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Interspecific Chemical Competition Between *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) Reduces Fecundity and Hastens Development Time

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Abstract

The flour beetles *Tribolium castaneum* (Herbst) and *Tribolium confusum* (du Val) are model organisms for studying the effects of intra- and interspecific competition. Both species are known to compete directly through egg cannibalism and indirectly through the density-dependent release of allelopathic chemicals. To better characterize these indirect interactions, recent work has focused on quantifying the per-capita effects of intraspecific chemical competition. However, the effects of interspecific chemical competition in this system have not previously been estimated. We used experimental microcosms to examine how interspecific chemical secretions affected the reproductive activity and development time of laboratory populations of *T. castaneum* and *T. confusum*. We created replicated habitats containing flour medium that had been occupied and chemically 'conditioned' by one of the two beetle species across a range of densities, then examined how beetles of the other species responded in terms of the number of eggs laid by females and, separately, the development time of offspring. We found that *T. castaneum* fecundity was reduced when beetles experienced flour conditioned by *T. confusum*. In contrast, fecundity of *T. confusum* was largely unaffected by flour conditioned by *T. castaneum*. Additionally, we found that interspecific conditioning decreased development times for beetles of both species, particularly the development of larvae to pupae. Our results indicate that interspecific chemical competition impacts the life history of *Tribolium* species and suggests that models incorporating chemical competition may more accurately describe the biology of flour beetle communities.

Key words: interspecific competition, *Tribolium*, chemical competition, fecundity, development rate

The flour beetles *Tribolium castaneum* (Herbst) and *Tribolium confusum* (du Val) have long been model organisms for understanding competition in populations and communities. Some of the earliest laboratory studies of these species focused on the role of toxic metabolites released by beetles growing at high densities—a phenomenon known to spoil flour for human consumption (Chittenden 1896) and suspected to be a form of chemically mediated competition. For example, Park (1934, 1935) exposed laboratory populations of *T. confusum* to previously inhabited (i.e., 'conditioned') flour, containing waste products and toxic metabolites, and found strong negative effects of conditioning on beetle fertility and development. Since then, numerous studies on both species have documented effects of intraspecific conditioning (i.e., conditioning by the same species) on beetle behavior and life-history traits (e.g., Park 1936, Park and Woolcott 1937, Loconti

and Roth 1953, Sonleitner 1961, Sokoloff 1977, Sonleitner and Gutherie 1991, Flinn and Campbell 2012, Khan et al. 2018).

While the effects of conditioning by conspecifics (i.e., intraspecific chemical competition) are well established, the effects of conditioning by heterospecifics (i.e., interspecific chemical competition) are less clear. The few studies of interspecific chemical competition involving *Tribolium* have focused on short-term, small-scale responses such as movement/orientation (Ghent 1963) or the number of visits to compounds isolated from conditioned flour (Suzuki et al. 1975, Levison and Mori 1983). Only two studies have directly examined the impact of interspecific conditioning on *Tribolium* life history, both of which found strong effects: Polnick (1960) examined how interspecifically conditioned flour affected *T. confusum* and a more distantly related flour beetle, *Latheticus oryzae* (Waterhouse),

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(Coleoptera: Tenebrionidae) finding strong negative effects on fecundity in both species. Similarly, Prus (1961) examined the effects of interspecifically conditioned flour on *T. castaneum* and *T. confusum* and found that the fecundity and development of both species were negatively affected, with *T. castaneum* populations experiencing stronger negative effects. Importantly, both Polnick (1960) and Prus (1961) conditioned flour using only a single, relatively high density of interspecifics. As a result, while they could demonstrate that fecundity was affected by the presence of interspecific conditioning, they were unable to estimate how it would be affected by varying levels of conditioning (i.e., per-capita effects of interspecific conditioning).

