

Development and Behavioral Ecology of *Tetrastichus jonsoni* (Hymenoptera: Eulophidae)

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Abstract

Tetrastichus johnsoni is a poorly known parasitoid wasp that attacks the larval stage of its host, the spider wasp, *Dipogon sayi*. After the host egg hatches *T. johnsoni* will lay its eggs inside the developing larva. As the host larvae mature, *T. johnsoni* will grow inside the host, killing it in the process. The parasitoid only completes its development when dozens of individuals burst from the fully-grown host larva. This type of egg-larval development is often associated with polyembryonic development of the parasitoid. In polyembryonic division a single parasitoid egg fragments (clones) within the host body to produce a large number of genetically identical individuals. In addition, because many siblings reach adulthood in close proximity, there is likely to be inbreeding because of brother–sister mating. Inbreeding is expected to have profound effects on the parasitoid's sex ratios.

Introduction

Polyembryonic wasps are known to occur in four families of parasitoid wasps; Encyrtidae, Platygasteridae, Braconidae, and Dryinidae. These are known as egg-larval parasitoids; a female

wasp lays one or a few eggs inside the egg of a host insect, but the parasitoid does not develop immediately. Instead, the host egg hatches and the host larva grow to full size with the parasite inside (Godfray 1994). The parasitoid embryo undergoes asexual polyembryonic division within the host and develops into a large number of separate individuals. When their development is complete, parasitic larvae emerge from their host, killing it in the process. One parasitoid egg develops into as many as 2,000 wasps (Strand 1989b). Because of the asexual polyembryonic development, all individuals arising from a single egg are genetically identical and of the same sex. In the case when a parasitoid female lays two eggs in a single host, one egg produces males and the other egg develops into females.

Related male wasps that develop from the same brood compete for females of the same brood. A corollary is that if males and females of the same brood mate there will be a strongly female biased sex ratio (Hamilton 1979). If mixed broods show a higher percentage of females than males, this will likely provide an example of local mate competition and inbreeding that favors female biased sex ratios (Hamilton 1967; Ode and Hunter 2002; Strand 1989a). When females and males mature in close proximity, males will most likely have reduced flight capacity because of limited mating opportunities away from the family group (Hamilton 1979). In addition to local-competition-for mates theory when multiple *T. johnsoni* females attack the same host individual, they should alter their sex ratio toward the production of more males. It is likely that polyembryonic life histories have evolved at least once in each of the four families with species exhibiting this trait (Godfray 1994).

Field Observations

Dipogon sayi is a common wasp that lives in the woodlands where it hunts spiders in the genus *Xysticus*. When nesting, a female *D. sayi* captures a spider, stings and paralyzes it, and then transports it to a hollow twig or empty insect tunnel in dead wood. There, she lays a single egg on the spider, and seals it behind a wall of debris that she collects. A female *D. sayi* will fill a deep cavity with multiple cells separated by debris wall, each containing a paralyzed spider and wasp egg. Female *T. johnsoni* gain access to a *D. sayi* egg by entering a nest cavity while the host is provisioning spiders and building debris barriers. Once sealed inside the cavity, the *T. johnsoni* female waits for *D. sayi* to hatch, and then lays her egg(s) inside the *D. sayi* larva.

Information about the wasp *Tetrastichus johnsoni*, in the family Eulophidae, is very fragmentary, but this insect is known to be a larval parasitoid that oviposits into the larval stage of the spider wasp *Dipogon sayi* (pompilidae), and several dozen full grown parasitoid larvae emerge from the mature host larva (Krombein 1967) (pers. obs.). It is unclear to how *T. johnsoni* develops within its host. Since *T. johnsoni* is a larval parasitoid there is reason to suspect that it may be polyembryonic. It could also be that a female *T. johnsoni* injects several dozen separate eggs into the host and each offspring develops independently. Because many young adult *T. johnsoni* larvae develop in close proximity, it is also possible that there is local competition for mates and female biased sex ratios. Our specific aims are to determine developmental patterns of *T. johnsoni*, its prevalence on its host *D. sayi*, whether *T. johnsoni* engages in local competition for mates, and whether its sex ratios conform to predictions based on local competition for mates theory.

Materials and methods

In order to test these hypotheses we placed fifty trap nests in the woodlands at Pierce Cedar Creek. Twenty-five trap nests were placed at the beginning of red trail near Batts cottage, and the remaining twenty-five trap nests were placed behind the wet lab. The spiders that *D.sayi* hunts live in the leaf litter of forests. We chose these areas because they are forested dry areas away from populated locations.

