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Reproductive Interference Explains the Competitive Disparity Between Congeneric Beanweevils Callosobruchus Maculatus and Callosobruchus Chinensis

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REPRODUCTIVE INTERFERENCE EXPLAINS THE COMPETITIVE DISPARITY BETWEEN CONGENERIC BEANWEEVILS CALLOS OBRUCHUS MACULATUS AND CALLOS OBRUCHUS CHINENSIS

By

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ABSTRACT

The system including the congeners *C. maculatus* and *C. chinensis* has long been a model for competition studies. Members of these two pest species often cohabit facilities designed for the storage of dried legumes, a crucial nutritional resource for the larvae of both species. In both natural and laboratory settings it has been observed that *C. chinensis* routinely drives *C. maculatus* to extinction. These observations convinced many ecologists that the trophic relationship between the two species presents an unequivocal case of competitive exclusion. Several predictively successful mathematical models based on resource competition alone accordingly estimated the competitive disparity between these two bean beetle species, suggesting that individual *C. chinensis* are approximately four times better competitors for a shared resource than their average *C. maculatus* counterparts. Recent studies (Kishi et al. 2009; Kyogoku and Nishida 2012, 2013; Kishi and Nakazawa 2013; Kishi and Tsubaki 2014) have called into question this apparently straightforward conclusion since there are few if any characteristic life history traits or behavioral mechanisms associated with exploitative or interference competition that would explain the competitive disparity. The authors of these studies contend that reproductive interference—a negative interspecific sexual interaction that reduces the fitness of at least one of the species involved—is a largely ignored but equally consequential factor when it comes to explaining interspecific dynamics. This study tests for the presence and explanatory importance of reproductive interference on per capita female fitness by simultaneously manipulating conspecific density and operational sex ratio in the presence (and absence) of heterospecific males. The experimental design also reveals the operative mechanism(s) behind reproductive interference. Results of this study clearly support outstanding claims for the causal significance of reproductive interference in this system.
CHAPTER 1
INTRODUCTION

Intraspecific and interspecific resource competition are often considered the primary drivers of numerical dynamics for species on the same trophic level (Schoener 1983; Lawton and Hassell 1984). *Heterospecific density* is accordingly thought to be one of the two major causes of decline in the population growth rate and per capita fitness of a competitor species. This is evident in early theoretical models such as the Lotka-Volterra competition model, which predicts that, when there is interspecific resource competition between two species, the growth rate of one competitor will decrease as heterospecific density increases (Tilman 1987; Grover 1997). Studies involving *Callosobruchus* bean beetles provide no exception in this regard (Bellows and Hassell 1984). Most studies of these model congeners unquestioningly adopted the assumption that resource competition drives observed population dynamics. It is widely known, however, that classic competition models are phenomenological in character. Even when accurately predicting population dynamics on the basis of heterospecific density, the models do not describe the manner in which competing species affect the dynamics that they do (Holt 1977). In some cases researchers are consequently ambivalent about which causal mechanisms are actually responsible for observed population dynamics. While alternative mechanisms can generate virtually indistinguishable dynamical patterns, it is also widely known that such mechanisms can cause divergent dynamical patterns at some (e.g., non-equilibrium) densities or relative frequencies. The unique controls afforded by laboratory experimentation can occasionally reveal the key components affecting the dynamics of interaction.

Recent laboratory competition studies involving *Callosobruchus* bean beetles have called into question the seemingly ubiquitous assumption that resource competition is the primary
driver of numerical population dynamics for competing dynamics (Kishi et al. 2009; Kyogoku and Nishida 2012, 2013; Kishi and Nakazawa 2013; Kishi and Tsubaki 2014). Studies on congeneric bean beetles have fit mathematical models of resource competition to experimental results and demonstrated that observed competition dynamics and outcomes could be described solely by reference to the density dependent effect of interspecific resource competition (Utida 1953; Bellows and Hassell 1984; Ishii and Shimada 2008; Kishi and Tsubaki 2014). Several of these studies also estimated competition coefficients for *Callosobruchus chinensis* and *Callosobruchus maculatus* from population dynamics data and were thereby able to gauge the intensity of interspecific resource competition. In one study (Ishii and Shimada 2008) the competition coefficient value for *C. chinensis* was 4.14. This implies that for every individual added to the population of one species (*C. chinensis*), the population of its competitor (*C. maculatus*) is reduced by approximately four individuals (Gotelli 2008). What calls this parameter value and the models from which it is derived into question is the rather striking fact that the strong asymmetric density effect between these competing species cannot as of yet be explained by any characteristic life history traits or behavioral mechanisms associated with exploitative or interference competition (Kishi and Tsubaki 2014). Since these two beetle species are similar in size and have generation times of approximately the same duration, it is unclear why one (*C. chinensis*) should use four times the amount of a limiting resource. This puzzle makes it well worth reconsidering whether resource competition is the proper means by which to explain competitive interaction in this model system.