Despite preliminary evidence from Polnick (1960) and Prus (1961) showing that interspecific chemical competition can affect demography in the *Tribolium* system, most studies involving multiple species of *Tribolium* consider only non-chemical interactions, particularly cross-species cannibalism (e.g., Park 1948, Park 1954, Park 1957, Park et al. 1965, Sokoloff and Lerner 1967, Leslie et al. 1968, Nathanson 1975, Mertz et al. 1976, Wade 1980, Van Allen and Rudolf 2016). Similarly, theoretical models describing population dynamics of flour beetles, such as the Larva-Pupa-Adult (LPA) model and stochastic Ricker model, tend to include cannibalism as the sole driver of intra- and interspecific competitive dynamics (Neyman et al. 1956, Leslie et al. 1968, Costantino and Desharnais 1991, Edmunds et al. 2003, Desharnais et al. 2005, Melbourne and Hastings 2008, Dallas et al. 2019). Developing more realistic models of *Tribolium* communities that can account for chemical competition first requires moving beyond presence/absence studies and quantifying the per-capita effects of conditioning. This requires examining demographic responses across a range of conditioning intensities (i.e., number of beetles conditioning flour), a step that has largely been completed for estimating the effects of intraspecific conditioning for *T. castaneum* (e.g., Khan et al. 2018) but is missing for *T. confusum* and the crucial interspecific case.

To address this gap, we examined how both *T. castaneum* and *T. confusum* fecundity and development responded to varying degrees of flour conditioning by the other species. To do so, we exposed *T. castaneum* and *T. confusum* beetles to flour conditioned by increasing densities of their interspecific counterpart, representing increasing levels of chemical release. To quantify the effects of chemical competition, we concentrated on the effective reproductive rate of adults exposed to conditioned flour, as well as the development time of eggs laid in such flour. We hypothesized that, similar to the previously observed effects of intraspecifically conditioned flour (e.g., Park 1935, Park 1936), and given the previous results of Prus (1961) and Polnik (1960), interspecifically conditioned flour would reduce fecundity and slow development time.

Materials and Methods

Stock Populations

Laboratory populations of *T. castaneum* and *T. confusum* were used for the experiments. For each species, individuals were sourced from six large (>5,000 individuals) stock populations (held > 2 yr at the University of Colorado at Boulder), all derived from long-running laboratory populations maintained by Robert F. Costantino (University of Arizona). Beginning 2 yr prior to the experiment, stock beetles were kept under constant environmental conditions (29.6°C and 60% relative humidity) and maintained on flour medium, consisting of 95% wheat flour and 5% brewer's yeast. Stock populations were maintained as nonoverlapping generations (Melbourne

and Hastings 2008) such that beetles in each population reached maturity and laid eggs for the next generation after 6 wk, at which time adults were removed, leaving only eggs to begin the next generation (flour medium was also refreshed at this time). To allow experimental work to be evenly spread out across the 6-wk life cycle, stock populations were separated in time such that each population matured on a different week. To minimize genetic drift between stock populations, each week approximately 5% of adult beetles were held over for 7 d and then mixed with the stock population of the subsequent week. Any remaining variation among stock populations was accounted for by a random effect of stock population identity in the statistical model described below.
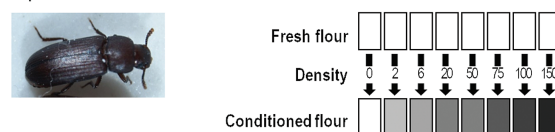
Conditioning Experiments

We conducted two simultaneous experiments: 1) *T. confusum* growing on flour conditioned by *T. castaneum* (the 'CONF-CAST' experiment), and 2) *T. castaneum* growing on flour conditioned by *T. confusum* (the 'CAST-CONF' experiment). Experimental populations were kept in acrylic boxes ('patches'; dimensions: 4.0 cm × 4.0 cm × 6.0 cm). Patches contained two tablespoons of flour medium (15 g; the same type of medium used for rearing the stock populations) and were maintained under the same temperature and humidity as the stock populations.
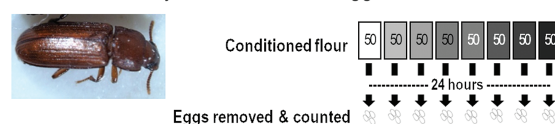
CONF-CAST Experiment

