 between treatments

DISCUSSION

We originally hypothesized that *Lytechinus variegatus* embryos derived from broodstock fed a carotenoid supplement would be protected from UVR. However, in some cases these embryos were shown to be at a disadvantage within the parameters of our study. We suggest parental carotenoid dietary intake affected the stress response of offspring exposed to differing levels of UVA and UVB radiation. Survival and development of both dietary treatments were impacted by UVR exposure. Embryos from both treatments were more sensitive to UVB radiation than UVA, most likely the result of higher levels of genomic disruption associated with UVB (Afaq and Mukhtar 2006). The initial hypothesis that carotenoids or metabolites stored within offspring (derived from carotenoid-supplemented broodstock) would protect against all levels of UVR exposure was not supported.

Parental carotenoid dietary intake affects the developmental rate of *Lytechinus variegatus* offspring between 0 and 55 hours post fertilization. Embryos from broodstock that received supplemental carotenoids developed at a slower rate than embryos from broodstock that received no supplemental carotenoids. We suggest that inclusion of supplemental carotenoids in the broodstock diet was responsible for a decrease in developmental rate of offspring as well as an increase in developmental abnormalities observed at both 6.5 and 34 hours post fertilization. It is difficult to determine if either an increase or decrease in developmental rate is a positive outcome reflecting embryonic health. However, since a decreased rate of development was coupled with increased abnormalities, we hypothesize that the supplementation of carotenoids at the level provided to the broodstock may have resulted in an accumulation of carotenoids or related metabolites in the eggs, which reduced developmental rates and increased abnormal development.

If this hypothesis is correct, then excessive dietary carotenoids can affect normal development as well as development in the presence of additional stressors, including UVR. Higher levels of normally developed embryos found in some UVR exposure treatments may also be due to an adaptive response to a low-level insult wherein UVR repair stimulates increased DNA synthesis and cell division rates (Skov, 1999).

The biochemical composition of the sea urchin eggs in the current study was not known, but broodstock were all fed a diet to excess shown previously to promote good growth and health (Hammer et al. 2012, Heflin et al. 2013), thus, it is doubtful that they were nutritionally restricted. Dietary carotenoids in sea urchins; however, are necessary for gamete health and development (Tsushima et al., 1991; de Jong Westman et al., 1995a, b). Kawakami et al. (1998) found that carotenoids promote phagocytic engorgement of foreign materials within the soma and gonad of the sea urchin *Psuedocentrotus depressus*. Nutritive phagocytes in the gonad aid in gamete development and β-echinenone is the principal facilitator of phagocytic activity in *P. depressus*. Concentrations of the carotenoids β, β-carotene and fucoxanthin were also positively correlated with successful gamete development.

Carotenoid sequestration in tissues can have negative effects in some organisms. Grether et al. (2008) hypothesized that excess carotenoids in the female guppy *Poecilia reticulata* may be transferred to developing eggs as a means of pigment disposal. They found carotenoid deposition in eggs was strongly affected by dietary carotenoid levels. These authors also proposed that excess carotenoid deposition within female *P. reticulata* negatively affects crypsis. We suspect that excess carotenoid in the urchin C+ diet may have been transferred to eggs and reduced developmental rate via unknown mechanisms. Toxic levels of carotenoid metabolites have also been suggested to induce developmental abnormalities. Vitamin A (a product of β-carotene metabolism) has been suggested to cause birth defects in humans when consumed at levels approximately as low as 25,000 IU/d (Hathcock et al., 1990). Levels of carotenoids that maximize reproductive health of broodstock, fecundity, and F1 embryonic health in *L. variegatus* have not been determined.

Light spectra and diet have been suggested to alter carotenoid deposition in the sea urchin gonad. In a previous study, prolonged blue actinic light exposure increased the redness phenotype of *L. variegatus* gonads when dietary β-carotene supplements are proffered *ad libitum* (Taylor et al., 2014). We subsequently hypothesized that this increase in redness is due to increased concentrations of carotenoids within the gonad. In the current study, sea urchins from both diet treatments were sequestered beneath actinic lamps for 5 months, potentially increasing carotenoid deposition into eggs during this time period.

Levels of carotenoids in the gonads or ova were not measured in either treatment. We cannot directly link the carotenoid concentration of the eggs to survival and developmental outcomes in the offspring. We only correlate these outcomes with the diet of the parents. A significant increase in mortality due to parental β-carotene supplementation was measured at 60, 80, and 100 mJ/cm^2 UVB exposure. We hypothesize that these intensities of UVB radiation promoted the formation of toxic byproducts from internal stores of β-carotene, which negatively impacted survival and development. Embryos from parents that did not receive β-carotene supplements may not have had significant stores of carotenoids within their soma. Siems et al. (2005) reported that β-carotene

exerts protective antioxidant activity during moderate oxidative stress. Heavy oxidative stress, however, will cleave carotenoids into oxidative products that accumulate within the cell and damage mitochondria, DNA, lipids, and the nucleus (Siems et al., 2005). Oxidative damage to DNA increases the risk of cancer and developmental abnormalities. These data support the findings of Siems et al. (2005), as well as those of the Alpha-Tocopherol, Beta-Carotene-Cancer-Prevention study, and the Beta Carotene and Retinol Efficacy Trial (Omenn et al., 1996, The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994).

While dietary carotenoids are necessary for successful gamete development, appropriate dietary levels and types of carotenoids have not been established for sea urchins. We hypothesize that levels of β-carotene and/or other carotenoids or xanthophylls in the C+ diet supersede the nutritional requirement of *L. variegatus* and are stored in excess. Excess β-carotene within the embryo may be toxic or increase its susceptibility to oxidative or other types of stress. In this study we were not able to evaluate the levels of carotenoids in the embryos and hypothesize that levels of inclusion within the eggs were proportional to those found in the gonads of broodstock fed supplemental dietary carotenoids. Changes in color phenotype of the gonads of broodstock fed supplemented dietary carotenoids confirm that carotenoids or their metabolites are incorporated into the gonad tissues and, presumably, transferred to the eggs (Taylor et al., 2014). Further research is necessary to evaluate the requirements and function of carotenoids in sea urchin tissues.

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Distribution and Structure of Torus-Bearing Membranes in the Wood of *Schisandra chinensis*

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ABSTRACT

Torus-bearing pit membranes control water movement between tracheary elements of vascular plants, while at the same time they inhibit spread of air embolisms. They are common in gymnosperms but relatively rare in angiosperms. A recent manuscript noted the presence of such membranes in *Schisandra chinensis*, a species of basal angiosperm. Building on this prior report, the present manuscript presents evidence for the presence of this membrane in four more species of the genus. The torus-bearing pit membranes are best developed between tracheids, a cell type that acts as a subsidiary conducting system to the vessel members. Detailed observations with an atomic force microscope show the torus to be deposited after formation of the subtending margo is complete.

Tracheary elements are the water-conducting cells of vascular plants (Evert, 2006). Bordered pit pairs in their walls provide a pathway by which water flows from one tracheary element to another (Pittermann *et al*., 2005). At the same time, the bordered pit pairs must inhibit movement of any air bubbles present in the conducting pathway to avoid the severing of water columns and subsequent cessation of flow (Pittermann *et al*., 2005).

Pit borders of a pit pair are separated by a pit membrane. Pit membranes associated with bordered pit pairs of tracheary elements are mostly of two types (Dute, 2015). One type, found in most gymnosperms, consists of a permeable margo with relatively large pores surrounding a central, impermeable, target-like thickening, the torus. In the absence of air bubbles, water flows through the margo pores. In the presence of air bubbles however, the pit membrane is displaced (or aspirated) such that the torus blocks one aperture and stops the spread of gas embolisms. In contrast to this system, bordered pit membranes in tracheary elements of most angiosperm species are homogeneous in that they lack a torus but have much smaller diameter pores. The smaller openings decrease water flow (relative to the situation in gymnosperms), but make passage across the membrane more difficult for air bubbles (Dute, 2015; Pittermann *et al.*, 2005).

Torus-bearing intervascular pit membranes were thought to be absent from angiosperms until Ohtani and Ishida (1978) discovered them in six species of flowering plants. These pit membranes presented a third type of anatomy in that a central, impermeable torus was surrounded by a margo with very fine pores as found in other angiosperms. Since their discovery, further work in other laboratories has increased the number of torus-bearing angiosperm species to 86 (Dute, 2015).

Looking at the systematic distribution of torus-bearing pit membranes among the genera of angiosperms (Table 3.1 Dute, 2015) clearly shows this feature to be homoplastic. This conclusion

is strengthened by the presence of multiple mechanisms of torus ontogeny in different angiosperm families (compare for example Dute and Rushing [1988] and Dute *et al.* [1990]). The most recent discovery of torus-bearing pit membranes in angiosperms is that of Sano *et al.* (2013) in *Schisandra chinensis* (Turcz.) Baill.

The aims of the present publication are to extend the observations of Sano *et al.* (2013) into the nature of the substructure of the margo and torus in *S. chinensis*, to survey other species of this genus for tori, and to survey species of the sister genus *Kadsura* (Denk and Oh, 2006) as well.

MATERIALS AND METHODS

The sampled herbarium specimens that were used in this study are listed in Table 1 [Included after Literature Cited]. A five millimeter-long piece of stem was removed from the cut base of each specimen. Such samples were prepared for observations by one of the following methods:

1. For light microscopy (LM) hand cut transverse and longitudinal samples of herbarium specimens were placed in 95% ethanol under a vacuum for two hours then left at ambient air pressure overnight. Samples were carefully infiltrated and embedded in JB-4 plastic resin over a two-day period. Some herbarium specimens were rehydrated in dH_2O overnight then dehydrated to 95 % ethanol prior to embedment in JB-4 or dehydrated to absolute ethanol and air dried and mounted for SEM (q.v. #3). Transverse and longitudinal sections of the wood were cut with a thickness of 3—8 μm using a Sorvall MT-2b ultramicrotome. Wood sections were stained with a benzoate buffered, aqueous toluidine blue O (TBO) (Ruzin, 1999). Photographs were taken either with a Nikon Biophot microscope or with a Nikon Eclipse 80 I epifluorescence microscope (using the brightfield setting) with a Qimaging Fast 1394 digital camera.
2. Wood macerations were carried out according to the procedure of Wheeler (1983). Briefly, wood slivers were put into a 1:1 mixture of hydrogen peroxide and glacial acetic acid at 60° C for three days. Next, a drop of the treated tissue was placed on a slide, the fluid replaced with dH_2O, then the addition of TBO followed by a dH_2O rinse. The stained tissue was teased to separate cells and a coverslip was added prior to viewing with a light microscope.
3. For scanning electron microscopy (SEM) branch segments of 5 mm in length were split with a razor blade to expose radial longitudinal surfaces (RLS). The segments (split surface up) were then attached to an aluminum stub with fingernail polish and sputter coated with gold vapor. Observations were made with a Zeiss EVO 50 operated at 20 kV.
4. For atomic force microscopy (AFM) of herbarium specimens, wood samples of 5 mm in length were split to expose the RLS. Samples then were attached to AFM discs using fingernail polish (Dute and Elder, 2011). AFM images were captured at 512X512 resolution using Veeco NanoScope 3D using TAP 150 tips with an amplitude set point being about 1.8 V. Nanoscope 5.3l rl was used to save height, amplitude and phase images.