The authors of several recent studies contend that the proximate biological mechanism responsible for reduced per capita fitness and thus lower population growth rate in *C. maculatus* appears to be *reproductive interference* (Kishi et al. 2009; Kyogoku and Nishida 2012, 2013;
Kishi and Nakazawa 2013; Kishi and Tsubaki 2014) Reproductive interference is a negative interspecific sexual interaction that reduces the fitness of at least one of the species involved (Gröning and Hochkirch 2008). By way of generic example, when a male of species X mistakenly recognizes a female of species Y as a conspecific and attempts to mate with her, the female might suffer from a loss of energy or time, reduced opportunities to mate with conspecifics, gamete wastage, genital damage, or hybrid formation (Gröning and Hochkirch 2008). Forms of reproductive interference have been documented in a wide range of taxonomic groups, including but not limited to ticks (Andrews et al. 1982), crayfish (Söderbäck 1994), frogs (Hettyey and Pearman 2003), geckos (Dame and Petren 2006), whiteflies (Liu et al. 2007), freshwater copepods (Thum 2007), shrubs (Thomson et al. 1981), wildflowers (Waser and Fugate 1986), grasses (Murphy and Aarsen 1995), sea urchins (Levitan 2002), corals (Fogarty et al. 2012), and guppies (Valero et al. 2008). Of particular interest for present purposes, behavior consistent with reproductive interference has been observed between the congeneric species of bean beetle, *Callosobruchus maculatus* and *Callosobruchus chinensis* (Kishi et al. 2009). Males of both species attempt to mate with heterospecific females indiscriminately even when conspecific females are present and there is no known prospect for hybridization.

While one might expect symmetric rates of reproductive interference to have a symmetric net effect on fecundity reduction in the case of *C. maculatus* and *C. chinensis*, there is actually an asymmetric relationship between these species. The males of both species clearly exhibit heterospecific sexual harassment (i.e., chasing and mounting) even in the presence of conspecific females (Kishi et al. 2009). But whereas *C. chinensis* males sometimes copulate with *C. maculatus* females, *C. maculatus* males seldom if ever copulate with heterospecific females (Kyogoku and Nishida 2012). This fact implicates interspecific copulation attempts, rather than
mere heterospecific harassment (i.e., chasing and mounting), as the mechanism of import for explaining competition dynamics. This claim has recently been supported by Kyogoku and Nishida’s (2013) study aimed at disentangling these oft-conflated mechanisms. They found that *C. chinensis* males with ablated genitals, which could harass *C. maculatus* females but not copulate with them, did not reduce the fecundity of *C. maculatus* females. As their (2013) study sought to elucidate the mechanism(s) of reproductive interference, it was clearly predicated on the assumption that reproductive interference was an important causal factor. This presumption is, however, questionable in light of the experimental design deployed in their previous studies (Kyogoku and Nishida 2012).

The asymmetrical net effect on fecundity reduction by way of interspecific copulation attempts provides an opportunity to test claims about the significance of reproductive interference as well as the biological mechanism(s) that underlie it. According to Kyogoku and Nishida (2012), reproductive interference can generate something akin to an Allee effect due to its positive frequency dependence. Reproductive interference shows frequency dependence since the higher the frequency of an interfering species X, the greater the intensity of the reproductive interference on species Y. As a corollary, if the density of the interfering species X remains constant, the per capita level of interference with species Y should become diluted as the density of species Y increases. Per capita fitness and population growth rate for species Y will subsequently increase with the addition of conspecific individuals. This runs counter to what would be expected in a “resource competition only” model, where the addition of conspecific or heterospecific individuals increases overall competition and thereby diminishes the fitness and population growth rate for each of the competing species. If attempted copulation (rather than mere harassment) is the mechanism by which reproductive interference reduces fitness and
recruitment, then one would expect (i) a positive frequency dependent effect in the experimental populations of *C. maculatus* which are subject to harassment by *C. chinensis* males and (ii) a corresponding absence of such an effect in experimental populations of *C. chinensis* subject to harassment by *C. maculatus* males.

We set out to determine if reproductive interference contributes to explaining the population dynamics of competing congeneric species of bean beetles *C. maculatus* and *C. chinensis*. Achieving as much required testing whether formal models that include reproductive interference better explain competition dynamics and outcomes than do models restricted solely to resource competition (for oviposition sites). We also sought a quantitative estimate of the magnitude of reproductive interference. This, too, was done by assessing the goodness of fit of formal models to data generated via replicated experimental laboratory populations. Similar studies done to date were refined by manipulating the operational sex ratio of the species experiencing reproductive interference. To the best of our knowledge, previous studies have not investigated this possibility. Kyogoku and Nishida’s (2012) experimental design, for example, altered only the number of female-male pairs of bean beetles (from one to five pairs in each treatment) subject to reproductive interference. The sex ratio of the species subject to sexual interference was consequently restricted to 1:1. While perhaps a prudent allocation of experimental effort, this constraint in design is nevertheless problematic because it can mask the presence and magnitude of reproductive interference and thus make it falsely appear as though resource competition is a more important factor than it actually is. In the generic case of sexual interference discussed above, if the density of the interfering species X is held constant then the per capita level of interference with species Y should become diluted as the density of species Y increases. But increasing the density of the species being interfered with (Y) only by increments
of one or several female-male pairs at a time might underestimate the actual importance of reproductive interference. An equivalent density increase involving a sex ratio skewed toward a greater frequency of females (e.g., 75% female, 25% male), for instance, could potentially generate an even more profound increase in per capita fitness and population growth rate for species Y. The potential for such confounding is particularly pressing for the species of bean beetle under study here, as one of them (*C. maculatus*) is known to experience maximal population growth at a strongly female-biased operational sex ratio (Miller and Inouye 2011).