The experimental protocol for the CONF-CAST experiment is illustrated in Fig. 1 and described here. To condition flour medium (Fig. 1, step 1), we added unsexed *T. castaneum* adults in eight possible densities (0, 2, 6, 20, 50, 75, 100, and 150 individuals) creating a 'conditioning intensity' treatment ranging from low to high intensity. These adults were allowed to lay eggs, consume flour, and release chemicals into the patches for 48 h. Density gradients such as this have been shown to produce a gradient of increasing chemical emissions into the flour (Faustini and Burkholder 1987, Verheggen et al. 2007, Duehl et al. 2011, Flinn and Campbell 2012). Conditioning densities were set up in a random order to remove any confounding effects related to order or timing. There were four replicates per

1. Multiple densities of **T. castaneum** conditioned flour for 48 hours.

2. For each conditioning intensity, 50 **T. confusum** were added. After 24 hours, they were removed & eggs were counted.

3. 50 **eggs** were returned to conditioned flour & development was tracked over 36 days.
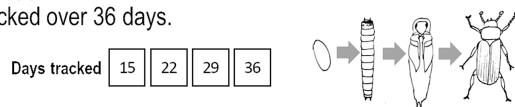
Fig. 1. Design of the CONF-CAST experiment for each of six weekly blocks of the study. For each conditioning intensity (i.e., density of beetles added in step 1), there were four replicates per week. The design of the CAST-CONF experiment was identical, except species identities were reversed.

week of each density for 6 wk. There were thus 24 replicates of each density, for a total of 192 experimental units. After conditioning, adults and eggs of *T. castaneum* were completely removed using a micro-sieve and the conditioned flour medium returned to the patch. To minimize cross-contamination, for both the conditioning phase and the measurement phase (see below), multiple micro-sieves were used and between uses sieves were cleaned with brushes and held at 80°C for at least 10 min.

To measure the effect of conditioning on fecundity (Fig. 1, step 2), 50 adult *T. confusum* were put into each patch with the conditioned flour. After 24 h, these patches were micro-sieved to remove adults and eggs. Adults were discarded. We used the number of eggs laid in a patch as a measure of fecundity. To estimate the number of eggs in a patch, we counted the first 50 eggs and weighed them to estimate the average mass of an egg, then weighed all eggs together, converting this mass into a count.

To measure the effect of conditioning on development (Fig. 1, step 3), 50 eggs from each patch were mixed back into the conditioned flour of the patch they came from (the same number of eggs was used in all patches to hold density constant). We then tracked development of the beetles (from eggs through to larvae, pupae, and adults) in the conditioned flour over the next 36 d using a destructive sampling design. After 14 d of undisturbed development, one of the four replicate patches per conditioning intensity was randomly selected and destructively sampled by sieving. Such sieving is necessary to census patches as individuals hide in flour and must be destructive because sieving patches is damaging to beetles. We repeated this process weekly for 4 wk (i.e., 15, 22, 29, and 36 d after adding eggs back to the flour). After sieving a patch, we counted the numbers of larvae, pupae, and adults.

CAST-CONF Experiment

The CAST-CONF experiment was the same as the CONF-CAST experiment, except the identities of the species were reversed.

Statistical Analyses

For both experiments, we estimated the effect of conditioning intensity on fecundity (total egg count) using a generalized linear mixed effects model (log link and negative binomial distribution representing over-dispersed count data) with stock population identity as a random effect (intercept only) and conditioning intensity (density of the interspecific competitor) as a fixed effect.

