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Susan Lesyk, Director, Teaching and Learning Center
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Acknowledgements

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appreciation the guidance and
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**University of Oregon
McNair Scholars Program
Research Journal**

2017

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**Flexibility and Stability
in a Task-Switching Paradigm**

Dagger Anderson
Psychology

Mentor: Ulrich Mayr
Psychology

Mentor: Jason Hubbard
Psychology

A common method to assess executive control is the task-switching paradigm. It is well known that reaction times are longer when subjects switch tasks compared to when the same task is performed over consecutive trials. This reduction in performance when flexibility is required is referred to as a switch cost. It is not well understood how switch costs are influenced by a rapidly changing environment that sometimes demands more flexibility (i.e., more switching) and other times requires more stability and resistance to distracting information. Prior research using between-subjects designs suggests that people can indeed adapt to a more flexible or stable state depending on switch rate. Unlike previous research, the current paper investigates how low and high switch-rate conditions influence the tendency to adopt a flexible or stable state as a within-subject manipulation. Results indicate that people can quickly adapt their behavior to meet the demands of both flexibility and stability in a sample of college students.

Passive Carryover and Conflict Triggered Regulation: Differences in Early Visual Components

Bradley Boyce

Psychology

Mentor: Ulrich Mayr

Psychology

Our cognitive system has the ability to adapt control settings to fit current task demands. One example of this is the conflict adaptation effect, which is the reduced response time-cost shown in a set of conflict trials that follows an earlier set of conflict trials. A previous study used participants' fixations on distractors to show that high levels of *experienced* conflict in trial $n-1$ led to a diminished conflict effect in trial n . Given the importance of *experienced* conflict and the involvement of the $N2pc$ event-related potential (ERP) in distractor suppression, we plan to conduct an electroencephalography to explore the $N2pc$ response and to measure participants' experience of conflict by using a traditional response-conflict task.

Pioneers of Planning: An Urban Planning Analysis of the Castro since 1985

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History of Art and Architecture

Mentor: Ocean Howell

History of Art and Architecture, Honors College

By focusing on the urban-planning practices of the Castro district in San Francisco, my research explores how urban planners began to include the Castro into their urban-planning practices as a recognized gay community. That process began after the election of Harvey Milk, the first openly gay person to be elected into public office in California. Beyond the history of gay rights, the story of the Castro is under researched. From a perspective of urban planning and analysis, the relationship between community housing, the aids epidemic, and continuing homophobia offers a narrative of the gay community's resilience. Texts by Castell, Godfrey, and Boyd recount the urban development of San Francisco's various ethnic and sexual communities. Important sources of documentary information were the San Francisco Library History Room, the James C. Hormel LGBTQIA Center, and the Bancroft Library in Berkeley. My research reveals that even after Harvey Milk, the Castro faced governmental oppression not only because of homophobia but also because of the aids epidemic of the 1980s-1990s. The Daughters of Bilitis helped the process of planning and organizing in the Castro, but not until the mid-1990s was an urban planning organization for the Castro founded—the Castro Area Planning and Action.

Age Differences in a “Short Long-term Memory” System for Visual Information

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Psychology

Mentor: Ulrich Mayr

Psychology

Mentor: Atsushi Kikumoto

Psychology

Age differences in memory are particularly pronounced when there is opportunity to actively consolidate and elaborate information during encoding. Endress and Potter (2014) developed a paradigm to assess memory for non-consolidated/non-elaborated information by testing recognition for visual objects that were presented at a very rapid pace (i.e., 4 objects per second). Younger adults exhibited a surprisingly large capacity for this type of information, correctly identifying 70-80% of images. Performance declined when the same set of images was used across trials, suggesting that some elaboration is required to counteract proactive interference. If age-related differences are due to elaboration, we expect no age differences in the low-interference condition but emerging age differences in the high-interference condition. We compared 30 college students to 30 older adults, aged 65-80, using the Endress and Potter paradigm. Initial results suggest a striking absence of age differences in the low-interference condition, but—surprisingly—also no age differences in the high-interference condition.

Markers of Cardiovascular Health in Chronic Marijuana Smokers

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Biochemistry

Mentor: Christopher Minson

Human Physiology

Chronic tobacco smoking is known to decrease cardiovascular health, but the long-term effects of chronic marijuana smoking have not been well studied in humans. Although the chemical cocktail in each type of smoke differs, there are many common factors, such as hot particulates and polycyclic aromatic hydrocarbons, that increase the risk of cardiovascular disease. Thus, the cardiovascular health of tobacco and marijuana smokers may be similarly impaired. However, recent studies of marijuana smoke suggest possible cardiovascular protective properties. Therefore, the purpose of this research is to examine markers of cardiovascular health in marijuana smokers in comparison with tobacco smokers and non-users. We expect to find decreased cardiovascular function and increased cardiovascular risk in marijuana smokers, but not to the extent of tobacco smokers.

Sequelae of Maternal Trauma: Attachment Relationships and the Development of Empathy in the Next Generation

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Psychology

Mentor: Jennifer C. Ablow

Psychology

Mentor: Jeffrey R. Measelle

Psychology

Mentor: Xiaoning Sun

Psychology

Mothers with trauma and adverse mental health histories are at risk for not accurately interpreting their infants' emotional bids and responding to their children in frightened or frightening ways. This contributes to attachment disturbances, and, potentially, to problems in the child's own development of empathy. Surprisingly, few studies have examined how child empathy develops within the context of the primary attachment relationship or how maternal trauma and depression contribute to individual differences in children's development of empathy. The current study originated with two central aims: (1) to determine whether infants' displays of empathy differ according to the quality of their attachment; and (2) to explore the extent to which maternal trauma and depression contribute to these differences. Thus, this study seeks to advance our understanding of how maternal characteristics impact children's empathy within the attachment context and to identify the mechanism(s) by which the capacity for empathy is transmitted across generations.

Fluid Borders, Neo-Liberal Displacements: Current Push and Pull Factors Driving Latin American Migration

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Sociology

Mentor: Aaron Gullickson

Sociology

Mentor: Jessica Vasquez Tokos

Sociology

An estimated 12 million undocumented migrants live in the United States, a number that continues to increase because of state-sanctioned violence, extreme poverty, and exploitation in the wake of US involvement in Latin America during the last 75 years. A new strategy has been deployed by Latino migrants over the last few years that involves surrendering themselves at the border seeking asylum in lieu of attempting to cross illegally. Once the migrants have been taken into custody, they are released in the U.S. pending a federal asylum hearing. While awaiting their hearings, these migrants are in a state of flux, attempting to navigate this grey area, being *documented* undocumented immigrants. A grounded theory approach using qualitative interviews with undocumented immigrants currently in custody and informal interviews with ICE agents will attempt to shed light on this new status of being a *documented* undocumented immigrant. The project explores whether this new shift in migration status while awaiting trial helps navigate the asylum process.

Leafcutter Ants Inside the Nest Have Sharper Mandibles than Ants Outside the Nest

David M. Lee

Biology

Mentor: Robert Schofield

Physics

Mandibular wear has a strong impact on leafcutter behavior and energetics. We report that the mandibles of ants outside the nest have higher wear on average than the mandibles of ants inside the nest by a factor of 2.14 ($p = 5E-18$). 95% of ants found outside the nest had wear that was more than .025 mm, and 70% of ants found inside the nest had wear that was less than .025 mm. This wear difference between ants inside the nest and outside the nest may indicate that ants do not go outside of the nest until their mandibles have worn, and that ants with the sharpest mandibles stay in the nest where most of the cutting occurs.

Feelings of Belonging and Future Persistence in STEM

Kristina Lowney

Psychology

Mentor: Sara Hodges

Associate Dean of the Graduate School, Psychology

The present research explores two variables that may contribute to women's underrepresentation in STEM fields: low feelings of belonging in STEM fields and aversion to the "science nerd" stereotype. Ninety-eight dyads composed of graduate and undergraduate students from the same STEM field held 10-minute video-recorded conversations about graduate school. Undergraduate students with an interest in graduate study in a STEM field asked questions of current STEM graduate students who offered feedback about grad school preparation. After the interaction, undergraduate students completed questionnaires about their feelings of belonging in STEM and intentions to pursue graduate study in STEM. We also coded the conversations and rated how well each graduate student fit the "science nerd" stereotype. We predicted that undergraduates who reported lower feelings of belonging in STEM and who interacted with more "nerdy" graduate students would report lower motivation to persist in STEM fields. Additionally, we predicted that the lowered motivation would be especially true for female undergraduates. Results indicated that there was no significant difference between male and female undergraduates' feelings of belonging and future persistence in their STEM field. Also, we found that being paired with a "nerdy" graduate student was not a significant predictor of an undergraduate's future persistence in STEM. However, feelings of belonging, regardless of gender, may be related to decisions to persist in STEM fields.

Transfer of Fungal Endophytes from Leaves to Woody Substrates

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Biology

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Biology and Institute of Ecology and Evolution

Fungal endophytes have been found in all plants surveyed to date, yet for many fungi the function of endophytism is still unknown. The Foraging Ascomycete Hypothesis (FAH) proposes that saprotrophic fungi utilize an endophytic stage in leaves to modify dispersal. Under this hypothesis, leaves can provide food and water during time of environmental scarcity, and they can transport the fungi to other substrates upon dehiscence. If the FAH is accurate, then some endophytes should have the ability to colonize saprobic substrates directly from a leaf-endophyte stage, though this has been little studied. To assess this ability, twelve surface-sterilized leaves of a tropical tree (*Nectandra lineatifolia* Mez) were placed directly on wood and incubated for six weeks. Fungi from the wood were subsequently cultured and identified by ITS sequences or morphology. 477 fungal isolates comprising 26 OTUs were cultured from the wood, the majority of which belong to saprotrophic genera (70.8% of OTUs, 82.3% of isolates). The mean OTU richness per leaf was 5.67. The term *viaphyte* (literally, “by way of plant”) has been introduced and defined as fungi that colonize living leaves as endophytes and use the leaves to transfer to another substrate, such as wood, when the leaves dehiscence. These results support the Foraging Ascomycete Hypothesis and expose the possibility that viaphytism plays a significant role in the dispersal of fungal saprotrophs.

Identifying Genes Required for *Caenorhabditis Elegans* Embryonic Morphogenesis

Thalia Padilla

Biology

Mentor: Bruce Bowerman

Biology, Institute of Molecular Biology

Mentor: Molly Jud

Institute of Molecular Biology

Morphogenesis is one of three fundamental aspects of developmental biology concerned with shaping tissues into organs and forming the bodies of organisms. Defects in morphogenesis can cause a number of developmental abnormalities in humans that can be disfiguring or lead to death. The cytoskeleton and cell adhesion are instrumental to cell shape changes and movement, but their regulation is only partially understood. *Caenorhabditis elegans* is a powerful morphogenetic model that can provide new insight to improve our understanding of this vital biological process. The Bowerman Lab has generated a large collection of ~1000 temperature-sensitive (TS), embryonic-lethal mutants to study embryonic development, including morphogenesis. The TS mutants are useful tools because they can bypasses earlier essential requirements for genes. By performing forward genetic screens to identify TS morphogenetic mutants in essential genes, I have identified mutations in two conserved genes, *rib-1* and *emb-4*. The former functions as a putative N-acetylglucosamine (GlcNAc) transferase gene, and the latter is likely important for regulating transcription. These two genes will be important tools for future investigations into cell shape changes and migration required for embryonic morphogenesis in *C. elegans* that, in turn, will improve our understanding of developmental abnormalities in humans.

Increasing the Efficiency of a Biotin-Streptavidin Pull Down for An Investigation of Pt(II)-Protein Interactions

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Biochemistry, Biology

Victoria DeRose, Chemistry, Institute of Molecular Biology

Rachael Cunningham, Institute of Molecular Biology

Platinum(II)-based chemotherapeutics such as cisplatin are utilized across a broad range of anti-cancer treatments. The accepted model for cisplatin's signaling of cell death is through DNA binding interactions, though recent findings have shown that cisplatin can induce cell death through stress in the endoplasmic reticulum (ER). Pt(II) may induce ER stress by binding to essential ER proteins such as protein-folding chaperones. This study seeks to identify the proteins that Pt(II) binds by using the copper catalyzed 1,3 dipolar cycloaddition, or click reaction, to label targeted proteins with biotin post-treatment. We show that by denaturing proteins prior to click and digesting the platinated proteins before introducing streptavidin for pull-down, the resultant biotin-tagged peptides will be more likely to be bound by streptavidin, and the non-specific binding elements will be greatly decreased. This protocol will greatly increase the yield and specificity of platinated protein pull-down assays.

**Indigenizing Kalihi:
Imagining Native Solidarities between
Native Hawaiians and Working-Class
Filipinos in the U.S. Colony of Hawai'i**

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Mentor: Stephanie Nohelani Teves
Ethnic Studies

Kalihi, Hawai'i, an urbanized home to a working-class immigrant community of Filipinos, holds stories of anger, frustration, and sorrow about the forced economic displacement to Hawai'i from the Philippines. Kalihi also holds the stories of struggle and perseverance of people residing in that ghettoized area. Filipinos in Kalihi build solidarity around the shared stories and develop a collective connection to that particular place. Using a "storied place" framework, I examine those connections and explore how Kalihi Filipinos stories of place obscure Native Hawaiians' indigenous connection to Hawai'i. That examination may also reveal how Filipino settler stories unwittingly contribute to Hawai'i's colonial multicultural narrative that makes it difficult for Kalihi Filipinos to build solidarity with Native Hawaiians in their struggle against the United States' illegal occupation of Hawaiian lands. By connecting Filipino stories of displacement to those of Native Hawaiians, this research may reveal the significance of the Filipino role in combatting settler colonialism in Hawai'i and help to advance the Native Hawaiian sovereignty movement.

Selected Papers

Flexibility and Stability in a Task-Switching Paradigm

Dagger Anderson

Psychology

Mentor: Ulrich Mayr

Psychology

Mentor: Jason Hubbard

Psychology

Introduction

Behaving in an efficient manner to achieve one's goals depends on executive control settings that specify what internal and external information is currently relevant in a particular context. The task-switching paradigm investigates how one's executive control settings can flexibly adapt to changing environmental demands. Subjects in this paradigm alternate between tasks based on cues that instruct them in which task is currently relevant when reaction time (RTs) is the primary dependent variable. A common finding of this paradigm suggests that flexible behavior is costly. The so-called switch cost is the difference between repeat (no switch) and switch trials, where RTs are larger for switch trials compared to no-switch trials (Mayr, 2006; Mayr, Kuhns, & Rieter, 2012; Kiesel et al., 2010).

Competing explanations of switch costs exist in the literature—the carry-over account and the long-term memory (LTM) account. The carry-over account suggests that the executive control settings of the task used prior to a switch subsequently carry over when a different task set is introduced. The resulting interference between competing tasks leads to switch costs (Gilbert & Shallice, 2002; Bryck & Mayr, 2008; Mayr, Kuhns, & Hubbard, 2014). Unlike the carry-over account, the LTM account suggests that interference comes from memory traces that are automatically encoded in LTM for every experience with a given task set (Bryck & Mayr, 2008; Mayr et al., 2014). Support for the latter explanation arises from research that provided evidence of a switch cost in the absence of an actual task switch (Bryck & Mayr, 2008). The LTM interference position of Bryck and Mayr (2008) gained additional support with O'Reilly and Frank's (2006) gating theory of working memory. This model suggests that when the gate to working memory is closed, no new sensory information can enter. Consequently, working memory is biased and operates in a maintenance mode during which current representations (e.g., task-sets) are maintained and protected from interference. When the gate is open, however, new sensory information can enter. This explanation corresponds to an "update mode," so that current representations can be updated to the relevant task

set, but they are prone to interference. Bryck and Mayr (2008) explain that when a task set needs to be updated (i.e., update mode), competing LTM traces of task sets can interfere with one another, resulting in switch costs.