Understanding the respective contributions of sexual interference and resource competition can extend well beyond the laboratory into areas such as conservation or invasion biology, especially as the differences between an experimental laboratory setup and the “wild” storage setting for interactions between competing bean beetles are less contrived than in other systems. Knowledge of the causal mechanisms involved in interspecific interactions is crucial for successful intervention. Sex differences in dispersal, for example, can lead to highly skewed sex ratios on the periphery of an invasive population’s range, which could in turn influence the speed and expansion potential of a biological invasion (Miller et al. 2011; Miller and Inouye 2013). Conservation biologists would of course choose a very different course of action if they suspected that the expansion potential of invasive species was influenced more heavily by reproductive interference than the amount of a limiting (food) resource for competing species. As increased biological reality is assumed to be a sought-after virtue when it comes to formal modeling, it is well worth investigating whether mathematical models that incorporate reproductive interference via distinct terms for sex ratio and stage-structure repay their increased computational complexity and experimental effort.
It is worth pausing to take stock of the questions that are under investigation in this study. First and foremost, we wanted to know whether reproductive interference is an important causal factor in explaining the competitive exclusion of *C. maculatus* by *C. chinensis*. Our approach to this question was to determine whether formal models that include reproductive interference (via explicit terms for sex-structure and stage-structure) better explain experimentally-generated data from a treatment condition where *C. chinensis* males were present than do models restricted solely to resource competition. Properly addressing this initial question also required the experimental manipulation of both density and operational sex ratio for the focal (victim) species in each treatment. The response surface design of our experiment (see Figure 1) enabled us to disentangle possible interactions between sex ratio and density in the presence of heterospecific sexual interference. A third and closely related aim was to ascertain the approximate magnitude of sexual interference when it occurs. Maximum likelihood methods were used to attain numerical estimates and confidence intervals for a parameter indicating the presence (or absence) of interfering male heterospecifics. Put simply, we created a formal analogue for competition coefficients within the likelihood framework. The fourth and final question that we sought to address was one concerning the operative mechanism—heterospecific copulation—behind reproductive interference. This required testing for the effects of reproductive interference by *C. maculatus* males on the per capita fitness of *C. chinensis* females even though heterospecific male harassment in this direction is thought to have a negligible effect on the fecundity of *C. chinensis* females.
CHAPTER 2

MATERIALS AND METHODS: EXPERIMENTAL METHODS

*Callosobruchus chinensis* [Coleoptera: Bruchidae] and *Callosobruchus maculatus* [Coleoptera: Bruchidae] are stored grain pests as well as model species in ecology in evolutionary biology (Bellows 1982; Møller et al. 1989; Sibly et al. 1991; Bonsall et al. 2002, Yanagi et al. 2013). We used the Yemen strain of *C. chinensis* and the Kenya strain of *C. maculatus*. Mature beetles do not need to eat and direct their efforts almost exclusively to locating mates and depositing eggs. Gravid females of both species deposit approximately 60 eggs on seeds of various cultivated legumes (i.e., beans) during their adult lifespan of around one week (Janzen 1977; Varma and Anadi 2010). Larvae of both species burrow into beans, pupate, and emerge as adults. The egg-to-adult developmental period is 30–35 days for the stock cultures maintained under our incubator conditions (25°C, 50–60% relative humidity, 16L8D). Stock cultures were fed mung beans (*Vigna radiata*). Adult sexes are readily distinguished by their size (females are larger), color, and patterning on their elytra. There are no known cases of the two species hybridizing (Yamane and Miyatake 2010).

Following the experimental design of Kyogoku and Nishida (2013), each experimental population was introduced into a square Petri dish (100mm x 100mm x 10mm) with 3.0 grams of black-eyed peas (*Vigna unguiculata*) and stored in an incubator. This amount of the resource suffices to meet the reproductive needs of approximately 26 female beetles\(^1\) and was chosen so as to minimize intraspecific resource competition for oviposition sites among females. As populations were initiated with adult beetles that are 6-36 hours old, we assumed that the chosen beetles were virgins. Since all beetles were reared under similar densities our analysis did not

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\(^1\) Miller and Inouye found that 5.0g of mung beans suffices to sustain 43 females. Although changing the type of bean, the treatments used never exceeded 18 female beetles per Petri dish.
include any effects of the parental generation's larval density on adult reproductive performance. After 35 days, all experimental populations were frozen for no less than 72 hours to halt any further development and subsequent emergence. We then tallied the total number of beetles in each Petri dish and inferred the number of offspring by subtracting the number of conspecific adults comprising the parental generation ($N_t$) and any heterospecific males. The resulting value, divided by the number of parental generation females in each dish, was taken as a proxy measure of female lifetime fitness (Miller and Inouye 2011).

The effect of *C. chinensis* males on per capita fecundity (i.e., emerged offspring per female) of *C. maculatus* was investigated under different *C. maculatus* density and operational sex ratio regimes (solid blue triangles in Figure 1). In this treatment condition four different density levels of *C. maculatus*, specifically 6, 10, 12, and 20 individuals, were housed with five *C. chinensis* males throughout their lives in a plastic Petri dish (100 mm x 100 mm x 10 mm) containing 3.0g of black-eyed peas. Heterospecific males, but no heterospecific females, were introduced in the *C. maculatus* treatment condition because we wished to investigate whether and how reproductive interference by heterospecific males affects the fecundity of the *C. maculatus* females. We concurrently manipulated the operational sex ratio at each density level. It ranged from 10% to 90% female within each of the aforementioned density levels for the focal species subject to reproductive interference. Since both sexes can mate multiply we assumed that all of the adult beetles constituting an experimental population were available for mating. The adult sex ratio thus corresponds directly with the operational sex ratio (Arnqvist et al. 2005). As the control (open blue triangles in Figure 1), we examined the per capita fecundity of *C. maculatus* females across changes in both density (6, 10, 12, or 20 individuals) and sex ratio (from 10% to 90% female) for individuals housed without any *C. chinensis* males. We performed
3-10 replications of each: the largest density (20) was replicated three times, the next-to-largest density (12) was replicated five times, the next-to-smallest density (10) was replicated six times, and the smallest density (6) was replicated ten times. Using the same density levels and operational sex ratios, we conversely examined the effect of harassment by *C. maculatus* males on the per capita fecundity of *C. chinensis* females (solid red circles in Figure 1).

