REPORT for Stiefel Foundation Small Grant Award, 2009

PROJECT: Ecology of slave-maker ants and their hosts:
The effect of geographic variation in parasite and host range on co-evolutionary
trajectories.

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Abstract

Slave-maker ants are specialized social parasites that repeatedly raid colonies of other ant species to acquire brood supplies, which become the slave-maker's work force. Once thought to have little impact on their host, recent work shows varied reciprocal selection strengths between slave-maker social parasites and hosts across populations. This geographic mosaic of co-evolutionary interaction strengths is likely related to variation in host and parasite range. My two recent studies of an Ohio population with two sympatric slave-makers that share a single host suggest high competition between slave-makers produced ecological shifts in both slave-makers and attenuated the co-evolutionary 'arms race' between the evolutionarily older slave-maker and shared host. During 2009, I carried out a laboratory study with slave-makers and their host from the proposed co-evolutionary 'hot-spot' Black Rock Forest[1] to test this hypothesis. Here, *P. americanus*, the prudent slave-maker species in Ohio, has exclusive access to the available host. I found that the demography of NY hosts and slave-makers differed substantially from the Ohio population. Where there is a 1:3:1 ratio of queenless to monogyne (single queen) to polygyne (multiple queen) colonies in Ohio, in BRF there were almost no polygyne colonies. The laboratory arena experiment revealed that *P. americanus* was indeed more virulent in this population, raiding a significantly greater proportion of brood from host colonies in a shortened time span. The production of slave-maker sexuals (gynes and males) in experimental colonies was also significantly fewer, an indication that co-evolutionary arms race in BRF is strong.

Introduction

Competition for shared resources is instrumental in structuring the abundance, distribution and resource use of plants and animals (Lack 1954). Most studies of competition focus on paired interactions between enemies (competitors), between targets (prey or host), or between single coevolutionary interactants (enemy and target). Only recently have theoretical and empirical studies begun to examine the interaction between direct (competitor) and indirect (target) effect of competing enemies on each other and on their targets (e.g., Benkman et al. 2001, Johnson & Herbers 2006, Johnson 2008). Enemy and target relationships are typically predator-prey or parasite-host and follow a coevolutionary 'arms race' trajectory, whereby virulence and defense levels cycle continually (Dawkins & Krebs 1979). Coevolutionary interactions between a single enemy and target tend to vary throughout a species' range due to intrinsic and extrinsic effects, and thus produce a geographic mosaic of reciprocal selection strengths (Thompson 1994, 1999, 2005). The outcomes of multipartite coevolutionary interactions are even more variable (e.g., Siddon & Witman 2003) as more enemy options, i.e., the available range (number) of targets (Thompson & Pellmyr 1992) typically temper exposure to selection and multiple enemies force trade-offs by their targets (Sih et al. 1998).

The importance of parasites in evolutionary biology and ecology has long been understood (Price 1980). However, social parasites have traditionally been overlooked as model organisms for studies of competition and co-evolutionary interactions. They occur in such diverse groups as birds, fish, and social insects, and negatively impact their host by parasitizing behaviors such as brood care, feeding and grooming towards themselves (Wilson, 1971) and manipulating sex ratios (Bourke, 1989). Social parasites are often closely related phylogenetically to their hosts and have similar generation times and

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[1] Separate funds were acquired for a replicate study in Huyck Preserve during 2009 that will eventually be analyzed with BRF findings, but these data are presented only marginally here.

ecological necessities. Thus, they are more susceptible to cyclical reciprocal selection dynamics than the traditional endo- and ecto-parasites. The avian brood parasites, such as the brown-headed cowbird, have been well studied partly because of their negative impact on songbird populations (Brittingham & Temple, 1983; Robinson et al., 1995). Hymenopteran social parasites are less well understood, but recent work on slave-maker ants dispels past assumptions that their effect on host populations due to their relative rarity is negligible (Hare & Alloway, 2001, Foitzik et al., 2001; Herbers & Foitzik, 2002; Johnson & Herbers, 2006; Johnson, 2008).

