THE EFFECTS OF DISPERSAL, INBREEDING, AND MUTUALISMS ON THE FITNESS OF FRAGMENTED POPULATIONS

By

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ABSTRACT

Anthropogenic global change has led to the decline and fragmentation of countless natural populations throughout the tree of life and across the globe. Understanding how these fragmented populations evolve and interact with their environments is a fundamental question in ecology and evolution with potential applications for biodiversity conservation. In this dissertation, I researched how dispersal, inbreeding, and mutualisms affect fitness in two systems: *Poecilia reticulata* (the Trinidadian guppy) and *Chamaecrista fasciculata* (the partridge pea). Using observations and experiments, I aimed to answer three main questions: (Chapter 2) What are the effects of dispersal on fitness in the Trinidadian guppy?; (Chapter 3) What are the individual and population-level drivers of dispersal in the Trinidadian guppy?; (Chapter 4) How do inbreeding and mutualisms interact to affect fitness in *Chamaecrista fasciculata*? I found that dispersal behavior in guppies is driven by complex interactions between population, individual, and seasonal conditions; that dispersal and inbreeding are important drivers of fitness in guppies and *C. fasciculata*; that mutualisms are affected by inbreeding in *C. fasciculata*; and that the expression of inbreeding depression in *C. fasciculata* is altered by the loss of mutualistic interactions.

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CHAPTER ONE:

Introduction

Habitat fragmentation is one of the defining human impacts of the Anthropocene (Haddad et al., 2015). Fragmentation affects biodiversity at all levels, from continents (Riitters *et al.* 2000; Crooks *et al.* 2011) to microbes (Mony *et al.* 2022), whole ecosystems (Mitchell *et al.* 2015) to single genes (McCartney-Melstad *et al.* 2018), ecology to evolution. As an example from a single biome, as of 2015, approximately 70% of all remaining forests on Earth lie within one kilometer of an edge (Haddad et al., 2015). Understanding the consequences of such extensive restructuring of natural landscapes requires research that connects across scales, considering the knock-on effects that one scale may have on another.

At the individual level, habitat fragmentation can decrease organisms' ability to disperse (e.g. Brooker et al., 1999; Coulon et al., 2010; Schtickzelle et al., 2006; Soons et al., 2005). When suitable patches are surrounded by a hostile matrix of human-modified environments, individual dispersal can become constrained. Decreased patch sizes, increased distance between patches, and increased patch isolation all contribute to disrupt dispersal (Niebuhr *et al.* 2015; Cote *et al.* 2017; Fletcher *et al.* 2018). Dispersal, however, is an important part of the life-history of many species—allowing for the establishment of new territories, release from competition, and inbreeding avoidance (Van Dyck & Baguette 2005; Clobert *et al.* 2012; Baguette *et al.* 2013). When individual dispersal is limited by fragmentation, ecological and evolutionary processes like range expansion, recolonization, mate choice, and gene flow also cease to function as they previously did.

As a result, population level processes can be deeply changed by habitat fragmentation. Ecologically, lower dispersal decreases colonization of new patches and prevents the rescue of declining populations, potentially leading to local extinctions (MacArthur & Wilson 1963; Hanski 1998; Mittelbach & Schemske 2015). Evolutionarily, decreased dispersal leads to lower rates of gene flow, without which populations become more differentiated from one another, and lose genetic diversity through increased drift and inbreeding (Slatkin 1987; Ronce 2007; Clobert *et al.* 2012). Increased inbreeding is linked to loss of fitness ("inbreeding depression") in many systems, and especially in small and declining populations (Brook *et al.* 2002; Armbruster & Reed 2005a; Kardos *et al.* 2016; Doekes *et al.* 2021). Inbreeding depression occurs due to genome-wide increases in homozygosity, which lead to the expression of previously masked recessive deleterious variants and loss of heterozygote advantage ("overdominance") (Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009; Keller & Waller, 2002). Similarly, the loss of genetic diversity through increased drift can lead to the fixation of deleterious alleles, decreasing fitness and eroding populations' ability to adapt to novel conditions over time (Wright 1966; Lande 1976; Willi *et al.* 2006; Bijlsma & Loeschcke 2012; Hoffmann *et al.* 2017; Spigler *et al.* 2017). Together, these factors can speed up declines and send populations into an extinction vortex (Gilpin & Soulé, 1986).

As populations and species are lost, whole ecological communities change. Local extinctions not only decrease biodiversity as a whole, but they create gaps in biotic interaction networks that can lead to further losses (Colwell *et al.* 2012; Bascompte *et al.* 2019). Tightly coevolved interactions, like many mutualisms and parasitisms, are increasingly at risk of coextinctions—once one of the partners is lost, the other is also likely to disappear (Dunn *et al.* 2009a). Obligate ant-following birds, for example, depend on patchily-distributed colonies of army ants, so as ants disappear from small forest fragments due to the lack of food resources, so do the birds (Harper 1989). Even in the absence of local extinctions, fragmentation can still disrupt biotic interactions. For instance, when differential migration ability through a human-modified landscape creates asynchrony in the interactions between plants and their seed dispersers (Rodríguez-Cabal *et al.* 2007), or when harmful pathogens are introduced to new locations through human movement (James *et al.* 2015). The reshuffling of biotic interactions that comes with fragmentation is often a concurrent stressor that species need to contend with alongside disrupted dispersal and lower gene flow.

The negative effects of fragmentation not only propagate up, as described above, from individuals to populations to communities, but the consequences of fragmentation at larger scales can also feed back down to lower levels of organization. For example, novel pathogens at the community level can be more successful at establishing in more genetically depauperate, inbred populations (Gibson & Nguyen 2020); and changes in population structure that increase overall relatedness can alter individual dispersal behavior (Daniels & Walters 2000). As a result, understanding *how* fragmentation negatively impacts biodiversity can be incredibly complex, requiring observation and experimentation across several levels of organization.

For my dissertation, I investigated how dispersal, inbreeding, and biotic interactions impact fitness in two species: *Chamaecrista fasciculata*, the partridge pea, and *Poecilia reticulata*, the Trinidadian guppy. *P. reticulata* is a model system in evolutionary ecology, naturally occurring in streams in northern South America (de Bragança *et al.* 2020). In Trinidad, these fish are a textbook example of rapid adaptation, evolving different life histories, behavior, and morphology in response to selective pressures related to their habitats, especially the predator community (Endler 1980, 1987; Reznick 1989; Magurran & Seghers 1991; Magurran *et al.* 1993; Endler & Houde 1995; Reznick *et al.* 2001). In higher elevation headwater streams, guppies coexist with few predators and are naturally fragmented by the distribution of streams across the landscape, waterfall barriers between stream reaches, and riffles that form between the pools where guppies spend their lives. As such, these small headwater populations can suffer from inbreeding depression (Van Oosterhout *et al.* 2003; Johnson *et al.* 2010; Smallbone *et al.* 2016), blinking in and out of existence as population sizes fluctuate and extinctions and recolonizations occur.

The first two chapters of my dissertation aim to better understand the causes and consequences of dispersal in small populations of *P. reticulata*. **Chapter 2** looks at the fitness consequences of individual dispersal, describing the movement patterns of these fish and how they correlate with reproductive success. For **Chapter 3**, I connect population-level factors, specifically local pool census size, sex ratio, and relatedness, to individual dispersal behavior. In Chapter 2, I find that most fish move little or not at all throughout their lifetimes, but the ones that do disperse accrue significant fitness benefits. Males, in particular, are more likely to produce offspring, have a higher number of offspring, and have more mates the more they disperse. In terms of dispersal drivers (Chapter 3), I find that census size is an important predictor of dispersal probability in both streams, and that guppies' response to census size vary with season and fish weight. Sex ratio and relatedness also predict dispersal probabilities, but did so differently in the two study streams. Overall, I find that population-level characteristics in these streams are driving individual guppy behavior, with consequences to their fitness. These chapters made use of an extensive, multi-year dataset and pedigree collected by Fitzpatrick et al. (2015, 2016, 2020) in two natural guppy populations, initially collected to test hypotheses about genetic rescue of headwater fish using divergent lower elevation populations.

Chamaecrista fasciculata is an annual legume native to eastern North America that occurs in disturbed prairie habitats in Michigan (Fenster 1991a; Fenster *et al.* 2003; Bueno *et al.*

2019). As such, its populations are naturally fragmented across the landscape, appearing in disturbed and early-successional sites and disappearing as prairies develop. That natural fragmentation, however, is exacerbated by the loss of prairie habitat in the U.S. Midwest (Samson & Knopf 1994). As an outcrossing but self-compatible plant, *C. fasciculata* suffers from inbreeding depression when occurring in small and isolated populations (Fenster 1991a; Fenster & Galloway 2000; Mannouris & Byers 2013). As a legume, *C. fasciculata* participates in a mutualism with nitrogen-fixing soil bacteria (rhizobia), as well as a mutualism with bodyguard ants.

In **Chapter 4**, I researched whether inbreeding in *C. fasciculata* affects the strength of its mutualistic interactions with ants and bacteria, and whether the presence of rhizobia alters the expression of inbreeding depression. Following two generations of controlled crosses in the greenhouse and an outdoor common garden experiment with over 2500 individuals, I found that inbreeding decreases the plants' investment in both mutualisms. Importantly, I find that inbreeding depression is steeper for plants that interact with rhizobia, as the fitness benefits provided by the mutualism are lost at high levels of inbreeding.

My dissertation aims to fill gaps in our understanding of the effects of fragmentation in natural systems, focusing on how dispersal, increased inbreeding, and mutualism loss affect fitness. The effects we find of population-level dynamics on individual dispersal behavior (Chapter 3), of dispersal on fitness (Chapter 2), and of inbreeding and mutualisms (Chapter 4) highlight the need to integrate across scales when considering how organisms will respond to the pervasive and ongoing fragmentation of their habitats.

CHAPTER TWO:

Reproductive benefits associated with dispersal in headwater populations of Trinidadian guppies (*Poecilia reticulata*)

Abstract

Theory suggests that the evolution of dispersal is balanced by its fitness costs and benefits, yet empirical evidence is sparse due to the difficulties of measuring dispersal and fitness in natural populations. Here, we use spatially-explicit data from a multi-generational capture-mark-recapture study of two populations of Trinidadian guppies (*Poecilia reticulata*) along with pedigrees to test whether there are fitness benefits correlated with dispersal. Combining these ecological and molecular datasets allows us to directly measure the relationship between movement and reproduction. Individual dispersal was measured as the total distance moved by a fish during its lifetime. We analyzed the effects of dispersal propensity and distance on a variety of reproductive metrics. We found that number of mates and number of offspring produced were positively correlated to dispersal, especially for males. Our results also reveal individual and environmental variation in dispersal, with sex, size, season, and stream acting as determining factors.

Introduction

Dispersal is one of the major processes shaping diversity in the natural world. Broadly defined, dispersal is a departure from a local environment, followed by movement and settlement in a new location (Clobert *et al.* 2012). Dispersal followed by successful breeding leads to gene flow, which alters the distribution of genotypes across space (Ronce 2007; Clobert *et al.* 2012). Dispersal, then, plays a crucial role in determining population structure, patterns of adaptive differentiation, and population dynamics (Kawecki & Holt 2002; Sexton et al. 2009; Kubisch et al. 2014; Bonte & Dahirel 2017). The effects of dispersal on these evolutionary and ecological processes crucially depends on the fitness of dispersing individuals, both in terms of their survival and reproduction. Yet, dispersal and fitness are two of the most challenging quantities to empirically measure in wild populations, and so our understanding of their relationship is tenuous (Doligez & Pärt 2008).

Theory suggests that the probability and extent of dispersal within a population are shaped by a balance between fitness costs and benefits incurred by dispersing individuals (Holt 2003). Dispersal costs may manifest though energetic costs of physically moving, and/or increased mortality associated with moving through unsuitable or dangerous environments (Bonte *et al.* 2012). Benefits may arise through a variety of mechanisms, including a reduction in competition among relatives (Hamilton 1977; Taylor 1988; Perrin & Mazalov 2000), escape from unfavorable natal conditions (Venable & Brown 1988; Meylan *et al.* 2002), resource acquisition (Clobert *et al.* 2012), and inbreeding avoidance (Pusey & Wolf 1996; Perrin & Mazalov 1999). Dispersal should evolve when dispersal benefits outweigh costs, yet we expect those benefits to differ between individuals and environments. Given observed variation in dispersal propensity, it is important to understand how fitness benefits of dispersal are distributed across individuals and environments to understand dispersal evolution. The extent to which fitness effects of dispersal depend on individual characteristics such as sex, size, and total distance dispersed, as well as environmental factors, remains an open question in most natural systems. **Here, we integrate whole-population mark-recapture and pedigree analyses in two natural populations of Trinidadian guppies (*Poecilia reticulata*) to quantify the relationship between dispersal and reproductive fitness at fine spatial and temporal scales.**

Poecilia reticulata are live-bearing fish inhabiting streams and rivers throughout Trinidad and Tobago. Decades of research on the guppy mating system have shown that female choice is important (Haskins & Haskins, 1950; Haskins & Haskins, 1949; Houde, 1987), often on the basis of phenotypic novelty (Brooks & Endler 2001; Hughes *et al.* 2013a). Given the genetic basis of *P. reticulata* coloration (Haskins & Haskins 1951; Houde 1992), this preference might be a mechanism for females to avoid mating with kin (Hughes *et al.* 1999; Daniel & Rodd 2016). *P. reticulata* populations in headwater streams are also strongly regulated by density dependence, with high population density increasing mortality and limiting reproductive success (Reznick *et al.* 2012). Thus, it is likely that guppy dispersal plays an important role in minimizing inbreeding and allowing fish to escape high density pools—potentially providing dispersing individuals with substantial fitness benefits.

To date, it is known that male guppies are more likely to disperse than females (Croft *et al.* 2003) and that density-dependent dispersal varies with guppy life stage (De Bona *et al.* 2019). Throughout Trinidad, guppies show strong patterns of population structure associated with drainage (Fraser *et al.* 2015), distance, and barriers to gene flow (Crispo *et al.* 2006), suggesting some dispersal limitation at large spatial scales. *P. reticulata* is also a prolific global invader, and its ability to establish new populations following dispersal or artificial introduction greatly impacts freshwater ecosystems worldwide (Deacon *et al.* 2011). However, no previous study has related dispersal to reproduction in the *P. reticulata* system, or quantified how reproductive benefits of dispersal vary among individuals or environments. As such, the crucial link between dispersal and fitness—and its consequences for dispersal evolution, gene flow, and population structure—remain unknown. Here, we aim to fill this gap with a long-term, multi-generational study to ask the overarching question: **Is dispersal correlated with reproductive success in *P. reticulata*?**

Our hypothesis, based on the natural history of guppy populations in headwater environments, is that dispersal confers a reproductive benefit at this scale, and that these benefits are greater for males than females. That is, guppies that move at all and those that disperse farther experience greater reproductive success than philopatric individuals, and reproductive benefits are higher for males as they compete for access to females. However, we predict that movement is only beneficial at small spatial scales within the low predation environment, as long-distance dispersal into downstream high predation reaches results in high mortality (Weese *et al.* 2011). These costs associated with local adaptation to heterogenous predation regimes may lead to the evolution of short dispersal distances over evolutionary time. We also analyzed how dispersal behavior vary with male body size, and across seasons. We hypothesize that smaller fish move more to escape conditions where they are competitively inferior, and all fish move more and in the downstream direction during the wet season due to floods and high stream flow which facilitate both passive and active movement. Our dataset allowed us to test for correlations between fine-scale dispersal and fitness for two natural populations, and contributes to the understanding of individual variation in dispersal for a model system in ecology and evolution.

Methods

Field capture-mark-recapture

We studied low predation populations of *P. reticulata* in neighboring streams, Taylor and Caigual, in the Guanapo drainage on the south slope of the Northern Range of Trinidad. Data used for this project were collected in a spatially explicit, monthly capture-mark-recapture study that spanned June 2009–July 2011. In April 2009, as part of a separate study (see Travis et al. 2014), guppies from downstream site within the same drainage were translocated upstream of our two focal sites. Translocated individuals eventually reached and bred with the focal resident populations (Fitzpatrick *et al.* 2016, 2020). However, our goal for this study was to focus on dispersal behavior of the resident population. Therefore, individuals reported on in this study were fish captured in the first 13 months of the mark-recapture study (June 2009–July 2010), when immigration of translocated individuals into the focal sites was minimal. To account for the presence of few immigrant and hybrids in our dataset, we included a hybrid index covariate in all statistical models. This hybrid index varied from 0 (pure resident) to 1 (pure immigrant) and was calculated using genetic data, as described below (see Fitzpatrick et al. 2020). Only 5% of fish in our dataset were classified as pure immigrants, suggesting that the vast majority of our dataset represents the resident populations of Taylor and Caigual prior to the onset of gene flow (Fitzpatrick *et al.* 2016).

Detailed capture-mark-recapture methods are described in Fitzpatrick et al. (2016). Briefly, we selected a portion of each stream that was the upstream-most extent of wild guppy populations uninterrupted by waterfall barriers. The sampled reach in Taylor was 240 m in length, and 80 m in length in Caigual. Each distinct pool or riffle within focal reaches was uniquely labelled and sampled monthly using a combination of hand nets and mesh traps. All guppies greater than 14 mm were captured, transferred to the lab, and placed in aerated tanks, separated by pool location and sex. Only mature individuals were included in the analyses, as determined by gonopodium morphology for males, and the presence of melanophores at the cloaca for females. These methods are commonly used in capture-mark-recapture studies of Trinidadian guppies, and have been shown to produce low lab mortality and high capture probabilities (Reznick et al. 1996). During lab processing, individuals were anesthetized with a dilute solution of MS-222, and new recruits were given a unique subcutaneous elastomer mark (Northwest Marine Technologies, Inc., Shaw Island, WA, USA). Recruits had three scales collected and dried for DNA extraction, and all individuals were weighed and photographed each month. All fish were returned to their exact capture location one to two days after processing. During their release, fish were acclimated to stream water and released into the lowest flow region of their capture location to minimize accidental passive downstream movement.

Quantifying dispersal variation

The exact pool locations of initial capture and subsequent recaptures were recorded every sampling event for all individuals. Although sizes and locations of pools and riffles change based on seasonal dynamics, they are always noted by the number of meters from the upstream extent of the reach. *P. reticulata* were considered philopatric (non-dispersing) if they were consistently captured less than 10 m from their initial capture location, the approximate length of the maximum pool size. Individuals were considered dispersers if at any point in the study they were captured 10 m or more from the pool where they were first captured, regardless of how many sampling occasions it took for that movement to occur.

We use the threshold of 10 m because that is the length of the largest pool in our study, such that any movement beyond it reflects that an individual departed its initial pool and settled at a new location for a period of time. Given that most guppy daily activity takes place at the within-pool scale and movement between pools places an individual in a new, non-local environment, this threshold matches our definition of dispersal as a “a departure from a local environment, followed by movement and eventual settlement in a new location.” A potential source of error in our estimate of distance could stem from individuals moving and backtracking between sampling occasions, such that total movement would be underestimated. Thus, we are only able to estimate *minimum* distance moved.

In addition to the categorical classification of philopatric or dispersing, we quantified the total distance travelled for all individuals as the cumulative distance the moved during our study (Figure 2.1). This estimate considers upstream and downstream movement equally. For example, a fish that moved upstream from 0 m to 10 m to 20 m would have the same dispersal distance (10 + 10 = 20 m) as a fish that moved upstream from 0 m to 10 m, then back downstream from 10 m to 0 m (10 – 0 + 10 = 20 m). Finally, we calculated the range of all dispersing fish, defined as the minimum non-cumulative distance spanning all of its locations across the study—in the example above, the first fish has a range of 20 m, but the second fish has a range of 10 m.

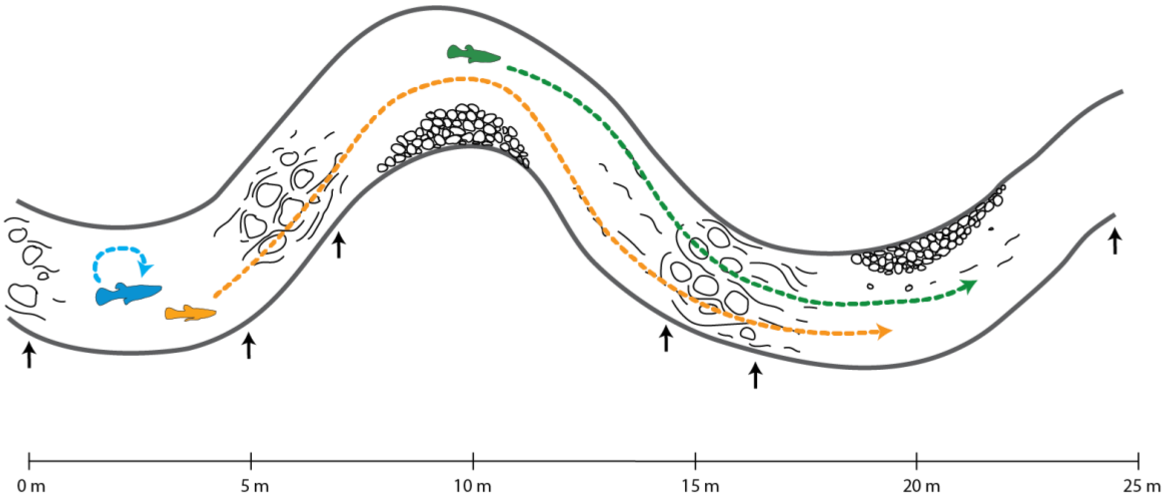

Figure 2.1. How dispersal distances were calculated. Arrows represent boundaries between pools and riffles along the stream. Dispersal distances for all fish were measures based on the distance between the pools where they were captured. Distances between pools were measured as the minimum distance an individual would have to move to change pools. For example, the yellow fish was initially found in a pool that extended from 0-5 m, then captured in a pool that extended from 17-25 m, and so was recorded to have moved 12 m (17 minus 5). The green fish, having moved from a pool extending from 7-14 m to the same 17-25 m pool, was considered to have moved only 3 m (17 minus 14). Our metric of total distance, then, reflects a conservative minimum distance that each fish moved during the study. Fish that were always captured in the same pool, like the blue fish represented in the figure, were not considered dispersers.

Analyses of dispersal patterns

Total estimated dispersal distances showed an excess of zero values, as 47.5% of fish did not disperse at all. As such, we modelled dispersal distance using zero-inflated negative binomial distributions, which accommodates separate analyses of zeros and total counts. We found this to be the most biologically appropriate model to understand both the probability of moving (zero vs. non-zero) as well as total distance moved. For all models described below, we used AIC (Akaike Information Criterion) for model selection (Johnson & Omland 2004) to choose between models including different independent variables and distributional assumptions, in addition to using the R package DHARMa (Hartig 2021) to assess model fit. Covariates in all models included stream, sex, hybrid index, and longevity. Longevity was calculated as the total number of months a fish was captured in our study, which likely underestimates the true number of months that individuals live due to imperfect detection probability and because it does not account for the time when fish are too small to be captured (less than 14 mm).

To test whether *P. reticulata* move more in the downstream direction and in the wet season, we first investigated whether movement differed in distance and direction between seasons and streams. We used generalized linear mixed models to test for the effect of season on the movement of fish for each capture occasion, with individual and month as random effects. Seasonal analyses were done separately for each stream.

To test the hypothesis that smaller males disperse more often and farther, we tested for relationships between standard length and dispersal status and total dispersal distance. Females size was not tested because females have indeterminate growth, making size and longevity strongly colinear. All models included stream, hybrid index and longevity as covariates, and the interaction between hybrid index and stream.

Effects of dispersal on fitness

Lifetime reproductive success was determined for each individual by reconstructing pedigrees for Caigual and Taylor populations using individual genotypes from 12 polymorphic microsatellite markers (see Fitzpatrick et al. 2016 for genetic data collection methods and Fitzpatrick et al. 2020 for details on pedigree reconstruction). Final pedigrees consisted of 1,106 individuals in Caigual (458 maternal links, 655 paternal links) and 1,725 individuals in Taylor (975 maternal links, 994 paternal links) spanning 4-6 overlapping generations. We used the pedigrees to estimate the number of offspring that reached a minimum size of 14 mm for each individual, as well as the total number of mates for each individual that produced at least one offspring that survived to 14 mm.

We tested for a relationship between dispersal and lifetime reproductive success using zero-inflated negative binomial regression because 55% of fish were assigned zero offspring in the pedigree analysis. As with distance, we were interested in how our variables affected the probability of zero vs. non-zero values of offspring as well as total counts. Number of mates was also modeled with a zero-inflated negative binomial regression for similar biological reasons. Dispersal status and total distance were used as predictor variables in separate models, with stream, hybrid index and longevity as covariates. We included the interaction between hybrid index and stream in these models, given higher immigration into the Taylor during the study period, and overall higher fitness of hybrid individuals (Fitzpatrick *et al.* 2020). We analyzed males and females separately when modelling reproductive success, because the offspring from each fish were not independent of the other parent. In models with males, standard length was also included as a covariate, while female size is accounted for by incorporating longevity in models of reproduction.

Finally, we explored whether guppies with a larger range have an increased number of mates, where a "mate" is defined as an individual that shared at least one surviving offspring with the focal individual. Range was used in this analysis because it directly reflects the extent to which fish explored the stream beyond their natal pools—total distance, on the other hand, includes backtracking and reflects overall movement (Figure 2.1). We again used a negative binomial zero-inflated model with stream, hybrid index, and longevity as covariates. All analyses were conducted in R 3.5.3 (R Development Core Team. 2019).

Results

Patterns of dispersal

A total of 1,357 fish were caught and recaptured at least once in our 13 sampling occasions from June 2009 to July 2010. On average, fish in our study were recaptured 3.6 times during that time period. A total of 525 (38.7%, Table S1) individuals in our dataset were classified as dispersers (i.e., moved ≥10 m during the study). Our data consisted overwhelmingly of resident genotypes, with 954 out of 1,357 fish having a hybrid index lower than 0.5, and 726 of those falling between zero and 0.1 (Figure S2.1). The distribution of dispersal distances was highly skewed, with 50% of all dispersing fish moving between 10-26 m, a mean dispersal distance of 41.5 m, and a maximum of 248 m (Figure 2.2, Table 2.1). The proportion of fish that dispersed was higher in Caigual (48.6%) than in Taylor (28.9%, Table S2.1).

We found that the likelihood of fish becoming a disperser increased with longevity (N=1357, z=7.825, p<0.001) and was higher for guppies of resident descent (N=1357, z=-2.848, p=0.004). Longer-lived fish also moved greater total distances (N=1357, z=9.667, p<0.001; Figure S2.2A), as did fish from Taylor (N=1357, z=11.709, p<0.001). We did not find evidence that fish in older age classes are more likely to move. That is, we found that most dispersal happens between a fish's first and fifth captures, and there does not seem to be an age threshold after which fish begin to disperse (Figure S2.3, Table S2.2).We also uncovered an interaction between stream and hybrid index, where fish moved higher total distances if they were from Taylor and had low hybrid indexes (N=1357, z=-4.247, p<0.001). Thus, we kept longevity, stream, hybrid index, and the interaction between stream and hybrid index as covariates in all subsequent models.

