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The Effects of Intra- and Interspecific Phenotypic Variations for Competition in Freshwater Zooplankton

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Biology Department from The College of William and Mary

by

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Accepted for Honors (Honors, High-Honors, Highest Honors)

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Williamsburg, VA
May 2, 2019
8. Reference.........................................................................................................................35
1. Introduction

Communities are structured by interactions between species and their environment and between one another. Because resources are typically limited in nature, competition (sensitivity to the presence of other individuals of the same or of another species) is an important determinant of whether or not species can coexist, and is also an important process to understand biodiversity (Tilman, 1987; Freckleton et al., 2009). Numerous studies have measured how the presence of competitors alters growth and survival (Connell, 1983; Ascheoug et al., 2016), and researchers are currently focused on effectively translating experimental measures of competition to the coexistence and biodiversity patterns observed in natural communities (Freckleton et al. 2009). Resource competition has been demonstrated to show sensitivity to temperature and other factors that influence productivity (Goldberg et al., 1999), and it is therefore likely that biodiversity patterns associated with competition will shift as a result of anthropogenic environmental changes such as altered rainfall patterns (Hautier et al., 2009; Clark et al., 2011) and urbanization (Shochat et al., 2010).

The effects of competition can be quantified in numerous ways (Weigelt et al., 2003). An effective method for quantifying competitive ability is to estimate parameter values from models that describe the effects of competitors for growth of populations (Beverton & Holt, 1957; May & Leonard, 1975). This method allows a precise, quantitative definition of competitive ability for a given species that can be compared across different species or conditions, which differs from approaches such as experimental removal of focal species (e.g. Oksanen et al. 2006). Generally, the models include a growth rate term, a term for intraspecific competition (interaction with individuals of the same species), and a term for interspecific competition (interaction with individuals of other species).
Despite the numerous models available for describing population dynamics in the presence of competitors, Chesson (2000; 2012) has shown that common determinants for competitive ability and coexistence emerge from these models. Across the range of models for competition, competitive dominant species are those that combine a high growth rate in the absence of competition and an ability to tolerate competition from both conspecific and heterospecific individuals in their shared location (Hart et al. 2017). If the parameters of these competition models are correctly estimated, it is possible to quantify competition, determine competitive hierarchies, and determine expected coexistence patterns for groups of potentially co-occurring species (Hart et al. 2017).

One important assumption for these models is that the parameters are traditionally treated as fixed for each species. This implies there is no intraspecific variation for the traits that underlie these parameters, which is unlikely to be the case in nature. The population growth rate parameter used in ecological models of population growth, \( r \), is ultimately the same as an individual’s realized fitness (Coulson et al. 2006), and fitness varies in the context of genes and environment. Furthermore, traits that influence competition (e.g. Jung et al. 2010; Edwards et al., 2013; Vogt et al. 2013; Kunstler et al. 2016) are often heritable and vary among populations (Ehlers et al., 2016). These findings are important for accurately understanding biodiversity because community dynamics can be influenced by the genetic composition of resident species (Vellend 2006). For example, one study demonstrated that the genetic composition of one species altered the colonization success of other immigrant species (De Meester et al., 2007), and another study found that rapid adaptation for one species in response to different environmental conditions caused entirely different zooplankton communities to assemble (Pantel et al., 2015). Another study in a plant- microbe system found that coevolution of microbes with their host Brassica rapa also led to microbial communities with distinct composition patterns (terHorst et al., 2014).
While studies have measured the consequences of genetic variation for competition, community assembly, and coexistence, it is currently not known whether coefficients for competition models demonstrate heritable intraspecific variation. The aim of this study was to determine whether the strength of competition between two species is a heritable trait. We used freshwater zooplankton as a model system to investigate this and had three main goals: (1) to estimate genetic variation in functional traits that might influence competitive ability; 2) to determine whether there is genetic variation for competitive ability itself (both intraspecific and interspecific competition); 3) and to determine if variable competition strength affects community dynamics and species coexistence in experimental mesocosms. We combined three experiments to achieve these goals. The first was measurement of grazing rates in multiple clones of two zooplankton species in a common garden environment. The second was a common garden experiment to quantify pairwise competition coefficients for multiple clones of each species. The third was a mesocosm experiment to determine whether intraspecific genetic variation in competition strength altered the outcome of community dynamics and whether this effect was temperature-dependent.

2. Background

2.1. Sampling Site

Zooplankton used in the experiments were collected from rock pools on the banks of the James River (Belle Isle, Richmond, Virginia) between June and July 2018. Rock pools are small depressions on the rock-bed that are initially filled after the last river flooding event (June 2018). Many pools retain water throughout the summer, and they are also transiently filled with rain water. Highly variable size, depth, location and water chemistry of rock pools create various
environments within a small geographic area, making them an ideal system to study community ecology.

![Figure 1. Rock pools filled with water on the bank of the James River (Belle Isle, Richmond, Virginia).](image)

### 2.2. Model System

Freshwater zooplankton are an ideal model system for studying competition and its influences for biodiversity. Freshwater pools are bounded, and thus distinct communities can be defined with ease. Numerous zooplankton taxa can coexist locally and regionally, and they compete for phytoplankton resources. Zooplankton are also an excellent model system for studying genetic variation. Cladoceran zooplankton are cyclical parthenogens. They produce clonal female offspring during the growing season. When environmental conditions decline, they produce males and then undergo sexual reproduction to produce diapausal eggs that are encased in a hard covering (an ephippia) that can withstand drying and freezing. Because of the parthenogenetic stage, clonal lineages can be maintained indefinitely in laboratory conditions. Zooplankton often harbor a substantial degree of among-population genetic variation (De Meester 1996; Lynch et al. 1999) and numerous studies document the impact of genetic variation for ecological processes such as grazing (e.g. Park & Post, 2017). Daphnia pulex is an evolutionary model organism. Its genome
has been fully sequenced (Colbourne et al. 2011) and numerous genetic tools have been developed (e.g. microsatellites: Colbourne et al. 2004).