Slave-maker ants are specialized social parasites that replenish their host supply by repeatedly raiding colonies of other ant species for their brood. Slave-maker species were once thought to have little impact on their host because of their relative rarity. However, behaviors of host and slave-maker, colony social structure, and sex allocation ratios have been shown to vary among populations, as does the number of interacting species, which indicate intimate co-evolutionary interactions between species (Foitzik & Herbers, 2001; Johnson & Herbers, 2006; Johnson 2008). The overall goal of my research is to examine how variation in host and slave-maker range (i.e., the number of interacting species) across the geographic landscape impacts respective co-evolutionary interaction strengths. The focal taxon is the acorn ants (Myrmicinae: Formicoxenini), which contains several species of slave-makers that nest inside preformed cavities of slightly aged sticks and various nuts found on the forest floor. Two of these slave-makers (*Protomognathus* [= *Harpagoxenus*] *americanus* [Emery], *Temnothorax duloticus* [Wesson]) are widely distributed in deciduous forests throughout the northeastern United States and southeastern Canada, where they share three closely related host species in the genus *Temnothorax*[2] in different frequencies (Talbot 1957, Alloway 1980, Alloway et al., 1982). My past research has shown that where these slave-makers are sympatric (co-occur) and share a single host, high competition between slave-makers produced ecological shifts in both slave-makers and attenuated the co-evolutionary 'arms race' between the evolutionarily older slave-maker and shared host (Johnson & Herbers, 2006; Johnson 2008): 1) initial raiding times were asynchronous, which afforded a priority advantage to the early raider, 2) intra-specific raiding occurred only among prudent parasite colonies that led to larger single prudent slave-maker colonies that may offer protection against the competing slave-maker species, and 3) there was a higher mutual tolerance of sexuals of both the slave-maker and the host in prudent parasite colonies, which suggests an attenuated arms race for the 'weaker' competitor.

During the summer of 2009 with funds from the Stiefel Foundation Small Grant Award, I carried out a study in the putative co-evolutionary 'hot-spot', Black Rock Forest, that was designed to test the above hypothesis with the slave-maker *P. americanus,* which has exclusive access to the available host. First, I found that the demography of NY hosts and slave-makers differs substantially from the Ohio population of hosts and slave-makers. In NY, both host and slave-maker colonies contained fewer individuals than in OH and a higher proportion of the population of host and slave-maker colonies were queenless. Host colonies in Black Rock Forest were also significantly less likely to have multiple host queens. Second, the laboratory arena experiment revealed that the NY *P. americanus* were more virulent than OH *P. americanus*. These results suggest that the relatively vast contiguous forest provides ample habitat for these ants and that without a competing slave-maker rival, the slave-maker species that is a prudent parasite in OH exerts strong selective pressure on the exclusive host in Black Rock Forest, indicating an active and strong co-evolutionary arms race.

Study System

The slave-maker ant *P. americanus* is widely distributed throughout the northeastern U.S. In Black Rock Forest, the slave-maker *P. americanus* has exclusive access to its host *Temnothorax longispinosus*. The lack of competitors (Sih et al., 1998) and restricted host use (Kawecki, 1994, 1998) make this site a likely co-evolutionary 'hot-spot', where reciprocal selection is strong. In central Ohio, the presence of the competing slave-maker *Temnothorax duloticus* appears to have shifted the ecological dynamics from those in the eastern population. In OH, *T. duloticus* raids earlier in the season than *P. americanus* and consequently, wiped out *P. americanus* in both laboratory and field experiments and quickly decimated its host before the seasonal production of host sexuals, leaving little opportunity for

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[2] These species along with *T. duloticus* have been recently transferred from the genus *Leptothorax* (Bolton, 2003).

