Optimization of host species raiding preference relative to temperature and host defensive capability by the kidnapper ant *Temnothorax americanus*

by

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Abstract

Kidnapper ants raid host-species colonies, abscond with brood, and raise host workers that work for the kidnapper colony. *Temnothorax americanus* uses *Temnothorax ambiguus*, *Temnothorax curvispinosus* and *Temnothorax longispinosus* as hosts. In southern Ontario, *T. curvispinosus* colonies are absent, while free-living *T. ambiguus* colonies occur in warm microhabitats and *T. longispinosus* colonies occupy cooler microhabitats, presumably achieving optimal performance in species-typical microhabitat. In choice tests *T. americanus* preferentially selects pupae of the host species whose temperature optimum is opposite to the temperature conditions of the *T. americanus* colony, possibly reflecting a preference to retrieve the least well-defended host species relative to temperature. I tested whether that temperature-dependent pupal retrieval preference extends to raiding preference for whole colonies and whether the defensive capability of *T. ambiguus* and *T. longispinosus* against *T. americanus* raids depends on temperature. I acclimatized *T. americanus* colonies and their hosts to 25°C or 15°C in controlled environment chambers and offered simultaneous choices of standardized nests of the two host species to assess any effect of temperature on host species raiding preference. I also allowed *T. americanus* to raid each host species within either temperature and recorded the numbers of host species casualties, kidnapper casualties, salvaged brood and captured brood, and the length of raids to assess any effects of temperature and species on defensive capability. I also tested for associations between kidnapper colony composition and raiding success. There was no effect of temperature or host species on raiding preference or host defensive capability, suggesting that host species are raided without preference and are equally well defended regardless of temperature. Recent prior raiding experience did increase kidnapper ant success in raids and larger *T. americanus* colonies captured more brood. Defensive capability does not appear to

drive the documented temperature-dependent pupal retrieval preference. It is possible that temperature instead influences the volatility of the cuticular-hydrocarbon profiles of the pupae of the host species differentially, thus affecting the attractiveness of the pupae to kidnapper colony members, though future work is needed to determine exactly what drives this temperature-dependent pupal retrieval preference.

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Chapter 1: General Introduction

Social parasitism is an alternative strategy to traditional parental care whereby the social parasite exploits the labour of their hosts to raise the parasite brood (Hölldobler & Wilson, 1990; Payne, 1977). Liberated from energetic investment in rearing young, the social parasite accrues a fitness benefit (Hölldobler & Wilson, 1990). Brood parasitism is known across a wide range of taxa including birds, fish and the eusocial Hymenoptera, and is particularly common among ants (Hölldobler & Wilson, 1990; Payne, 1977; Taborsky, 2001). Within the ants there are varying degrees of social parasitism but the most developed forms include the workerless inquilines and dulotic kidnapper ants, formerly known as slave-making ants (Buschinger, 1986). Kidnapper ants are social parasites that send out raiding parties to nearby host nests, where they abscond with host brood and, typically, return that brood to their own colony (Alloway, 1979; Hölldobler & Wilson, 1990; Wilson, 1975). When host brood eclose within kidnapper ant colonies, they become fully functioning members of the colony, performing duties such as foraging, nest construction, raiding of host colonies and rearing of both host and kidnapper brood (Hölldobler & Wilson, 1990; Stuart & Alloway, 1985). There are both facultative kidnappers where the colony can survive on its own without a host workforce and obligate kidnappers which require a host workforce for survival (Mori, Grasso, Visicchio, & Le Moli, 2001). In obligate kidnapper ants an evolutionary process known as domestic degeneration has occurred where kidnapper workers have lost the ability to perform any domestic tasks and instead are specialized exclusively for raiding (Stuart & Alloway, 1985). Thus, other than social parasite worker replenishment of the host workforce, colony productivity depends almost exclusively on the host workforce. There are many kidnapper species that have multiple potential hosts, but some, like *Polyergus lucidus* Mayr, will prioritize one host over others, and never use both hosts at once

(Goodloe, Sanwald, & Topoff, 1987), while others, such as *Temnothorax americanus* (Emery), exploit multiple host species in their colony simultaneously (Alloway, 1979).

Animals are expected to behave in a manner that optimizes their fitness (Stephens & Krebs, 1987; Maynard Smith, 1978; Orians & Pearson, 1979). Ant colonies are characterized by mechanisms that optimize the performance of various tasks or collection of resources (Hölldobler & Wilson, 1990). Ants optimize the way they forage (Detrain, Tasse, Versaen, & Pasteels, 2000; Shaffer, Sasaki, & Pratt, 2013), find new nest sites (Visscher, 2007), arrange their social networks and organization (Pinter-Wollman, Wollman, Guetz, Holmes, & Gordon, 2011; Wakano, Nakata, & Yamamura, 1998) and partition tasks (Burd & Howard, 2008). In kidnapper ants, host colonies serve as a resource for potential future host workers and thus raiding behaviour should be optimized. *T. americanus* colonies from communities with dense host populations or that have high degrees of cooperation are more likely to guard the nest entrance of host nests during raids and subsequently capture more brood, thus optimizing their raiding behaviour compared to colonies from low host density communities or with low degrees of cooperation (Foitzik, DeHeer, Hunjan, & Herbers, 2001; Miller, 2017). With multiple host species available, *T. americanus* colonies preferentially raid minority hosts compared to the numerically dominant host species, which have evolved superior defensive capability due to higher parasite pressure (Brandt & Foitzik, 2004). Brandt and Foitzik (2004) also showed that when presented with two host species from different communities, *T. americanus* colonies would preferentially raid the host species from the community with less parasite pressure and thus less adapted defenses, suggesting that the kidnappers are able to make the optimal choice of raiding the least well-defended host even when they do not have prior experience with both host species. Thus, when there are multiple host species available, kidnapper ants likely optimize their raiding preference for which host species they select based on host defensive capability. Other ecological and abiotic factors besides parasite pressure may also influence host species defensive capability and thus kidnapper ant raiding preference, however, to my knowledge, this has never been studied.

According to the geographic mosaic of coevolution theory, the interactions of populations will vary across space due to changes in the abiotic and biotic environment (Thompson, 1999). This variation in population interactions across spatial scales leads to differing selection pressures in isolated populations, such that each population will evolve along separate coevolutionary trajectories (Thompson, 1999). Spatial variation in the ratios of hosts to kidnappers and in host and kidnapper fauna creates a geographic mosaic of host-parasite selection pressures, resulting in different coevolutionary trajectories (Brandt & Foitzik, 2004; Foitzik et al., 2001; Johnson & Herbers, 2006). Ant colonies also experience spatial variation in microhabitat patches related to temperature, humidity, light intensity and other factors that can affect their performance and productivity (Queiroz, Ribas, & França, 2013). As ectotherms, the performance of ants depends heavily on temperature in particular (Porter & Tschinkel, 1987). Behaviourally dominant species operating outside of their thermal optima can even be outcompeted by subordinate species (Bestelmeyer, 2000; Cerdá, Retana, & Cros, 1997). More productive and better performing colonies are presumably better at defense, allowing kidnapper ants to take advantage of these microhabitat temperature preferences and optimize their raiding preference based on differential defensive capabilities.

Temnothorax americanus is an obligate kidnapper ant native to eastern North America which uses *T. ambiguus* (Emery), *T. longispinosus* (Roger) and *T. curvispinosus* (Mayr) as hosts, though populations in the northern limits of its range use only *T. ambiguus* and *T. longispinosus* as they are the only host species present (Alloway, 1979; Creighton, 1950). All three host species also engage in facultative intraspecific and interspecific kidnapping (Alloway, 1980). *T. americanus* colonies commonly contain multiple host species simultaneously (Alloway, 1979). Long-term collection records from Thomas M. Alloway and his students in Southern Ontario reveal that *T. ambiguus* is found predominantly in open areas, where it is warmer due to direct sun exposure, and that *T. longispinosus* colonies are more abundant in shaded areas, where it is cooler (T.M. Alloway, personal communication, May 2019), presumably achieving optimal performance in species-typical microhabitat. In choice tests, *T. americanus* preferentially selects pupae of the host species whose temperature optimum is opposite to the temperature conditions experienced by the *T. americanus* colony (Novotny and Hare, in prep). This documented preference may reflect a preference of the kidnapper to raid the least well-defended host species relative to the microhabitat temperature (Novotny and Hare, in prep). These ants thus offer a perfect model system to test for the first evidence of optimization of raiding preference based on differences in host microhabitat temperature optima and defensive capability. In the second chapter of my thesis, I investigate whether *T. americanus* shows a temperature-dependent raiding preference for one of its two host species, *T. longispinosus* and *T. ambiguus*, by using simultaneous presentation choice trials in both warm and cool temperature conditions. In the third chapter of my thesis, I quantitatively measure host species defensive capability and *T. americanus* raiding success relative to temperature using raids on single host colonies in both warm and cool temperature conditions.

References

Alloway, T. M. (1979). Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). *Animal Behaviour*, *27*, 202–210. https://doi.org/10.1016/0003-3472(79)90140-4

Alloway, T. M. (1980). The origins of slavery in Leptothoracine ants (Hymenoptera: Formicidae). *The American Naturalist*, *115*, 247–261. https://doi.org/10.1086/283557

Bestelmeyer, B. T. (2000). The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. *Journal of Animal Ecology*, *69,* 998–1009. https://doi.org/10.1046/j.1365-2656.2000.00455.x

Brandt, M., & Foitzik, S. (2004). Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*, *85,* 2997–3009. https://doi.org/10.1890/03-0778

Burd, M., & Howard, J. J. (2008). Optimality in a partitioned task performed by social insects. *Biology Letters*, *4*, 627–629. https://doi.org/10.1098/rsbl.2008.0398

Buschinger, A. (1986). Evolution of social parasitism in ants. *Trends in Ecology and Evolution, 1*, 155–160. https://doi.org/10.1016/0169-5347(86)90044-3

Cerdá, X., Retana, J., & Cros, S. (1997). Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, *66*, 363–374. https://doi.org/10.2307/5982

Creighton, W. S. (1950). The ants of North America. *Bulletin of the Museum of Comparative Zoology of Harvard College*, *104,* 1–585.

Detrain, C., Tasse, O., Versaen, M., & Pasteels, J. M. (2000). A field assessment of optimal foraging in ants: trail patterns and seed retrieval by the European harvester ant *Messor barbarus*. *Insectes Sociaux, 47*, 56–62. https://doi.org/10.1007/s000400050009

Foitzik, S., DeHeer, C. J., Hunjan, D. N., & Herbers, J. M. (2001). Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society B: Biological Sciences*, *268*, 1139–1146. https://doi.org/10.1098/rspb.2001.1627

Hölldobler, B., & Wilson, E. O. (1990). *The Ants*. Cambridge, Massachusetts: Harvard University Press.

Johnson, C. A., & Herbers, J. M. (2006). Impact of parasite sympatry on the geographic mosaic of coevolution. *Ecology, 87*, 382–394. https://doi.org/10.1016/j.jmwh.2006.05.004

Maynard Smith, J. (1978). Optimization theory in evolution. *Annual Review of Ecology and Systematics*, *9*, 31–56. https://doi.org/10.1201/b11866-5

Miller, J. S. (2017). *Collective behavior in slave-making ants: how ecology and social structure shape raiding strategies* (Doctoral thesis). Ithaca, NY: Cornell University.

Mori, A., Grasso, D. A., Visicchio, R., & Le Moli, F. (2001). Comparison of reproductive strategies and raiding behaviour in facultative and obligatory slave-making ants: the case of *Formica sanguinea* and *Polyergus rufescens*. *Insectes Sociaux*, *48,* 302–314. https://doi.org/10.1007/PL00001782

Novotny, N. C., & Hare, J. F. (in prep). Temperature affects retrieval preference of host species pupae by the slave-making ant *Temnothorax americanus* (Hymenoptera: Formicidae).

Orians, G. H., & Pearson, N. E. (1979). On the theory of central place foraging. In D. J. Horn, G. R. Stairs, & R. D. Mitchell (Eds.), *Analysis of Ecological Systems* (pp. 154–177). Columbus: Ohio State University Press.

Payne, R. B. (1977). The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics*, *8*, 1–28. https://doi.org/10.1146/annurev.es.08.110177.000245

Pinter-Wollman, N., Wollman, R., Guetz, A., Holmes, S., & Gordon, D. M. (2011). The effect of individual variation on the structure and function of interaction networks in harvester ants. *Journal of the Royal Society Interface*, *8*, 1562–1573. https://doi.org/10.1098/rsif.2011.0059

Porter, S. D., & Tschinkel, W. R. (1987). Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology*, *16*, 802–808. https://doi.org/10.1093/ee/16.3.802

Queiroz, A. C. M., Ribas, C. R., & França, F. M. (2013). Microhabitat characteristics that regulate ant richness patterns: the importance of leaf litter for epigaeic ants. *Sociobiology*, *60*, 367–373. https://doi.org/10.13102/sociobiology.v60i4.367-373

Shaffer, Z., Sasaki, T., & Pratt, S. C. (2013). Linear recruitment leads to allocation and flexibility in collective foraging by ants. *Animal Behaviour*, *86*, 967–975. https://doi.org/10.1016/j.anbehav.2013.08.014\

Stephens, D. W., & Krebs, J. R. (1987). *Foraging Theory*. Princeton, N.J: Princeton University Press.

Stuart, R. J., & Alloway, T. M. (1985). Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). *Animal Behaviour*, *33*, 1080–1088. https://doi.org/10.1016/S0003-3472(85)80166-4

Taborsky, M. (2001). The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *Journal of Heredity*, *92*, 100–110. https://doi.org/10.1093/jhered/92.2.100

Thompson, J. N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *The American Naturalist*, *153*, 1–14. https://doi.org/10.1086/303208

Visscher, P. K. (2007). Group decision making in nest-site selection among social insects. *Annual Review of Entomology*, *52*, 255–275. https://doi.org/10.1146/annurev.ento.51.110104.151025

Wakano, J. Y., Nakata, K., & Yamamura, N. (1998). Dynamic model of optimal age polyethism in social insects under stable and fluctuating environments. *Journal of Theoretical Biology*, *193*, 153–165. https://doi.org/10.1006/jtbi.1998.0697

Wilson, E. O. (1975). *Leptothorax duloticus* and the beginnings of slavery in ants. *Evolution*, *29*, 108–119. https://doi.org/ 10.2307/2407145

Chapter 2: No evidence of a temperature-dependent raiding preference in the kidnapper ant *Temnothorax americanus*

Introduction

Animals constantly make decisions that influence their fitness, so there is strong selective pressure for animals to optimize their decision-making behaviour and make optimal choices (Stephens & Krebs, 1987; Maynard Smith, 1978; Orians & Pearson, 1979). Social insects, such as ants, are unique in that they predominantly use collective decision making whereby the colony as a whole makes a decision by integrating the information of individual colony members (Beckers, Deneubourg, Goss, & Pasteels, 1990; Franks et al., 2008; Robinson, Smith, Sullivan, & Nigel, 2009). However, ants are still able to make individual decisions when it is more profitable to do so (Cerdá, Angulo, Boulay, & Lenoir, 2009). These complex decision-making behaviours allow ants to optimize a variety of tasks, such as foraging (Beckers et al., 1990; Detrain et al., 2000; Shaffer et al., 2013) and nest site relocation (Franks et al., 2008; Visscher, 2007).

Kidnapper ants are social parasites that raid nearby colonies of other ant species, their hosts, abscond with their brood and return it to their own colony where the captured brood will eclose and become fully functioning workers for the kidnapper colony (Hölldobler & Wilson, 1990). In obligate kidnapping species, the kidnapper workers themselves are specialized for raiding and have lost the ability to perform domestic tasks such as foraging and caring for brood (Stuart & Alloway, 1985). Thus, the kidnappers cannot survive without a host workforce (Stuart & Alloway, 1985). Host colonies therefore represent a critically valuable resource, the use of which kidnapper ants would be expected to optimize. Host colonies are a unique resource, however, as unlike many potential food sources or nest sites, colonies will defend themselves and can kill considerable numbers of attacking kidnapper workers (Foitzik et al., 2001). Thus, kidnapper ants should optimize their use of the host species resource based on the defensive capability of their target host colonies. *Temnothorax americanus* colonies achieve optimal raiding outcomes at moderately-sized host colonies, avoiding large colonies, which are well defended, and small colonies, where there are small amounts of brood available to be captured (Miller, 2020). Pohl and Foitzik (2011) showed that in choice trials, *T. americanus* preferred these moderately-sized host colonies (median 25 workers) over small host colonies (median 10 workers). Some kidnapper ant species, such as *T. americanus*, have multiple acceptable host species and can use them simultaneously within the host workforce (Alloway, 1979), allowing the opportunity to optimize host species choice. In choice tests, *T. americanus* preferentially raids the host species who has experienced lower parasite pressure and thus has evolved fewer defensive adaptations (Brandt & Foitzik, 2004). However, besides host species parasite pressure, other ecological and abiotic factors affecting kidnapper ant raiding preference in a multiple host species context have not been studied. There is considerable variation in the microhabitats that ant colonies experience, even on a small spatial scale, as factors such as humidity, light intensity and temperature can all vary. The microhabitat of an ant colony strongly affects its productivity and performance, and ants perform best within their preferred microhabitats (Queiroz et al., 2013). Temperature in particular has a strong effect on ant colony performance (Porter & Tschinkel, 1987). Behaviourally dominant species operating outside of their thermal optima can even be outcompeted by subordinate species (Bestelmeyer, 2000; Cerdá et al., 1997). More productive and better performing colonies are presumably better at defense, allowing kidnapper ants to take advantage of these microhabitat temperature preferences and optimize their raiding preference based on differential defensive capabilities.