![Figure 2. Life cycle of Daphnia magna. The left column illustrates major events of embryonic development which takes place in the dorsal brood pouch of the mother. Scale bars: 100 μm. Daphnia follows two different reproduction strategies. The parthenogenetic (I) and sexual (II) life cycle are pictured. Figure is reproduced from Wolff & Gerberding 2015 (Figure 2.2).](image)

2.3. Rock Pool Survey

In order to identify candidate species for inclusion in competition experiments, we first needed to determine which zooplankton species were present in the rock pool system. We surveyed zooplankton community composition throughout the growing season of 2018 for thirty-four Bell Isle rock pools. The pools were initially chosen from the first section exposed after the last flooding event, but some sites were added later in the summer as they became exposed. A variable volume was sampled from each pool every two weeks from June 7th to September 16th, 2019. This time span included two flooding events, which means pools likely experienced different stages of community assembly after being initially stocked with a random sub-sample of zooplankton remaining in the pools after the flooding event. The eventual goal is to use this data to determine the relative importance of time since flooding, pool size, and pool isolation for turnover in zooplankton species composition. The data set also included pools that zooplankton used for
subsequent experiments were drawn from. Water samples were filtered through a 63µm zooplankton net, stored in 60ml plastic bottle, placed on ice during transport to the lab, and fixed in absolute ethanol.

*Daphnia* and *Simocephalus* were identified to species, Copepods were identified to order, and other zooplankton were identified to genus (Thorpe & Covich 2010). The presence-absence for the first two weeks of the survey (Figure 3) indicates new species are continuing to emerge in some sites while others went extinct, suggesting the beginning stages of community assembly.

**Figure 3.** Presence (black dots) and absence (circles) of zooplankton taxa in surveyed rock pools. Each column represents a pool and each row represents a species, genus, or order.

### 2.4. Identification of unique clonal lineages

Based on the taxa present in Bell Isle, we chose to focus on competition between Cladocerans in the genus *Daphnia* and *Simocephalus*. These genera are both in the family Daphniidae and are the most similar in body size within that family. Body size is an important determinant of resource competition in zooplankton (Hall *et al.* 1976). We supplemented the
environmental survey with rock pools from other regions on Bell Isle to collect genetically diverse *Daphnia* and *Simocephalus*, isolated all collected individuals in a 100ml beaker filled with 90ml of filtered (30 µm) water from Lake Motoaka, and transferred the clonal lineages into 250ml beakers filled with 200ml filtered lake water after the field-collected individuals produced their first clutch. Care was taken to distinguish between two *Daphnia* species, *Daphnia pulex* and *Daphnia ambigua*, which were identified based on the presence (*D. pulex*) or the absence (*D. ambigua*) of teeth on the post-abdominal claw (Figure 4). In total 65 lineages of *D. pulex* were identified and maintained for subsequent genotyping. Two *Simocephalus* species, *S. vetulus* and *S. serrulatus*, were identified and we kept 22 lineage of *S. vetulus* for subsequent genotyping.

Figure 4. Post-abdominal claws of *D. pulex* (left, teeth present) and *D. ambigua* (right, teeth absent).

The parthenogenetic life cycle of both *D. pulex* and *S. vetulus* means that many individuals in the survey could be genetically identical to one another and required investigation of molecular markers. We extracted DNA for 65 *D. pulex* and 22 *S. vetulus* lineages using the Qiagen DNeasy Blood and Tissue Kit. Numerous molecular tools were available to genotype the well-studied *D. pulex*, including microsatellite markers (short regions of repetitive DNA with particular motifs; Colbourne *et al*, 2004). Repeats of microsatellites often cause errors in DNA replication machinery and thus can easily be deleted or added during DNA replication, making the number of microsatellites repeats a reliable method to determine genetic variation even in closely related
individuals (Vieira et al., 2016). We chose 3 markers, Dp433, Dp27, and Dp102 that have previously been used to study North American D. pulex (Pantel et al. 2011; Steiner et al. 2016).

Primers for each microsatellite marker were obtained from wFleaBase (Daphnia Water Flea Genome Database; http://wfleabase.org). Microsatellite Dp27 was amplified using primers Dp27-F: 5’-TCAAACCAGCCAACAACCCAAG-3’ and Dp27-R: 5’-GAATAACGGCCCACCCCTTTTC-3’. Microsatellite Dp433 was amplified using primers Dp433-F: 5’-GACACTCTCCACGCCTGCTT-3’ and Dp433-R: 5’-ACCAAGGCGAGGTTTTC-3’. PCR conditions are described in Pantel et al. (2011).

Microsatellites extracted by marker Dp102 did not amplify well and were not included in clonal identification. Fluorescently labeled fragment size is given for each lineage in Table 1. We identified ten genetically distinct haplotypes out of the 65 lineages.

Table 1. Fragment size for microsatellite markers Dp433 and Dp27 for the 10 genetically distinct haplotypes. Each unique allele combination is given a unique color.

*S. vetulus* is less well-studied than *D. pulex*, so fewer molecular techniques are available that have been shown to work successfully in this species. Sequences of mitochondrial cytochrome c oxidase subunit I (COI) were previously used for *Simocephalus* in China (Huang et al., 2014). COI is among the most conservative protein-coding genes in the mitochondrial genome of animals,
which makes it an ideal gene to detect genetic differentiation over longer periods of time (Folmer et al, 1994; Leray et al. 2013). The sequenced COI gene segment of *S. vetulus* is around 330-340 base pairs long. The gene was amplified using forward primer mlCOIIntF and the reverse primer HCO2198 (Leray et al. 2013). Ten genetically unique clones of *S. vetulus* were identified based on the sequence of COI gene.

To select the most genetically diverged clones of both *D. pulex* and *S. vetulus*, we also sequenced the COI gene of *D. pulex* using the same method. Our goal was to choose three genetically distinct lineages from each species for subsequent experiments. We chose our 3 focal clones for each species based on sequence quality and number of SNPs (single-nucleotide polymorphism). Focal clones of *D. pulex* are Dp472.19, Dp472B.1, and Dp473B.1. Focal clones for *S. vetulus* are Sv.367.3, Sv472.1 and Sv471.9.