Each trap nest was in a bundle of six sticks. The sticks are six inches long with an area of one square inch. Each stick was drilled in to a depth of five inches, and each was drilled with a different size drill bit. The bit sizes range from 5/32, 3/16, 7/32, 1/4, 9/32, and 5/16. Newly completed *D.sayi* nests were collected on a daily basis throughout the summer, and replaced with new unoccupied sticks. Occupied sticks were split open, and species were identified and recorded.

Polyembryonic Development Analysis

D.sayi nests were collected and opened. Spiders with a *D.sayi* egg were weighed, and then placed in rearing chambers. Overwintered *T. johnsoni* were used to start experimentation. Overwintering *T. johnsoni* were allowed to finish their development, mate, and then were placed individually in rearing chambers containing a *D.sayi* egg on a spider. Rearing chambers were checked in fifteen minute intervals daily until oviposition was observed. Periodically, parasitized hosts were placed in buffered formalin to prepare for microscopy in order to determine how *T. johnsoni* eggs were developing inside *D.sayi*. Also, we dissected *T. johnsoni* adult mated females in order to determine how fertilized eggs appear upon oviposition.

Local- Competition- for- Mates Theory

Various *T. johnsoni* were allowed to continue development inside *D.sayi*. Matured *T. johnsoni* were fed honey in order to develop eggs, and allowed to mate. *D.sayi* eggs with a spider were collected and placed in rearing chambers with two to four mated females of different broods. When development completed brood sizes were counted

Results

Tetrastichus Behavior

Information about the species *T. johnsoni* is scarce. Any type of observations about behavior, appearance and social communication of *T. johnsoni* can provide new insights to this species. The systematic differences in form of the male and female species of *T.johnsoni* were limited. Males of the species *T. johnsoni* were smaller than their female counterparts, and displayed yellow coloration on the underside of their abdomens. Courtship displays of male *T. johnsoni* involved mounting of females, and rapidly flap their wings and antennae. Male behavior was also different from females. Males emerged from the pupa stage earlier than females, and were also more active and social.

Female behavior was readily seen when placed in rearing chambers with additional females. When placed in rearing chambers with *D. sayi* females would compete with each other for a position on *D.sayi*. Superparasitism of multiple foundresses was prevalent. When two females were found on a single host both females were witnessed ovipositing into the host. When females were placed in rearing chambers with *D.sayi* in its egg stage, they would wait until *D.sayi* was hatched to oviposit. After *D.sayi* hatched, female *T.johnsoni* would wait a week or two to oviposit. Female *T. johnsoni* chewed on tissue of *D.sayi*, or pierced their ovipositor into

D.sayi to feed on hemolymph. Female *T. johnsoni* parasitizes *D.sayi* by piercing and placing her ovipositor deep inside *D.sayi*. Oviposition has lasted from 2 minutes to 8 minutes, and in some cases occurred multiple times.

Additional information that we were able to collect during the summer includes characteristic of *T. johnsoni* that are new to science. We were able to survey the attack of one two, three, four and sister *T.johnsoni* on a single host. Table one demonstrates the percent rate of attack on *D.sayi* compared to the number of *T.johnsoni* that was placed with the host. Only 15-32% of reared *D.sayi* that was exposed to *T.johnsoni* produced parasitoids. The percent rate of attack reveals that the number of *T.johnsoni* placed on a host didn't affect the likelihood that young *T. johnsoni* would be produced.

	One <i>T.johnsoni</i>	Two <i>T.johnsoni</i>	Three <i>T.johnsoni</i>	Four <i>T.johnsoni</i>	Sister <i>T.jonsoni</i>
Sample size	232	85	43	16	59
Number of attacked <i>D. sayi</i>	35	18	14	3	10
% Rate of Attack	15%	21%	32%	18%	16%

Table 1: The sample sizes taken of attack of one, two, three, four and sister T. johnsoni on D.sayi. This table determines the rate of attack to each of the four scenarios.

When opening up trap nests we observed that *D.sayi* larva feed on different size larva. We wanted to determine if the weight of the spider will determine the size of the *D.sayi* larva, and thus determine brood sizes of *T.johnsoni*. The larger the spider, the larger *D.sayi* will grow, and the weight of the spider will ultimately determine the amount of young *T.johnsoni* produced.

Figure one depicts that as the weight of the spider increases the brood sizes of *T.johnsoni* increases. Also, this graph demonstrates success of *T.johnsoni* is with in brood sizes of twenty to thirty *T.johnsoni* offspring.