T. americanus is an obligate kidnapper ant native to eastern North America and in Canadian populations uses *T. ambiguus* and *T. longispinosus* as hosts (Alloway, 1979; Creighton, 1950). *T. americanus* colonies can include both host species in their host workforce simultaneously (Alloway, 1979). In Southern Ontario *T. ambiguus* is found predominantly in open areas, where it is warmer due to direct sun exposure, and *T. longispinosus* colonies are more abundant in shaded areas, where it is cooler (T.M. Alloway, personal communication, May 2019), presumably achieving optimal performance in species-typical microhabitat. In choice tests, *T. americanus* preferentially retrieves pupae of the host species whose temperature optimum is opposite to the temperature conditions experienced by the kidnapper colony (Novotny and Hare, in prep). This documented preference may reflect a preference of the kidnapper colony to raid the least well-defended host species relative to the microhabitat temperature (Novotny and Hare, in prep). These ants thus offer a perfect model system to test for the first evidence of optimization of raiding preference based on differences in host microhabitat temperature optima. The objective of this study was to test the hypothesis that the raiding preference for host species colonies by *T. americanus* depends on temperature. I predicted that *T. americanus* would preferentially raid the host species that was cultured outside of its thermal optimum compared to host species cultured within their thermal optimum. Therefore, I predicted that if a temperature-dependent raiding preference occurs, then a *T. americanus* colony in a warm temperature will preferentially raid *T. longispinosus* colonies over *T. ambiguus* colonies, and vice versa for cool temperatures, all other factors being equal.

Methods

T. americanus, *T. ambiguus* and *T. longispinosus* are native to eastern North America (Creighton, 1950) so a collecting trip was undertaken from 26 May to 1 June 2019 to acquire colonies for study. Ants were collected from wooded areas in the Rural Municipality of Halton, Ontario. These *Temnothorax* species live primarily in white oak (*Quercus alba*) acorns and shagbark hickory (*Carya ovata*) nuts evacuated by coleopteran or lepidopteran larvae. Nuts found on the forest floor in areas of abundant nutfall were cracked open and if they contained any of the three study species, the ants were collected in a small (16.5 x 14cm), plastic sandwich bag within their nut of origin. *T. americanus*, *T. ambiguus* and *T. longispinosus* are facultatively polydomous (Del Rio Pesado & Alloway, 1983) so individual nests cannot be deemed separate colonies as non-nestmates collected from the same site may still be members of the same colony. Thus, I assumed that nests of the same species were unrelated only when collected from different sites (Hare, 1996). Nests were labelled with a number according to site of origin and assigned a unique identification number within each site number. The collected nests were stored in a refrigerator at ~4°C from when they were collected until when they were couriered overnight on icepacks back to the lab on the Fort Garry Campus of the University of Manitoba on 2 June 2019.

In the lab, I transferred the ants to artificial nests, which allow the observation of behaviours inside and outside the nest, as described by Alloway (1979). A 10 x 40 mm plastic petri dish lid, painted green (211360; Rust-Oleum, Vernon Hills, Illinois), was used as the base of the nest with a small hole (ca. 1.5 mm) made in the side of the dish with the hot tip of a soldering iron to act as the nest entrance. The nest was covered with a custom-milled orange acrylic lid which has a groove in it, allowing it to snap firmly onto the nest base. These artificial nests were placed in a clear plastic 15 x 150 mm petri dish, which acted as the ants' foraging arena. This larger petri dish also contained a 15 x 45 mm glass vial, filled with water and stoppered with absorbent cotton. Water was provided *ad libitium* to all nests in culture. A strip of wax paper was also placed in the large petri dish as a food strip to which a small cube of artificial ant diet, developed by Bhatkar and Whitcomb (1970), was applied three times a week. The foraging area was covered with a clear plastic petri dish lid to prevent ants from escaping. Nests were maintained at approximately 21°C in the laboratory on a natural photoperiod via windows to the outside.

I censused *T. americanus* nests before acclimation to treatments for their demographic characteristics to allocate nests to treatments in a balanced manner and censused them again one day before trials to use their demography as covariates in later analyses to control for and assess any effect of current workforce composition on host species raiding preference. Demographic measures were: the presence of a queen, number of *T. americanus* workers, total number of host workers, number of *T. ambiguus* workers, number of *T. longispinosus* workers, total number of brood pieces, number of pupae, number of *T. ambiguus* pupae, number of *T. longispinosus* pupae, number of *T. americanus* pupae, number of larvae, number of eggs, number of alate queens and number of males. Approximately 440 ant nests were collected from 22 unique sites (minimum inter-site distance = 0.39km) . Thirty-five nests of *T. americanus* were collected but only 25 were viable for use in the study. The ten unviable nests either lost their *T. americanus* workers or were unresponsive in experimental trials. Unresponsive nests failed to mount a raid on one of the host species nests within ten hours and trials were aborted.

I allocated *T. americanus* nests to temperature treatments in a balanced manner by matching nest demographic composition across treatments. Nests were blocked into groups of two based upon similarity in composition then randomly assigned by coin-flip to temperature treatments. The target host species nests were also exposed to the temperature treatments. Target host species nests used in trials were from the same site of origin but from a different site than the *T. americanus* nest was collected from. Two temperature treatments were established using temperature-controlled environment chambers (Conviron Model C1009, Conviron Technologies Ltd., Winnipeg MB) at the University of Manitoba. The warm treatment was set to 25°C to simulate the average summer raiding conditions of a warm, open habitat where *T. ambiguus* is commonly found. The cool treatment was set to 15°C to simulate the average summer raiding conditions of a cool, shady habitat where *T. longispinosus* is commonly found. Photoperiod of the chambers was held constant at 15:9 hour light to dark regime, light onset 0700 hrs CDT, with light levels of 1400 lumen at the level of the ant nests for the entire light period. Relative humidity was set at 55%. Although only two temperature-controlled environment chambers were used there are limited concerns regarding pseudoreplication as ant nests were contained separately within their petri dish foraging arenas or experimental arenas and thus acted as independent units. Nests were acclimated to treatments for two weeks from 29 July to 5 August 2019.

After acclimation, trials were started by presenting *T. americanus* nests with a simultaneous choice assay. Nests were tested in a random order in each treatment. Prior to a trial, a *T. americanus* nest was placed into a 60 x 60 x 10cm clear acrylic experimental arena which had a 40cm wall extending from one side to partially divide the arena in two and a removeable 60cm divider to divide the arena into three sections, as seen in Figure 2.1. The outside walls of the experimental arenas were covered in ~1cm thick white foam to prevent any visual cues from outside the arenas from affecting the ants, and the inside walls were covered in a thin layer of fluon (Insect-a-Slip Insect Barrier, BioQuip Products, Rancho Dominguez CA) to prevent ants escaping. The *T. americanus* nests were conditioned to scout the areas where the target host nests would be present in trials by placing small chunks of mealworms (*Tenebrio molitor*) on wax-paper strips in these areas. Nests were conditioned in this manner for three days prior to trials and a quarter chunk of mealworm was placed on the wax strips each day. Test fragment nests were created from the acclimated host species nests by transferring 15 workers, one queen and ten pieces of brood (late instar larvae or pupae) with a camel-hair brush into a new petri dish containing an artificial nest one day prior to trials. Using standardized test fragments as targets for raids prevented the number of workers and brood from confounding differences in defensive capability due to temperature. Camel-hair brushes, test fragment artificial nests and experimental arenas were washed in 70% ethanol followed by distilled water and dried before use with another separate nest to prevent the transfer of chemical cues. A separate brush was used for each species. After conditioning, the experimental arenas containing *T. americanus* nests were moved into room temperature (~20°C). The removeable divider was placed into the arena and any scouting workers were moved into the section of the arena containing the *T. americanus* nest using a camel-hair brush. Test fragments of the host species (*T. ambiguus* and *T. longispinosus*) were placed into the arena and the divider was removed. The positions of the host species (left versus right) was balanced over the trials for each treatment. A gooseneck lamp with a 100-watt incandescent bulb was oriented such that the bulb was ~15cm directly above the *T. americanus* nest to heat the nest and stimulate scouting behaviour (Buschinger, Ehrhardt, & Winter, 1980). I recorded the first host species worker(s) to be encountered, which host species was raided, and the time taken for the first piece of brood to be retrieved to the *T. americanus* nest. The first host species nest encountered was recorded, however, for only 19 of the 25 trials due to errors in record keeping. Host nests were filmed during trials with cameras (Sony Handycam DCR-SR68, Sony Corporation, Tokyo Japan) mounted on tripods above the experimental arena. There was always contact with both host species workers before raids occurred so the *T. americanus* nest should have gained simultaneous information about the presence of both host species, allowing an informed choice and providing a test of preference.

I used a Pearson chi-square test with Yates correction to test the independence of host species raiding preference from temperature. I used a Fisher's exact test to test the independence of host species raiding preference and the first host species worker encountered. I used a Fisher's exact test to test the independence of the first host species nest encountered and host species raiding preference. I used a Pearson chi-square goodness-of-fit test to test if overall host species raiding preference across both temperature treatments differed from random selection (50% probability of raiding either host species). I used a Fisher's exact test to test the independence of the first host species encountered and temperature. I also used generalized linear models (GLMs) to assess the effects of temperature, the first encountered host species, the number of days post acclimation the trial occurred on, the number of males in the colony and the proportion of *T. longispinosus* within the host-workforce on host species raiding preference. Within the models, host species raiding preference was measured as a binary variable, so a Bernoulli distribution and logit link function were used where a success equated with the raiding of a *T. longispinosus* colony and a failure equated with the raiding of a *T. ambiguus* colony (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). I built models representing five *a priori* hypotheses (Table 2.1). Data exploration and fitting of the global model were done according to the approach of Zuur et al. (2010). Days post acclimation was centered around its mean to reduce collinearity with the interaction term (Zuur et al., 2009). Model assumptions were checked visually by plotting the residuals according with the recommendations of Zuur and Ieno (2016). Models were ranked using AICc, and their Akaike weights (w_i) were calculated. Due to high model selection uncertainty, I performed full model averaging using the R package "MuMIn" (Bartoń, 2019; Burnham & Anderson, 2002). All statistical tests were performed in R version 3.6.2 (R Core Team, 2019) with differences considered statistically significant where $P < 0.05$.

Results

Host species raiding preference did not depend on the treatment temperature (chi-square test of independence with Yates correction: $X_c^2 = 0$, $P = 1$; Table 2.2), nor was there any overall preference for one host species over the other across both temperatures (chi-square goodness of fit test: $X^2 = 0.36$, df = 1, $P = 0.5485$). The host species worker encountered first within trials was independent of the treatment temperature (Fisher's exact test: $P = 0.411$). Host species raiding preference was independent of which host species worker was encountered first within trials (Fisher's exact test: $P = 0.6766$). *T. americanus* colonies raided the host species whose nest they encountered first 68% of the time, but this did not depart significantly from the chance expectation of 50% (Fisher's exact test: $P = 0.5573$). Mean ± SE time for *T. americanus* colonies to retrieve the first brood piece was 177.2 ± 17.64 minutes. The number-of-males model (g_4) was selected as the best model, but there was considerable model selection uncertainty ($w_4 = 0.29$). All models had strong support (ΔAICc < 2), and the best-ranked model only had 2.64 times more support in the data than the worst-ranked model. After model averaging the unconditional standard errors of the parameter estimates were large, severely limiting power and precluding valid inference (Table 2.3). Thus, I was not able to assess any impacts of colony demography on raiding preference.

Discussion

T. americanus did not exhibit a raiding preference among its two host species based on temperature, counter to my prediction that temperature would influence raiding preference and that the host species outside its thermal optimum would be preferentially raided. In fact, *T. americanus* exhibited no raiding preference overall regardless of temperature, raiding each host species in nearly equal numbers. *T. americanus* preferentially raids more weakly-defended host species when offered a raiding choice between host colonies from different communities with different levels of parasite pressure or between the numerically dominant host species and the minority host species within a community, who also experience different levels of parasite pressure (Brandt & Foitzik, 2004). Thus, it is possible that there is no difference in defensive capability between *T. ambiguus* and *T. longispinosus* populations in southern Ontario and that existing within their respective species-specific microhabitat temperature optima does not alter defense. Overall level of parasite pressure may more strongly influence the defensive capability of host species than microhabitat temperature. *T. ambiguus* is more common than *T. longispinosus* in the southern Ontario community (Del Rio Pesado & Alloway, 1983), but my collection records from 2018-2020 show that *T. americanus* uses both host species roughly equally with 49 colonies using *T. ambiguus* hosts and 53 using *T. longispinosus* hosts, with 56.92% of colonies containing both host species. Thus, parasite pressure is likely equal for the two host species and they would presumably have evolved similar levels of defensive adaptations. *T. americanus* pupal retrieval preference for host species is affected by temperature, however, suggesting that temperature somehow influences the attractiveness of the host species to the kidnappers (Novotny and Hare, in prep). It is puzzling why this pupal retrieval preference does not extend to a raiding preference.

It is also possible that not exerting a raiding preference may result in optimal outcomes for the kidnappers. *T. americanus* raiding range has been observed to be up to three metres (Miller, 2017). Within a raiding range of this size there could be considerable variability in the microhabitats present. Thus, it may not be optimal for *T. americanus* colonies to base their raiding preference upon the temperature of their nest site as the host colony encountered may not be within the same microhabitat. Microhabitats may also vary throughout the season as leaf litter can be disturbed and changes in light exposure due to changes in surrounding vegetation could occur, altering temperature conditions. Thus, as the current temperature conditions may not reflect future temperature conditions, it would not be optimal to base raiding preference upon current temperature.

T. americanus has optimal raiding outcomes against medium-sized host colonies (Miller, 2020), but in simultaneous choice experiments does not exert a raiding preference for these optimal medium-sized colonies over other-sized colonies (Miller, 2017). Host species also represent a very valuable and rare resource, so it may be optimal to raid any encountered host colony that meets a certain threshold of acceptance (Miller, 2017). Miller (2017) calculated a theoretical encounter rate for *T. americanus* colonies discovering host colonies of an average of seven days per encounter. As *T. americanus* has only a limited raiding season in mid-summer where there are sufficient amounts of host pupae available and a low encounter rate, it would be optimal to raid every acceptable colony encountered (Miller, 2017). The low encounter rate and limited raiding season prevents scouting all possible host colonies or even a subset of all host colonies from being a viable strategy. Thus, *T. americanus* colonies would be expected to raid the first encountered host; however, neither the first contacted host species worker nor the first encountered host species nest had an effect on which host was raided, though the first encountered nest was raided in 68% of trials. It is possible that the first encountered nest was preferentially raided, and I did not have adequate power to detect an effect. In other simultaneous choice trials, *T. americanus* predominantly raided the host colony discovered first (Miller, 2017; Pohl & Foitzik, 2011). *T. americanus* has been shown to exhibit a raiding preference in laboratory experiments where they raid the more weakly-defended host species who have experienced less parasite pressure (Brandt & Foitzik, 2004). Additionally, Pohl & Foitzik (2011) showed that *T. americanus* preferentially raids medium sized host colonies over small colonies. The presence of a raiding preference shown by Brandt & Foitzik (2004) and Pohl & Foitzik (2011) but not by Miller (2017) and my study may be explained by encounter rate. Brandt & Foitzik (2004) and Pohl & Foitzik (2011) used smaller 20 x 20 cm square and 2.7 x 15 cm Y-shaped arenas respectively, while Miller (2017) and my study used larger arenas of 45 x 20 cm and 60 x 60 cm respectively. Encounter rates would presumably be much higher in the smaller arenas, preventing encounter rate from being a limiting factor underlying raiding preference. It took a long time for *T. americanus* colonies to retrieve the first piece of brood during raids in my study (mean ± SE: 177.2 ± 17.64 minutes), so encounter rates were likely a limiting factor. Larger arenas more closely represent the density of host nests that would be found in the field, where host density is low (Miller, 2017). Additionally, prior contact with a kidnapper scout increases the aggression of host species colonies, so scouting all possible colonies to assess quality and choose the best among those would likely increase colony defenses and reduce kidnapper raiding success (Kleeberg, Pamminger, Jongepier, Papenhagen, & Foitzik, 2014).

T. americanus exhibited no temperature-dependent or overall raiding preference. This lack of preference suggests that there may be no temperature-dependent differences in host species defensive capability or kidnapper outcomes. However, it is also possible that it is optimal for *T. americanus* to not exhibit a raiding preference even if a temperature-dependent defensive difference between host species exists, as low encounter rates limit the opportunities to raid. Thus, kidnappers should raid the first encountered host colony of sufficient quality. Ultimately, a quantitative test of the defensive capability of the host species *T. ambiguus* and *T. longispinosus* in different temperature conditions is needed to support either of these possible explanations and is the focus of Chapter 3.

References

Alloway, T. M. (1979). Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). *Animal Behaviour*, *27*, 202–210. https://doi.org/10.1016/0003-3472(79)90140-4

Bartoń, K. (2019). MuMIn: Multi-model inference. R package version 1.43.17. Retrieved from https://cran.r-project.org/package=MuMIn

Beckers, R., Deneubourg, J. L., Goss, S., & Pasteels, J. M. (1990). Collective decision making through food recruitment. *Insectes Sociaux, 37*, 258–267. https://doi.org/10.1007/BF02224053

Bestelmeyer, B. T. (2000). The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. *Journal of Animal Ecology, 69*, 998–1009. https://doi.org/10.1046/j.1365-2656.2000.00455.x

Bhatkar, A., & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *The Florida Entomologist*, *53*, 229–232. https://doi.org/10.2307/3493193

Brandt, M., & Foitzik, S. (2004). Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*, *85*, 2997–3009. https://doi.org/10.1890/03-0778

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference : a practical information-theoretic approach* (2nd ed.). New York, NY: Springer.

Buschinger, A., Ehrhardt, W., & Winter, U. (1980). The organization of slave raids in dulotic ants — a comparative study (Hymenoptera; Formicidae). *Zeitschrift für Tierpsychologie,* *53*, 245–264. https://doi.org/10.1111/j.1439-0310.1980.tb01053.x

Cerdá, X., Angulo, E., Boulay, R., & Lenoir, A. (2009). Individual and collective foraging decisions: a field study of worker recruitment in the gypsy ant *Aphaenogaster senilis*. *Behavioral Ecology and Sociobiology*, *63*, 551–562. https://doi.org/10.1007/s00265-008-0690-5

Cerdá, X., Retana, J., & Cros, S. (1997). Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, *66*, 363–374. https://doi.org/10.2307/5982

Creighton, W. S. (1950). The ants of North America. *Bulletin of the Museum of Comparative Zoology of Harvard College*, *104*, 1–585.