Figure 5. Maximum Likelihood phylogenetic tree for clones of *S. vetulus* and *D. pulex* sequenced at a portion of the COI gene. Trees were generated using the Tamura-Nei method in Mega 7. The unit of branch length is nucleotide substitutions per site, a measure of lineage divergence. The length of 0.01 on the maximum likelihood tree represents 1 nucleotide substitution per 100 nucleotide sites. Focal clones are circled.

**2.5. Purging Maternal Environmental Effects**
Phenotypic traits of zooplankton can be influenced by the environment as well as genes. Defensive traits such as helmets and neck spines can be induced in the presence of predators, and environment also influences life history traits such as body size and age at maturity (e.g. Black 1993; Scheiner & Berrigan 1998; Beckerman et al. 2010). To ensure that genetic variation is the only source of the phenotypic variation among clones, the environmental effects of the experimental animals must be purged prior to common garden experiments.

All lineages that were purged of maternal effects were placed in cultures using COMBO medium. COMBO is a nutritious medium that supports the growth of various species of freshwater zooplanktons and phytoplankton (Kilham et al., 1998). It was prepared in lab according to the original recipe by Kilham et al., 1998 (without adjusting the phosphate level). The N:P ratio of the COMBO medium is 20:1. It consists of 4 major components made from 24 chemicals. The first major part are the seven major elements, including NaNO\(_3\), NaHCO\(_3\), K\(_2\)HPO\(_4\), CaCl\(_2\) \(2\)H\(_2\)O, MgSO\(_4\) \(7\)H\(_2\)O, Na\(_2\)SiO\(_3\) \(9\)H\(_2\)O, and H\(_3\)BO\(_3\). Stock solutions of the seven major chemicals were prepared separately and stored in sterilized 1L plastic bottles at room temperature. The other major components are algal trace elements (ATE), animal trace elements (ANIMATE), and vitamins (VIM). Working solution of these elements and their chemical stock solution were stored in sterilized Nalgene plastic bottles at 4°C. VIM was filter sterilized prior to storage. One milliliter of the stock solutions of the seven major chemicals, and 1ml working solutions of ATE, ANIMATE, and VIM were each added in deionized water to make 1L of COMBO medium. The final COMBO medium was filter sterilized prior to use.

The three focal _D. pulex_ clones and three focal _S. vetulus_ clones were raised in standardized laboratory conditions (100ml beakers filled with 80 ml of COMBO medium at 20 °C on a 16:8 hour day:night light regime) for four generations. We included two replicates of each clone. 80% of the COMBO medium was refreshed every Monday, Wednesday, and Friday (maternal effects
cultures were set up on August 27th and the last clone finished its fourth generation on November 20th, 2019). To ensure both species had sufficiently high food levels, we fed cultures with 9.72 µg carbon / ml of Shellfish Diet 1800 (Reed Mariculture: a mix of six marine microalgae - *Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*) every Monday, Wednesday, Friday and Sunday. The other days, cultures were fed at half of this food level.

After the individuals collected directly from rock pools produced their first clutch, a single female from this culture was inoculated into a 100ml beakers filled with 80 ml of COMBO medium as the starting generation (P) (note this was repeated for two replicates per clonal lineage). The first and second clutches of the P generation were removed. Three juveniles from the third clutch were kept after checking that they are female, and all other individuals were then removed from the culture. When these juveniles were 4-5 days old, one of the three individuals was chosen randomly to retain, making up the first lab generation (F1). The same process was repeated to get a second (F2) and third (F3) lab generation. The third clutch of the third lab generation (F4) was used as the initial females of the stock cultures with maternal effects purged (i.e. used for all subsequent experiments).

2.6. Production of experimental juveniles

The three main experiments were all initiated using age-standardized *D. pulex* and *S. vetulus* individuals. To ensure that a sufficient number of age-standardized juveniles were available, 90 individuals from each of the six chosen clones were isolated from stock cultures (i.e. with maternal effects purged) and kept isolated in 250ml beakers filled with 200ml COMBO medium (3 individuals per beaker to avoid crowding). Juveniles were removed from these ‘farm’ cultures every 3-5 days to avoid overcrowding and to ensure that no males were produced.
3. Grazing Experiment

3.1. Introduction

The grazing rate of individual zooplankton fundamentally impacts its body size and growth rate (Cyr & Curtis, 1999). Studies have shown body size of *Daphnia* is linked to fitness, survival, and competition (Lampert & Trubetskova, 1996), since body size influences resource exploitation and susceptibility to predators (Riessen and Young 2005). Grazing is significantly influenced by the ecology of primary producers, such as the time of emergence and community composition of phytoplankton (Hairston et al., 1999; Park & Post, 2018). Since zooplankton can evolve rapidly in response to temporal environment conditions such as composition of phytoplankton (Hairston et al., 1999), variation in grazing ability may be likely in focal clones because the rock pools harbor diverse algal communities.

In this experiment, we quantify genetic variation in *D. pulex* and *S. vetulus* grazing rate (defined here as the population growth rate of the phytoplankton *Scenedesmus obliquus*) in a common garden environment. Competition for algal resources is an important factor determining zooplankton species composition (Hall et al. 1976; Romanovsky & Feniova 1985). We hypothesize that both species will harbor substantial genetic variation for this trait and that the keystone herbivore *D. pulex*, which has been shown to have strong impacts on algal grazing in numerous studies (Sarnelle 2005), will be a stronger grazer than *S. vetulus*.