**FIG.1.** Experimental design including control and treatment conditions for both species.

This contrast class was necessary in order to address questions about the actual mechanism(s) behind reproductive interference. As only *C. chinensis* males regularly attempt to copulate with (rather than merely harass) female heterospecifics, Kyogoku and Nishida (2013) argue that copulation attempts are the operative mechanism behind reproductive interference and the
otherwise seemingly inexplicable difference in competitive ability between the species. A contrast class testing for the presence and magnitude of reproductive interference exerted upon C. chinensis females by C. maculatus males should consequently demonstrate a much diminished effect on the per capita fitness of C. chinensis due to a dearth of actual heterospecific copulation attempts. The control condition in this case (open red circles in Figure 1) examined the per capita fecundity of C. chinensis females across changes in both density (6, 10, 12, or 20 individuals) and sex ratio (from 10% to 90% female) for individuals housed without C. maculatus males.

Our experiment had a response-surface design insofar as bean beetle dynamics were quantified over a range of operational sex ratios ($\rho = 0.1 – 0.9$ female) and population densities ($N_i = 6, 10, 12, \text{or} 20$ individuals). The response variable in each experimental condition was per capita female fitness ($W = N_{t+1}/F_t$). While treatments were distributed across the response surface (with the constraint that female and male densities were assigned integer values), we concentrated treatments near the low end of the density gradient to capture the transition from exponential to density-dependent changes to per capita fitness (Miller and Inouye 2011). Of the various operational sex ratios implemented, only the 1:1 sex ratio ($\rho = 0.5$) was tested on all four density levels. Doing so enables a direct comparison between our findings and those of Kyogoku and Nishida (2013), wherein density (number of female-male pairs) but not the sex ratio was subject to experimental manipulation. Each species takes a turn as the victim of reproductive interference and control treatments exclude interfering heterospecific males (Figure 1). This design enabled us to examine the possibility of interactions between sex ratio and density dependence (Inouye 2001).
CHAPTER 3

MATERIALS AND METHODS: MODEL SELECTION AND ANALYSIS

In order to determine whether reproductive interference has a significant effect and what
the magnitude of its effect might be we first had to choose an appropriate density-dependent
model structure for bean beetle population dynamics. The fits of three commonly used density-
dependent recruitment models to pooled data \( N = F + M \); where \( F \) is number of females and \( M \)
number of males) were compared: the Ricker model, \( N_{t+1} = \lambda N \exp(-bN_t) \) (Ricker 1954), the
Beverton-Holt model, \( N_{t+1} = \lambda N(1+bN_t) \) (Beverton and Holt 1957), and a generalized form of
Beverton-Holt model, \( N_{t+1} = \lambda N(1+[bN_t]^\gamma) \) (Shepherd 1982). The parameter \( b \) appears in all of
these models. It signifies the per capita contribution to density dependence, with greater values
indicating lower maximum recruitment. The parameter \( \gamma \) occurs only in the generalized form of
the Beverton-Holt function. This function can exhibit a range of dynamical behavior depending
on the value of \( \gamma \). When \( \gamma > 1 \), the function exhibits classic over-compensatory Ricker dynamics
whereupon recruitment decreases with increasing initial density past the peak of recruitment.
This signifies interference or aggression between conspecifics at high density. When \( \gamma < 1 \),
recruitment increases monotonically as initial density increases. When \( \gamma = 1 \), the generalized
form reduces to the standard Beverton-Holt model in which recruitment asymptotes as initial
density increases. This reflects a fixed amount space or resources available to new recruits. The
shape of the recruitment function is largely determined by the natural history of the organism
under study (Miller and Inouye 2011).

Using a Gaussian likelihood function and estimating the standard deviation of the data as
a free parameter, we evaluated sex-structured versions of these alternative functions using the
Akaike information criterion (AIC). We calculated \( \Delta \text{AICs} \) and AIC weights \((w)\), the latter of
which sum to one and give the proportional weight of evidence in favor of each candidate model (Burnham and Anderson 2002). The generalized version of the Beverton-Holt function was the only alternative to receive a substantial amount of support in all relevant partitions of our pooled data (discussed below). In no partition did the score for the generalized Beverton-Holt function exceed ΔAICs = 2.33 or fall below \( w = 0.15 \). Each of the other candidate functions exhibited ΔAICs > 5.00 and \( w < 0.15 \) in at least one partition of our pooled data. We thus concluded that the generalized Beverton-Holt function made for the most realistic density-dependent context within which to inquire about the appropriate two-sex demographic model or “mating function.”