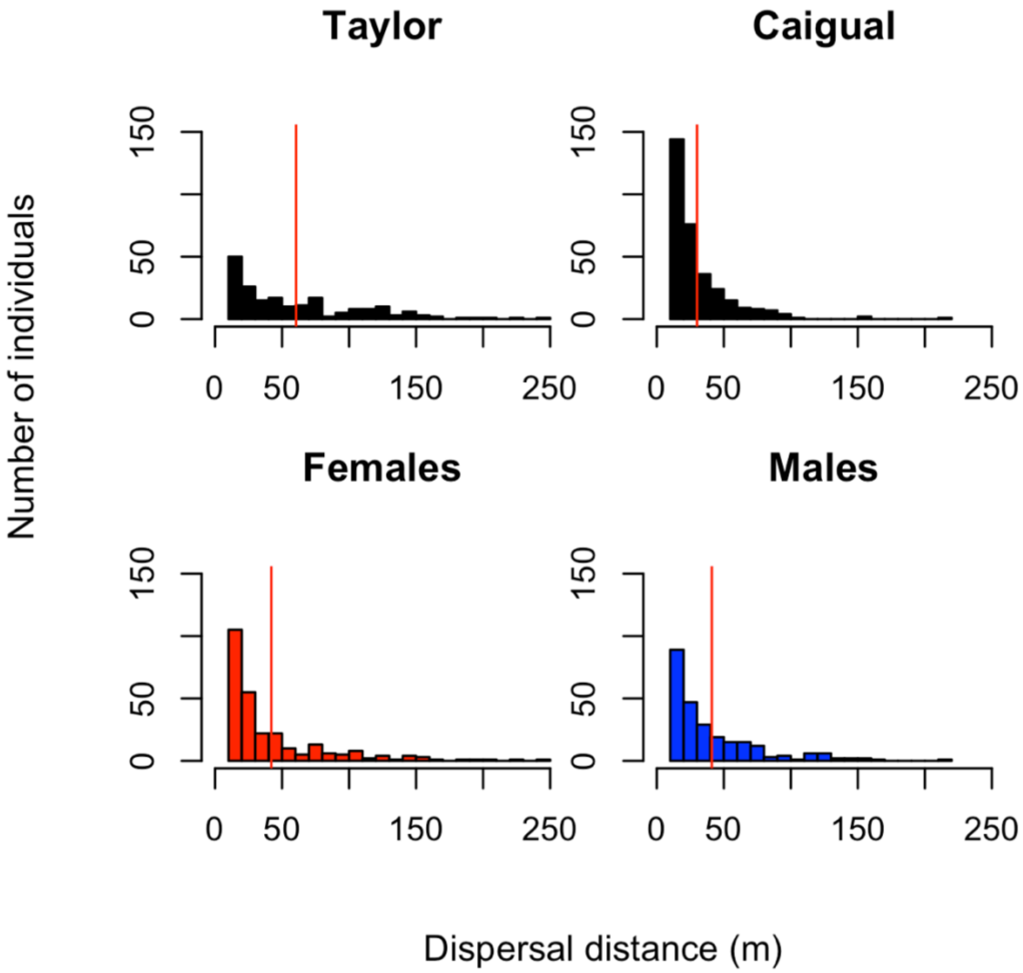

Figure 2.2. Total movement of fish considered dispersers (total distance moved ≥ 10 m) grouped by sex and stream. Red lines indicate means for each group.

Individual and environmental correlates of dispersal

Supporting our hypothesis about male-biased dispersal, we found that males were 12% more likely to disperse at all than females (N=1357, z=4.425, p<0.001; Table S2.1). Males also moved farther total distances (Figure 2.2; N=1357, z=2.451, p=0.014). Contrary to our hypothesis, we found positive correlations between male body size and likelihood of dispersing (N=604, z=4.142, p<0.001) as well as total distance moved (N=604, z=5.429, p<0.001; Figure S2.4).

The probability of movement was higher in the wet season for the Taylor population (N=469, z=6.499, p<0.001; Figure S2.5A), but was consistent across seasons in Caigual (N=651, z=-0.41, p=0.685; Figure S2.5B). The Caigual population also showed no seasonality in the extent of upstream (N=651, z=-0.698, p=0.485) versus downstream (N=651, z=-0.278, p=0.781) dispersal, indicating fish did not have a directional bias throughout the year of the study. However, as expected, fish in the Taylor population moved more in both directions during the wet season, with a stronger effect of the wet season on increasing downstream dispersal distances (N=469, z=11.36, p<0.001), compared to upstream movement (N=469, z=5.036, p<0.001).

Fitness consequences of dispersal

We observed different effects of dispersal status and distance on reproductive success between the sexes. In all models, longevity (Figure S2.2B), male standard length, higher immigrant ancestry, and being from Taylor had positive effects on reproductive success, so these covariates were always included. Male dispersers had a higher chance of having at least one surviving offspring than philopatric males (N=604, z=-2.877, p=0.004; Table 2.1), as well as a non-significant trend towards more total offspring (N=604, z=1.693, p=0.090; Table 2.1). The chance of males having a non-zero number of offspring also increased with total distance moved (N=604, z=-2.243, p=0.025, Figure 2.3A), though there was no effect of total distance on the number of offspring for males (N=604, z=1.002, p=0.316). Unlike males, a female's chance of having at least one surviving offspring was not influenced by dispersal status (N=753, z=-1.527, p=0.127), or dispersal distance (N=753, z=-1.204, p=0.229). Being classified as a disperser did have marginally-significant positive effect on the number of offspring in females (N=753, z=1.864, p=0.062), as did total dispersal distance (N=753, z=1.845, p=0.065, Figure 2.3A). Given the tight biological links between longevity and reproductive success, we re-ran our analyses on subsets of individuals of the same age, and found qualitatively similar results across longevities (Figures S2.6 and S2.7). We also conducted fitness analyses using absolute distance (distance between first and last capture locations) as a predictor, which gave us qualitatively similar results to our metric of total distance, and these analyses are described in Supplemental Materials Appendix 2.I.

We also analyzed the effects of range on number of mates (Figure 2.3B). In these models, longevity, hybrid index, and the interaction between lower hybrid index and being from Taylor all significantly increased number of mates. Range size had a positive effect on number of mates for males (N=604, z=2.754, p=0.006, Table 2.2), and a marginally-significant effect on number of mates for females (N=753, z=1.809, p=0.070, Table 2.2). For both sexes, we found no effect of range size on the chance of having at least one mate (N=753, z=1.224, p=0.221 for females, N=604, z=-0.970, p=0.332 for males). Tables 1 and 2 describe summary statistics for dispersal distance, range, reproductive success, and number of mates. Detailed model outputs are provided in Tables S2.3-6.

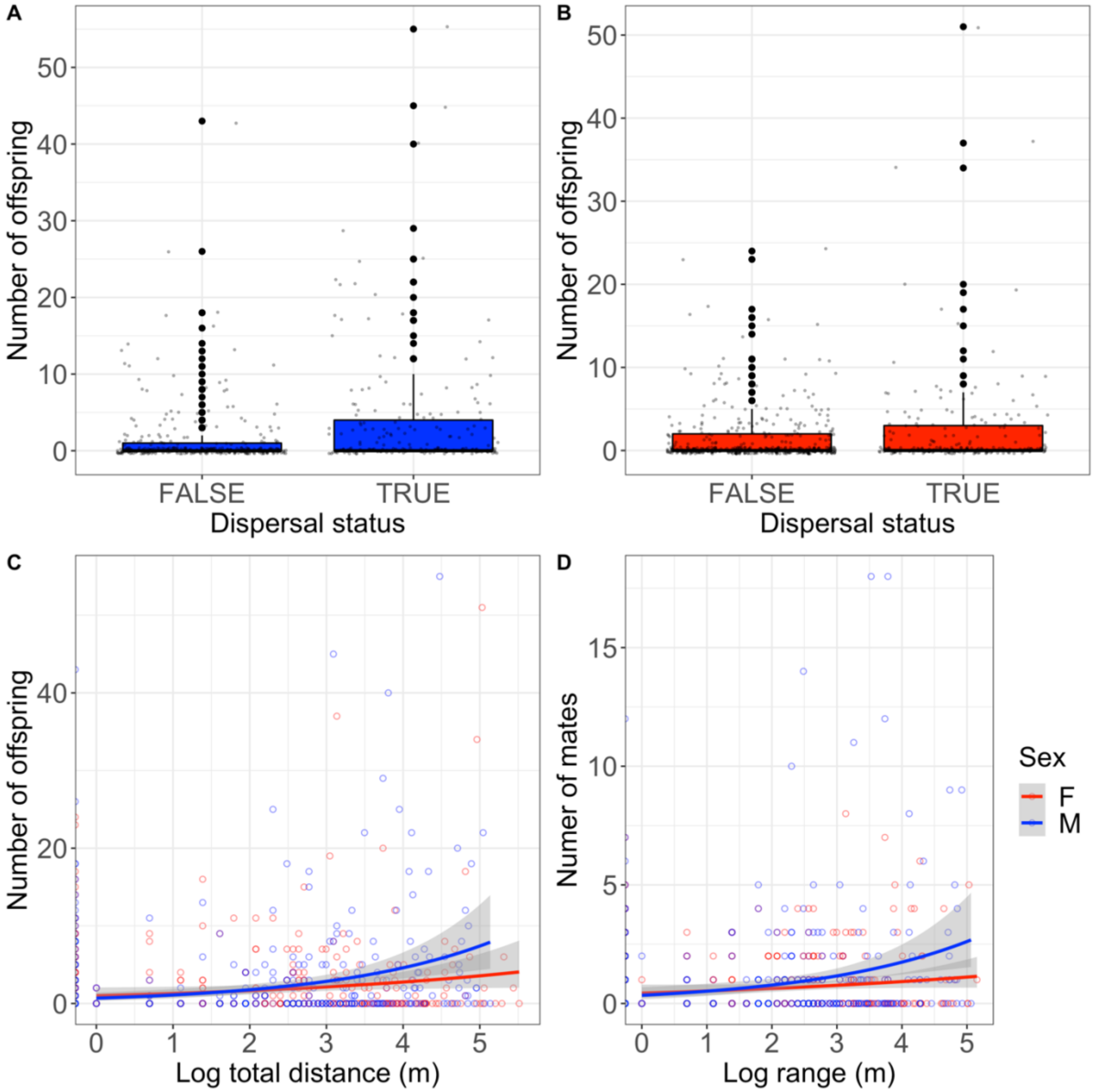

Figure 2.3. Response of fitness metrics to dispersal status, distance, and range. (A) Males that disperse are more likely to have at least one surviving offspring, and show a trend towards having more offspring; (B) Female dispersers do not show trends toward increased reproductive success compared to females that do not disperse. (C) Males have higher chance of having offspring with longer distance moved and females show a trend towards more offspring with higher distance moved; (D) Males with larger ranges have an increased number of mates, and that effect was not statistically significant for females. Lines show negative binomial regression and shading as 95% confidence interval.

Discussion

This study provides novel insights into the ecological drivers and evolutionary consequences of dispersal in two populations of the Trinidadian guppy. Our results reveal variation in dispersal propensity and distances within and between our study populations, with size, genetic ancestry, and seasonality influencing dispersal. We found strong evidence for sex-specific reproductive benefits of dispersal consistent with observed patterns of male-biased dispersal behavior, thus exemplifying how dispersal evolution can occur in natural populations.

Characterization of dispersal behavior

Our study directly measured fine-scale dispersal distances for all adult individuals in two populations of *P. reticulata* for over one year, adding to what is known about dispersal behavior in natural settings and the specific characteristics of movement in this system. Overall, we observed some variation in dispersal between seasons and streams, but mostly consistent patterns: large male guppies were most likely to disperse, and the majority of fish moved short distances or not at all. Not surprisingly, the overall proportion of dispersers we observed over the course of a year (39% across both sexes and streams, Figure 2,2) was significantly higher than what was observed for a single recapture event (Reznick *et al.* 1996; Croft *et al.* 2003), but similar to estimates from a 5 year period (De Bona *et al.* 2019), suggesting that variation in dispersal can be adequately captured over the course of a year.

We also detected seasonal variation in dispersal distance in Taylor, where guppies were more likely to disperse downstream in the wet season, but not in Caigual (Figure S2.3). This was expected given that Taylor has a higher probability of flooding in the wet season, as it is a steeper and more channelized stream (Fitzpatrick *et al.* 2014). Floods, which primarily occur in the wet season, may therefore increase the prevalence of long distance dispersal in the wet season in Taylor. We cannot determine whether increased wet season dispersal by *P. reticulata* is primarily active, due to floods and high water levels reducing dispersal barriers, or passive, due to fish being carried by stronger stream flows. We also note that while upstream dispersal can be interpreted as active, downstream movement could be either active or passive. There were no other strong biases in up- vs. downstream dispersal, consistent with previous studies of *P. reticulata* dispersal (Croft *et al.* 2003; Crispo *et al.* 2006) and findings of positive rheotaxis in this system (Blondel *et al.* 2020). As a whole, these data suggest that guppy dispersal is often active, raising questions about the environmental and individual triggers of this behavior.

In this study we cannot draw conclusions about the causes of dispersal, but are instead interested in characterizing dispersal patterns within these streams and understanding their fitness consequences. Similar to previous studies of *P. reticulata* (Croft *et al.* 2003; De Bona *et al.* 2019), we found that dispersal was male-biased: 42% of males and 36% of females in our study were classified as dispersers. This corroborates studies on a wide range of other species, supporting the hypothesis that polygynous mating systems tend to have male-biased dispersal (Trochet *et al.* 2016). The "resource competition hypothesis" for the evolution of sex-biased dispersal (Greenwood 1980) proposes that competition for local resources and mates leads to increased dispersal of the sex that most strongly competes for mates, while extensions of this hypothesis suggest that systems in which female choice is prevalent should evolve male-biased dispersal (Dobson 1982). Though abundant evidence supports these hypotheses in mammals and birds, data on other taxa remain scarce (Trochet *et al.* 2016), making *P. reticulata* an important example of how these ideas may apply to other species. Finally, contrary to our expectations, but in line with previous results (Croft *et al.* 2003), we found that larger males dispersed more often and farther—suggesting that larger fish are more likely to leave local pools or survive dispersal.

This may be because larger fish are less susceptible to predation by gape-limited predators (Gosline & Rodd 2008), better at acquiring resources (Potter *et al.* 2018), or stronger swimmers (more able to resist passive downstream movement and/or complete active upstream movement; Kieffer, 2000; Radinger & Wolter, 2014).

Fitness correlates of dispersal

Quantifying the fitness of dispersing individuals is a fundamental step towards understanding how dispersal shapes the ecology and evolution of natural populations. Successful gene flow between populations, colonization of new environments, and maintenance of range boundaries all crucially depend on the ability of dispersing individuals to reproduce. We found strong effects of dispersal on reproductive success in our study: 60.25% of non-dispersers had zero offspring, compared to only 37.79% of dispersers. We also saw a trend towards increased number of offspring for dispersers, where dispersing males had 35% more offspring than non-dispersing males when holding all other variables constant. Given that most fish do not leave behind any successful offspring, this metric reflects the most important reproductive achievement in these populations, namely, having at least one offspring survive to the minimum markable size of 14 mm (Figure S2.1A). Thus, our results demonstrate that dispersal is a major factor in male reproductive success.

Several aspects of guppy natural history and mating biology may contribute to the increased reproductive output of dispersing males. Theoretical models for the evolution of sex-biased dispersal suggest that the strength selection for male dispersal in polygynous systems depends on female choice, kin recognition, and the strength of inbreeding depression in the system (Lehmann & Perrin 2003). In *P. reticulata*, female choice plays a major role in determining male reproduction, such that increasing encounters with females is in itself not sufficient to increase number of successful matings (Pitcher *et al.* 2008; Zajitschek & Brooks 2008; Daniel & Rodd 2016). Rather, females prefer unrelated males and those with novel phenotypes, and are able to differentiate between kin and non-kin individuals (Hughes *et al.* 1999, 2013a; Hampton *et al.* 2009; Daniel & Rodd 2016, 2020). Female preference may be important for inbreeding avoidance in this system, given ample evidence that guppy populations in small headwater streams such as Caigual and Taylor can suffer from significant inbreeding depression (Van Oosterhout *et al.* 2003; Crispo *et al.* 2006; Fitzpatrick *et al.* 2014, 2016). Male color, a key phenotypic marker used in female choice, is highly heritable (Endler 1980; Endler & Houde 1995; Kemp et al. 2009), such that dispersal between pools may offer males a chance to exploit unrelated females' preference for novelty. Overall, the correlation between fitness and dispersal we observed for males reflects a selective pressure for increased dispersal resulting from the combination of female choice and inbreeding avoidance in this system, providing an empirical example for how these factors interact to shape dispersal evolution.

Unlike males, females' chances of having at least one surviving offspring was not affected by any dispersal metric. If female choice is adaptive (e.g., inbreeding avoidance or "good genes"; Anderson, 1982; Kodric-Brown & Brown, 1984; Perrin & Mazalov, 1999; Pusey & Wolf, 1996; Zahavi, 1977), we would expect females that dispersed farther to benefit from a larger pool of available mates, potentially leading to the observed trend in higher number of offspring for dispersing females. However, female reproduction in polygynous systems is more directly limited by resources than mates (Greenwood 1980; Perrin & Mazalov 2000; Magurran 2005). For example, a study on density-dependent dispersal in *P. reticulata* found that females were likely to disperse out of high density pools (De Bona *et al.* 2019). Again, these findings are in line with hypotheses for the evolution of sex-biased dispersal in polygynous species: increased philopatry in resource-limited females and increased dispersal in mate-limited males (Greenwood 1980; Perrin & Mazalov 2000; Lehmann & Perrin 2003).

Given the observed reproductive correlates of dispersal, males in the studied populations are under strong selection to disperse. Yet, dispersal was generally limited and occurred over short distances, even for males (Figure 2.2). One potential explanation for these contrasting results is the relationship between local adaptation, habitat heterogeneity, and dispersal in this system. *P. reticulata* populations adapted to low predation levels are likely to incur severe survival costs if they disperse into stream sections with higher predation, as will any of their offspring that retain low-predation phenotypes such as brighter coloration and weaker antipredator behaviors (Haskins *et al.* 1961; Endler 1980, 1987; Magurran & Seghers 1991; Reznick *et al.* 1996, 2001). As a consequence, this local adaptation to a gradient in predation regime should limit dispersal propensities through selective deaths of maladapted dispersers and/or adaptive dispersal decisions by individuals (McPeek & Holt 1992; Bolnick & Otto 2013; Berdahl *et al.* 2015). In other words, if dispersal is beneficial at the within-reach, between-pool scale—where we observed reproductive benefits—but costly between steep environmental gradients, most individuals should disperse only small distances.

In natural systems, it is notoriously difficult to directly determine causality between a behavior, such as dispersal, and reproductive success. In an observational study such as this, it is always possible that unaccounted for variables may lead to the observed correlation between reproductive success and dispersal. We dealt with this issue by including two important drivers of fitness and dispersal, longevity and size, as covariates in all our models of reproductive success. Though longevity and size are significant predictors of both dispersal distance and lifetime reproductive success in our system (Figures S2.2), accounting for them in our models allows us to statistically separate their effects on fitness from those of dispersal status and distance as best as possible. Within the constraints of an observational study of natural populations, our approach strongly suggests a causative link between dispersal and reproduction, yet experiments that directly manipulate dispersal and longevity would be needed to fully disentangle these factors.

Conclusions

We provide empirical evidence that fine-scale dispersal correlates to reproductive benefits in *Poecilia reticulata*. Our study highlights the value of multi-generational capture-mark-recapture data for understanding individual consequences of dispersal, especially when paired with fitness estimates from molecular data. Our characterization of dispersal and reproductive success sheds light on how the benefits of dispersal behavior vary between individuals and environments. Given drastic reductions in population connectivity caused by anthropogenic changes to the environment across the globe (Haddad *et al.* 2015b), understanding patterns of dispersal and its consequences for fitness in natural populations will be vital to understanding population persistence in the future (Kokko & López-Sepulcre 2006; Crispo *et al.* 2011)

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CHAPTER THREE:

Rollin’ on the river: Local census size, sex ratio, and relatedness drive dispersal behavior in the Trinidadian guppy (*Poecilia reticulata*)

Abstract

Dispersal is a major determinant of individual fitness, population structure, and range dynamics in natural populations. In organisms that disperse actively, the decision to move from one place to another is the fundamental building block of all dispersal dynamics that follow. Understanding what drives individual movement behavior, then, is fundamental to understanding dispersal. For this study, we asked how seasonal, local, and individual characteristics affects dispersal probabilities in two populations of Trinidadian guppies (*Poecilia reticulata*). We were especially interested in how the local composition of pools affected dispersal, focusing on the effects of local census size, sex ratio, and average relatedness. We found that guppies were more likely to disperse out of pools with fewer individuals, and pools with lower average relatedness. In one of the study streams, fish were also more likely to disperse out of male-biased pools, especially females. Overall, we find that intrinsic and extrinsic factors interact to drive dispersal behavior, explaining some of the dispersal variation we observe between individuals in the wild.

Introduction

Dispersal is an important ecological and evolutionary force that structures populations and influences individual fitness (Bowler & Benton 2005; Clobert *et al.* 2009, 2012). While some organisms disperse randomly, many show non-random dispersal behaviors driven by both intrinsic individual characteristics and extrinsic environmental conditions (Clobert *et al.* 2009). These small-scale individual movement decisions, multiplied across individuals, space, and time, scale up to define population- and species-level patterns of dispersal and gene flow across a landscape. From the perspective of individual organisms, multiple factors can potentially drive dispersal behavior. For example, poor habitat conditions can lead individuals to search for higher quality patches, high competition can push out less competitive individuals, and searching for available and/or unrelated mates can motivate reproductive-age individuals to search for new locations and opportunities. These different drivers will have consequences for where dispersers establish, how their fitness is affected by dispersal, and how populations are structured across a landscape (Morales *et al.* 2010; Kubisch *et al.* 2014; Spiegel *et al.* 2017).

It is often difficult to determine the relative importance of different drivers of dispersal in natural populations. Local context (e.g. conspecific abundance, sex ratio, predator presence, etc.), individual characteristics (e.g. sex, age, body condition), and seasonality lead to within-population variation in dispersal decisions and distances, with important consequences to ecological and evolutionary processes (Peniston *et al.* 2023). For instance, increased temporal variation in dispersal within a metapopulation framework can decrease population growth rates (Watson *et al.* 2012), while in an evolutionary context that same increased variation is expected to facilitate local adaptation (Peniston *et al.* 2019)

Studies that explore drivers of dispersal in natural populations often look at how individuals with differences in traits vary in their dispersal responses to local conditions. For example, small milkweed beetle (*Tetraopes tetrophthalmus*) males are more likely to leave male-biased patches than larger males, suggesting that avoidance of male-male competition is a driver of dispersal in this system (Lawrence 1987); while female red deer (*Cervus elaphus*) disperse more frequently when populations sex ratios become highly female-biased and male dispersal decreases (Pérez-González & Carranza 2009), suggesting an important role of inbreeding avoidance in this species. Competition and inbreeding avoidance may affect dispersal decisions differently for males compared to females, and for individuals of different sizes, depending on the mating system and resource availability of different populations. As such, variation in dispersal may be explained by temporal changes in intensity of competition or mating behavior. By analyzing the effects of individual, local, and seasonal variation on dispersal behaviors, we can better understand which drivers are most important in different scenarios.

Here, we make use of a multi-year capture-mark-recapture study of Trinidadian guppies (*Poecilia reticulata*) to ask: **What are the extrinsic and intrinsic factors influencing dispersal decisions, and how do they change between seasons?** We do this within two established headwater guppy populations, looking at factors affecting movement between patches ("pools") *within* the same population. As these animals are highly philopatric and rarely disperse long distances (Croft *et al.* 2003; De Bona *et al.* 2019; Borges *et al.* 2022), this within-population movement reflects the scale at which guppies are interacting with one another: competing, mating, and reproducing. We define dispersal as any movement that can lead to gene flow (Ronce 2007), and given our lack of information about where and when matings take place in our dataset, we use the terms "dispersal" and "movement" interchangeably. In this study, we do not look at long-distance, between-population dispersal, range expansion, or habitat colonization dynamics. Instead, **we asked how the local composition of pools, alongside individual size and sex, affected monthly dispersal decisions for over a thousand unique individuals across two streams** (Figure 3.1).

Our dataset allows us to assess the effects of three variables related to local guppy composition: local fish abundance ("census size"), sex ratio, and relatedness. In a previous study of the same two populations, we described dispersal variation between sexes, sizes, and across seasons, finding that dispersal has fitness benefits in these streams, especially for males (Borges *et al.* 2022). Here, we shift our focus from the consequences of dispersal to its causes. Though, to our knowledge, only two other studies have looked at dispersal in the Trinidadian guppy (Croft *et al.* 2003; De Bona *et al.* 2019), decades of research in this important model system allow us to make predictions about how dispersal decisions will be affected by local and individual factors. Understanding the relative importance of different drivers of dispersal in guppies not only offers an example of how dispersal occurs in natural systems, but also fills an important gap in our understanding of this model species, connecting existing knowledge on competition, mate choice, and seasonality to their effects on the ecological and evolutionary force that is dispersal.

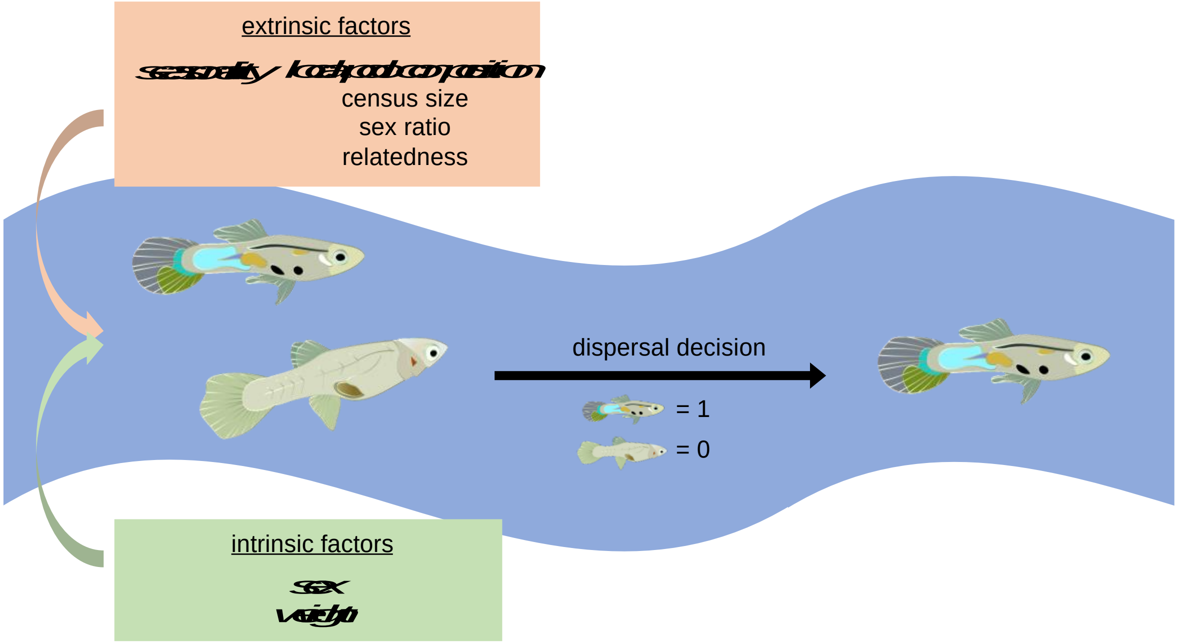

Figure 3.1. Conceptual figure of dispersal drivers analysed in this study. Both extrinsic and intrinsic factors affect individuals' dispersal decisions, in isolation and in interaction with one another. In this example, the colorful male does disperse (1), while the female does not (0).

For one, density-dependence has a major influence on guppy life-history: higher guppy density leads to decrease offspring size, delayed maturation, and higher mortality in low-predation headwater environments (Reznick *et al.* 2012; Bassar *et al.* 2013; Travis *et al.* 2023). In the context of dispersal, DeBona et. al (2019) showed that in the process of colonizing new sections of a stream, guppies respond differently to density based on life stage, sex, and the progression of colonization. Importantly, guppy densities vary predictably with season, increasing during the dry season (January–May) and decreasing during the wet season (June–December) when resources are scarce, competition is stronger, and mortality is higher (Reznick 1989; Fitzpatrick *et al.* 2014, 2016). We expect, then, that increased conspecific density will increase individual dispersal probabilities, as guppies avoid competitors, and that this effect will be stronger in the wet season.

In addition to density, sex ratio and relatedness may also be important drivers of dispersal in *P. reticulata*. In the guppy mating system, males actively search and compete for mates, while females tend to choose their mates based on male phenotypes such as color (Houde 1987; Endler & Houde 1995; Zajitschek & Brooks 2008). This female choice is thought to be a mechanism for inbreeding avoidance (Johnson *et al.* 2010), as females have the ability to recognize kin, male color patterns are heritable, and females show preference for novelty and rarity (Zajitschek & Brooks 2008; Hughes *et al.* 2013a; Daniel & Rodd 2016). Given this mating system, we expect that males disperse most often out of male-biased pools in the search for mates, potentially explaining males' observed higher rates of dispersal (Reznick *et al.* 1996; Croft *et al.* 2003; Borges *et al.* 2022). In contrast, we expect that females respond more strongly to high relatedness within pools, dispersing in search of less-related mates. Ultimately, differences in how the sexes respond to sex ratio and relatedness may also explain why males accrue more fitness benefits than females from movement within streams (Borges *et al.* 2022).