We considered the number of individuals in different life stages of complete metamorphosis (from least to most developed: larva, pupa, adult) to be the result of the overall development time within a replicate. We assumed that replicates with a higher proportion of individuals in older life stages were developing faster than those with most individuals in younger life stages. For day 22, individuals in CAST-CONF and CONF-CAST replicates were spread across only one or two life stages (larva and pupa). For day 29, nearly all individuals were spread across two life stages (pupa and adult), with 8 CONF-CAST and 3 CAST-CONF replicates containing only 1 or 2 larvae. Therefore, we modeled the impact of conditioning intensity on development by setting the number of 'successful' transitions to the oldest life stage as the response variable in a generalized linear mixed effects model (logit link and binomial distribution) with stock population identity as a random effect (intercept only) and conditioning intensity (density of the interspecific competitor) as a fixed effect. All day 15 replicates contained only larvae and all but one day 36 replicates contained only adults, and thus, we did not fit models for these data.

All statistical models were estimated using the Bayesian applied regression modeling package 'rstanarm' (Goodrich et al. 2018) in R (R Core Team 2018). We used the default weakly informative priors (Goodrich et al. 2018), as follows: the prior distribution for the intercept was a normal distribution with mean 0 and standard deviation equal to 10, and for the fixed effect was a normal distribution with mean 0 and standard deviation equal to 2.5. Models were run with 4 chains, each with 5,000 iterations (2,500 warmup, 2,500 sampling). Convergence was determined by examining values (Gelman and Rubin 1992), which were always below 1.002. Models were checked using posterior predictive checking (Gelman and Hill 2007, Gelman et al. 2013) using the 'rstanarm' function 'pp_check', which interfaces with the 'bayesplot' package (Gabry and Mahr 2018).

Results

Egg Count

For the CONF-CAST experiment, where *T. confusum* experienced flour conditioned by *T. castaneum*, the 95% highest posterior density interval (HPDI) for the effect of conditioning intensity included 0, suggesting that the effect of interspecific conditioning on reproductive success could not be distinguished from random variation in the data (Table 1, a, Fig. 2a). In contrast, for the CAST-CONF experiment, where *T. castaneum* experienced flour conditioned by *T. confusum*, the 95% HPDI for the effect of conditioning intensity did not include 0 with mean effect –0.0008 per conditioning beetle (log odds; Table 1, b). At the highest conditioning intensity (i.e., conditioning by 150 *T. confusum*), this effect would reduce *T. castaneum* reproduction by 20 eggs compared to zero conditioning (Fig. 2b, Table 1, b).

Development Time

When *T. confusum* experienced flour conditioned by *T. castaneum* (CONF-CAST), 22-d-old beetles were more likely to be pupa than larva, indicating a negative effect of conditioning on development time (Fig. 3a, Table 2, a). However, we did not find a significant effect of interspecific conditioning on the probability of being an adult 7 d later (day 29 HPDI included 0), indicating that such conditioning did not change total egg to adult development time (Fig. 3b, Table 2, b). In the CAST-CONF experiment, however, conditioning intensity had a significant positive effect on the probability that individuals were in an older, more developed stage for both on days 22 (larva to pupa) and 29 (pupa to adult), indicating an overall hastening of development time (Fig. 3c and d and Table 2, c and d).

Discussion

While early studies of *Tribolium* species found evidence of interspecific chemical competition, modern experimental studies involving *Tribolium* as well as mathematical models of their population dynamics (e.g., LPA model; Neyman et al. 1956) rarely consider such interactions. Here, we extend prior work on interspecific conditioning in the *Tribolium* system with two highly replicated experiments across a large range of conditioning intensities, quantifying the per-capita effects of conditioning on both fecundity and development. In our study, when flour was conditioned by *T. confusum* ('CAST-CONF' experiment), there was a significant reduction in the number of *T. castaneum* eggs laid—approximately 20 fewer eggs at the highest conditioning intensity (Fig. 2b). This was consistent with our hypothesis that interspecific chemical conditioning would have

Table 1. Summary of generalized linear mixed effects model of fecundity after 24 h where (a) *T. confusum* experienced flour conditioned by *T. castaneum* (CONF-CAST), and (b) *T. castaneum* experienced flour conditioned by *T. confusum* (CAST-CONF)