3.2. Method

3.2.1. Grazing experiment

We established and maintained cultures of *S. obliquus* (UTEX number 393) in COMBO medium in 1L glass bottles at room temperature and a 16:8 light:dark photoperiod. The day of the
In the experiment, we estimated phytoplankton culture densities by calculating the average of three cell counts inside a 0.04 mm$^3$ hemocytometer grid. We then calculated the volume of stock phytoplankton culture needed to obtain 9.72 µg carbon / ml, based on the carbon content and cell size of *S. obliquus* (Rocha & Duncan 1985). We also generated standard curves of phytoplankton cell counts at five density levels (9.95 × 10$^6$, 6.75 × 10$^6$, 3.6 × 10$^6$, 1 × 10$^6$, 4.2 × 10$^5$ cells/ml). Phytoplankton densities are inferred from the level of chlorophyll A in a sample, which is in turn inferred from the level of fluorescence at 430 nm. The standard curves pair readings of fluorescence at 430 nm on a Turner AquaFluor handheld fluorometer with observed cell counts.

24 hours prior to the start of the grazing experiment, all juveniles were removed from the ‘farm’ cultures. Juveniles isolated in the subsequent 24 hours period were collected from ‘farm’ cultures to use as age-standardized experimental animals. We prepared 4 replicates per clone, with 8 individuals in each replicate. Four replicates without zooplankton were set up as a control. To prepare the experiment, we transferred 9.72 µg carbon / ml into a 20ml glass vial and used COMBO medium to make a total experimental volume of 20ml. Experimental containers were 24 sterilized 20ml glass vials. 8 individuals for each replicate were transferred into each vial prior to adding the phytoplankton. Right after the phytoplankton was added, glass vials were loosely capped and placed on a shaking table in a dark 20°C environmental chamber for 24 hours to allow grazing and minimize phytoplankton growth. Eight replicates without zooplankton were created. Four of them were randomly chosen to be placed with experimental treatments for 24 hours. The remaining vials were used to record 3 fluorometer readings each, to measure the initial algae density of the grazing experiment. After 24 hours, 3 fluorometer readings for each remaining vial were recorded.

3.2.2. *Statistical analysis*
The goal of our analysis was to estimate the population growth rate of *S. obliquus* after 24 hours in each treatment, compared among species and clones. As of the time of this writing, data was analyzed using fluorescence readings, although this will be converted to μg C / ml using the phytoplankton standard curves. All data was analyzed using R (R 3.4.2, 2017) and using the R package ‘lme4’ (Bates *et al.* 2015). One replicate of a *D. pulex* clone (clone Dp472B.4) had dead individuals and was omitted from analysis. We first generated a single estimate of fluorescence across the four replicates for each time point (0 and 24 hours) by using the intercept value and the fixed effect estimate of time treatment estimated by a linear mixed-effects model with time as a fixed effect and replicate as a random effect. We then calculated the population growth rate, $r$, for each experimental bottle using ln(chlA$_{24}$ / chlA$_{0}$) and used a mixed effects model with species as a fixed effect and clone nested within species and replicate as random effects to estimate treatment $r$ values.

### 3.3. Result

The model-estimated fixed effect for intercept (± standard deviation: -0.6715 ± 0.1453) and species (0.5037 ± 0.2054) did not overlap with 0, indicating that species identity did influence the growth rate $r$ of *S. obliquus* (Figure 6). Species estimates were strongly influenced by variation due to clone (estimate of random effect standard deviation for clone nested within species: 0.250740) and less affected by variation due to replicate (0.003303). Residual standard deviation for the model was 0.067924. These results are more easily interpreted by giving the intercept $r$ values for each clone and replicate (Table 2). The AIC value of the model (-132.8261) was compared to the AIC value of a null model with no predictors (50.07793) using a likelihood ratio test (comparing the ratio of log-likelihood values to a chi-square distribution with 2 degrees of freedom, equal to the difference in the number of parameters estimated in the models), indicating the model likelihood was significantly higher for the explanatory model ($\chi^2 = 188.9$, $p << 0.001$).
<table>
<thead>
<tr>
<th>clone</th>
<th>intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp472.19</td>
<td>-1.0654706</td>
</tr>
<tr>
<td>Dp472B.4</td>
<td>-0.4923189</td>
</tr>
<tr>
<td>Dp473B.1</td>
<td>-0.4568109</td>
</tr>
<tr>
<td>Sv367.3</td>
<td>-0.5673722</td>
</tr>
<tr>
<td>Sv471.9</td>
<td>-0.7332317</td>
</tr>
<tr>
<td>Sv472.1</td>
<td>-0.7139964</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>replicate</th>
<th>intercept</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>-0.6708928</td>
</tr>
<tr>
<td>3</td>
<td>-0.6718378</td>
</tr>
<tr>
<td>4</td>
<td>-0.6711078</td>
</tr>
</tbody>
</table>

Table 2. Estimates of intercepts for each clone and each replicate for \( S. \) obliquus growth rate (\( r \), which was used as a proxy for zooplankton grazing rate). Estimates are based on random effects (for clone nested within species and replicate) from a mixed effects model. The estimate for the effect of species (0 = D. pulex, 1 = S. vetulus) was 0.5037 ± 0.2054, which means that \( r \) depended on species, with variation associated with both clone and replicate.
3.4. Discussion

The two zooplankton species, *D. pulex* and *S. vetulus*, had substantially different grazing rates, with strong effects of among-clone variation as well. Our analysis used a mixed effects model with fixed effects for species and random effects for clones (nested within species) and for replicates. Treating clone as a random effect means that instead of using the same intercept for each clone, we instead model clones as each having a distinct intercept. The results of the mixed effects model are thus reporting how the intercept of each clone varies, as well as reporting the effect size of species. Because our three clones per species are only random samples of all potential clones, it is appropriate to model clone as a random effect (see Bolker et al. 2008 for more information on this type of statistical model).

While the results are potentially unsurprising given that many traits in *D. pulex* show heritable variation (e.g. Lynch et al. 1989), and given that grazing efficiency is an important component of zooplankton community structure (e.g. Hall et al. 1976), there are relatively few studies that have measured genetic variation in *Daphnia* grazing (Park & Post 2017) and no study...
showing this for *Simocephalus*. Researchers have previously shown that intraspecific variation for life history traits such as body size can cause increased interactions between competing zooplankton species (Leibold & Tessier 1991). However, this has not yet been shown for a functional trait such as grazing. Grazing of zooplankton is an important top-down control of phytoplankton community structure (Ives et al. 1999). Our results suggest that community dynamics (i.e. population dynamics for algal species) could vary depending on the clonal identity of zooplankton grazers. This has previously been shown for the model organism *D. pulex* (Walsh et al. 2012), but has not been shown for other zooplankton taxa.