Three seedlings of *S. chinensis* were purchased from Horizon Herbs (Williams, Oregon, USA). Living stem segments (2 mm diameter) were cut into five mm lengths and treated for LM, SEM and AFM. Some specimens were stripped of their bark, dried for two days, then split to expose the RLS and mounted for either SEM or AFM. Other specimens were dehydrated through absolute ethanol, infiltrated with HMDS (Nation, 1983) and air dried for either SEM or AFM. Yet other living specimens were cut into 2 X 2 pieces and preserved in 3 % glutaraldehyde in 0.05 M

phosphate buffer. Fixation was followed by dehydration to 95 % ethanol followed by JB-4 embedment, sectioning, staining and observation using LM.

RESULTS
[The referenced figures are included at the end of the article.]

Wood Anatomy of *Schisandra*

Schisandra chinensis was selected for overall anatomical study due to the presence of living material as well as to the quality and quantity of herbarium specimens. Figure 1 is a trans-section of a young, living stem portion collected during its second year of growth and preserved in ethanol. The herbarium specimens that were used consisted of narrow diameter branches, which also contained few growth rings. Cytoplasm in the wood parenchyma cells was preserved better in chemically fixed specimens than in air-dried, herbarium samples. Figure 2 provides a detailed view of cell types in wood near the vascular cambium. There are two types of wood parenchyma cells: 1) axial, which partially ensheath vessel members, and 2) ray, which as the name implies, run radially through the wood from pith to ray initial cells of the vascular cambium. Rays are narrow, typically being only one cell wide (or uniseriate) as seen in material sectioned in the tangential longitudinal plane.

There are two distinct water-conducting systems in the wood of *Schisandra*. One of them consists of vessel members stacked end to end to form vessels. Measurements of cells from macerated wood of *S. chinensis*, show vessel member size to range from 75--112.5 μm in width and 325—550 μm in length (N = 25). Vessels are dead at maturity and in cross section usually are found as solitary cells in contact with both types of parenchyma as well as with the other type of water-conducting cell, the tracheid (Fig. 2). At maturity, vessel members are dead and devoid of cytoplasm and are connected by distinctly slanted end walls containing numerous, scalariform perforations (as seen tangential longitudinal section, Fig. 3). Figures 4--6 show a scalariform perforation plate in radial longitudinal section using SEM. Figures 5 and 6 are enlargements of the area indicated with an asterisk in Fig. 4 showing how individual perforations have developed from scalariform pits.

The second water-conducting system involves tracheids. Tracheids, like vessel members, are devoid of cytoplasm at maturity. They are distinguished from vessel members by being narrower (12.5--37.5 μm) (Fig. 2) and longer (400--1500 μm, N = 25). In addition, tracheids differ from vessel members in *Schisandra* by having a thicker wall (Fig. 3). Wall chemistry is indicated in a general way by color of the bound TBO, a metachromatic dye (O'Brien *et al.*, 1964). The inner layer of the secondary wall in tracheids of *S. chinensis* stains a deep purple. This layer is distinct even in black and white photographs (Fig. 3). Such a layer is lacking from vessel members of the same wood (Fig. 3). Tracheids are found in both early wood and late wood and are imperforate; that is, they lack perforations in their walls and are dependent exclusively on pit pairs to move water from one tracheid to the next. It is on the pit membrane of such pit pairs that tori are most likely to occur.

The torus is a thickening located in the center of the pit membrane. In sectional view under the light microscope, it appears as a fusiform object, which absorbs TBO heavily, resulting in a dark blue stain (Fig. 7). In face view the torus is circular (Figs. 5, 8). Position of the torus (and of the pit membrane) generally is midway between the two apertures of a pit pair when fresh material is

processed and viewed (Fig. 7), whereas in air-dried herbarium material, the torus is often displaced (aspirated) such that it occludes one of the apertures (Fig. 9).

Since tracheid walls are rather thick, each aperture associated with a pit pair has an inner and outer surface (Fig. 7), the former is located closer to the cell lumen and the outer closer to the pit membrane and torus surface. The outer surface of the aperture tends toward a circular outline and has a diameter less than that of the torus (Fig. 8, arrow). This observation of the torus diameter being greater than the outer aperture surface diameter holds true generally, but there are exceptions as shall be seen.

Distribution of Tori within the Wood

As indicated, bordered pit pairs connecting tracheids (both in early wood and late wood) always have tori. However, pitting among cells in the wood of *Schisandra* is complex due to the different cell types that are involved. As a case in point, consider Figs. 10 and 11, which represent the same vessel member isolated by maceration. By changing the focal plane, two different longitudinal walls (surfaces) are brought into focus. With the exception of the scalariform perforation to the bottom right in Fig. 11, the other openings represent pits leading from the vessel member to different cells that encircle it. Tori are a consistent feature of intertracheary pit membranes. Tori are an inconsistent feature of pit membranes separating vessel members and tracheids. Sometimes the torus is present and appears normal with the light microscope (Fig. 12); sometimes the torus is present but in a vestigial state (Fig. 13); sometimes it is absent (Fig. 14). All other types of pit membranes in the wood lack a torus.

Figures 4 and 5 summarize visually the differences in structure and pit type of the two water-conducting systems in wood of *Schisandra*.

Structure of the Intertracheary Pit Membrane--SEM

Structure of the intertracheary (torus-bearing) pit membrane was investigated in air-dried specimens of *S. chinensis* and *S. sphenanthera* Rehder & E. H. Wilson, and in HMDS-dried specimens of *S. chinensis*.

There is a clear distinction between torus and margo components of the pit membrane. The margo of air-dried membranes shows crossed microfibrils (Fig. 15). In exceptional views of air-dried material (Fig. 16), and in typical views in HMDS-dried specimens (Figs. 17 and 18), the margo is penetrated by pores of different diameters. In some instances (Figs. 16--18) many of the large openings and tears are artifactual, induced in many cases by specimen preparation and/or by heat of the electron beam (Nguyen *et al.*, 2017).

In all instances, the torus thickening is deposited atop the microfibrils of the margo and appears impermeable (*e.g*. Fig. 16). Note that any tearing found in the margo does not extend through the torus (Figs. 17 and 18).

Structure of the Intertracheary Pit Membrane--AFM

Detailed (high resolution) images of torus-bearing pit membranes were provided by atomic force microscopy (AFM). Wood of both *S. chinensis* and *S. sphenanthera* was investigated in air-dried and HMDS-dried conditions. The least physical damage was associated with HMDS-dried membranes of *S. chinensis*, so information from those pit membranes is provided herein.

The pit membrane clearly consists of two components: the fibrillar margo and the impermeable-appearing torus. The torus pad clearly sits atop the margo, which would indicate that the former is synthesized after the latter (Figs. 19, 20).

The margo consists of multiple layers of microfibrils of different diameters (Fig. 21). The torus is deposited atop a margo layer of parallel fibrils (Fig. 19); density of these microfibrils varies from one pit membrane to the next.

Morphology of the torus surface (as seen with AFM) varies, but it is generally pustular and not smooth (*e.g*. Fig. 20). In complex examples, there appear to be three regions to the torus: 1) a modified ring of pustules encircling the edge (1 in Figs. 20, 22), 2) a relatively smooth-surfaced region within 1 (2 in Figs. 20, 22), and 3) a large cluster of distinct pustules (3 in Figs. 20, 22) in the center (and highest point) of the torus. For the most part, the torus pad consists of non-fibrillar (matrix) material, but occasionally, there is evidence that microfibrils might also participate in torus construction (Fig. 23).

Pit borders enclosing the pit membrane on either side are constructed of concentrically arranged microfibrils (Fig. 24).

Torus Distribution in Spp. of *Schisandra*

Wood of various species of *Schisandra* was searched using both SEM and LM for presence of torus-bearing pit membranes. The following species had tori in addition to *S. chinensis* (Table 1): *S. micrantha* A. C. Sm., *S. sphenanthera*, *S. sphaerandra* Stapf (Fig. 25), and *S. pubinervis* (Rehder & E. H. Wilson) R. M. K. Saunders. Wood from *S. pubinervis* contained minitori in which torus diameter did not equal or surpass that of the pit apertures (Fig. 26).

The wood from some of the herbarium specimens proved refractory to preparation for microscopy. Perhaps future work on newly dried specimens will show tori in species now marked with an "N" in Table 1.

Torus Distribution in Spp. of *Kadsura*

None of the *Kadsura* species that were investigated possessed tori (Table 1).

DISCUSSION

Bailey and Nast (1948) refer to imperforate tracheary elements as "tracheids" in the related genera of *Illicium*, *Schisandra,* and *Kadsura* and comment upon the high concentration of distinct, bordered pits in these cells. In contrast, Metcalfe & Chalk (1950) refer to the same cells in the wood of *S. chinensis* as "fibers" although they note the cells' "conspicuous bordered pits." Saunders (1997), in a review of the Schisandraceae, labels the imperforate elements as "tracheids." Carlquist's (1999) study of the wood anatomy of *Kadsura* and *Schisandra* identifies the imperforate elements as (true) tracheids, and he found, as we have in the present study, that tracheid length is much greater than that of the associated vessel members. He also found tracheid wall thickness to be greater than wall thickness of the neighboring vessels. He had already identified the elements as true tracheids in 1988. Sano *et al.* (2013), in their article on pit membranes in ancestral angiosperms, opt for the term "fiber" when discussing torus-bearing pit membranes of *S. chinensis*.

Based on the high number of well-developed, circular bordered pits on the lateral walls of these cells (*q.v*. Fig. 4, this study), we would agree that their designation as "tracheids" is appropriate. This dual system of conductive elements (tracheids and vessel members) is reminiscent of the situation encountered in wood of *Cercocarpus* spp. (Dute *et al.*, 2010). Carlquist (1988) also considers this latter genus to possess true tracheids.

Pit membrane remnants in scalariform perforations have previously been illustrated for both *Schisandra* and *Kadsura* (Carlquist, 1999) as well as for a species of *Illicium* (*I. floridanum* J. Ellis) and other genera of the basal angiosperms (q.v. discussion in the introduction of Schneider and Carlquist, 2003). This situation also is apparent in various angiosperm families and is thought to represent a symplesiomorphic feature in the flowering plants (Schneider and Carlquist, 2003).