Following Miller and Inouye (2011), we subsequently tested four candidate two-sex demographic models (TABLE 1) for goodness of fit against data acquired from our experiment with *C. maculatus* and *C. chinensis*. The models in this set have in common a two-sex demographic function (occurring as the numerator) that predicts the density of mating based on the harmonic means of female and male densities. This choice is justified on the grounds that a harmonic mating function is taken to be the most biologically realistic for the congeneric species of beetle under study (Miller and Inouye 2011). The difference among the models is in the form of generalized Beverton-Holt density dependence that they incorporate (as the denominator). The models included either unstructured (model 1) or sex-structured (model 3) density dependence. The sex-structured models (models 3 and 4) include explicit terms for females and males (i.e., \( N = F + M \)). Two-sex mating functions also made it possible to fit models in which offspring (larval) density was added to the unstructured (model 2) or sex-structured (model 4) parental terms. Since larvae develop within beans we did not quantify their densities directly. We instead used the mating terms as a proxy for larval density on the grounds that these terms are
proportional to larval densities. Models extended in this fashion effectively include stage structure.

The primary difference between Miller and Inouye’s (2011) models and our own is that the significance of reproductive interference was measured in terms of its effect on per capita female fitness (an individual-level parameter) rather than recruitment (a population-level parameter). Fitness can be a perplexing concept at the best of times (De Jong 1994; Sober 2001). Momentarily setting aside the vexing issues that accompany this notion, we decided to quantify per capita fitness as the number of emerged (adult) beetles in the offspring generation per female beetle in the parental generation (i.e., \( W = N_{t+1}/F_t \)).

In TABLE 1, the variable \( N_t \) represents initial density, while \( W \) designates fitness or the arithmetic mean number of emerged offspring per female of the parental generation. The variable \( \lambda \) signifies the per-mating birth rate (i.e., the per capita net rate of increase). The variables \( F \) and \( M \) represent the number of females and the number of males. The parameter \( b \) controls the per capita contribution to density dependence (greater values correspond to lower maximum recruitment). The parameter \( \gamma \) describes the shape of the recruitment function and \( h \) represents “harem size.” Two-sex mating functions can be generalized to represent non-monogamous social mating systems by scaling female density (\( F \)) by harem size. Values of \( h \) can be less than, equal to, or greater than one, in which case they are interpreted as signifying polyandry, monogamy, and polygamy, respectively. The density dependent parameters \( b \) and \( \gamma \) are subscripted to specify females (\( F \)), males (\( M \)), and offspring (\( O \)) (Shepherd 1982, Caswell 2001, Kot 2001, Miller and Inouye 2011).
TABLE 1. Candidate two-sex demographic models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Mating Function (numerator)</th>
<th>Density Dependence (denominator)</th>
<th>Equation</th>
<th>K (Θ of parameters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Harmonic mean</td>
<td>Unstructured</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( W = \frac{\lambda \left( \frac{2M_t}{F_t h^{-1} + M_t} \right)}{1 + (bN_t)^\gamma} )</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Harmonic mean</td>
<td>Stage-structured</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( W = \frac{\lambda \left( \frac{2M_t}{F_t h^{-1} + M_t} \right)}{1 + (bN_t)^\gamma + \left( b_0 \frac{2F_t h^{-1} M_t}{F_t h^{-1} + M_t} \right)^\gamma_0} )</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Harmonic mean</td>
<td>Sex-structured</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( W = \frac{\lambda \left( \frac{2M_t}{F_t h^{-1} + M_t} \right)}{1 + (bF_r F_t)^\gamma_r + (bM M_t)^\gamma_M} )</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Harmonic mean</td>
<td>Sex- and stage-structured</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( W = \frac{\lambda \left( \frac{2M_t}{F_t h^{-1} + M_t} \right)}{1 + (bF_r F_t)^\gamma_r + (bM M_t)^\gamma_M + \left( b_0 \frac{2F_t h^{-1} M_t}{F_t h^{-1} + M_t} \right)^\gamma_0} )</td>
<td></td>
</tr>
</tbody>
</table>

As in the case of choosing between alternative models for density-dependence, these models were evaluated by way of the Akaike information criterion (AIC). Using a Gaussian likelihood function and estimating the standard deviation (\( \sigma \)) of the data as a free parameter, we calculated \( \Delta \text{AICs} \) and AIC weights (\( \omega \)) (Burnham and Anderson 2002). All statistical analyses were conducted with R software version 3.2.0 (R Development Core Team 2015).
CHAPTER 4

RESULTS

Four partitions of our pooled data were relevant for addressing whether reproductive interference explains the interspecific competitive superiority of *C. chinensis*: (i) a control condition for *C. maculatus* that excluded heterospecific *C. chinensis* males; (ii) a treatment condition for *C. maculatus* that included five heterospecific *C. chinensis* males; (iii) a control condition for *C. chinensis* that excluded heterospecific *C. maculatus* males; (iv) a treatment condition for *C. chinensis* that included five heterospecific *C. maculatus* males. It bears repeating that the same density and sex-ratio regimes were established in each of these conditions. The four candidate two-sex demographic models (models 1-4 in Table 1) were tested for goodness of fit against the data in each of these conditions.

The model with unique contributions to density dependence by females and males (model 3) provided the best fit to data in the *C. maculatus* control condition (i). AIC weights indicate that it received the majority of support (*w* = 0.54). In contrast, the model including unique contributions to density dependence by females, males, and offspring (model 4) proved to be the best fit to data in the *C. maculatus* treatment condition (*w* = 0.54). Table 2 provides a summary of these results. Figure 2 shows the data for per capita fitness and the predictions of the best-fitting models for control and treatment conditions using the maximum-likelihood parameter estimates in Table 4. As the model prediction is a surface in *N* vs. *ρ* space, the lines in Figure 2 (and Figure 3 below) represent two-dimensional slices of the surface that was fit for continuous variation in female-bias (*ρ*).
TABLE 2. Fit of candidate models in Table 1 to *C. maculatus* control and treatment data.