4. Quantifying Clonal Variation in Competitive Ability

4.1. Simulating Clonal Variation in Competition Model Coefficients

4.1.1. Introduction

Competition between individuals can have consequences for their growth and reproduction and can be expressed via a model describing the dynamics of competing species. We chose four mathematical models for competition between two species (summarized in Hart et al. 2017) to compare to experimental data for competition between *D. pulex* and *S. vetulus*. The models are:

<table>
<thead>
<tr>
<th>Competition Models</th>
<th>Competitive Ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A [ \frac{N_{i,t+1}}{N_{i,t}} = \lambda_i e^{-a_{ii}N_i - a_{ij}N_j} ]</td>
<td>[ \frac{\ln (\lambda_i)}{\sqrt{a_{ii}a_{ij}}} ]</td>
</tr>
<tr>
<td>B [ \frac{N_{i,t+1}}{N_{i,t}} = \frac{\lambda_i}{1 + a_{ii}N_i + a_{ij}N_j} ]</td>
<td>[ \frac{\lambda_i - 1}{\sqrt{a_{ii}a_{ij}}} ]</td>
</tr>
</tbody>
</table>
These models incorporate both intraspecific competition ($\alpha_{ii}$, the per-capita effect of an individual of species $i$ on species $i$) and interspecific competition ($\alpha_{ij}$, the per-capita effect of an individual of species $j$ on species $i$). The above formulas are used to predict population growth rate of a species ($N_{i,t+1} / N_{i,t}$), but our experiment uses the juvenile growth rate, $g$, which is the change in body length each day from birth to sexual maturity ($\mu$m day$^{-1}$). This parameter is highly correlated ($r^2 > 0.9$) with $r$, the rate of population increases, in experimental *Daphnia* populations (Lampert & Trubetskova 1996). We therefore evaluate the impact of intraspecific and interspecific competitors for the juvenile growth rate $g$ of each species after five days ($m_{5} / m_{0}$). The competitive ability of each species under these models are derived in Hart *et al.* (2017; using methods given in Chesson 2012). The numerator in the competitive ability expression indicates the species’ growth rate in the absence of competitors, and the denominator in the term is the geometric mean of the interaction coefficients and indicates the average sensitivity of the species to competition (i.e. its ability to maintain growth when conditions are crowded). The species (or clone) with the highest value for this term is the competitive dominant.

The goal of this project was to simulate data that will resemble the results of our competition experiment, fit the simulated data to statistical models estimating the parameters of the above mathematical models, and use statistical model comparisons to determine which model best fit the simulated data. Because our competition experiment considers the possibility of
heritable genetic variation in competition, we developed extensions of the competition models given above that estimate model parameters for each of the three clones per species. We also estimated competitive ability using the most likely model coefficients for each clone of each species.

4.1.2. Method

4.1.2.1 Experimental design

Competition strength between two species can be measured in the laboratory using a response surface experiment (Inouye 2001), which quantifies competitive strength by measuring the change in density of species $i$ when species $j$ is present, and vice-versa, at varying densities (Figure 7). Being both additive and multiplicative, response surface designs can provide more information about how competitor density affects population growth rate of a species, which cannot be described efficiently in other density treatment designs (Inouye 2001). We designed a 3 $D. pulex$ clones $\times$ 3 $S. vetulus$ clones $\times$ 10 density levels $\times$ 2 replicates factorial design to estimate intraspecific and interspecific competition coefficients for each clone in the presence of each other clone. This response surface design was used for model simulated data generation and for the common garden competition experiment.

Figure 7. 10 initial density levels of $D. magna$ and $S. vetulus$ in the response surface experiment. Axes represent the number of individuals of each species in each experimental container.
4.1.2.2 Simulated Data and Model Comparison

Simulations and model comparison were conducted in the statistical programming language R (version 3.5.0, 2018). To simulate data that mimics our competition experiment, we drew base rates of juvenile growth rate ($\lambda_i$ and $\lambda_j$ for D. pulex and S. vetulus, respectively) for each clone from random normal distributions with mean $\lambda_i = 1.4$ and $\lambda_j = 1.3$, respectively, and standard deviation 0.1. Intraspecific competition coefficients were treated as fixed for each species ($\alpha_{ii} = \alpha_{jj} = 0.05$) and interspecific competition coefficients were drawn from random normal distributions as well ($\alpha_{ij} \sim N(0.015,0.001), \alpha_{ji} \sim N(0.025,0.001)$). The initial body length $m_0$ was $N(0.8,0.01)$ for D. pulex and $N(0.5,0.01)$ for S. vetulus. Values for $m_5$ were generated using the Ricker model (Model A), using the parameterization found in Inouye 2001 and Hart et al. 2017, and random error was added to each estimate of $g$ ($m_5/m_0 \sim N(0,0.01)$). The calculation of $m_5$ values and addition of random error to $g$ estimates was repeated to simulate our second experimental replicate.

The parameters in the competition model ($\lambda_i, \lambda_j, \alpha_{ii}, \alpha_{jj}, \alpha_{ij}, \alpha_{ji}$) were estimated by fitting the simulated data to each of the four competition models using non-linear least squares estimation (using the R package ‘nlme’, Pinheiro et al. 2018), and these were estimated for each of the three clones per species. Akaike Information Criterion (AIC, which is $-2\times$log-likelihood + $2n$, where $n$ is the number of parameters in the model) was calculated for the model fit for each clone, and these model fits were compared among the four sets of models.