Sano *et al.* (2011) correlate water movement in imperforate tracheary elements with conductive cells that show complete pit membranes and a larger pit diameter and greater pit density than nonconductive imperforate tracheary elements. Our AFM work provides the necessary high resolution showing undamaged pit membranes between tracheids in *Schisandra* that are of a size and density to qualify as water-conducting, although dye movement was not attempted. Carlquist (1999) and Carlquist & Schneider (2002) consider such tracheid systems as found in *Schisandra* as providing a back-up or subsidiary water-conducting system having a high degree of conductive safety compared to that of the vessel system. We would agree.

When dealing with a structure as fragile as a pit membrane, one must always be alert to the problem of artifacts, especially in SEM imaging. These structural abnormalities can be induced in various ways, for example, during splitting of the wood specimens, chemical processing of the specimens, or by heat of the electron beam (Schneider and Carlquist, 2003; Jansen *et al.*, 2008; Jansen *et al.*, 2009; Nguyen *et al.*, 2017). A case in point is provided by comparing Figs. 16—19 in the present manuscript. The first three figures are SEM images of seriously damaged pit membranes. The porosity of the margo differs vastly among them, and all three margos in turn differ from the AFM image in Fig. 19. How are investigators able to determine the real anatomy of the pit membrane *versus* artifact? In some instances (as with the heat of the electron beam), the damage to the pit membrane occurs in real time, and one can quickly associate cause and effect (Nguyen *et al.*, 2017). Jansen and associates (Jansen *et al.,* 2008; 2009; Li *et al.*, 2016) have carefully catalogued

the effects of various treatments on intervascular pit membrane structure. They have concluded, among other things, that alcohol has a "clear dehydrating effect on the samples, resulting in more porous pit membranes" (Jansen *et al.*, 2008). They were referring to SEM samples, yet material prepared for TEM is routinely passed through an alcohol or acetone series prior to embedment in resin (e.g. Dute, 1994). Alcohol is an intermediary fluid that is miscible with both the aqueous fixative solution and the hydrophobic plastic resin.

Another instance of membrane change involves air drying and pit membrane aspiration. Aspirated pit membranes are known to be thinner and denser than non-aspirated pit membranes (Dute, 1994; Pesacreta *et al.*, 2005; Li *et al.*, 2016); but even in such instances, aspiration occurring naturally to the intrtracheary pit membranes of *Gingko* wood *versus* aspiration produced by air drying leads to different sectional images using the TEM (Dute, 1994).

In truth, pit membranes are so fragile that any preparatory technique and/or microscope combination is likely to introduce artifacts of some sort. Added to this is the fact that a given research laboratory is often limited in its equipment holdings, and so it is restricted in the types of observations that it can accomplish.

Recent pit membrane studies have been carried out using field emission scanning electron microscopes. However, the atomic force microscope provides atomic level resolution (Hanley *et al.*, 1992), and the specimen avoids being coated with metal and avoids interaction with a hot electron beam (Dute & Elder, 2011). Investigation of hydrated pit membrane with the AFM has, in our opinion, the best chance of observing torus-bearing pit membranes in their natural state (Pesacreta *et al.*, 2005).

The presence of minitori in pit membranes of *S. pubinervis* is surprising. Wheeler (1983) noted that torus diameter is less than aperture diameter in *Celtis reticulata* Torr. Individual instances of this situation have been observed in other torus-bearing species (e.g. Dute *et al.*, 2004). In such cases the torus diameter would not be sufficient to provide a tight seal during aspiration. Nevertheless, such thickenings would strengthen the pit membrane at its center and prevent or reduce the likelihood of membrane tearing at that site during aspiration (Wheeler, 1983). It is unfortunate that we had but one specimen of *S. pubinervis* on which to base our observations.

A recent review (Dute, 2015) lists all known dicot species whose intervascular pit membranes possess a torus. The list includes 86 species from six families (Oleaceae, Thymelaeaceae, Rosaceae, Ulmaceae/Cannabaceae, Schisandraceae). To this list we must add 4 more species from the present study. Although occasional species might be added to this list in the future, we doubt that the numbers will increase drastically. As noted in the review, it appears as if the appearance of torus-bearing pit membranes is homoplastic at the level of the family. This hypothesis is supported by different mechanisms of torus ontogeny among the families (q.v. Dute, 2015). Sano *et al.* (2013) correctly note that their report of tori in *S. chinensis* represents the first such observation from the basal angiosperms. The other torus-bearing species are located within the eudicots. As a point of interest, a study of tracheary elements of *Amborella trichopoda* Baill. shows no evidence of torus-bearing pit membranes (Feild *et al.*, 2000). *Amborella* is considered the sister group of all other angiosperms (Feild *et al.*, *2000*).

Material of the torus thickening is deposited as a secondary wall late in pit ontogeny. In this respect, development is akin to that of *Osmanthus*, *Daphne* and *Cercocarpus* and unlike torus development in *Ulmus*, *Celtis*, conifers and *Ginkgo* (Dute, 2015).

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We thank directors, curators and staff of the following herbaria for kindly providing loans of specimens and material for this study: AUA, L, MO, NY, PH and US. This research was supported by an Undergraduate Research Award (to J. R.) from the Fund for Excellence of the Department of Biological Sciences, Auburn University. This is contribution no. 746 of the Auburn University Museum of Natural History.

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TABLES AND FIGURES

Table 1. Specimens of *Kadsura* and *Schisandra* used in this study. AUA = Auburn University Herbarium; L= Leiden branch of National Herbarium of the Netherlands; MO = Missouri Botanical Garden; NY = New York Botanical Garden; PH = Academy of Natural Sciences, Philadelphia; US = Smithsonian

Species	Herbarium	Date of Collection	Collector(s) No.	Torus (Y/N)
***K. coccinea* (Lem.) A.C. Sm.**	PH	10 Jun 1995	*Qi 239*	N
***K. coccinea* (Lem.) A.C. Sm.**	US	1932-1933	*Chun & Tso 44188*	N
***K. heteroclita* (Roxb.) Craib**	PH	8 Aug 1970	*Huang & Kao 5435*	N
***K. heteroclita* (Roxb.) Craib**	US	24 Aug 1970	*Hu 10888*	N
***K. scadens* Blume**	PH, US	4 Jun 1972	*Stone 10777*	N
***S. arisanensis* Hayata subsp. *viridis* (A.C. Sm.) R.M.K. Saunders**	NY	1 Aug 1932	*Tsang 21423*	N
***S. arisanensis* Hayata subsp. *viridis* (A.C. Sm.) R.M.K. Saunders**	NY		*Tsui 825*	N

***S. bicolor* W.C. Cheng**	MO	9 Aug 1963	*Chiu s.n.*	N
***S. chinensis* (Turcz.) Baill.**	MO			Y
***S. chinensis* (Turcz.) Baill.**	L		*anon. 10781*	Y
***S. chinensis* (Turcz.) Baill.**	L		*anon. 254*	Y
***S. elongata* Baill.**	PH		*Thoms. s.n.*	N
***S. elongata* Baill.**	US	1885-1888	*Henry 6383*	N
***S. glabra* (Brickell) Rehder**	AUA	2 Jul 1960	*Ahles 53722*	N
S. henryi* C.B. Clarke subsp. *henryi	NY	23 Aug 1988	*Boufford & Bartholomew 24074*	N
S. henryi* C.B. Clarke subsp. *henryi	NY	9 Jul 1985	*Yao 9514*	N
***S. lancifolia* (Rehder & E.H. Wilson) A.C. Sm.**	NY	May-Oct 1922	*Rock 4299*	N
***S. micrantha* A.C. Sm.**	NY		*Henry 11211*	Y
***S. neglecta* A.C. Sm.**	NY		*Henry 10697*	N
***S. nigra* Maxim.**	NY	13 Jul 2003	*Watanabe et al. s.n.*	N
***S. perulata* Gagnep.**	US	Apr 1925	*Tonkin s.n.*	N
***S. plena* A.C. Sm.**	NY		*A. Henry 12192*	N
***S. propinqua* (Wall.) Baill. subsp. *intermedia* (A.C. Sm.) R.M.K. Saunders**	NY		*A. Henry 13023*	N
***S. propinqua* (Wall.) Baill. subsp. *sinensis* (Oliv.) R.M.K. Saunders**	NY	18 Jun 1934	*Chow 567*	N
***S. pubescens* Hemsl. & E.H. Wilson**	NY	1928	*Fang 2632*	N
***S. pubinervis* (Rehder & E.H. Wilson) R.M.K. Saunders**	NY	21 Jul 1989	*Zhao Qing-Sheng 1069*	Y
***S. repanda* (Siebold & Zucc.) Radlk.**	US	12 Jul 1889	*Watanabe s.n.*	N
***S. sphaerandra* Stapf**	NY	7 Aug 2005	*Gaoligong Shan Biodiversity Survey 25699*	Y
***S. sphenanthera* Rehder & E.H. Wilson**	NY	1 Jun 1994	*Boufford, Liu, Ying, C. J. Zhang*	Y

			& X.C. Zhang 26380	
***S. sphenanthera* Rehder & E.H. Wilson**	NY	15 May 2007	*Boufford & Jia 37614*	Y, but uncommon
***S. sphenanthera* Rehder & E.H. Wilson**	MO	May 2005	*Wang s.n.*	Y

FIGURES REFERENCED IN RESULTS
[FIGURES ARE NUMBERED IN THE LOWER RIGHT CORNER OF EACH IMAGE]

Abbreviations used in the figures of this study. A = axial parenchyma cell; AP = aperture in pit border; B = circular pit border; IA = inner aperture; M = margo; OA = outer aperture; P = perforation plate; PH = secondary phloem; PI = pith; R = ray parenchyma (cell); T = tracheid; TO = torus; V = vessel (member); VC = vascular cambium. XY = secondary xylem or wood. All figures depict *S. chinensis* except for Figure 16, which is *S. sphenanthera*, and Figures 25 and 26, which are *S. sphaerandra* and *S. pubinervis*, respectively.