Del Rio Pesado, M., & Alloway, T. M. (1983). Polydomy in the slave-making ant, *Harpagoxenus americanus* (Emery) (Hymenoptera: Formicidae). *Psyche*, *90*, 151–162. https://doi.org/10.1155/1983/63051

Detrain, C., Tasse, O., Versaen, M., & Pasteels, J. M. (2000). A field assessment of optimal foraging in ants: trail patterns and seed retrieval by the European harvester ant *Messor barbarus*. *Insectes Sociaux*, *47*, 56–62. https://doi.org/10.1007/s000400050009

Foitzik, S., DeHeer, C. J., Hunjan, D. N., & Herbers, J. M. (2001). Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society B: Biological Sciences*, *268*, 1139–1146. https://doi.org/10.1098/rspb.2001.1627

Franks, N. R., Hardcastle, K. A., Collins, S., Smith, F. D., Sullivan, K. M. E., Robinson, E. J. H.,

& Sendova-Franks, A. B. (2008). Can ant colonies choose a far-and-away better nest over an in-the-way poor one? *Animal Behaviour*, *76*, 323–334. https://doi.org/10.1016/j.anbehav.2008.02.009

Hare, J. F. (1996). Discrimination of nestmate larvae by the ant *Leptothorax longispinosus*. *Canadian Journal of Zoology*, *74*, 2055–2061. https://doi.org/10.1139/z96-233

Hölldobler, B., & Wilson, E. O. (1990). *The Ants*. Cambridge, Massachusetts: Harvard University Press.

Kleeberg, I., Pamminger, T., Jongepier, E., Papenhagen, M., & Foitzik, S. (2014). Forewarned is forearmed: aggression and information use determine fitness costs of slave raids. *Behavioral Ecology*, *25*, 1058–1063. https://doi.org/10.1093/beheco/aru084

Maynard Smith, J. (1978). Optimization theory in evolution. *Annual Review of Ecology and Systematics*, *9*, 31–56. https://doi.org/10.1201/b11866-5

Miller, J. S. (2017). *Collective behavior in slave-making ants: how ecology and social structure shape raiding strategies* (Doctoral thesis). Ithaca, NY: Cornell University.

Miller, J. S. (2020). Not too big, not too small: raids at moderately sized hosts lead to optimal outcomes for a slave-making ant. *Behavioral Ecology and Sociobiology*, *74*, 18. https://doi.org/10.1007/s00265-019-2797-2

Novotny, N. C., & Hare, J. F. (in prep). Temperature affects retrieval preference of host species pupae by the slave-making ant *Temnothorax americanus* (Hymenoptera: Formicidae).

Orians, G. H., & Pearson, N. E. (1979). On the theory of central place foraging. In D. J. Horn, G. R. Stairs, & R. D. Mitchell (Eds.), *Analysis of Ecological Systems* (pp. 154–177).

Columbus: Ohio State University Press.

Pohl, S., & Foitzik, S. (2011). Slave-making ants prefer larger, better defended host colonies. *Animal Behaviour*, *81*, 61–68. https://doi.org/10.1016/j.anbehav.2010.09.006

Porter, S. D., & Tschinkel, W. R. (1987). Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology*, *16*, 802–808. https://doi.org/10.1093/ee/16.3.802

Queiroz, A. C. M., Ribas, C. R., & França, F. M. (2013). Microhabitat characteristics that regulate ant richness patterns: the importance of leaf litter for epigaeic ants. *Sociobiology*, *60*, 367–373. https://doi.org/10.13102/sociobiology.v60i4.367-373

R Core Team, A. (2019). R: A language and environment for statistical computing. Vienna, Austria.: R Foundation for Statistical Computing.

Robinson, E. J. H., Smith, F. D., Sullivan, K. M. E., & Nigel, R. (2009). Do ants make direct comparisons? *Proceedings of the Royal Society B*, *276*, 2635–2641. https://doi.org/rspb.2009.0350

Shaffer, Z., Sasaki, T., & Pratt, S. C. (2013). Linear recruitment leads to allocation and flexibility in collective foraging by ants. *Animal Behaviour*, *86*, 967–975. https://doi.org/10.1016/j.anbehav.2013.08.014

Stephens, D. W., & Krebs, J. R. (1987). *Foraging Theory*. Princeton, N.J: Princeton University Press.

Stuart, R. J., & Alloway, T. M. (1985). Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). *Animal*

Behaviour, *33*, 1080–1088. https://doi.org/10.1016/S0003-3472(85)80166-4

Visscher, P. K. (2007). Group decision making in nest-site selection among social insects. *Annual Review of Entomology*, *52*, 255–275. https://doi.org/10.1146/annurev.ento.51.110104.151025

Zuur, A. F., & Ieno, E. N. (2016). A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, *7*, 636–645. https://doi.org/10.1111/2041-210X.12577

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1,* 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. (M. Gail, K. Krickeberg, J. Samet, A. Tsiatis, & W. Wong, Eds.) (1st ed.). New York, NY: Springer Science+Business Media. https://doi.org/10.4324/9780429201271-2

Tables and Figures

Table 2.1: *A priori* models representing alternative hypotheses for the probability of *Temnothorax americanus* raiding a *T. longispinosus* nest in simultaneous choice trials between *T. longispinosus* and *T. ambiguus*. Temp = Temperature, DaysPostAcclim = number of days post acclimation, PElongi = proportion of host-workforce which is *T. longispinosus*, FirstContact = first encountered host species worker.

Model	Parameters	Hypothesis
g_1	Temp	Temperature affects host species defense
g_2	Temp + Days + (Temp x DaysPostAcclim)	Longer the nests experience temperature conditions the stronger the effect (interaction)
g_3	PElongi	The proportion of species in the host-workforce will affect which species is raided
g_4	Number of males	Number of males in the nest will influence which species is raided (Novotny and Hare, in prep)
g_5	FirstContact	The first encountered host species will influence which species is raided

Table 2.2: Number of trials where host nests of *Temnothorax ambiguus* or *T. longispinosus* were raided in simultaneous choice trials by *T. americanus* nests in two temperature treatments.

Host species raided	Temperature	
	15°C	25°C
T. ambiguus	7	7
T. longispinosus	6	5

Table 2.3: Results of GLM multi-model averaging for the probability of *Temnothorax americanus* raiding a *T. longispinosus* nest in simultaneous choice trials between *T. longispinosus* and *T. ambiguus.*

Variable	Parameter estimate	Unconditional standard error	Confidence interval (95%) 2.5, 97.5
Intercept	-0.23439	0.66633	-1.6025, 1.1338
Number of males	-0.07659	0.19295	-0.4677, 0.3146
First Contact (*T. longispinosus*)	0.16736	0.51873	-0.8876, 1.2223
Temperature (25°C)	-0.05701	0.46221	-1.0134, 0.8994
Proportion of host workers *T. longispinosus*	-0.04216	0.48428	-1.0421, 0.9578
Days post acclimation	-0.01205	0.03972	-0.0912, 0.0671
Days post acclimation x Temperature	0.01480	0.05052	-0.0862, 0.1158

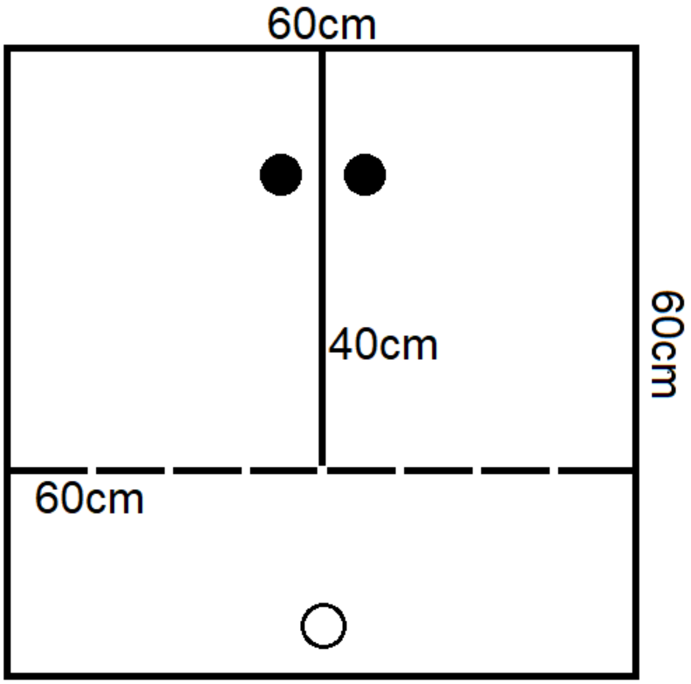

Figure 2.1: Experimental test arena for raiding preference. 60 x 60 x 10cm clear acrylic with 40cm dividing wall and 60cm removeable divider (dashed line). Location of host nests represented by filled circles and location of the *Temnothorax americanus* nest represented by open circle.

Chapter 3: Defensive capability of *Temnothorax ambiguus* and *T. longispinosus* colonies against raids by the kidnapper ant *T. americanus* is not temperature-dependent

Introduction

Kidnapper ants are social parasites that raid nearby colonies of other ant species, their hosts, abscond with their brood and return it to their own colony where the captured brood will eclose and become fully functioning workers for the kidnapper colony (Hölldobler & Wilson, 1990). In obligate kidnapping species the kidnapper workers themselves are specialized for raiding and have lost the ability to perform domestic tasks such as foraging and caring for brood such that they cannot survive without a host workforce (Stuart & Alloway, 1985). Thus, host colonies represent a critically valuable resource for the kidnapper ants. Ants in general are known for their ability to optimize the collection and use of resources (Beckers et al., 1990; Detrain et al., 2000; Hölldobler & Wilson, 1990). Thus, kidnapper ants should be expected to optimize their collection of the host resource by selecting the optimal host colonies to raid. Host colonies are a unique resource, however, as kidnapper ants must overcome the defenses of the colony to access the brood. While kidnapper workers are specialized for raiding, host colony workers can still injure and kill a considerable number of kidnappers in raids (Foitzik et al., 2001). Thus, kidnapper ants should optimize their use of the host species resource based on the defensive capability of their target host colonies.

Many factors are known to influence the defensive capability of host colonies against kidnapper raids (Grüter, Jongepier, & Foitzik, 2018). The degree of worker specialization within a colony can influence host defense, as generalist workers are more adept at defense than specialists (Jongepier & Foitzik, 2016). During raids kidnapper ants use propaganda pheromones to induce infighting within host colonies (Lenoir, D'Ettorre, & Errard, 2001), but host colonies can vary in their ability to resist the propaganda pheromone's effects (Foitzik, Fischer, & Heinze, 2003; Jongepier, Kleeberg, & Foitzik, 2015). Host colonies better able to resist the effect of propaganda pheromone are more successful in defending against kidnapper raids (Jongepier et al., 2015). The level of parasite pressure experienced by hosts also influences host defensive capability as hosts who experience higher levels of parasite pressure have evolved more defensive adaptations and increased aggression (Brandt & Foitzik, 2004; Kleeberg, Jongepier, Job, & Foitzik, 2015). Prior contact with a kidnapper scout also increases the aggression of host species colonies and increases their ability to defend against kidnapper raids (Kleeberg et al., 2014; Pamminger, Scharf, Pennings, & Foitzik, 2011). Additionally, the overall defensive strategy used by host colonies can vary across communities (Jongepier, Kleeberg, Job, & Foitzik, 2014). However, other ecological and abiotic factors affecting host species defensive capability against kidnapper raids have not been studied.

There is considerable variation in the microhabitats that ant colonies experience, even on a small spatial scale, as factors such as humidity, light intensity and temperature can all vary. The microhabitat of an ant colony strongly affects its productivity and performance, and ants perform best within their preferred microhabitats (Queiroz et al., 2013). Temperature in particular has a strong effect on ant colony performance (Porter & Tschinkel, 1987). Behaviourally dominant species operating outside of their thermal optima can even be outcompeted by subordinate species (Bestelmeyer, 2000; Cerdá et al., 1997). More productive and better performing colonies are presumably better at defense, allowing kidnapper ants to take advantage of these microhabitat temperature preferences and optimize their raiding preference based on these differential defensive capabilities.

Temnothorax americanus is an obligate kidnapper ant native to eastern North America and in Canadian populations uses *T. ambiguus* and *T. longispinosus* as hosts (Alloway, 1979; Creighton, 1950). *T. americanus* colonies can contain both host species in their host workforce simultaneously (Alloway, 1979). In Southern Ontario *T. ambiguus* is found predominantly in open areas, where it is warmer due to direct sun exposure, and *T. longispinosus* colonies are more abundant in shaded areas, where it is cooler (T.M. Alloway, personal communication, May 2019), presumably achieving optimal performance and defensive capability in species-typical microhabitat. Both host species experience roughly equal parasite pressure and thus should have evolved similar defensive adaptations (Chapter 2). These ants thus offer a perfect model system to test if temperature influences the defensive capability of hosts against kidnapper raids. In choice tests, *T. americanus* preferentially retrieves pupae of the host species whose temperature optimum is opposite to the temperature conditions experienced by the kidnapper colony (Novotny and Hare, in prep). This documented preference may reflect a preference of the kidnapper colony to raid the least well-defended host species relative to the microhabitat temperature (Novotny and Hare, in prep). However, in Chapter 2, I showed that temperature did not affect the raiding preference of *T. americanus* when offered a choice between nests of *T. ambiguus* and *T. longispinosus*. It is unclear whether this lack of temperature-dependent raiding preference for nests is due to the lack of a difference in host defensive capability relative to temperature or if it is optimal to instead raid any host nest of sufficient quality. Thus, the objective of this study is to quantitatively test the hypothesis that the defensive ability of *Temnothorax* colonies against kidnapper-raids from *T. americanus* is dependent upon temperature. I predicted that the host species within its temperature optimum would have superior defensive capability to the host species outside its temperature optimum and would experience lower injury rates, salvage more brood and injure more *T. americanus* colony members. I also hypothesized that *T. americanus* colonies will show different rates of raiding success against its two host species, *T. ambiguus* and *T. longispinosus*, depending on temperature. I predicted that *T. americanus* colonies will complete raids in less time and capture more brood against the host species whose temperature optimum is opposite to the temperature of their current environment.

Methods

T. americanus, *T. ambiguus* and *T. longispinosus* are native to eastern North America (Creighton, 1950) and thus cannot be collected in Manitoba. Due to COVID-19 travel restrictions I was unable to collect ants myself and paid three field assistants to collect colonies for me. Ants were collected from wooded areas in the Regional Municipality of Halton, Ontario from 23 – 24 and 30 – 31 May 2020, and 13 – 14 June 2020. These *Temnothorax* species live primarily in white oak (*Quercus alba*) acorns and shagbark hickory (*Carya ovata*) nuts evacuated by coleopteran or lepidopteran larvae. Nuts were cracked open and if they contained any of the three study species, the ants were collected in a small (16.5 x 14cm), plastic sandwich bag within their nut of origin. *T. americanus*, *T. ambiguus* and *T. longispinosus* are facultatively polydomous (Del Rio Pesado & Alloway, 1983) so individual nests cannot be deemed separate colonies as non-nestmates collected from the same site may still be members of the same colony. Thus, I assumed that nests of the same species were unrelated only when collected from different sites (Hare, 1996). Nests were labelled with a number according to site of origin and assigned a unique identification number within each site number. The collected nests were stored in a fridge at ~4°C from the day they were collected until they were couriered overnight on icepacks back to the lab on the Fort Garry Campus of the University of Manitoba. The nests collected in May 2020 were shipped on 2 June 2020 and the nests collected in June 2020 were shipped on 15 June 2020. Voucher specimens were deposited in the Wallis Roughley Museum of Entomology at the University of Manitoba.

In the lab, I transferred the ants to artificial nests, which allow the observation of behaviours inside and outside the nest, as described by Alloway (1979). A 10 x 40 mm plastic petri dish lid, painted green, was used as the base of the nest with a small hole (ca. 1.5 mm) made in the side of the dish with the hot tip of a soldering iron to act as the nest entrance. The nest was covered with a custom-milled orange acrylic lid which has a groove in it, allowing it to snap firmly onto the nest base. These artificial nests were placed in a clear plastic 15 x 150 mm petri dish which acted as the ants' foraging arena. This larger petri dish also contained a 15 x 45 mm glass vial, filled with water and stoppered with absorbent cotton. Water was provided *ad libitium* to all nests in culture. A strip of wax paper was also placed in the large petri dish as a food strip to which a small cube of artificial ant diet, developed by Bhatkar and Whitcomb (1970), containing agar, chicken egg, honey and vitamins, was applied three times a week.
T. americanus colonies were also given a quarter chunk of mealworm (*Tenebrio molitor*) weekly to supplement their diet with protein and other nutrients, thus reducing the probability that brood retrieved in trials were being taken as food (Hare, 1996). The foraging area was covered with a clear plastic petri dish lid to prevent ants from escaping. Nests were maintained at approximately 21°C in the laboratory on a natural photoperiod via windows to the outside.

I censused *T. americanus* nests before acclimation to treatments for their demographic characteristics to allocate nests to treatments in a balanced manner and to use in later exploratory analyses to identify any factors of current colony composition that may influence raiding outcomes. Demographic measures were: the presence of a queen, number of *T. americanus* workers, total number of host workers, number of *T. ambiguus* workers, number of *T. longispinosus* workers, total number of brood pieces, number of pupae, number of *T. ambiguus* pupae, number of *T. longispinosus* pupae, number of *T. americanus* pupae, number of larvae and number of eggs (Table 3.1). There were 307 ant nests collected for study from 6 unique sites (minimum inter-site distance: 1.27km), including 245 *T. ambiguus*, 45 *T. longispinosus* and 17 *T. americanus* nests. Of the 17 collected *T. americanus* nests only 15 were viable for use in the study; unviable nests did not conduct a raid within 24 hours.