Understanding local drivers of dispersal behavior in natural environments is inherently difficult because it requires fine scale, individual, multi-site and multi-year datasets. The Trinidadian guppy system offers just such an opportunity, supported by a thorough understanding of other ecological and evolutionary processes in this organism. Disentangling the relative importance of local, individual, and temporal factors in driving dispersal in this system will shed light not only on how these fish move across their environments, but on how factors such as inbreeding avoidance and competition influence dispersal in natural settings.

Methods

Field capture-mark-recapture

This study was conducted on two populations of *P. reticulata* in neighboring headwater streams, Taylor and Caigual, in the Guanapo drainage in northern Trinidad. The data were collected in a monthly capture-mark-recapture study between July 2009–October 2010, with the full mark-recapture methods described in Fitzpatrick et al. (2016). To summarize, we selected reaches in each stream that were the upstream-most extent of wild guppy populations uninterrupted by waterfall barriers (240 m in length for Taylor, and 80 m in length in Caigual). Every pool within these reaches was uniquely labeled based on its distance to the upstream limit of the reach, such that labels remained consistent across season as pools changed in size. For every month of the study, each pool was sampled using hand nets and mesh traps. Every guppy larger than 14 mm was captured, temporarily moved to a field laboratory, marked, and measured. Here we only include sexually mature individuals in our analyses, with maturity being determined by gonopodium morphology for males, and the presence of melanophores at the cloaca for females. These mark-recapture methods are commonly used in studies of wild Trinidadian guppies, showing low lab mortality and high capture probabilities (Reznick et al. 1996). For each sampled fish, we also collected tissue for DNA extraction, and all individuals were weighed and photographed each month. All fish were returned to their exact capture location one to two days after processing.

Quantifying dispersal probability

The pool location of each capture was recorded for every individual at every sampling occasion. For this study, every movement between two distinct pools was considered as a dispersal event, regardless of the distance between pools. Given that sampling occasions were separated by one month, it is possible that individual fish moved away then backtracked between sampling dates, and we are not able to observe such movement in our data, such that we are quantifying the *minimum* dispersal between pools for these individuals.

For each timepoint in which an individual fish was captured, we included a variable in the dataset recording whether it was captured at a different pool in the following month (i.e. whether or not it dispersed from that timepoint to the next), regardless of the distance between pools. This is in contrast with our analyses of the same dataset in Borges et al. (2022), where we were interested in the distances traveled between pools and only considered movement above 10 m to be indicative of dispersal. Here, we were not interested in dispersal distances and therefore considered any movement between distinct pools to be reflective of a dispersal decision made by an individual in response to the local pool composition. We then used this variable as a binomial response variable in our "probability of dispersal" models to determine which individual and environmental characteristics affected dispersal probability. We found that in Caigual, 31% of individuals never moved between pools, while that number was 70% for Taylor (Figure 3.2). The maximum number of dispersal events within a fish's lifetime within our study was 7, achieved by 12 individuals in Caigual.

Modeling dispersal response to pool characteristics

We were interested in how guppy dispersal probabilities respond to the local, biotic, intraspecific composition of the pools, specifically: number of guppy individuals in that pool (“census size”), sex ratio, and average relatedness from the focal individual. Sex ratio was calculated as the number of males divided by the number of females in a given pool at a given capture occasion. Both census size and sex ratio values were the same for all fish in a given pool in a given month. In contrast, average relatedness was calculated for each individual fish at each pool in each timepoint as the average relatedness value for that focal fish when compared to all other fish present in that pool at that time. Relatedness values were extracted from a relatedness matrix based on 12 microsatellite loci genotyped for each individual fish in the study. Relatedness between two individuals was calculated as the Jaccard similarity between alleles at all microsatellite loci, and a matrix was populated with all pairwise relatedness values between individuals. Average within-pool relatedness was calculated as the mean pairwise relatedness of the focal individual to all other individuals present in a pool at a specific time. Methods for obtaining the microsatellites is described in detail in Fitzpatrick et al. 2016. Relatedness and sex ratio were only tested as drivers of dispersal for pools that contained more than one individual, since in those pools the sex ratio variable is equal to the sex of the that individual, and its relatedness to itself is equal to one.

To test for individual characteristics driving dispersal probabilities, we included fish sex and weight as dependent variables in all models. Every model also included fish ID as a random effect to account for individual behavioral consistency in dispersal from one timepoint to the next. We also tested how dispersal probabilities change between the wet and dry seasons by including season as a dependent variable.

All our models include dispersal probability as our response variable, modeled with a binomial distribution. We modeled each stream separately, given extreme differences in population sizes between the two locations, which would not only create an imbalance in terms of sample size within a joint model, but also may lead to different biological interpretations of dispersal behavior in response to the local factors we investigated. For each potential local driver of dispersal (e.g. census size), we also build separate models for males and females to assess the effects of each driver in isolation, as well as its interactions with season and fish size. We decided to separate the sexes due to the correlations between fish size and sex (females guppies are larger and grow indeterminately, while males stop growing after they reach maturity; Constanz 1989) and the different biological interpretations of some of our parameters of interest. For example, females dispersing out of male-biased pools suggest that they may be avoiding male harassment, while males dispersing out of male-biased pools might be avoiding competition with other males. We attempted to build a full model containing all potential drivers of dispersal, yet that model did not converge for either stream. Therefore, we present results for models that tested each local driver of dispersal separately (six total models, three for each stream). For example, the census size model included as variables: census size, fish sex, weight, and season, but not sex ratio or relatedness (Table 3.1). All statistical analyses were conducted in R 1.3.1093 (R Development Core Team. 2019). We used AIC (Akaike Information Criterion) for model selection (Johnson & Omland 2004) and the R package DHARMa (Hartig 2021) to assess the fit of candidate models.

Results

Individual and temporal drivers of dispersal

In agreement with a different analyses of this same dataset (Borges *et al.* 2022), we found that male guppies are more likely to disperse, and that fish weight is also positively correlated with dispersal probability. Similarly, we found that fish were more likely to disperse during the wet season, when water volumes in these streams are higher and resource abundance is lower, potentially leading to increased incidental and intentional dispersal, respectively.

We found that a significant portion of the variation in dispersal probabilities in both Caigual and Taylor was due to the two variables we modeled as random effects: month and individual. In Taylor, random effects accounted for between 68-89% of the proportion of the variance explained by our models, while in Caigual that range was 49-84% of the R^2 values in our models (Table 3.1). This suggests that variation between individuals, and consistency within individuals, regardless of sex and weight, are important components of dispersal behavior variability. Similarly, variation between months, even when accounting for seasons, was a driver of dispersal variability.

Local drivers of dispersal

We were primarily interested in the effects of local pool composition on the probabilities of dispersal. The measured variables were census size, sex ratio, and relatedness, which changed between each pool and time point. In Caigual, pool census sizes varied more widely and were up to an order of magnitude larger than in Taylor. Sex ratio also differed between the streams, with pools in Caigual being more female-biased. Average relatedness within pools was higher in Caigual than Taylor (Figure 3.2).

Census size. We found negative effects of census size on dispersal probabilities in both Caigual (N=2367, z=-4.765, p<0.001) and Taylor (N=460, z=-2.725, p=0.006; Figure 3.3C). Guppies in a pool by themselves in Caigual had a 91% probability of dispersal in the next time step, compared to 47% for guppies in a pool of average Caigual census size, and 4% at the maximum census size. The full census model explained 40% of the variance in dispersal probabilities in Caigual (marginal R^2 = 0.404, conditional R^2=0.170), but only 5.4% of the variation in Taylor (conditional $R^2 = 0.210$). In Taylor, guppies alone in a pool have a 13% probability of dispersal in the next time step, compared to 3% for guppies in a pool of average Taylor census size, and 0.3% at the maximum. In both streams, the negative effect of census size was also significant in the separate male (Taylor: N=244, z=-2.196, p=0.028; Caigual: N=669, z=-2.182, p=0.029) and female models (Taylor N=216, z=-1.942, p=0.052; Caigual: N=1698, z=-4.205, p<0.001).

Sex ratio. We also found a significant positive effect of sex ratio on dispersal probability in Caigual (N=2336, z=7.478, p<0.001; Figure 3.4A), with the full sex ratio model explaining about 42% of variance in dispersal (marginal $R^2 = 0.419$, conditional $R^2=0.110$). Guppies in female-only pools disperse 41% of the time, and that probability grows to 88% in even sex-ratio pools, and 99% in male-only pools. That effect was significant for the full and separate sex models in Caigual, suggesting both males (N= 659, z=4.413, p<0.001) and females (N=1677, z=6.364, p<0.001) are more likely to move out of male-biased pools. In Taylor, sex ratio did not significantly affect dispersal probabilities (N=335, z=0.380, p=0.704; Figure 3.4B).

Relatedness. In Caigual, we found a significant negative effect (N= 2313, z=-3.736, p<0.001) of relatedness on movement probability (Figure 3.5A). This model had the lowest marginal R^2 of the three Caigual models, with fixed effects explaining only 7.6% of the variance (though the conditional R^2 was still high at 48.7%). In pools with zero average relatedness, dispersal probabilities were 81%, increasing to 68% at an average relatedness of 0.17, and decreasing to 9% when relatedness equals 1. We see similar results in Taylor, with a significant negative effect of dispersal on relatedness (N= 288, z=-2.993, p=0.003; Figure 3.5D). In the separate sex models, there is no significant effect of relatedness itself on dispersal for males (Caigual: N=603, z=1.679, p=0.093, Figure 3.5B; Taylor: N=138, z=1.265, p=0.206, Figure

3.5E), and in the female-only models the negative effect remains for Caigual (N=1610, z=-3.418, p<0.001, Figure 3.5C) but not for Taylor (N=150, z=-1.688, p=0.091, Figure 3.5F).

Interactions between local, individual, and temporal drivers of dispersal

We were also interested in how individual characteristics mediate guppies' dispersal behavior in response to local conditions. We tested for this by looking for significant interactions between local, temporal, and individual predictors of dispersal probability, and again found varying results depending on local drivers and stream.

Caigual. We found a significant positive interaction between census size and season in explaining dispersal probabilities (N=2367, z=2.571, p=0.010), suggesting that fish are responding more strongly to census during the dry season, when guppy populations in general are more abundant in response to higher resource availability (Figure 3.3A). This interaction was significant in the full model, almost significant in the female model (N=1698, z=1.803, p=0.071), and not significant for males. We also found a significant interaction between census size and fish weight (N=2367, z=3.507, p<0.001). This interaction is significant in the full model, and for males (N=669, z=2.252, p=0.024) and females (N=1698, z=5.120, p<0.001) separately. As a positive interaction, it suggest that larger guppies' dispersal probabilities are less responsive to local pool census (Figure 3.3B).

When looking at sex ratio, we found a significant interaction between pool sex ratio and individual sex in Caigual (N=2336, z=-2.109, p=0.035). As a negative interaction, it suggests that males' dispersal probabilities are less responsive to pool sex ratio than females (Figure 3.4A). Finally, we found a significant interaction between relatedness and season for Caigual males (N=603, z=-2.194, p=0.028, Figure 3.5B), but not in the full relatedness model or the female-only model.

Taylor. We found no significant interactions between census size or sex ratio and season or individual characteristics in Taylor. For relatedness, we again saw a significant interaction with season in the male-only model (N=138, z=-2.328, p=0.019, Figure 3.5E).

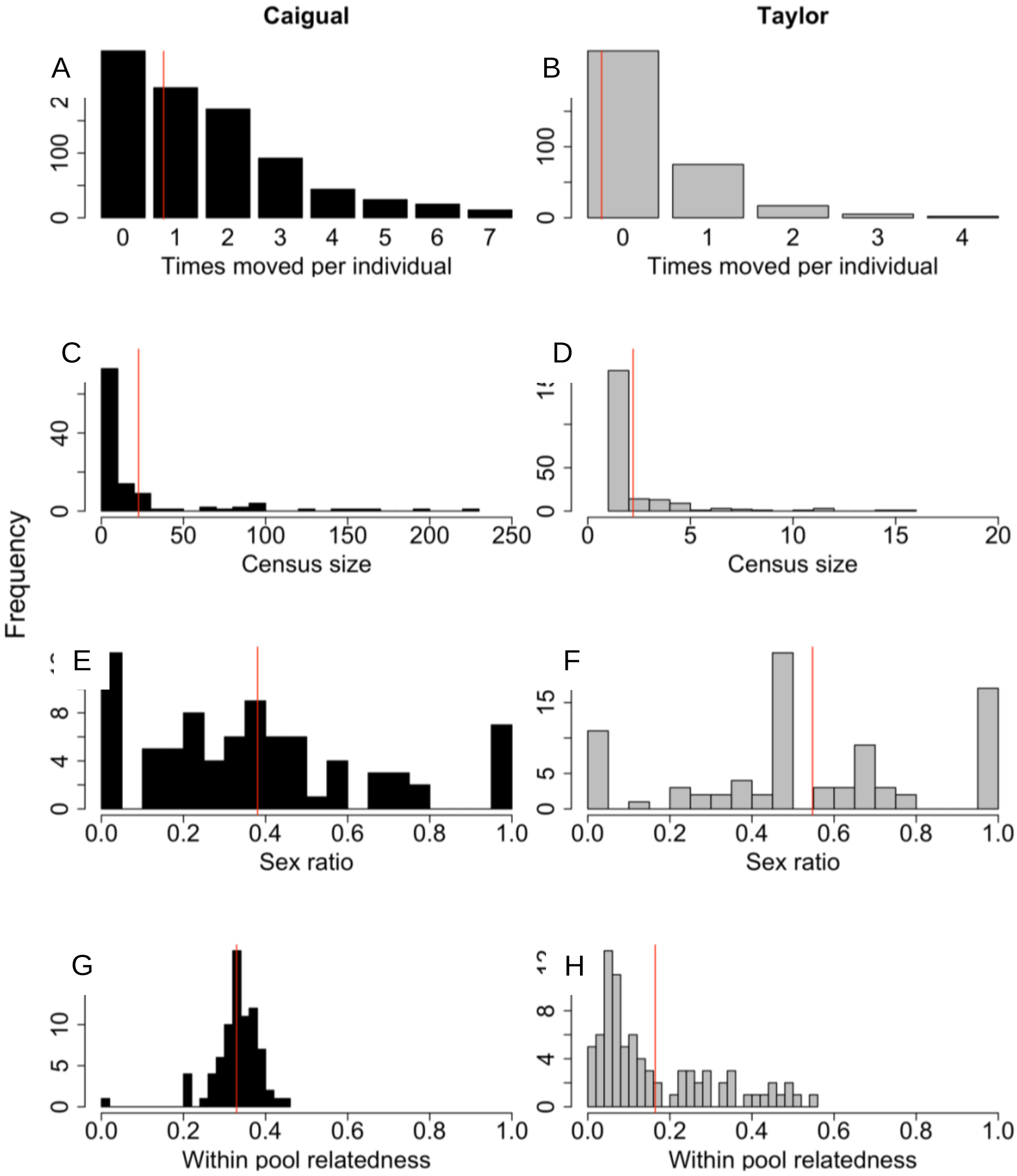

Figure 3.2. Distribution of individual movement and local pool composition. Red lines show average value of each distribution. Left (black) plots represent Caigual data, right (gray) plots represent Taylor. A and B) How many times individuals moved, reflecting that most fish move little or not at all. C and D) Caigual census sizes (and overall population size) are much larger and more widely distributed than in Taylor census sizes. E and F) Sex ratio in Caigual is more female biased (lower than 0.5, which represents a 1:1 ratio), while in Taylor it is more even. G and F) Average relatedness of a focal individual to the rest of its pool (excluding pools with a single individual) is more widely distributed in Taylor, with a higher average in Caigual.

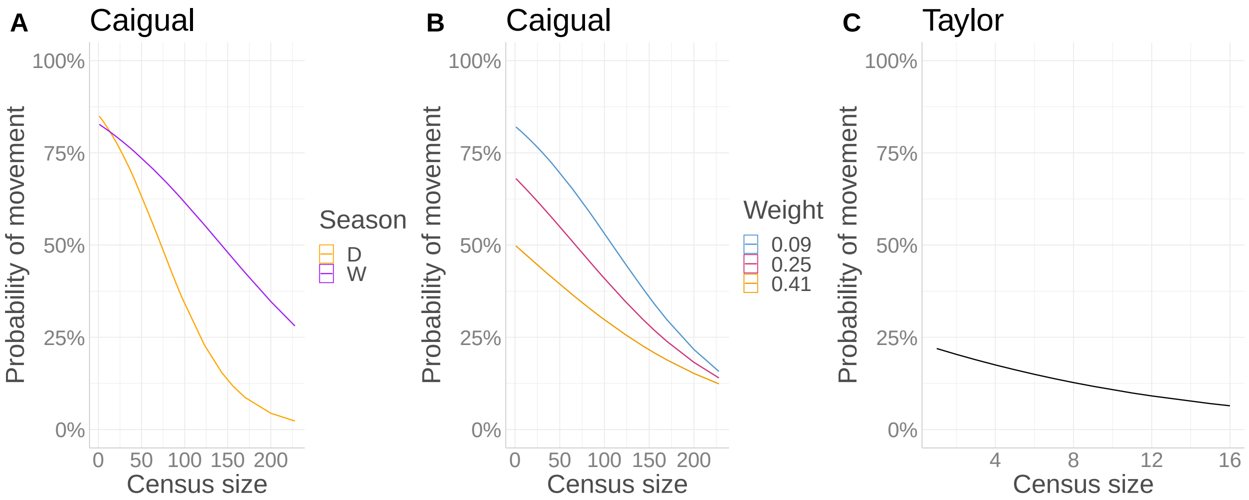

Figure 3.3. Probability of dispersal out of a pool in response to local census size. A) In Caigual, the response to census size differs between seasons, and is stronger in the dry season. B) Representing the interaction between census size and fish weight, different color lines represent small, average, and large fish. The dispersal response to census size is more extreme for smaller fish. C) In Taylor, we see that increased census size decreases dispersal probabilities, and does so with no interactions with season, weight, or sex.

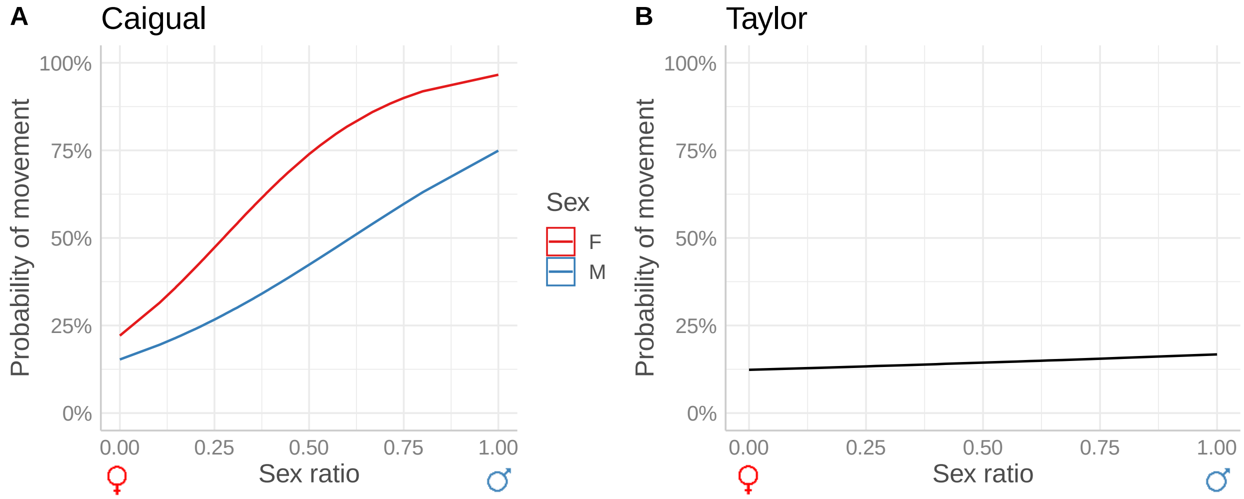

Figure 3.4. Probability of moving out of a pool in response to local sex ratio. A) In Caigual, all fish disperse more out of male-biased pools, and the response from females to sex ratio is steeper than males’. B) Local sex ratio did not affect dispersal probabilities in Taylor.

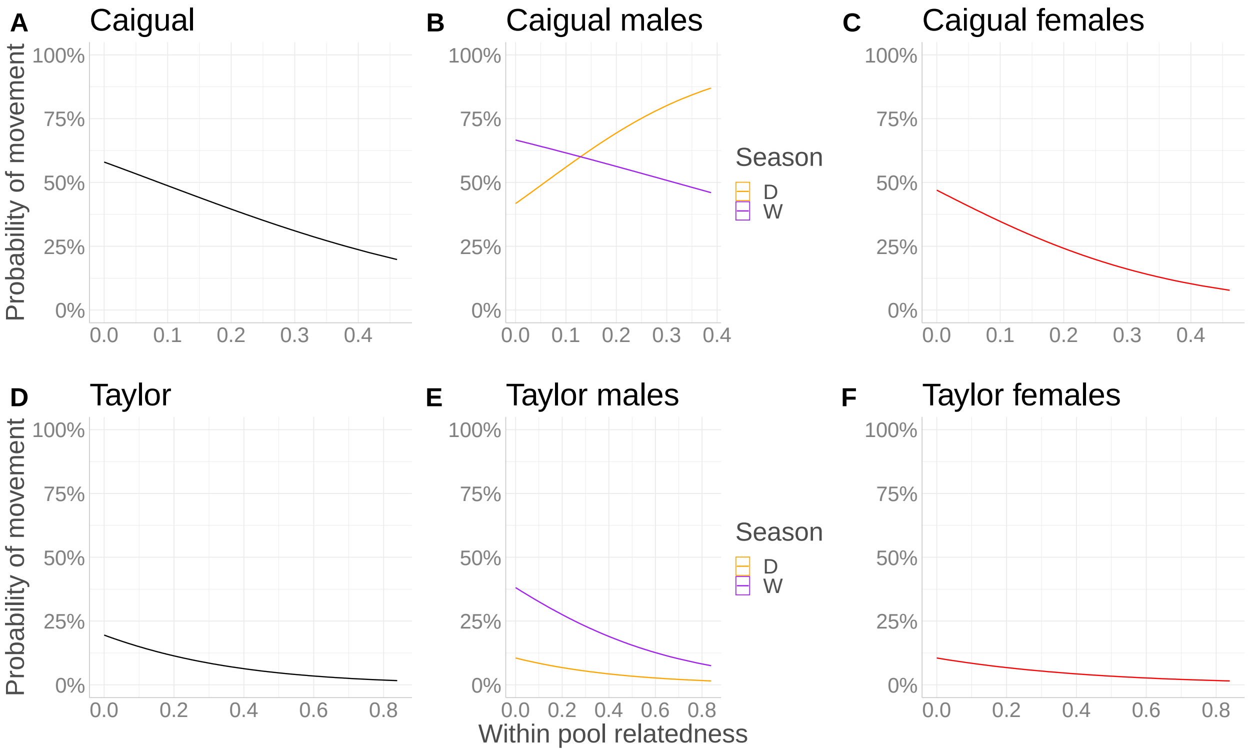

Figure 3.5. Probability of moving out or in of a pool in response to local average relatedness relative to the focus individual. A) In Caigual, fish are most likely to move out of pools with lower average relatedness. B) For males, there was an interaction between relatedness and season, with the negative pattern flipped during the dry season, making makes more likely to disperse out of pools with the most relatives. C) When modeling Caigual females alone, the negative effect was still significant, though it did not interact with season. D) Similarly to Caigual, guppies in Taylor were more likely to disperse out of lower average relatedness pools. E) We also observed an interaction between seasonality and relatedness in Taylor males, but unlike in Caigual, the relationship was not flipped in the dry season, only weaker. F) In Taylor females, we observe a similar trend of decreased dispersal with increased relatedness, though that effect was not statistically significant.

Discussion

We found a consistent effect of local census size and relatedness on dispersal probabilities in Trinidadian guppies, where individuals were more likely to move out of emptier pools and pools with lower average relatedness in both of our study streams. In Caigual, we found that fish were most likely to move out of male-biased pools. We also find evidence that dispersal responses to census size and sex ratio in Caigual vary based on season, focal fish weight, and focal fish sex. Overall, these results suggest that guppies are making dispersal decisions based on local stream conditions, and that these decisions can differ systematically between the sexes, seasons, fish size, and streams.

Our census size results show that dispersal behavior is another life-history trait affected by local population sizes. We were not able to measure density directly, as we lacked depth information for pools at our two study sites, but we assume that our metric of guppy census size and density are correlated. The negative effects of census size on dispersal probability across both streams suggest that guppies disperse most out of low-density pools, a surprising result given the known negative effects of competition on fitness at high densities in these low-predation populations (Reznick *et al.* 2012). We also find that the smallest fish are the ones most likely to move out of emptier pools, and are not dispersing out of crowded pools with a higher probability than larger (and assumed to be more competitive) individuals, as would be predicted if competition was a strong driver of movement behavior.

We propose some potential explanations for these non-intuitive results. First, this study looked only at dispersal in sexually mature guppies, where resource competition may not be as strong as it is for juveniles, who are gape-limited to fewer potential food sources (Dial *et al.* 2017) and more susceptible to cannibalism by adults (Loekle *et al.* 1982; Magurran & Seghers 1990). A previous study looking at dispersal dynamics during colonization of low-predation habitat by guppies from high-predation habitats found that juveniles disperse most out of more crowded pools (positive density-dependence), while adults changed density-dependent dispersal based on the stage of colonization (De Bona *et al.* 2019). In that study, adult guppies show negative density-dependence at the beginning of colonization of a new habitat, which is attributed in part to schooling behavior in high-predation guppies in a novel environment (Magurran & Seghers 1991; Magurran *et al.* 1993; De Bona *et al.* 2019). That, however, is not expected to be a factor in our study of headwater fish populations. Another potential mechanism behind the negative-density dependent dispersal we observed is census size being an honest indicator of habitat quality. That is, the pools with more individuals are the ones with the most resources, lower mortality, and higher reproductive output, effectively attracting, producing, and retaining more individuals. When moving between pools, conspecific attraction as a means to assess habitat quality can be an effective strategy when animals are unable to directly determine quality (Fletcher 2006).

It is also possible that the increased mating opportunities in pools with more individuals outweigh the costs of higher resource competition for adult guppies. Dispersing *P. reticulata*, especially those who move longer distances, show increased reproductive output (Borges *et al.* 2022). For males, dispersal significantly increases the likelihood of having any offspring at all (Borges *et al.* 2022), suggesting that finding mates is an important consequence—and therefore potential driver—of dispersal for these animals. Alongside our results on the effects of sex ratio and relatedness, discussed below, the increased dispersal of guppies from empty pools and the increased reproductive output of dispersers points to the importance of reproduction over competition in driving individual dispersal decisions.

Comparing differences in response to census size between the seasons , we found some evidence of competition influencing dispersal. As predicted, the probability of dispersal from crowded pools is higher in the wet season, when resources are more scarce (Reznick 1989; Fitzpatrick *et al.* 2014, 2016). This points to temporal variation in the role of competition as a driver of dispersal, in line with what is known about the effects of seasonality on other aspects of guppy life history.

These mechanisms—decreased competition in adults when compared to juveniles, high census sizes as an honest signal of habitat quality, heightened importance of finding mates, and seasonal fluctuations in competitive pressures—are not mutually exclusive, and may all contribute to the negative effect of census on dispersal probability in the studied streams. Our results add nuance to what is known about the importance of competition driving dispersal in this model system. Future studies linking census size and density, and directly assessing the role of competition on dispersal could further our knowledge of density-dependent dispersal in guppies.

In contrast to density, less is known about the effects of sex ratio and relatedness on guppy dispersal behavior. The mating biology of *P. reticulata* in low-predation habitats is defined by vibrantly colored males pursuing females, who then choose their partners based primarily on color pattern, showing strong preferences for novelty (Houde 1987; Endler & Houde 1995; Hughes *et al.* 2013b). Males compete to attract mates, indirectly by displaying their colors for females to choose and directly by interfering with other males' displays (Liley 1966; Houde 1987; Jirotkul 1999; Auld *et al.* 2015). When sex ratios are more male-biased, interference behaviors between males are more common and individual males copulate less often (Jirotkul 1999). Females can also incur negative fitness consequences from excessive mating attempts from males, foregoing foraging to escape courtship displays and sneaky mating attempts, especially in more male-biased pools (Magurran & Seghers 1994). Given this natural history, we expected both sexes to avoid male-biased pools: males to avoid competition for mates, and females to avoid harassment. In Caigual, we observed that exact pattern, with female avoidance of male-biased pools being stronger than males'. This suggests that male harassment of females in these streams may be more detrimental to female fitness than male-male competition is to males.