host recovery. Thus, in OH, there is strong selection pressure on the host to evolve defenses in response to *T. duloticus* and being parasitized by *P. americanus* may offer relative benefits. The delayed and attenuated impact on the experimental host population when subjected to both slave-makers along with an inverse relationship in the proportion of the two slave-maker species in natural populations in OH suggest direct asymmetrical antagonism between slave-makers, the advantage of which belonged to *T. duloticus* (Johnson & Herbers, 2006). The asymmetrical antagonism between slave-makers appears to have weakened the antagonistic co-eveolutionary relationship between *P. americanus* and the shared host and selected for a more mutualistic relationship compared to the eastern population (Foitzik & Herbers, 2001). The prudent parasite has a less destructive impact on its host and protects future host supplies by rearing host sexuals (Johnson & Herbers, 2006, Johnson, 2008). However, when raiding 'motivation' of both slave-makers were synchronized, *P. americanus* emerged as the better direct competitor, decimating *T. duloticus* colonies and taking the brood. This indicates that raiding phenology provides *T. duloticus* the competitive advantage and that reduced virulence in *P. americanus* is a function of virulent parasite pressure. In NY, there is no antagonism from a competing slave-maker and therefore the dynamics should differ and the co-evolutionary arms race between slave-maker and host should be strong.

Methods
Colony collections
      During May 2009, colonies of the slave-maker *P. americanus* and of its primary free-living host in the region, *T. longispinosus,* were collected from Black Rock Forest, censused and settled between two glass slides (7.5 cm x 2.5 cm) separated by semi-translucent rubber (1.5 mm thickness) that had been carved out to create a small cavity. Slide nests were placed into Petri dishes (8.5 cm diameter), where ants had access to food and water until testing time. From each colony, several ants were preserved in 75% or 95% ethyl alcohol for voucher or future genetic analysis, respectively.

Laboratory Experiment

Experimental design
      To assess the impact of parasite and intra-parasite competition on the host, one or two parasite nests were introduced to laboratory arenas (16 cm h x 52 cm l x 35 cm) that contained one queenless and three monogyne (single-queen) nests of *T. longispinosus* of similar size. Slave-maker nests were placed in the arena center or off-center approximately 4 cm from the walls lengthwise in the paired slave-maker treatment facing outwards. Feeding stations that consisted of a 0.5 cm^2 piece of aluminum foil were placed alongside each nest and replenished with food every other day. Each arena also contained additional glass tube nesting sites (6.5 cm l; 4 mm o.d.) to provide ants the opportunity to relocate. Arena bottoms consisted of Plaster of Paris and sides were coated with Vaseline and double-sided tape to prevent ant escapes. Ants were fed the Bhatkar and Whitcomb (1970) diet and frozen *Drosophila.*
      Treatments were replicated four times for each population for a total of 24 trials (12 for each population). All treatments of a single replicate were activated on a single day, with the four replicates for each population starting within seven days of each other. Arenas were censused daily for the number of queens, workers, and juveniles of each species 1) in each nest to determine the parasite impact on free-living and captive hosts; 2) in the arena and on container sides to account for missing individuals within nests; and 3) in the arena and on container sides clasping another. Mortality counts along with changes in brood number between census dates relative to total brood loss provided a measure of colony raiding in the absence of direct observation.
      Trial lengths varied within and among treatments. Trials were terminated when worker numbers from free-living host colonies fell 90% below original numbers. If both slave-maker colonies remained in an arena, the trial was continued until one slave-maker colony raided the other colony. Some trials never met these criteria but were terminated eventually.

Statistical Procedures
      Distributions were tested for normality and heteroscedasticity. Data that violated these assumptions were transformed or subjected to non-parametric tests accordingly. Colony demographics were analyzed using repeated measures analysis of variance (RMA) on the proportion of individuals

(workers or juveniles) that remained in nests over time to assess the impact of parasite on parasite or host colonies (workers and brood). The square roots of proportional data were arcsine transformed to normalize the data; 'arena' was a random effect and 'condition' and time were fixed effects.

Multivariate regressions were used to determine what initial factor (ratio of captive host workers to parasites, sum of captive host workers and parasites, or juveniles in parasite nests) determines the impact on the enclosure host population. Tukey's HSD (honestly significant differences) and sequential Bonferroni correction (Rice 1989) were used for pairwise comparisons and multiple comparisons, respectively.