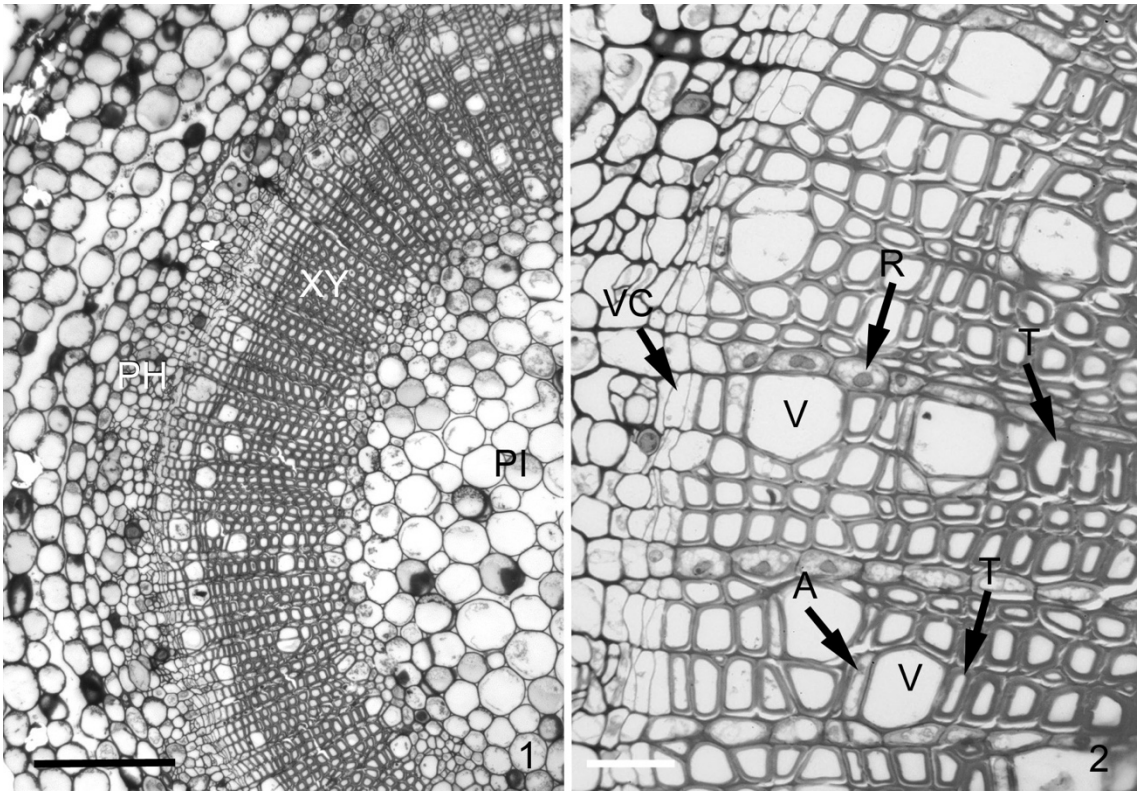


Figure 1. Trans-section of a branch showing secondary xylem and phloem. The portion of the branch was in its second year of growth when collected. **Figure 2**. A detail of the wood (secondary xylem) from Figure 1. A uniseriate ray is indicated, and vessel members and tracheids are distinguished one from the other. Scale bars = 100 µm (Fig.1) and 30 µm (Fig. 2).

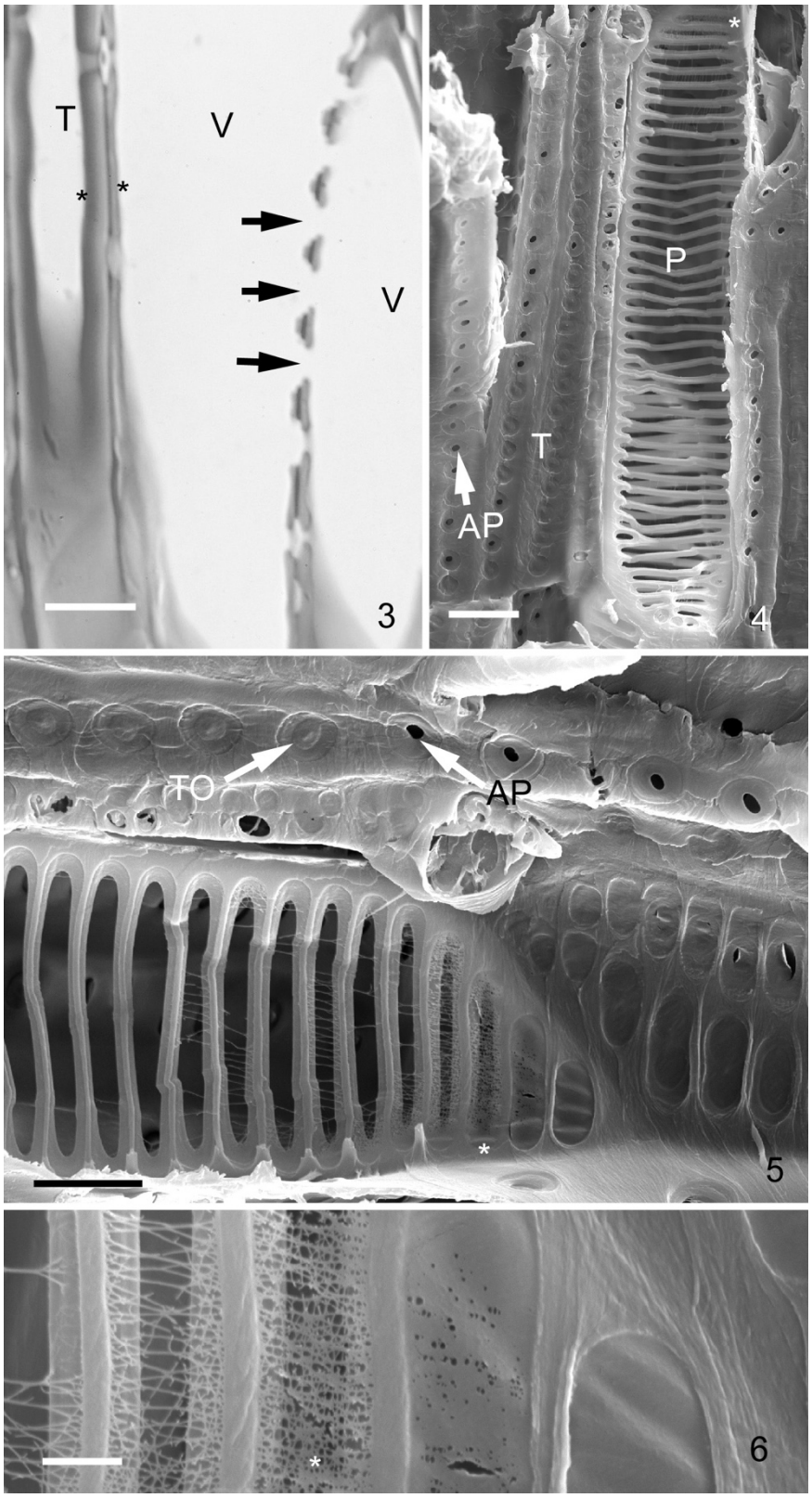
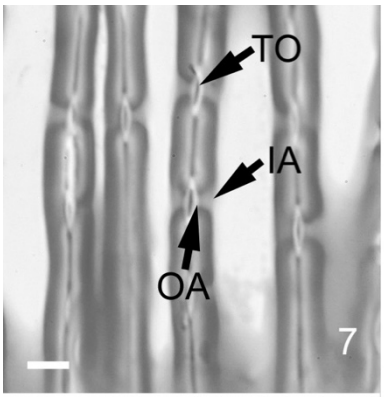
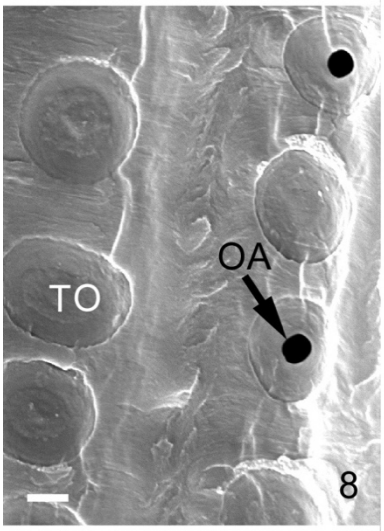


Figure 3. A perforation plate (seen in tangential longitudinal section) connecting two vessel members. Arrows indicate individual perforations. The difference between tracheids and vessel members as regards wall thickness and chemistry is evident (asterisks). **Figure 4**. A scalariform perforation plate seen in radial longitudinal section using a scanning electron microscope (SEM). The perforation plate is flanked to the left by three tracheids. Asterisks in Figures 4—6 indicate the same position in each figure. **Figure 5** is an enlargement of Figure 4 that is rotated 90º. A torus and a bordered pit aperture are denoted in a tracheid. **Figure 6** is a further enlargement of Figure 5. Note how the perforations grade into scalariform pits. Scale bars = 10 µm (Fig. 3), 10 µm (Fig. 4), 10 µm (Fig. 5) and 2 µm (Fig. 6).