We did not observe the same effects of sex ratio on dispersal in Taylor, which could be due to lower sample sizes decreasing the power of our analyses. Interestingly, average sex ratios between Taylor (0.55) and Caigual (0.38) were different—almost even (1:1) in Taylor and more female-biased in Caigual (Figure 3.2). Population-level sex ratios are known to vary between populations and over time in *P. reticulata* adults, with potential consequences for sexual selection (Pettersson et al., 2004; Rodd & Reznick, 1997; Seghers, 1983). Given that juvenile sex ratios tend to be even in this species (Pettersson *et al.* 2004), this suggests increased mortality of males in Caigual, or it may be a consequence of their higher overall dispersal abilities (moving out of the studied reach entirely; Borges et al. 2022). Male guppies are known to increase investments in courtship in female-biased environments (Jirotkul 1999), which may increase both male-male competition and male harassment of females, driving the increased sex-biased dispersal we see in Caigual compared to Taylor. Future studies linking sex ratio variation with mating behaviors and sex-biased dispersal could help explain the differences we observed between streams.

We also find that relatedness affected dispersal probabilities in both Caigual and Taylor. Our results go against expectations based on inbreeding avoidance in the guppy mating system (Pitcher *et al.* 2008; Zajitschek & Brooks 2008; Johnson *et al.* 2010), with fish being more likely to disperse out of pools with lower average relatedness (Figure 3.5). De Bona et al. (2019) found similar results in their study of dispersal during colonization, suggesting a potential benefit to guppies sharing a pool with kin. Habitat quality may also be at play, where individuals that encounter high quality pools have more offspring and tend to stay in those pools, leading to a pattern of increased relatedness to their neighbours alongside a decrease in dispersal probabilities (De Bona *et al.* 2019). In both that study and ours, however, the effects of relatedness on dispersal probabilities were substantially weaker than the effects of census size, which appears to be the main driver of dispersal dynamics in these streams.

Interestingly, we found an interaction between seasonality and relatedness driving male dispersal in both Caigual and Taylor (Figure 3.5). This interaction shows that the negative effects of relatedness on dispersal are only present for males during the wet season, while for females that effect remains constant throughout the year. For males in the dry season, when resources are more abundant, the direction of this effect is reversed in Caigual and becomes flat in Taylor. If males avoid pools with more relatives in search of females to whom they appear more novel, and therefore more attractive, this pattern may be driven by variation in reproductive behaviors between seasons. Specifically, if females are more receptive during the dry season, males should disperse out of pools with more relatives then. Future studies linking reproductive behavior and seasonality in *Poecilia reticulata* will increase our understanding of how relatedness drives dispersal patterns in these populations.

Finally, across all of our models, we consistently found that individual and monthly variation explained a lot of the variance in dispersal probabilities. This was true even when accounting for between-individual differences in sex and weight (marginal vs. conditional R^2 values, Table 3.1). This suggests that there may be individual consistency in behavior that is driven by factors we did not include in our models. The study of guppy "personalities," individual consistency in behavior across time and context (Réale *et al.* 2010; Carter *et al.* 2013; Kaiser & Müller 2021), has shown that these animals can vary in boldness, exploratory behavior, and sociability (Budaev 1997; Harris *et al.* 2010; Kniel & Godin 2019). These personality traits are expected to influence how individuals respond to competition and mates, as well as their dispersal behavior (Cote *et al.* 2010b, 2010a; Spiegel *et al.* 2017). Though guppy personalities are partially shaped by their exposure to predation, as well as their sex and body size (Harris *et al.* 2010), our study suggest that these factors may not be enough to explain individual consistency in dispersal behavior in natural low-predation settings. This points to a gap in our understanding of the links between individual behavior variation in guppies and their effects on movement and dispersal (Spiegel *et al.* 2017).

As a whole, our results highlight the complexity of dispersal decision-making in natural habitats. The local composition of habitat patches, reflected in metrics like census size, sex ratio, and relatedness vary dynamically across space and time, and different individuals will respond differently to them based on their own traits, shedding light on the selective pressures driving dispersal in this system. Overall, we see that finding mates may be more important than resource competition for determining local dispersal behavior in *Poecilia reticulata*, with variation across season and streams. Knowing the drivers of animals' movement decisions at this fine scale is necessary for a thorough understanding of the selective forces behind patterns of dispersal at the population level.

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CHAPTER FOUR:

Mutualism benefits are lost at high inbreeding levels

Abstract

Inbreeding depression is a major concern for the persistence of small, isolated populations. In plants, the severity of inbreeding depression often depends on the environment in which fitness is assessed. Here, we explore how inbreeding interacts with mutualisms to affect plant fitness in an outcrossing legume, *Chamaecrista fasciculata*. We crossed plants for two generations to produce seeds that differ in their extent of inbreeding, and conducted a common garden experiment where plants were exposed to mutualistic nitrogen-fixing bacteria (rhizobia) and bodyguard ants. We found that plant inbreeding decreased plants' investment in the mutualisms with rhizobia and with ants. Plant fitness also decreased with inbreeding, yet the slope of that decline was different for plants that did or did not receive rhizobia. The benefits of rhizobia for biomass and total seed set were lost at high inbreeding values, such that inbreeding depression was steeper in the presence of this mutualist. Overall, we found that mutualisms are affected by inbreeding, and that rhizobia can change the severity of inbreeding depression in *C. fasciculata*.

Introduction

Understanding the fitness consequences of inbreeding is a longstanding goal in ecology and evolutionary biology, with deleterious effects of inbreeding having been documented since the inception of evolutionary research (Darwin 1876; Charlesworth & Charlesworth 1987). Here we define inbreeding as mating with relatives more often than expected by chance (Keller & Waller 2002). As anthropogenic changes drive population declines and habitat fragmentation, increased rates of inbreeding in small populations are now a major threat to the conservation of biodiversity (O'Grady *et al.* 2006). Studies that have tested for inbreeding depression, the reduction in fitness associated with inbreeding, have found it to be common in small, isolated populations (e.g. Spielman et al. 2004; Armbruster & Reed 2005a; Honnay & Jacquemyn 2007). However, the severity of fitness declines associated with inbreeding vary widely depending on the environmental context in which fitness is assessed (Cheptou & Donohue 2011; Fox & Reed 2011; Reed *et al.* 2012).

Greenhouse and laboratory studies generally detect less inbreeding depression than studies of the same populations in natural environments, where multiple environmental stresses are present (Fenster & Galloway 2000; Armbruster & Reed 2005a; Reed *et al.* 2012; Springer *et al.* 2020). This points to the context-dependency of fitness declines, where the effects of inbreeding can be greatly modified by the presence of abiotic stress (Keller *et al.* 2002; Reed *et al.* 2003, 2012; Armbruster & Reed 2005a), competition (Schmitt & Ehrhardt 1990; Cheptou *et al.* 2000; Meagher *et al.* 2000; Richardson & Smiseth 2017), herbivory (Carr & Eubanks 2002; Campbell *et al.* 2013; Kalske *et al.* 2014), disease (Haag *et al.* 2003; Calleri *et al.* 2006; Ross-Gillespie *et al.* 2007), or predation (Auld & Relyea 2010). These environmental factors and biotic interactions have the potential to relieve or exacerbate inbreeding depression in natural settings. For example, there is ample evidence that parasitism and disease prevalence are higher in populations with lower genetic diversity and higher inbreeding (Smallbone *et al.* 2016; Gibson & Nguyen 2020). As a consequence, the presence of parasites can exacerbate fitness loss in inbred populations (Smith *et al.* 2009).

Mutualisms are ubiquitous biotic interactions that positively affect the fitness of both participating organisms (Kiers *et al.* 2010). Though the genetic bases of various mutualisms are well-established (Gorton *et al.* 2012; Boutin *et al.* 2014; Marcionetti *et al.* 2019), there are no general hypotheses for the effects of inbreeding on mutualisms—in contrast with established predictions for these dynamics in other biotic interactions. In the context of plants, many of the physiological pathways involved in the defense against pathogens and herbivores are similar to those that mediate the recruitment and maintenance of mutualisms, such as root exudates (Bais *et al.* 2006; Narula *et al.* 2009; Huang *et al.* 2014), volatile organic compounds (Kost & Heil 2008; Dudareva *et al.* 2013), and immune loci (Wood *et al.* 2018). Many of the mechanisms through which inbreeding affects other biotic interactions can also alter mutualisms, although these dynamics remain largely unexplored. Two studies have explicitly looked at the effects of plant inbreeding (defined by one generation of selfing vs. outcrossing) on resource mutualisms, finding that it can affect plants' interaction with mycorrhizal fungi (Botham *et al.* 2009) and with nitrogen-fixing bacteria (Rehling *et al.* 2019). Here, we build on this work by controlling inbreeding values along a continuum, and testing effects of inbreeding depression when plants are or are not exposed to mutualists.

Beyond the basic gap in understanding of the interactions between mutualism and inbreeding, there is also an applied urgency to this question. The loss of mutualistic interactions often results from many of the same global change factors that lead to increased inbreeding in natural populations: fragmentation, habitat deterioration, and climate change (Dunn *et al.* 2009b; Hegland *et al.* 2009; Jorge & Howe 2009; Aslan *et al.* 2013; Renner & Zohner 2018). When predicting extinctions, accounting for the disruption of biotic interactions significantly increases predicted biodiversity loss (Colwell *et al.* 2012; Bascompte *et al.* 2019). Notable examples include increased asynchrony in the phenology of plants and pollinators due to differential responses to warming (Hegland *et al.* 2009; Forister *et al.* 2010), and coral bleaching due to temperature stress in warming oceans (Knowlton 2001). The loss and reshuffling of these mutualisms directly affects fitness of participant species and may interact with inbreeding in these same organisms, potentially speeding up biodiversity declines when these stressors occur concurrently.

In this study, we answer two related questions: (1) **What are the effects of host inbreeding on the presence and strength of mutualisms?**; and (2) **How does the presence or absence of a mutualist affect the expression of host inbreeding depression (i.e. the loss of fitness that comes from inbreeding)?** We conducted a common garden experiment to test how inbreeding in the legume *Chamaecrista fasciculata* altered two of its mutualisms: a resource mutualism with nitrogen-fixing soil bacteria (rhizobia), and a defense mutualism with nectar-feeding ants (Figure 4.1A). The mutualism between legumes and rhizobia is a widespread, economically important interaction known to affect plant fitness (Kiers *et al.* 2010; Masson-Boivin & Sachs 2018) and vary with plant and bacterial genotype (Heath *et al.* 2010; Gorton *et al.* 2012; Burghardt *et al.* 2017). This mutualism can be inferred by the presence of specialized nodules on plant roots, where rhizobia provide plants with nitrogen in exchange for carbohydrates. *Chamaecrista fasciculata* also participates in a defense mutualism with ants, where plants provide nectar through extra-floral nectaries on their leaves in exchange for ant-mediated defense against herbivores (Bentley 1977; do Nascimento & Del-Claro 2010).

Previous studies have shown that fragmentation of natural habitats and increased rates of selfing cause inbreeding depression in *C. fasciculata*, which is primarily outcrossing (Fenster 1991b, 1991a; Sork & Schemske 1992; Mannouris & Byers 2013). However, the extent to which plant inbreeding affects plants' mutualism with rhizobia and ants, and the consequences of these effects on plant fitness, have not yet been explored in *C. fasciculata* or other systems. For question (1), we tested the effect of plant inbreeding on the number of mutualism structures (root nodules and extra-floral nectaries), with two alternative predictions: **(1A)** inbred plants increase investment in mutualism structures to attract mutualists and potentially compensate for the physiological stress of inbreeding; **(1B)** inbred plants are less able to invest in mutualism structures, such that they produce fewer nodules and EFNs (Figure 4.1B). For question (2), we tested whether the presence of mutualistic rhizobia affects the expression of inbreeding depression, with three potential predictions: **(2A)** resources provided by the presence of a rhizobia buffer plant fitness declines, such that plants can at least partially compensate for other physiological costs of inbreeding and maintain high fitness; **(2B)** the mutualism breaks down with inbreeding, as plants lose the ability to recruit, control, or provide for rhizobia, causing the benefits of the interaction to decrease as inbreeding increases; or **(2C)** the mutualisms are not affected by inbreeding, such that inbred and outbred plants invest similarly in the interactions and accrue similar benefits from them (Figure 4.1C).

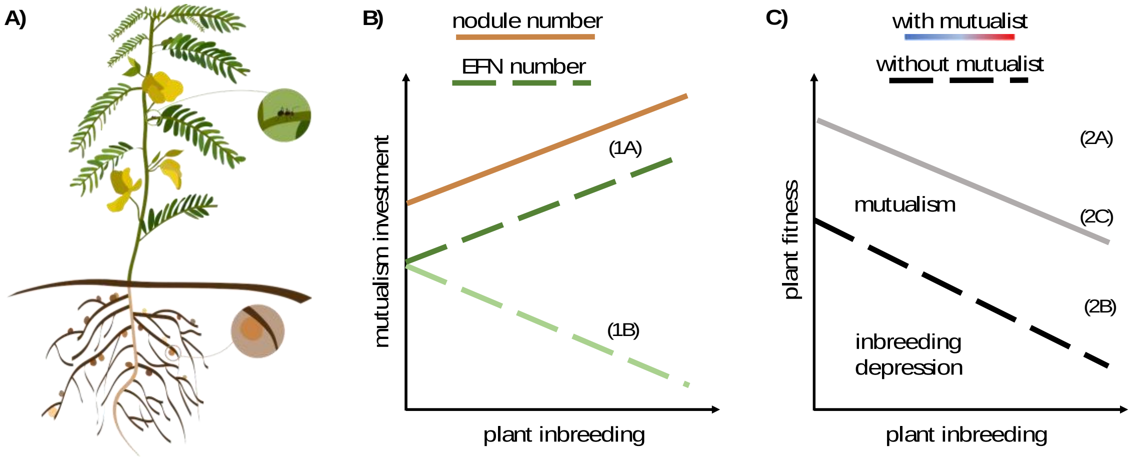

Figure 4.1. Description of system and predictions of results. A) Illustration of *C. fasciculata* showing the two mutualisms studied here: the aboveground ant defense mutualism and the belowground nitrogen-fixing rhizobia mutualism. B) Predicted effects of inbreeding on mutualism investment. The number of nodules is represented by orange solid lines, reflecting plant investment in the rhizobia mutualist. The number of extra-floral nectaries (EFNs) is represented by green dashed lines, reflecting plant investment in the ant mutualist. Darker lines with a positive slope show hypothesis (1A), with investment in mutualisms increasing with inbreeding. Lighter lines with a negative slope depict hypothesis (1B), where more inbred plants invest less in the mutualism. C) Predicted effects of inbreeding on plant fitness with and without rhizobia. The black dashed line represents an expected decline in fitness with inbreeding.

Figure 4.1 (cont'd) The solid lines in different colors represent different predictions for how the addition of a mutualist may affect the expression of inbreeding depression (i.e. the slope of the line). It is assumed that the presence of mutualists increases plant fitness, shown as the higher intercept of the solid lines. In blue (2A) the rhizobia partially rescue plants from inbreeding depression by maintaining similar fitness across different levels of inbreeding; in red (2B), the mutualism benefit is lost as inbreeding increases; and in gray (2C) the mutualism does not affect the slope of inbreeding depression, offering similar benefits to plants across the whole gradient of inbreeding.

Methods

This study was conducted with the annual legume *Chamaecrista fasciculata*, and its *Bradyrhizobium sp.* and ant mutualists. Using an outdoor common garden experiment, we first tested whether plant inbreeding affected the presence and number of mutualistic structures involved in the interactions with rhizobia and ants (root nodules and extra-floral nectaries, respectively). Then, we asked whether the presence of rhizobia modified the expression of inbreeding depression in *C. fasciculata*.

To obtain seeds for this experiment, we manipulated two generations of plant crosses for *C. fasciculata* individuals collected from two natural populations in the US Midwest (Westland, MI, and Sandusky, OH). Four maternal families from each population were used to generate all seeds used in the experiment, and crosses were conducted manually in a greenhouse at Kellogg Biological Station in Hickory Corners, MI (Figure 4.2). See Supplemental Materials for details. After two generations, we had a total of 3423 F2 seeds varying from twice-selfed to outbred, which we used in the common garden experiment described below.

All *C. fasciculata* seeds were geminated in the greenhouse, then planted into 2.5 x 10 inch "cone-tainers" (Stuewe & Sons, Inc., Tangent OR, USA) filled with Premier Pro-Mix FPX 2.8cf soil from BFG Supply Co. (Grand Rapids MI, USA). Parental and F1 generations were kept in the greenhouse for the duration of their lifecycle, while the experimental F2 plants remained in the greenhouse only until rhizobia inoculations were completed, then were moved to an outdoor common garden.

For the common garden experiment, we attempted to geminate 3209 seeds, resulting in 2571 live seedlings. Out of those experimental plants, 1715 *C. fasciculata* individuals of all crossing backgrounds were inoculated with *Bradyrhizobium sp.*, while 856 plants did not receive any rhizobia. We used two rhizobia strains that were obtained from previous collections of naturally occurring *C. fasciculata* at the Kellogg Biological Station, which did not differ in their ability to nodulate with experimental plants or the number of nodules they produced (Figure S4.1). The strains were collected in 2017 from nodules in *C. fasciculata* grown at the Kellogg Biological Station, such that both experimental plant populations were naïve to the strains used in the common garden. We added 2mL of liquid culture to each plant, with control plants receiving the same volume of sterile media (see Supplemental Materials for full culturing methods).

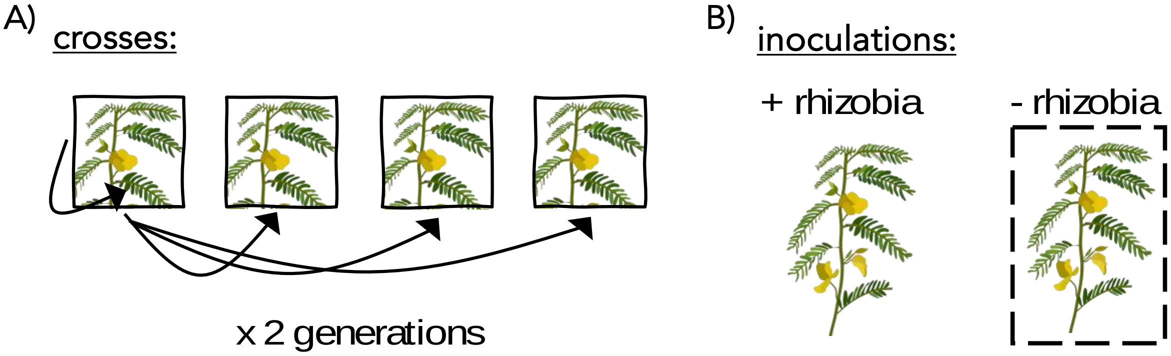

Figure 4.2. Outline of crossing design and inoculations. A) For crosses, two populations were used. Each box depicts a maternal line from one population, with different sizes representing the two different populations. From left to right, cross types were: between populations, within population between maternal lines, within maternal lines, and selfed. All cross types were applied to all maternal lines in both populations, for two generations. B) Individuals resulting from these crosses were then divided into two inoculation treatments: one with rhizobia (full gradient line) and one without rhizobia (dashed black line).

Common garden:

All individuals were then moved to a fenced outdoor common garden at the Kellogg Biological Station. The common garden allowed for natural levels of insect herbivory and pollination, while preventing mammalian herbivory. We scored the day of first flower for each individual, and height at flowering on that date.

To study the plant-ant mutualism, we selected 58 individuals of *C. fasciculata* that spanned our inbreeding gradient. For the month after plants were placed outside, we conducted two surveys of the number of extra-floral nectaries (EFN) on each of those 58 individuals and quantified insect herbivory once as the percentage of plant leaves with any herbivore damage. We conducted seven ant surveys on those plants, where we counted the number of ants on each plant, then calculated an average number of ants per survey per plant. Once plants began to set seed (late August), we manually collected seed pods daily. Plants started being harvested in October, once they senesced and no longer had any green leaves or flowers. At time of harvest, we separated above and belowground biomass. Plant fitness was quantified as the total number of viable seeds produced throughout the growing season, as well as above and belowground biomass at senescence. We also estimated the strength of plants' interactions with rhizobia by counting the number of nitrogen-fixing nodules on the roots of each plant at the end of the experiment.

Data analyses:

We used statistical modeling to test how inbreeding and mutualisms affected plant traits. For all models described below, we used the R package DHARMa (Hartig 2021) to assess model fit. For comparing candidate models that varied in explanatory variables and distributional assumptions, we selected best models based on AIC (Akaike Information Criterion, Johnson & Omland 2004).

For all plant fitness and trait responses, our main explanatory variable of interest was plant inbreeding, which we calculated using the pedigree obtained from two generations of crosses. We used the R package pedtools (Vigeland 2021) to build the pedigree from our cross data, and the R package ribd (Vigeland 2021) to calculate inbreeding coefficients from the constructed pedigree. To do so, we assumed no initial relatedness between the populations, no differences in baseline levels of inbreeding, and treated all maternal lines within populations as being equally related to one another. To account for between-population differences that may affect traits and fitness, we also calculated ancestry proportion by counting how many of the four grandparents of each focal F2 individual originated from a parental population, divided by four. We chose the large population from Ohio as our reference, such that an ancestry proportion equal to 1 reflects an individual with all grandparents originating from the Ohio population and 0 reflects an individual with all grandparents from the Michigan population.

- Effects of plant inbreeding on mutualisms: We first tested for the effects of plant inbreeding on the mutualism traits and responses: number of root nodules (indicator for the legume-rhizobia mutualism), number of EFNs (indicator of plant investment in ant mutualist recruitment), and average number of ants on plants across seven ant surveys (direct measure of mutualist recruitment). Number of nodules and ants were modeled with a negative binomial distribution. Probabilities of contamination (plants that were not inoculated but produced nodules) and nodulation failure (plants that were inoculated but did not produce nodules) were modeled with a binomial distribution, and number of EFNs was approximately normal.

Herbivory was measured once during the season as the percentage of plants' leaves that had any herbivory damage, and was modeled with a zero-inflated beta distribution.

The independent variables of interest tested in these models were pedigree inbreeding (both linear and quadratic effects) and inoculation status (i.e. whether or not it received rhizobia). We also included proportion of ancestry from each population in all models. For herbivory, we included average number of ants as a predictor. All models included planting date as a random effect, which was removed from final models if it did not improve model fit or increased conditional R^2. For all nodulation variables, we also included harvesting date as a random effect, as it could affect nodule senescence and counts.

<u>- Effects of inbreeding on plant traits:</u> We then tested the extent to which inbreeding affected the different plant traits and fitness. To do so, we modelled: total seed set, aboveground biomass, belowground biomass, plant height, and individual seed mass. The independent variables tested in these models were pedigree inbreeding (both linear and quadratic effects), rhizobia inoculation, ancestry proportion, and interactions between these variables. All potential two-way interactions between independent were tested, and dropped from the model if they were not significant.

<u>- Effect of mutualisms on inbreeding depression:</u> Due to small sample sizes in the ant portion of the study, we could only examine the effect of rhizobia on the slope of inbreeding depression (Figure 4.1B). This was done using the same models described above, where a significant interaction between inoculation status (i.e. rhizobia presence) and inbreeding was interpreted as an effect of this mutualism on the expression of inbreeding depression.

Results

Effects of plant inbreeding on mutualisms

We first examined the effect of plant inbreeding on plant interactions with mutualistic ants and rhizobia. For rhizobia, we used root nodule number as a proxy for the investment from the plants in this mutualism. For ants, we used the number of extra-floral nectaries as proxy of plant investment, average number of ants across all surveys as a direct measurement of the strength of the interaction, and measured herbivory as a proxy for the effectiveness of bodyguard ant mutualists.

We found a significant negative effect of inbreeding on EFN number (N=50, z=-2.900, p=0.004; model conditional R^2=0.845; marginal R^2=0.577; Figure 4.3A), even when accounting for the positive effect of plant size on ENFs (aboveground biomass; z=5.577, p<0.001; Figure 4.3B). EFN number, in turn, had a significant positive effect on the average number of ants that visited plants across seven ant surveys (N=58, z=3.542, p<0.001; model R^2=0.311; Figure 4.3C). For herbivory (model conditional R^2=0.509; marginal R^2=0. 472), we detected no significant effect of average ant number (N=58, z=0.075, p=0.94043), but did find significant positive effects of inbreeding (linear: z=2.022, p=0.043; and quadratic: z=3.056, p=0.002), as well as a significant negative interaction between quadratic inbreeding and rhizobia (z=-2.072, p=0.038; Figure 4.3D). As a whole, an increase in pedigree inbreeding from the minimum to the maximum value in our dataset is predicted to decrease EFN number by approximately 20%, decrease ant numbers by 2%, and lead to a 48% increase in herbivory.

We then examined the effects of inbreeding on the plant-rhizobia mutualism. For nodule number, our best supported model (conditional R^2=0.269; marginal R^2=0.071) found nearly significant negative effects of plant inbreeding on nodule counts (N=1432, z=-1.821, p=0.068) and a significant positive effect of inbreeding on the likelihood of plants producing zero nodules (z=2.788, p=0.005; Figure 4.4A). Supporting the effectiveness of our rhizobia treatments, we found that the strongest effect in these models was inoculation status—a positive effect on nodule counts and (z=6.901 p<0.001) a negative effect on the likelihood of producing zero nodules (z=-17.354, p<0.001). An increase in inbreeding from minimum to maximum values in our dataset is predicted to decrease nodule number by approximately 17%.

We observed some plants that were not inoculated yet did form nodules (i.e. contamination, 176 out of 856 non-inoculated plants, 20%), as well as a lower number of plants that did not form nodules despite being inoculated (i.e. failure to nodulate, 40 out of 1715 inoculated plants, 2%). We modeled the probability of contamination and failure to nodulate and found a nearly significant negative effect of inbreeding on the probability of contamination (N=1546, z=-1.726, p=0.084), and a significant positive effect of inbreeding on the probability of nodulation failure (N=1512, z=2.162, p=0.031). That is, more inbred plants were less likely to recruit rhizobia from the environment when they were not inoculated (Figure 4.4B), and plants that failed to nodulate despite being inoculated tended to be more inbred (Figure 4.4C). Ancestry proportions were not significant predictors of either contamination or failure to nodulate, suggesting that both populations used in the study were similarly naïve to and compatible with the used rhizobia strains.