Results

Slave-makers were found in 13% of the 192 colonies collected from Black Rock Forest and 5% of the 416 colonies collected from Huyck Preserve during May and June 2009 (Table 1).

Table 1. The number and proportion of slave-maker and host colonies collected from Black Rock Forest (BRF) and Hyuck Preserve (HP).

	Colonies	
	Slave-maker	Host
2009		
BRF	25 (13%)	167 (87%)
HP	20 (5%)	396 (95%)
2008		
BRF	56 (16%)	295 (84%)
HP	22 (8%)	240 (92%)

Black Rock Forest had fewer queenless colonies of *T. longispinosus* than Hyuck Preserve, which approximated the proportion of queenless, single queen and multiple queen colonies found in OH, suggesting the relatively large contiguous forest provides ample nesting sites and reduced competition for those sites. There were also fewer queenless slave-maker colonies in BRF (Table 2).

Table 2. Number and proportion of queenless, single queen and multiple queen colonies of the host *T. longispinosus* and average number of workers in these colonies from Black Rock Forest (BRF) and Hyck Preserve (HP) in 2008 and 2009 and central Ohio 2005-2006.

		Queen Number		
2008		**0**	**1**	**>1**
	N	165 (49%)	149 (44%)	22 (7%)
BRF	Mean	14.78	30.77	24.45
	SD	10.71	34.85	30.89
	N	89 (26%)	162 (46%)	98 (28%)
HP	Mean	15.64	27.18	36.77
	SD	8.83	23.50	37.36
2009				
	N	102 (62%)	63 (38%)	1 (.006%)
BRF	Mean	16.23	28.48	43
	SD	10.76	20.95	
	N	106 (27%)	212 (54%)	78 (20%)
HP	Mean	16.11	23.97	26.96
	SD	9.66	16.12	17.47
2003 - 2005				
	N	346 (22%)	926 (60%)	282 (18%)
Ohio	Mean	26.85	41.87	56.11
	SD	21.72	35.82	48.98

Parasite impact on host colonies

Slave-makers had a significant effect on the decline of juveniles in arena host nests (RMA: $F_{2, 94} =$ 25.13, $P < 0.0001$) (Figure 1a). The end average loss in arena host colonies with a slave-maker present was 1 1/2 (single slave-maker colony) to 6 two slave-maker colonies) times more than the end loss of host colonies in arenas with no slave-maker present (Tukey's HSD = 2.39, $P = 0.05$). The majority of juvenile loss in host colonies occurred within one day after the onset of each trial (RMA: $F_{7, 89} = 9.35$, $P < 0.0001$, Tukey's HSD = 3.11, $P = 0.05$).