4.1.3. Result

The four competition models ultimately differ in the functional form for how the juvenile growth rate decreases with an increasing number of competitors. Model comparison using AIC successfully identified the Ricker model as the most likely model given the data (Table 3; note one
exception is the AIC value for Model B, *S. vetulus* Clone 1). The functional form for how $g$ varies with density of intraspecific and interspecific competitors for the Ricker model (using simulated data) is pictured in Figure 7. The results of the non-linear least squares estimates using the Ricker model are given in Table 4.

<table>
<thead>
<tr>
<th>model</th>
<th>AIC (D. pulex)</th>
<th>AIC (S. vetulus)</th>
<th>Comp sens&lt;sub&gt;DP&lt;/sub&gt;</th>
<th>Comp sens&lt;sub&gt;SV&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>-78, -82, -32</td>
<td>-51, -43, -131</td>
<td>13.5, 9.6, 14</td>
<td>6.4, 10.3, 5.7</td>
</tr>
<tr>
<td>C</td>
<td>-57, -57, -15</td>
<td>-41, -40, -117</td>
<td>4.5, 4, 4.6</td>
<td>3.1, 3.6, 3</td>
</tr>
<tr>
<td>D</td>
<td>-78, -75, -30</td>
<td>-40, -42, -133</td>
<td>13.2, 9.8, 13.8</td>
<td>6.6, 10.3, 5.8</td>
</tr>
</tbody>
</table>

Table 3. AIC values for each of four competition models fit to data for *D. pulex* and *S. vetulus* (values are given for each of the 3 clones per species). The sensitivity to competition is also given using the model estimates for each model coefficient ($\lambda$, $\lambda_i$, $a_{ii}$, $a_{ij}$, $a_{ji}$). Simulated data was generated using the Ricker model (Model A).

*D. pulex*

<table>
<thead>
<tr>
<th>clone</th>
<th>$\lambda$</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.588517</td>
<td>0.00202073</td>
</tr>
<tr>
<td>2</td>
<td>1.385608</td>
<td>0.00432458</td>
</tr>
<tr>
<td>3</td>
<td>1.495493</td>
<td>0.00446309</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>$a_{ii}$</th>
<th>Estimate</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05016896</td>
<td>0.00041419</td>
</tr>
<tr>
<td>2</td>
<td>0.05058417</td>
<td>0.00101673</td>
</tr>
<tr>
<td>clone</td>
<td>Estimate</td>
<td>Std.</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>1</td>
<td>0.01517077</td>
<td>0.00035911</td>
</tr>
<tr>
<td>2</td>
<td>0.01535848</td>
<td>0.00088097</td>
</tr>
<tr>
<td>3</td>
<td>0.01484325</td>
<td>0.00084197</td>
</tr>
</tbody>
</table>

**S. vetulus**

<table>
<thead>
<tr>
<th>clone</th>
<th>$\lambda$</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.361142</td>
<td>0.00226406</td>
</tr>
<tr>
<td>2</td>
<td>1.439725</td>
<td>0.00132727</td>
</tr>
<tr>
<td>3</td>
<td>1.11309</td>
<td>0.00305645</td>
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</table>

<table>
<thead>
<tr>
<th>clone</th>
<th>$\alpha_{ij}$</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04959223</td>
<td>0.00052391</td>
</tr>
<tr>
<td>2</td>
<td>0.05003363</td>
<td>0.00028902</td>
</tr>
<tr>
<td>3</td>
<td>0.04822037</td>
<td>0.00085177</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>clone</th>
<th>$\alpha_{ji}$</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0240319</td>
<td>0.00047286</td>
</tr>
</tbody>
</table>
Table 4. Estimates and standard error of estimates for non-linear least square model of Ricker competition for *D. pulex* (Residual standard error: 0.03314952, degrees of freedom: 39) and *S. vetulus* (Residual standard error: 0.009739718, degrees of freedom: 39).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.02539707</td>
<td>0.00026198</td>
</tr>
<tr>
<td>3</td>
<td>0.02745795</td>
<td>0.00078395</td>
</tr>
</tbody>
</table>

Figure 8. Change in *D. pulex* juvenile growth rate (y-axis) with varying density (x-axis) of intra- (black lines) and inter-specific (red lines) competitors. B is the same, but for *S. vetulus*. Values are given for all 3 clones of each species.

4.1.4. Discussion

The AIC-based model comparison accurately indicated the model used to generate the simulated data. This means that we can use the AIC-based model comparison to analyze the data that results from the competition experiment to identify the functional form of *D. pulex* and *S. vetulus* growth. An important consideration that can be addressed in future simulations is how sensitive the models are to increased variance (which is likely to be the case in experimental data). Possible alternative scenarios in this instance include treating the intraspecific competition coefficient as a constant (and thus not estimated in the model) or developing a Bayesian alternative to model estimation. If competition coefficients are found to not be sensitive to density, it is
possible in future experiments to increase the number of clones and replicates and decrease the number of density-varying treatments. Another important development for this analysis is determining the degree of uncertainty in estimates of competition sensitivity. This involves deriving a formula for the propagation of error, which can thus make use of the model estimated coefficients ($\lambda_i$, $\lambda_j$, $\alpha_{ij}$, $\alpha_{ji}$, $\alpha_{ii}$, $\alpha_{jj}$) and their associated standard errors.

4.2. Common Garden Competition Experiment

4.2.1. Introduction

The purpose of this experiment was to determine whether there is intraspecific genetic variation in terms that are traditionally treated as fixed in community ecological models of competition. The novelty of the experiment is that there is currently no knowledge of whether or not interspecific competition is a heritable trait that varies across within populations of different species.