<table>
<thead>
<tr>
<th>Model</th>
<th># Parameters</th>
<th>ΔAIC (control)</th>
<th>AIC-weight (control)</th>
<th>ΔAIC (treatment)</th>
<th>AIC-weight (treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Unstructured</td>
<td>5</td>
<td>18.11</td>
<td>0.00</td>
<td>45.38</td>
<td>0.00</td>
</tr>
<tr>
<td>(2) Stage-structured</td>
<td>7</td>
<td>0.68</td>
<td>0.38</td>
<td>2.99</td>
<td>0.12</td>
</tr>
<tr>
<td>(3) Sex-structured</td>
<td>7</td>
<td>0.00</td>
<td><strong>0.54</strong></td>
<td>0.91</td>
<td>0.34</td>
</tr>
<tr>
<td>(4) Sex- and stage-structured</td>
<td>9</td>
<td>3.82</td>
<td>0.08</td>
<td>0.00</td>
<td><strong>0.54</strong></td>
</tr>
</tbody>
</table>

FIG. 2. Bean beetle data (points) and predictions of best fitting models (lines) for *C. maculatus* control condition (top) and treatment condition (bottom).

The sex-structured model with unique contributions to density dependence by females and males (model 3) provided the best fit to data in both the *C. chinensis* control and treatment conditions. AIC weights indicate that it received the greatest support in both conditions (w =
0.50 and \( w = 0.49 \), respectively). Table 3 summarizes the results. Figure 3 shows the data for per capita fitness and the predictions of the best-fitting model for control and treatment conditions using the maximum-likelihood parameter estimates in Table 4.

To determine the magnitude of the effects of heterospecific males on per capita female fitness, we had to create an analogue for competition coefficients within the maximum-likelihood framework. This required two notable changes to our analysis. First, the fit of the four two-sex demographic models (TABLE 1) had to be reassessed within more inclusive data sets.

**TABLE 3. Fit of candidate models in Table 1 to *C. chinensis* control and treatment data.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>( \Delta \text{AIC} ) (control)</th>
<th>AIC-weight (control)</th>
<th>( \Delta \text{AIC} ) (treatment)</th>
<th>AIC-weight (treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Unstructured</td>
<td>5</td>
<td>56.80</td>
<td>0.00</td>
<td>46.36</td>
<td>0.00</td>
</tr>
<tr>
<td>(2) Stage-structured</td>
<td>7</td>
<td>0.26</td>
<td>0.44</td>
<td>0.13</td>
<td>0.46</td>
</tr>
<tr>
<td>(3) Sex-structured</td>
<td>7</td>
<td>0.00</td>
<td><strong>0.50</strong></td>
<td>0.00</td>
<td><strong>0.49</strong></td>
</tr>
<tr>
<td>(4) Sex- and stage-structured</td>
<td>9</td>
<td>4.00</td>
<td>0.07</td>
<td>4.38</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**FIG. 3.** Bean beetle data (points) and predictions of best fitting models (lines) for *C. chinensis* control condition (above) and treatment condition (below).
Pooled experimental data were initially partitioned into four conditions (i-iv above) when testing for the effects of reproductive interference. The pooled data were subsequently repartitioned so as to combine data from the control and the treatment conditions for a focal (victim) species. This yielded two data sets: one containing all data on per capita female fitness for treatments where *C. chinensis* was the focal species and another which contained all data on per capita female fitness for treatments where *C. maculatus* was the focal species. The second step was to reconfigure the four candidate two-sex demographic models so as to incorporate a term that estimates the impact of heterospecific males and thus reproductive interference. This was accomplished by including an additional parameter within each of the four models and, then, testing these expanded models within the more inclusive repartitions of the pooled data. When models were outfitted with this additional parameter to measure the competitive equivalency of heterospecific males, we once again calculated ΔAICs and AIC weights (w) to determine which model was the best fit to the data. After determining which model was the best fit to data, we used maximum likelihood methods to acquire numerical estimates of competitive equivalency.

The stage-structured model which included a unique contribution to density dependence by offspring but not sex (model 2) received the majority of support in both repartitions of the pooled data. When the per capita fecundity of *C. maculatus* females was tested for the effect of reproductive interference, model 2 received the vast majority of support as indicated by an AIC-weight of w = 0.75. Model 2 also received the majority of support, w = 0.51, when the per capita fecundity of *C. chinensis* females was tested for the effect of reproductive interference. With the best-fitting model in hand, maximum likelihood methods were used to attain numerical estimates and confidence intervals for the parameter indicating the competitive equivalency of heterospecific males. The per capita effect *C. chinensis* males on the fitness of *C. maculatus*
females was equivalent to the effect of approximately 2.8 conspecifics \([\alpha_{12} = 2.767 (1.057-4.511)]\). In contrast, the per capita effect of *C.maculatus* males on the fitness of *C.chinensis* females was equivalent to the effect of approximately 1.7 conspecifics \([\alpha_{21} = 1.672 (1.047-2.295)]\). These results are summarized in TABLE 5.