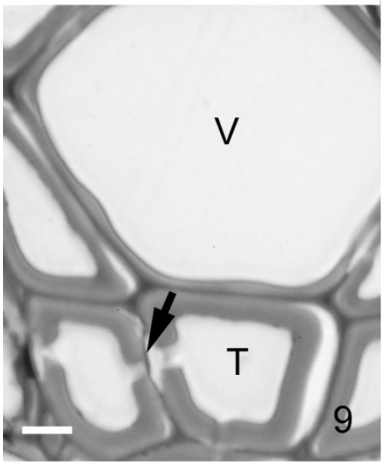
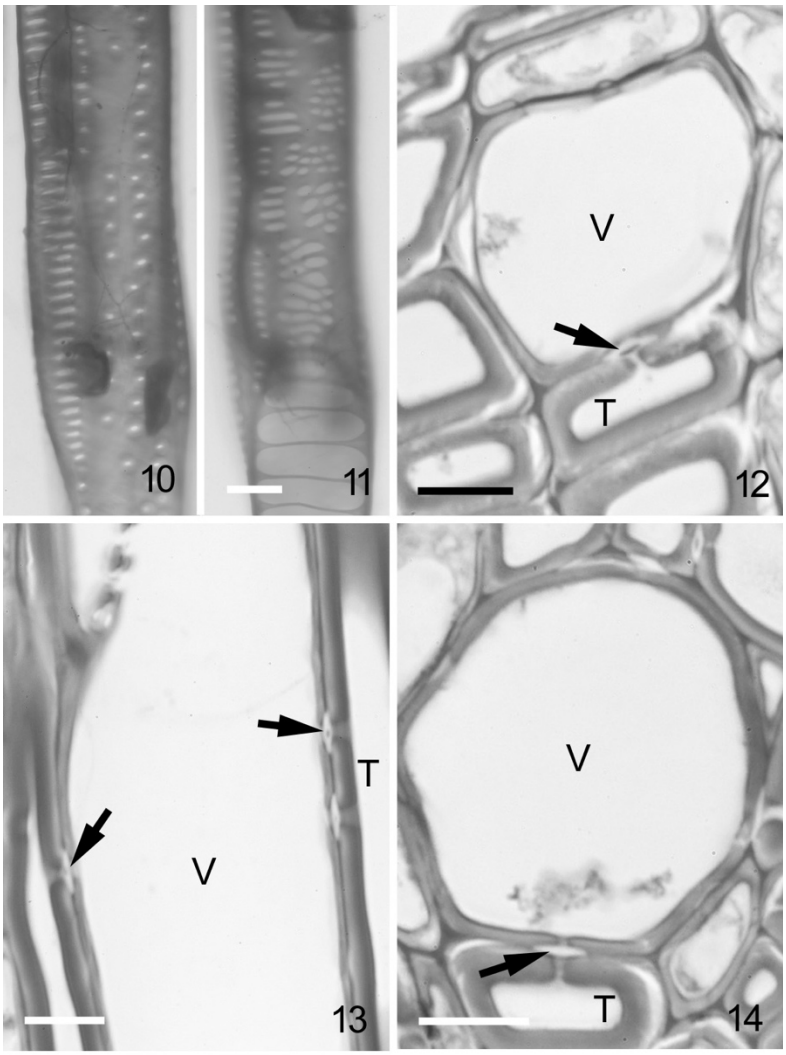
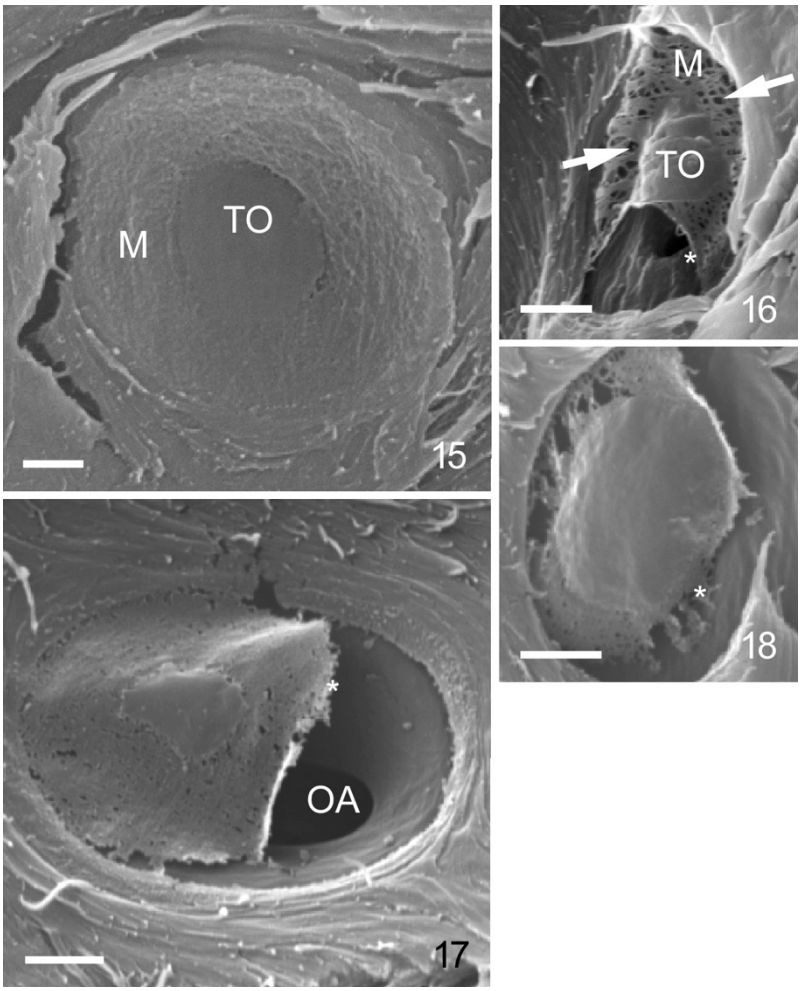


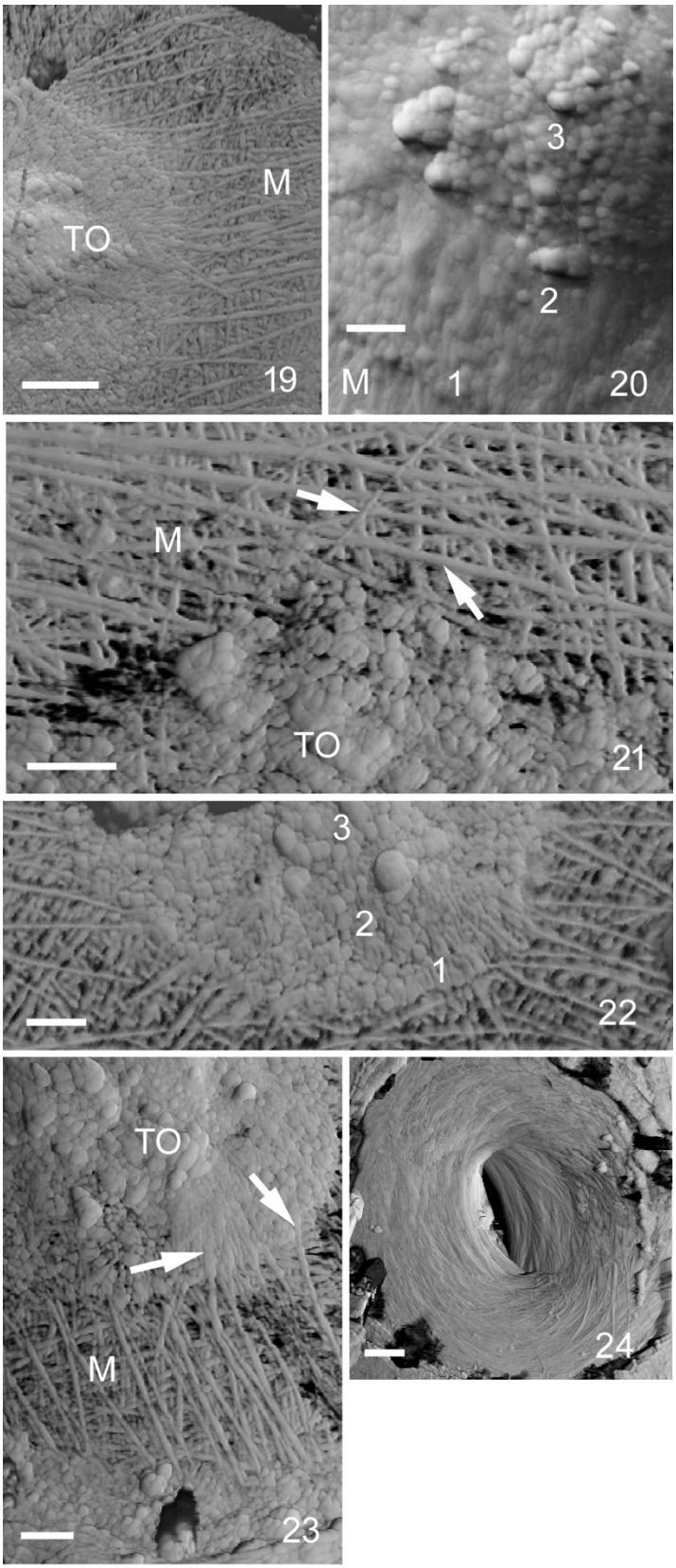
Figure 7. Light micrograph of a preserved specimen showing nonaspirated tori in sectional view. The outer *versus* inner aperture of a pit canal are indicated. Tori generally are of a greater diameter than the outer aperture. **Figure 8**. SEM of bordered pits in a tracheid wall of a herbarium specimen. In some pits the pit membrane and torus are exposed, in others, the pit membrane has been removed exposing the pit border with the outer aperture. **Figure 9**. Herbarium material in which the pit membrane is aspirated (unlabeled arrow) show how the torus completely occludes an aperture. Scale bars = 5 µm (Fig. 7), 2 µm (Fig. 8) and 5 µm (Fig. 9).



Figures 10, 11. Two focal planes of the same vessel member showing different pits on each cell surface. **Figure 12.** Wood transsection showing a well-developed torus (arrow) between a vessel member and a tracheid. **Figure 13**. A longitudinal section in which tori between vessel member and tracheids are poorly developed. **Figure 14**. A bordered pit pair between vessel member and tracheid in which the torus is absent (arrow). Scale bars = 20 µm (Figs. 10, 11), 10 µm (Fig. 12), 10 µm (Fig. 13) and 10 µm (Fig. 14).



Figures 15—18. Scanning electron micrographs of torus-bearing pit membranes in *Schisandra* wood. **Figure 15**. Air-dried specimen. Note the difference in surface texture between torus and margo. The woven nature of the latter is evident. **Figure 16**. An unusual air-dried specimen in which the pit membrane is damaged, but distinct openings are present in the margo (arrows). **Figures 17 & 18**. Both pit membranes were dried with HMDS. Neither pit membrane is aspirated, but considerable damage is present in each (asterisks). Very small openings are present in the margos. Scale bars = 1 μm (Fig. 15), 1μm (Fig. 16), 1 μm (Fig. 17) and 1μm (Fig. 18).



Figures 19—23 represent atomic force microscope images of torus-bearing pit membranes. **Figure 19**. An overall view of a pit membrane. Torus and margo are clearly distinct. The surface layer of the margo has microfibrils that run parallel to one another. **Figures 20 & 22** show the three regions of the torus. **Figure 21** is a detail of the pit membrane in which the margo is shown to consist of microfibrils of different diameters (arrows). The torus is clearly situated on the surface of the margo. **Figure 23.** A case in which microfibrils seem to take part in torus construction (arrows). **Figure 24**. This image reveals the fibrillar nature of the pit border. Scale bars = 0.5 µm (Fig. 19), 0.25 µm (Fig 20), 0.25 µm (Fig. 21), 0.25 µm (Fig. 22), 0.25 µm (Fig. 23) and 0.5 µm (Fig. 24).

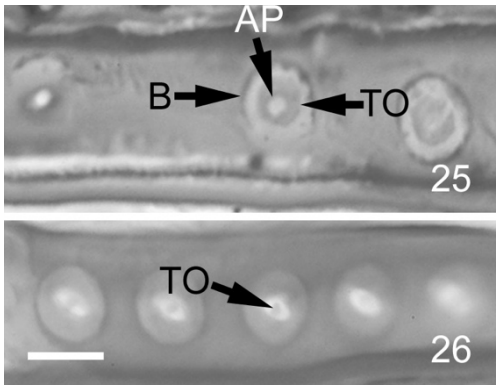


Figure 25. Circular bordered pits with distinct tori in wood of *S. sphaerandra*. **Figure 26**. Wood of *S. pubinervis* with minitori whose sizes were confirmed in trans-section. Scale bar = 5 µm (Figs. 25 and 26).

Wayne and Sara Finley: Alabama's Trailblazers in Medical Cytogenetics

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Wayne House Finley, MD, PhD (April 7, 1927) and Sara Crews Finley, MD (February 26, 1930 – February 20, 2013) were medical trailblazers and co-founders in 1962 of the Laboratory of Medical Genetics, the first such center in the Southeastern U.S. It was at that center that much genetic research was conducted over the years and patients and their families from throughout the Southeast were counseled regarding information on current offspring and the likelihood of genetic problems in future offspring.