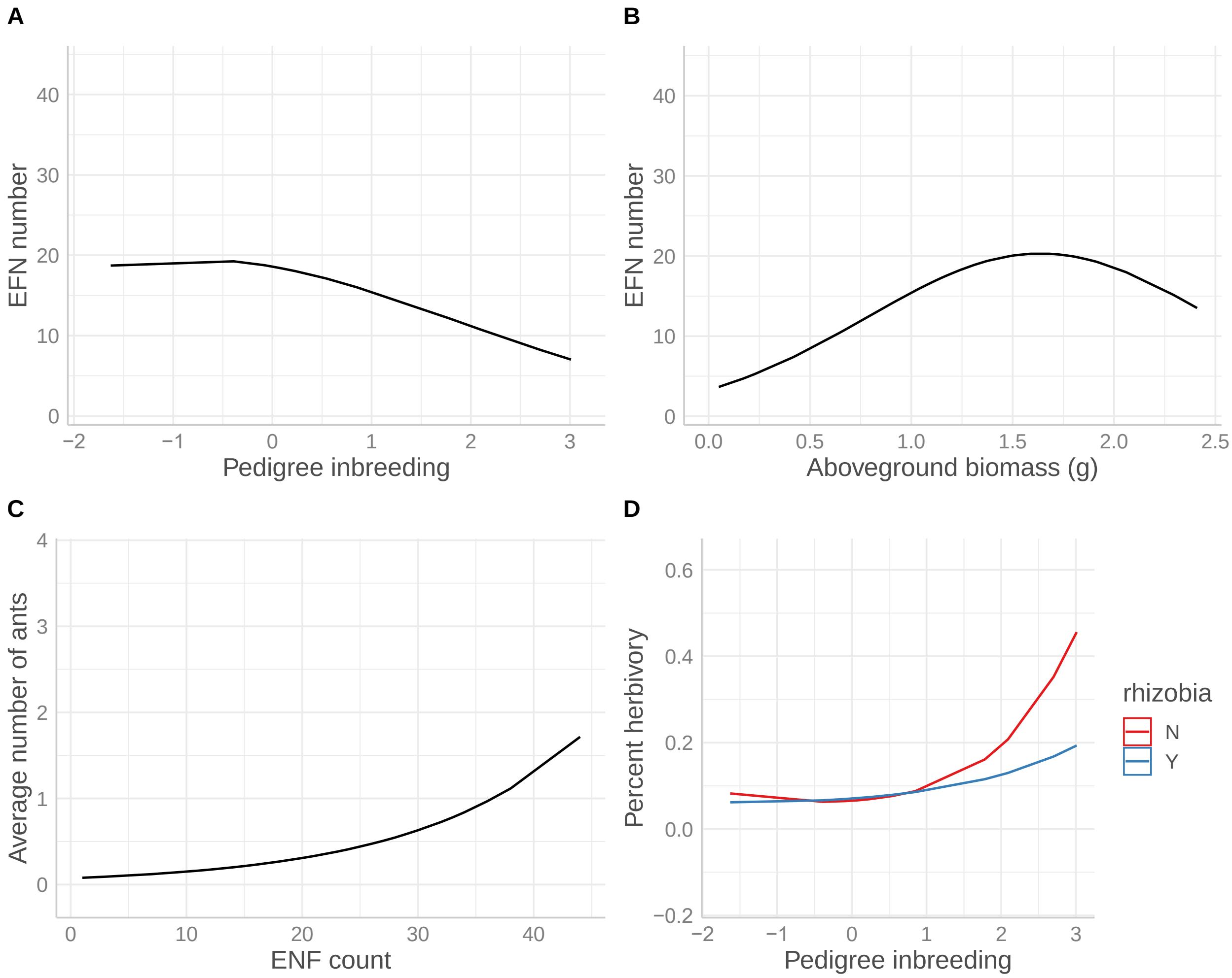

Figure 4.3. Predicted values based on best models. A) EFN number decreases with increasing inbreeding, even when accounting for biomass. B) EFN number peaks at intermediate values of aboveground plant biomass. C) The average number of ants across three survey occasions during the growing season increases with increased number of EFNs. D) The percent of leaves with herbivory increases with increased plant inbreeding, and does so more steeply for plants that are not inoculated with rhizobia (red).

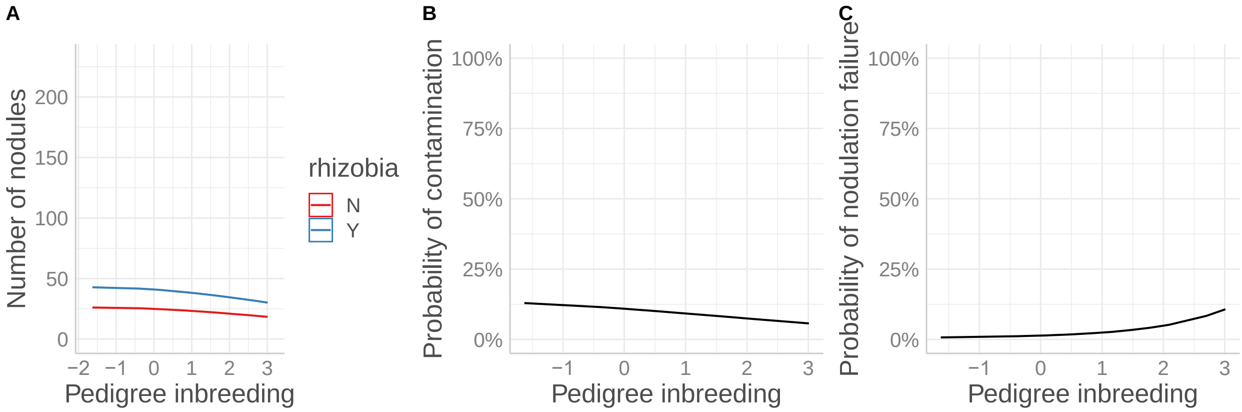

Figure 4.4. Predicted values based on best models. A) Number of rhizobia root nodules decreases with increased plant inbreeding, plants inoculated with rhizobia produced more nodules than those that did not, but both rhizobia treatments are shown due to contamination. B) Probability of contamination (producing nodules despite not being inoculated) decreases with increased inbreeding, though this effect is not significant. C) Probability of nodulation failure (not producing nodules despite inoculation) increases with increased inbreeding.

Effects of inbreeding on plant traits

As expected, we found that inbreeding in *Chamaecrista fasciculata* generally led to decreases in plant fitness. Effects differed by trait, but most showed some response to inbreeding, and those responses were generally negative (i.e. more inbred plants having lower values). Some of these effects (e.g. seed set) were also present in the first generation of crosses (F1, Borges personal obs.), but here we only show and discuss the effects for the experimental generation, F2.

The best fit model for total seed set explained over 80% of the variation in this metric (marginal R^2=0.813), and showed a significant decrease in seed counts with increased inbreeding (N=2566, z=-6.78, p<0.001). We also found a significant negative quadratic effect of pedigree on seed number (N=2566, z=-5.18, p<0.001), suggesting that plants with intermediate inbreeding values produce the highest number of seeds. Inoculation with rhizobia (N=2566, z=27.08, p<0.001), average number of ants (N=57, z=2.464, p=0.014), and ancestry proportion (N=2566, z=5.07, p<0.001) all had significant positive effects on seed counts. The interaction between inbreeding and rhizobia was also significantly positive (N=2566, z=3.12, p=0.002), suggesting that plants that receive rhizobia show steeper declines in seed set with increased inbreeding (Figure 4.5A).

For aboveground biomass, our best fit model explained approximately a third of the variation (marginal R^2= 0.182, conditional R^2= 0.301). Inbreeding had a significantly negative effects on biomass (N=1619, linear: z=-3.428, p<0.001; and quadratic: z=-3.357 p<0.001). Ancestry proportion also had significantly negative effects on biomass (z=-4.943, p<0.001), while rhizobia had strong positive effects on it (z=17.284, p<0.001). We found a significant interaction between inbreeding and rhizobia presence (z=-1.930, p=0.054) in this model, suggesting that the biomass benefits that rhizobia provide to plants decrease with increased inbreeding (Figure 4.5C).

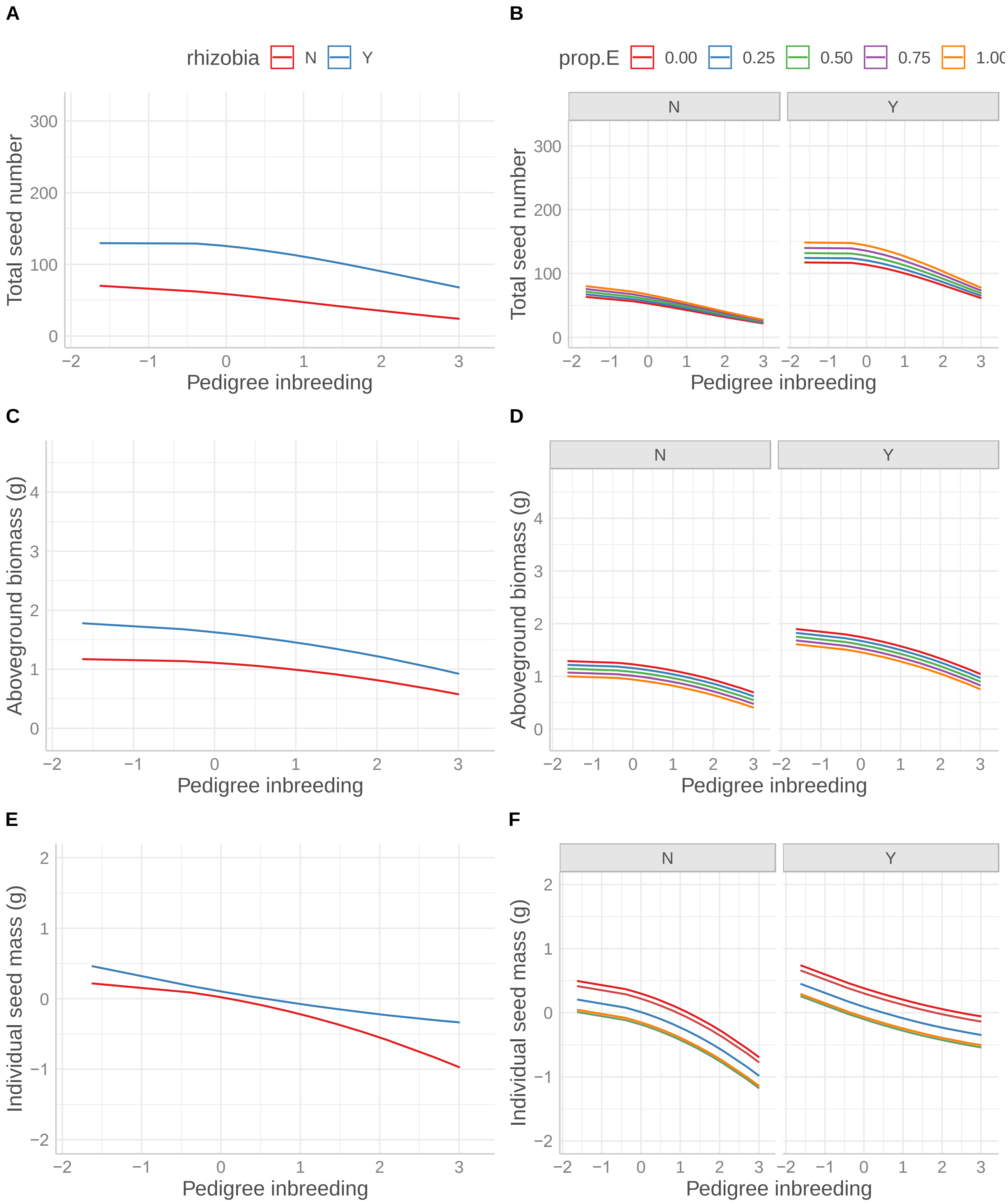

Figure 4.5. Predicted values based on best models. A) Total seed number (fitness) decreases with increased plant inbreeding, and does so more steeply for plants that were inoculated with rhizobia. B) Ancestry proportions affect number of seeds produced, with values closer to 0 (higher ancestry from smaller Michigan population) producing fewer seeds. Left panel shows trends for plants that were not inoculated with rhizobia, right panel shows trends for plants that did get inoculated. C) Similarly to the trend we see for seed set, aboveground biomass decreases

Figure 4.5 (cont'd) with increased plant inbreeding, and the decrease is steeper for plants that received rhizobia. D) Ancestry proportions also affected aboveground biomass, but in the opposite direction of seed set. Plants with more Michigan ancestry (lower values) were larger. E) The mass of individual seeds (averaged per envelope) decreases with increased inbreeding, and in contrast to total seed set and aboveground biomass, the decrease is less steep for plants that were inoculated with rhizobia. F) Similarly to biomass, plants with increased Michigan ancestry produce on average larger seeds.

For belowground biomass, a generalized linear model with a gamma distribution best fit the data, though it had a low explanatory power (marginal R^2=0.006). We found significant positive effects of rhizobia on belowground biomass (N=1436, z=-5.106, p<0.001), and significant negative effects of inbreeding (z=3.080, p=0.002), but no interaction between the two (Figure 4.6A).

We found significant negative effects of inbreeding (N=1973, z=--5.695, p<0.001) on plant height at flowering, with our best model explaining almost half of the variation in height (marginal R^2= 0.265, conditional R^2= 0.492). We also see that ancestry (z=-16.193, p<0.001) affects height, with plants that have more ancestors from the Ohio population being smaller. We also included age at flowering (i.e. days from planting until first flower) as a variable in this model and found significant positive effect of it on height (z=18.102, p<0.001; Figure 4.S2). There was also no significant effect of rhizobia on height at flowering (Figure 4.6B).

We also modeled individual seed mass, which we obtained by dividing the total weight of seeds from seed pods collected from the same plant each day by the number of seeds in those pods (one value per plant per day where seeds where collected). Due to this methodology, this response variable had low variation, and our best fit linear model had low R^2 (conditional=0.040). This model found significant negative effects of inbreeding (N=1951, z=-6.469, p<0.001) and ancestry proportions (z=-3.876, p<0.001). We also found a significant interaction between inbreeding and rhizobia (z=2.344, p<0.001), suggesting that the loss in seed mass that comes with increased inbreeding is less severe when plants interact with rhizobia (Figure 4.5E). There was no significant effect of rhizobia alone in this model.

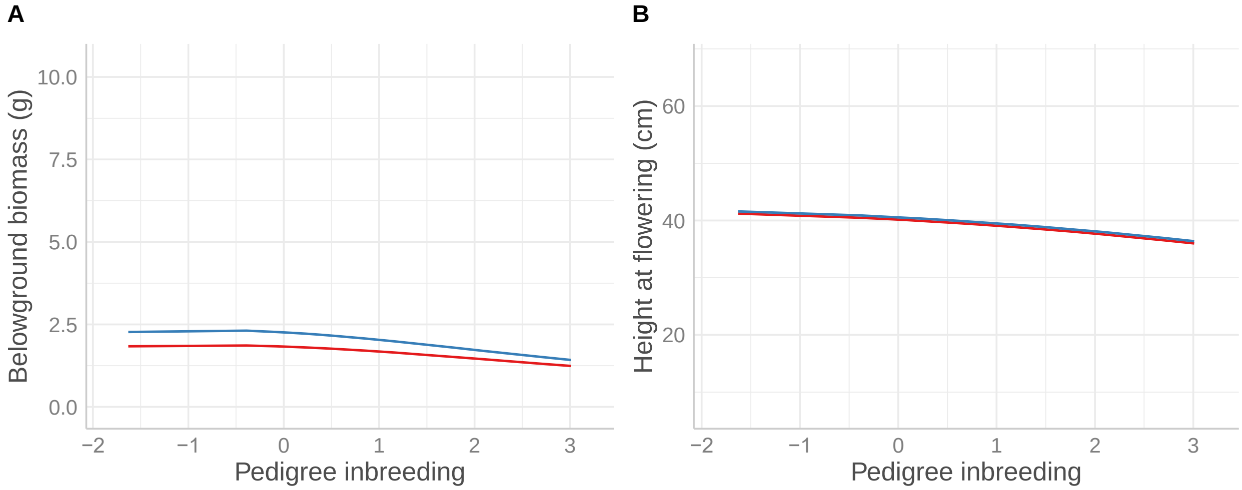

Figure 4.6. Predicted values based on best models. A) Belowground biomass decreases with increased inbreeding, and though plants that received rhizobia have higher belowground biomass, there is no statically significant interaction between inbreeding and inoculation with rhizobia. B) There was no effect of rhizobia inoculation on plant height at flowering, but height did decrease with increased plant inbreeding.

Effects of mutualisms on inbreeding depression

For each trait, we tested for interactions between inbreeding (both linear and quadratic) and rhizobia to determine whether this mutualism altered the slope of inbreeding depression. For total seed set and aboveground biomass, we found interactions between inbreeding and rhizobia where plants with rhizobia had a steeper decline in these traits compared to plants that did not (Figures 4.5A and 4.5C). In contrast, the interactions we observe for individual seed mass (Figure 4.5E) and herbivory (Figure 4.3D), show that plants that received rhizobia had a shallower slope for these traits, and so did not lose seed mass or increase herbivory as much as their non-inoculated counterparts.

Discussion

We found that inbreeding decreased *Chamaecrista fasciculata*'s investment in the mutualism structures involved in the interactions with both nitrogen-fixing rhizobia and ant bodyguards. The presence of rhizobia, in turn, affected the expression of inbreeding depression in the plants, but did so differently for different plant traits. Interactions between rhizobia and plant inbreeding for the most important fitness metrics, total seed counts and aboveground biomass, suggest that the benefits of the rhizobia mutualism are lost at high levels of inbreeding.

In accordance with previous studies of inbreeding depression in *C. fasciculata*, we generally found that inbred plants have smaller stature and lower fecundity (Fenster 1991b; Fenster & Galloway 2000; Erickson & Fenster 2006). There was substantial variation in the strength of inbreeding depression for different traits, with total seed set, height, and aboveground biomass being strongly negatively affected by inbreeding. This suggests that inbred individuals are not as capable of acquiring or maintaining resources as their outbred counterparts (Paige 2010; Kariyat & Stephenson 2019)—potentially increasing plants' need for defense and resource mutualisms. For example, drought, another stress that generally decreases plant biomass and resource availability, has been shown to increase the effectiveness and importance of ant and rhizobia mutualisms in legumes (Staudinger *et al.* 2016; Leal & Peixoto 2017). However, we found that inbreeding decreased the investment of plants' in the two mutualisms we studied. That is, more inbred plants generally produced fewer extra-floral nectaries, attracted fewer ants, produced fewer root nodules, and were more likely to fail to nodulate when inoculated with rhizobia.

Our study adds to the small body of evidence that plant inbreeding affects belowground mutualisms (e.g. arbuscular mycorrhizal fungi in Botham et al. 2009; rhizobia in Rehling et al.

2019). The potential mechanisms behind the decrease in mutualism structures and benefits are multifold. Broadly, it is possible that inbred plants' decreased access to resources lead to a decreased ability to invest in these interactions. Providing rhizobia with carbon is an energy-intensive process (between five and ten grams of plant carbon input to one gram rhizobia nitrogen output; Xu & Wang 2023), and though the costs of extra-floral nectaries and nectar are less well-defined (Bentley 1977; Rutter & Rausher 2004; Marazzi *et al.* 2013; Pyke & Ren 2023), both interactions can be assumed to impose metabolic costs on plants. In root nodules, rhizobia are dependent on the carbon provided by plants, and are not able to multiply and fix nitrogen in the absence of these resources (Xu & Wang 2023). Ants, in turn, are active partners in the ant-plant mutualism, showing preference for nectar with specific sugar and amino-acid compositions, and visiting plants and leaves with higher sugar concentrations (Ness 2003). An inbred plant that cannot provide adequate rewards to its mutualists may lose those interactions and their benefits as a result.

A second suite of potential mechanisms for the decrease in mutualism structures with inbreeding pertains to the biochemical and physiological complexity involved in attracting ants and rhizobia. Inbreeding is known to affect plant chemical exudates related to pollination and herbivore defense (Ferrari *et al.* 2006; Kariyat *et al.* 2012; Campbell *et al.* 2013), and plant-rhizobia and plant-ant interactions are mediated through similarly specific chemical signals (Heil 2008; Kost & Heil 2008; Dudareva *et al.* 2013; Cao *et al.* 2017; Xu & Wang 2023). The legume-rhizobia interaction, in particular, is incredibly complex, involving genotype-by-genotype interactions between the two partners (Sachs & Simms 2006; Heath & Tiffin 2009; Gorton *et al.* 2012; Burghardt *et al.* 2017; Nobarinezhad & Wallace 2020). Root nodules are plant organs that get formed in response to these specific lock-and-key signals between plants and rhizobia, relying on several chemical cues in order to produce an environment where nitrogen fixation can occur (Xu & Wang 2023). Similarly to how host-pathogen interactions can be modified by inbreeding when immunity is disrupted with increased homozygosity (e.g. Reid et al. 2003, 2007; Stevens et al. 1997), it is possible that some of these mutualism molecular signals get disrupted by the intrinsic genomic changes that come from inbreeding. Our finding that more inbred plants are more likely to fail at nodulation when inoculated with rhizobia provides some support for this idea.

Another important facet of the ant-plant and plant-rhizobia mutualisms is their dependence on the local environments experienced by plants. Plants' interaction with rhizobia will be favored in nitrogen-poor environments where plants cannot acquire sufficient nitrogen from root foraging alone (Akçay & Simms 2011; Keller & Lau 2018). In our common gardens, we used nitrogen-poor soil and did not make any nitrogen additions, so that plants were more likely to nodulate when inoculated with rhizobia. This low nitrogen availability can be assumed to be a main driver of the fitness benefits we observed with nodulation, and it likely modulated the fitness declines we observed when inbred plants produced fewer nodules. Similarly with ants, the number of extra-floral nectaries and the amount of nectar produced by plants can be induced by herbivore damage (Mondor & Addicott 2003; Koricheva & Romero 2012), and the benefits of ant defense will only manifest in the presence of herbivores that can be deterred by ants (Bentley 1976; Kelly 1986).

In our common garden, mammalian herbivory was fully absent and insect herbivory was low (on average, each plant had evidence of herbivore damage on only 7% of its leaves), such that the fitness loss that followed from decreased investment in extra-floral nectaries was likely weaker than in environments with higher herbivore pressure. Ultimately, the interactions we found between inbreeding depression and mutualisms will be dependent on the environments that plants experience. The decrease in mutualism benefit we see at high inbreeding levels is more likely to be observed, and to be relevant to plant population dynamics, in stressful (i.e., low nitrogen and high herbivory) environments. Importantly, the anthropogenic changes that lead to increased inbreeding and mutualism loss in natural populations often also increase other kinds of stress, like disrupted soil nutrient cycles (Mosier 1998; Sardans & Peñuelas 2012; Smith *et al.* 2016) and increased insect herbivory at habitat fragment edges (Urbas *et al.* 2007; Guimarães *et al.* 2014; Murphy *et al.* 2016). As such, the relevance of the interactions we observe between mutualism loss and inbreeding depression may become increasingly relevant as global change progresses.

Beyond the environmental dependency of these interactions, they are also likely to vary across plant populations with different evolutionary histories. Local adaptation to mutualists and herbivores can change the population-level investment of plants in the ant and rhizobia interactions (Bronstein 1998; Vittecoq *et al.* 2012; Pringle & Gordon 2013; Keller & Lau 2018; Magnoli & Lau 2020), and a previous history of inbreeding may change the expression of inbreeding depression in plants (e.g. purging; Byers & Waller 1999; Fowler & Whitlock 1999; Glémin 2003; Keller et al. 2002). In this study, we used two populations assumed to be naïve to experimental rhizobia strains, not likely to be in contact via gene flow, and with very different estimated population sizes (small Michigan roadside population vs. large Ohio preserve population). Interestingly, many of our models revealed significant negative quadratic effects of inbreeding on plant traits, suggesting some outbreeding depression between these two populations, as the lowest pedigree inbreeding values could only result from between-population crosses. We also used a metric of ancestry proportion in all of our models to account for any effects of population, and found significant ancestry effects on plant traits but not on nodulation. We then tested for interactions between ancestry and inbreeding for all traits, as they would suggest differences in the expression of inbreeding depression between the two populations—potentially indicative of purging in the small Michigan population. However, those interactions were never significant and were dropped from final traits models. These results suggest that our two experimental populations suffered similarly from inbreeding depression and interacted similarly with rhizobia, despite population-specific trait differences.

Broadly, our results highlight the ecological dependencies and consequences of inbreeding depression. Inbreeding is increasingly recognized and managed as a stressor in small and fragmented population populations, but we still know little about how it affects and is affected by biotic interactions. Even in a system like *Chamaecrista fasciculata*, where inbreeding depression has been previously characterized (Fenster & Galloway 2000; Erickson & Fenster 2006), considering the ant and rhizobia mutualisms changes our understanding of how inbreeding manifests in this species. Incorporating realistic ecological interactions into inbreeding studies could provide better predictions for how inbreeding depression plays out in natural environments, where interactions are often being disrupted by the same anthropogenic pressures that cause inbreeding (Liao & Reed 2009). Our results suggest that the presence of a mutualist does not alleviate inbreeding depression, making mutualism loss and inbreeding compounding stressors that need to be considered jointly for predicting the fate of populations (Rudgers *et al.* 2020; Kiesewetter & Afkhami 2021). Our study also points to the potential consequences of inbreeding in one species scaling to whole communities: two generations of inbreeding in our focal plant impacted ant presence, nodulation (and consequentially soil nitrogen), and herbivory. Considering the full spectrum of ecological consequences of inbreeding has the potential to improve our predictions about the fate of declining and fragmented populations and communities in the face of global change.

Acknowledgements

Susan Magnoli provided *Chamaecrista* seeds collected across the Midwest to kick off this project. Mark Hammond and Jeff Conner managed the KBS greenhouses and Plant Ecology Field Lab, offering much needed advice at every stage of the common garden experiment. Sam Pendrick's help in the field, alongside the whole Fitzpatrick Lab, made seed collections possible. The Fitzpatrick Lab and KBS Writing Group provided valuable feedback on previous versions of this manuscript. This project was funded by a J. S. Karling Award from the Botanical Society of America and a Student Research Award from the American Society of Naturalists awarded to ILB. This is KBS contribution X.

TABLES:

Table 1.1. Average and maximum values for dispersal distance of both sexes in both streams, and for number of offspring of dispersing (moved ≥ 10 m) and non-dispersing fish.

Stream	Sex	Average dispersal distance (m)	Maximum Dispersal Distance (m)	Average Number of Offspring per Individuals		Maximum Number of Offspring	
				Non-Dispersers	Dispersers	Non-Dispersers	Dispersers
Taylor	F	17.57	248	1.74	4.03	24	51
	M	19.12	170	1.42	4.66	26	22
Caigual	F	13.88	157	0.75	1.02	11	20
	M	16.94	216	1.31	3.38	43	55

Table 1.2. Average and maximum values for range of both sexes in both streams, and for number of mates of dispersing (moved ≥ 10 m) and non-dispersing fish.

Stream	Sex	Average Range (m)	Maximum Range (m)	Average Number of Mates per Individuals		Maximum Number of Mates	
				Non-Dispersers	Dispersers	Non-Dispersers	Dispersers
Taylor	F	14.38	173	0.64	1.17	7	8
	M	17.19	158	0.52	1.54	7	9
Caigual	F	9.65	56	0.42	0.48	4	7
	M	11.69	61	0.45	1.32	12	18

Table 3.1. Model summaries for best models of probability of dispersal in Caigual and Taylor.

Response: binary variable describing whether individuals moved in the next time step						
	Caigual			Taylor		
Predictors	*Odds Ratios*	*CI*	*p*	*Odds Ratios*	*CI*	*p*
Census	**0.15**	**0.07 – 0.32**	**<0.001**	**0.66**	**0.49 – 0.89**	**0.006**
Sex (M)	**0.54**	**0.40 – 0.72**	**<0.001**	1.35	0.76 – 2.38	0.308
Season (W)	3.51	0.58 – 21.24	0.172	2.08	0.69 – 6.32	0.196
Weight	**0.08**	**0.03 – 0.19**	**<0.001**	0.53	0.04 – 7.04	0.63
Census * Season	**2.83**	**1.28 – 6.26**	**0.01**	–	–	–
Marginal R-squared = 0.170				Marginal R-squared = 0.054		
Conditional R-squared = 0.404				Conditional R-squared = 0.211		
Sex ratio	**99.75**	**29.86 – 333.28**	**<0.001**	1.43	0.23 – 8.91	0.704
Sex (M)	0.64	0.34 – 1.18	0.152	1.29	0.28 – 5.93	0.74
Season (W)	1.56	0.27 – 9.15	0.623	1.83	0.45 – 7.39	0.394
Weight	**0.03**	**0.01 – 0.07**	**<0.001**	4.93	0.19 – 129.81	0.339
Sex ratio * Sex	**0.17**	**0.03 – 0.88**	**0.035**	1.49	0.13 – 16.42	0.746
Marginal R-squared = 0.110				Marginal R-squared = 0.027		
Conditional R-squared = 0.419				Conditional R-squared = 0.247		
Relatedness	**8404.6**	**63.56 – 1111356.20**	**<0.001**	0.69	0.00 – 1201.26	0.922
Quadratic relatedness	**0**	**0.00 – 0.00**	**<0.001**	0.00	0.00 – 328.02	0.22
Sex (M)	**0.49**	**0.35 – 0.70**	**<0.001**	1.18	0.52 – 2.71	0.691
Season (W)	1.96	0.24 – 16.19	0.531	3.19	0.53 – 19.30	0.207
Weight	**0.02**	**0.01 – 0.04**	**<0.001**	3.23	0.10 – 103.22	0.507
Marginal R-squared = 0.075				Marginal R-squared = 0.112		
Conditional R-squared = 0.474				Conditional R-squared = 0.410		

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APPENDIX A:

Supporting information: Reproductive benefits associated with dispersal in headwater populations of Trinidadian guppies (*Poecilia reticulata)*

Supplementary Appendix 2.I.

We explored whether differences between the metrics of cumulative lifetime distance moved (“total distance” in the manuscript) and absolute distance moved (first vs. last capture locations) affected reproduction. To do so, we calculated absolute distance as the minimum distance between the pools where individuals were first and last captured in our study. We note that this metric cannot be interpreted as the distance between locations of birth and death, given that we capture fish at a minimum size of 14 mm and cannot determine timing or location of death.