**FIG.4.** Predictions of best-fitting two-sex demographic model in each experimental condition for the relationship between the dependent variables operational sex ratio (proportion female, \(F_t/N_t\)) and initial density \(N_t\) and the independent variable per capita female fitness \((N_{t+1}/F_t)\).
TABLE 4. Maximum likelihood estimates for *C. maculatus* and *C. chinenis* demographic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maculatus Control (model 3)</th>
<th>Maculatus Treatment (model 4)</th>
<th>Chinensis Control (model 3)</th>
<th>Chinensis Treatment (model 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>50.458</td>
<td>74.236</td>
<td>73.633</td>
<td>47.968</td>
</tr>
<tr>
<td>$h$</td>
<td>189.990</td>
<td>13.217</td>
<td>67.428</td>
<td>41.899</td>
</tr>
<tr>
<td>$b_F$</td>
<td>0.487</td>
<td>2.146</td>
<td>1.728</td>
<td>0.999</td>
</tr>
<tr>
<td>$b_M$</td>
<td>1.805</td>
<td>0.059</td>
<td>0.301</td>
<td>11.818</td>
</tr>
<tr>
<td>$b_O$</td>
<td>N/A</td>
<td>1.138</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>$\gamma_F$</td>
<td>1.113</td>
<td>1.106</td>
<td>0.834</td>
<td>0.906</td>
</tr>
<tr>
<td>$\gamma_M$</td>
<td>0.168</td>
<td>-0.250</td>
<td>0.060</td>
<td>-0.426</td>
</tr>
<tr>
<td>$\gamma_O$</td>
<td>N/A</td>
<td>0.840</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

TABLE 5. Maximum likelihood parameter estimates from revised models for competitive equivalency of heterospecific males.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\alpha_{12}$</th>
<th>AIC-weight</th>
<th>$\alpha_{21}$</th>
<th>AIC-weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Unstructured</td>
<td>0.552</td>
<td>0.00</td>
<td>1.369</td>
<td>0.00</td>
</tr>
<tr>
<td>(2) Stage-structured</td>
<td>2.767 (1.057-4.511)</td>
<td>0.75</td>
<td>1.672 (1.047-2.295)</td>
<td>0.51</td>
</tr>
<tr>
<td>(3) Sex-structured</td>
<td>4.016</td>
<td>0.21</td>
<td>1.754</td>
<td>0.49</td>
</tr>
<tr>
<td>(4) Sex- &amp; stage-structured</td>
<td>3.669</td>
<td>0.04</td>
<td>7.038</td>
<td>0.00</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION

The models that best fit the data in conditions (i)-(iv) predict that per capita fitness declines with increasing initial density. This is unsurprising from a theoretical standpoint since intraspecific resource competition typically increases at higher densities when holding the amount of a limiting resource fixed. The fitness decline that accompanies increased initial density becomes ever more pronounced as the proportion of females in the initial population increases. This, too, was not unexpected because the pivotal resource, namely oviposition sites, is that over which only female bean beetles directly compete. Even though the amount of resource provided was more than sufficient to meet the needs of all females in each of the experimental populations, females still vie with one another for what they see as optimal sites. It should be remembered that the reproductive burden also becomes heavier for the few conspecific males when female bias is high. As the time and energy of male beetles are limited, any intrinsic impediments to their effectively locating and copulating with female beetles will be magnified in the resulting measures of female per capita fitness.

Reproductive interference appears to be the means by which *C. chinensis* gains competitive leverage over *C. maculatus*. This is especially evident when focusing on changes in the relationship between fitness and density at low female bias (i.e., \( \rho \leq 0.33 \)) in the *C. maculatus* control and treatment conditions. In the control condition excluding heterospecific *C. chinensis* males, per capita fitness declines with increased density in a nearly monotonic fashion. At similarly low female bias in the treatment condition, the decline of fitness with increased density is even more dramatic at low density due to heterospecific harassment. But the more pronounced decrease in the per capita fitness of *C. maculatus* females at low density tapers off quickly and
does not differ significantly from the control condition at higher density levels (≈20 individuals). This result suggests a pivotal threshold at which heterospecific male harassment begins to have a diminished effect. While fitness generally decreases with higher initial density within both experimental conditions, the functional relationship between per capita fitness and density in the treatment condition for *C. maculatus* shows greater concavity (at $\rho \leq 0.33$) than that which appears in the corresponding control condition. Bearing in mind that number of heterospecific males in the treatment condition is held constant, greater density entails increased relative frequency. In the context of this experimental arrangement such positive frequency dependence is the hallmark of reproductive interference: If the density of the interfering species X (e.g., *C. chinensis*) remains constant, the per capita level of interference with species Y (e.g., *C. maculatus*) should and does become diluted as the density and thus relative frequency of species Y increases.

A second general trend emerged in the treatment condition for *C. maculatus*. The gradual decline in per capita fitness with ever larger proportions of female beetles occurred over the entire spectrum of female bias in the treatment condition but not in the control condition. When the proportion of females in a population was greater than $\rho = 0.5$, the predictions of the best fit model (model 3) for per capita fitness in the control condition were virtually indistinguishable from a qualitative standpoint. Contrast this with the treatment condition, where the predictions of the best fit model (model 4) show a gradual but uninterrupted decrease in per capita fitness with increased female bias. The maximum likelihood estimates for the parameter $b$, which occurs in all four of the models tested (Table 4) and is sex-indexed within the sex-structured models (3 and 4), quantifies the disparity between the treatments. This parameter controls the per capita contribution to density dependence with greater values corresponding to lower maximum
recruitment \((N_{t+1})\) and thus lower per capita fitness \((N_{t+1}/F_t)\). For the control condition, \(b_F\) was estimated at a value of 0.487 (0.422-0.561), while \(b_M\) had a value of 1.805 (0.715-4.544). The estimates of \(b_F\) and \(b_M\) in the treatment condition yielded values of 2.146 (1.827-2.528) and 0.059 (0.047-0.065), respectively. In the presence of heterospecific male harassment the negative impact of adding each female conspecific is thus more than four times what it was in the control condition, whereas the negative impact of adding each male conspecific is less than \(\frac{1}{30}\) of what it is in the control.