Slave-makers also had a significant effect on the decline of workers in arena host nests (RMA: $F_{2, 81} = 24.44$, $p < 0.0001$) (Figure 1b). However, a single colony of slave-maker had much smaller effect than two slave-maker colonies (Tukey's HSD = 2.39, $P = 0.05$). The majority of juvenile loss in host colonies occurred within the first two days after the onset of each trial (RMA: $F_{7, 89} = 9.35$, $P < 0.0001$, Tukey's HSD = 3.11, $P = 0.05$). The impact of two slave-maker colonies on the survival of host colonies queens was also significantly greater than a single or no slave-maker colony (RMA: $F_{2, 23} = 15.77$, $p < 0.0001$, Tukey's HSD = 2.55, $P = 0.05$).
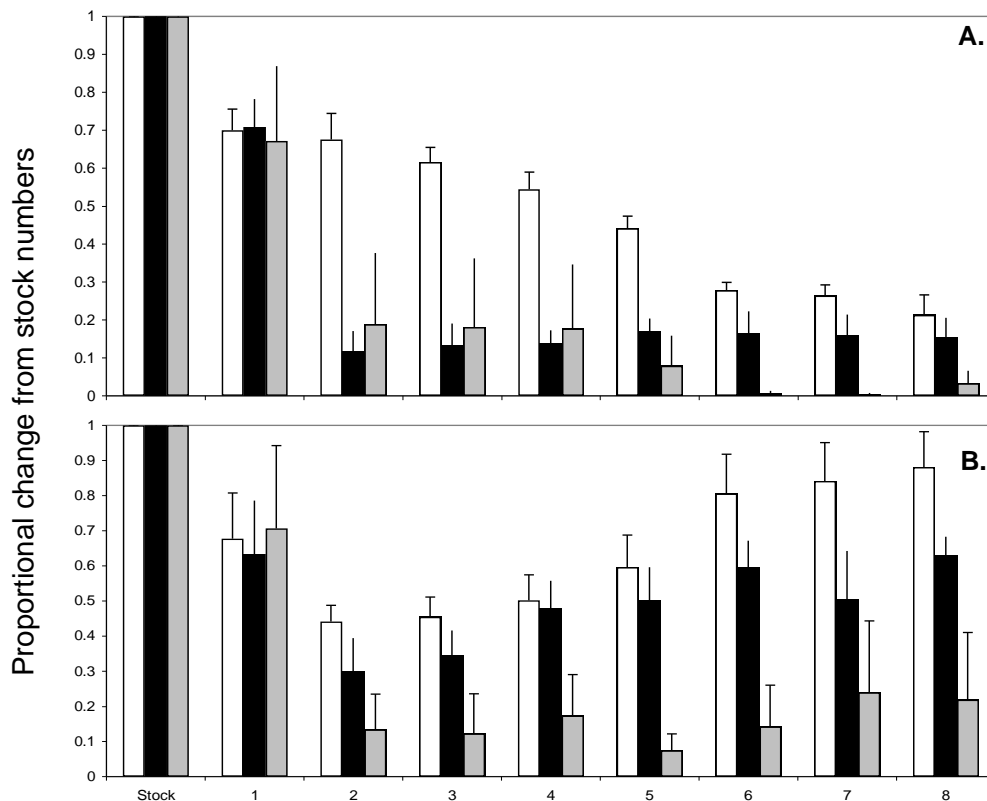

Figure 1. Mean proportion of stock **A.** juveniles and **B.** host workers in arena host colonies over time. White bars = no slave-maker arenas; black bars = 1 slave-maker colony arenas; grey bars = 2 slave-maker colony arenas. colony arenas.

Population differences in parasite impact on host colonies

The comparison of parasite impact on host juveniles and host workers between NY and OH revealed that NY slave-makers had a significantly greater impact on host than OH slave-makers, which included the virulent slave-maker *Temnothorax duloticus* (RMA-juveniles: $F_{1, 143} = 36.3$, $p < 0.0001$;