Absolute distance is very strongly correlated to cumulative distance (adjusted R-squared: 0.5063; Figure S2.1) and range (adjusted R-squared: 0.7809; Figure S2.2). This is not surprising given that most individuals in our study move little or not at all, such that for many fish the movement from initial to final locations is the same as their total dispersal distance.

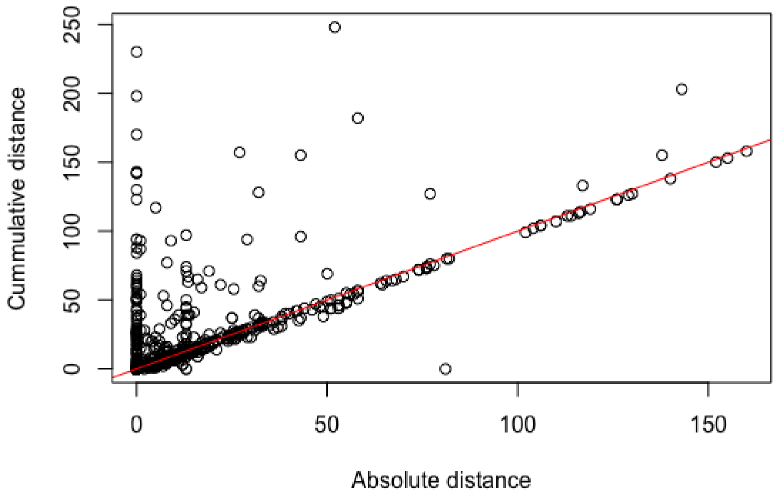

Figure S2.1. Correlation between absolute and cumulative distance in dataset.

We then reran all reproductive models using absolute distance as an independent variable in the place of cumulative distance (“total distance” in the manuscript) or range. Absolute distance did not significantly predict reproductive success (count or zero-inflation) for males. This result is in contrast to what we found for cumulative distance, where it significantly increased the chances of males having non-zero offspring. The dispersal status models currently in the manuscript also show a significant effect of disperser status on the chance of males having non-zero offspring and a marginally significant effect on their number of offspring. As such, it seems that dispersal status and cumulative distance are correlated to reproductive success for males in a way that absolute distance is not.

For females, increases in absolute distance led to significant increases in total number of offspring, and no effect on the chances of having non-zero offspring. This is similar to the outcomes of using the "total distance" metric (as presented in the manuscript). That is, cumulative distance and dispersal status both have marginally significant effects on number of offspring, and no effect on zero-inflation. Thus, for females, the metric of absolute distance captures a similar trend as cumulative distance and status.

When comparing absolute distance to range, and their effects on number of mates, we again find qualitatively similar patters between the metrics. Both range and absolute distance have significantly positive effects on number of mates for males. For females, the effect of absolute distance does not significantly alter number of mates, compared to a nearly significant effect of range.

These results raise interesting questions about how the effects of absolute and cumulative distance differ from the perspective of a dispersing guppy, and what the potential underlying biological causes and consequences of those differences may be. For example, the correlation between cumulative and absolute distances is actually higher for males (R-squared = 0.5781) than females (R-squared = 0.4521), yet they have a more similar effect on reproduction in females. This could be due to differences in exploratory behavior and territoriality between the sexes, and their effects on reproduction. Though males travel farther cumulative distances than females, they do not significantly differ in their absolute distances. This suggests that males explore new pools more than females, moving more throughout the entirety of our study reach before reaching their location of last capture.

Ultimately, the similarity in most of the results between these analyses that use absolute distance and the ones presented in the manuscript that use cumulative distance, in addition to the strong correlation between the different predictors, suggests that our current metrics of dispersal adequately capture the aspects of guppy dispersal that we are most interested in. Though this analysis does raise interesting questions about sex differences in guppy dispersal behavior, the main results linking reproductive benefits to dispersal still hold when considering either total or absolute distance.

Supplementary Appendix 2.II.

We are extremely grateful to the field assistants who contributed to the capture-mark-recapture data collection in Trinidad during 2009 – 2011. Their names are listed in alphabetical order with project managers highlighted in bold: Jeremy Austin, **Brian Ayers**, Ron Bassar, Nicole Bedford, Dale Broder, Ethan Brown, Elizabeth Brunner, Mark Burton, Nichole Bushey, Becky Chong, Jacob Dillon, **Connor Fitzpatrick**, Courtney Fitzpatrick, Dylan Fitzpatrick, Eva Fischer, Andrew Furness, **Stephanie George**, Cameron Ghalambor, Katherine Gleason, Ryan Godfrey, Michael Grundler, Guanapo, Corey Handelsman, **Justa Heinen**, Maria Hernandez, Emily

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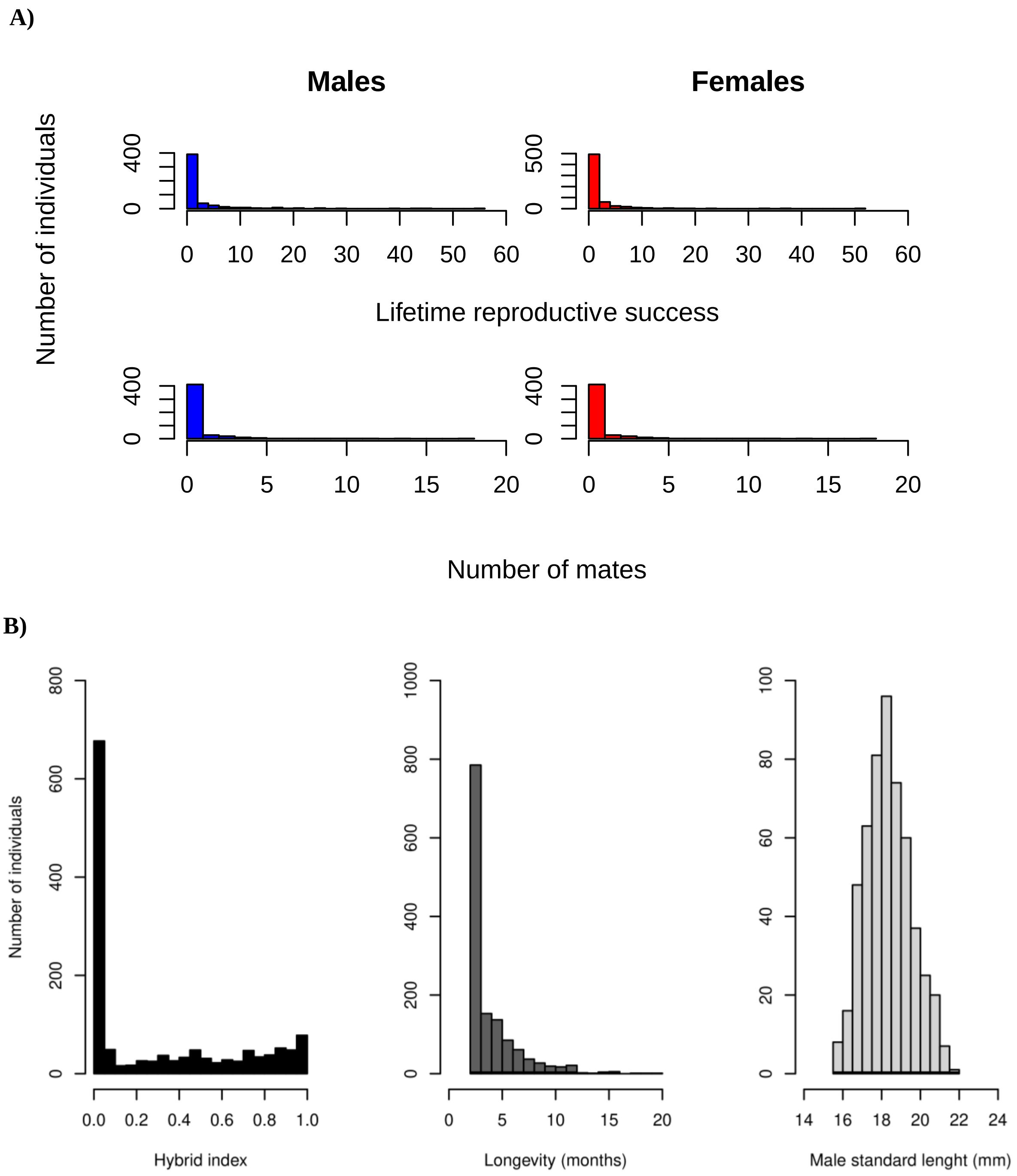

Figure S2.3. Distributions of variables included in models of fitness and dispersal. A) Lifetime reproductive success and number of mater for males and females. B) Hybrid index and longevity for all fish, and male standard length.

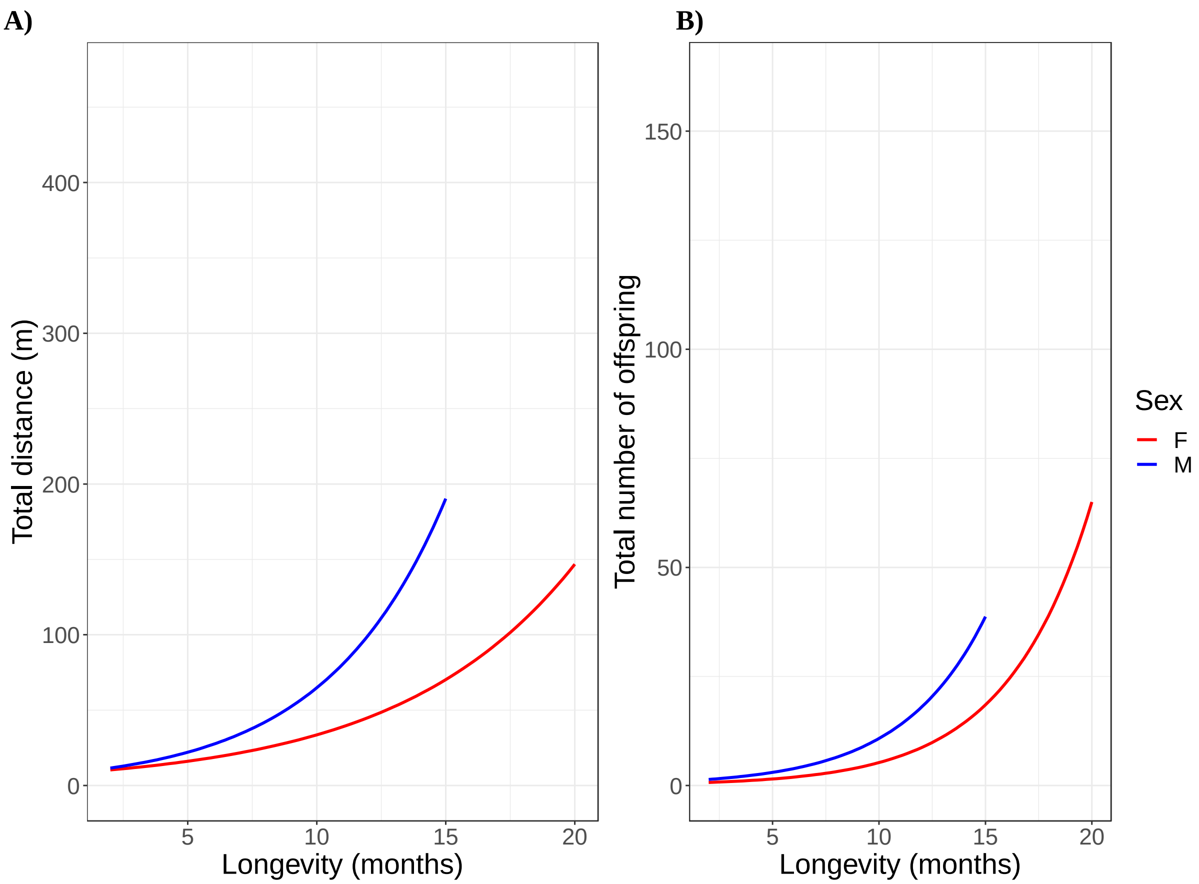

Figure S2.4. Effect of longevity on total dispersal distance (A) and lifetime reproductive success (B). Lines show negative binomial regression and shading as 95% confidence interval, colored by sex.

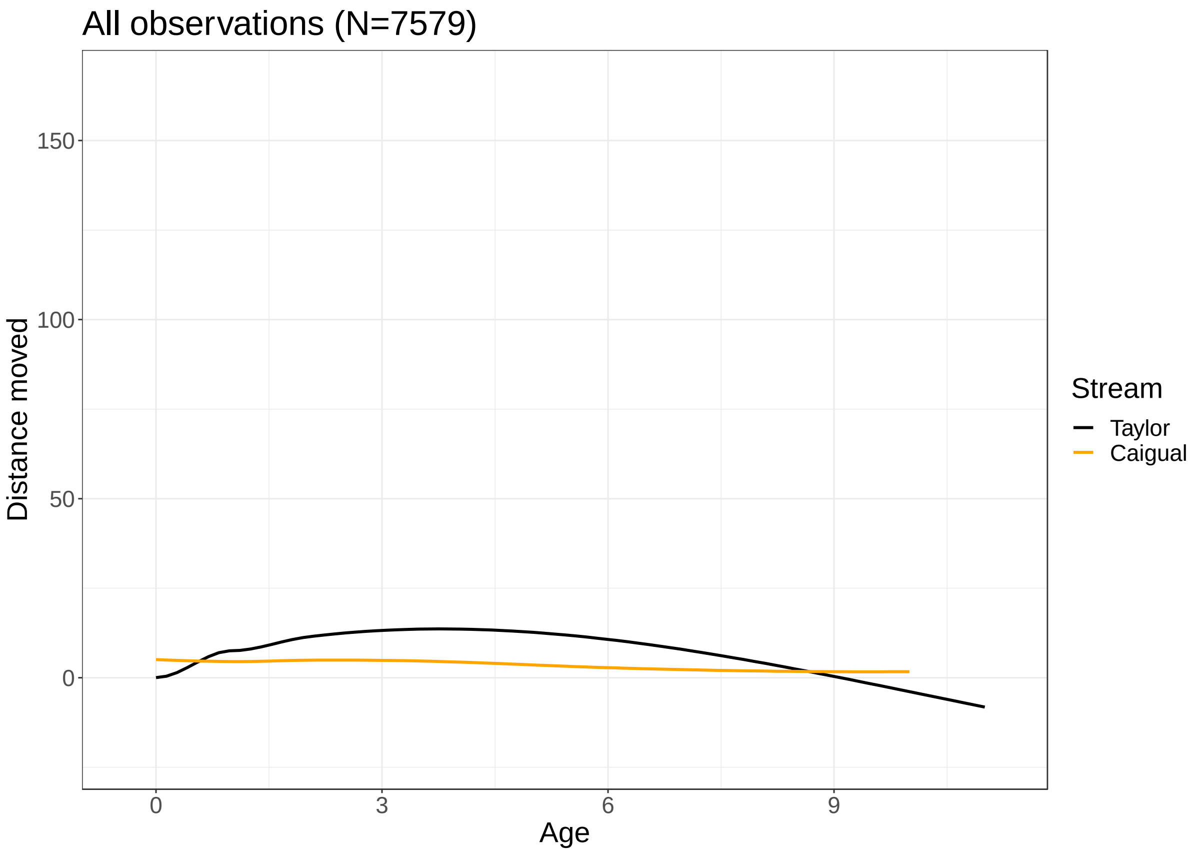

Figure S2.5. Effect of age (months since first capture) on monthly dispersal distance. Lines show loess fit with shading as 95% confidence intervals, colored by stream.

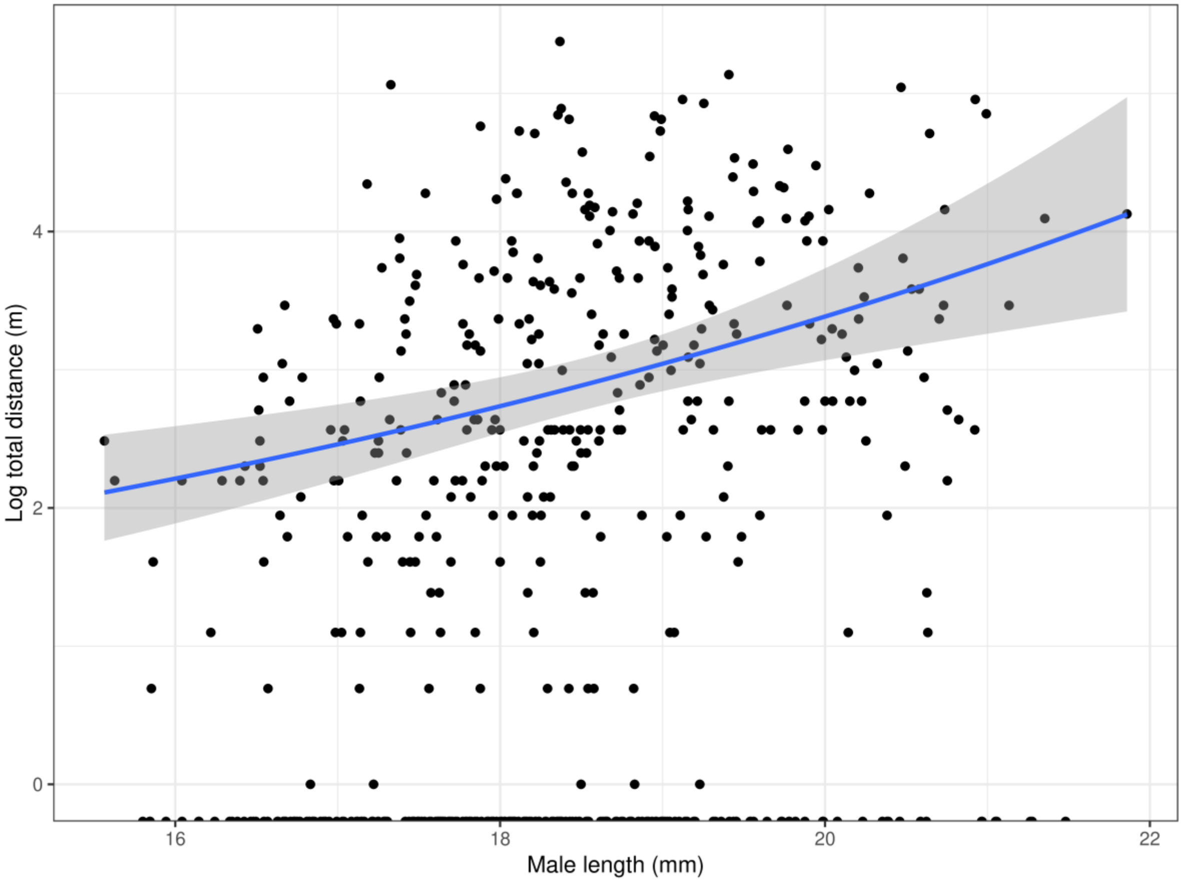

Figure S2.6. Effect of male standard length on total dispersal distance. Line shows negative binomial regression and shading as 95% confidence interval.

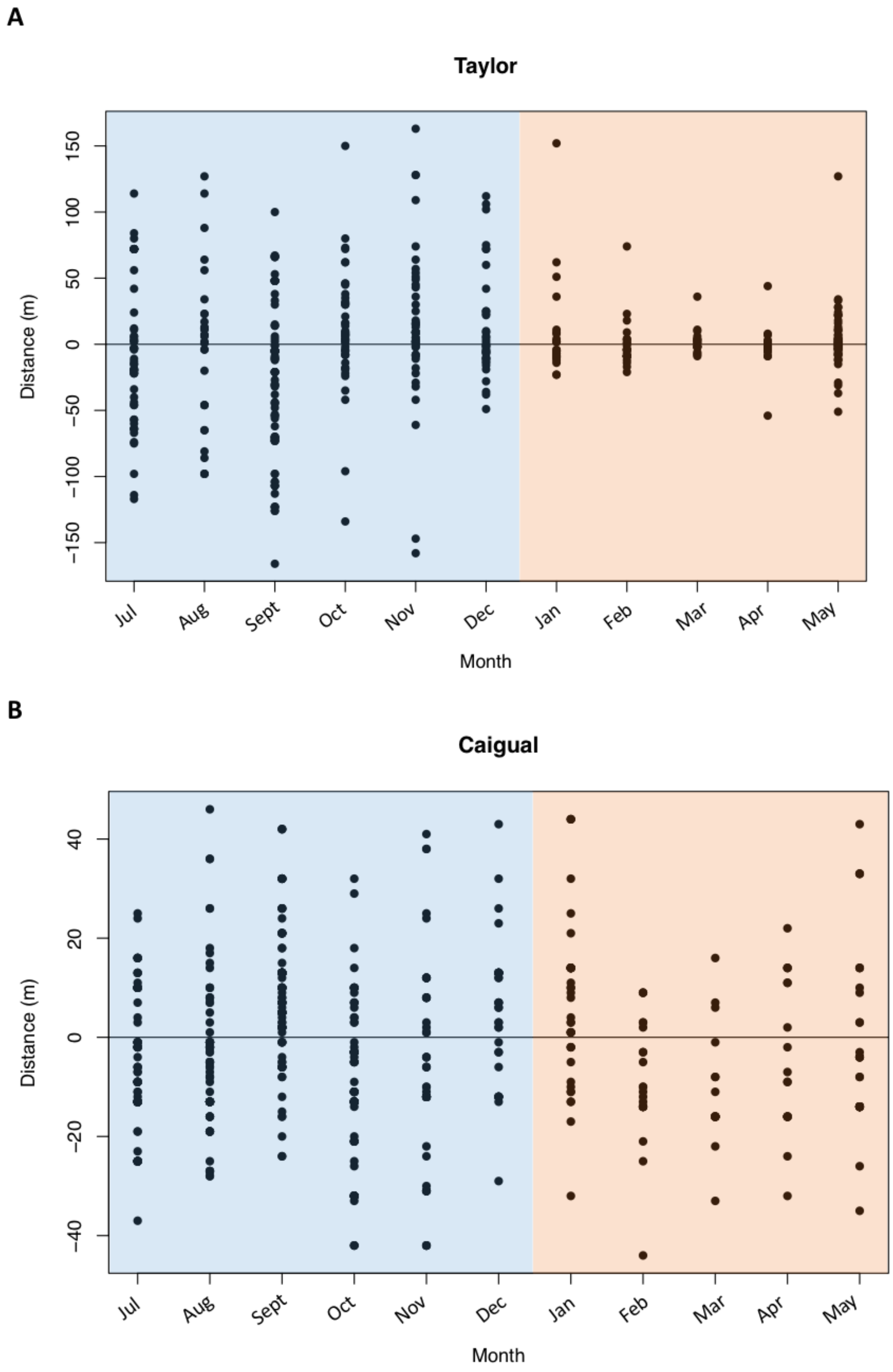

Figure S2.7. Monthly seasonal dispersal over the course of the capture-mark-recapture study, wet season in blue and dry season in orange. Each point represents an individual capture, with positive values representing upstream movement and negative values showing downstream movement. Fish in Taylor (A) had decreased movement in the dry season, while Caigual (B) showed uniform dispersal throughout the year.

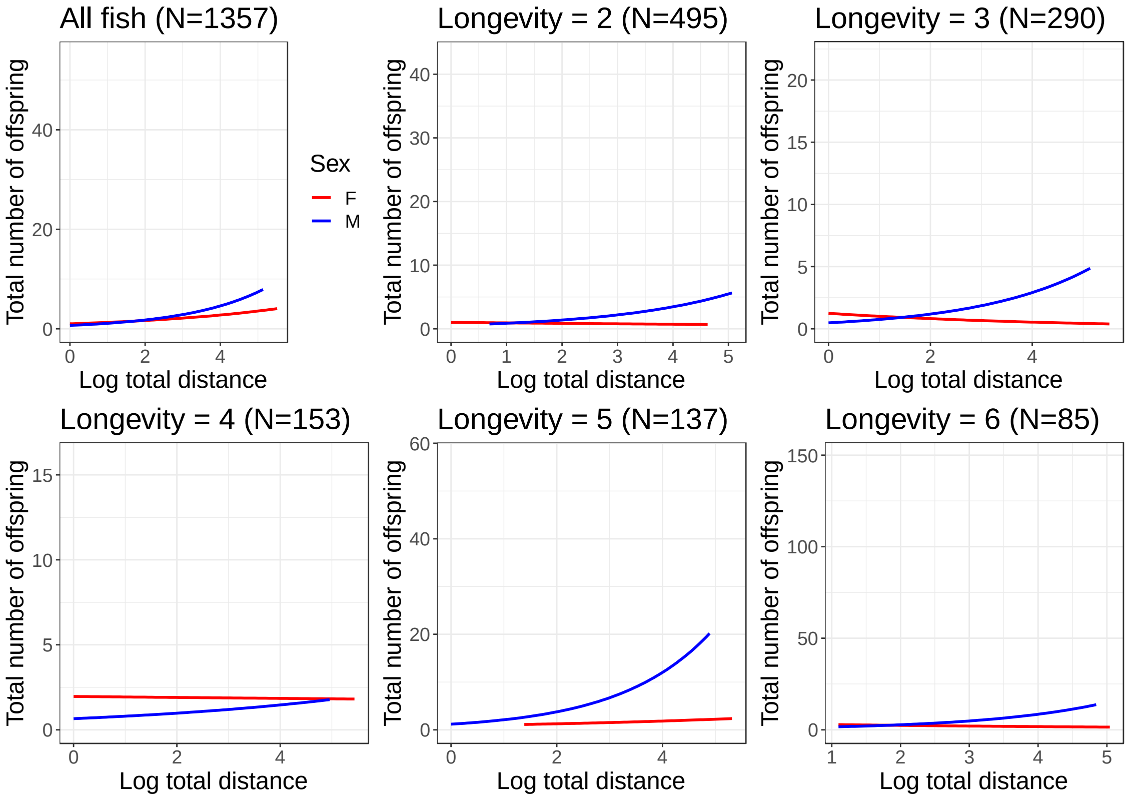

Figure S2.8. Effect of total dispersal distance on total number of offspring. Panels represent the same analyses on subsets of the data that include only individuals of the same longevity. Despite decreasing sample sizes with increasing longevity, trends are qualitatively similar. Line shows negative binomial regression and shading as 95% confidence interval, colored by sex.

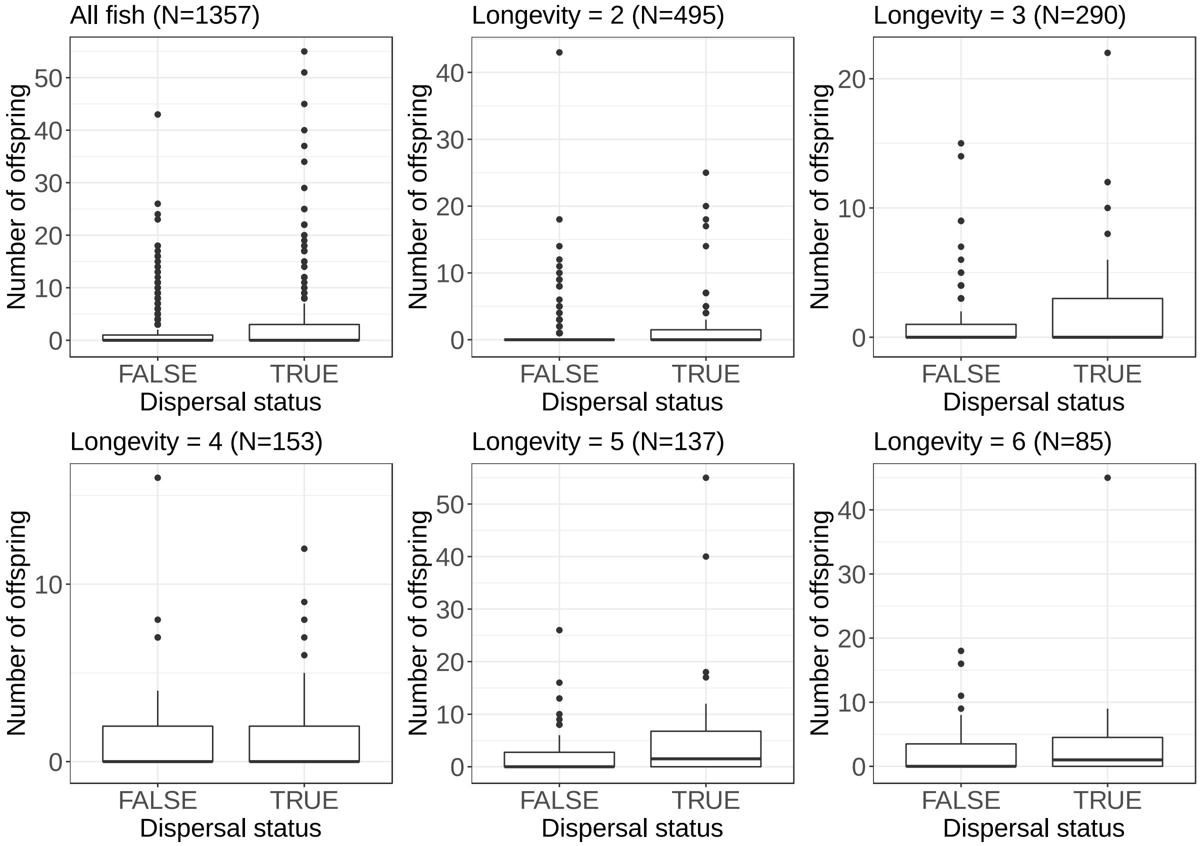

Figure S2.9. Effect of dispersal status (moving **≥ 10 m)** on total number of offspring. Dispersal status “FALSE” represents individuals that moved less than 10 m during the course of our study. Panels represent the same analyses on subsets of the data that include only individuals of the same longevity. Despite decreasing sample sizes with increasing longevity, trends are qualitatively similar.