This finding suggests that countering the negative effects of reproductive interference by means of increased relative frequency can come with a steep ecological and evolutionary price when females alone account for such a density increase. One possible interpretation of this result is that conspecific resource competition is heightened when density increases are due primarily to the addition of female bean beetles. That cannot, however, be a sufficient explanation since we would accordingly expect to see more or less the same global reduction in per capita fitness within the control condition for \(C.\text{maculatus}\). Our analyses revealed the absence of such a trend in the control condition when \(\rho > 0.5\). It is, therefore, likely that the additional fitness reductions we observed at greater female bias in the treatment condition are due to the heterospecific harassment.

In order to address questions about the operative mechanism behind reproductive interference it was necessary to examine the results for the experimental treatments involving \(C.\text{chinensis}\) as the focal (victim) species. Bearing in mind that \(C.\text{maculatus}\) males harass but do not copulate with \(C.\text{chinensis}\) females, we would at minimum expect a much diminished effect of heterospecific males upon the per capita fitness of \(C.\text{chinensis}\) females. Another way to comprehend this point is just to restate it in terms of a hypothetical “decision”: if given a choice
between being housed with either (i) 10 conspecific males and five *C. maculatus* males or (ii) 15 conspecific males, a *C. chinensis* female would on average enhance its fitness by choosing to cohabit with heterospecific males as in option (i). While heterospecific harassment is clearly unwelcome since fruitless, the toll it takes upon the reproductive efforts of a female *C. chinensis* beetle is less than that excised by redundant mating with conspecific males. Our expectations in this regard were borne out by the data (Figure 3 and Figure 4). One prominent example occurs at a low female bias of 0.1. At a density of ten individuals (9 males, 1 female), per capita fitness in the control condition is approximately 40 offspring per female. This increases approximately 20% to nearly 48 offspring per female in the treatment condition. This difference becomes even more profound if we use our data and best fit models to make the hypothetical “decision” discussed above concrete. When female bias is 0.5 in the treatment condition, ten *C. chinensis* individuals are paired with five heterospecific males. This makes for a situation in which five females are faced with 10 male “suitors” (5 conspecific, 5 heterospecific). Female bias drops to 0.33 in this set of circumstances and per capita fitness is approximately 20 offspring per female. The appropriate contrast class within the control condition is one in which \( \rho = 0.33 \) and initial density is 15 individuals. Females are, once again, faced with 10 male “suitors.” But in this case all of the suitors are conspecifics and per capita fitness drops to approximately 17 offspring per female. Such results unequivocally support the findings of previous studies in which actual copulation attempts, as opposed to mere harassment, are implicated as the causal mechanism by which *C. chinensis* gains its competitive superiority over *C. maculatus* (Kishi et al. 2009; Kyogoku and Nishida 2012, 2013; Kishi and Nakazawa 2013; Kishi and Tsubaki 2014).

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2 This imaginary scenario assumes, of course, that there are a sufficient number of conspecific males with whom to copulate and thus assure insemination.
Models that included sex-structure clearly provide a better fit to data in all experimental
conditions. Only in the treatment condition for *C. maculatus* did the sex- and stage-structured
model (4) prove a better overall fit. This model uses mating terms as a proxy for larval density
on the grounds that these terms are proportional to larval densities. Our findings show that
heterospecific reproductive interference between these competing congeners has a negative
effect on the per capita fitness of *C. maculatus* females via a reduction in the number or viability
of eggs oviposited per female. This is, of course, what explains the better overall fit of model 4
to the data in the treatment condition for *C. maculatus*. Further studies are required to determine
why larval competition in the presence of reproductive interference affects emergence since it is
typically high larval densities that are known to negatively impact adult emergence. One is
tempted to speculate that female *C. maculatus* beetles experiencing reproductive interference and
thus fewer or shorter opportunities for oviposition tend to overexploit their host resource by
laying too many eggs per bean when given a chance. There is some extant data to support this
speculation. Kishi and Tsubaki (2014) have found that *C. maculatus* females often enter gaps
between beans to avoid the mating attempts of heterospecific males and *C. maculatus* females
housed with heterospecific males are more likely to oviposit on areas of a bean accessible only
by way of such gaps than *C. chinensis* female counterparts. At any rate, there is clearly much
room for further inquiry. Although the models that we tested introduce additional computational
complexity, our analyses suggest that they do in fact repay the effort by providing a more
realistic formal description of this model system’s observed dynamics. This study also lends
support to recent calls for refining our understanding of interspecies competition. Grether et al.
(2009), for instance, claim the following: “The extent to which interspecific interference
competition has contributed to character evolution is one of the most neglected problems in
evolutionary biology.” Based on our findings as well as those which motivated our study, we find it increasingly difficult to disagree with this general sentiment.
REFERENCES


BIOGRAPHICAL SKETCH

Peter Takacs received a B.A. in Philosophy with a minor in English Literature from the University of West Florida in 2002. He subsequently pursued and acquired a M.A. through the History and Philosophy of Science Program at Florida State University in 2010. The work for this M.S. in the Department of Biological Science (Ecology and Evolution) was completed while concurrently pursuing a Ph.D. in the Department of Philosophy at Florida State University.