RMA-workers: $F_{1, 143} = 32.19$, $p < 0.0001$) (Figure 2). A between population comparison of juveniles remaining in host colonies on Census Day 6 only with two *P. americanus* colonies also showed that OH *P. americanus* had a much greater effect on host colonies (t-test = $|-6.18|$, $p = 0.0002$). Slave-maker impact on host workers on Census Day 6 was not as great (t-test = $|-2.33|$, $p = 0.052$).
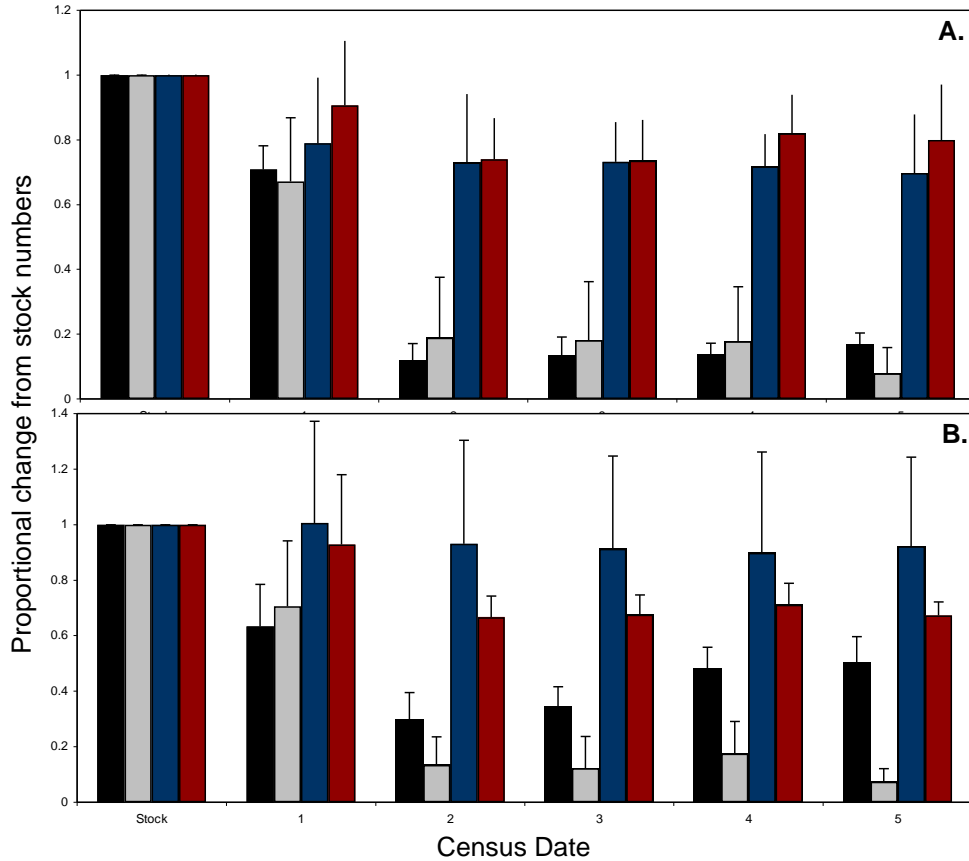

Figure 2. Average proportion of stock **A.** host workers and **B.** juveniles in arena slave-maker colonies over time. Black bars = one **NY** *P. americanus* ('prudent' slave-maker) colony arenas; grey bars = two **NY** *P. americanus* ('prudent' slave-maker) colony arenas; blue bars = two **OH** *P. americanus* ('prudent' slave-maker) colony arenas; red bars = one **OH** *Temnothorax duloticus* ('virulent' slave-maker) colony arenas. Note the significantly greater impact of *P. americanus* in NY compared to OH. The impact is even greater than the more virulent OH slave-maker species.

Although the average colony size of the host species in Ohio (x = 41, SD = 37.3) is greater than the colony size of the host species in NY (x = 23, SD = 15.6) (t = 11.29, df = 2098, $p = 0.0001$), the mean ratio of slave-maker colony individuals to host colony size is the same (t-test = $|-0.18|$, $p = 0.86$) in parasitized colonies (Table 3). Thus, it is likely that the probability for interactions among individuals is equal in both populations.

Table 3. Number of queenless, single queened and newly-founded slave-maker colonies and the average number of slave-maker and host workers in theses colonies.

	N	Queen N	Slave-makers	Host Workers
	14	0	1.9 (0.8)	15 (11.7)
NY BRF	5	1	4 (1.22)	55.2 (26.6)
	5	Newly Founded		
	7	0	4.4 (3.7)	50 (33.4)
NY HP	9	1	4.8 (2.6)	51.3 (37.1)
	3	Newly Founded		
	41	0	4.7 (4.4)	31 (21.4)
	55	1	8.3 (5.2)	71.3 (43)
OH PA	1	2	24	42
	10	Newly Founded		
	29	0	9.6 (7.8)	37.8 (39.7)
OH TD	31	1	15 (10.3)	54.6 (36.3)
	3	Newly Founded		

Production of new queens & males.