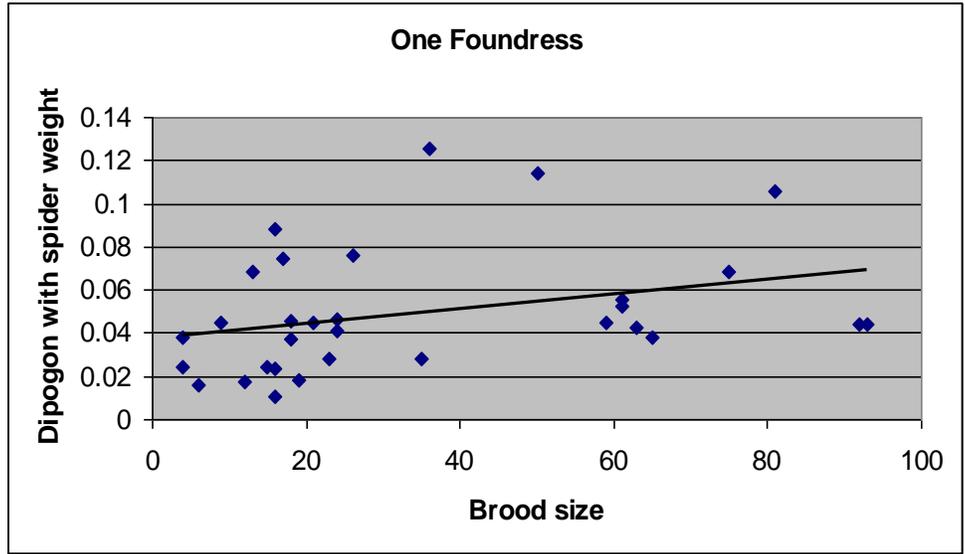


Figure one: Brood size as a function of the weight of spider with *D.sayi*.

Discussion

The lack in knowledge and research on the species *T. johnsoni* supplied many obstacles to overcome while trying to determine polyembryonic development. When *T. johnsoni* was observed ovipositing into *D.sayi*, attacked *D.sayi* was placed into a buffered formalin solution. The formalin was used to preserve the *D.sayi*, and also to maintain the growth of the *T. johnsoni* egg at the exact time that *D.sayi* was parasitized. The formalin preserved and toughened the tissue of *D.sayi* in a way that made it difficult to separate and to observe under the microscope. To try and correct this problem we prepared live *D.sayi* that had been parasitized for microscopy. We could not confidently determine eggs from *D.sayi* tissue from this method. Alternatively, we dissected mated females in order to verify how fertilized *T. johnsoni* eggs would materialize upon attack of the host. When trying to determine how fertilized eggs appear inside mated

female *T. johnsoni*, it was difficult to distinguish between internal fluids or actual eggs.

Required resources or different research approaches might have aided in our understanding of egg development.

Optimal brood sex ratios of *T. johnsoni* should shift from fewer males to more males as the number of attacking females increases. We placed two to four females of *T. johnsoni* on the same host egg to observe their development and the sex ratios of their broods. Table two shows that in nature 88% of females found attacking *D. sayi* are not competing directly with other females for the same host, while 13% of females are directly competing with other females for the same host.

Total sample size: 510, 3.1% of sample size population		
Number of <i>T. johnsoni</i>	One	Two
<i>T. johnsoni</i> found attacking <i>D. sayi</i> in nature	14	2
% found	88%	13%

Table 2: Attack events in nature

We could ask that if it is more likely for females not to encounter one another, then there would be no shift in sex ratios to males if another female was present. By looking at brood sex ratios compared to the number of attacking females we could determine how females compensate for competing females on the same host. We discovered that females allow their offspring to go into diapause in early July. Disappointingly when waiting for *T. johnsoni* to pupate development stopped at the larval stage, and will not be fully developed until spring of next year.

References

Godfray, H. D. J. 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton.

Hamilton, W. D. 1967. Extraordinary Sex Ratios. *Science* 156:147-488.

Hamilton, W. D. 1979. Wingless and fighting Males in Fig Wasps and Other Insects. Pp. 167-220 in M.S. Blum and N.A. Blum, eds. *Sexual Selection and Reproductive Competition in Insects*. Academic Press, New York

Humason, G.L. 1979. *Animal Tissue Techniques*. W. H. Freeman

Krombein, K. V. 1967. *Trap-Nesting Wasps and Bees: Life Histories, Nests, and Associates*. Smithsonian Press, Washington, D. C.

Ode, P. J., and M. Hunter. 2002. Sex Ratios of Parasitic Hymenoptera with Unusual Life histories in I. C. W. Hardy, ed. *Sex Ratios, Concepts and Research Methods*. Cambridge University Press, Cambridge.

Strand, M. R. 1989a. Clutch Size, Sex Ratios and Mating by the Polyembryonic Encyrtid *Copidosoma floridanum* (Hymenoptera: Encyrtidae). *Florida Entomologist* 72: 32-42.

Strand, M. R. 1989b. Development of the Polyembryonic Parasitoid *Copidosoma floridanum* in *Trichoplusia ni*. *Entomologia Experimentalis et Applicata* 50: 37-46