	Mean	Standard deviation	95% HPDI[a]
(a) CONF-CAST			
Intercept	5.1252	0.0590	(5.0031, 5.2407)
Conditioning intensity	−0.0003	0.0004	(−0.0010, 0.0004)
Reciprocal dispersion[b]	16.8744	1.8880	(13.3365, 20.6356)
Stock ID standard deviation (random effect)	0.0172	0.0254	(0.0000, 0.0543)
(b) CAST-CONF			
Intercept	5.1907	0.1080	(4.9537, 5.3963)
Conditioning intensity	−0.0008	0.0003	(−0.0014, −0.0001)
Reciprocal dispersion[b]	20.5202	2.3310	(16.1117, 25.1910)
Stock ID standard deviation (random effect)	0.0650	0.0924	(0.0046, 0.1980)

Net fecundity was modeled as a negative binomial random variable, reflecting overdispersed count data. Intercept and fixed-effect estimates are on the log scale.
[a]Highest posterior density interval (i.e., credible interval).
[b]Reciprocal dispersion is a parameter of the negative binomial distribution and can be thought of as a measure of the overdispersion of the data relative to a Poisson distribution (higher values indicate greater overdispersion).
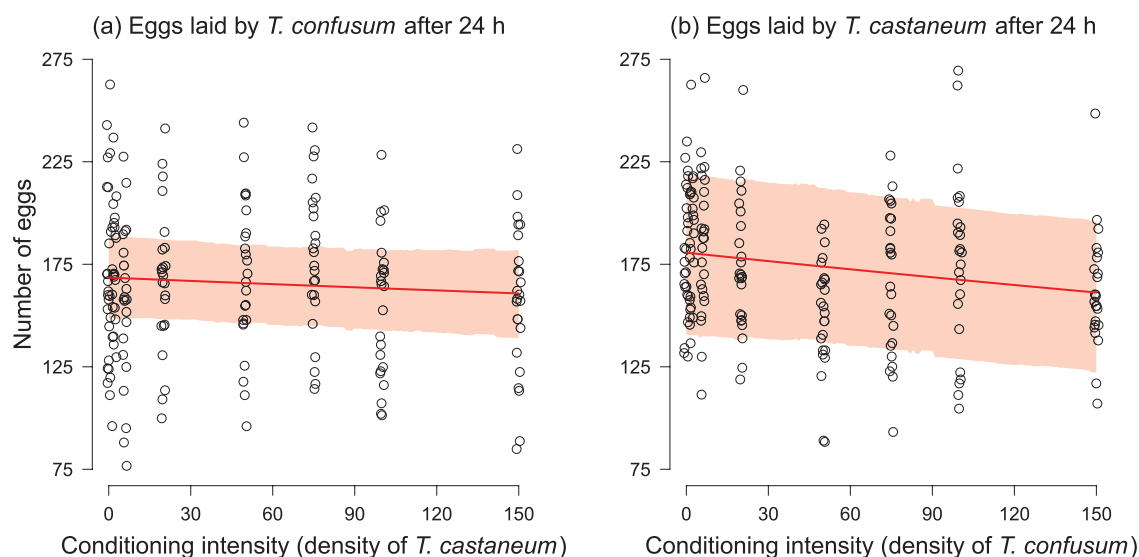
Fig. 2. The effect of interspecific conditioning on fecundity of both species. Points are the number of eggs laid by a population of 50 individuals after 24 h on flour medium with different conditioning intensity (density of interspecific competitor). Panel (a) shows the effect of conditioning by *T. castaneum* on *T. confusum* reproductive activity (CONF-CAST) and (b) shows the effect of conditioning by *T. confusum* on *T. castaneum* reproductive activity (CAST-CONF). Lines represent the mean effect while shading shows the 95% credible intervals for the fitted model (summarized in Table 1). The random effect of stock population identity was added to each point to help visualize the marginal effect of conditioning intensity.

a negative effect on fecundity. In contrast, the 'CONF-CAST' experiment found negligible effects of conditioning by *T. castaneum* on the number of eggs laid by *T. confusum* (Table 1, a; Fig. 2a). With respect to development time, the effects of flour conditioning by an interspecific competitor were more consistent between species, but also counter to our initial hypothesis: interspecific conditioning hastened development of both *T. castaneum* and *T. confusum* (Fig. 3).