4.2.2. Method

4.2.2.1 Common Garden Competition Experiment

Based on the response surface experimental design, single clone treatments used to quantify intraspecific competition of each clone are repeated 3 times in the factorial design. ‘Farm’ cultures were cleared of juveniles 12 hours prior to experimental setup. On March 23rd 2019, I isolated juveniles for each clone that were less than 12 hours old from ‘farm’ cultures and placed these into a 250ml beaker with 200ml COMBO medium (without food to prevent growth). Because all three $S$. vetulus clones did not produced sufficient juveniles within 12 hours to set up all the treatments, I selected single clone treatments (i.e. intraspecific competition only) of $S$. vetulus and randomly selected a subset of interspecific competition treatments to set up in a first experimental
block on March 23rd. All the intraspecific competition treatments of *D. pulex* were also set up in the same block. For each treatment, I inoculated *D. pulex* and/or *S. vetulus* individuals into a 100ml beaker filled with 90ml of COMBO medium (at densities according to the response surface design). For each beaker, I added 4.89μg carbon/ml of Shellfish Diet. This food level is sufficient for one individual *D. pulex* and *S. vetulus*, but imposes food limitation for individuals in treatments with increasing density. I then added a sterilized synthetic aquatic plant to each beaker, as this provides a substrate preferred by *S. vetulus* (the species spends most of its time while not grazing adhered to a surface, whereas *D. pulex* swims constantly in the water column). Treatments were placed in a 20°C environmental chamber with a 16 Light: 8 Dark photoperiod. I fixed 10 randomly selected individuals of each clone in absolute ethanol, to later measure body length for use as the day 0 measure for each clone. I then cleared the ‘farm’ culture again the evening of March 23rd and repeated the same process on March 24th to set up the remaining treatments. All treatments were fed every 48 hours for 5 days. Five days after the treatments were set up, I preserved individuals from each treatment in absolute ethanol. I plan to measure the body length (µm) of each individual using Image J after photographing all the individuals under a microscope.

4.2.2.2 Data Analysis

Body length data of day 0 and day 5 individuals will be fit using the four competition models described previously (but modified to incorporate clonal variation, as described in Section 4.1.2.2). We will select the best fit model based on AIC values, and use the estimates for λi, λj, αii, αjj, αij, and aji generated from the best-fit model to determine whether clones vary in values of each coefficient, which are heritable traits associated with clone under our experimental design.

5. Community Dynamic Mesocosm Experiment
5.1. Introduction

Community dynamics are typically predicted using species as the fundamental units of observation (Vellend 2006). These predictions have a basic assumption that individuals of the same species have identical traits. However, genetic difference among individuals within species may have important ecological consequences for community dynamic such as competitive interactions (Pimentel 1968; Vasseur et al. 2011; Kremer & Klausmeier 2013). While there are experimental studies demonstrating that the outcomes of community assembly can differ depending on the genetic identity of some resident species (e.g. De Meester et al. 2007; terHorst et al. 2014; Pantel et al. 2015), there are fewer studies that monitor genetic and community dynamics over time in the same experiment. This research is valuable because changes in clonal composition and changes in species composition are both potential responses to environmental selection pressures (Govaert et al. 2016).

We placed diverse populations (3 clones each) of *D. pulex* and *S. vetulus* into experimental mesocosms with diverse bacterio- and phytoplankton communities at two experimental temperatures. Temperature can influence grazing rates (Park & Post 2017). The goal of this experiment is to evaluate how intraspecific and interspecific growth is influenced by temperature in diverse algal communities. We will track the change of underlying genetic structure over time in competing species influenced by variation in clonal competitive strength.

5.2. Method

5.2.1. Experimental design

We designed and set up a mesocosm experiment with 3 population treatments × 2 temperature × 4 replicates, resulting in 24 experimental units. Population treatment 1 is *D. pulex* in isolation, Population treatment 2 is *S. vetulus* in isolation, and Population treatment 3 is the
competition treatment with both species present. Each species in each treatment was inoculated with mixtures of all 3 clones. Temperature treatment levels were 20 °C and 25 °C, which was included because grazing is often temperature dependent (Park & Post 2017) and the competitive dynamics between *D. pulex* and *S. vetulus* may vary if this is the case. Experimental containers are plastic 18.9 L buckets filled with 15 L of COMBO medium and a diverse bacterio- and phytoplankton community to serve as a resource base for the zooplankton community. We are sampling zooplankton in 1L samples weekly and preserving them in absolute ethanol. We plan to extract DNA from samples and use FREQ-Seq, which uses next-generation sequencing for quantitative allele frequency detection (Chubiz et al. 2012), to monitor clonal frequencies for both species based on the sequence of mitochondrial COI region (Leray et al. 2013). The experiment will be run for 8 eight weeks after *D. pulex* and *S. vetulus* are inoculated in the mesocosms.

5.2.2. *Mesocosm experiment*

In spring of 2019, we sampled ponds and lakes in Williamsburg, VA for a diverse initial bacterio- and phytoplankton community. We chose 5 ponds/lakes based on water quality (R. Chambers, personal communication): 3 ponds on William & Mary campus (Crim Dell, Grim Dell, and Swem Dell), Lake Matoaka, and Overlook Pond. On March 15th, 2019, twelve 18.9 L plastic buckets were each filled with 15L of filter sterilized COMBO medium and placed into two Thermo Scientific™ Precision Plant Growth Chambers at 25°C to warm COMBO medium to 25 °C prior to experiment set up. On March 16th, twelve 18.9 L plastic buckets were each filled with 15L of filter sterilized COMBO medium and placed in two Thermo Scientific™ Precision Plant Growth Chambers at 20°C. I then I took 3L of water samples from each of the 5 ponds, excluded zooplankton using a 35 μm filter, and homogenized the water sample. I inoculated 100ml of this mixed water sample into each experimental bucket as the starting diverse bacterio- and phytoplankton community. On March 30th, two weeks after the algal communities were
introduced, I collected individuals of *D. pulex* and *S. vetulus* clones that were less than 24 hours old (i.e. after clearing ‘farm’ cultures 24 hours prior), and added 4 individuals for each clone into the experimental buckets.

For each experimental mesocosm, I recorded 3 fluorometer readings (to monitor chlorophyll A levels), scraped off periphyton attached to the sides of the buckets, and refreshed 1L of COMBO medium in each mesocosm each week beginning March 16th. COMBO medium evaporates, so buckets were refilled to 15L when medium was refreshed. For the first week after the zooplankton inoculation (April 6th), when refreshing the COMBO medium, I collected zooplankton using a 35µm filter and replaced them in the mesocosm to prevent disturbance of initial population growth. From April 13th onwards (weekly, until the 8th sample to be collected on May 18), I collected zooplankton from the 1L water sample of each mesocosm and preserved them in absolute ethanol for subsequent DNA extractions.