Additional research on executive control has investigated how switch probability influences one's cognitive flexibility and the working memory processing modes mentioned above. Generally, as the probability of a task change increases, switch costs are reduced, a result typically caused by an increase in repeat trial RTs rather than because switch trial RTs decrease (Monsell & Mizon, 2006; Mayr, 2006; Mayr et al., 2012). Researchers have tended to interpret that pattern of results as support for an internal reconfiguration process. High switch rate conditions may influence subjects toward updating representations to reflect the relevant task set because of a greater frequency of switch trials compared to repeat trials. That result may be an optimal solution in times of an actual task switch, but in the cases of a non-switch, a RT cost would result (Monsell & Mizon, 2006; Mayr, 2006, 2012). On the other hand, the low switch rate conditions may encourage subjects to stay in maintenance mode because repeat trials occur more frequently than switch trails. This circumstance may be beneficial in times of a no-switch, but would be costly in times of a switch (Monsell & Mizon, 2006; Mayr, 2006;

Mayr et al., 2012). This explanation in the literature adequately accounts for the observed pattern of decreasing switch costs as the probability of a switch increases. Additionally, the hallmark finding of reduced switch costs as preparation intervals increase also seems to generalize across switch probability conditions (Rogers & Monsell, 1995, Mayr et al., 2012).

Prior research investigating the influence of switch rate on switch costs utilized between-subject designs. The question of how well people adapt their behavior to meet the demands of stability or flexibility within the same experimental session has not been investigated. To explore this issue, we adopted a typical task-switching paradigm with varying switch-rate conditions as a within-subject manipulation to promote either flexibility or stability. We predicted that people can quickly adapt to the changing environmental demands. Consequently, we expected to observe the typical pattern seen in previous between-subjects work involving changing switch rates. We expected that high switch rate conditions would be associated with reduced or absent switch costs because of an increase in no-switch RTs. Similarly, we expected that low switch rate conditions would be associated with significant switch costs. In addition, we predict that longer preparation intervals would reduce switch

costs in both switch probability conditions.

Method

Participants

A total of 24 participants from the University of Oregon participated for course credit.

Task and Procedure

A 4:2 cue-task design was adopted to reduce cue repetitions and possible cue-priming effects that previous research has highlighted (Mayr & Kliegl, 2003). In this design, 2 different cues are used for instructing each task. Participants in the current experiment were cued aurally in preparation for the upcoming trial. The cue-stimulus interval (CSI) was either 300ms or 1000ms. For each trial, subjects were presented with three circular stimuli arranged along a virtual equilateral triangle on a computer monitor. One of these stimuli was a colored circle that was either light blue or purple. A second item was a blue, segmented circle with gaps on opposing arcs of the circle, oriented towards the upper-left, lower-right, upper-right, or lower-left. The final circle stimulus was a neutral circle that was the same color as the segmented circle. Across trials, the virtual triangle was randomly rotated, so that the positions of the stimuli were not predictable. There were two tasks: one associated with the uniquely

colored item, and one associated with the gap stimulus. In the *Color/Hue* task, subjects responded to the light blue or purple stimulus. If the circle was light blue, the left arrow key was the correct response. If the stimulus was purple, the right arrow key was the correct response. In the *Gap/Space* task, the segmented circle was the target circle. A gap pointing to the left was associated with the left arrow key, and a gap pointing to the right was associated with the right arrow key. Subjects practiced each task separately, followed by a practice block with alternating tasks. After practice was completed, subjects began the experimental session. See *Figure 1* for an outline of one completed experimental trial and the stimuli used.

For the switch rate manipulation, a 75% switch probability was used for the high switch rate condition, and a 25% switch probability was used for the low switch rate condition. Subjects switched conditions every 4 blocks, and order was counterbalanced across subjects. Subjects were provided visual instructions about the switch rate condition prior to each block: “Task switches in 1 out of 4 trials”/ “Task switches in 3 out of 4 trials.” The CSI changed halfway through the experiment, and the order was counterbalanced across subjects.

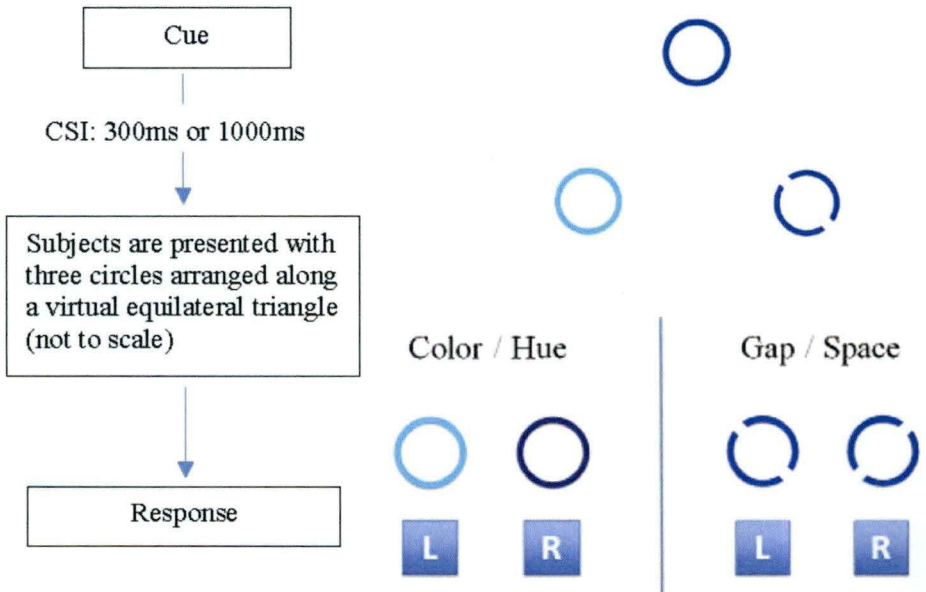


Figure 1: Subjects are presented with three circular stimuli arranged along a virtual equilateral triangle. If performing the color/hue task, a left arrow response is correct for a light blue circle, whereas a right arrow response is correct for a purple circle. If performing the gap/space task, a left key response is correct if the segment is pointing to the left, whereas a right key response is correct if the segment is pointing to the right.

Results

Trials with reaction times greater than 4000ms, errors, and post-error trials were discarded from analyses. We ran a Repeated Measures Analysis of Variance (ANOVA) with CSI (300ms vs 1000ms), Switch (switch vs no-switch), and Switch Rate (high vs low) as within-subject factors. Providing support for the hallmark finding from the task-switching paradigm was a main effect of switch ($F(1,23) = 65.556, p < .001$) where RTs were larger in switch trials compared

to no-switch trials ($M_{No-Switch} = 670$, $M_{Switch} = 739$). In addition, there was a main effect of switch rate ($F(1,23) = 32.336$, $p < .001$) where a higher switch probability is associated with larger RTs than a lower switch probability ($M_{Low-Switch} = 688$, $M_{High-Switch} = 741$). Lastly, longer preparation intervals (CSI) were associated with faster RTs than shorter preparation intervals in both switch rate conditions ($M_{Long\ CSI} = 652$, $M_{Short\ CSI} = 757$, $F(1,23) = 265.433$, $p < .001$). All 2 and 3 way interactions were significant at the .05 level. For the interaction of switch and switch rate (see *Figure 2*), a large switch cost was ob-

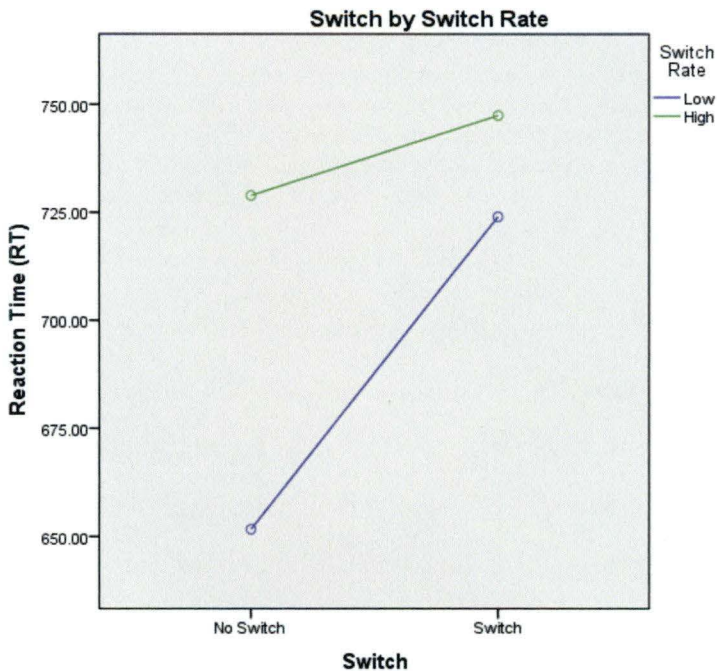


Figure 2: Large switch costs resulted when switch probability was low, and this cost was reduced when the switch probability increased.

served in the low switch rate condition, but this cost was reduced in the high switch rate condition ($F(1,23) = 30.104, p < .001$). The switch cost reduction in the high switch rate condition resulted from an increase in repeat trial RTs not because switch trial RTs decrease. In addition, the longer CSI condition resulted in reduced switch costs compared to the shorter CSI condition (see *Figure 3*, $F(1,23) = 13.486, p = .001$). Lastly, the three-way interaction of CSI, switch, and switch rate was significant (see *Figure 4*, $F(1,23) = 13.283, p = .001$). This suggests that the longer CSI condition had the most beneficial impact on switch costs when the switch rate was low.

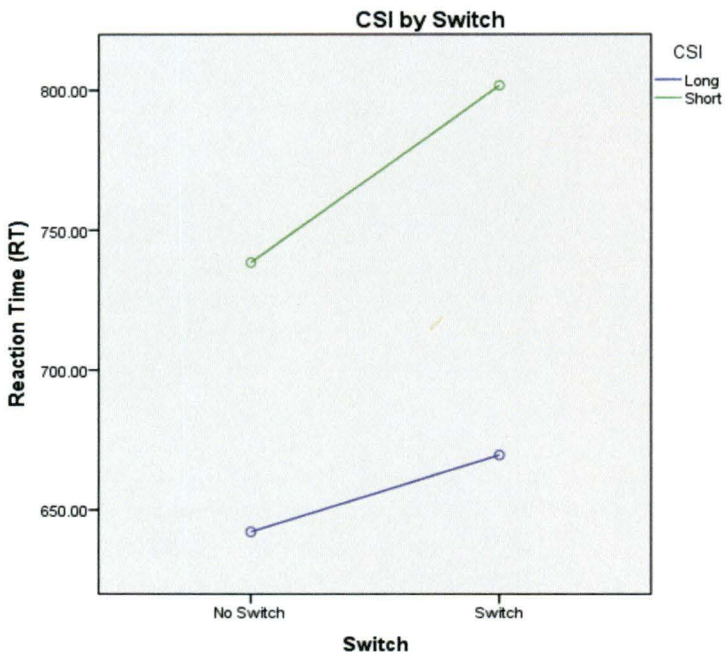


Figure 3: Reduced switch costs in longer CSI condition compared to the shorter CSI manipulation.

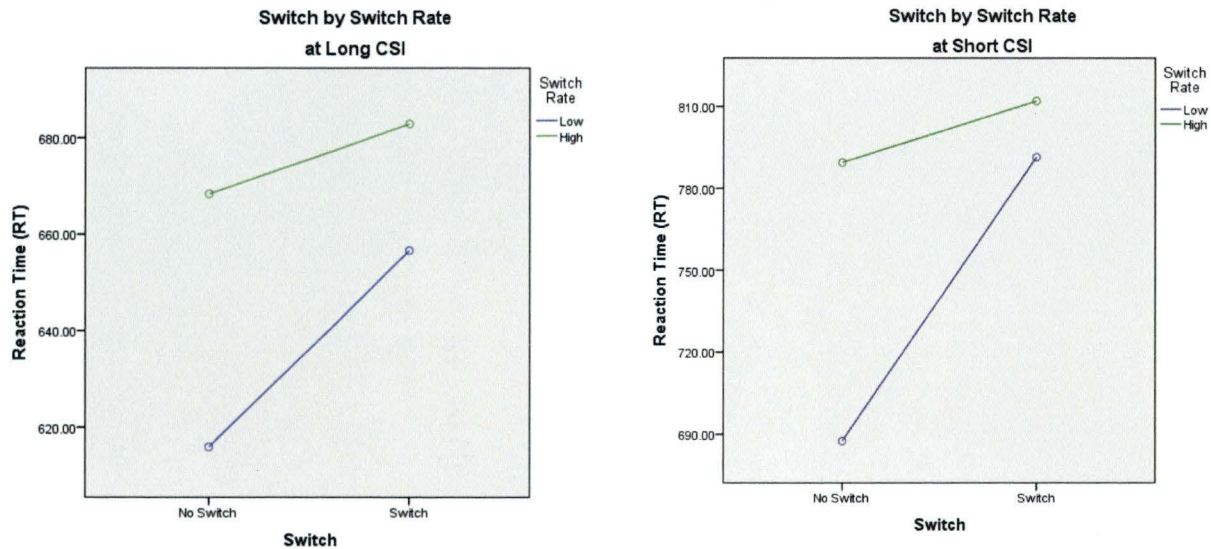


Figure 4: Longer preparation intervals seemed to have the greatest impact at reducing switch costs in the low switch rate condition.

Discussion

It was predicted that people would be able to rapidly adapt to the demands of flexibility and stability in a task-switching paradigm that involved changing control demands. This prediction was supported by a significant increase in no-switch RTs when the switch rate was high and when the switch rate manipulation seemed to promote the working memory processing modes outlined in O'Reilly & Frank (2006). The low switch rate conditions seemed to facilitate a bias toward maintenance mode, as indicated by large switch costs. On the other hand, high switch rate conditions seemed to promote a bias toward an update mode associated with increased no-switch RTs and reduced switch costs. To our knowledge, this was the first study to use switch probability as a within-subject manipulation, which suggests that this internal reconfiguration process (Mayr, 2006) can rapidly adjust to the demands of flexibility and stability of the environment. High switch rate conditions seem to orient our executive control system to remain in a flexible state, which is beneficial when the task changes. However, low switch rate conditions seem to orient our executive control processes to remain in a more rigid state. The latter case may be costly in times of a task switch, but beneficial when the task remains the same across subsequent trials. It was also

predicted that the benefit of longer preparation intervals, which was replicated in the current experiment, would generalize across switch rate conditions. It seems, however, that this benefit was greater in the low switch rate condition, even though this effect was present in both switch rate conditions. This result supports the idea that longer preparation intervals help orient our cognitive system, and that the preparation process helps us overcome switch-costs, especially when the task switches infrequently.

One limitation of the current project concerns generalizability. How well does this rapid reconfiguration process seen in a sample of college students generalize to different age groups? There is evidence that there are age differences in terms of executive control. For example, older adults show larger local switch costs (the RT difference between repeat and switch trials within heterogeneous task blocks only) than younger adults (Kray & Lindenberger, 2000; Mayr, 2001). In addition, older adults seem to benefit from longer preparation intervals than younger adults, even though preparation reduces switch costs in general (Kray & Lindenberger, 2000). The extent to which these age differences influence performance in low and high switch rate conditions is a topic for future work. Perhaps this effect would be seen only in the low switch rate condition in a sample of older adults.