I allocated *T. americanus* nests to temperature treatments in a balanced manner by matching nest demographic composition across treatments. Nests were blocked into groups of two based upon similarity in composition then randomly assigned via coin-flip to treatments. Within temperature treatments, *T. americanus* nests were evenly allocated to two target host nest treatments of raiding *T. ambiguus* and raiding *T. longispinosus*, creating a 2x2 factorial design with treatments of: 1) 15 °C raiding *T. ambiguus*, 2) 15 °C raiding *T. longispinosus*, 3) 25 °C raiding *T. ambiguus* and 4) 25 °C raiding *T. longispinosus*. The target host species nests were also placed in the temperature treatments. Target host species used in trials were from a different site of origin than the *T. americanus* nest was collected from. Two temperature treatments were established using temperature-controlled environment chambers (Conviron Model C1009, Conviron Technologies Ltd., Winnipeg MB) at the University of Manitoba. The warm treatment was set to 25°C to simulate the average summer raiding conditions of a warm, open habitat where *T. ambiguus* is commonly found. The cool treatment was set to 15°C to simulate the average summer raiding conditions of a cool, shady habitat where *T. longispinosus* is commonly found. Photoperiod of the chambers was held constant at 15:9 hour light to dark regime, light onset 0700 hrs CDT, with light levels of 1400 lumen at the level of the ant nests for the entire light period. Relative humidity was set at 55%. Although only two temperature-controlled environment chambers were used there are limited concerns regarding pseudoreplication as ant nests were contained separately within their petri dish foraging arenas and thus acted as independent units. Nests were acclimated to treatments for two weeks from 1 - 15 July 2020.

After acclimation trials were begun starting 16 July 2020. Nests were tested in a random order by selecting nests from a list with a random number generator (https://www.random.org/) alternating between the four treatment conditions. I placed a *T. americanus* nest into a 60 x 60 x 10 cm clear acrylic experimental arena, which had a 40cm wall extending from one side to partially divide the arena in two, 24 hours prior to the start of a trial to allow scouts to become accustomed to the arena (Figure 3.1). The inside walls of the arena were covered in a thin layer of fluon (Insect-a-Slip Insect Barrier, BioQuip Products, Rancho Dominguez CA) to prevent ants escaping and the outside bottom surface of the arenas were painted with white primer (Behr Co., Santa Ana, California) to allow ants to be easily seen. A water vial and food strip were placed against the outer wall of the arena equidistant from the host nest and *T. americanus* nest. The arenas were at room temperature of ~20°C and exposed to a natural photoperiod via windows to the outside. Trials began when I placed the target host nests in the arena. Target host nests were made into standardized test fragments by transferring 10 workers, one queen and ten pieces of brood (late instar larvae or pupae) with a camel-hair brush into a new petri dish containing an artificial nest one day prior to trials. Using standardized test fragments as targets for raids prevented the number of workers and brood from confounding differences in defensive capability due to temperature. I also marked host nest workers with a small dab of red enamel paint (1104; Testors Co., Rockford, Illinois) on the central dorsal surface of their gaster 24 hours prior to trials to allow me to discriminate between free-living hosts and host-workers of the *T. americanus* nests (Miller, 2017). Host nests were placed into a fridge at ~4°C for 10 minutes prior to being marked to slow their movement and make marking workers easier. I also placed an additional empty artificial nest in the arena next to the host nest to provide an escape option for the hosts after raids, as they would likely have access to in nature. A 6V 15W microscope light was placed ~10cm above the *T. americanus* nest and focused on the nest center to heat the nest and stimulate scouting behaviour (Buschinger et al., 1980). Trials were run for 24 hours and were recorded with webcams (Logitech C922, Logitech International S.A., Lausanne, Switzerland) clipped onto the walls of the arena to record the nests of *T. americanus* and free-living hosts.

Due to a small sample size, I used each *T. americanus* nest in two trials. In both rounds of raids the *T. americanus* nests were in the same temperature treatment but the target host species treatment was switched between trials such that half of the kidnapper nests within a temperature raided *T. ambiguus* in round one then *T. longispinosus* in round two, and vice versa. Five *T. americanus* nests in each temperature treatment successfully raided in both rounds. Two trials were run a day from 16 July – 5 August 2020. After a trial was complete, I censused all ants in the arena. I recorded the number of host brood guarded by host workers (either in the escape nest or elsewhere in the arena) as salvaged brood and the number of injured (i.e. lost antennae or legs) and killed workers of each nest as casualties. Using the recorded footage, I recorded the number of host brood retrieved to the *T. americanus* nest as captured brood, the time from when a *T. americanus* nest member discovered the host nest entrance until the first piece of brood was retrieved to the *T. americanus* nest in minutes as the length of a raid, and the total time from the trial start until the first brood piece was retrieved to the *T. americanus* nest in minutes. The length of raids and time until first brood was only able to be recorded for 20 of the 25 successful trials as either the camera lost focus or the raid occurred when it was dark in the early morning or late night for the other 5 trials. Camel-hair brushes, test fragment artificial nests, escape nests and experimental arenas were washed in 70% ethanol followed by distilled water and dried before use with another separate nest to prevent the transfer of chemical cues. A separate brush was used for each species.

To assess the effects of temperature, host species and their interaction on the defensive capability of free-living hosts against kidnapper raids generalized linear models (GLMs) were used. I considered the temperature treatment, the host species, and the interaction of temperature and host species as fixed effects and also included the raiding round as a fixed effect to control for that aspect of the experimental design. I analyzed the number of free-living host casualties using a Poisson GLM with a log link function. The number of *T. americanus* nest member casualties was examined using a Poisson GLM, but due to overdispersion (c-hat = 1.838) and a linear mean-variance relationship I used a negative binomial distribution (Ver Hoef & Boveng, 2007). A Poisson GLM for the number of salvaged brood was also over-dispersed (c-hat = 3.474) but had a non-linear mean-variance relationship so a quasi-Poisson model was used (Ver Hoef & Boveng, 2007). I analyzed the number of captured brood using a Poisson GLM with a log link function. I took the $\log_{10}$ of the length of a raid so it fit a normal distribution and then used a Gaussian GLM (i.e. multiple linear regression using maximum likelihood). I also took the $\log_{10}$ of the time until first brood so it fit a normal distribution and used a Gaussian GLM.

Since each *T. americanus* nest raided each host species within its respective temperature treatment I used paired analyses to test if kidnapper outcomes changed when they raided the two different host species. If the variable met the parametric assumptions (differences are normally distributed) I used a paired t-test and if they did not meet the parametric assumptions (differences are not normally distributed) I used a Wilcoxon signed-rank test. I used a paired t-test to test if the difference in the number of free-living host casualties between raiding *T. ambiguus* and *T. longispinosus* at 15°C differed from zero. The difference in the number of *T. americanus* nest member casualties between raiding *T. ambiguus* and *T. longispinosus* at 15°C did not meet the parametric assumptions, so I used a Wilcoxon signed-rank test to assess if it was different from zero. I used a paired t-test to test if the difference in the number of salvaged brood between raiding *T. ambiguus* and *T. longispinosus* at 15°C differed from zero. I used a Wilcoxon signed-rank test to test if the difference in the number of captured brood between raiding *T. ambiguus* and *T. longispinosus* at 15°C differed from zero. I used a paired t-test to test if the difference in the length of raids between raiding *T. ambiguus* and *T. longispinosus* at 15°C differed from zero. I used a Wilcoxon signed-rank test to test if the difference in the time until first brood between raiding *T. ambiguus* and *T. longispinosus* at 15°C differed from zero. I used a paired t-test to test if the difference in the number of free-living host casualties between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. I used a Wilcoxon signed-rank test to test if the difference in the number of *T. americanus* nest member casualties between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. I used a paired t-test to test if the difference in the number of salvaged brood between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. I used a paired t-test to test if the difference in the number of captured brood between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. I used a

Wilcoxon signed-rank test to test if the difference in the length of raids between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. I used a Wilcoxon signed-rank test to test if the difference in the time until first brood between raiding *T. ambiguus* and *T. longispinosus* at 25°C differed from zero. Differences were considered statistically significant where $P \leq 0.05$.

I used an “all-subsets” model selection approach to identify any colony demographic measures (Table 3.1) that are associated with host defensive capability variables. This technique does not identify any causality and only identifies variables of interest (Burnham & Anderson, 2002; Burnham, Anderson, & Huyvaert, 2011). Variables are considered to be associated with the response variable when $P \leq 0.05$. I used GLMs with the same distributions and link functions for the defensive capability measures as in the previous analyses with the colony demographic variables and the number of days after acclimation when the trial was done as fixed effects. I checked the correlation coefficients and variance inflation factor of each fixed effect to eliminate multicollinearity among factors and eliminated a fixed effect where $r > 0.7$ or VIF > 3. I added the fixed effect proportion of host workers which were *T. ambiguus*, in favour of the numbers of the individual host species. As a result, the final global model included the fixed effects: days post acclimation, queenright status, number of *T. americanus* workers, number of host workers, proportion of host workforce which is *T. ambiguus* and number of brood. To determine which factor(s) contribute most strongly to host defensive capability I performed multi-model averaging using the R package “MuMIn” (Bartoń, 2019) using the selection criterion of AICc < 4 or QAICc < 4 if the distribution was quasi-Poisson (Burnham & Anderson, 2002). Full model averaging was used (Burnham & Anderson, 2002; Lukacs, Burnham, & Anderson, 2010). I also measured the relative importance (rel. imp) of each fixed effect as the sum of the Akaike weights of the models where the fixed effect was included (Burnham & Anderson, 2002). All statistics were performed in R version 3.6.2 (R Core Team, 2019).

Results

There was no effect of temperature (β = -0.02732 ± 0.21300, P = 0.8979), target host species (β = -0.08956 ± 0.22383, P = 0.6891), or the interaction between temperature and target host species (β = 0.16451 ± 0.31032, P = 0.5960) on the number of free-living host casualties in raids (Table 3.2; Figure 3.2A). There was a significant, positive effect of raiding round on the number of free-living host casualties however, with more host casualties in the second round of raids (β = 0.38139 ± 0.15511, P = 0.0139; Table 3.2; Figure 3.3A). There was no effect of temperature (β = 0.1621 ± 0.3471, P = 0.640), target host species (β = 0.3290 ± 0.3462, P = 0.342), or the interaction between temperature and target host species (β = -0.2742 ± 0.4850, P = 0.572) on the number of *T. americanus* nest member casualties in raids (Table 3.3; Figure 3.2B). There was also no effect of raiding round on the number of *T. americanus* nest member casualties (β = -0.2288 ± 0.2465, P = 0.353; Table 3.3; Figure 3.3B). There was no effect of temperature (β = 0.1850 ± 0.7084, P = 0.7967), target host species (β = -0.6401 ± 0.9138, P = 0.4916), or the interaction of temperature and target host species (β = -0.2380 ± 1.2665, P = 0.8528) on the number of salvaged brood in raids (Table 3.4; Figure 3.4A). There was also no effect of raiding round on the number of salvaged brood, though there was considerably less variability in the second round (β = -1.4344 ± 0.7899, P = 0.0844; Table 3.4, Figure 3.5A). There was no effect of temperature (β = -0.04551 ± 0.21143, P = 0.8296), target host species (β = 0.03035 ± 0.21496, P = 0.8877), or the interaction between temperature and target host species (β = 0.14869 ± 0.29963, P = 0.6197) on the number of captured brood in raids (Table

3.5, Figure 3.4B). *T. americanus* nests captured more brood in the second round of raids (β = 0.35664 ± 0.14986, *P* = 0.0173; Table 3.5; Figure 3.5B). There was no effect of temperature (β = 0.35160 ± 0.31630, *P* = 0.2838), target host species (β = 0.25494 ± 0.32361, *P* = 0.4431), or the interaction between temperature and target host species (β = -0.74351 ± 0.42480, *P* = 0.1005) on the $\log_{10}$ of the length of raids (Table 3.6; Figure 3.6A). There was also no effect of raiding round on the $\log_{10}$ of the length of raids (β = 0.00527 ± 0.20512, *P* = 0.9798; Table 3.6; Figure 3.7A). There was no effect of temperature (β = -0.10567 ± 0.20785, *P* = 0.619), target host species (β = -0.04122 ± 0.21265, *P* = 0.849), or the interaction between temperature and target host species (β = -0.02673 ± 0.27915, *P* = 0.925) on the $\log_{10}$ of the total time until the first brood piece was retrieved to the *T. americanus* nest (Table 3.7, Figure 3.6B). There was also no effect of raiding round on the $\log_{10}$ of the total time until the first brood piece was retrieved to the *T. americanus* nest (Table 3.7: Figure 3.7B).

There was no difference in free-living host casualties (paired t-test: t = -0.2325, df = 4, *P* = 0.8276) or *T. americanus* nest member casualties (Wilcoxon signed-rank test: V = 1.5, n = 5, *P* = 0.5862) between raiding *T. ambiguus* and *T. longispinosus* nests at 15°C (Figure 3.8). There was also no difference in the number of salvaged brood (paired t-test: t = 1.4142, df = 4, *P* = 0.2302) or captured brood (Wilcoxon signed-rank test: V = 7.5, n = 5, *P* = 1) between raiding *T. ambiguus* and *T. longispinosus* nests at 15°C (Figure 3.8). There was no difference in the length of raids (paired t-test: t = -0.28695, df = 2, *P* = 0.8011) or the total time until the first brood piece was retrieved (Wilcoxon signed-rank test: V = 4, n = 3, *P* = 0.75) between raiding *T. ambiguus* and *T. longispinosus* nests at 15°C (Figure 3.9). At 25°C there was no difference in the number of free-living host casualties (paired t-test: t = 1.633, df = 4, *P* = 0.1778), the number of *T. americanus* nest member casualties (Wilcoxon signed-rank test: V = 4, n = 5, *P* = 0.8501), the number of salvaged brood (paired t-test: t = 0, df = 4, *P* = 1), or the number of captured brood (paired t-test: t = 0.30151, df = 4, *P* = 0.778) between raiding *T. ambiguus* and *T. longispinosus* nests (Figure 3.10). There was also no difference in the length of raids (Wilcoxon signed-rank test: V = 7, n = 3, *P* = 0.625) or the total time until the first brood piece was retrieved (Wilcoxon signed-rank test: V = 5, n = 3, *P* = 1) between raiding *T. ambiguus* and *T. longispinosus* nests at 25°C (Figure 3.11).

There were 30 candidate models with considerable support in the data (AICc<4) for the exploratory Poisson GLMs for the number of free-living host casualties. After model averaging no variables were associated with the number of free-living host casualties (Table 3.8). There were 9 candidate models with considerable support for the exploratory negative binomial GLMs for the number of *T. americanus* nest member casualties. Model averaging identified the number of brood as very important (rel. imp = 0.95) and positively associated with the number of *T. americanus* nest member casualties (*P* = 0.0137; Table 3.9). There were 9 candidate models with considerable support (QAICc<4) for the exploratory quasi-Poisson GLMs for the number of salvaged brood. There were no variables associated with the number of salvaged brood after model averaging (Table 3.10). There were 8 candidate models with considerable support for the exploratory Poisson GLMs for the number of captured brood. Model averaging identified the number of *T. americanus* workers as important (rel. imp = 1.00) and positively associated with the number of captured brood (*P* = 0.010, Table 3.11). There were only 3 candidate models with considerable support for the exploratory Gaussian GLMs for the $\log_{10}$ length of raids but model selection uncertainty was still high (max w = 0.323). No variables were associated with the $\log_{10}$ length of raids (Table 3.12). There were 5 candidate models with considerable support for the exploratory Gaussian GLMs for the $\log_{10}$ time until first brood and model averaging did not identify any associated variables (Table 3.13).

Discussion

There was no effect of temperature or of the interaction between temperature and host species on the number of free-living host casualties, *T. americanus* nest member casualties, salvaged brood, captured brood, the length of raids or the total time until the first brood piece was retrieved in raids. Additionally, there was no difference in host defensive capability or kidnapper success for *T. americanus* colonies when raiding the two host species at both 15°C and 25°C. These results suggest that there is no effect of temperature on the ability of *T. americanus* to conduct raids or on the defensive capability of *T. ambiguus* and *T. longispinosus* against kidnapper raids from *T. americanus*, counter to my predictions that temperature would influence host species defense and that the host species outside of its thermal optimum would be less well defended than the host species within its thermal optimum. There was also no effect of host species on defensive capability or kidnapper outcomes regardless of temperature, suggesting that overall *T. ambiguus* and *T. longispinosus* have roughly equal defensive capability in the southern Ontario community. This lack of difference in overall defensive ability regardless of temperature is expected as the two host species experience roughly equal parasite pressure (of *T. americanus* colonies collected from 2018-2020: 49 colonies used *T. ambiguus* hosts and 53 used *T. longispinosus* hosts, with 56.92% of colonies containing both host species), and parasite pressure is known to be a major factor influencing host defense (Brandt & Foitzik, 2004; Foitzik et al., 2001). However, due to my low sample size and thus low power, I ultimately cannot take these results as conclusive evidence for or against these hypotheses, though if there is any effect of temperature on host species defensive capability it is small. While operating outside of a species thermal optimum has been shown to affect the outcome of inter-specific conflicts in other species of ants (Cerdá et al., 1997), it does not appear to affect the fighting performance of these *Temnothorax* species. Perhaps in these *Temnothorax* species operating within their thermal optima may influence other behaviours or characteristics besides those related to defense, such as foraging or brood care. It is also possible, however, that the observed distributions and niche partitioning of *T. ambiguus* and *T. longispinosus* colonies relative to microhabitat temperature in southern Ontario are due to other "hidden" factors besides temperature. A wide range of microhabitat characteristics beyond temperature are known to affect ant performance (Queiroz et al., 2013), and these other characteristics may be driving these observed distributions. These results suggest that the lack of a temperature-dependent raiding preference demonstrated in Chapter 2 is due to a lack of a temperature-dependent difference in host defensive capability, though it is still likely optimal to raid any host colony of acceptable quality. *T. americanus* pupal retrieval preference for the two host species is affected by temperature, however, suggesting that temperature somehow influences the attractiveness of the host species to the kidnappers (Novotny and Hare, in prep). Host species defensive capability does not appear to drive this documented temperature-dependent pupal retrieval preference.