Production of new sexuals (queens and males) is a primary measure of survival fitness. In BRF, it appears as though there is reduced tolerance of sexuals by both host and slave-maker alike as compared to the OH *P. americanus* slave-maker and host (Table 4). This too suggests that there are strong antagonistic co-evolutionary interactions between BRF *P. americanus* and its host; the BRF host can detect and destroy slave-maker reproductive juveniles in defence against the more virulent BRF slave-maker, whereas the OH host rears OH *P. americanus* sexuals as the host needs and evolved the ability to detect sexuals of the more virulent slave-maker or host fitness is higher in colonies of the OH *P. americanus*.

Table 4. Number of new slave-maker gynes and males reared.

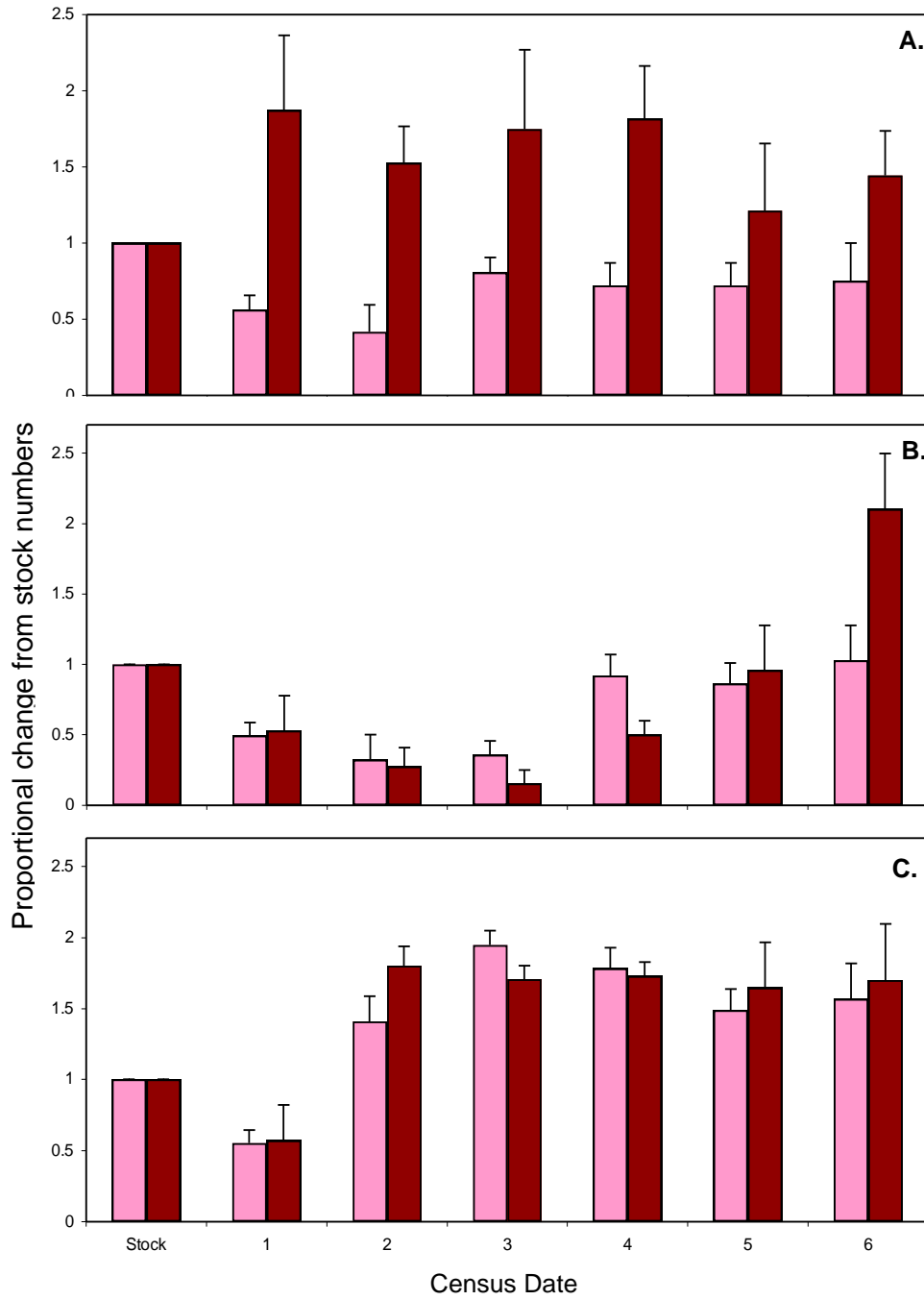| Population	Treatment	Gynes	Males
NY	Single *P. americanus*	0	0
	Paired *P. americanus*	1	6
OH	Single *P. americanus*	NA	NA
	Paired *P. americanus*	143	155
	Single *T. duloticus*	0	0
	Paired *T. duloticus*	`1	76