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Leafcutter Ants Inside The Nest Have Sharper Mandibles Than Ants Outside The Nest

David M. Lee

Biochemistry, Biology

Mentor: Robert Schofield

Physics

Introduction

Leafcutter ants are both dominant consumers and valuable contributors to ecosystems throughout the neotropics.¹⁻³ They harvest small disks of leaves (and other materials) from their environment, cut them into smaller fragments, and use the fragments as substrate for the gardens of fungi they grow as a food source.⁴⁻⁷ Therefore, leafcutters as producers of a crop are farmers. The amount of energy provided by the crop limits the total net growth of the colony, much like the growth of a factory is limited by the amount of money brought in from production. The colony must be aware of how much energy it uses⁸⁻¹¹ versus the amount of energy the fungal gardens provide.^{10,11} With positive net energy the colony grows, but with negative net energy growth stops. If the energy budget goes too far into the red, the colony begins to collapse. Leafcutter ants

have made their living in environments of bustling tree canopies and grasslands. Even with the heavy labor of harvesting materials, processing the substrate, and tending meticulously to their fungal gardens, leafcutter ants and their mycological symbiont have conquered most of Latin America.⁴⁻⁶

Of all the cutting that leafcutters do, 90% occurs inside of the nest.¹¹ Leafcutters use a pair of toothed mandibles to do their cutting and rely on two techniques: leading-lagging for cutting soft materials and saw-action for cutting tough materials.^{10,11} As a leafcutter ages, its mandibles wear down without regeneration. Consequently, an ant with worn mandibles will spend twice the energy and will cut half as fast as an ant with sharp mandibles.¹⁰ Smaller organisms, such as leafcutter ants, are able to prolong the sharpness and efficiency of their biotools in part because those critical body parts are enriched by heavy metal and halogen (Zn, Mn, Br).¹³ Moreover, the allocation of tasks based on wear may also play a significant role in prolonging the optimal cutting efficiency of a leafcutter's mandibles while contributing to the viability of the colony.

Methods

We suspected that ants inside the nest, where most of the cutting happens, would have the sharpest mandibles. To investigate this

hypothesis, we analyzed four groups of media *Atta cephalotes* collected from a two-year-old colony in Minca, Colombia (2012): one group from inside the nest and three groups from outside the nest. Our study compared the tooth lengths of each ant to the lengths of a similar-size late-stage pupa. Late-stage pupae are ideal for comparing tooth wear because they have pristine, fully developed mandibles. By subtracting the tooth lengths measured in the lab from the tooth lengths of a late-stage pupa and averaging the wear values of all measured teeth per ant, we calculated the average amount of tooth that had worn over the ant's life.¹⁰ We then compared the results from individual groups to one another.

Specimens

Groups consisted of *Atta cephalotes* collected from fungal gardens inside the nest (Inside Ants, $n = 79$) and three groups from outside the nest: 1) ants cutting leaves (Leaf Cutters, $n = 81$), 2) ants cutting a mango (Mango Cutters, $n = 43$), and 3) ants carrying leaves (Carriers, $n = 79$). Our selection criteria called for only media ants to be collected. Groups were stored in a glass vial with ethanol as a storage medium. Using similar-sized late-stage pupae as comparators, tooth lengths were calculated using the methods described in Schofield et al.¹⁰

Dissection and Photography Protocol

Each head and mandible was photographed using a Fujifilm FinePix S2950 digital camera shot through a Zeiss dissection microscope. The head and mandibles were photographed separately with their superior surfaces parallel to the plane of the stage.

Head Width Photo (Figure 1). Each ant was decapitated, and the antennae and mouthparts were removed with tweezers. The head was oriented in the middle of the optics, and a millimeter scale was placed behind it. A “head-width” photo was captured. The closure apodeme for each mandible was then severed using a No. 11 scalpel. Severing the apodeme was necessary to allow the mandible to be manipulated across its full range of motion.

Mandible Photo (Figure 1). The head was placed in a groove on a clay pedestal, and the left mandible was positioned using a toothpick. Proper orientation required that the superior surface of the mandible be parallel to the plane of the stage. To assure that the mandible was positioned correctly, strobing light from an LED flashlight was shone through the eyepiece to allow a visual confirmation that the mandible was level. Strobing light glinting evenly across the surface of the mandible acted as a visual indicator that the sample was near-parallel to the plane of the stage. With the sample

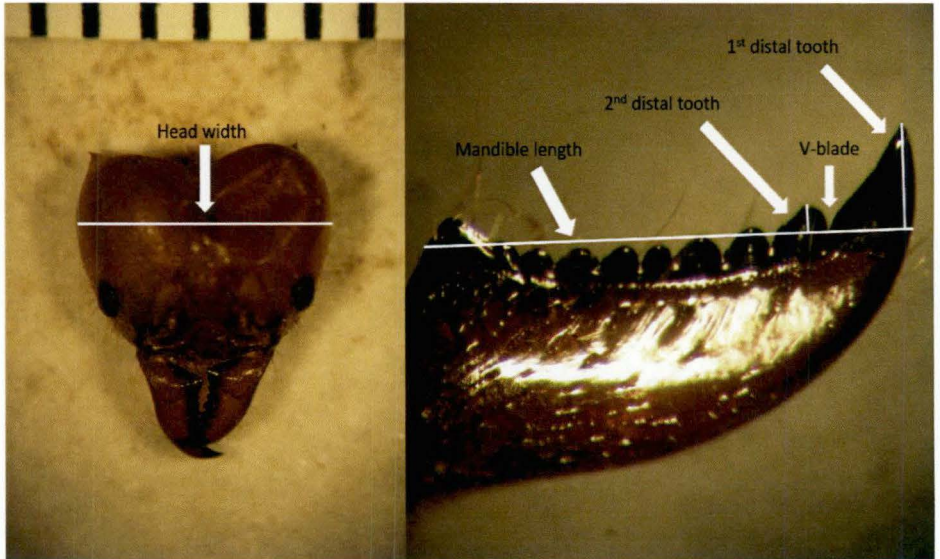


Figure 1. Measuring head width (left) and mandible (right) photos. Solid lines represent measurements taken on ImageJ. Dashed lines represent scalpel cuts.

oriented correctly, a “left mandible photo” was captured using the optical zoom of the camera at 1.8X and the microscope zoom at 5X. A photograph of a millimeter scale was taken at this zoom for data analysis. The mandible photo process was repeated for the right mandible.

Photographic Analysis

Photographic measurements were recorded using ImageJ photo analysis software version 1.50i. A head width measurement was considered the length of the longest line that could be drawn across the top of the head while remaining parallel to a line connecting the

center of the eyes (Figure 1.a). A mandible length measurement was considered the length of a line that connected the anterior edge of the first distal tooth to the proximal edge of the last tooth by passing through the bottoms of the valley created by the V-blade and the valley formed at the interface of the last two teeth. Distal tooth measurements were considered the length of a line connecting the tip of the tooth perpendicularly to the mandible length line (Figure 1.b).

Data Analysis

First and second distal tooth measurements were compared to a similar-size late-stage pupa using Equations 1 and 2, respectively. The difference between the tooth lengths from our measured sample (T_1 & T_2) and those calculated for a late-stage pupa (bracketed portion of Equations 1 & 2) quantified the amount of wear per tooth (W_1 & W_2). Wear computed from the first distal tooth of the left and right mandible was regressed to the status of a second distal tooth with proportional levels of wear to account for biases in wear rates. The regressed teeth were averaged in Equation 3 with the measured levels of wear from the second distal teeth to compute the average wear ($\overline{W}$). Groups were binned per average mandible length, .225 mm to .900+ mm in .075 mm increments. Bins were normalized using an online random number generator (<https://www.random.org>) to elim-

Weighted Average Wear Computation

L_M = mandible length, T_1 and T_2 = measured tooth lengths,
 W_1 and W_2 = calculated tooth wear, $\overline{W}$ = weighted average wear

$$1. [.2648L_M - .0308] - T_1 = W_1 \quad \text{for } W_{1L} \text{ \& } W_{1R}$$

$$2. [.0533L_M + .0301] - T_2 = W_2 \quad \text{for } W_{2L} \text{ \& } W_{2R}$$

$$3. \frac{\frac{W_{1L} + W_{1R}}{2.61} + W_{2L} + W_{2R}}{4} = \overline{W}$$

Figure 2. Wear equations from Schofield et al.¹⁰

Equation 1. Difference in length, late-stage pupa minus measured: 1st tooth.

Equation 2. Difference in length, late-stage pupa minus measured: 2nd tooth.

Equation 3. Average Wear: regressed 1st teeth and 2nd teeth.

inate individual data points. Wilcoxon-Mann-Whitney Test results were computed using an online calculator (www.ccb.uni-saarland.de/wtest).

ESEM Protocol

Electron micrographs were captured on an FEI Quanta 200 ESEM/VPSEM in low vacuum mode ($P \leq 20$ Pa) with low voltage ($V \leq 10$ kV) over a 90 second scanning interval. The bottom 10% least worn mandibles from individuals of each group were selected to compare minute levels of wear at higher magnification. The mandibles

were dissected completely from the head and mounted parallel to each other on a circular mounting pin with the plane of the first distal tooth's proximal edge oriented normal to the stage. Uncoated samples had been air dried prior to scanning, and the mount had been prepared with an adhesive carbon disk to aid in stabilizing the sample and decreasing charge levels. Electron micrograph measurements were recorded using ImageJ photo analysis software version 1.50e.

Results

Preliminary findings revealed that 95% of ants outside the nest had wear that was more than .025 mm, and 70% of ants inside the nest had wear that was less than .025 mm (Figure 3). Ants found inside the nest had mandibles that were sharper than those of ants found outside the nest by a factor of 2.14 ($p = 5E-18$) (Table 1).

Wear Comparison	<i>n</i> per group	Test	<i>p</i> -value	$\frac{\overline{W}_{out}}{\overline{W}_{in}}$
Inside Ants vs. Outside Ants	72	$H_1 = in < out$	4.67^{-18}	2.14
Inside Ants vs. Carriers	34	$H_1 = in < out$	1.70^{-16}	2.42
Inside Ants vs. Leaf Cutters	34	$H_1 = in < out$	1.80^{-10}	2.32
Inside Ants vs. Mango Cutters	34	$H_1 = in < out$	9.60^{-08}	1.98
Table 1. Results from Wilcoxon-Mann-Whitney Test are shown with exact <i>p</i> -values and group average wear ($\overline{W}$) ratios (outside over inside).				

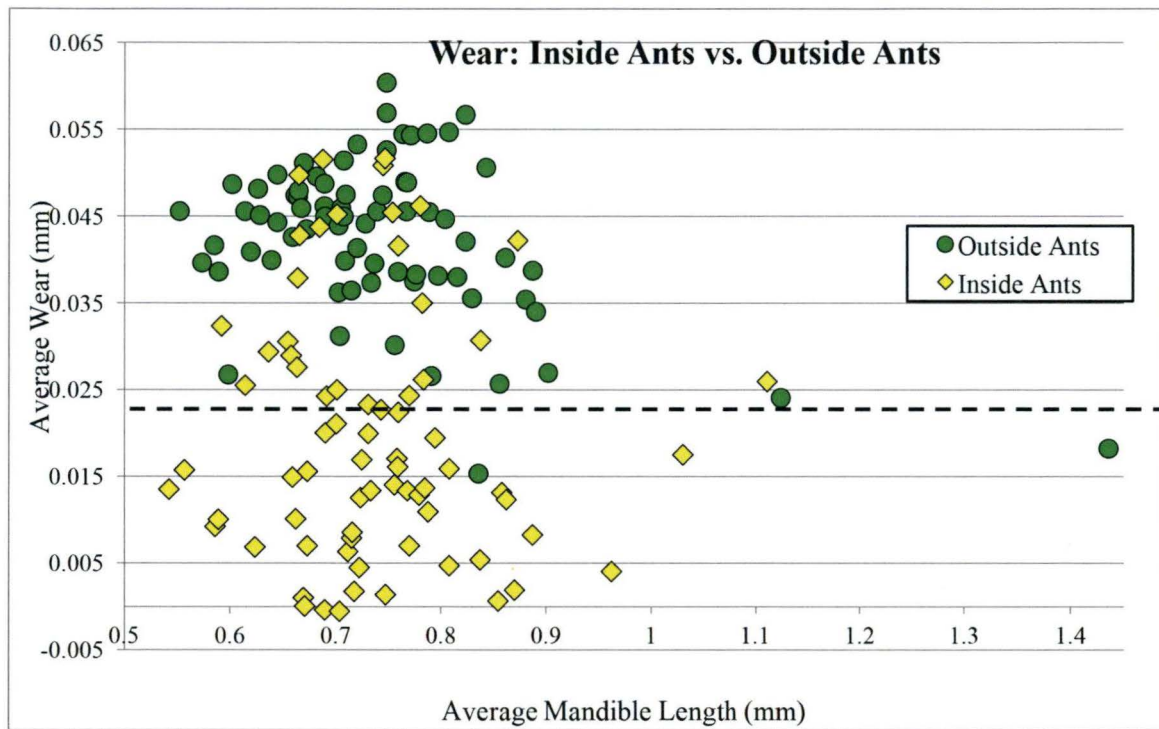


Figure 3. Scatterplot showing distribution of wear between Inside Ants and Outside Ants.
Dashed line = .025 mm average wear. n = 72 per group.

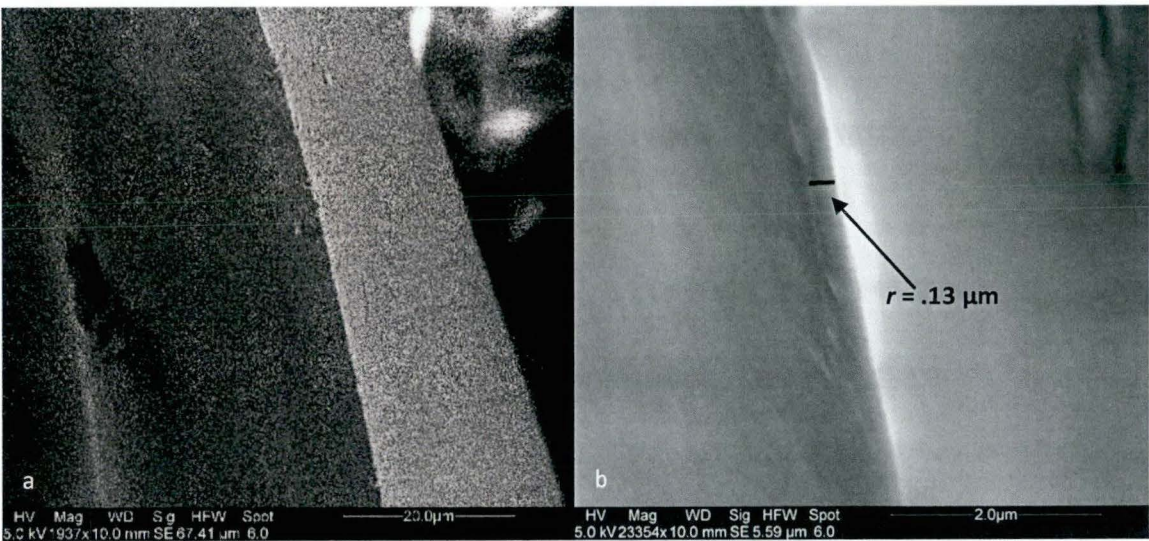


Figure 4. Electron micrographs: Inside Ant. Proximal edge of 1st distal tooth. Distant (a) and close-up (b) zooms. Black line represents diameter measurement taken on ImageJ.