Ants use cuticular hydrocarbon (CHC) profiles to recognize and distinguish between nestmates and non-nestmates, and between species (van Zweden & D'Ettorre, 2010). Brood retrieval in *Temnothorax* ants appears to be based on CHCs (Hare, 1996), thus *T. americanus* likely uses CHC profiles to distinguish between their host species. The CHC profiles of *T. longispinosus* and *T. ambiguus* are similar but have distinct species-specific differences (Brandt, Heinze, Schmitt, & Foitzik, 2005; Kleeberg, Menzel, & Foitzik, 2017). Even within species, CHC profiles can vary with temperature (Wagner, Tissot, & Gordon, 2001), and between colonies from different microhabitat patches (Nielsen, Boomsma, Oldham, Petersen, & Morgan, 1999). Differences in CHC profiles affects their physical properties, and can change their overall volatility (Gibbs & Pomonis, 1995). The CHC profiles of the host species may be adapted to their microhabitat temperature optimum, such that the overall volatility of their CHC profiles is reduced. As kidnapper ants could detect the CHC profiles from large colonies there is the possibility for selection on hosts to conceal their chemical presence. Thus, host species outside of their temperature optimum would have increased CHC volatility, which could increase their attractiveness to kidnappers, possibly explaining the temperature-dependent pupal retrieval preference shown by Novotny and Hare (in prep). However, the CHCs which change most with temperature are associated with anti-desiccation and rarely play an important role in recognition (van Zweden & D'Ettorre, 2010). It is also possible that the temperature-dependent pupal retrieval preference arose simply due to chance via type I error. Future work should attempt to confirm the documented temperature-dependent pupal retrieval preference and if it is robust, assess any effects of temperature optima on the host species CHC profiles.

There was a higher number of free-living host casualties, and kidnappers captured more brood in the second round of raids, suggesting that recent prior experience increases kidnapper ant raiding success. Additionally, the variability in the number of *T. americanus* nest member casualties and salvaged brood was considerably lower in the second round of raids, and I likely would have detected a negative effect with more power. Prior contact with a kidnapper scout increases the aggression of *T. longispinosus* colonies and allows them to salvage more brood in raids (Kleeberg et al., 2014; Pamminger et al., 2011). A similar mechanism is likely occurring in *T. americanus* as well, where recent prior contact with a host colony likely increases the raiding motivation and aggression of the kidnapper colony and allows them to capture more brood.

However, as the second round of raids were all completed after the first round of raids there is a possibility this effect could be due to seasonality. *T. americanus* has a very short raiding season in mid-summer of 35 – 50 days and host-encounter rate is low (Miller, 2017). It is possible that diminishing future opportunities for raiding as the season progresses may increase the raiding motivation of *T. americanus* colonies and cause them to increase their aggression. All raids were completed within a 21-day period which constitutes a large portion of the total raiding season. However, the number of days post acclimation that the trial was completed on was not identified as being associated with the number of host casualties or the number of captured brood in the exploratory colony composition analysis and had low relative importance, suggesting that this effect of round is likely due to prior experience. Future work on elucidating the mechanism of how prior experience increases raiding success and determining how long the effect lasts would be useful in understanding *T. americanus* raiding behaviour and colony dynamics, especially since colonies raid 6 times a season on average (Foitzik & Herbers, 2001). As I was able to detect the effect of prior experience despite the low power of my experiment, if there is a small undetected effect of temperature on host defensive capability it is likely not biologically relevant, as other factors like prior experience and parasite pressure would presumably outweigh it.

My exploratory colony composition analysis identified a positive association between the number of *T. americanus* workers within the colony and the number of captured brood in raids. Larger *T. americanus* colonies have higher numbers of active scouts, allowing them to more quickly locate potential host colonies to raid (Pohl & Foitzik, 2013). Additionally, larger kidnapper colonies would presumably be able to send out larger raiding parties and the closer the size of the raiding party to the size of the target host nest the more brood is captured (Miller, 2020). Larger brood counts in the *T. americanus* colony were positively associated with the number of *T. americanus* casualties in raids. The majority of brood in the colonies at the time of censusing were eggs (Table 3.1), which would be *T. americanus* as host eggs are rarely taken in raids. It is possible that colonies with the largest amount of brood also have high levels of worker reproduction, which is common in other kidnapper ants (Heinze, 1996; Herbers & Stuart, 1998). *T. americanus* colonies with high levels of worker reproduction use different raiding strategies than colonies with lower levels, and are more likely to conduct raids with just a single scout rather than recruiting a raiding party (Miller, 2017). It is possible that this alternative raiding strategy with no recruitment is more likely to cause casualties, though I do not have any data to support this hypothesis. The estimated effect size for the number of brood influencing the number of *T. americanus* casualties is very small, so it is also possible that this association represents a spurious effect detected by the "all-subsets" modelling approach.

In summary, there was no evidence that temperature influences the defensive capability of *T. ambiguus* and *T. longispinosus*, suggesting that some other factor, such as CHC volatility, is driving the documented temperature-dependent pupal retrieval preference of *T. americanus* (Novotny and Hare, in prep). Additionally, the two host species have roughly equal defensive capability in the southern Ontario community. Recent prior raiding experience increases kidnapper raiding success and future work should focus on uncovering the mechanism and length of this effect. Larger *T. americanus* colonies were associated with capturing more brood and colonies with larger brood stores were associated with increased kidnapper casualties. These findings contribute to our understanding of kidnapper-host interactions and how they vary across communities, and the factors which affect raiding success in kidnapper ants.

References

Alloway, T. M. (1979). Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). *Animal Behaviour*, *27*, 202–210. https://doi.org/10.1016/0003-3472(79)90140-4

Bartoń, K. (2019). MuMIn: Multi-model inference. R package version 1.43.17. Retrieved from https://cran.r-project.org/package=MuMIn

Beckers, R., Deneubourg, J. L., Goss, S., & Pasteels, J. M. (1990). Collective decision making through food recruitment. *Insectes Sociaux, 37*, 258–267. https://doi.org/10.1007/BF02224053

Bestelmeyer, B. T. (2000). The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. *Journal of Animal Ecology, 69*, 998–1009. https://doi.org/10.1046/j.1365-2656.2000.00455.x

Bhatkar, A., & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *The Florida Entomologist*, *53*, 229–232. https://doi.org/ 10.2307/3493193

Brandt, M., & Foitzik, S. (2004). Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*, *85,* 2997–3009. https://doi.org/ 10.1890/03-0778

Brandt, M., Heinze, J., Schmitt, T., & Foitzik, S. (2005). A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. *Journal of Evolutionary Biology*, *18,* 576–586. https://doi.org/10.1111/j.1420-9101.2004.00867.x

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference : a* *practical information-theoretic approach* (2nd ed.). New York, NY: Springer.

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*, 23–35. https://doi.org/10.1007/s00265-010-1029-6

Buschinger, A., Ehrhardt, W., & Winter, U. (1980). The organization of slave raids in dulotic ants — a comparative study (Hymenoptera; Formicidae). *Zeitschrift für Tierpsychologie*, *53*, 245–264. https://doi.org/10.1111/j.1439-0310.1980.tb01053.x

Cerdá, X., Retana, J., & Cros, S. (1997). Thermal disruption of transitive hierarchies in Mediterranean ant communities. *Journal of Animal Ecology*, *66*, 363–374. https://doi.org /10.2307/5982

Creighton, W. S. (1950). The ants of North America. *Bulletin of the Museum of Comparative Zoology of Harvard College*, *104*, 1–585.

Del Rio Pesado, M., & Alloway, T. M. (1983). Polydomy in the slave-making ant, *Harpagoxenus americanus* (Emery) (Hymenoptera: Formicidae). *Psyche*, *90*, 151–162. https://doi.org/ 10.1155/1983/63051

Detrain, C., Tasse, O., Versaen, M., & Pasteels, J. M. (2000). A field assessment of optimal foraging in ants: trail patterns and seed retrieval by the European harvester ant *Messor barbarus*. *Insectes Sociaux*, *47*, 56–62. https://doi.org/10.1007/s000400050009

Foitzik, S., DeHeer, C. J., Hunjan, D. N., & Herbers, J. M. (2001). Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the*

Royal Society B: Biological Sciences, *268*, 1139–1146. https://doi.org/10.1098/rspb.2001.1627

Foitzik, S., Fischer, B., & Heinze, J. (2003). Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behavioral Ecology*, *14*, 80–88. https://doi.org/10.1093/beheco/14.1.80

Foitzik, S., & Herbers, J. M. (2001). Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution*, *55*, 316–323. https://doi.org/10.1111/j.0014-3820.2001.tb01296.x

Gibbs, A., & Pomonis, J. G. (1995). Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. *Comparative Biochemistry and Physiology -- Part B: Biochemistry and Molecular Biology*, *112*, 243–249. https://doi.org/10.1016/0305-0491(95)00081-X

Grüter, C., Jongepier, E., & Foitzik, S. (2018). Insect societies fight back: the evolution of defensive traits against social parasites. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*. https://doi.org/10.1098/rstb.2017.0200

Hare, J. F. (1996). Discrimination of nestmate larvae by the ant *Leptothorax longispinosus*. *Canadian Journal of Zoology*, *74,* 2055–2061. https://doi.org/10.1139/z96-233

Heinze, J. (1996). The reproductive potential of workers in slave-making ants. *Insectes Sociaux*, *43*, 319–328. https://doi.org/10.1007/BF01242933

Herbers, J. M., & Stuart, R. J. (1998). Patterns of reproduction in slave-making ants. *Proceedings of the Royal Society B: Biological Sciences*, *265*, 875–887.

https://doi.org/10.1098/rspb.1998.0373

Hölldobler, B., & Wilson, E. O. (1990). *The Ants*. Cambridge, Massachusetts: Harvard University Press.

Jongepier, E., Kleeberg, I., & Foitzik, S. (2015). The ecological success of a social parasite increases with manipulation of collective host behaviour. *Journal of Evolutionary Biology*, *28*, 2152–2162. https://doi.org/10.1111/jeb.12738

Jongepier, E., & Foitzik, S. (2016). Fitness costs of worker specialization for ant societies. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20152572. https://doi.org/10.1098/rspb.2015.2572

Jongepier, E., Kleeberg, I., Job, S., & Foitzik, S. (2014). Collective defence portfolios of ant hosts shift with social parasite pressure. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140225. https://doi.org/10.1098/rspb.2014.0225

Kleeberg, I., Jongepier, E., Job, S., & Foitzik, S. (2015). Geographic variation in social parasite pressure predicts intraspecific but not interspecific aggressive responses in hosts of a slavemaking ant. *Ethology*, *121*, 694–702. https://doi.org/10.1111/eth.12384

Kleeberg, I., Menzel, F., & Foitzik, S. (2017). The influence of slavemaking lifestyle, caste and sex on chemical profiles in *Temnothorax* ants: insights into the evolution of cuticular hydrocarbons. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20162249. https://doi.org/10.1098/rspb.2016.2249

Kleeberg, I., Pamminger, T., Jongepier, E., Papenhagen, M., & Foitzik, S. (2014). Forewarned is forearmed: aggression and information use determine fitness costs of slave raids.

Behavioral Ecology, *25*, 1058–1063. https://doi.org/10.1093/beheco/aru084

Lenoir, A., D'Ettorre, P., & Errard, C. (2001). Chemical ecology and social parasitism in ants. *Annual Review of Entomology*, *46*, 573–599. https://doi.org/10.1146/annurev.ento.46.1.573

Lukacs, P. M., Burnham, K. P., & Anderson, D. R. (2010). Model selection bias and Freedman's paradox. *Annals of the Institute of Statistical Mathematics*, *62*, 117–125. https://doi.org/10.1007/s10463-009-0234-4

Miller, J. S. (2017). *Collective behavior in slave-making ants: how ecology and social structure shape raiding strategies* (Doctoral thesis). Ithaca, NY: Cornell University.

Miller, J. S. (2020). Not too big, not too small: raids at moderately sized hosts lead to optimal outcomes for a slave-making ant. *Behavioral Ecology and Sociobiology*, *74*, 18. https://doi.org/10.1007/s00265-019-2797-2

Nielsen, J., Boomsma, J. J., Oldham, N. J., Petersen, H. C., & Morgan, E. D. (1999). Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum*. *Insectes Sociaux*, *46*, 58–65. https://doi.org/10.1007/s000400050113

Novotny, N. C., & Hare, J. F. (in prep). Temperature affects retrieval preference of host species pupae by the slave-making ant *Temnothorax americanus* (Hymenoptera: Formicidae).

Pamminger, T., Scharf, I., Pennings, P. S., & Foitzik, S. (2011). Increased host aggression as an induced defense against slave-making ants. *Behavioral Ecology*, *22*, 255–260. https://doi.org/10.1093/beheco/arq191

Pohl, S., & Foitzik, S. (2013). Parasite scouting and host defence behaviours are influenced by colony size in the slave-making ant *Protomognathus americanus*. *Insectes Sociaux, 60*, 293–301. https://doi.org/10.1007/s00040-013-0293-7

Porter, S. D., & Tschinkel, W. R. (1987). Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology*, *16,* 802–808. https://doi.org/10.1093/ee/16.3.802

Queiroz, A. C. M., Ribas, C. R., & França, F. M. (2013). Microhabitat characteristics that regulate ant richness patterns: the importance of leaf litter for epigaeic ants. *Sociobiology, 60,* 367–373. https://doi.org/10.13102/sociobiology.v60i4.367-373

R Core Team, A. (2019). R: A language and environment for statistical computing. Vienna, Austria.: R Foundation for Statistical Computing.

Stuart, R. J., & Alloway, T. M. (1985). Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). *Animal Behaviour*, *33*, 1080–1088. https://doi.org/10.1016/S0003-3472(85)80166-4

van Zweden, J. S., & D'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons. In G. Blomquist & A. G. Bagnères (Eds.), *Insect hydrocarbons: biology, biochemistry, and chemical ecology* (pp. 222–243). Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/CBO9780511711909.012

Ver Hoef, J. M., & Boveng, P. L. (2007). Quasi-Poissonvs. negative binomial regression: how should we model overdispersed count data?. *Ecology*, *88*, 2766–2772. https://doi.org/10.1890/07-0043.1

Wagner, D., Tissot, M., & Gordon, D. (2001). Task-related environment alters the cuticular hydrocarbon composition of harvester ants. *Journal of Chemical Ecology*, *27*(9), 1805–1819. https://doi.org/10.1023/A:1010408725464

Tables and Figures

Table 3.1: Mean ± SD of demographic measures from *Temnothorax americanus* nests (n=14) collected from wooded areas in the Rural Municipality of Halton, Ontario from 23 – 24 and 30 – 31 May 2020, and 13 – 14 June 2020.

Demographic measure	Mean ± SD	Range
Number of *T. americanus* workers	4.00 ± 3.038	0 - 10
Number of host workers	30.79 ± 20.104	12 - 89
Number of *T. ambiguus* workers	16.71 ± 16.771	0 - 65
Number of *T. longispinosus* workers	14.79 ± 9.390	0 - 28
Total number of brood	20.07 ± 17.362	0 - 55
Total number of pupae	4.86 ± 5.815	0 - 19
Number of *T. ambiguus* pupae	0.29 ± 0.726	0 - 2
Number of *T. longispinosus* pupae	1.43 ± 3.390	0 - 12
Number of *T. americanus* pupae	3.07 ± 3.709	0 - 13
Number of larvae	1.50 ± 1.870	0 - 5
Number of eggs	13.71 ± 14.751	0 - 41
Queenright status	5 queenright nests : 9 queenless nests	

Table 3.2: Results of Poisson GLM for the number of free-living host casualties in raids from *Temnothorax americanus* nests against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	z - value	*P* - value
Intercept	1.77791	0.16938	10.497	<2e-16
Temperature (25°C)	-0.02732	0.21300	-0.128	0.8979
Target host species (*T. longispinosus*)	-0.08956	0.22383	-0.400	0.6891
Temperature x Host species	0.16451	0.31032	0.530	0.5960
Raiding round (2nd)	*0.38139*	*0.15511*	*2.459*	*0.0139*

Table 3.3: Results of negative binomial GLM for the number of *Temnothorax americanus* nest member casualties in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	z - value	*P* - value
Intercept	1.2780	0.2659	4.806	1.54e-06
Temperature (25°C)	0.1621	0.3471	0.467	0.640
Target host species (*T. longispinosus*)	0.3290	0.3462	0.950	0.342
Temperature x Host species	-0.2742	0.4850	-0.565	0.572
Raiding round (2nd)	-0.2288	0.2465	-0.928	0.353

Theta = 10.8.