Table S2.1: Summary of dispersing and non-dispersing individuals by stream and sex.

	Taylor			**Caigual**			**Both Streams**		
	Disperser	Non-Disperser	All	Disperser	Non-Disperser	All	Disperser	Non-Disperser	All
F	100 (27%)	276 (73%)	376	171 (45%)	206 (55%)	377	271 (36%)	482 (64%)	753
M	98 (32%)	210 (68%)	308	156 (53%)	140 (47%)	296	254 (42%)	350 (58%)	604
All	198 (29%)	486 (71%)	684	327 (49%)	346 (51%)	673	525 (39%)	832 (61%)	1357

Table S2.2. Model output for monthly dispersal distance in response to age.

All fish:

```
Call:  glmmTMB.all.age.distance <- glmmTMB(min_dist ~ Age + I(Age^2) + Sex + Stream +
(1|FishID) + (1|Capture_event), ziformula = ~ Age + I(Age^2) + Sex + Stream + (1|FishID) +
(1|Capture_event), family=nbinom2)

    AIC       BIC        logLik     deviance   df.resid
 16055.7  16156.9     -8012.9     16025.7     6282

Count model coefficients:
                        Estimate              Std. Error        z value            Pr(>|z|)
(Intercept)             2.490851              0.145333          17.139           < 2e-16 ***
Age                     0.262037              0.049465          5.297            1.17e-07 ***
I(Age^2)                -0.018425             0.005784          -3.185           0.00145 **
SexM                    0.079378              0.062582          1.268            0.20466
StreamCaigual           -0.590060             0.068015          -8.675           < 2e-16 ***

Zero-inflation model coefficients:
(Intercept)             3.990996              0.334676          11.925           <2e-16 ***
Age                     -1.110323             0.065618          -16.921          <2e-16 ***
I(Age^2)                0.126267              0.009068          13.925           <2e-16 ***
SexM                    -0.864999             0.092355          -9.366           <2e-16 ***
StreamCaigual           -0.821609             0.098619          -8.331           <2e-16 ***
```

Table S2.3. Model output for dispersal status and dispersal distance. Results presented separately for status and distance, and for all fish vs. males only.

a) Dispersal status (moving ≥ 10 m):

All fish:

```
Call: glm(formula = disp.status ~ Sex.x + Stream * hindex + Longevity, family = "binomial")

Deviance Residuals:
    Min       1Q      Median      3Q      Max
-1.9845  -0.9666  -0.5946   1.1534   2.2671

Coefficients:
                        Estimate          Std. Error         z value          Pr(>|z|)
(Intercept)             -0.98609           0.15184           -6.494           8.35e-11 ***
Sex.xM                  0.54703            0.12362           4.425            9.64e-06 ***
StreamTaylor            -0.09329           0.16197           -0.576           0.56462
hindex                  -1.27910           0.44920           -2.848           0.00441 **
Longevity               0.18699            0.02390           7.825            5.06e-15 ***
StreamTaylor:hindex     -0.50577           0.51282           -0.986           0.32401

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 1811.1  on 1356  degrees of freedom
Residual deviance: 1627.1  on 1351  degrees of freedom
AIC = 1639.1
```

Males only:

```
Call:  glm(formula = disp.status ~ Stream * hindex + Longevity + Male_SL, family = "binomial")

Deviance Residuals:
    Min       1Q      Median      3Q      Max
-2.4790  -0.9492  -0.5145   1.0221   2.3136

Coefficients:
                        Estimate          Std. Error        z value          Pr(>|z|)
(Intercept)             -7.48376           1.57885          -4.740           2.14e-06 ***
StreamTaylor            -0.28026           0.28297          -0.990           0.322
hindex                  -0.11827           0.58155          -0.203           0.839
Longevity               0.31263            0.05385           5.805           6.42e-09 ***
Male_SL                 0.35313            0.08525           4.142           3.44e-05 ***
StreamTaylor:hindex     -1.14974           0.71108          -1.617           0.106

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 733.89  on 535  degrees of freedom
Residual deviance: 620.32  on 530  degrees of freedom
(68 observations deleted due to missingness)
AIC: 632.32
```

Table S2.3. (cont'd)

b) Dispersal distance:

All fish:

```
Call:  glmmTMB(total_dist ~ Sex.x + Stream*hindex + log(Longevity),
                       ziformula = ~ Sex.x + Stream*hindex + log(Longevity),
                       family=nbinom2,data=d)

   AIC       BIC     logLik   deviance   df.resid
  7896.1   7963.8  -3935.0   7870.1       1344

Count model coefficients:
                        Estimate          Std. Error       z value        Pr(>|z|)
(Intercept)             2.03783           0.12447          16.372         < 2e-16 ***
Sex.xM                  0.18125           0.07394           2.451         0.0142 *
StreamTaylor            1.13730           0.09713          11.709         < 2e-16 ***
hindex                  0.03830           0.27245           0.141         0.8882
log(Longevity)          0.68927           0.07130           9.667         < 2e-16 ***
StreamTaylor:hindex    -1.27645           0.30052          -4.247         2.16e-05 ***

Zero-inflation model coefficients:
(Intercept)             0.7509            0.1937            3.877         0.000106 ***
Sex.xM                 -0.5877            0.1239           -4.744         2.09e-06 ***
StreamTaylor            0.3969            0.1670            2.376         0.017499 *
hindex                  1.8705            0.4571            4.092         4.27e-05 ***
log(Longevity)         -0.9323            0.1195           -7.803         6.06e-15 ***
StreamTaylor:hindex    -1.0837            0.5058           -2.143         0.032147 *
```

Males only:

```
Call: glmmTMB(total_dist ~ Male_SL + Stream*hindex + log(Longevity),
                       ziformula = ~ Male_SL + Stream*hindex + log(Longevity),
                       family=nbinom2)

   AIC       BIC     logLik   deviance   df.resid
  3394.0   3449.7   -1684.0   3368.0      523

Count model coefficients:
                        Estimate          Std. Error       z value        Pr(>|z|)
(Intercept)            -2.7612            0.9054           -3.050         0.00229 **
Male_SL                 0.2633            0.0485            5.429         5.67e-08 ***
StreamTaylor            1.0921            0.1533            7.122         1.06e-12 ***
hindex                  0.4356            0.2988            1.458         0.14491
log(Longevity)          0.6945            0.1049            6.618         3.65e-11 ***
StreamTaylor:hindex    -1.4285            0.3581           -3.989         6.63e-05 ***

Zero-inflation model coefficients:
(Intercept)             2.89838           1.56897           1.847         0.0647 .
Male_SL                -0.12482           0.08528          -1.464         0.1433
StreamTaylor            0.63306           0.28889           2.191         0.0284 *
hindex                  1.08094           0.60279           1.793         0.0729 .
log(Longevity)         -1.33409           0.23015          -5.797         6.77e-09 ***
StreamTaylor:hindex    -0.76240           0.70513          -1.081         0.2796
```

Table S2.4. Model output for lifetime reproductive success (LRS). Results presented separately for status and distance, and for males and females.

a) Dispersal status (moving ≥ 10 m):

Male LRS by dispersal status:

```
Call: glmmTMB(LRS ~ disp.status + Stream*hindex + Longevity + Male_SL,
    ziformula = ~ disp.status + Stream*hindex + Longevity + Male_SL, family=nbinom2)

  AIC     BIC      logLik     deviance    df.resid
 1409.5  1470.9   -689.8      1379.5      428

Count model coefficients:
                      Estimate        Std. Error    z value      Pr(>|z|)
(Intercept)           -1.01081        1.24334       -0.813       0.41623
disp.statusTRUE       0.29813         0.17608       1.693        0.09042 .
StreamTaylor          0.76258         0.30297       2.517        0.01183 *
hindex                3.13134         0.46996       6.663        2.68e-11 ***
Longevity             0.10351         0.03638       2.846        0.00443 **
Male_SL               0.05261         0.06767       0.777        0.43690
StreamTaylor:hindex   -2.53136        0.56414       -4.487       7.22e-06 ***

Zero-inflation model coefficients:
(Intercept)           6.93705         2.10281       3.299        0.00097 ***
disp.statusTRUE       -0.81742        0.28415       -2.877       0.00402 **
StreamTaylor          -0.40665        0.42960       -0.947       0.34385
hindex                -1.56677        0.77881       -2.012       0.04425 *
Longevity             -0.16920        0.07276       -2.325       0.02005 *
Male_SL               -0.28269        0.11392       -2.481       0.01308 *
StreamTaylor:hindex   0.72034         0.91030       0.791        0.42875
```

Female LRS by dispersal status:

```
Call: glmmTMB(LRS ~ disp.status + Stream*hindex + Longevity,
     ziformula = ~ disp.status + Stream*hindex + Longevity, family=nbinom2)

  AIC     BIC      logLik     deviance    df.resid
 1675.1  1732.7   -824.5      1649.1      610

Count model coefficients:
                      Estimate        Std. Error    z value      Pr(>|z|)
(Intercept)           -0.20817        0.19590       -1.063       0.287941
disp.statusTRUE       0.25069         0.13447       1.864        0.062285 .
StreamTaylor          0.93491         0.20469       4.567        4.94e-06 ***
hindex                1.85030         0.55458       3.336        0.000849 ***
Longevity             0.12263         0.01868       6.565        5.21e-11 ***
StreamTaylor:hindex   -1.63847        0.59084       -2.773       0.005552 **

Zero-inflation model coefficients:
(Intercept)            2.11033        0.33952       6.216        5.11e-10 ***
disp.statusTRUE       -0.36899        0.24166       -1.527       0.1268
StreamTaylor          -0.30166        0.31628       -0.954       0.3402
hindex                -1.58791        0.93383       -1.700       0.0891 .
Longevity             -0.24491        0.04682       -5.230       1.69e-07 ***
StreamTaylor:hindex   0.73478         0.98627       0.745        0.4563
```

Table S2.4. (cont'd)

b) Dispersal distance:

Male LRS by dispersal distance:

Call: glmmTMB(LRS ~ total_dist + Stream*hindex + Longevity + Male_SL,
ziformula = ~ total_dist + Stream*hindex + Longevity + Male_SL, family=nbinom2)

AIC	BIC	logLik	deviance	df.resid
1409.5	1470.9	-689.8	1379.5	428

Count model coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.088168	1.319158	-0.825	0.40943
total_dist	0.002548	0.002542	1.002	0.31619
StreamTaylor	0.674386	0.305067	2.211	**0.02706 ***
hindex	3.149473	0.477491	6.596	**4.23e-11 *****
Longevity	0.106751	0.037071	2.880	**0.00398 ****
Male_SL	0.061621	0.070457	0.875	0.38179
StreamTaylor:hindex	-2.480057	0.568054	-4.366	**1.27e-05 *****

Zero-inflation model coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	6.056602	2.145451	2.823	**0.00476 ****
total_dist	-0.017379	0.007748	-2.243	**0.02490 ***
StreamTaylor	-0.193640	0.447262	-0.433	0.66505
hindex	-1.590957	0.777393	-2.047	**0.04070 ***
Longevity	-0.170592	0.073341	-2.326	**0.02002 ***
Male_SL	-0.243500	0.115808	-2.103	**0.03550 ***
StreamTaylor:hindex	0.725659	0.914829	0.793	0.42765

Female LRS by dispersal distance:

Call: glmmTMB(LRS ~ total_dist + Stream*hindex + Longevity,
ziformula = ~ total_dist + Stream*hindex + Longevity, family=nbinom2)

AIC	BIC	logLik	deviance	df.resid
1676.6	1734.3	-825.3	1650.6	610

Count model coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.107259	0.191226	-0.561	0.574865
total_dist	0.003079	0.001669	1.845	0.064965 .
StreamTaylor	0.872491	0.207320	4.208	**2.57e-05 *****
hindex	1.785193	0.546174	3.269	**0.001081 ****
Longevity	0.120411	0.018863	6.384	**1.73e-10 *****
StreamTaylor:hindex	-1.586753	0.584534	-2.715	**0.006636 ****

Zero-inflation model coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.998890	0.324641	6.157	**7.40e-10 *****
total_dist	-0.003880	0.003224	-1.204	0.229
StreamTaylor	-0.214694	0.321891	-0.667	0.505
hindex	-1.388891	0.909127	-1.528	0.127
Longevity	-0.247760	0.047679	-5.196	**2.03e-07 *****
StreamTaylor:hindex	0.553283	0.973838	0.568	0.570

Table S2.5. Model output for number of mates as a response to net range. Results presented separately for males and females.

Mates by range for males:

```
Call: glmmTMB(NumMates ~ net_range + Stream*hindex + Longevity,
          ziformula = ~ net_range + Stream*hindex + Longevity, family=nbinom2)

   AIC     BIC       logLik      deviance     df.resid
 1009.3  1063.8     -491.7       983.3        474

Count model coefficients:
                       Estimate          Std. Error          z value          Pr(>|z|)
(Intercept)            -1.860020         0.423770            -4.389           1.14e-05 ***
net_range              0.007493          0.002721            2.754            0.005881 **
StreamTaylor           0.397865          0.398920            0.997            0.318591
hindex                 4.004606          0.556801            7.192            6.38e-13 ***
Longevity              0.142510          0.038921            3.661            0.000251 ***
StreamTaylor:hindex    -2.715788         0.659690            -4.117           3.84e-05 ***

Zero-inflation model coefficients:
(Intercept)            0.93879           1.00815             0.931            0.3518
net_range              -0.06256          0.06451             -0.970           0.3321
StreamTaylor           -0.17207          1.09044             -0.158           0.8746
hindex                 0.31275           1.68424             0.186            0.8527
Longevity              -0.34331          0.20066             -1.711           0.0871 .
StreamTaylor:hindex    -0.40510          1.90798             -0.212           0.8319
```

Mates by range for females:

```
Call: glmmTMB(NumMates ~ net_range + Stream*hindex + Longevity,
           ziformula = ~ net_range + Stream*hindex + Longevity, family=nbinom2)

   AIC     BIC       logLik      deviance     df.resid
  1164.8 1222.1    -569.4       1138.8        597

Count model coefficients:
                       Estimate          Std. Error          z value          Pr(>|z|)
(Intercept)            -1.343364         0.246658            -5.446           5.14e-08 ***
net_range              0.005223          0.002887            1.809            0.070442 .
StreamTaylor           0.899758          0.240047            3.748            0.000178 ***
hindex                 3.127215          0.610870            5.119            3.07e-07 ***
Longevity              0.094350          0.022458            4.201            2.65e-05 ***
StreamTaylor:hindex    -3.043065         0.662271            -4.595           4.33e-06 ***

Zero-inflation model coefficients:
(Intercept)            3.420125          0.983719            3.477            0.000508 ***
net_range              0.011949          0.009765            1.224            0.221056
StreamTaylor           0.642681          0.753834            0.853            0.393909
hindex                 3.083049          2.130647            1.447            0.147897
Longevity              -1.364230         0.455567            -2.995           0.002748 **
StreamTaylor:hindex    -4.491290         2.325537            -1.931           0.053447 .

Theta = 2.3921
Number of iterations in BFGS optimization: 71; Log-likelihood: -569.4 on 13 Df
```

Table S2.6. Output from models of total distance and upstream and downstream distances in response to season.

a) Generalized linear mixed models of distance moved in each capture occasion, with individual and month as random effects. Analyses done separately for each stream.

Taylor

```
Family: nbinom2  ( log )
Formula:  min_dist ~ Season + (1 | FishID_nodash) + (1 | month)


Conditional model:
Groups          Name           Variance     Std.Dev.
FishID_nodash   (Intercept)    0.41736      0.6460
month           (Intercept)    0.03679      0.1918

Number of obs: 469, groups:  FishID_nodash, 334; month, 11
Overdispersion parameter for nbinom2 family (): 1.45


Conditional model:
                Estimate       Std. Error   z value      Pr(>|z|)
(Intercept)     2.3071         0.1358       16.993       < 2e-16 ***
SeasonW         1.0994         0.1692       6.499        8.07e-11 ***


Log-likelihood: -2006.9; AIC = 4023.9
```

Caigual

```
Family: nbinom2  ( log )
Formula:  min_dist ~ Season + (1 | FishID_nodash) + (1 | month)


Conditional model:
Groups          Name           Variance     Std.Dev.
FishID_nodash   (Intercept)    0.145766     0.38179
Month           (Intercept)    0.006882     0.08296

Number of obs: 651, groups: FishID_nodash, 376; month, 12
Overdispersion parameter for nbinom2 family (): 3.24


Conditional model:
                Estimate       Std. Error   z value      Pr(>|z|)
(Intercept)     2.53176        0.07835      32.31        <2e-16 ***
SeasonW         -0.03655       0.09012      -0.41        0.685


Log-likelihood: -2265.6; AIC = 4541.1
```

Table S2.6. (cont’d)

b) Negative binomial models of upstream and downstream movement in response to season. Analyzes done separately for each direction in each stream.

Taylor upstream

Call: glm.nb(formula = upstream ~ Season, data = t.disp, init.theta = 0.8605303544, link = log)

Null deviance: 282.05 on 227 degrees of freedom

Residual deviance: 259.48 on 226 degrees of freedom

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.7874	0.1351	20.626	**< 2e-16 *****
SeasonW	0.8066	0.1602	5.036	**4.75e-07 *****

2 x log-likelihood: -1992.1410

AIC = 1998.1

Taylor downstream

Call: glm.nb(formula = downstream * -1 ~ Season, data = t.disp, init.theta = 1.126641728, link = log)

Null deviance: 373.10 on 241 degrees of freedom

Residual deviance: 266.47 on 240 degrees of freedom

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.1959	0.1110	19.77	**<2e-16 *****
SeasonW	1.5235	0.1341	11.36	**<2e-16 *****

2 x log-likelihood: -2048.1020

AIC = 2054.1

Table S2.6. (cont'd)

b) Negative binomial models of upstream and downstream movement in response to season. Analyzes done separately for each direction in each stream.

Caigual upstream

Call: glm.nb(formula = upstream ~ Season, data = c.disp, init.theta = 2.184139022, link = log)

Null deviance: 278.02 on 261 degrees of freedom

Residual deviance: 277.53 on 260 degrees of freedom

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.64309	0.09990	26.456	**<2e-16 *****
SeasonW	-0.07814	0.11198	-0.698	0.485

2 x log-likelihood: -1837.824

AIC = 1843.8

Caigual downstream

Call: glm.nb(formula = downstream * -1 ~ Season, data = c.disp, init.theta = 2.163640678, link = log)

Null deviance: 419.89 on 388 degrees of freedom

Residual deviance: 419.82 on 387 degrees of freedom

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	2.59027	0.08299	31.213	**<2e-16 *****
SeasonW	-0.02581	0.09285	-0.278	0.781

2 x log-likelihood: -2725.339

AIC = 2731.3

APPENDIX B:

Supporting information: Rollin' on the river: Local census size, sex ratio, and relatedness drive dispersal behavior in the Trinidadian guppy (*Poecilia reticulata*)

Supplemental Tables:

Table S3.1. Model output for best model of dispersal probability in response to census size in Caigual.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.78	0.14 – 4.24	0.773
census scaled	0.15	0.07 – 0.32	**<0.001**
Season [W]	3.51	0.58 – 21.24	0.172
Sex [M]	0.54	0.40 – 0.72	**<0.001**
Weight	0.08	0.03 – 0.19	**<0.001**
census scaled * Season [W]	2.83	1.28 – 6.26	**0.010**
Random Effects			
σ^2	3.29		
$\tau_{00\ \mathrm{FishID}}$	0.63		
$\tau_{00\ \mathrm{Month}}$	0.67		
ICC	0.28		
N_{Month}	7		
N_{FishID}	823		
Observations	2367		
Marginal R^2 / Conditional R^2	0.170 / 0.404		

Table S3.2. Model output for best model of dispersal probability in response to sex ratio in Caigual.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.71	0.13 – 3.84	0.688
sex ratio	99.75	29.86 – 333.28	**<0.001**
Sex [M]	0.64	0.34 – 1.18	0.152
Season [W]	1.56	0.27 – 9.15	0.623
Weight	0.03	0.01 – 0.07	**<0.001**
sex ratio * Sex [M]	0.17	0.03 – 0.88	**0.035**
Random Effects			
σ^2	3.29		
$\tau_{00\ \mathrm{FishID}}$	1.08		
$\tau_{00\ \mathrm{Month}}$	0.67		
ICC	0.35		
N_{Month}	7		
N_{FishID}	817		
Observations	2336		
Marginal R^2 / Conditional R^2	0.110 / 0.419		

Table S3.3. Model output for best model of dispersal probability in response to relatedness in Caigual.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	1.28	0.16 – 10.11	0.814
relatedness jaccard	8404.60	63.56 – 1111356.20	**<0.001**
relatedness jaccard^2	0.00	0.00 – 0.00	**<0.001**
Season [W]	1.96	0.24 – 16.19	0.531
Sex [M]	0.49	0.35 – 0.70	**<0.001**
Weight	0.02	0.01 – 0.04	**<0.001**
Random Effects			
σ^2	3.29		
$\tau_{00\ \mathrm{FishID}}$	1.52		
$\tau_{00\ \mathrm{Month}}$	0.96		
ICC	0.43		
N_{Month}	7		
N_{FishID}	807		
Observations	2313		
Marginal R^2 / Conditional R^2	0.075 / 0.474		

Table S3.4. Model output for best model of dispersal probability in response to census size in Taylor.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.22	0.07 – 0.69	**0.009**
Season [W]	2.08	0.69 – 6.32	0.196
census scaled	0.66	0.49 – 0.89	**0.006**
Sex [M]	1.35	0.76 – 2.38	0.308
Weight	0.53	0.04 – 7.04	0.630
Random Effects			
σ^2	3.29		
$\tau_{00\ \mathrm{FishID\_nodash}}$	0.00		
$\tau_{00\ \mathrm{Month}}$	0.65		
$N_{\mathrm{FishID\_nodash}}$	331		
N_{Month}	11		
Observations	460		
Marginal R^2 / Conditional R^2	0.064 / NA		

Table S3.5. Model output for best model of dispersal probability in response to sex ratio in Taylor.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.10	0.02 – 0.59	**0.011**
Season [W]	1.83	0.45 – 7.39	0.394
sex ratio	1.43	0.23 – 8.91	0.704
Sex [M]	1.29	0.28 – 5.93	0.740
Weight	4.93	0.19 – 129.81	0.339
sex ratio * Sex [M]	1.49	0.13 – 16.42	0.746
Random Effects			
σ^2	3.29		
$\tau_{00\ \mathrm{FishID\_nodash}}$	0.00		
$\tau_{00\ \mathrm{Month}}$	0.96		
ICC	0.23		
N_{Month}	11		
$N_{\mathrm{FishID\_nodash}}$	267		
Observations	335		
Marginal R^2 / Conditional R^2	0.027 / 0.247		

Table S3.6. Model output for best model of dispersal probability in response to relatedness in Taylor.

	moved in next step		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.14	0.02 – 0.82	**0.030**
Season [W]	3.19	0.53 – 19.30	0.207
relatedness jaccard	0.69	0.00 – 1201.26	0.922
relatedness jaccard^2	0.00	0.00 – 328.02	0.220
Sex [M]	1.18	0.52 – 2.71	0.691
Weight	3.23	0.10 – 103.22	0.507
Random Effects			
σ^2	3.29		
$\tau_{00\ \text{FishID\_nodash}}$	0.00		
$\tau_{00\ \text{Month}}$	1.66		
ICC	0.33		
N_{Month}	11		
$N_{\text{FishID\_nodash}}$	250		
Observations	314		
Marginal R^2 / Conditional R^2	0.112 / 0.410		

APPENDIX C:

Supporting information: Mutualism benefits are lost at high inbreeding

Supplemental Methods

Crosses:

To obtain seeds for this experiment, we manipulated two generations of plant crosses for *C. fasciculata* individuals collected from two natural populations in the US Midwest (Westland, MI, and Sandusky, OH). The Ohio population is a large population located at the Erie Sand Barrens Preserve, while the Michigan population was a small marginal road-side population. Maternal seeds used to initiate crosses for this experiment were the result of several generations of greenhouse crossings of field-collected seeds. Four maternal families from each population were used to generate all crosses described below. Our first generation of crosses was organized into four different cross types: between the two populations, between maternal lines within the same population, within maternal lines, and selfed (Figure 4.2). Flowers of *C. fasciculata* are buzz pollinated and open for approximately one day, with plants flowering for approximately 4 months in the greenhouse environment. Germination rate for the parental generation was low, with only one third (101 out of 303) of individual seeds surviving to the seedling stage. During their flowering period, plants were checked for flowers daily, and every open flower was hand-pollinated using a vibrating device and a toothpick to obtain pollen and place it on anthers, respectively. We attempted to maximize cross variety by randomly choosing pollen donor and recipients from the available open flowers each day, and conducting reciprocal crosses for each plant pair that produced more than one flower on any given day. Given the lower fecundity of selfed crosses, we also attempted to self-pollinate at least one flower per plant per flowering day. Because *C. fasciculata* has explosive seed dispersal, all maturing seed pods were bagged with mesh bags to prevent seed loss, and seeds were collected once pods had opened within bags. We obtained over 300 F1 seeds from the initial parental plants.

F1 seeds were then germinated in the greenhouse in the following summer (2020). 249 F1 individuals germinated, with 146 (58%) surviving to flowering. We applied the same protocol of crosses above for this generation: once plants started flowering we checked them for flowers daily and pollinated all open flowers with randomly chosen individuals that also had open flowers on that date, and selfed all plants each day when possible. We obtained a total of 3423 seeds from the F1 crosses, which varied from twice-selfed to outbred. These F2 seeds were then used in the common garden experiment described below.

Germination:

The germination protocol was the same for plants in the parental, F1, and F2 generations. All *C. fasciculata* seeds were geminated by first being submerged in a solution of 10% bleach for one minute, then scarified with a sterile razor blade and put in petri dish with deionized water. Petri dishes contained up to 12 scarified seeds, always from the same seed pod (assumed full siblings), and were placed on a bench in a greenhouse on a 90-75F, 16 light-8 dark cycle. Every day for 15 days, seeds were checked for cotyledon and radicle development. Individuals were planted once cotyledons were green and radicles were longer than 0.5 cm, into 2.5 x 10 inch “cone-tainers” (Stuewe & Sons, Inc.) filled with Premier Pro-Mix FPX 2.8cf soil from BFG Supply Co. Seeds that did not sprout cotyledons or radicles within 15 days were discarded and counted as failed germinants. Parental and F1 generations were kept in the greenhouse for the duration of their lifecycle. The experimental F2 plants remained in the greenhouse only until rhizobia inoculations were completed, then were moved to an outdoor common garden.