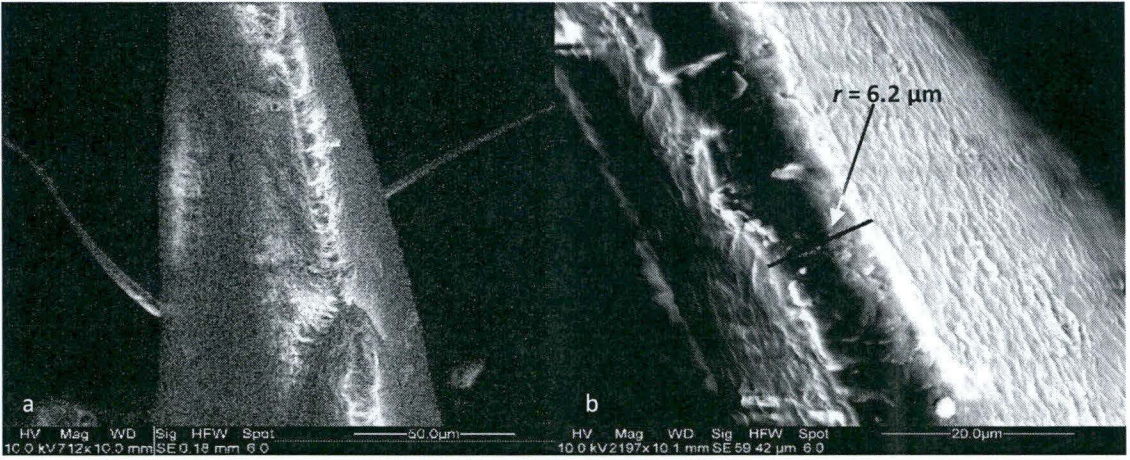


Figure 5. Electron micrographs: Outside Cutter. Proximal edge of 1st distal tooth. Distant (a) and close-up (b) zooms. Black line represents diameter measurement taken on ImageJ.

Discussion

Preliminary findings reveal that leafcutter ants inside the nest have sharper mandibles than ants outside the nest. The result we obtained was simple but carries large implications. It costs a lot of energy to make and maintain an ant.⁸⁻¹¹ The colony would risk losing that investment of energy by having the ant immediately leave the safety of the nest after ecdysis to work outside, but the colony still needs to be continually sending workers out to harvest materials for substrate to feed to their fungal gardens. Leafcutters may spend the first portion of their lives working inside the nest when they are the most efficient at cutting. Once their mandibles wear to a certain point, it will take an ant twice the energy to cut the same amount of material.¹⁰ This decreased efficiency may be enough for the colony to risk losing the ant to the dangers of the outdoors.

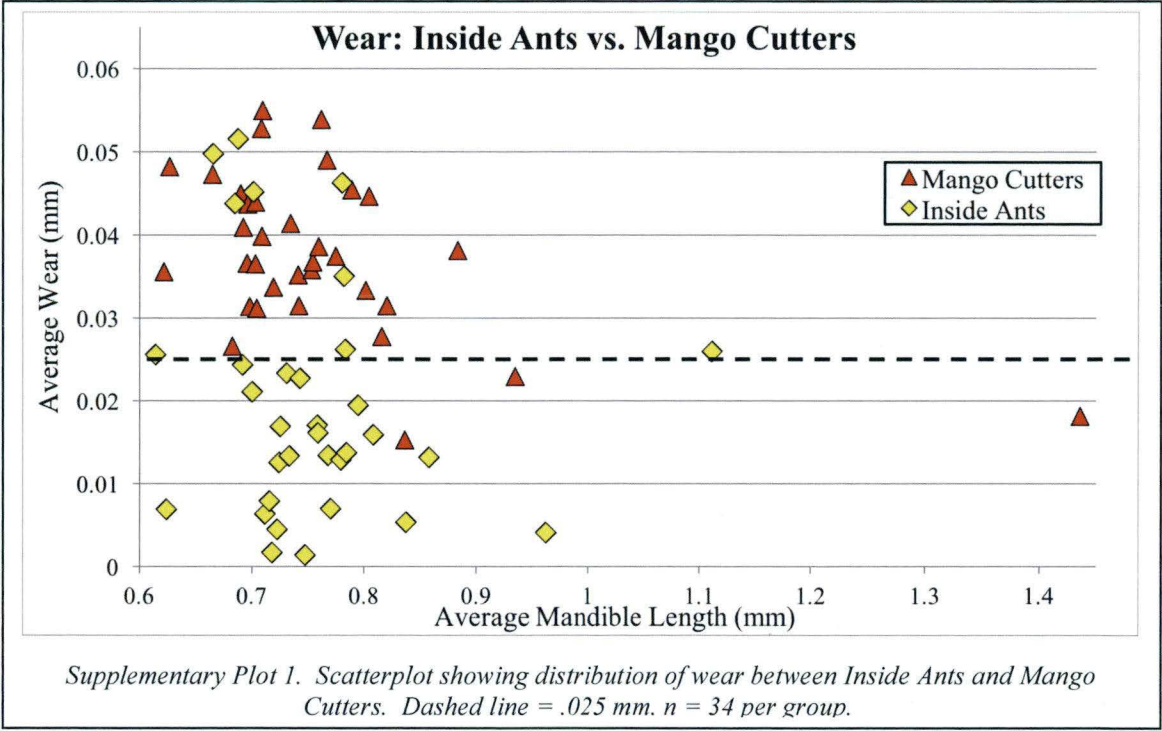
The electron micrographs in Figure 4 and Figure 5 show the difference in wear between an ant selected from inside the nest and an ant selected from outside the nest. The sharpness of a blade is dependent on the radius of the blade's tip. A smaller radius will contact less material, constituting a sharper blade.¹² In this comparison, both ants possess mandibles that are in the bottom 10% (least worn) from their respective groups although the inside ant has a cutting

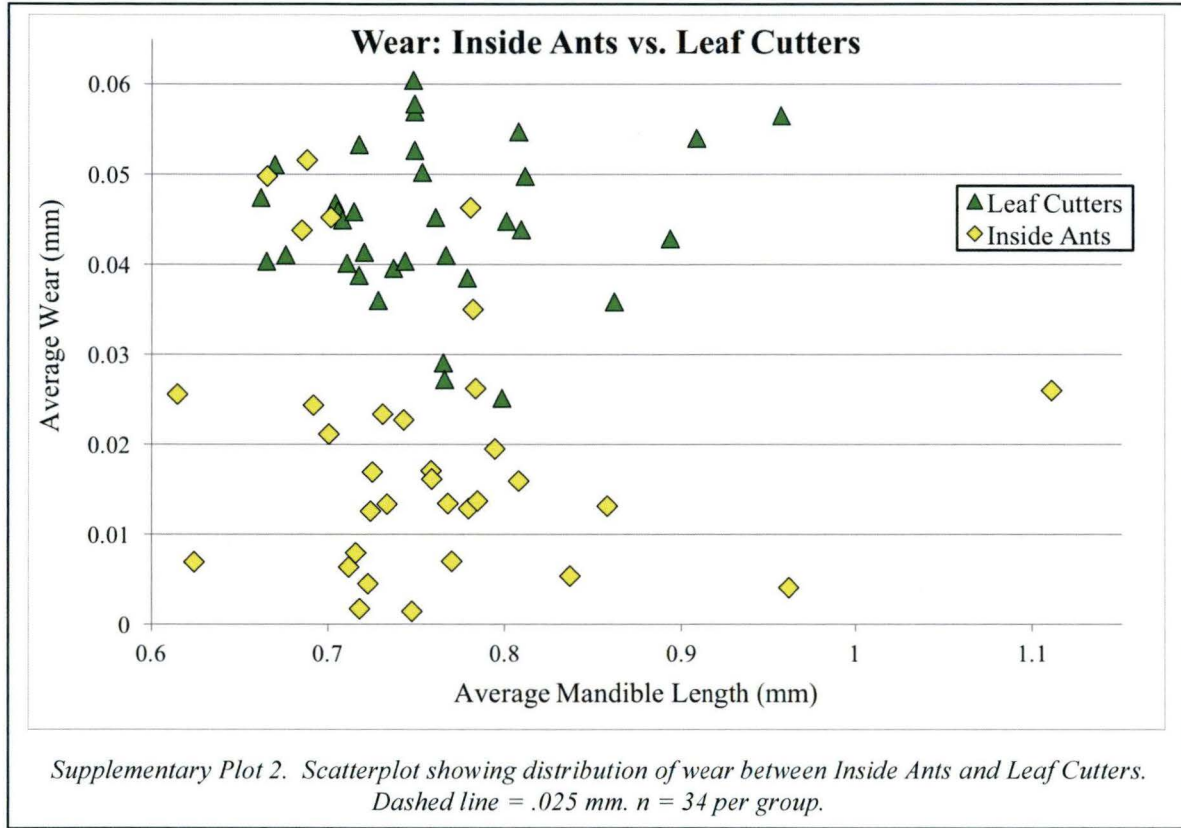
blade that is 50 times sharper than the cutting blade of the outside ant. This difference in sharpness is comparable to cutting a steak using a sharp steak knife versus a dull butter knife. The sharper knife requires less energy and allows the task to be completed in less time. Keeping the sharpest mandibles inside the nest may be one way the colony chooses which ants work inside the safety of the nest and which ants must risk their lives by foraging outside of the nest. Our data suggest that an ant starts to work outside the nest after .025 mm of its mandibles have worn away. However, we are uncertain whether this observed behavior is in response to wear or in response to the longevity an ant has left to give to the colony. One could test this by seeing if young ants with especially worn mandibles leave the nest to forage.

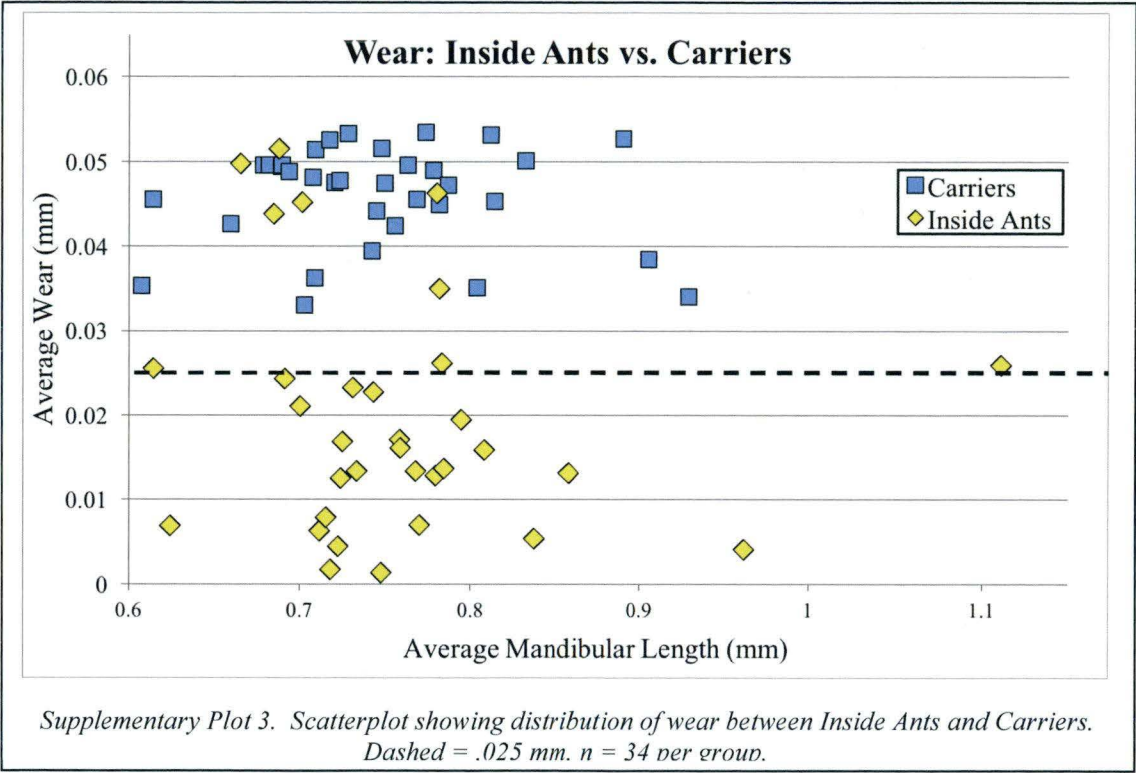
Acknowledgements

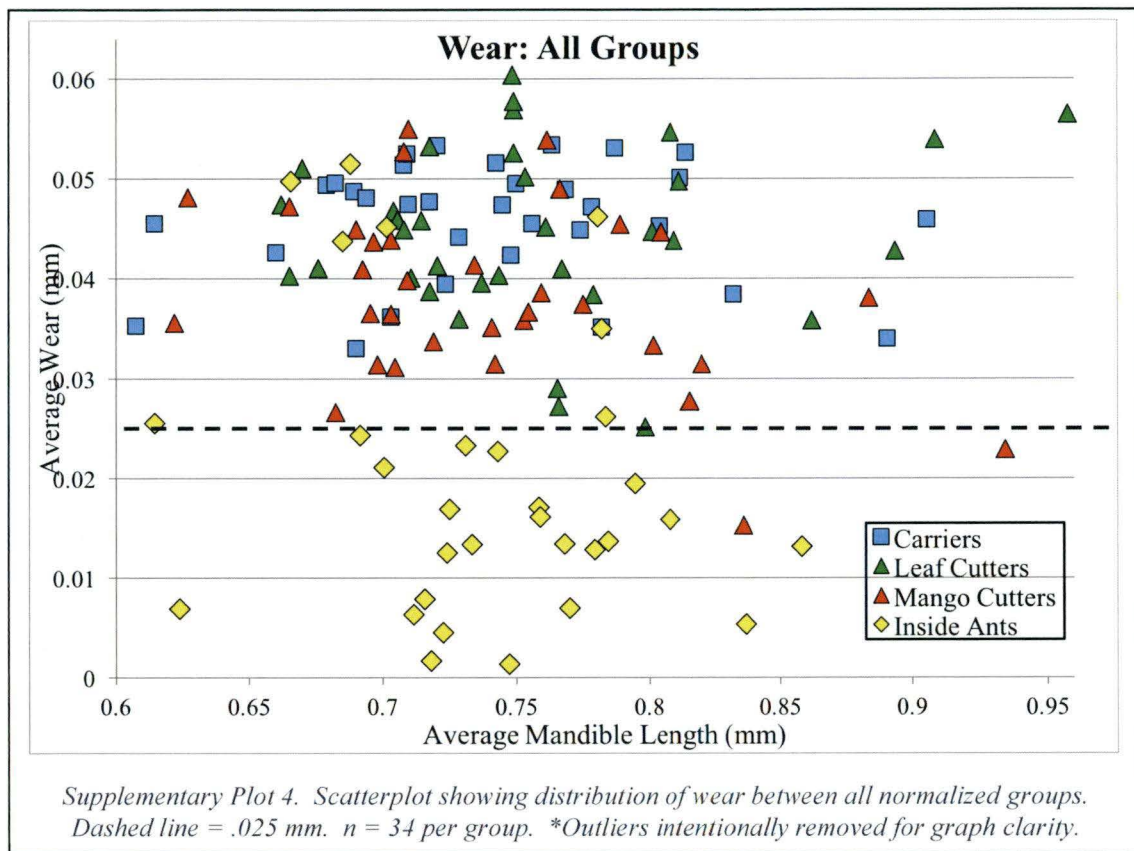
I would like to thank Dr. Robert Schofield for birthing the project and guiding me toward its completion; Kate Carlson, Ryan Garrett, and Matthew Goggans for their intellectual contributions; my fellow McNair scholars for taking this ride with me; and Dr. Gail Unruh for always going above and beyond.