Table 3.4: Results of quasi-Poisson GLM for the number of salvaged brood by free-living hosts in raids by *Temnothorax americanus* nests against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	t - value	*P* - value
Intercept	1.2737	0.5097	2.499	0.0213
Temperature (25°C)	0.1850	0.7084	0.261	0.7967
Target host species (*T. longispinosus*)	-0.6401	0.9138	-0.701	0.4916
Temperature x Host species	-0.2380	1.2665	-0.188	0.8528
Raiding round (2nd)	-1.4344	0.7899	-1.816	0.0844

$\Phi = 4.0434$

Table 3.5: Results of Poisson GLM for the number of captured brood by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	z - value	*P* - value
Intercept	1.81239	0.16646	10.888	<2e-16
Temperature (25°C)	-0.04551	0.21143	-0.215	0.8296
Target host species (*T. longispinosus*)	0.03035	0.21496	0.141	0.8877
Temperature x Host species	0.14869	0.29963	0.496	0.6197
Raiding round (2^{nd})	*0.35664*	*0.14986*	*2.380*	*0.0173*

Table 3.6: Results of Gaussian GLM for the $\log_{10}$ of the length of raids in minutes by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	t - value	*P* - value
Intercept	1.329983	0.261261	5.091	0.000133
Temperature (25°C)	0.351605	0.316304	1.112	0.283809
Target host species (*T. longispinosus*)	0.254948	0.323610	0.788	0.443073
Temperature x Host species	-0.743510	0.424807	-1.750	0.100500
Raiding round (2nd)	0.005271	0.205125	0.026	0.979837

Table 3.7: Results of Gaussian GLM for the $\log_{10}$ of the total time in minutes until the first brood piece was retrieved by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	t - value	*P* - value
Intercept	2.16871	0.17168	12.632	2.14e-09
Temperature (25°C)	-0.10567	0.20785	-0.508	0.619
Target host species (*T. longispinosus*)	-0.04122	0.21265	-0.194	0.849
Temperature x Host species	-0.02673	0.27915	-0.096	0.925
Raiding round (2nd)	-0.15687	0.13479	-1.164	0.263

Table 3.8: Results of exploratory Poisson GLM multi-model averaging for the number of free-living host causalities in raids by *Temnothorax americanus* nests against *T. ambiguus* and *T. longispinosus* nests at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	1.4526280	0.4284700	(0.61284, 2.29241)	3.390	0.000698	
Number of *T. americanus* workers	0.0257428	0.0413017	(-0.05520, 0.10669)	1.508	0.131487	0.40
Days post acclimation	0.0286562	0.0483429	(-0.06609, 0.12340)	1.443	0.149049	0.38
Number of brood	-0.0005482	0.0031632	(-0.00674, 0.00565)	0.572	0.567296	0.12
Number of host workers	0.0003241	0.0022156	(-0.00401, 0.00466)	0.487	0.626357	0.11
Queenright status (Yes)	0.0115055	0.1008635	(-0.18618, 0.20919)	0.365	0.714992	0.11
Proportion of host workers *T. ambiguus*	-0.0254057	0.1477344	(-0.31495, 0.26414)	0.579	0.562895	0.11

Table 3.9: Results of exploratory negative binomial GLM multi-model averaging for the number of *Temnothorax americanus* nest member casualties in raids against *T. ambiguus* and *T. longispinosus* nests at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	0.6984301	0.4692851	(-0.22135, 1.61821)	1.488	0.1367	
Number of brood	*0.0240954*	*0.0097699*	*(0.00494, 0.04324)*	*2.466*	*0.0137*	*0.95*
Number of host workers	0.0051401	0.0082733	(-0.01107, 0.02135)	0.621	0.5344	0.36
Proportion of host workers *T. ambiguus*	0.0268025	0.1681879	(-0.30283, 0.35644)	0.159	0.8734	0.07
Queenright status (Yes)	0.0140439	0.0936337	(-0.16947, 0.19756)	0.150	0.8808	0.06
Days post acclimation	-0.0016456	0.0162930	(-0.03357, 0.03028)	0.101	0.9196	0.05
Number of *T. americanus* workers	0.0005794	0.0124742	(-0.02386, 0.02502)	0.046	0.9630	0.05

Theta = 41914

Table 3.10: Results of exploratory quasi-Poisson GLM multi-model averaging for the number of salvaged brood by free-living hosts in raids by *Temnothorax americanus* nests against *T. ambiguus* and *T. longispinosus* nests at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	1.436	6.973e-01	(0.06979, 2.80309)	2.060	0.0394	
Number of *T. americanus* workers	-6.108e-02	1.261e-01	(-0.51225, 0.01477)	0.484	0.6281	0.246
Number of host workers	-4.524e-03	1.505e-02	(-0.08598, 0.01763)	0.300	0.7638	0.132
Queenright status (Yes)	-1.179e-01	4.230e-01	(-2.45391, 0.75080)	0.279	0.7806	0.138
Days post acclimation	-6.644e-03	4.431e-02	(-0.35464, 0.18116)	0.150	0.8808	0.077
Proportion of host workers *T. ambiguus*	1.396e-02	2.954e-01	(-2.01111, 2.43632)	0.047	0.9623	0.066
Number of brood	-6.896e-05	5.459e-03	(-0.04307, 0.04094)	0.013	0.9899	0.065

$\Phi = 6.133974$

Table 3.11: Results of exploratory Poisson GLM multi-model averaging for the number of captured brood by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	1.3168065	0.2927926	(0.74294, 1.89066)	4.497	6.9e-06	
Number of T. americanus workers	*0.1072700*	*0.0416427*	*(0.02565, 0.18888)*	*2.576*	*0.010*	*1.00*
Queenright status (Yes)	0.0941438	0.2108762	(-0.15413, 0.89942)	0.446	0.655	0.25
Number of host workers	0.0003464	0.0020576	(-0.00754, 0.01458)	0.168	0.866	0.10
Proportion of host workers *T. ambiguus*	0.0118966	0.1155239	(-0.59133, 0.87180)	0.103	0.918	0.08
Number of brood	0.0001582	0.0022982	(-0.01346, 0.01738)	0.069	0.945	0.08
Days post acclimation	0.0001231	0.0127749	(-0.08822, 0.09139)	0.010	0.992	0.08

Table 3.12: Results of exploratory Gaussian GLM multi-model averaging for the $\log_{10}$ of the length of raids in minutes by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	1.54955	0.42525	(0.71608, 2.38301)	3.644	0.000269	
Number of *T. americanus* workers	-0.05489	0.06839	(-0.22139, -0.00477)	0.803	0.422183	0.49
Queenright status (Yes)	0.15500	0.32966	(-0.09576, 1.36924)	0.470	0.638213	0.24

Table 3.13: Results of exploratory Gaussian GLM multi-model averaging for the $\log_{10}$ of the total time in minutes until the first brood piece was retrieved by *Temnothorax americanus* nests in raids against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Significant effects in italics.

Variable	Effect Size (β)	± Standard Error	95% Confidence interval (2.5, 97.5)	z - value	*P* - value	Relative importance
Intercept	2.0409841	0.1787020	(1.69073, 2.39123)	11.421	<2e-16	
Proportion of host workers *T. ambiguus*	0.0895456	0.2290523	(-0.23801, 1.05601)	0.391	0.696	0.219
Number of brood	-0.005196	0.0027544	(-0.01817, 0.00777)	0.211	0.833	0.112
Number of *T. americanus* workers	-0.020222	0.0114365	(-0.08264, 0.04219)	0.167	0.867	0.094

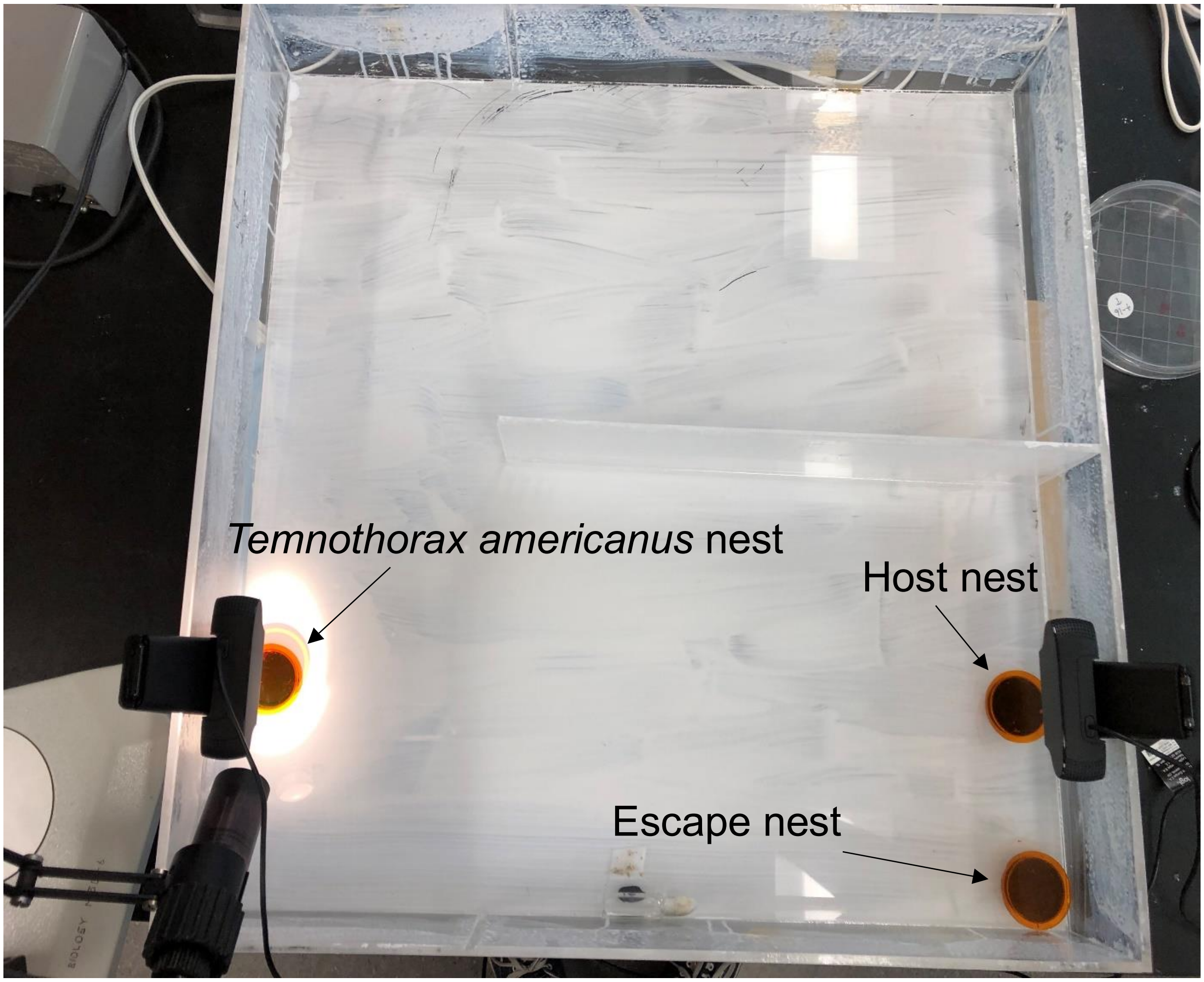

Figure 3.1: Experimental test arena for defensive capability. 60 x 60 x 10cm clear acrylic with 40cm dividing wall.

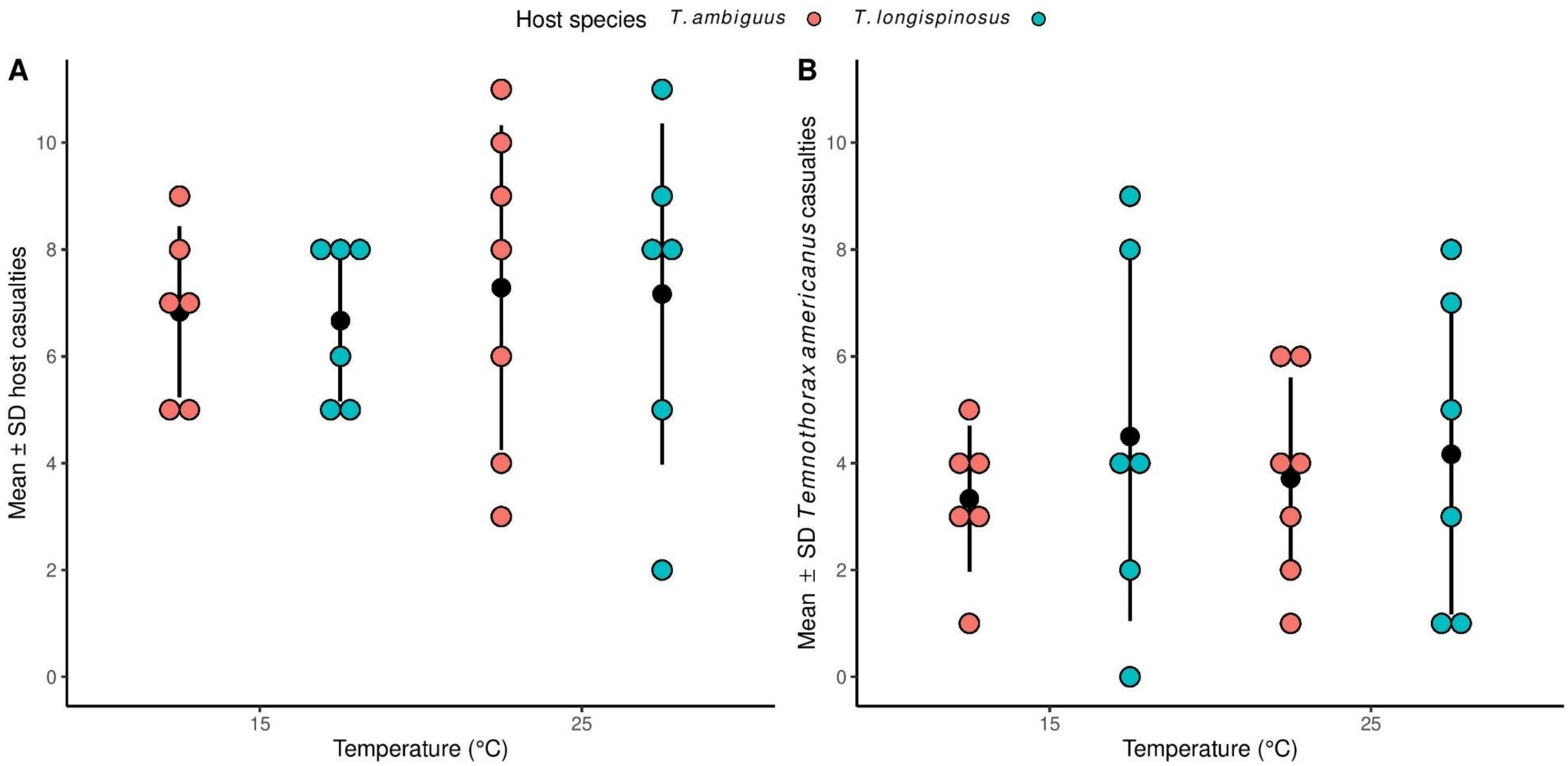

Figure 3.2: Mean ± SD of: A) the number of free-living host casualties, and B) the number of *Temnothorax americanus* nest member casualties, in raids by *T. americanus* nests against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Coloured circles represent the value for a single raid.

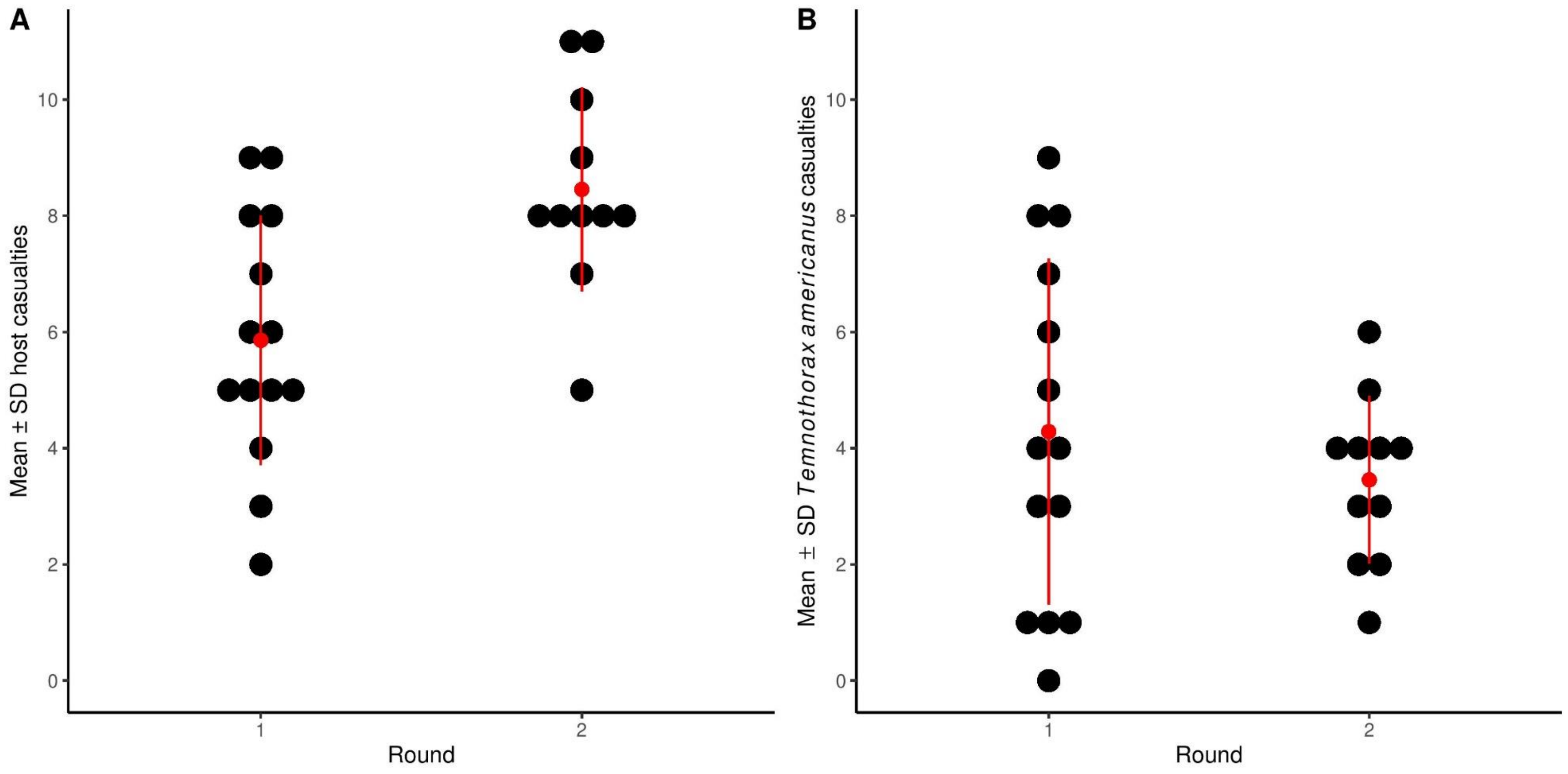

Figure 3.3: Mean ± SD of: A) number of free-living host casualties, and B) number of *Temnothorax americanus* nest member casualties, in the first and second rounds of raids by *T. americanus* nests. Black circles represent the value for a single raid.