Wayne was born at Goodwater, Alabama to Byron Bruce and Lucille House Finley. He was educated in the schools of Coosa County and graduated from Bibb Graves High School in Millerville, Alabama. He attended Jacksonville State University, where, after having his education plans interrupted (1945-46) by service in the U.S. Army Infantry's occupation forces in Germany, he received his B.S. in 1947. Following his active service in the military, he entered the University of Alabama, where he received the M.A. in 1950 and met Sara Crews, whom he married on July 6, 1952. From 1951-53, he was on active duty as an officer on the Staff and Faculty of the U.S. Army Chemical Corps. Despite these military interruptions, he completed the M.S. degree in Biochemistry (1955), and the PhD in Biochemistry (1958), both at the University of Alabama's Birmingham campus.

Shortly thereafter, he was admitted to the MD program at the Medical College of Alabama, later known as the University of Alabama School of Medicine (UASOM), which was located at what was to become the University of Alabama at Birmingham (UAB). He received the M.D. in 1960. Throughout his educational years, he remained in the military service, serving from 1946-74 as a member of the U.S. Army Reserve. Following the awarding of the M.D. degree, he completed in 1961 an internship in Pediatrics at the Jefferson-Hillman Hospital and Clinics, which was part of the Medical College of Alabama.

Sara was born at Lineville, Alabama to J. B. and Jessie Matthews Crews. She was educated in the public schools of Clay County, at Lineville High School, and at the University of Alabama, where she obtained the B.S. degree in 1951. Immediately after graduation, she was admitted to the M.D. program of the Medical College of Alabama, being one of the earliest women to be admitted to that medical school. She completed the M.D. degree in 1955, served an internship at Lloyd Noland Hospital in Birmingham in 1955-56 and did a National Institutes of Health Fellowship in Pediatrics at the UASOM in 1956-59.

Soon after completing her NIH fellowship in 1959, she and Wayne accepted faculty positions at the UASOM. Shortly thereafter, Dr. Joseph Volker, President of UAB, urged them to apply for National Institutes of Health-sponsored traineeships in medical genetics at the Institute for Medical Genetics at the University of Uppsala, Sweden. With their two young children, they traveled to Sweden and participated for one year in this fruitful and career-directing learning experience. It was there that they studied under Professor Jan Böök, Director of the Institute and an internationally known medical geneticist.

Establishment in 1946 that each person has 46 chromosomes, breakthroughs in the 1950s in the cultivation of human cells, and the demonstration in 1959 of the association between chromosome aberrations and abnormal clinical findings all resulted in the development of medical genetics research and service programs worldwide. Returning to Birmingham and the UASOM in 1962, the Finleys established the first medical genetics program in the southeastern U.S. Recognizing the valuable outcome of this work, the UASOM and the UA School of Dentistry supported this program, which, over the years, was also supported by the National Institutes of Health (Medical Genetics program and, later, its Pediatrics Oncology Group), several state agencies, private foundations, and some families who had a family member who had been treated at the clinic. On the basis of research based partly on UAB Medical Genetics Center data, Blue Cross-Blue Shield increased its reimbursement for medical genetics services, an action that greatly helped patients' families.

Believing that exposure of students early in their careers to a new field of clinical study might be most beneficial, the Finleys guided the development of a regional research, training, and service program for 35 years, and, in the process, trained both medical genetics professionals and physicians who would use medical genetics information to advise their patients and families, thereby preparing 57 PhDs, 1 DrPh, and 35 M.A.s (8 of whom later became MD's).

Elements of the medical genetics program consisted of genetics and genetic counseling clinics and several specialized laboratories for diagnosis of suspected genetic disorders. The laboratories evolved for general and cancer cytogenetics, clinical biochemical genetics, prenatal diagnosis, and clinical molecular and fluorescent *in situ* hybridization studies. Through the efforts of Jerry Thompson, PhD, a laboratory to detect mucopolysaccharidoses was developed, another first in the Southeast. It soon became a national reference lab. Over the years, the Medical Genetics Clinic provided care to thousands of patients and counseled thousands of parents about their child's current genetic problem and the likelihood of a genetic problem in a subsequent child. Initially, these clinics became and continued over the decades to be the busiest in the Southeast.

In addition, as a team and in coordination with their graduate students and employees, the Finleys engaged in genetic-related scientific research resulting in 247 publications. While being heavily involved in clinical services, graduate student training, and medical student education, the Finleys were also involved in the advancement of medical genetics nationally as a field of medical study. In support of that effort, the UAB Medical Genetics Program hosted several meetings: International Seminar on Medical Genetics (1966), National Medical Genetics Meeting (1970), 15th Annual March of Dimes Birth Defects Conference (1982), and the Annual Meeting of the American College of Medical Genetics (1996). Over the years and courtesy of the UAB Medical Genetics program, 103 professionals were sponsored for participation in professional meetings, and 91 professionals came to UAB to present and interact with graduate students in the UAB Medical Genetics program. The medical genetics instruction which UAB medical students received resulted in a large number of referrals over the years from those physicians and other physicians in Alabama and elsewhere in the Southeast.

In addition to their commitment to addressing genetically-related problems in patients, the Finleys participated in numerous local, state, regional, national, and international organizations. In addition, Wayne served as president of the UAB Chapter of Sigma XI, chairman of the

Alabama Healthcare Hall of Fame, the first director of the graduate study program in medical genetics at UAB, chairman of Medical Student Research Day at UAB for 10 years, chairman of the Reynolds Historical Library Associates Steering Committee for 25 years, and chairman of the Carey Phillips Travel Fellowship committee for medical students for 30 years. Over his long career, his honors were extensive and included key honors from his undergraduate institution, the UASOM Medical School, and the Academy of Pediatrics.

Sara's honors were equally noteworthy: the XXXI's 31 Most Outstanding University of Alabama Alumnae Award, Distinguished Alumna Award of the University of Alabama National Alumni Association, Distinguished Alumni Award of the University of Alabama Medical Alumni Association, American Medical Women's Association/National Library of Medicine Legends Award, Phi Beta Kappa, National Outstanding Alumna Award of Zeta Tau Alpha Women's Fraternity, 1989 Top 10 Women in Birmingham, Will Gaines Holmes Award from the Children's Aid Society, and Southern Women's Committee of 50.

In addition to her commitment to genetically-related problems in patients, Sara served for 20 years on the UASOM Admissions Committee and participated in numerous civic and religious activities, being the first woman member of the Rotary Club of Birmingham and a member of its Board of Directors, member of the Board of Directors of Compass Bank, the United Way of Central Alabama, and Girl's Inc. In addition, she was the first woman to serve as President of both the University of Alabama Medical Alumni Association and the Jefferson County Medical Society. She was also a member of the University of Alabama President's Cabinet for more than 10 years.

Recognition that they were indeed a team, resulted in them receiving numerous honors jointly: UAB Distinguished Faculty Lecture Award, Distinguished Service Award, and the Martha Myers Role Model Award (both by the University of Alabama Medical Alumni Association), Samuel Buford Word Award by the Medical Association of the State of Alabama, Gardner Award of the Alabama Academy of Science, Alabama Healthcare Hall of Fame, Brother Bryan Humanitarian Award by the Women's Committee of One Hundred, Birmingham Business Journal's Lifetime Achievement Award, establishment by UAB of the Finley-Compass Bank Conference Center, and establishment by the University of Alabama Board of Trustees of the Wayne H. and Sara Crews Finley Chair in Medical Genetics. A portrait of them is located in the Genetics Conference Center which adjoins the Kaul Genetics Building. Both retired in 1996 as Professor emeriti in 1996.

Additional Reading

Tennant S. McWilliams, New Lights in the Valley: The Emergence of UAB, The University of Alabama Press, Tuscaloosa, 2007), 114, 186, 245, 285, 370, 376.

Who's Who in America, 1974 (Wayne H. Finley, MD, PhD)

Who's Who in America, 1974 (Sara C. Finley, MD)
Alabama Health Care Hall of Fame, 2001 (Wayne H. Finley, MD, PhD & Sara C. Finley, MD) http://www.healthcarehof.org/honorees01/finley.html

Alabama Academy of Science, 2002 (Wayne H. Finley, MD, PhD & Sara C. Finley, MD)
Lifetime Achievement Award, Birmingham Business Journal, October 17, 2003 (Wayne H. Finley, MD, PhD & Sara C. Finley, MD)

Distinguished Faculty Lecturers, University of Alabama at Birmingham, 1983 (Wayne H. Finley, MD, PhD & Sara C. Finley, MD

Buford Word Award, Medical Association of the State of Alabama, 2003 (Wayne H. Finley, MD, PhD & Sara C. Finley, MD

Alabama Medical Alumni Bulletin (Wayne H. Finley, PhD, MD)
Bob Shepard, "Sara Finley, Pioneering UAB Geneticist, Dies at 82," UAB News, February 20, 2013. https://www.uab.edu/medicine/news/latest/item/61-sara-finley-pioneering-UAB-geneticist-dies-at-82

National Library of Medicine, Local Legends: Celebrating America's Local Women Physicians (Sara Finley, MD)

Distinguished Service Award, Alabama Medical Alumni Association, 2012 (Sara Crews Finley, MD)
http://www.alabamamedicalalumni.org/clientuploads/documents/2012awardwinnerswebsite.pdf

"The Passing of Pioneers" (Sara Finley, MD), UAB Medicine, vol. 39, no. 2, Summer 2013.

The Technological Imperative and Medicine

Dennis Samson, Samford University, Chair of the Philosophy Department, 800 Lakeshore Dr, Birmingham, AL 35229 dlsansom@samford.edu

My argument is this. Because of technology's success and dominance in medicine, our dependency on it has created a "technological imperative," and this imperative objectifies and frames people according to mechanistic rules. However, such a view of people lacks the ability to account for people's unique, moral, and spiritual dimensions. This aspect of the patient's identity requires an ethic that guides medical technology to serve the comprehensive purposes of human life; it needs a teleological ethic.

The Ethical Dilemma of Using Technology in Healthcare

The use of technology in healthcare can mean a stethoscope, needle, hammer, or scalpel. These instruments, over which we do not have moral quandaries, are natural extensions of the hands, ears, and eyes.

However, sophisticated machines such as EKGs, MRIs, CAT Scans exercise diagnostic and therapeutic influence on patients that are more than natural extensions. They replace the user.[1] They discover and decipher information and some attempt correction and therapy. The ethical worries about technology are about it being an independent force from the user.