Slave-maker interactions.

Figure 3. Average proportion of stock **A.** slave-makers, **B.** host workers and **C.** juveniles in arena slave-maker colonies over time. Pink bars = one *P. americanus* colony arenas; red bars = two **NY** *P. americanus* colony arenas.

DISCUSSION

The results from this study reveal that the slave-maker *P. americanus* has a significantly greater negative impact on its host when not in competition with the more virulent slave-maker *T. duloticus*. In other words, *P. americanus* is in a hot-spot of co-evolutionary interactions with its host in the Black Rock Forest area, where it is released from inter-specific slave-maker interactions and competition for access to its host.

In central Ohio, *P. americanus* competes for a shared host with *T. duloticus*. In this population, *T. duloticus* raids earlier than *P. americanus*, is therefore able to and does carry out pre-emptive raids against *P. americanus*, and reaches and decimates host colonies prior to the onset of raiding by *P. americanus*. The impact of this virulent competitor on the shared host and on *P. americanus* (directly and indirectly) appears to have selected for an attenuated parasite-host relationship between *P. americanus* and host (Johnson & Herbers, 2006; Johnson 2008). The results of this study show that *P. americanus* in Black Rock Forest is not only more virulent than the *P. americanus* in OH, but is also more virulent than the virulent slave-maker *T. duloticus*. BRF *P. americanus* raided significantly more juveniles than OH *P. americanus* (Figure 3). BRF *P. americanus* also had comparatively a much earlier impact on the decline of the laboratory host population as intra-specific *P. americanus* interactions occurred either later in the experiment or did not have priority over raiding hosts (Figure 3). Because BRF slave-makers had significantly lower tolerance for host reproductives, earlier raiding as a function of lower intra-specific slave-maker interactions leaves the host with fewer opportunities to 'reproduce' and produce new colonies.

The low rate of polygynous (multiple queen) host colonies in BRF compared to OH and Huyck Preserve suggests an abundance of forest in BRF, where there is little competition for suitable nest sites. This may account for a greater number of slave-maker colonies and the higher proportion of slave-maker colonies in BRF than in Huyck Preserve. It also is likely to account for the greater degree of queenlessness in BRF host colonies, as single queen colonies are more likely to become queenless than multiple queen colonies. This would suggest that the polygyny of host colonies associated with slave-maker hosts may be due primarily to nest-site limitation but secondarily serve to facilitate host colony survival.

In sum, demographic differences and the results from this laboratory experiment suggest a strong arms race between prudent slave-maker and host in NY. This also supports the hypothesis that the addition of a more virulent competitor can attenuate this evolutionarily older relationship between slave-maker and shared host. Clearly the number of interacting parasites and hosts affects co-evolutionary outcomes.

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Budget Justification

Below is details how funds were spent.

Accounting for Black Rock Preserve Funds

batteries	$ 6.52
car rental	$2,271.84
forceps	$ 274.11
gas	$ 589.92
misc	$ 32.25
plaster of paris	$ 30.98
plastic bags	$ 82.32
plastic boxes	$ 260.81
tape/plaster	$ 48.92
toll	$ 114.25
tools	$ 5.39
vaseline, tape	$ 8.88
Total	**$3,726.19**