Inoculations:

For the common garden experiment, we attempted to geminate 3209 seeds, resulting in 2571 live seedlings following the germination protocol described above. Out of those experimental plants, 1715 *C. fasciculata* F2 individuals of all crossing backgrounds were inoculated with *Bradyrhizobium sp.*, while 856 plants did not receive any rhizobia. We used two rhizobia strains which were obtained from previous collections of naturally occurring *C. fasciculata* at the Kellogg Biological Station (KBS, Hickory Corners, MI), which did not differ in their ability to nodulate with experimental plants or the number of nodules they produced (Figure S4.1). The strains were collected in 2017 from nodules in *C. fasciculata* grown at the Kellogg Biological Station, such that both experimental plant populations should be naïve to the strains used in the common garden. The strains had been maintained at -80C until 2021, and were revived first on solid tryptone yeast media. They were incubated on that solid media for 2 days at 30ºC degrees, then re-plated on fresh solid media and incubated again for 2 days at 30ºC degrees. The isolates were then transferred to a liquid modified arabinose gluconate media where they were incubated at 30ºC degrees on a MaxQ 4000 Orbital Shaker (Thermo Scientific). On the 3rd day of growth on liquid media, we measured optical density of each culture with a spectrophotometer set to OD600 (Eppendorf BioPhotometer plus) and diluted all cultures to the concentration of the least concentrated sample. We then added 2ml of liquid culture to each plant, with control plants receiving the same volume of sterile liquid arabinose gluconate media that had been incubated for the same amount of time.

We conducted a pilot experiment using these inoculation protocols, rhizobia strains, F2 seeds, and soil mixture in the greenhouse in January of 2021 to confirm that the strains would produce nodules on these plants. In this pilot, we also tested for the effects of autoclaving soil on rhizobia contamination to inform the design of our main experiment. This pilot confirmed that our rhizobia strains did generally nodulate in our F2 plants. Out of 137 pilot plants in non-autoclaved soil, half of which were inoculated with rhizobia, all inoculated plants formed nodules and only one uninoculated plant was contaminated (produced one nodule). Based on that pilot, we decided to not autoclave soil for our common garden experiment, as that would alter nutrient content and availability for plants (Alphei & Scheu 1993; Endlweber & Scheu 2006).

Given the large number of experimental plants, inoculations were conducted with the methods described above on two separate dates, June 14th for individuals that were planted between May 1st and May 28th, and June 21st for individuals planted between May 29th and June 13th. We organized experimental plants into racks so that individuals with the same inoculation treatment were placed together, to minimize contamination between pots of different treatments while plants were watered in the greenhouse. Plants were later reshuffled when moved to the field. We also measured the height of each plant at the time of inoculation. We included planting date on all statistical models of mutualism and fitness traits described below.

Common garden:

All experimental (F2) plants were germinated, planted, and inoculated in the greenhouse. On July 1st, all individuals were moved to a fenced outdoor common garden at the Kellogg Biological Station in Hickory Corners, MI, USA. Outdoors, plants were organized into 18 blocks, each containing 9 racks. Each rack contained 12 plants of the same rhizobia treatment, and each block contained 3 racks of each treatment. Every 3 weeks, all racks were rotated to minimize microenvironmental effects of block placement. The common garden allowed for natural levels of insect herbivory and pollination, while preventing mammalian herbivory. Given a history of vole infestations in our field site, we also used deterrents (Solar Powered Mole

Groundhog Repellent Stakes Sonic Gopher Chaser Vole Deterrent and Shake Away 5006258 Fox Urine Granules) in addition to the fencing to avoid destructive rodent herbivory. Six sonic repellent stakes were placed in regular intervals along a corridor between blocks of experimental plants and left running for the duration of the experiment, while the urine granules were applied monthly around the perimeter of the experiment, approximately 3m away from the edge of the blocks. We did not observe any herbivory from rodents, deer, or other mammals in our experiment.

We watered plants daily to avoid desiccation in the common garden, except on days with natural precipitation. We scored the day of first flower for each individual, and height at flowering on that date. To study the plant-ant mutualism, 58 individuals of *C. fasciculata* that spanned our inbreeding gradient were selected to undergo weekly ant surveys. For the month after plants were placed outside, we conducted two surveys of the number of extra-floral nectaries (EFN) on each of those 58 individuals and quantified insect herbivory as the percentage of plant leaves with any herbivore damage. We also conducted seven ant surveys on those plants, were we counted the number of ants on each plant, then calculated an average number of ants per survey per plant. Once plants began to set seed (late August), we manually collected seed pods daily before seed pods dehisced and seeds were dispersed into the environment. Plants started being harvested in October, once they senesced and no longer had any green leaves or flowers, with harvest being completed in November. At time of harvest, we separated above and belowground biomass for weighting and counting rhizobia root nodules. Plant fitness was quantified as the total number of viable seeds produced throughout the growing season, as well as above and belowground biomass at senescence. Seed viability was visually estimated, any seeds were very thin, small, or hollow (indications that they did not have developed endosperm) were not counted or weighted. We also estimated the strength of plants' interactions with rhizobia by counting the number of nitrogen-fixing nodules on the roots of each plant at the end of the experiment.

Supplemental Figures:

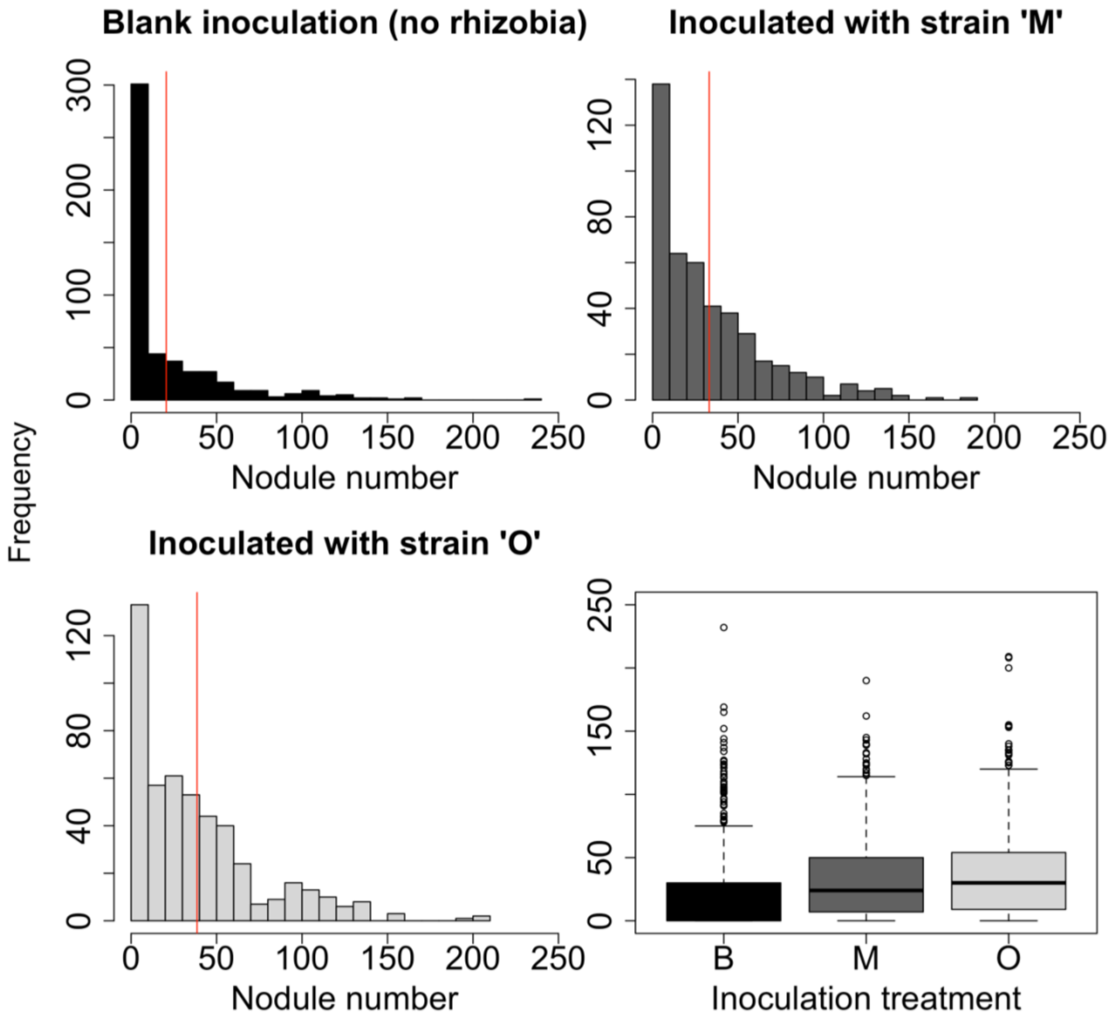

Figure S4.1. Variation in nodule number based on inoculation treatment, with red lines on histograms representing average nodule number for that treatment. Plants that did not receive rhizobia ("blank" treatment, or "B") had a majority of zero values, but some contamination made the average number of nodules non-zero. Plants inoculated with "M" and "O" strains produced a similar distribution of nodules. Therefore, all analyses in the main text do not consider strain variation, instead grouping "M" and "O" treatments into "inoculated with rhizobia," while the blank treatment plants are considered "not inoculated with rhizobia."

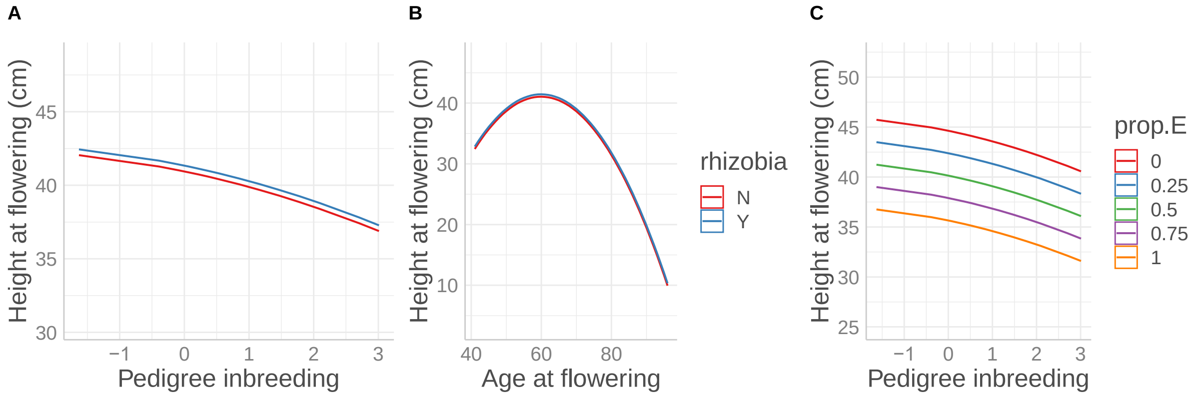

Figure S4.2. Predicted relationships based on best models. A) There was no effect of rhizobia inoculation on plant height at flowering, but height did decrease with increased plant inbreeding B) Plants that flowered at intermediate ages (in days) were taller. B) Plants with higher proportion of Ohio ancestry (higher values) were shorter.

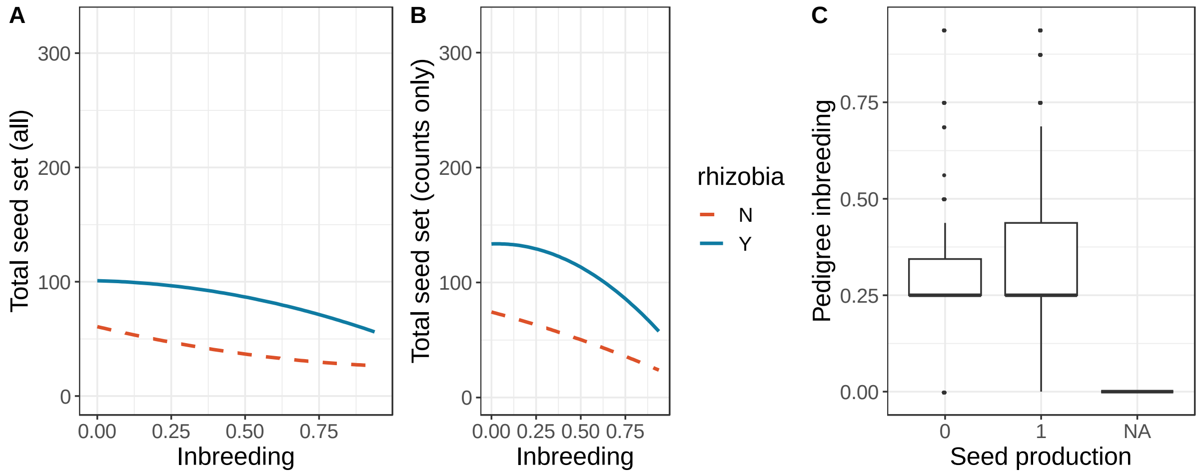

Figure S4.3. Visualization of actual data, corresponding to model results in Figure 4.5A in the main text. Different lines show different rhizobia inoculation treatments. A) Quadratic lines of best fit for complete dataset. B) Quadratic lines of best fit for only the subset plants that produced non-zero seeds. C) Boxplot of inbreeding values for plants that produced no seeds (“0”) vs. plants that produced at least 1 seed (“1”).

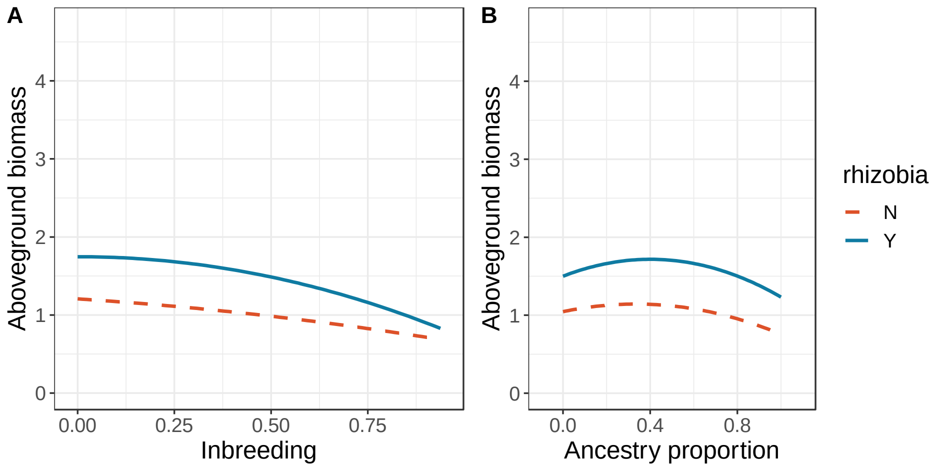

Figure S4.4. Visualization of actual data, corresponding to model results in Figure 4.5C and 4.5D in the main text. Different lines show different rhizobia inoculation treatments. A) Quadratic lines of best fit for inbreeding effects on aboveground biomass. B) Quadratic lines of best fit for ancestry effects on aboveground biomass.

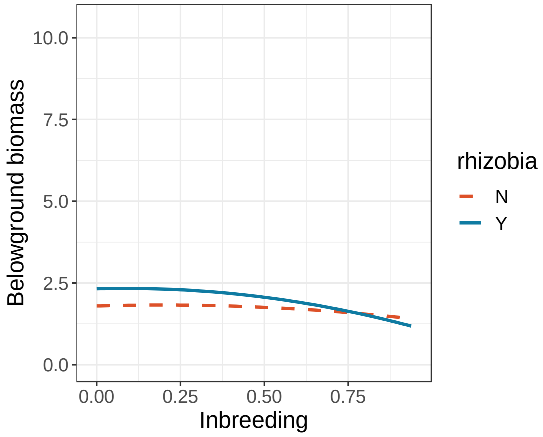

Figure S4.5. Visualization of actual data, corresponding to model results in Figure 4.6A. Quadratic lines of best fit for inbreeding effects on belowground biomass, different lines show different rhizobia inoculation treatments.

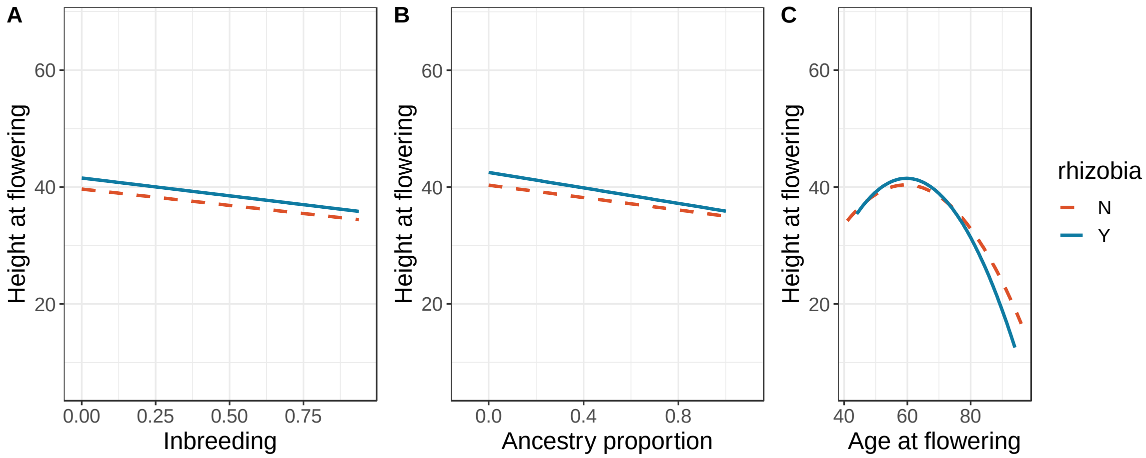

Figure S4.6. Visualization of actual data, corresponding to model results in Figure 4.6B. Different lines show different rhizobia inoculation treatments. A) Quadratic lines of best fit for inbreeding effects on height at flowering. B) Quadratic lines of best fit for ancestry effects on height. C) Quadratic lines of best fit for age effects on height.

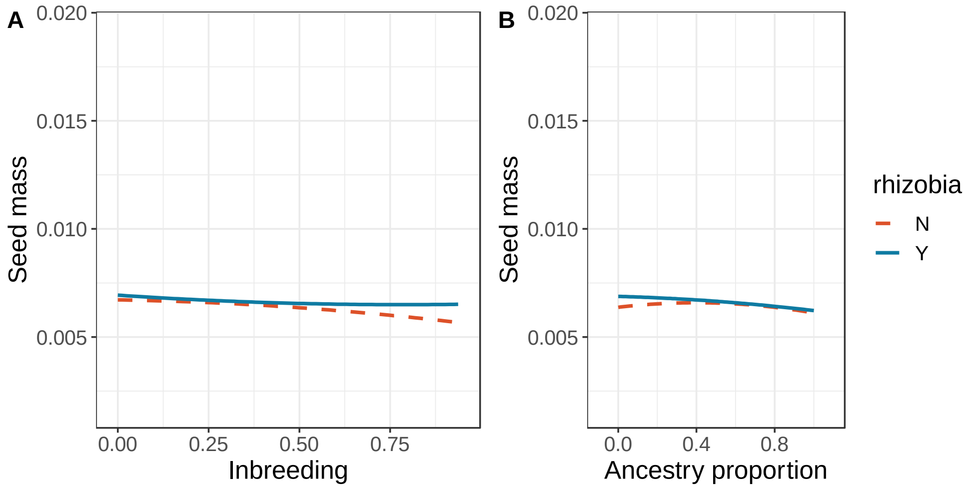

Figure S4.7. Visualization of actual data, corresponding to model results in Figure 4.6B. Different lines show different rhizobia inoculation treatments. A) Quadratic lines of best fit for inbreeding effects on individual seed mass. B) Quadratic lines of best fit for ancestry effects on individual seed mass.

Supplemental Tables:

Table S4.1. Model output for best model of total seed set.

	[illegible]		
Predictors	*Estimates*	*CI*	*p*
[illegible]			
(Intercept)	52.93	49.62 – 56.46	[illegible]
ped inbreeding c	0.84	0.80 – 0.88	[illegible]
rhizobia [Y]	2.15	2.03 – 2.27	[illegible]
prop E	1.27	1.16 – 1.39	[illegible]
ped inbreeding c^2	0.96	0.95 – 0.98	[illegible]
ped inbreeding c * rhizobia [Y]	1.09	1.03 – 1.16	[illegible]
(Intercept)	720.03		
[illegible]			
(Intercept)	0.21	0.17 – 0.25	[illegible]
prop E	2.36	1.62 – 3.45	[illegible]
ped inbreeding c	0.93	0.84 – 1.03	0.161
Observations	2566		

Table S4.2. Model output for best model of total seed set, including only individuals for which we conducted ant surveys.

	[illegible]		
Predictors	*Estimates*	*CI*	*p*
[illegible]			
(Intercept)	69.59	52.59 – 92.08	[illegible]
T ant	1.21	1.04 – 1.40	[illegible]
ped inbreeding c	0.85	0.73 – 0.99	[illegible]
rhizobia [Y]	1.47	1.18 – 1.84	[illegible]
prop E	1.49	1.06 – 2.12	[illegible]
ped inbreeding c^2	0.96	0.90 – 1.02	0.204
ped inbreeding c * rhizobia [Y]	1.24	1.03 – 1.49	[illegible]
(Intercept)	68.96		
[illegible]			
(Intercept)	0.05	0.01 – 0.30	[illegible]
prop E	1.05	0.07 – 14.71	0.973
ped inbreeding c	2.20	0.99 – 4.90	0.053
Observations	57		

Table S4.3. Model output for best model of aboveground biomass.

	aboveground biomass		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.23	1.10 – 1.35	**<0.001**
prop E	-0.29	-0.40 – -0.17	**<0.001**
ped inbreeding c	-0.09	-0.14 – -0.04	**0.001**
rhizobia [Y]	0.52	0.46 – 0.58	**<0.001**
ped inbreeding c^2	-0.03	-0.05 – -0.01	**0.001**
ped inbreeding c * rhizobia [Y]	-0.06	-0.11 – 0.00	0.054
Random Effects			
σ^2	0.33		
$\tau_{00\ \mathrm{planting\_date\_julian}}$	0.05		
ICC	0.14		
$N_{\mathrm{planting\_date\_julian}}$	21		
Observations	1619		
Marginal R^2 / Conditional R^2	0.182 / 0.301		

Table S4.4. Model output for best model of belowground biomass.

	belowground biomass		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.73	1.67 – 1.79	**<0.001**
ped inbreeding c	1.03	1.01 – 1.05	**0.002**
rhizobia [Y]	0.90	0.87 – 0.94	**<0.001**
ped inbreeding c^2	1.02	1.01 – 1.03	**0.003**
Observations	1436		
R^2 conditional / R^2 marginal	NA / 0.006		

Table S4.5. Model output for best model of height at flowering.

	[illegible]		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-42.34	-52.64 – -32.03	[illegible]
age at flowering in days	2.88	2.56 – 3.19	[illegible]
age at flowering in days^2	-0.02	-0.03 – -0.02	[illegible]
rhizobia [Y]	0.40	-0.16 – 0.97	0.163
ped inbreeding c	-0.90	-1.22 – -0.59	[illegible]
prop E	-8.93	-10.01 – -7.85	[illegible]
ped inbreeding c^2	-0.16	-0.33 – 0.01	0.066
[illegible]			
σ^2	35.14		
$\tau_{00\ \text{planting\_date\_julian}}$	15.66		
ICC	0.31		
$N_{\text{planting\_date\_julian}}$	21		
Observations	1973		
Marginal R^2 / Conditional R^2	0.266 / 0.492		

Table S4.6. Model output for best model of individual seed mass.

	seed ratio by envelope c		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.30	0.14 – 0.46	**<0.001**
prop E^2	0.94	0.19 – 1.70	**0.014**
prop E	-1.39	-2.10 – -0.69	**<0.001**
ped inbreeding c	-0.19	-0.25 – -0.14	**<0.001**
ped inbreeding c^2	-0.05	-0.09 – -0.00	**0.044**
rhizobia [Y]	0.08	-0.02 – 0.19	0.127
ped inbreeding c^2 * rhizobia [Y]	0.06	0.01 – 0.11	**0.019**
Observations	1951		
R^2 / R^2 adjusted	0.040 / 0.037		

Table S4.7. Model output for best model of nodule number.

·	[illegible]		
Predictors	*Incidence Rate Ratios*	*CI*	*p*
[illegible]			
(Intercept)	25.75	20.86 – 31.79	[illegible]
prop E	0.93	0.76 – 1.13	0.462
ped inbreeding c	0.95	0.90 – 1.00	0.069
rhizobia [Y]	1.64	1.43 – 1.89	[illegible]
ped inbreeding c^2	0.98	0.95 – 1.02	0.315
(Intercept)	4.92	0.01 – 0.03	
[illegible]			
(Intercept)	1.55	1.09 – 2.20	[illegible]
prop E	1.11	0.57 – 2.15	0.760
ped inbreeding c	1.32	1.08 – 1.60	[illegible]
ped inbreeding c^2	1.08	0.98 – 1.20	0.139
rhizobia [Y]	0.02	0.01 – 0.03	[illegible]
[illegible]			
σ^2	0.62		
$\tau_{00\ \mathrm{harvesting\_date}}$	0.17		
ICC	0.21		
N $_{\mathrm{harvesting\_date}}$	36		
Observations	1432		
Marginal R^2 / Conditional R^2	0.071 / 0.269		

Table S4.8. Model output for best model of contamination.

	contamination		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.12	0.08 – 0.18	**<0.001**
prop E	1.00	0.52 – 1.93	0.994
ped inbreeding c	0.85	0.71 – 1.02	0.084
ped inbreeding c^2	0.98	0.88 – 1.08	0.643
Random Effects			
σ^2	3.29		
$\tau_{00\ \text{harvesting\_date}}$	0.19		
ICC	0.05		
$N_{\text{harvesting\_date}}$	39		
Observations	1546		
Marginal R^2 / Conditional R^2	0.010 / 0.063		

Table S4.9. Model output for best model of nodulation failure.

	no nod		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.01	0.01 – 0.03	**<0.001**
prop E	0.82	0.26 – 2.55	0.728
ped inbreeding c	1.69	1.05 – 2.73	**0.031**
ped inbreeding c^2	1.07	0.87 – 1.31	0.534
Random Effects			
σ^2	3.29		
$\tau_{00\ \text{harvesting\_date}}$	1.28		
$\tau_{00\ \text{planting\_date\_julian}}$	0.21		
ICC	0.31		
$N_{\text{harvesting\_date}}$	38		
$N_{\text{planting\_date\_julian}}$	21		
Observations	1512		
Marginal R^2 / Conditional R^2	0.075 / 0.363		

Table S4.10. Model output for best model of average ant number.

	T ant		
Predictors	*Incidence Rate Ratios*	*CI*	*p*
(Intercept)	0.07	0.02 – 0.23	**<0.001**
EFN Count 7 9	1.07	1.03 – 1.12	**<0.001**
Observations	58		
R^2 conditional / R^2 marginal	NA / 0.242		

Table S4.11. Model output for best model of EFN count.

	EFN Count 7.9		
Predictors	*Incidence Rate Ratios*	*CI*	*p*
(Intercept)	3.26	1.81 – 5.87	**<0.001**
aboveground biomass	9.27	4.24 – 20.28	**<0.001**
aboveground biomass^2	0.51	0.39 – 0.66	**<0.001**
ped inbreeding c	0.89	0.82 – 0.96	**0.004**
ped inbreeding c^2	0.93	0.85 – 1.02	0.144
rhizobia [Y]	0.89	0.69 – 1.14	0.345
ped inbreeding c^2 * rhizobia [Y]	1.11	1.00 – 1.24	0.056
Random Effects			
σ^2	0.07		
$\tau_{00\ \text{planting\_date\_julian}}$	0.12		
ICC	0.63		
$N_{\text{planting\_date\_julian}}$	16		
Observations	50		
Marginal R^2 / Conditional R^2	0.577 / 0.845		

Table S4.12. Model output for best model of herbivory.

·	[illegible]		
Predictors	*Estimates*	*CI*	*p*
[illegible]			
(Intercept)	0.07	0.04 – 0.12	[illegible]
T ant	1.02	0.64 – 1.63	0.940
ped inbreeding c	1.21	1.01 – 1.46	[illegible]
ped inbreeding c^2	1.23	1.08 – 1.41	[illegible]
rhizobia [Y]	1.08	0.59 – 2.00	0.800
ped inbreeding c^2 * rhizobia [Y]	0.86	0.75 – 0.99	[illegible]
(Intercept)	13276649987.50	0.41 – 1.13	
[illegible]			
(Intercept)	0.32	0.15 – 0.65	[illegible]
T ant	1.32	0.51 – 3.40	0.567
ped inbreeding c	0.68	0.41 – 1.13	0.135
[illegible]			
σ^2	0.28		
$\tau_{00\ \text{planting\_date\_julian}}$	0.02		
ICC	0.07		
$N_{\text{planting\_date\_julian}}$	17		
Observations	58		
Marginal R^2 / Conditional R^2	0.472 / 0.509		