Technology, undoubtedly, contributes significantly to healing, and this should continue, but, to be so successful, it by design treats the patient as an object explainable by algorithmic analyses. The user thus looks upon the patient in the same way that technology is applied upon the patient— organism following mechanistic laws so that the use of technology can yield measurable and quantifiable results. Of course, the chemical-physiological aspects of the person follow law-like patterns and hence are measurable and somewhat predictable. Yet, the laws cannot measure people's personal identity; that is, our sense of being the same person across time, our emotional makeup, and the defining relationships with others and the world. This personal aspect is as affected by disease, injury, and psychological duress as is the individual's chemical components. However, the more the use of technology succeeds, the more its users must objectify the patients, and, consequently, the more it either overlooks, ignores or deemphasizes the personal identity of patients.

Here is the ethical dilemma. To contribute to people's health through technology, medicine must view patients as organisms measurable by nomological patterns (that is, law-like patterns). Because their success as healthcare providers depends on the successful application of technology, the providers may acknowledge the patient's personal aspects, but they cannot make them a primary focus of their treatments.

In that technology, due to its accomplishments, dominates medical practices, it has also become domineering.[2] Medicine, thus, must view the patient in ways amenable to technological

Notes

[1] I am borrowing from Stanley Reiser's definition of technology, "material inventions developed to extend or replace human capabilities," (Cole, 2015, 75).

analysis and description. Technology's success generates a demand, that is, to continue to improve healthcare, we must increase the use of technology. This is the "technological imperative."[3] We are logically and professionally forced to think that if we do not increase the use of technology, we are not as committed to increasing the health and healing of patients. Subsequently, we anthropomorphically confer onto technology an agency. We say, "what does the instrument tell us" or "machines don't lie." Technology does not talk or tell lies. It cannot. Its output, which we read, is strictly algebraic, the conferring of symbols upon data. The mystique of technology transforms it from a blind and soulless machine into an intelligent agent who engages our minds in a dialogue about the patient.

Moreover, for those inclined toward philosophical materialism, the technological imperative makes the assumptions of reductionist materialism even more convincing. If we can heal without taking into consideration the personal aspects of a person, we can treat these aspects as epiphenomenal to the body. They are not actual parts of what are treatable, and thus we need not factor them into the scientific treatment of patients. Others can address these aspects, but they are beyond medicine's therapeutic interests. The person is reduced to physical-chemical explanations, and healing becomes the rectifying of the physical-chemical problems. In this respect, healthcare providers are always under the pressure to become in their practice and outlook upon patients thoroughgoing philosophical materialists. Consequently, their professions become expressions in tangible practices of a tendentious metaphysical position, one that disregards the anomological aspects of human identity.

Ethics Serving Technology or Technology Serving Ethics

Because technology naturally tends to dominate the diagnostic and therapeutic actions of healthcare, it de-emphasizes the personal identity of patients by framing them into technicized categories and by making any encompassing purpose greater than the successful application of technology to be epiphenomenal to the person. Thus, we are faced with a choice—does ethics serve the power and promise of technology so that medicine can continue to fulfill its moral commitment to heal or should technology help medicine contribute to the societal goal of experiencing the profound happiness achieved in realizing a sense of human well-being, a final aim?

This choice arises from within technology's essence.[4] On one hand, technology determines its application upon patients and also defines its own successful application, and, on the other hand,

[2] Albert Jonsen is right when he says, "The crucial ethical problem, then, is to measure the use of technology to the fragility of life. The fragility of life had dominated medicine since its inception; now the power of technology has assumed priority" (Jonsen, 2006, 670).

[3] The phrase is common, but for a description of it see Weissman, 2016, 2.

[4] The question of "what is technology's essence" has generated much discussion, and, although an important topic for this paper's concern, unpacking that discussion would distract from the paper's argument. However, behind much of what I say in the paper about technology's role and power are arguments found in the following books: Jacques Ellul's *The Technological Society*, *The Technological System*, and *The Technological Bluff* ("The abstraction, Man, is only an epiphenomenon . . . a natural secretion of technical progress" [Ellul, 1964, 390]; Martin Heidegger's "The Question Concerning Technology" ("Unlocking, transforming, storing, distributing, and switching about are ways of revealing" [Heidegger, 1977, 16]); and Hans Jonas' *Philosophical Essays: from*

technology is an artifact created by people and thus manifests the nature of causal forces—the efficient, material, and formal dimensions of causal efficacy. However, because the technological imperative mandates a materialistic assessment and understanding of the patient, it obstructs the formulation of a final cause or purpose. A final cause, having the capacity to fulfill an overarching number of pursuits than the specific act of technology, has greater ontological significance than what may be the efficient, formal, and material cause of an action or artifact. It compels moral actions toward a surpassing and comprehensive reality by creating ways to actualize the natural desire to seek a completed action.

A final aim is beyond what technology can articulate. Even though we should further the success of technology, we must also realize that it cannot contribute to an understanding of a moral purpose comprehensive enough to serve as a final aim of human pursuits.

What kind of ethic would best serve the therapeutic and caring purposes of healthcare and at the same time control and guide the technological imperative?
It would not be preferential utilitarianism. Such cannot correct the potential of technology to de-humanize people. This is the ethic of the Princeton ethicists, Peter Singer. Singer's basis for ethics is simple—that is, an action is desirable and permissible if it maximizes a person's interests or preferences,[5] which he believes expresses the natural biological impulses, making irrelevant any appeal to God, substance, natural law, or intuitions. Our interests are as identifiable to us as are our natural urges, so Singer contends, and hence are easily indexical, enabling us to take guesswork and mystery out of ethical decisions.

However, preferential utilitarianism cannot contain the technological imperative. Because our interactions with objects, social experiences, and future prospects shape our specific preferences, the preferences become a way of measuring and forming which objects etc. can actually formulate a preference for us. The greater we can create and measure preference-creating events, the more preferences we identify and chose. The application of technology increases our ability to create and measure preference-creating experiences and consequently, the more we use technology, the more preferences we can maximize.

However, by conferring such power to technology, it subsequently frames persons into objects amenable to technological measurements, into objects defined by what can be technologically analyzed. Thus, the best preferential utilitarianism can do in facing the dilemma of medical technology is perhaps to offer guidelines but never a goal, and, thus, it becomes an acolyte to the technological imperative.[6]

Ancient Creed to Technological Man and *The Imperative of Responsibility: In Search of an Ethics for the Technological Age* ("In other words, technology, apart from its objective works, assumes ethical significance by the central place it now occupies in human purpose" [Jonas, 1980, 11]).

[5] Singer reduces the search for the criterion of morality to interest or preferences. Animals and humans are equal in that they can have interests (Singer, 1985, 9). He claims that we should consider something morally significant if it can suffer because the "capacity of suffering and enjoying things is a pre-requisite for having interest at all (Singer, 1986, 221)." In fact, we should consider the pre-born human embryo morally significant only when it become sentient, can fell pain; "until that point is reached, the embryo does not have any interests and . . . cannot be harmed—in a morally relevant sense (Singer, 1990, 73)."

However, ethical decisions are not merely identifying preferences and interests. They are more like what University of Pittsburgh philosopher Michael Thompson calls "life-forms" (Thompson, 2008, 62); that is, the internal directivity of certain actions compelled to act in ways properly called ethical, like the DNA of an animal compels it to be the species it is. There are four reasons to agree with this explanation. First, ethical actions are unique because of their particular directivity and intentionality. We distinguish (at least try) them from other actions.

Second, this type of activity is goal-oriented, aimed toward comprehensive aims that justify the actions. We do them for reasons.

Third, these comprehensive-fulfilling goals determine which actions are proper ones and whether the actions are acting properly. For example, the reality of a baseball game describes which actions by the players should occur and whether they occur successfully. And fourth, the properness of these actions is thus attributive, not predicative, to the actions, in that they describe the internal orientation of the actions, rather than an addition to the action's innate directivity. Therefore, it is cogent to maintain that ethical actions are aimed toward a more comprehensive aim.[7]

Moreover, it follows that the final aim fulfills all the life-forms aimed toward more and more comprehensive fulfilling experiences. If indeed it is a final aim indicated by the directivity of life-forms that aim for it, then consequently there is the expectation of completion, of fulfillment of human purpose. Furthermore, a telos that fulfills also cannot be conditioned by a more comprehensive reality; its completing power cannot depend upon another reality. Due to the fact that the telos fulfills the immanent process towards fulfillment, it must be an unconditional reality, not one in process or becoming toward another reality. Thus, it is reasonable to maintain that the telos exists, fulfills the inner directivity of human nature to find happiness, and is an unconditional reality.[8]

Conclusion

[6] Samuli L. Saarni et al conducted a study to determine the influence of ethical deliberation upon the selection and use of technology in medicine. They concluded, "The work is based on the insight that ethics seen as an 'add on' to solve the moral issues of a technology is likely to have little effect on the implementation of the technology. Ethical analysis performed in isolation of the HTA [that is, Health Technology Assessment] process appears to be too narrow and come too late (Saari, 2008, 620)."

[7] My claim needs to acknowledge and also reject what a Kantian-ethicist would say at this point. A Kantian would say strict adherence to the "categorical imperative" is enough to safeguard humanity from the potential abuses of the technological imperative. All we need is "to treat oneself and all others *never merely as means* but always *at the same time as ends in themselves*" (Kant, 1996, 83). Even though to determine the source of ethical obligation Kant focuses on the autonomous individual rather than the utilitarian consequences, his definition of such a person as a rational agent who can formulate a universal and necessarily true moral claim narrows the ethical field of players too much. The very young, the feeble old, the mentally disable, the comatose lives crippled by illness cannot reason this way, and hence are not under ethical obligations, and consequently are treated by those who can follow the "categorical imperative" as only recipients of good-will but not as rational agents with dignity, thereby making them more vulnerable to technological abuses than would be the adult rational agent.

[8] The Thomistic influence here should be obvious, "Final and perfect happiness can consist in nothing else than the vision of the Divine Essence." (Aquinas, 1990, 381).

Medicine needs a teleological ethic to control the technological imperative and to help medicine contribute to human well-being.

A teleological ethic has two aspects—that is, horizontal and vertical. Although they are distinct in their aims, they define each other. The horizontal aspect refers to the immediate aims of an action compelled by a particular person's life-form. For instance, health is the immediate aim for eating good food.

However, health is not the only compelling reason why we should eat good food. We reasonably believe that health is a necessary means to gain a greater aim than health itself—that is, happiness in the sense of human well-being. Hence, we determine heath to be a legitimate aim because we realize that to fulfill our innate drive for fulfillment, we should seek happiness. Moreover, though happiness as well-being can justify many pursuits as necessary means to greater goals (for example, courage, generosity, friendship, and so on), happiness must also have a purpose. We can fulfill all the social virtues but still realize there is a greater reality than the sum of our actions. Aristotle would call this dimension the contemplation on "a reality that cannot be otherwise;" Aquinas would call it our worship of the perfectly good reality, God. God would thus be the vertical aspect of the teleological directivity that adjudicates which aims finally lead to human fulfillment.