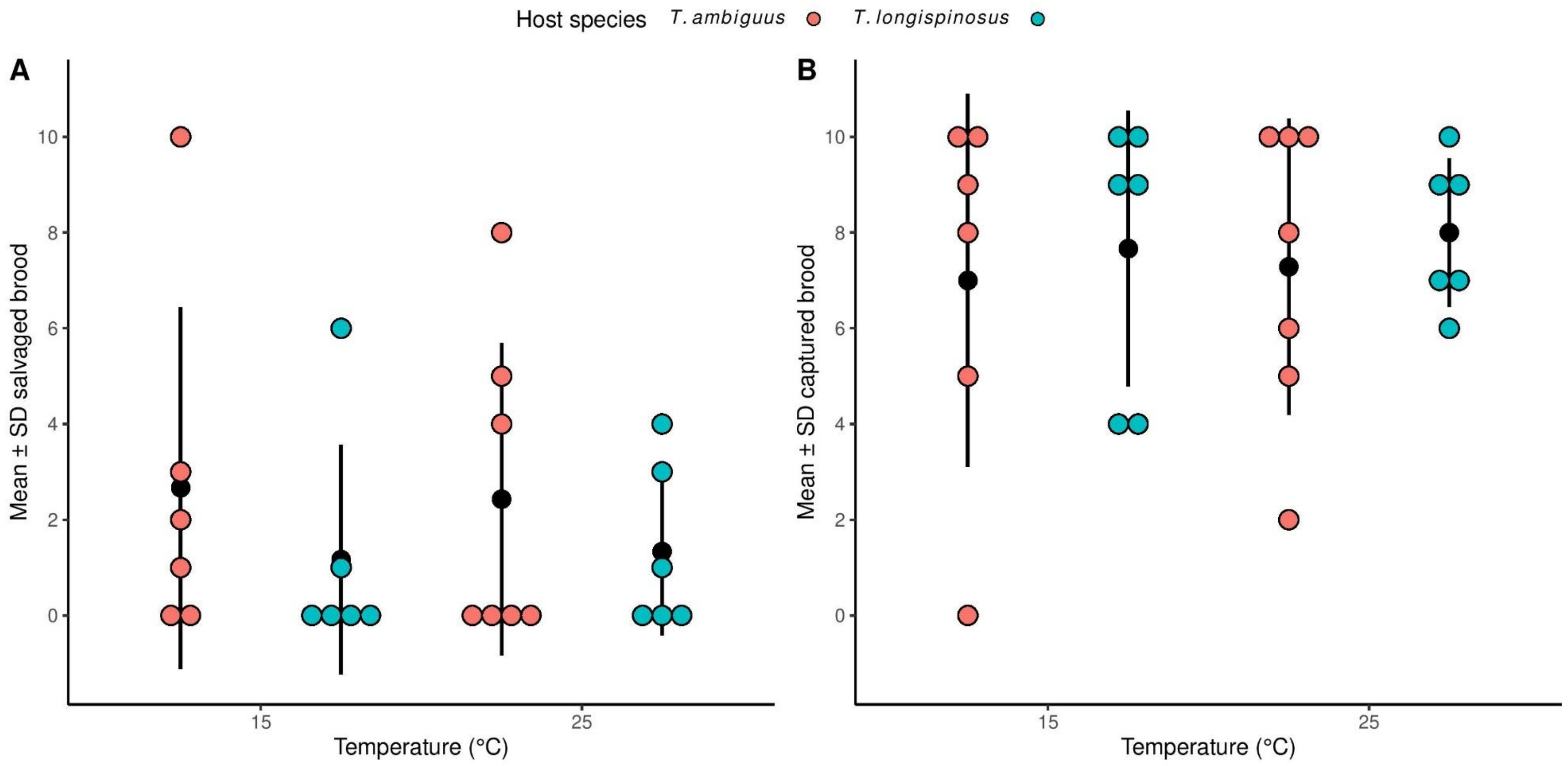

Figure 3.4: Mean ± SD of: A) the number of salvaged brood by free-living hosts, and B) the number of captured brood by *Temnothorax americanus*, in raids by *T. americanus* nests against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Coloured circles represent the value for a single raid.

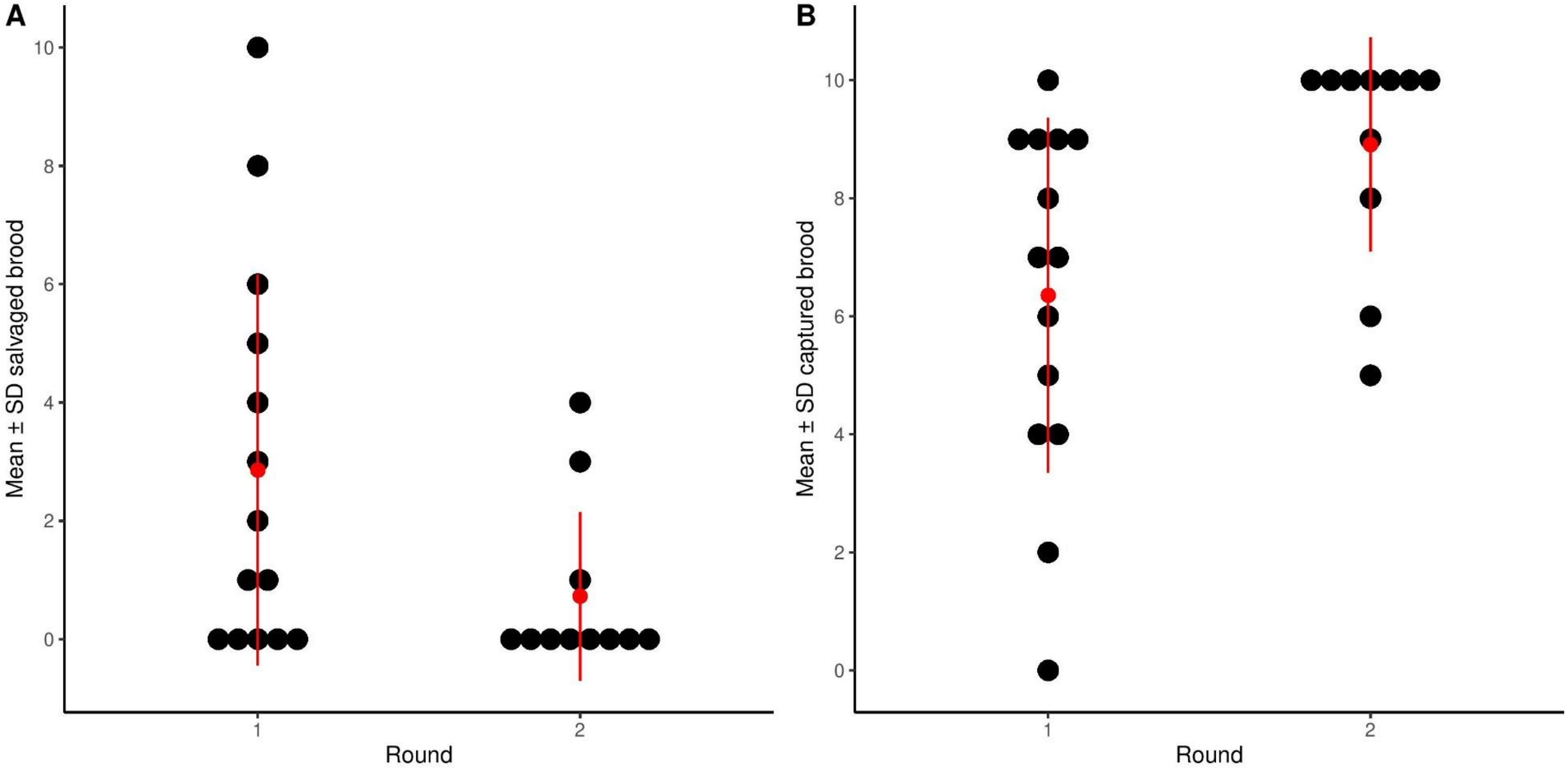

Figure 3.5: Mean ± SD of: A) number of salvaged brood by free-living hosts, and B) number of captured brood by *Temnothorax americanus,* in the first and second rounds of raids by *T. americanus* nests. Black circles represent the value for a single raid.

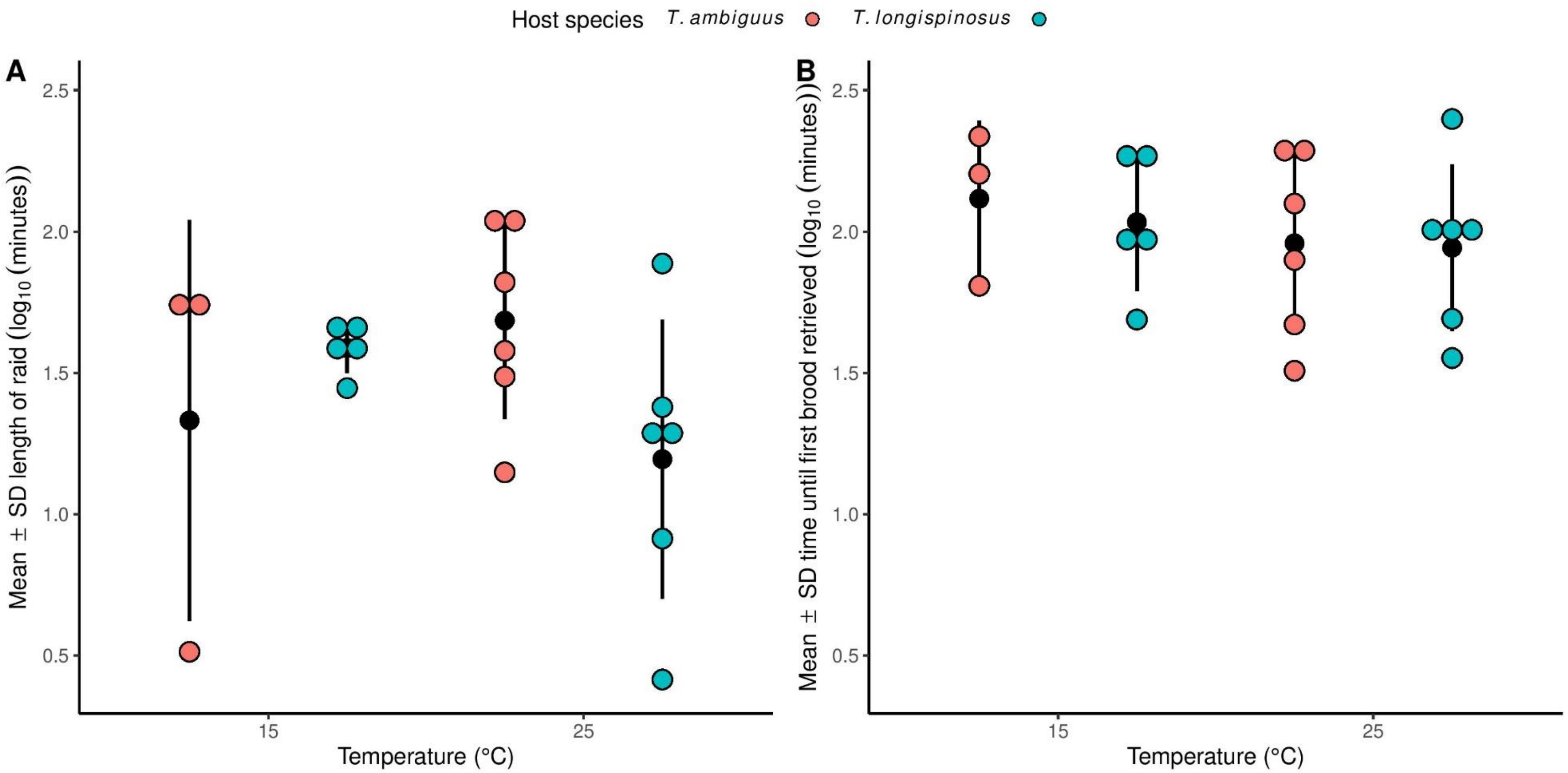

Figure 3.6: Mean ± SD of: A) the $\log_{10}$ of the length of raids in minutes, and B) the $\log_{10}$ of the total time until the first brood piece was retrieved, in raids by *Temnothorax americanus* nests against *T. ambiguus* and *T. longispinosus* at 15°C and 25°C. Coloured circles represent the value for a single raid.

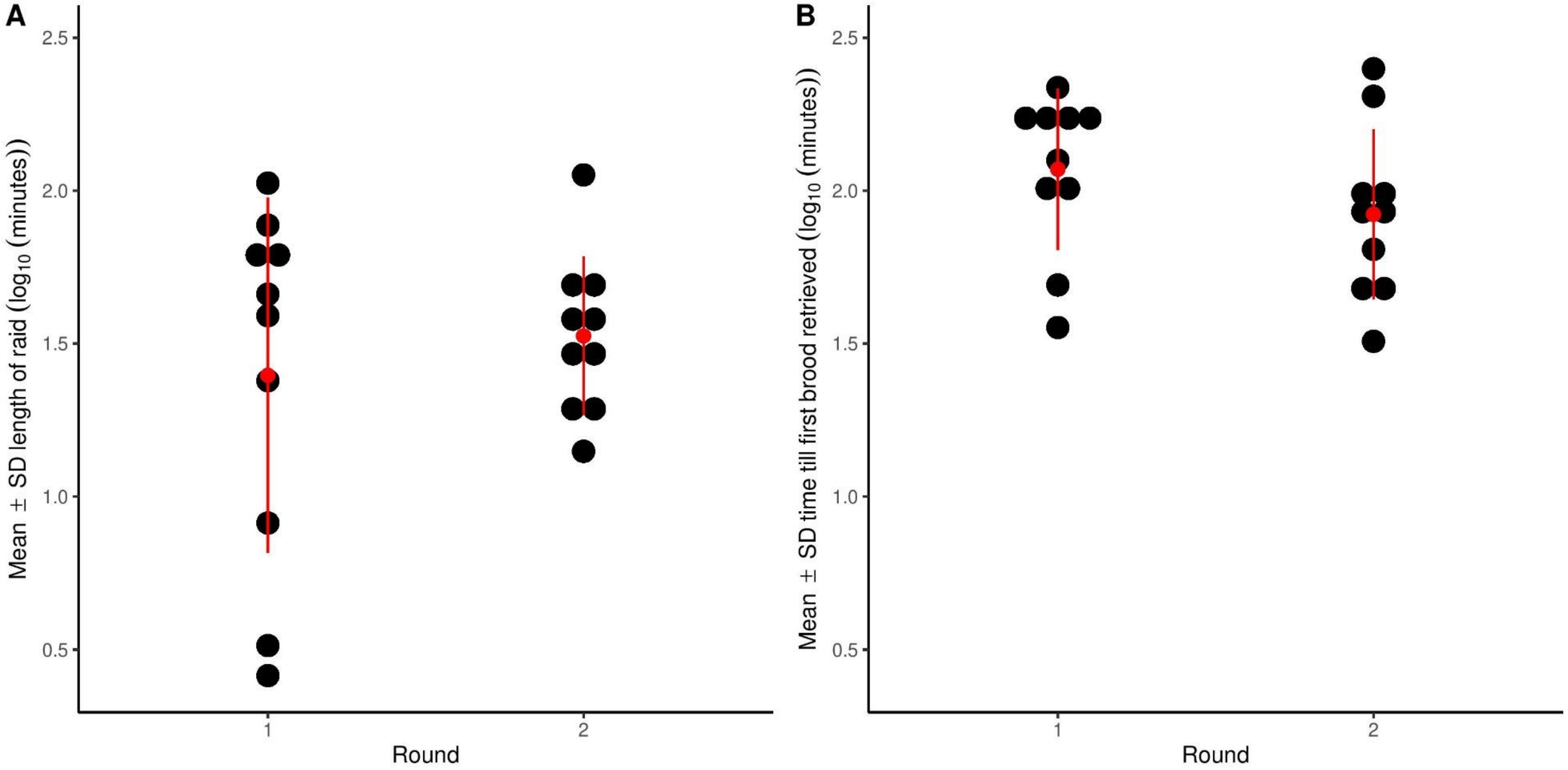

Figure 3.7: Mean ± SD of: A) the $\log_{10}$ of the length of raids in minutes, and B) the $\log_{10}$ of the total time until the first brood piece was retrieved, in the first and second rounds of raids by *Temnothorax americanus* nests. Black circles represent the value for a single raid.

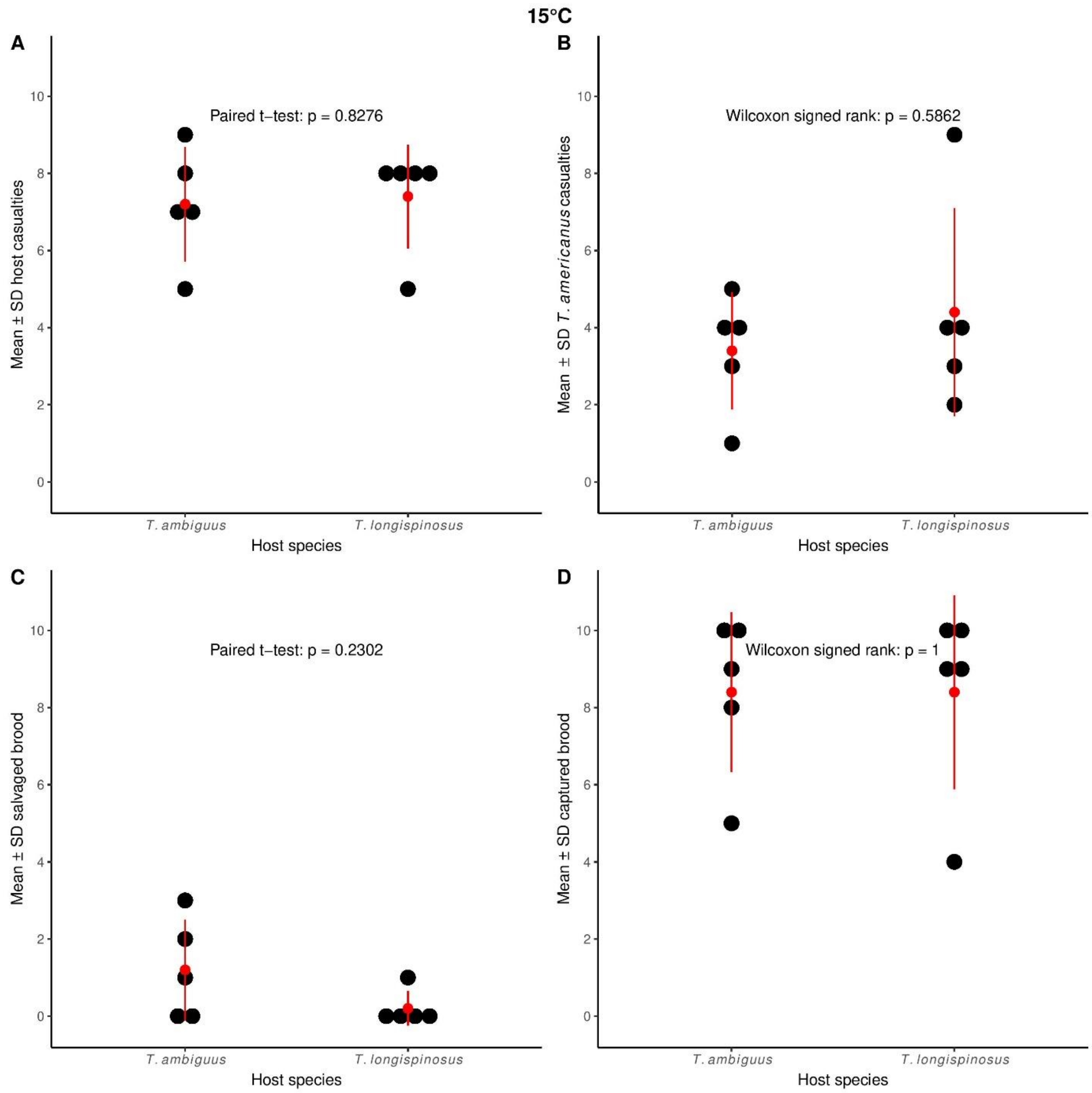

Figure 3.8: Mean ± SD of: A) the number of free-living host casualties, B) the number of *Temnothorax americanus* nest member casualties, C) the number of salvaged brood by free-living hosts, and D) the number of captured brood by *Temnothorax americanus*, by *T. americanus* nests who have raided both *T. ambiguus* and *T. longispinosus* at 15°C. Black dots represent a single raid.