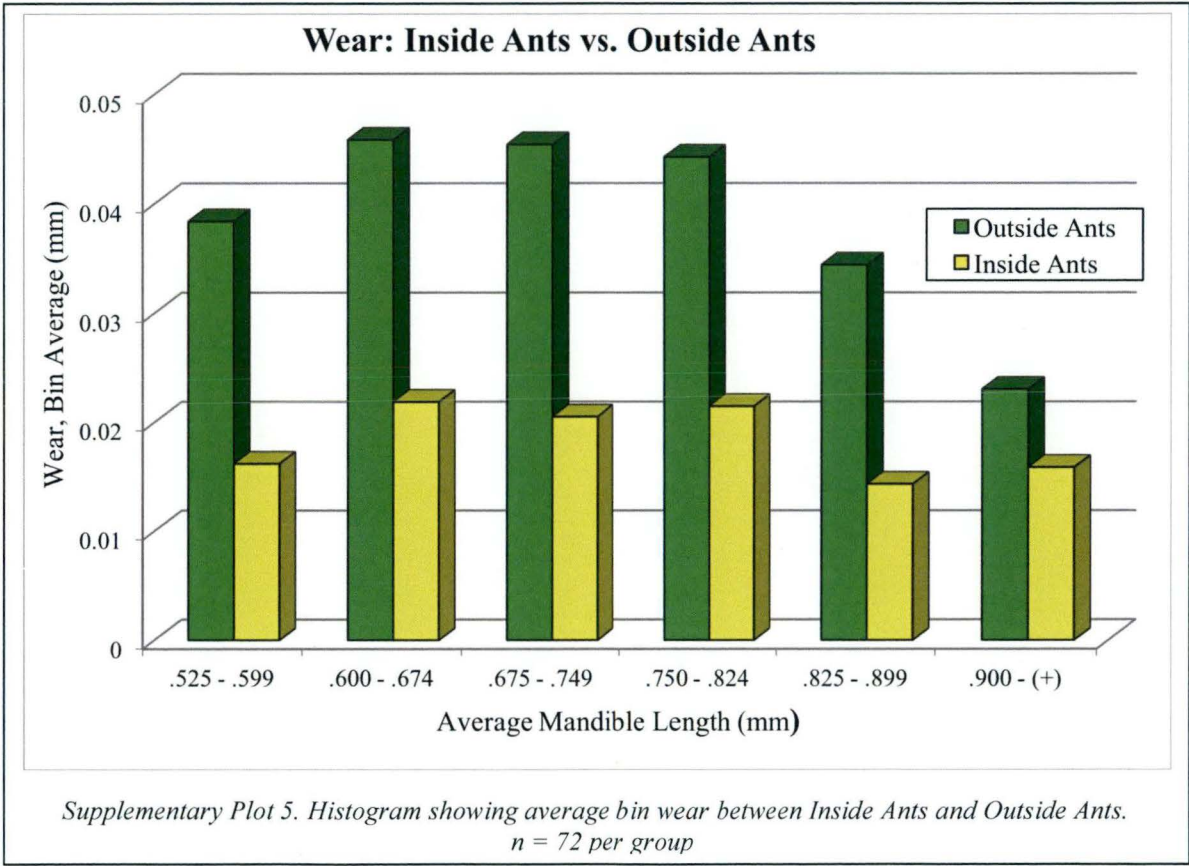
Supplemental Materials

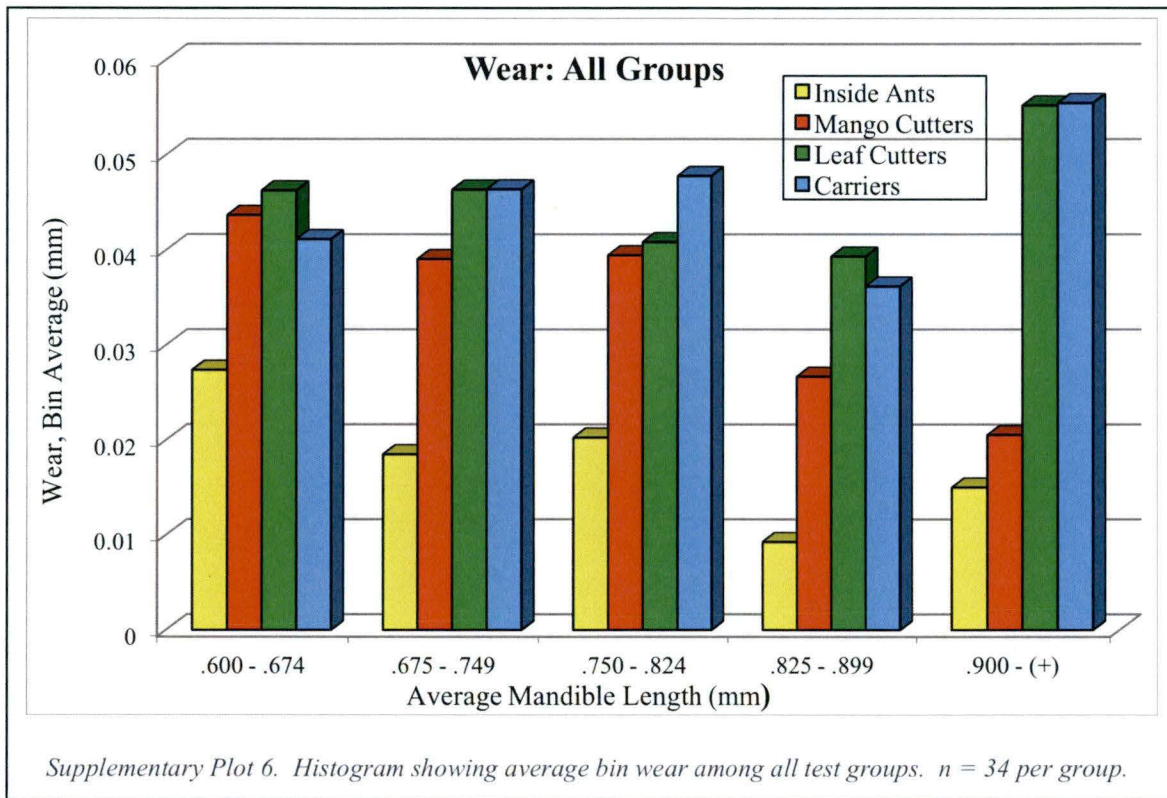












Wear Comparison (a vs. b)	<i>n</i> per group	Test	<i>p</i> -value	$\frac{\overline{W}_b}{\overline{W}_a}$
Inside Ants vs. Outside Ants	72	$H_1 = a < b$	4.67^{-18}	2.14
Inside Ants vs. Carriers	34	$H_1 = a < b$	1.70^{-16}	2.42
Inside Ants vs. Outside Cutters	62	$H_1 = a < b$	2.31^{-14}	2.07
Inside Ants vs. Leaf Cutters	34	$H_1 = a < b$	1.80^{-10}	2.32
Inside Ants vs. Mango Cutters	34	$H_1 = a < b$	9.60^{-08}	1.98
Mango Cutters vs. Carriers	34	$H_1 = a \neq b$	5.25^{-05}	1.22
Mango Cutters vs. Leaf Cutters	34	$H_1 = a \neq b$	0.0119	1.17
Outside Cutters vs. Carriers	62	$H_1 = a \neq b$	0.0346	1.07
Leaf Cutters vs. Carriers	34	$H_1 = a \neq b$	0.699	1.05

Supplementary Table 1. Results from Wilcoxon-Mann-Whitney Test are shown with exact *p*-values (*p* > .05 denoted in red) and group average wear ($\overline{W}$) ratios (b over a) for an array of comparisons

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Identifying Genes Required for *Caenorhabditis Elegans* Embryonic Morphogenesis

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Introduction

During embryogenesis of all multicellular organisms, cells must propagate, undergo differentiation, and migrate to give rise to tissues, organs, and the general body plan. The three tenets of development (cell growth, differentiation and morphogenesis) are imperative to produce a complete organism. Morphogenesis, originating from the Greek word meaning “beginning of shape,” refers to coordinated tissues movements including migration, contraction, folding, and fusion to shape an embryo and form its organs. Disruption of morphogenesis causes a variety of birth defects in humans, including vascular irregularities, neural tube closure, and limb developmental defects (Roberts et al., 2004; Sabapathy et al., 1999;

Dudley et al., 1995). Therefore, understanding the genes and signaling pathways required for morphogenesis to properly occur is of great importance.

Caenorhabditis elegans is a small nematode that for a number of reasons is an excellent genetic model for studying morphogenesis. Perhaps the most compelling reason to view *C. elegans* as a powerful model organism for genetic studies of genome function is that it has high genetic conservation with humans: 83% of the worm proteome is homologous to that of humans (Lai et al., 2000). Second, the worms can be easily grown in a petri dish. The nematode has two sexes, hermaphrodites and males. Hermaphrodites produce both sperm and eggs; therefore, they procreate by either self-fertilization or cross-fertilization. Self-fertilization can simplify the maintenance of stocks because hermaphrodites can give rise to an entire population without the need of males. Further, *C. elegans* has large brood sizes, and a single hermaphrodite can give rise to about 300 progeny. These worms can be stored as frozen stocks making them cheap and easy to maintain. Not only are the worms easy to cultivate and require low maintenance, they also have quick generation times and rapid life cycles, taking only 4 days at 20°C to go from a fertilized egg to a reproductive adult. This rapidity reduces

the waiting periods necessary to obtain reproductive adults, and the rapid generation time allows for faster genetic results. They are also completely transparent, another key virtue that allows us to easily image and analyze morphogenesis. All these features make *C. elegans* a powerful model for genetic dissection of morphogenesis, which relates directly to human health and disease (Ahringer, 2006).

The tenet of morphogenesis is the process of cell shape changes and migration. Cells can change shape, migrate, or rotate, and each change alters the way tissues are organized. Thus, small changes at the cellular level can lead to large changes at the tissue level in forming the shape of an organism's body and organs. Although morphogenesis in *C. elegans* remains only partially understood, many of the basic processes have been characterized and are shared with other organisms, including epiboly, cell intercalation, and apical constriction. Development of the epidermis has been the main tissue studied to understand morphogenesis in *C. elegans* (Andrew and Hardin, 2005), which is broken into three main stages: gastrulation, ventral closure of the epidermis, and elongation (Figure 1).

During early animal development, most embryos go through a process called gastrulation (Figure 1). During gastrulation, cells divide and invaginate to create a tiny blastopore (Wassarman, 2011);

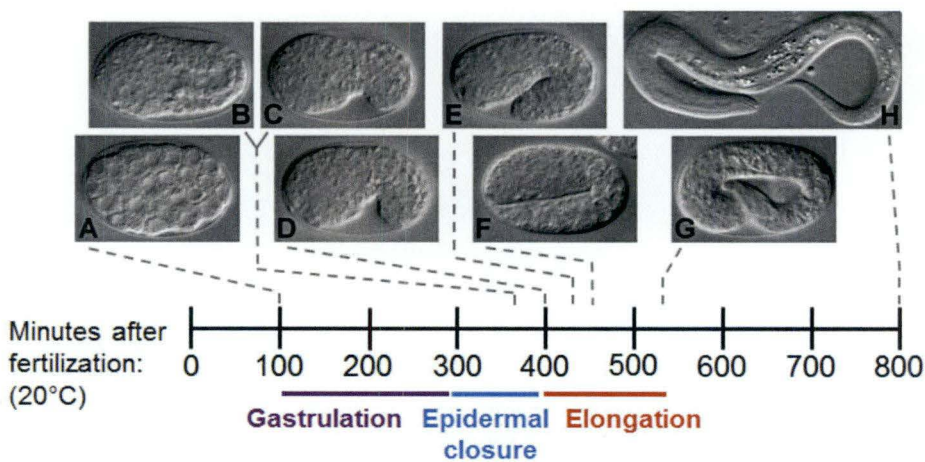


Figure 1: Morphogenesis stages during embryogenesis at 20° C. (A) 26-cell embryo, (B and C) bean stage [1.25-fold], shown ventrally (B) and laterally (C), (D) comma stage, (E) tadpole stage [1.5-fold], (F) plum stage [2-fold], (G) pretzel stage [3-fold], and (H) fully elongated L1 worms that hatched from their eggshell. Timeline based on Sulston et al (1983).

thus, cell movement is essential for gastrulation. Transcription factors are necessary to specify tissue precursors and activate actin cytoskeleton regulating genes. (Wassarman, 2011). Gastrulation in *C. elegans* involves the specification of three germ layers that are the initial tissue layers of the embryo: (1) ectoderm that gives rise to hypodermis and neurons, (2) mesoderm that generates pharynx and muscle, and (3) endoderm that gives rise to germ line and intestine (Wassarman, 2011).

Following gastrulation is epidermal morphogenesis, which is broken down into two events: dorsal cell intercalation and ventral

enclosure (Figure 1), both of which require actin microfilaments and microtubules for cell shape changes. The first morphogenetic event to occur in the epidermis is intercalation of dorsal epidermal cells. Epidermal cells are born at the end of gastrulation as a group of cells on the posterior and dorsal surface of the embryo and form two cellular rows (Heid et al., 2001; Schierenberg et al., 1983). These dorsal cells become wedge-shaped as they begin intercalation, eventually adopting a “ladder like” arrangement in the dorsal midline. After completion of dorsal intercalation, the lateral epidermal cells on each side of the embryo migrate toward the ventral midline and fuse to enclose the embryo in epidermis. Ventral enclosure takes place in two phases (Raich et al., 1999). In the first phase, the two most anterior epidermal cells on each side of the embryo, called the “leading cells,” migrate by extending long protrusions toward the ventral midline. They eventually meet and establish contact. In the second phase, the posterior cells form a continuous actin-rich border around the “ventral pocket.” The pocket cells become wedge-shaped and initiate contractions to fully enclose the epidermis through a “purse-string” mechanism.

Once ventral enclosure is completed, the embryo elongates from the lima bean shape to the characteristic worm shape, resulting in

a fourfold increase in length (Figure 1). Elongation is the least understood of the three morphogenetic developmental stages in *C. elegans*. During elongation, epidermal cells modify their shape. About midway through elongation, muscles start to contract, an change that is essential because muscle-defective embryos are paralyzed and their development arrested at the 2-fold stage (Waterson, 1989; Williams and Waterson, 1994). Elongation can be divided into an early and late phase relative to the beginning of muscle contractions, with each stage mediated by distinct molecular pathways. During early elongation, genetic and molecular data suggest that actomyosin contractility is essential, and that actin filaments are necessary for cell shape changes and include the actin cytoskeleton as well as proteins that regulate actin dynamics, such as Rac, WASP, and Arp2/3 (Andrew and Hardin, 2005). During late phase elongation, muscle cells play a central role, and actomyosin contractibility in the epidermis is thought to be less important (Wassarman, 2011). Muscle contractions create a longitudinal tension that is transmitted to the epidermis, causing the dorsal and ventral cells to become locally compressed and stretched longitudinally (Wassarman, 2011), and allowing the body shape of the worm to lengthen.

One reason that morphogenesis in *C. elegans* remains only par-

tially understood is that many genes have dual roles in its development. If a gene has a requirement earlier in development before morphogenesis occurs, that gene may be hard to link to morphogenetic processes. Although RNAi has provided a rich tool for conditionally knocking down genes to study morphogenesis, that method can be inefficient and create weak phenotypes. Furthermore, forward genetic screens that are labor intensive have likely not been saturated. (Andrew and Hardin, 2005). Thus, there is a lack of conditional, loss-of-function, stable genetic mutants in the field.