Our findings of reduced fecundity due to conditioning are broadly consistent with previous work on chemical competition in the *Tribolium* system, including studies of both intraspecific (e.g., Park 1936, Park and Woolcott 1937) and interspecific conditioning (Polnick 1960, Prus 1961). Several mechanisms have been proposed to explain such effects. In a study of *T. castaneum*, Faustini and Burkholder (1987) found that quinones released by beetles dissolved sex-attractant pheromones, an effect that could reduce matings between beetles and thereby lower fecundity. Multiple studies have also found that *T. castaneum* are 'repelled' by (intraspecific)

conditioned flour and will disperse away from such flour when given the choice (Naylor 1961, Ghent 1963, Ogden 1970). In contrast, dispersal of *T. confusum* from (intraspecific) conditioned flour appears to be depressed relative to unconditioned controls (Ghent 1963, Ogden 1970). Sonleitner and Guthrie (1991) found *T. castaneum* that could not disperse away from conditioned flour laid fewer eggs, suggesting a possible direct effect of conditioning on egg-laying behavior. This direct effect was largely confirmed by Khan et al. (2018), which showed that *T. castaneum* ovipositioning was significantly reduced following only 6 h of exposure to synthetic ethyl and methyl benzoquinones, compounds found in *Tribolium* stink glands and associated with conditioning. Therefore, at the intraspecific level, the effects of chemical release appear to be multifaceted, affecting mating behavior, dispersal propensity, and modulating egg-laying behavior. Our findings suggest at least one of these processes—reduced egg-laying—also occurs at the interspecific level and that its impact scales linearly with conditioning intensity. Mating and dispersal
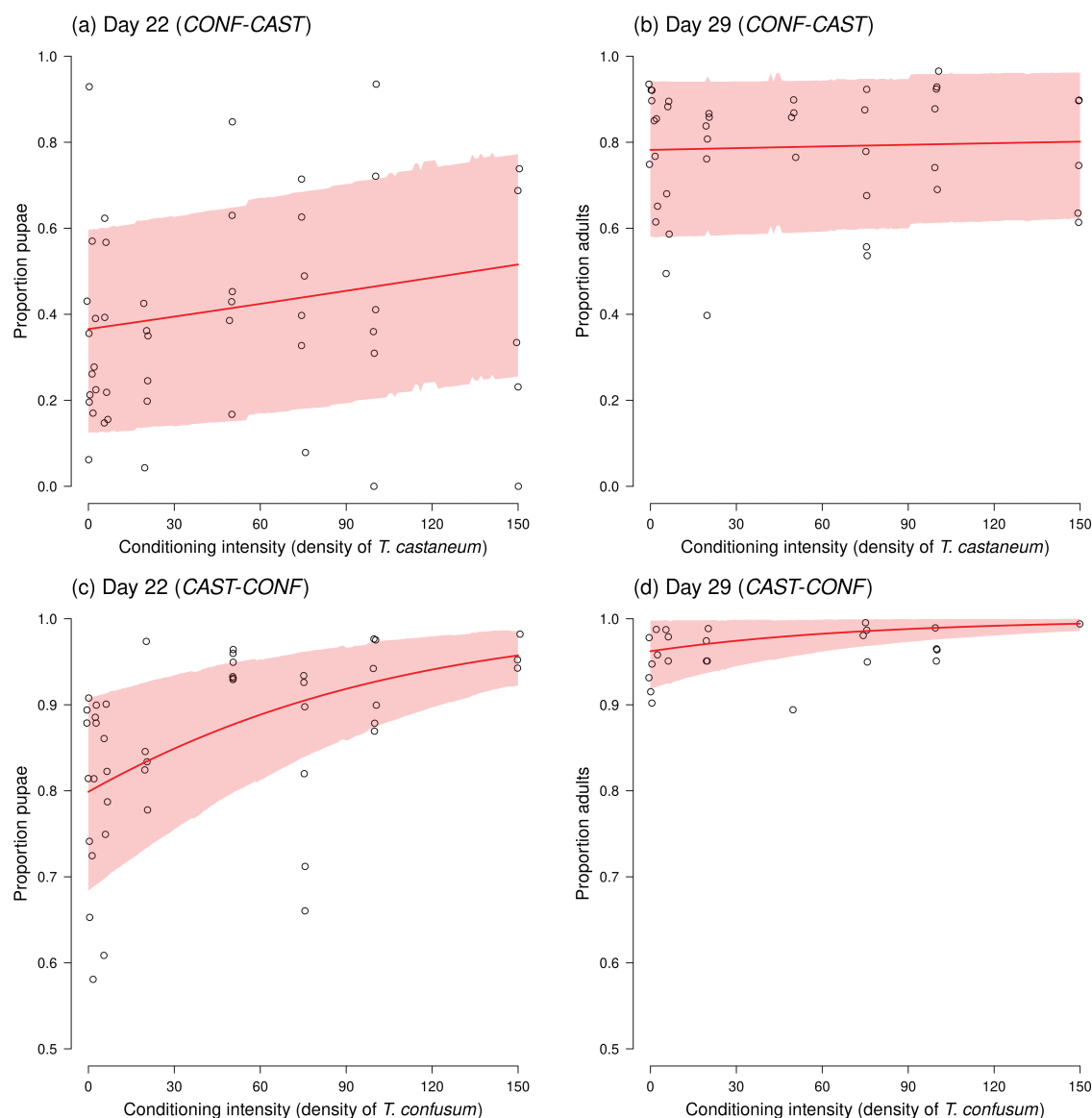
Fig. 3. The effect of conditioning on proportions of beetles in older life stages. Points are the proportion of individuals from populations in the oldest of two life stages on flour medium with different conditioning intensity (density of interspecific competitor). The top row shows the proportion of *T. confusum* in the oldest life stage after (a) 22 d (oldest life stage = pupa) and (b) 29 d (oldest life stage = adult). The second row shows the proportion of *T. castaneum* in the oldest life stage after (c) 22 d (oldest life stage = pupa) and (d) 29 d (oldest life stage = adult). Lines represent the mean effect while shading shows the 95% credible intervals for the fitted model (summarized in Table 2). The random effect of stock population identity was added to each point to help visualize the marginal effect of conditioning intensity.