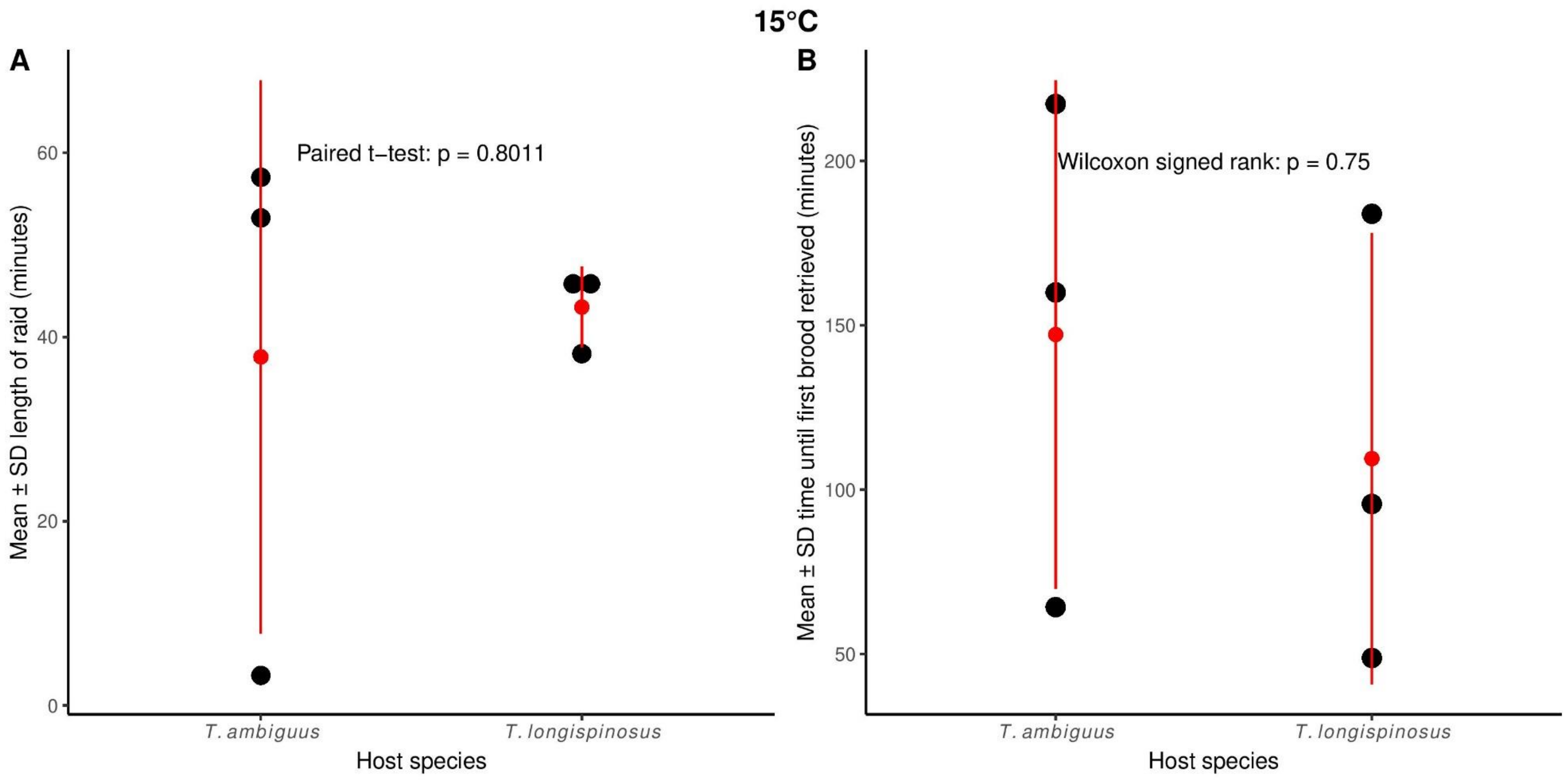

Figure 3.9: Mean ± SD of: A) the length of raids in minutes, and B) the total time until the first brood piece was retrieved, by *Temnothorax americanus* nests who have raided both *T. ambiguus* and *T. longispinosus* at 15°C. Black dots represent a single raid.

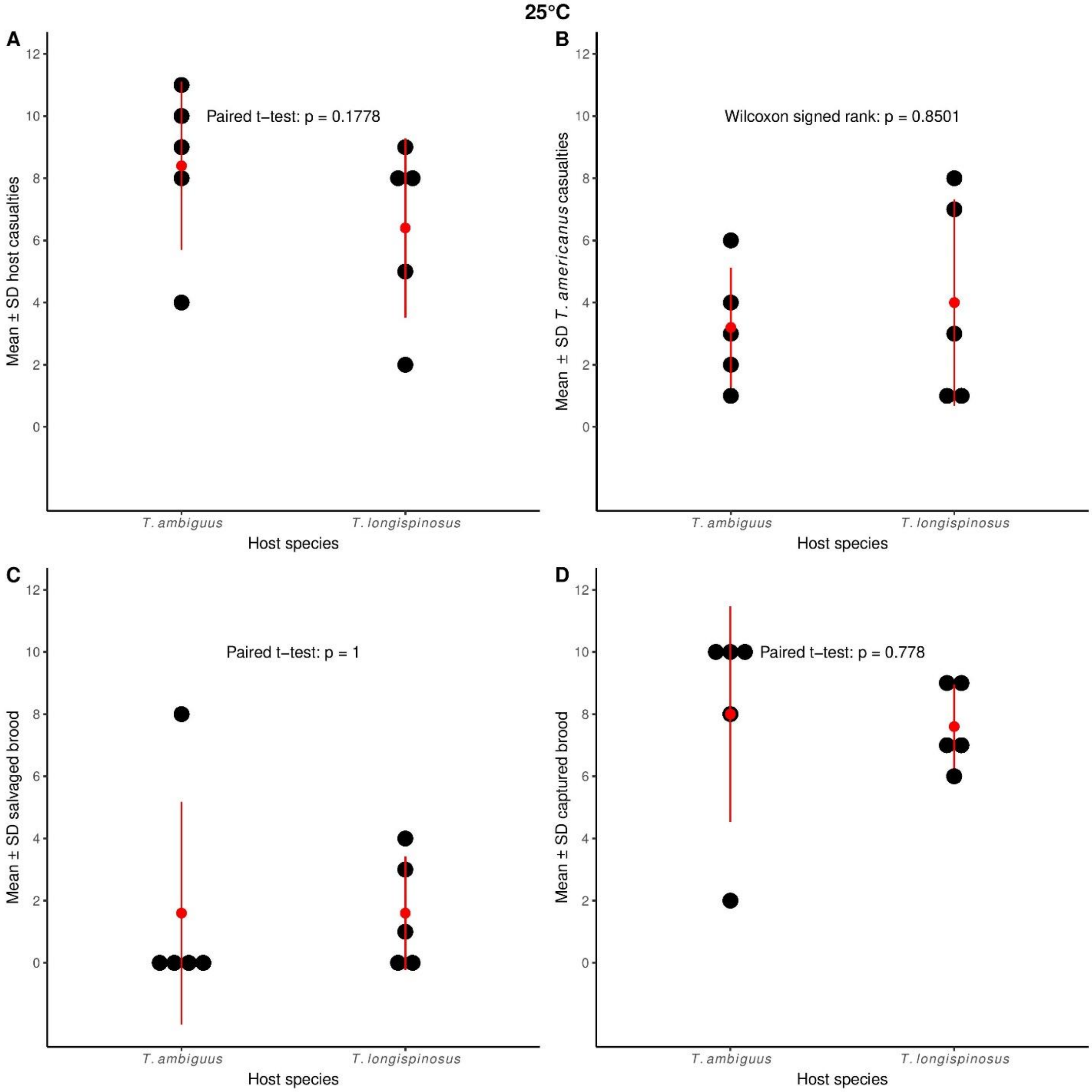

Figure 3.10: Mean ± SD of: A) the number of free-living host casualties, B) the number of *Temnothorax americanus* nest member casualties, C) the number of salvaged brood by free-living hosts, and D) the number of captured brood by *Temnothorax americanus*, by *T. americanus* nests who have raided both *T. ambiguus* and *T. longispinosus* at 25°C. Black dots represent a single raid.

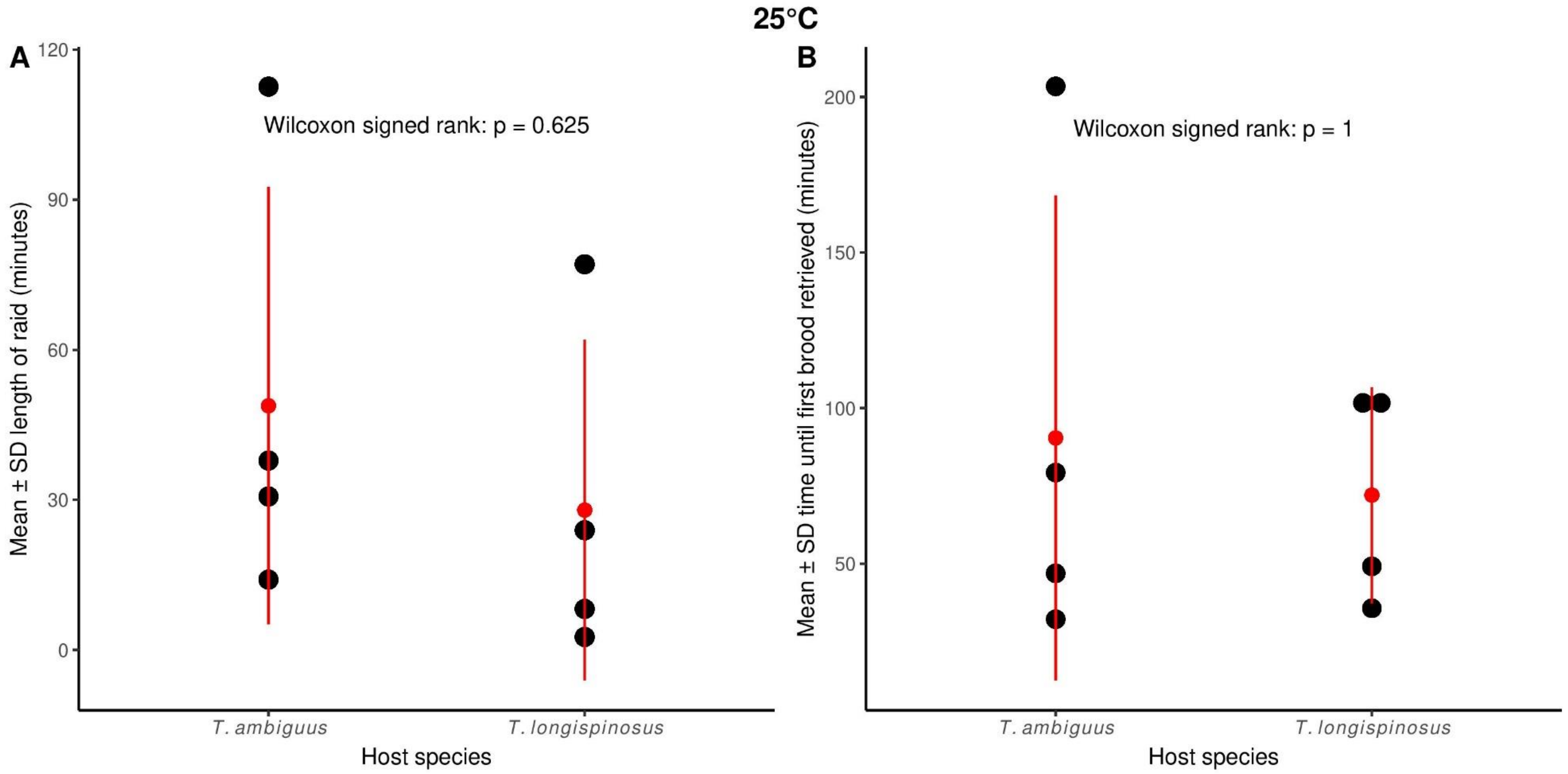

Figure 3.11: Mean ± SD of: A) the length of raids in minutes, and B) the total time until the first brood piece was retrieved, by *Temnothorax americanus* nests who have raided both *T. ambiguus* and *T. longispinosus* at 25°C. Black dots represent a single raid.

Chapter 4: Conclusions

Previous research has shown that the kidnapper ant *Temnothorax americanus* shows a temperature-dependent pupal retrieval preference for its two host species, *T. ambiguus* and *T. longispinosus* (Novotny and Hare, in prep). *T. americanus* preferentially retrieved pupae of the host species whose temperature optimum is opposite to the temperature conditions experienced by the kidnapper colony, possibly reflecting a preference of the kidnapper colony to raid the least well defended host species relative to temperature (Novotny and Hare, in prep). Based upon these results I undertook a series of experiments to investigate the optimization of raiding preference relative to temperature and host defensive capability by *T. americanus* from a community in southern Ontario, Canada.

In Chapter 2, I investigated whether *T. americanus* shows a temperature-dependent raiding preference for one of its two host species, by using simultaneous presentation choice trials in both warm and cool temperature conditions. *T. americanus* exhibited no temperature-dependent or overall raiding preference for either of the host species. This lack of preference suggests that there may be no temperature-dependent differences in host species defensive capability or kidnapper outcomes. However, it is also possible that it is optimal for *T. americanus* to not exhibit a raiding preference even if a temperature-dependent defensive difference between host species exists, as low encounter rates limit the opportunities to raid. Thus, kidnappers should raid the first encountered host colony of sufficient quality. The data presented in this chapter contributes to our understanding of the optimization of host-choice in kidnapper ants in multiple-host species contexts.

In Chapter 3, I quantitatively measured host species defensive capability and *T. americanus* raiding success relative to temperature using raids on single host colonies in both warm and cool temperature conditions. There was no evidence that temperature influences the defensive capability of *T. ambiguus* and *T. longispinosus*, suggesting that the lack of a temperature-dependent raiding preference demonstrated in Chapter 2 is due to a lack of a temperature-dependent difference in host defensive capability, though it is still likely optimal to raid any host colony of acceptable quality. Additionally, I showed that the two host species have roughly equal defensive capability in the southern Ontario community. Recent prior raiding experience increased kidnapper raiding success and future work should focus on uncovering the mechanism of this effect and determining how long it lasts. Larger *T. americanus* colonies were associated with capturing more brood and colonies with larger brood stores were associated with increased kidnapper casualties. The data presented in this chapter helps contribute to the base of knowledge on the defensive-capability of hosts against kidnapper ant raids and on the factors that influence raiding success in kidnapper ants.

Taken together, my findings suggest that some factor other than the defensive capability of the host species *T. ambiguus* and *T. longispinosus* is driving the documented temperature-dependent pupal retrieval preference of *T. americanus*. It is possible that temperature influences the cuticular hydrocarbon profiles of the host species, such that its volatility is lowest within the species' temperature optimum to reduce the likelihood that kidnappers can detect and recognize the hosts. Thus, species outside of their thermal optima would have more volatile cuticular hydrocarbon profiles and therefore be more attractive to kidnapper workers. It is also possible that this temperature-dependent pupal retrieval preference is simply a type I error detected by chance. Future work should attempt to confirm the documented temperature-dependent pupal retrieval preference and if it is robust, assess any effects of temperature optima on the host species CHC profiles. I provided additional evidence to support the hypothesis proposed by Miller (2017) that *T. americanus* should not exert a raiding preference and that it is more optimal to raid the first encountered host species. Additionally, the data presented in this thesis adds to the large body of work on the geographic mosaic of kidnapper-host interactions by showing that in the southern Ontario community the host species *T. ambiguus* and *T. longispinosus* experience roughly equal parasite pressure and thus have evolved equally effective defenses against *T. americanus* raids. This lack of a difference in defensive capability between the host species contrasts with results found in other communities (Brandt & Foitzik, 2004; Foitzik et al., 2001). My results have also provided additional insight into the mechanisms which affect kidnapper ant raiding success by showing that recent prior experience increases the amount of brood they can capture. Very little work has been done on host species preference in multiple-host species contexts and the work presented in this thesis is the first to investigate optimization of raiding preference in relation to microhabitat temperature and its effects on host defensive capability. My results provide further insight into how kidnapper ants choose their host species when there are multiple species available and contribute to our understanding of kidnapper-host interactions, how they vary across communities, and which factors affect raiding success in kidnapper ants.

References

Brandt, M., & Foitzik, S. (2004). Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*, *85*, 2997–3009. https://doi.org/10.1890/03-0778

Foitzik, S., DeHeer, C. J., Hunjan, D. N., & Herbers, J. M. (2001). Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society B: Biological Sciences*, *268*, 1139–1146. https://doi.org/10.1098/rspb.2001.1627

Miller, J. S. (2017). *Collective behavior in slave-making ants: how ecology and social structure shape raiding strategies* (Doctoral thesis). Ithaca, NY: Cornell University.

Novotny, N. C., & Hare, J. F. (in prep). Temperature affects retrieval preference of host species pupae by the slave-making ant *Temnothorax americanus* (Hymenoptera: Formicidae).