Bruce Bowerman's lab is performing a forward genetic screen for temperate-sensitive (TS) mutant alleles that are powerful tools for elucidating gene function. These TS alleles are conditional, based on temperature and loss-of-function genetic mutations. Worms grown at the permissive temperature of 15°C develop and reproduce like wild-type worms, but worms grown at the restrictive temperature of 26°C lay embryos that fail to hatch. Simply by controlling the temperature of the TS mutant alleles, researchers can induce a mutant phenotype at different stages of development. In this manner, early essential requirements can be by-passed by growth at a permissive temperature, with later essential functions then studied after temperature increases. About 1000 TS mutant alleles have already been

generated by chemical mutagenesis in the Bowerman Lab and given a unique *or* number, such as *or1330ts*. This collection has been successfully screened for early cell division mutant alleles (Connolly et al., 2014, Connolly et al., 2015) and for germline membrane and eggshell defective mutant alleles (Lowry et al., 2015).

By screening the Bowerman Lab's TS mutant collection for morphogenetic mutant alleles, I aim to achieve two goals: (1) to discover new alleles of genes known to affect morphogenesis, and (2) to discover new genes that have never been identified to affect morphogenesis. Currently, I have found two morphogenetic genes: *rib-1* (*or1193ts*) that functions in heparan sulfate proteoglycan biosynthesis and *emb-4* (*or1330ts*) that may have a role in regulating transcription. These findings may contribute to the endeavor to better understand the genes and pathways that control morphogenesis.

Materials & Methods:

Worm maintenance and generation of mutants

All strains were maintained following standard procedures (Brenner, 1974). The TS alleles were generated in a *lin-2* (*e1309*) mutant strain using chemical mutagenesis (ENU) as previously described (Kemphues et al., 1988; Encalada et al., 2000; O' Rourke et

al., 2011). Mutant *lin-2 (e1309)* worms produce a defective vulva and are unable to lay eggs, thus leading to a classic “bag of worms” phenotype (Kenyon et al., 1983). This mutation makes it easy to decipher TS embryonic lethal strains at the restrictive temperature of 26°C.

Phenotypic analysis

Morphogenetic alleles were screened and mapped to genes according to Lowry et al. (2015). Briefly, I first screened the mutant collection for alleles that exhibit defective morphogenesis at the restrictive temperature of 26°C using Nomarski optics. The L4 worms were upshifted to 26°C for a minimum of eight hours. Eggs were harvested and 1-8 cell-stage embryos were mounted on 4% agarose pad slides before being returned to 26°C. About 16 hours later, embryos were scored for developmental arrest and penetrant alleles were kept for genetic testing.

Genetic Tests

Standard genetic tests were performed to identify recessive and mendelian segregating alleles of each strain. Genetic tests were performed by setting a cross between homozygous mutant hermaphrodites and N2 (Bristol) wild-type males (Figure 2A). This cross is

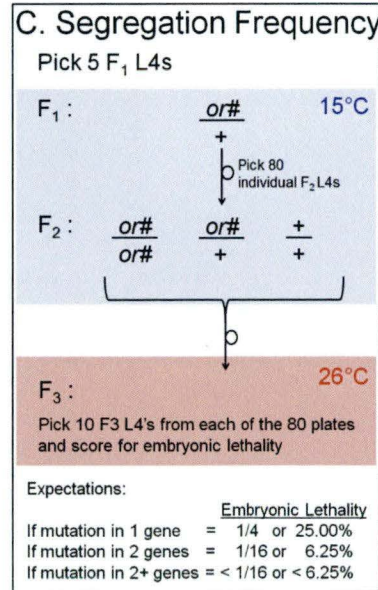
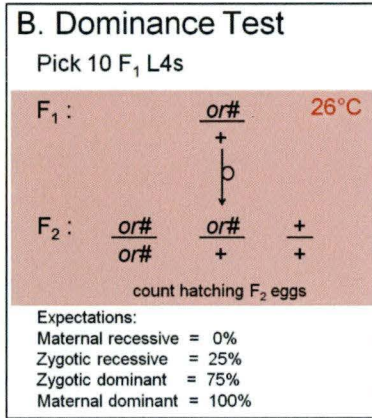
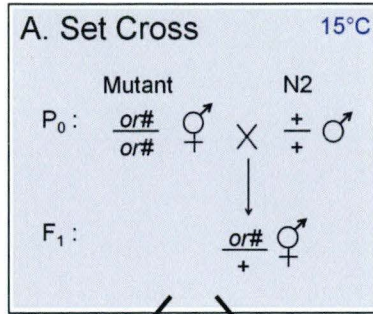


Figure 2: Crossing scheme for genetic characterization. (A) For both the dominance test (B) and the segregation frequency (C), and initial cross is set between a homozygous mutant hermaphrodite and a wild-type (N2) male to generate heterozygous F₁ hermaphrodites. (B) Dominance test. 10 heterozygous F₁ hermaphrodites are upshifted to 26°C and embryonic lethality is scored. Expectations of embryonic lethality for recessive, dominant, maternal and zygotic requirements are listed. (C) Segregation frequency. 5 heterozygous F₁ hermaphrodites are picked and allowed to self at 15°C. 80 individuals F₂ L4s are picked from a single F₁ and allowed to self at 15°C. 10 F₃ L4s are picked from each F₂ parent, upshifted to 26°C, and scored for embryonic lethality. Homozygous F₃ mutants will have 100% embryonic lethal progeny. Expectations for mutation at a single locus versus at multiple loci are listed

important for three reasons: (1) it allowed the leaky *lin-2* (*e1309*) mutation to be removed from the genetic background of the TS allele, (2) it permitted the identification recessive versus dominant alleles (Figure 2B), and (3) it distinguished strains that have one mutant allele instead of multiple mutant alleles (Figure 2C). After crossing to N2, I confirmed that the mutant phenotype of the allele was maintained by scoring embryonic lethality at 15°C and 26°C.

Identification of causative genes

After the genetic testing, I mapped the causative gene for each TS allele by whole genome sequencing according to Lowry et al (2015). Homozygous mutant hermaphrodites were outcrossed to CB4856 males, a strain derived from Hawaii (Hw) with a unique array of single nucleotide polymorphisms (SNPs) compared to the N2 (Bristol) strain (Figure 3). About eighty F₂ worms were singled out and homozygous mutants identified by picking ten F₃ L4 hermaphrodites from each F₂ plate and incubating at 26°C for three days. Synchronized L1s were obtained by pooling gravid adults from homozygous mutant F₂ plates and destroying contaminant bacteria DNA by hypochlorite treatment. These L1 worms were used for genomic DNA extraction (Qiagen DNeasy Kit). Libraries were prepared using the KAPA Hyper Plus Library Preparation Kit

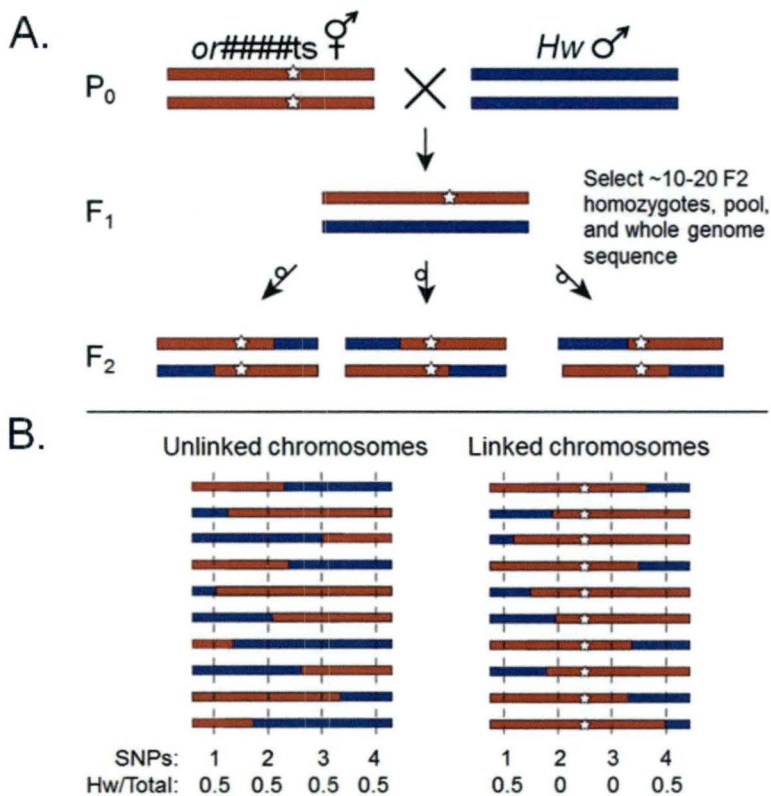


Figure 3: Crosses scheme and analysis for whole genome SNP mapping. (A) A hermaphrodite harboring a homozygous mutation (star) in a wild-type (N2) background (red chromosome) is mated with a Hawaiian (Hw; blue chromosome) male. P_0 will yield F_1 worms that are heterozygous for all chromosomes. The F_1 worms will then self-fertilize, and during meiosis, recombination will occur randomly to give rise to the F_2 generation. We select for 10-20 homozygous F_2 worms and send their progeny for whole genome sequencing. (B) Interpretation of mutationally unlinked and linked chromosome analysis. In an unlinked chromosome, we will see 50% Hw/Total SNPs. In our linked chromosome, we will see 0% Hw/Total SNPs (i.e., where the mutation of interest is located).

and Illumina TruSeq Adapters. Sequencing was performed by using the Illumina HiSequation 2000 at the Genomics and Cell Characterization Core Facility at the University of Oregon. Alleles were mapped using the Galaxy platform (Giardine et al., 2005; Goecks et al., 2010; Menevich et al., 2012; Langmead and Salzberg, 2012; DePristo et al., 2011; Cingolani et al., 2012; Quinlan and Hall, 2010). Candidate genes were identified as the causative genes by classic complementation genetic tests with null deletion alleles whenever possible.

Results

Terminal phenotypes

Morphogenesis refers to the development of structure and form of the body and organs of multicellular eukaryotes. Because morphogenesis remains partially understood, I sought to identify temperature-sensitive (TS) morphogenetic mutants as a tool for the community. The first step involved screening the Bowerman Lab collection of mutants with defective morphogenesis by analyzing embryos and scoring the stage at which they arrest at the restrictive temperature of 26°C. Most mutants arrest early during morphogenesis with no elongation past the 1-fold stage (Figures 4, 5). When

greater than 70% of the embryos produced by a mutant exhibit a consistent morphogenesis phenotype, the mutant is classified as penetrant. Thus far, penetrant mutants include *or870ts*, *or896ts*, *or922ts*, *or1006ts*, *or1193ts*, *or1214ts*, *or1330ts*, *or1354ts*, *or1404ts*, *or1742ts* and *or1755ts*; most of these alleles arrest with no elongation while a smaller number of alleles arrest with some elongation (Figures 4, 5A). Some alleles have a variable phenotype, where greater than 50% but less than 70% of embryos exhibit a single embryonic terminal phenotype. Alleles *or893ts*, *or1164ts*, *or1300ts*, *or1395ts*, *or1672ts* arrest at multiple stages of morphogenesis (Figures 4, 5B).

There are three categories of terminal phenotypes that warrant expulsion of the mutant from further study. First, alleles that can survive and fully elongate through the restricted temperature of 26°C are not considered morphogenetic mutants (data not shown). Second, *or1167ts* is an example of an allele taken out of our future studies because it is too variable and did not have a definite phenotype (Figures 4, 5C). Third, alleles *or1257ts* and *or1424ts* display large undifferentiated cells and are not considered morphogenetic mutants but are likely weak cell division mutants (Figures 4, 5C). We initially categorized *or1424ts* incorrectly as a mutant arresting with no elongation, and as a result, we performed the genetic test

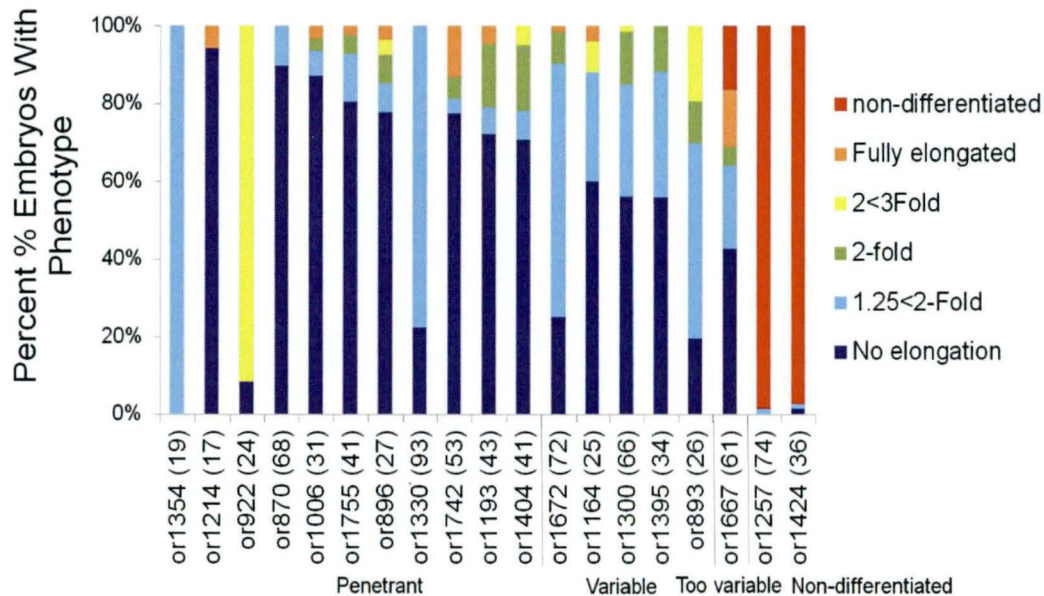


Figure 4: Quantification of morphogenetic terminal phenotypes percent of embryos that arrest at specific stages are color coded (see key). Alleles categorized as penetrant display a single phenotype 70% of the time or more. Alleles categorized as variable display 50%-70% of a single phenotype. Alleles categorized as too variable show all phenotypes less then 50%. Alleles categorized as non-differentiation mutants do not produce differentiated cell types (such as skin, pharynx, gut, etc.) and arrest with large undifferentiated cells. Temperature sensitive alleles are listed on the x-axis in order of decreasing penetrance from left to right. The total numbers of embryos scored are in parenthesis.