propensity may also be affected by interspecific chemical release, but to our knowledge no studies have tested for such effects.

Surprisingly, we found that development time, particularly larva-to-pupa development, was hastened by increasing levels of interspecific conditioning. In contrast, Park (1935, 1936) found that beetles developing on conditioned flour matured later than beetles developing on fresh flour. However, in Park's studies, the conditioned flour used in the experiment had been taken from long-running cultures (>100 d). Therefore, in addition to containing compounds associated with chemical competition, the flour was likely also of reduced nutritional quality due to feeding and waste secretion. In contrast, the flour we used had been inhabited for only 48 h and likely differed from unconditioned flour primarily in the concentration of released compounds (i.e., chemical competition) rather than nutritional quality. Thus, our experiment more effectively isolated the

effects of chemical competition. One possible explanation for why development was faster in the conditioned patches for both species is that the compounds associated with conditioning—which would also increase in concentration with increasing densities—are cues for beetles to develop more quickly. Such plastic responses could be advantageous for escaping cannibalism from beetles in older life stages, which removes as much as 99% of new recruits in dense laboratory cultures (Park et al. 1965, Mertz and Robertson 1970, Dawson 1975).

Importantly, *T. castaneum* and *T. confusum* responded differently to interspecific conditioning. Specifically, *T. castaneum* was more strongly affected than *T. confusum* in terms of both fecundity and development time, though clearly conditioning had effects on both species. Absent further data, it is difficult to infer the physiological or behavioral factors that drive this asymmetry. We note that

Table 2. Summary of generalized mixed effects model of development time for *T. confusum* in flour conditioned by *T. castaneum* (CONF-CAST) and *T. castaneum* in flour conditioned by *T. confusum* (CAST-CONF) at two different time periods

	Mean	Standard deviation	95% HPDI[a]
(a) Day 22 (CONF-CAST)			
Intercept	−0.5897	0.5639	(−1.6845, 0.5852)
Conditioning intensity	0.0044	0.0010	(0.0024, 0.0063)
Stock ID standard deviation (random effect)	1.8783	1.4955	(0.3308, 4.6870)
(b) Day 29 (CONF-CAST)			
Intercept	1.3688	0.5868	(0.1665, 2.5024)
Conditioning intensity	0.0008	0.0011	(−0.0014, 0.0029)
Stock ID standard deviation (random effect)	2.0881	1.6135	(0.3754, 5.1113)
(c) Day 22 (CAST-CONF)			
Intercept	1.4188	0.3722	(0.6478, 2.1307)
Conditioning intensity	0.0118	0.0016	(0.0086, 0.0148)
Stock ID standard deviation (random effect)	0.7846	0.6525	(0.1047, 2.0627)
(d) Day 29 (CAST-CONF)			
Intercept	3.4259	0.6532	(2.1444, 4.7710)
Conditioning intensity	0.0134	0.0034	(0.0071, 0.0202)
Stock ID standard deviation (random effect)	2.1706	1.8584	(0.2309, 5.3955)

Proportion of beetles developing was modeled as a binomially distributed response variable reflecting the probability of being in the oldest of two life stages. Intercept and fixed-effect estimates are on the logit scale.

[a]Highest posterior density interval (i.e., credible interval).

in other experiments involving beetles from the same laboratory stock populations, we have found *T. confusum* to be less responsive than *T. castaneum* to high conspecific and heterospecific densities, in terms of population growth rate and dispersal propensity (i.e., achieving both higher population sizes and dispersing less at high densities compared to *T. castaneum*). Thus, while some of the mechanisms remain unclear, for flour beetles raised under our laboratory conditions—which follow one of Park's famous treatments (Park 1948, Park 1954, Park 1957)—asymmetrical responses between species appear to be standard.

Our experiment intentionally did not examine the effects of intraspecific conditioning, which is known to be important in the *Tribolium* system. However, experiments simultaneously testing both interspecific and intraspecific chemical interactions would allow for a more complete understanding of competition between *T. castaneum* and *T. confusum* species and could help determine the relative importance of chemical competition compared to other important drivers such as cannibalism. Detailed knowledge of competitive interactions of *Tribolium* species is particularly important given the historical and continued significance of the *Tribolium* system in population and community ecology. While the most common population models of *Tribolium*, the LPA model (Neyman et al. 1956) and the stochastic Ricker model (Melbourne and Hastings 2008), include density-dependent effects on population growth, these effects are generally considered to correspond to intra- and interspecific cannibalism only. Neither approach explicitly incorporates any effects of intra- or interspecific chemical competition. If the effects of chemical competition are strong, as suggested by our results and the intraspecific results of Khan et al. (2018), and if such effects scale nonlinearly with cannibalism (which we did not test), accurate *Tribolium* models may require additional parameters for the per-capita effects of chemical competition and/or different functional forms. Given that the concentration of released chemicals in a habitat is likely to increase over time, it may also be necessary to develop *Tribolium* models that can account for any time-dependent effects of chemical competition. Future work considering these questions would improve our general understanding of *Tribolium* competition and could aid the development of general multispecies models that could be validated in the

Tribolium system and later applied to the many other systems where chemical competition is important.

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