### 5.2.3. Data Analysis

Assuming the clonal lineages experience no substantial mutations in the relatively short duration of the experiment, the change in total grazing rate of the two-species community can be decomposed into three elements: the component due to species sorting \((\Delta \bar{x}_s)\), the component due to evolution \((\Delta \bar{x}_e)\), and the interaction of species sorting and evolution \((\Delta \bar{x}_{s*e})\); see Govaert et al. 2016 for a potential method of decomposing trait change).

We will use a hierarchical Bayesian model to model the resulting clonal and species frequency time series data (Ovaskainen et al. 2017). The statistical model will include treatment as a fixed effect and mesocosm as a random effect. We will use each of the fractions \((\Delta \bar{x}_s, \Delta \bar{x}_e, \text{ and } \Delta \bar{x}_{s*e})\) as response variables to determine whether the importance of these fractions varies for temperature treatments.
5.3. Expected Results & Discussion

Previous studies in Daphnia have shown that both clonal and species frequencies shift in response to food quality and quantity (Weider et al. 2008; Govaert et al. 2016). This indicates there may be different conditions that lead to clonal vs. species selection. We expect the relative importance of clonal and species sorting to differ depending on temperature treatments. We plan to repeat the grazing experiment described in Section 3.2.1 at 25°C to help interpret our results. We expect that clonal variation in grazing across temperatures measured in a common garden will be reflected in the relative clonal frequencies observed in this mesocosm experiment.

6. Discussion

The influence of ecology on population genetic dynamics and genetic diversity has been well studied. Functional trait variation can arise rapidly from adaptive responses to diverse ecological conditions. An smaller but increasing number of studies consider the effects of evolution on ecology in community assembly. For example, a number of studies showed that genetic differences within populations can influence community composition. In the study of Pantel et al. (2015), *D. magna* populations that adapted to various environmental conditions over the course of a single growing season significantly altered the community assembly of zooplankton. Genetic differentiation in plant hosts was also found to impact the community structure and diversity of arthropod consumers (Wimp et al. 2005). Genetic composition of *D. magna* populations altered the establishment success of immigrant cladoceran species, impacting the zooplankton community assembly trajectory (De Meester et al., 2007). Rapid radiative adaptation in Hawaiian spiders altered the local spider community via inter- and intraspecific competition (Gillespie 2004).
While these studies have contributed greatly to the field of eco-evolutionary dynamics, the mechanisms for how genetic variation can alter community composition are less well studied. One possible mechanism is that evolutionary changes in one species impacts population dynamics of other species in the community by altering the interspecific interaction rates. A rotifer-algae predator-prey system in a chemostat experiment showed that an emerging defensive trait of prey can change the population dynamics of the predator (Becks et al., 2012). A wasp-housefly parasite-host system showed that increased resistance in the housefly host led to the reduction of the parasite wasp population by almost one-half (Pimentel, 1968). It is important to note, however, that species interactions can also influence the species evolutionary trajectory as well (Barraclough, 2015).

Both theoretical and experimental studies have shown that the presence of a competitor can impact a population’s evolution. Since species competition alters the population growth rate and population sizes determine the rate of evolution (Barraclough, 2015), most theoretical studies predict reduced evolutionary rates in competing populations. Some empirical studies did find evidence supporting the restrictive effect of competition on evolutionary rate (Fukami et al., 2007). However, with partial niche overlap, evolution rate can be enhanced (Osmond & de Mazancourt, 2013). Species with partial niche overlap experience both selection towards the trait optimum in that environment as well as selection pressure to avoid competition with the other species, which can overall enhance the rate of evolution.

*D. pulex* and *S. vetulus* could have partial niche overlap due to their different movement patterns. *D. pulex* actively swim in the water column, while *S. vetulus* tend to rest on the surface of sediments and macrophytes (aquatic plants). Different algal species exist in different areas of water bodies, so *D. pulex* and *S. vetulus* may have evolved different grazing preferences. Since we provided a single food resource in the common garden competition experiment, we expect that *D. pulex* and *S. vetulus* will not show strong niche differentiation. However, in the mesocosm
experiment with diverse bacterio- and phytoplankton communities, *D. pulex* and *S. vetulus* could occupy different areas of the mesocosms and consume different phytoplankton species as their preferred food source. Thus, they might compete less strongly in the mesocosms than in the common garden competition experiment. This could lead to different population dynamics than the outcome predicted based on the competition coefficients measured in the common garden competition experiment. Various studies also have shown that intra- and interspecific competitive ability are trade-offs of each other (e.g. Lankau, 2008). We could compare competition strength estimated from both experiments by fitting data from the mesocosm experiment to a competition growth model and evaluate whether niche partitioning affects the relationship between intra- and interspecific competition.

Interspecific competition is predicted to reduce over time, allowing species coexistence. Future studies can put *D. pulex* and *S. vetulus* in an experiment where both can experience rapid coevolution (*Daphnia* can show rapid adaptation to environmental conditions in as little as 3 months; Pantel et al. 2015), and evaluate how competition alters the evolutionary trajectories and population dynamics of each species. The relative importance of ecology (population dynamics of competitors) and evolution (genetic diversity or genetic identities of individuals within species) can be partitioned using a method developed by Ellner et al. (2011). This would be an important development, because it is currently unknown how to predict the relative importance of ecological and evolutionary processes for community assembly.
Acknowledgement

There are several people who deserve acknowledgement and thanks for their contribution to my project. First, I would like to thank my advisor Jelena Pantel for all her support and guidance in my all research. Second, I would like to thank Helen Murphy for supervising me in the second semester of senior year and supporting me for my project. I would also like to thank Zhengtian Hao for his help in various lab work. I would also like to thank Randolph Chambers and Leah Shaw for reviewing my thesis as members of my committee.

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Reference