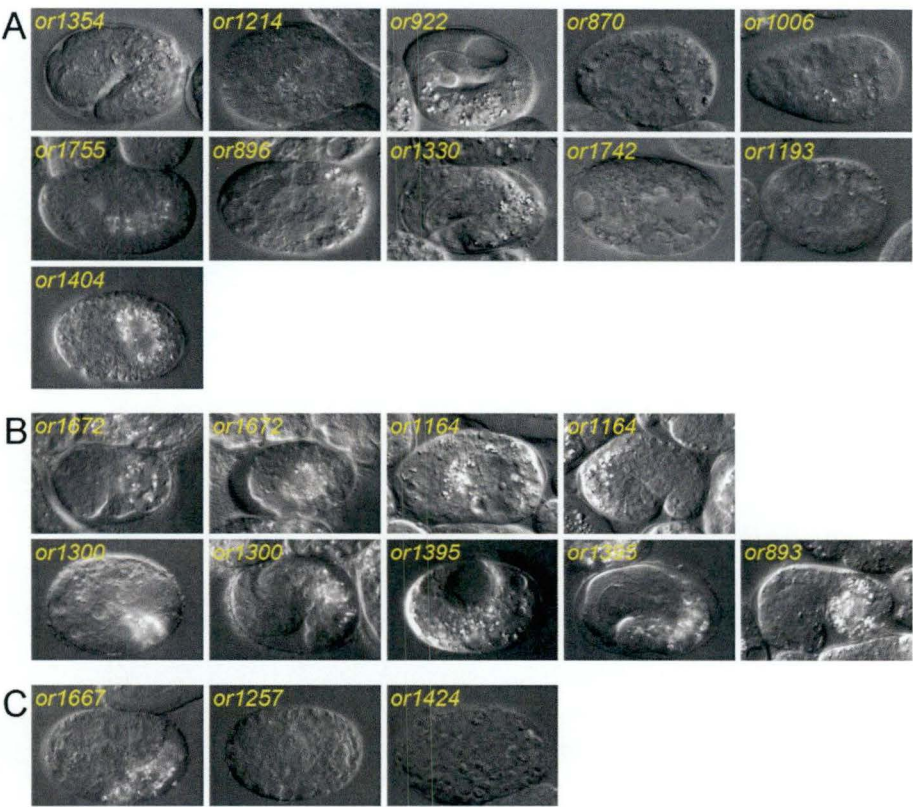


Figure 5: Nomarski images of terminally differentiated mutant embryos. (A) Penetrant alleles, (B) variable alleles, and (C) too variable and undifferentiated alleles as defined in Figure 4. Alleles are organized in decreasing penetrance from left to right (as in Figure 4).

and mapping crosses. Although *or1424ts* is not a morphogenetic mutant, its data is included in Table 1 below. In our study, we focused on penetrant morphogenetic alleles because these phenotypes are most likely caused by mutations in essential genes.

Table 1. Genetic characterization and embryonic lethality for temperature-sensitive alleles

Allele	Dom. Test (n)	Seg. Freq (n)	Embryonic Lethal	
			15°C (n)	26°C (n)
<i>or870</i>	7.4% (269)	27.5% (80)	0 % (91)	99.2% (130)
<i>or896</i>	2.0% (147)	25.0% (80)	1.9% (262)	90.8% (412)
<i>or922</i>	4.8% (272)	17.5% (80)		
<i>or1193</i>	0.9% (473)	21.8% (78)	1.9% (782)	91.8% (538)
<i>or1330</i>	1.2% (330)	10.0% (80)	46% (791)	100% (847)
<i>or1354</i>	3.3% (456)	22.5% (80)		
<i>or1424</i>	1.1% (533)	23.8% (80)	0.4% (272)	97.2% (671)
<i>or1672</i>	4.1% (291)	22.5% (80)	14.9% (341)	100% (456)
<i>or1742</i>	1.2% (450)	20.0% (80)	1.82% (177)	80.0% (235)
<i>or1755</i>	7.2% (454)			

Genetic Characterization

The TS conditional mutants can reproduce at the permissive temperature of 15°C and exhibit embryonic lethality only when L4 parents are upshifted to the restrictive temperature of 26°C. Therefore, using complicated genetics, such as balancers, is unnecessary to maintain the alleles. As genes that influence morphogenesis were

identified, mutants were characterized for being recessive and occurring at a single locus (Mendelian segregation).

First, a dominance test provided a general and superficial characterization of whether a gene is recessive or dominant and whether it has a maternal or zygotic requirement. We wish to work with only recessive mutants to study the “normal” function of a gene. Recessive mutations tend to be loss-of-function null or hypomorphic alleles, whereas dominant mutations tend to be gain-of-function or neomorphic alleles and do not provide insight into a gene’s normal function. Moreover, early embryos contain genes expressed from two distinct origins: those expressed from the mother’s genome and deposited in the oocytes (maternal) and those expressed from the embryo’s genome after fertilization (zygotic). *C. elegans* may have essential morphogenetic genes that have multiple requirements during development from either or both pools. Whether a gene is required maternally, zygotically, or both, analysis of progeny from upshifted homozygous recessive mutants allows us to bypass earlier requirements for a gene and reduce both maternal and zygotic functions. To perform a dominance test, a mutant hermaphrodite is mated to wild-type (N2) males (Figure 2A). The resultant F₁ heterozygous L4 hermaphrodites are upshifted to the restrictive temperature and

the F₂ embryos scored for lethality (Figure 2B). I expected the following: (1) 0% embryonic lethality for recessive, maternal effect mutations, (2) 25% embryonic lethality for recessive, zygotic mutations, (3) 75% embryonic lethality for dominant, zygotic mutations, and (4) 100% for dominant, maternal effect mutations. Alleles that have embryonic lethality under 25% are considered recessive, while alleles with embryonic lethality over 25% are considered dominant. Therefore, alleles *or870ts*, *or896ts*, *or922ts*, *or1006ts*, *or1193ts*, *or1330ts*, *or1354ts*, *or1404ts*, *or1672ts*, *or1742ts*, and *or1755ts* display close to 0% embryonic lethality, characterizing them as recessive and likely maternal-effect mutations (Table 1).

Second, to evaluate whether each allele represents a mutation at a single locus versus at multiple loci, I scored each mutant's segregation frequency. When investigating the function of a gene, it is important to study the gene in isolation so that the mutant phenotype can be attributed to a single gene. In studying a double mutant, it would be difficult to distinguish how each gene contributes to the phenotype. To measure the segregation frequency, a homozygous recessive mutant hermaphrodite was mated to wild-type (N2) males, and the embryonic lethality of the F₃ progeny of 80 upshifted F₂ L4 hermaphrodites was scored (Figure 2A, C). If the homozygous re-

cessive allele is a mutant in a single gene, about 1/4 (or 25%) of the F₂ parents will have embryonic lethal progeny at 26°C. If, however, the homozygous recessive allele is a double mutant, 1/16 (or 6.25%) of the F₂ parents will have embryonic lethal progeny at 26°C. Alleles *or870ts*, *or896ts*, *or922ts*, *or1193ts*, *or1354ts*, *or1424ts*, *or1672ts*, and *or1742ts* have a segregation frequency around 25%, falling between the acceptable range of 15-35% (Table 1), indicating these alleles are single genetic mutants. When a segregation frequency is below 10%, the strain is likely a double mutant. Therefore, it is unclear whether *or1330ts* is a double mutant or whether the frequency is low due to early picking of single F₂ worms, which can cause an overabundance of phenotypically wild-type worms (Table 1). Since *or1330ts* has a penetrant morphogenetic phenotype, the analysis moved forward with all nine alleles.

After the dominance and segregation frequency analyses, I needed to ensure that the N2-outcrossed strain has maintained the TS mutant phenotype of embryonic lethality, similar to the original strain in the *lin-2* (*e1309*) mutant background. I performed a test scoring for percent of embryonic lethality at 15°C and 26°C, which should give 0% and 100% embryonic lethality, respectively. Alleles *or870ts*, *or896ts*, *or1193ts*, *or1330ts*, *or1424ts*, *or1672ts*,

and *or1742ts* have acceptable embryonic lethality compared to the expected levels (Table 1). Although alleles *or1330ts* and *or1672ts* display embryonic lethality higher than the expected 0% at the permissive temperature of 15°C, there is still a drastic increase in embryonic lethality from 15°C to 26°C for these alleles. Therefore, the high embryonic lethality at 15°C is acceptable and simply indicates these mutants will be slow growers at the permissive temperature. Therefore, all these alleles are acceptable for genetic mapping analysis because they are homozygous recessive, single mutant alleles that exhibit high embryonic lethality 26°C. I will perform the same genetic tests on the remaining alleles: *or922ts*, *or1006ts*, *or1214ts*, *or1354ts*, *or1404ts*, and *or1755ts*. The results gained from performing genetic characterization (dominance test, segregation frequency, and mutant maintenance) give a preliminary characterization of the mutants to determine which strains to map.

Mapping and Gene Identification

To study morphogenesis, the causative gene needs to be identified for each mutant allele. The process of identifying candidate genes in a chromosomal location uses whole-genome, single nucleotide polymorphism (SNP) sequencing by mating homozygous mutant N2-outcrossed hermaphrodites to Hawaiian (Hw; *CB4856*) males, a

strain with a unique array of SNP's roughly spaced at 1kb intervals across its genome compared to the N2 wild-type strain (Figure 3). The cross yields an F_1 heterozygous generation in which the worms each have an N2 and Hw chromosome for each chromosomal pair. Meiotic recombination will occur randomly in the genomes of the F_1 heterozygous generation, and the F_1 worms will self-fertilize to give rise to an F_2 generation with a recombined N2-Hw genome. From that F_2 generation, homozygous mutants are selected by picking F_3 L4s, upshifting them to 26°C, and screening for embryonic lethality three days later. Ten to twenty homozygous mutant F_2 worms are isolated and their progeny sent for whole genome sequencing. Analysis using the Galaxy platform (Giardine et al., 2005; Goecks et al., 2010; Menevich et al., 2012; Langmead and Salzberg, 2012; DePristo et al., 2011; Cingolani et al., 2012; Quinlan and Hall, 2010) compares the N2 and Hw SNPs across all chromosomes of the genome (Figure 3). An N2 chromosome that is unlinked to the mutation will have random recombination with the Hw chromosome for every SNP; therefore, I expect the ratio of Hw over total SNPs to be about 50% across the entire unlinked chromosome. An N2 chromosome that is linked to a mutation will have low recombination with the Hw chromosome at SNPs physically close to the location of the

mutation; therefore, I expect the ratio of Hw over total SNPs to be 0% at the site of the mutation. This technique allows us to identify the chromosomal region where the mutation lies and also gives rise to a list of gene candidates.

Mapping was completed, and candidates were identified for two alleles: *or1193ts* and *or1330ts*. Allele *or1193ts* maps to the 5-12 Mb region on chromosome IV, and *or1330ts* maps to the 11-21 Mb region on chromosome V (Figure 6). Two potential gene candidates were identified on chromosome IV for *or1193ts*: (1) *rpn-1*, which encodes a subunit of the 26S proteasome (Takahashi et al., 2002), and (2) *rib-1*, which encodes an N-acetylglucosamine (GlcNAc) transferase, important for heparan sulfate proteoglycan (HSPG) synthesis (Franks et al., 2006; Kitagawa et al., 2007). Three potential gene candidates were identified on chromosome V for *or1330ts*: (1) *emb-4*, which encodes a nuclear protein with a putative ATPase domain (Checchi and Kelly, 2006); (2) *Y39B6A.25*, which is a ring finger protein and involved in body morphogenesis during embryo development; and (3) *F10A3.16*, which regulates *csr-1*, which in turn encodes an Argonaute protein involved in RNAi, and *sir-2.1*, which encodes a deacetylase protein involved in the metabolism (Harris et al., 2014).

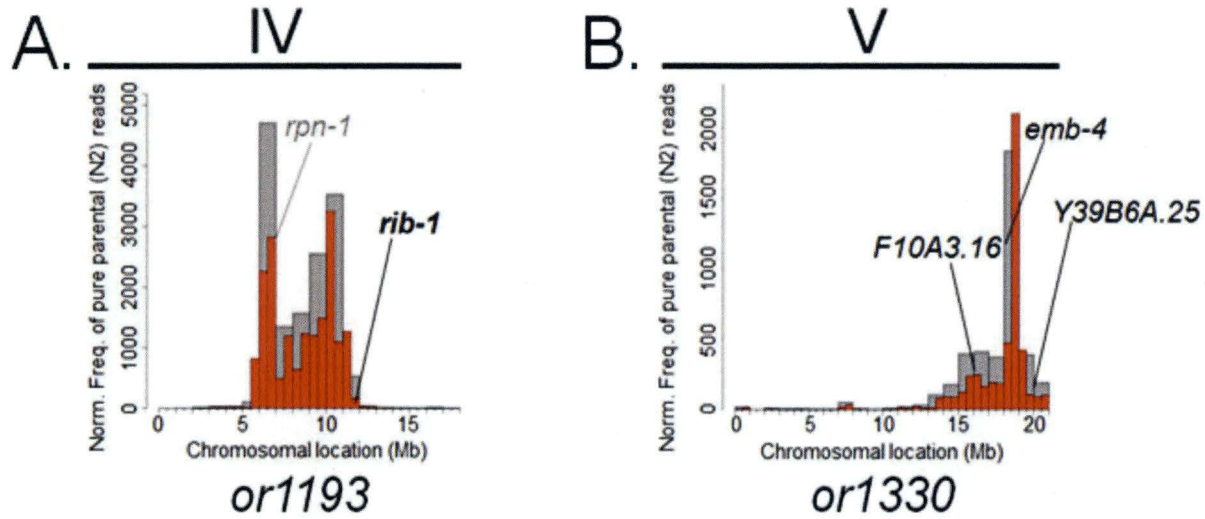


Figure 6: Genomic location of temperature sensitive mutants. Normalization frequencies of Hawaiian/Total SNPs across chromosomes IV and V for mutants *or1193ts* (A) and *or1330ts* (B), respectively. Candidate genes and their location are indicated.

Once candidate genes are identified, complementation tests using existing, known alleles identify the causative gene for the mutation. To determine the causative gene for *or1193ts*, complementation tests were performed by mating *or1193ts* hermaphrodites to *rpn-1* (*ok259*) and *rib-1* (*ok556*) deletion allele males and scoring for embryonic lethality at 26°C. The *rpn-1* (*ok259*) gene complemented *or1193ts*, with only 2.7% (n = 990) embryonic lethality, indicating *rpn-1* is not the causative gene for *or1193ts* (Table 2). Alternatively, *rib-1* failed to complement *or1193ts*, with 97.1% (n = 1514) embryonic lethality, indicating *or1193ts* is a mutant allele of the gene *rib-1* (Table 2).

Whole genome sequencing identified the nucleotide change of *or1193ts* to be an A to T transition, resulting in the non-synonymous substitution of amino acid 126 from a histidine (H) to a leucine (L) in the gene *rib-1* (Table 2; Figure 7A). There are two existing alleles of *rib-1*: (1) *ok556* causing an in-frame deletion of 55 amino acids, and (2) *tm516* in which 468 bp are deleted and 32 bp are inserted into *rib-1*, causing an immediate premature stop. A comparison of the terminal phenotype of the isolated allele of *rib-1*, *or1193ts*, to the previously published results for the two existing alleles revealed that *or1193ts* mutant embryos primarily arrest with no elongation

Table 2. Gene identification among *or1193ts* and *or1330ts*.

Allele	Gene Tested (allele)	% Emb. Leth (26° C)	(n)	Sequence change (genome/protein)	Human ortholog	Gene function
or1193ts	rpn-1 (ok2259)	2.7%	990	N/A	RPN-1	Non-ATPase subunit of the 26S proteasome
or1193ts	rib-1(ok556)	97.1%	1518	A>T/H126L	EXT1	GlcNAc transferase for synthesis of heparin sulfate proteoglycans
or1330ts	emb-4 (hc60ts)	100%	1111	atc>aAt/ I967N; this frameshift causes a premature stop at amino acid 1004	Aquarius	Nuclear protein with putative AAA. ATPase domain

(72%, n = 42; Figure 7B). Similarly, *tm516* mutant embryos arrest primarily at the 1 to 1.25-fold stage (68%, n = 92; Kitagawa et al., 2007; Figure 7B). Alternatively, *ok556* mutant embryos primarily arrest at a later stage compared to *or1193ts* and *tm516* so that 57% of embryos arrest at the 1 to 1.25-fold and 19% embryos arrest at 3-fold or as hatched larva (n=37%; Franks et al., 2006; Figure 7B).