The horizontal aspect of a teleological ethic would ask of any application of technology, does its use promote a sense of a fulfilled life greater than the mere the successful application of the technology? The application should contribute, for example, to the health of the patient. Health then is the horizontal aim of technology. A teleological use of technology would not consider using technology just to use it; would not describe a person primarily as a machine-like organism reducible in analysis to the aims of the successful application of technology. We thus would justify technology in medicine only if its aim is the health of patients.

Consequently, this restriction would reject using technology in medicine primarily to secure and increase the use of technology and also would alert us to the domineering effects of the technological imperative. For example, it would be unethical to implant nanochips in a person just to see how they would work or use them in a non-remedial sense to make a person smarter. These would be unethical because the values of such implants are merely technological values, values relative to a machine and not relative to humans with a life-form. The principle is this—the applicative value of technology in medicine must be whether it enhances the values of a life-form aimed at health, not whether it makes for a more productive or effective machine-like organism. However, we must also understand the aim of health.

Our recognition of the vertical aspect is just as needed and important to curb the technological imperative. Because with technology we assume we can conform nature to our preconceived goals for it, the basic motivation of the technological imperative is "knowledge is power." It confers tremendous control to those who apply technology and those who benefit from it. Yet, this aim reduces people to materialistic systems, measurable by algorithms and laws of mechanical engineering. This reduction is a constant temptation to medicine and should be countered with what philosopher Hans Jonas calls the sacred (Jonas, 1980, 19), with those

objects and practices that indicate an unsurpassable reality in which people are ultimately oriented in their life-forms. It would be consistent with the goal of medicine (that is, to contribute to people's health so that they can experience happiness in relation to the final aim) to find ways to incorporate the objects and practices that indicate a transcendent purpose; for example, religious symbols in hospital and clinical rooms, chapels and prayer rooms, chaplains playing a vital part in healthcare, acknowledgment of patient and family's religious lives, and so on.

This vertical aspect would focus the various aims of healthcare toward an ontologically surpassing reality inclusive of all human actions directed towards comprehensive fulfillment. Of course, some people may be indifferent or ignorant of sacred objects and practices, but their presence alongside the practices of medicine would indicate that the practitioners serve a greater and more comprehensive purpose than their immediate technological actions, thereby encouraging and enabling people to better pursue and realize their internal directivity towards what properly describes an ethical person.

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MINUTES OF THE
ALABAMA ACADEMY OF SCIENCE
Executive Committee Meeting
Samford University
Room 033, William Propst Hall
October 6, 2018

Meeting was called to order at 8:35 am by President, Drew Hataway. Those in attendance were:

Ellen Buckner
Cameron Gren
Drew Hataway
Mark Jones
Akshaya Kumar
Larry Krannich
Ken Marion
Prakash Sharma
Jack Shelley-Tremblay
Chris Stopera
Brian Toone

Jack Shelley-Tremblay moved to approve the minutes and Ken Marion seconded. Minutes were approved.

The following is an update of the Action Items from the Spring Executive Committee meeting was done.

Action Items from Spring 2018 Executive Committee Meeting			
Action Item	**Person Responsible**	**Due Date**	**Action**
Hiring of videographer for development of videos to showcase the activities of the AAS.	Public Relations Committee (Brian Burnes)	Fall 2018	Video Produced, to be viewed and discussed
Distinguished Service and Outstanding Leadership Award.	The Long-Range Planning Committee (Akshaya Kumar, Acting Chair)	Fall 2018	Carried forward to Spring 2019
Role of Graduate Students in AAS Governance**	Special topic for discussion raised by AAS member	Fall 2018	New Business
Appoint a second Vice-President for 2018-19**	Cameron Gren to present a nominee slate	Fall 2018	See report, C15
Action Item from Spring 2018 Business Meeting			

Explore possibility of an Associate Executive Director (see meeting minutes)**	Akshaya Kumar and Long-Range Planning Committee	Fall 2018	See report, C5

**Added since Spring 2018 Executive Committee Meeting

Ellen moved that Jack Shelley-Tremblay be appointed as Second Vice-President. The Executive Committee concurred.

President Hataway then proceeded to go through the Committee reports. The minutes reflect the report discussions that occurred.

B-6: The Treasurer's report was approved. Ellen notified the Committee that the net revenue to the Academy from the 2018 Annual meeting was $4,556.45.

B-7: Brian Toone, JAAS editor, noted that the electronic edition of the 2017 issue is online at www.aasjournal.org and a link will be added to the Academy website. Use is being made of the Open Journal System for reviewing and publishing the Journal. Soon an email will go to all members with the PDF version of the journal and a message that the Academy has moved to electronic versions of the journal. Members will be asked to reply to the email should they opt in for the hardcopy versions of future issues of the journal. [Action Item] Drew asked that the editor develop a list of duties and responsibility of the associate editors. [Action Item] A suggestion was made that the editor, associate editors, and interested individuals hold a working group meeting at the annual meeting on Thursday (February 21, 2019) at noon. [Action Item].

B-8: Mark reviewed the major issues in the report. The video to highlight Academy activities was viewed. Although the Video was to showcase the Academy (both AAS and AJAS), the produced product focused only on the AJAS. The committee was impressed with the quality of the video and discussed distributing it to school district throughout the state. It will be put onto the Academy Facebook page. There is potential in using it for development and breaking it up into snippets that can be used to enliven the Academy website. Also, it should be distributed to all Academy members. [Action Item] Further discussion about additional video development will occur at Fall 2019 Executive Committee meeting. The Academy approved $500 to support video acquisition of AAS activities at the Tuskegee 2019 meeting.

B-12: The Committee noted that very few section chairs attend the Executive Committee meetings. There was a brief discussion about developing a virtual aspect to the Exec. Comm. meeting to remove barriers to attendance and increase participation. [Action Item for Spring]

B-13: The Call for Papers was approved. This will be distributed to members and posted on the Academy website in mid-November.

C-1: The Provost at Tuskegee has graciously picked up the cost of the Kellogg Conference Center and some vendors have committed financial support for the meeting. The first 50 completed registrations will receive a conference portfolio. In the past, many student presenters have not registered for the meeting and presenter registration will be emphasized. The Abstract submission site should include a statement that "all presenters must be registered for the meeting." [Action Item] Dr. Kumar made a Power Point presentation on conference details, including food, breaks, room layout, etc. The committee recommended that Banquet fee be set at $40 for members and nonmembers prior to Feb. 11th and $50 thereafter. Other registration fees were approved as per

the draft registration form. There will be extensive advertisement of the meeting with special emphasis on the Nobel Laureate banquet speaker.

C-5: Approved a motion to create a position of Associate Executive Director as per the guidelines and the guidelines be included in the By-Laws. The position will not be filled until after the Spring 2019 Executive Committee meeting. [Action Item]

C-9: Need to contact individuals about hosting the 2022 meeting.

C-12: Approved the motion to change the By-Laws (Article IV, Section 1q) with a modification: remove "not later than December 1" and "very" before "…exceptional individual."

C-18: Jack indicated that the web-analytics received are not very helpful. He felt there was a need to modify the website to provide more active interaction with users. We may want to have a "subscribe to updates" option. This would send any updates in our blog posts to members of the site using email, automatically.

New Business: The Committee discussed the creation of a "graduate student at large" member of the Executive Committee position. Cameron Gren will develop a draft amendment that addresses this issue. [Action item]

Questions were raised about the titles for Sections V and VIII and whether alternatives may be more attractive to potential and current members. Also, there was discussion about the role and responsibilities of Section Chairs/Vice-Chairs. This discussion will continue at the Spring 2019 meeting and include a consideration of possible Chair/Vice-Chair training. [Action Item]

The meeting was adjourned at 12:20 pm.

Action Items from Fall 2018 Executive Committee Meeting			
Action Item	**Person Responsible**	**Due Date**	**Action**
Send JAAS Issues to members with opt-in hardcopy instructions	Larry Krannich	Oct. 2018	
Distinguished Service and Outstanding Leadership Award.	The Long-Range Planning Committee (Akshaya Kumar, Acting Chair)	Carried forward to Spring 2019	
Role of Graduate Students in AAS Governance**	Special topic for discussion raised by AAS member	Spring 2019	
Develop a list of duties & responsibilities of associate editors.	Brian Toone	Spring 2019	
Schedule a working meeting of editor, associate editors, and interested individuals for noon, Thursday, February 21, 2019.	Brian Toone	Spring 2019	
Distribute produced video to state school districts	Ellen Buckner	Fall 2018	
Post produced video on Academy website	Jack Shelley-Tremblay	Fall 2018	

Distribute produced video to all Academy members	Ellen Buckner/Larry Krannich	Fall 2018	
Discussion of additional video development	Ellen Buckner	Fall 2019	
Discuss implementation of virtual Fall Exec. Committee meetings	Exec. Committee	Spring 2019	
Place "All presenters must be registered for the meeting." Statement on the Abstract/Title Submission site	Jack Shelley-Tremblay	Fall 2018	
Update the By-Laws with Associate Executive Director guidelines	Larry Krannich	Fall 2018	
Update the By-Laws with amended Article IV, Section 1q approved at Fall 2018 Exec. Comm. meeting	Larry Krannich	Fall 2018	
Post updated By-Laws on Academy website	Larry Krannich & Jack Shelley-Tremblay	Fall 2018	
Develop a draft amendment to the By-Laws for a "graduate student at large" Executive Committee position	Cameron Gren	Spring 2019	
Discuss and formulate the roles and responsibilities of Section Chair/Vice-Chairs	Executive Committee	Spring 2019	
Discuss utility of Section V and VIII titles	Executive Committee and Section V and VIII chairs	Spring 2019	

Alabama Academy of Science Journal

Scope of the Journal:

The Alabama Academy of Science publishes significant, innovative research of interest to a wide audience of scientists in all areas. Papers should have a broad appeal, and particularly welcome will be studies that break new ground or advance our scientific understanding.

Information for the Authors:

- Manuscript layout should follow the specific guidelines of the journal.
- The authors are encouraged to contact the editor (E-mail: brtoone@samford.edu) prior to paper submission to obtain the guidelines for the author.
- At least one author must be a member of the *Alabama Academy of Science* (except for Special Papers).
- The author(s) should provide the names and addresses of at least two potential reviewers.
- Assemble the manuscript in the following order: Title Page, Abstract Page, Text, Brief acknowledgments (if needed), Literature Cited, Figure Legends, Tables, Figures.

Review Procedure and Policy:

Manuscripts will be reviewed by experts in the research area. Manuscripts receiving favorable reviews will be tentatively accepted. Copies of the reviewers' comments (and reviewer-annotated files of the manuscript, if any) will be returned to the correspondent author for any necessary revisions. The final revision and electronic copy are then submitted to the *Alabama Academy of Science Journal* Editor. The author is required to pay $100 for partial coverage of printing costs of the article.