To determine the causative gene for *or1330ts*, I performed a complementation test. Of the three gene candidates, *emb-4* is the best option for *or1330ts* because previous studies indicated that *emb-4* is a morphogenetic mutant (Checchi and Kelly, 2006). The other two alleles, *Y39B6A.25* and *F10A3.16*, have no existing alleles, and prior analysis was performed using RNAi. For the complementation test, the *emb-4* (*hc60ts*) mutant hermaphrodite was crossed with wild-type (N2) males to generate heterozygous F₁ males. Then, a homozygous mutant *or1330ts* hermaphrodite was crossed to *hc60ts/+* males to generate cross progeny of two expected genotypes, *or1330ts/hc60ts* and *or1330/+*, and scored for embryonic lethality at 26°C. If *or1330ts* is a mutation of the *emb-4* gene, half of the cross progeny (those of the genotype *or1330ts/hc60ts*) should fail to complement and display 100% embryonic death at 26°C. Alternatively, if *or1330ts* is not a mutation of the *emb-4* gene,

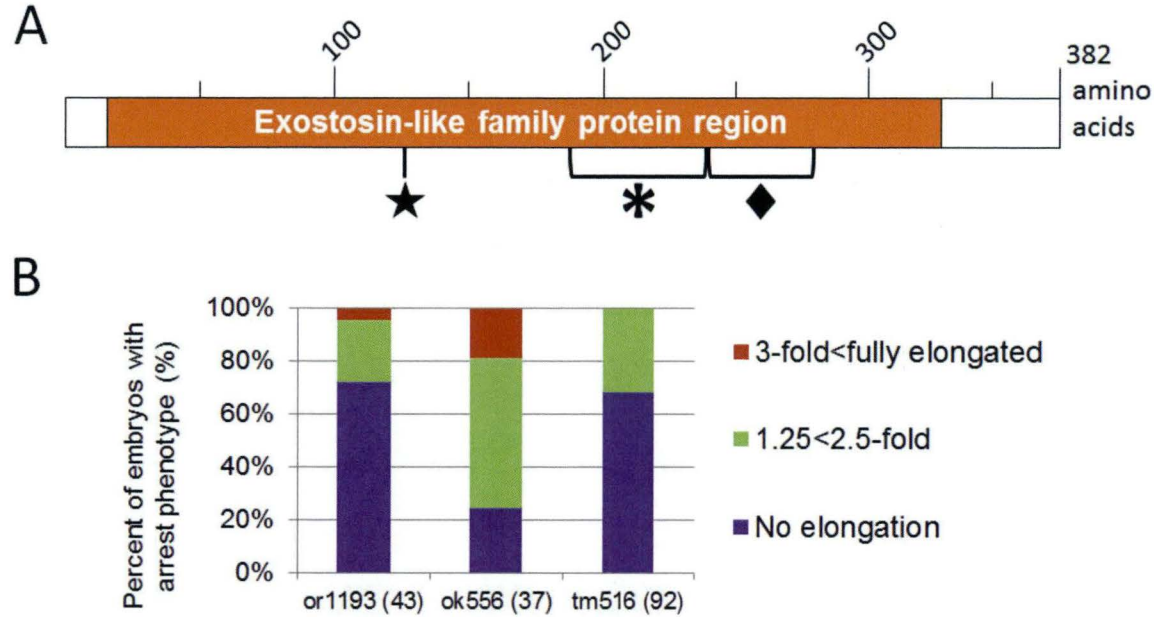


Figure 7: Comparison of the rib-1 alleles: orl193ts, ok556, and tm516. (A) Protein structure of RIB-1. orl193ts (star) mutation is a non-synonymous change between a histidine to a leucine in amino acid 126 . ok556 (asterisk) is a 55 amino acid in-frame deletion of amino acids 189-243. tm516 (diamond) is a complex mutation of a 35 amino acid deletion amino acids (244-278) and a 32 bp insertion causing a frame shift and a premature stop at amino acid 245 (B) Terminal phenotypes of orl193ts compared to ok556 and tm516 (ok556 data from Franks et al., 2006; tm516 data from Kitagawa et al., 2007).

all of the cross progeny should yield 100% hatched larva at 26°C. Results showed that *emb-4* (*hc60ts*) failed to complement *or1330ts*, and that 35/78 cross progeny had 100% embryonic lethality ($n = 1111$ embryos) indicating that *or1330ts* is a mutant allele of the gene *emb-4* (Table 2).

Whole genome sequencing identified *or1330ts* to be an insertion of a single adenine in codon 967 (atc to aatc) that causes a frame shift and premature stop at codon 1004 (Table 2; Figure 8A). Previous studies have indicated there are other existing mutant alleles of *emb-4*, the strongest and most studied being *hc60ts*. The nature of the mutation of *hc60ts* has not been well curated, although Checchi and Kelly (2006) indicate that there is a non-synonymous change of an amino acid to a premature stop somewhere in exon 5 (Figure 8A). I compared the embryonic lethality and terminal phenotypes of alleles *emb-4*, *or1330ts* to the previous pre-existing allele, *hc60ts*. Both alleles have about 50% embryonic lethality at 15°C, 100% embryonic lethality at 26°C, and both terminally arrest primarily in the 1.25 to 2 fold stage (Figure 8B, C).

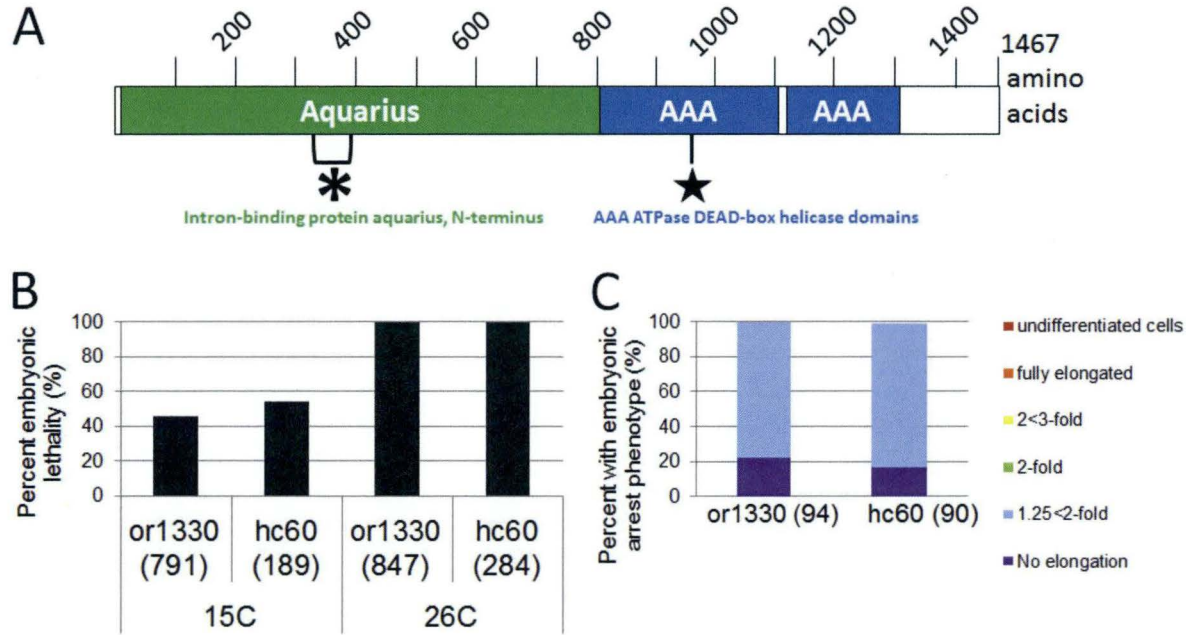


Figure 8: Comparison of the *emb-4* alleles: *or1330ts* and *hc60ts*. (A) Protein structure of EMB-4. *or1330ts* (star) is a single nucleotide insertion in codon 967 (atc to aatc) causing a frame shift and premature stop at amino acid 1004. *hc60ts* (asterisk) is a non-synonymous change of an amino acid located somewhere in exon 5 (amino acids 321-395) to a stop. (B) Embryonic lethality at 15°C and 26°C. (C) Terminal phenotypes of 1330ts compared to hc60ts.

Discussion

The basic genetic and molecular changes that occur at the cellular basis and result in gross tissue morphogenesis are only partially understood. One challenge in studying morphogenesis is that genes often have multiple roles during their development; thus, if a gene has an earlier role in embryogenesis before morphogenesis occurs, its role in morphogenesis often remains unstudied. In order to study morphogenesis, I have screened for conditional, temperature-sensitive (TS), morphogenetic mutants in the model organism *C. elegans* seeking to identify alleles with penetrant defects in morphogenesis and identify their genetic mutations. I have discovered several penetrant alleles with severe morphogenetic defects, including *or870ts*, *or896ts*, *or922ts* *or1006ts*, *or1193ts*, *or1214ts*, *or1330ts*, *or1354ts* *or1404ts*, *or1742ts*, *or1755ts*. Currently, I have mapped two morphogenetic mutant alleles, *or1193ts* and *or1330ts*, and identified one to be the gene *rib-1* and one to be the gene *emb-4*.

Allele or1193ts is the gene rib-1, known to affect sugar modification biosynthesis

We have identified the allele, *or1193*, to be a mutation in the gene *rib-1*. The human ortholog of *rib-1* is EXT1, a member of the Exostosin (EXT) gene family of glycosyltransferases. The EXT1 gene

functions in heparan sulfate proteoglycan (HSPG) biosynthesis. In their turn, HSPGs are composed of heparan sulfate (HS) chains, alternating glucuronic acid and *N*-acetylglucosamine (GlcNAc) residues, that are covalently linked to a core protein at serine amino acids (reviewed in Poulain and Yost, 2015). EXT1 is the enzyme responsible for the addition of GlcNAc sugar molecules to the elongating HS chain. The structure of HS chains is highly diverse because the glucuronic acid and GlcNAc residues can undergo extensive sulfation and epimerization (Lindahl et al., 1998; Esko and Selleck, 2002; Bulow and Hobert, 2006; Bishop et al., 2007; Kreuger and Kjellen, 2012). The HS chains are attached to proteins located at the cell surface (receptors) or in the extracellular space (ligands); thus, HSPGs have been implicated in modulating signal transduction (including Wnt, Hh, Dpp, EGF, and FGF signal pathways), important for cell survival, division, migration, and differentiation in vertebrates and invertebrates (Poulain and Yost, 2015; Humphreys et al, 2013). According to the “sugar code” hypothesis (Holt and Dickson, 2005), HS modifications within core proteins in specific tissues at specific times within the embryo control signaling pathways and coordinate morphogenesis and development. Loss of *EXT1* gene function in mice does, in fact, lead to embryonic death and gastrulation defects

(Lin et al., 2000; Mitchell; et al., 2001; Shimokowa et al., 2011), along with neurogenesis defects (Inatani et al., 2003). Furthermore, deletion of *EXT1* in humans has been linked to mental disorders and autism (Lie et al., 2002), and disruption of *EXT* genes result in hereditary multiple exostoses, a rare medical disorder in which boney spurs develop in embryos/fetuses, children and adults (Ahn et al., 1995).

Characterization of *rib-1* in *C. elegans* has identified a role for HS modification in embryonic morphogenesis. Like *EXT1*, *RIB-1* likely functions as glycosyltransferase in HS biosynthesis because loss of *rib-1* in embryos results in decreased levels of HS (Franks et al., 2006; Kitagawa et al., 2007). Analysis of two previously existing alleles, *ok556* and *tm516*, indicates that both result in maternal effect embryonic lethality and exhibit variable penetrance for developmental arrest (Franks et al., 2006; Kitagawa et al., 2007). In addition to body elongation defects, *ok556* also exhibits pharyngeal isthmus elongation defects. These data taken together suggest that *rib-1* is required for morphogenetic events. By comparing terminal phenotypes of the *rib-1* allele to the two preexisting alleles, I found that *or1193ts* is stronger than *ok556* and as strong as *tm516*. Intuitively we would expect the deletion alleles to cause a more penetrant phe-

notype, but my data indicate that the single amino acid substitution in *or1193ts* causes a phenotype as strong as the complex mutation in *tm516*. This result is a surprise because deletions usually result in stronger alleles than single nucleotide substitutions causing a non-synonymous amino acid change. Allele *or1193ts* is the only known temperature sensitive allele for gene *rib-1* and, therefore, may provide a better tool for studying morphogenesis.

Allele or1330 is the gene emb-4, a known morphogenetic gene with unclear function

We identified the allele, *or1330ts*, to be a mutation in the gene *emb-4* that encodes a nuclear protein with a helicase-like domain, meaning it could potentially affect the unwinding of the DNA or RNA (Checchi and Kelly, 2006). Studies by Katic and Greenwald (2006) confirmed that *emb-4* is homologous to the Aquarius family. The *emb-4* gene is conserved within a wide array of species other than *C. elegans*, including *Arabidopsis*, *Saccharomyces pombe*, *Drosophila*, mice, and humans. The function of *emb-4* is still unclear and has yet to be identified; thus, *emb-4* has multiple listed roles and potential mechanisms. First *emb-4* is involved in the regulation of the notch-signaling pathway post-embryonically in vulva development in *C.elegans* (Katic and Greenwald, 2006). Second, the *S.*

pombe emb-4 ortholog, Cwf11p, co-purifies with Cdc5p, which is a member of the pre-mRNA splicing complex (Katic and Greenwald, 2006). Therefore, *emb-4* could potentially have a role in RNA splicing. Moreover, the best line of evidence suggests that *emb-4* regulates histone modification. Specifically, *emb-4* suppresses H3K4 methylation, which marks transcriptionally competent chromatin, in the germ line lineage cells Z2/Z3 (Checchi and Kelly, 2006). Therefore, it is most likely that *emb-4* function globally to suppress transcription. Any one of the three proposed functions is a potential mechanism by which *emb-4* could control gene expression, perhaps by regulating the cytoskeleton or cell adhesion genes important to cell shape changes and/or migration.

The *emb-4* gene is a known morphogenetic gene because although its cell fate patterning is fine, its cell placement is impaired in mutants. Specifically, E cell lineage cells were found to divide 33% slower in *emb-4* mutants than in wild-type embryos (Schierenberg et al., 1980). Furthermore, E lineage cells mark by ETL-7::GFP indicated that the number of gut cells specified were not different from wild-type, but their location within the embryo was altered, and in general, many tissues were poorly organized (Checchi and Kelly, 2006). Taken together, these data indicate that *emb-4* is

a gene required for morphogenesis. In comparison to the preexisting allele *hc60ts*, our allele *or1330ts* behaves similarly; both have strong TS embryonic lethality and penetrant terminal phenotype. While both alleles result in premature stop, the mutation in *hc60ts* truncates the protein at an earlier location than *or1330ts*; therefore, we would have expected *hc60ts* to be a stronger mutant. However, both mutations have equally strong phenotypes. Because *emb-4* is clearly an important morphogenetic gene, the use of either of the TS alleles may help better understand regulation of cell shape changes and migration important to morphogenetic events.

Conclusion

Alleles *or1193ts* and *or1330ts* show great promise as morphogenetic mutants. Both alleles have interesting phenotypes that could allow us to better investigate cell shape changes and migration. Additionally, genes identified by my forward genetic screen aid our basic understanding of morphogenesis in *C. elegans* and may also be applicable to our understanding of morphogenesis in other species, such as humans. These studies may help to eventually provide insight and suggest therapeutic approaches to morphogenetic defects during human embryogenesis. Furthermore, the alleles and genes identified by this forward genetic screen will be shared with

the research community through the Caenorhabditis Genetic Center (CGC) to more broadly help the community study questions regarding morphogenesis.

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The McNair Scholars Program is made possible through a grant from the U.S. Department of Education awarded to the University Teaching and Learning Center (TLC) and substantial institutional commitment.

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