THE *ANAGRUS EPOS* COMPLEX: A LIKELY SOURCE OF EFFECTIVE CLASSICAL BIOLOGICAL AGENTS FOR GLASSY-WINGED SHARPSHOOTER CONTROL

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ABSTRACT

What appear to be eight or nine different *Anagrus* species were obtained from 18 collection sites for morphological and molecular examination. Confirming our hypothesis that there might be cryptic species hidden in this complex, an *Anagrus* species in Mexico, two species in Colorado, and one in the state of Washington were found to be genetically different from the Minnesota strain of *A. epos*. Genetic and morphological analyses are nearly complete and we are preparing a manuscript on this work (Triapitsyn et al. 2008). Rearing *A. epos* on GWSS eggs for field release has been problematic and to help solve this, several studies were undertaken (Krugner et al. 2007, 2008) which compared alternative rearing hosts (i.e. eggs of various leafhopper species) and investigated the basic biology of *A. epos*. Limited field releases have been made during summer 2006 and 2007 (due to rearing difficulties) but to date, *A. epos* has not been recovered. Based on this work, we have started rearing aster leafhopper as an alternative *A. epos* host to allow greater numbers of parasitoids to be released and field sleeve cage releases are planned for next year.

INTRODUCTION

Anagrus epos is a common and seemingly widespread egg parasitoid of leafhoppers (Cicadellidae) in North America. Location records for this species include Colorado, Kentucky, New Mexico, and New York in the U.S. as well as Baja California and Sonora in Mexico (Triapitsyn 1998). While commonly collected as a parasitoid of grape leafhopper species (*Erythroneura* spp. and *Erasmoenura* spp.), a recent collection of *A. epos* from the egg mass of the sharpshooter genus *Cuerna* in Minnesota was the first time this species had been collected from a sharpshooter species (Hoddle & Triapitsyn 2004). Wasps from this collection have been reared continuously since June 2004 in the UC Riverside Quarantine facility on eggs of the glassy-winged sharpshooter.

Like many minute parasitoids, identification to species in this group is exceedingly difficult because of the lack of adult morphological features. Species identifications have been made using light microscopy to determine the presence of key morphological features for *A. epos.* A recent taxonomic revision of the genus *Anagrus* associated with vineyards in North America (Triapitsyn 1998) has shown that: 1) More species are present than previously thought, 2) Some species have a wide geographic distribution, and 3) Relatively few morphological characters are available for distinguishing these species, leaving several authors to think that *A. epos* is not a single species but a complex of different species.

OBJECTIVES

1. Examination of Male and Female *A. epos* Complex Populations for Unique Morphological Characters.
2. Molecular Characterization of Mitochondrial and Ribosomal DNA *of A. epos* Populations.
3. Mating Compatibility Studies Between *A. epos* strains.
4. Field Release and Evaluation of the "Minnesota strain" of *A. epos*.

RESULTS

Progress - Objective 1. Examination of Male and Female *A. epos* Complex Populations for Unique Morphological Characters.

Proposed research is complete and we were able to collect and examine specimens from a larger number of locations that we anticipated. Line drawings are complete and a manuscript will be finished and submitted soon.

Table 1 summarizes the specimens we had available in several collections or that were collected to date and the results of genetic examination. We were fortunate that a number of the specimens Dr. Triapitsyn had collected earlier (a number as far back as 1994) were preserved in very good condition allowing genetic study followed by slide-mounting for morphological examination. In addition, due to a number of productive trips by Dr. Triapitsyn and the assistance from several cooperators (special thanks are due Dr. Larry Wright, Washington State University, Prosser and Dr. Kent Daane, UC Berkeley) we have filled in all major "holes" in the *A. epos* species complex.

Scanning electron micrographs (SEMs) of the antennae and bodies were taken for the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), and *A. epos* (Minnesota origin). Digital photographs (using the Automontage system) of the antennae, forewings, and bodies were taken for the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), *A. epos* (Minnesota origin), and *A. epos* (Illinois). Certain body part measurements were taken from the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), *A. epos* (Minnesota, both original and CA progeny), and *A. epos* (Illinois). Morphometric studies of these specimens have also been completed. Genetic examination is complete (results are discussed below) as is morphological examination and Dr. Triapitsyn has prepared line drawings of all relevant specimens. We are currently discussing what journal would be most appropriate for a combined taxonomic/ genetic presentation of our data in which a number of new species will be described.

Table 1. Summary of specimens collected for morphological (Objective 1) and genetic (Objective 2) research.

Collection	Genus	Species	Collection site
1	*Anagrus*	*epos*	UCR culture, originally collected near Glyndon, Clay Co., MN, 2004
2	*Anagrus*	*sp.*	Campo Experimental INIFAP, Sonora, Mexico, 1994
3	*Anagrus*	*sp.* (same as 2)	Near Caborca, Sonora, Mexico, 1994
4	*Anagrus*	*nigriventris*	UCR, Riverside, Riverside Co., CA, 2004
5	*Anagrus*	*daanei*	Kingsburg, Fresno Co., CA, 2005
6	*Anagrus*	*erythroneurae*	WSU-Prosser Research Center, Prosser, Benton Co., WA, 2005
7	*Anagrus*	*erythroneurae*	Oasis, Coachella Valley, Riverside Co., CA, 1994
8-10, 12	*Anagrus*	*tretiakovae*	Albuquerque, Bernalillo Co., NM, 2005 (ex. *Erythroneura triapitsyni* eggs)
11	*Anagrus*	*erythroneurae*	Temecula, Riverside Co., CA, 2006
13	*Anagrus*	*tretiakovae*	Pavich vineyard, Harquahala Valley, Maricopa Co., AZ, 1994 (ex. *Erasmoneura variabilis* eggs)
14	*Anagrus*	*new species*	Grand Junction, Mesa Co., CO, 2006 (ex. *Erasmoneura vulnerata* eggs)
15	*Anagrus*	sp. 1 near *A. daanei*	Palisade, Mesa Co., CO, 2006 (ex. *Erythroneura ziczac* eggs)
16	*Anagrus*	sp. 2 near *A. daanei*	WSU-Prosser Research Center, Prosser, Benton Co., WA, 2006
17	*Anagrus*	*new species*	Grand Junction, Mesa Co., CO, 2007 (ex. *Erasmoneura vulnerata* eggs)
18	*Anagrus*	sp. 1 near *A. daanei*	Palisade, Mesa Co., CO, 2007 (ex. *Erythroneura anfracta* eggs)

In September 2007, Dr. Triapitsyn made another trip to Colorado to collect *Anagrus* spp., ship them alive under permit to UCR's Quarantine Facility, and determine in Quarantine if they would attack GWSS eggs. Collections were made on 4 September 2007 and were sent to Quarantine the same day. Numerous parasitoids emerged 7-9 September and were exposed in two or three replicates each, to fresh GWSS eggs on *Euonymus japonica* leaves at 22-23°C. By 26 September, GWSS nymphs had started to emerge and it was clear there were no signs of parasitism. Collection #17 from Grand Junction, CO was determined by Dr. Triapitsyn to be a new *Anagrus* species, obtained from eggs of *Erasmoneura vulnerata* (Fitch) on wine grapes. A manuscript describing this species is in preparation based on the 2006 specimens. A second *Anagrus* species tested in Quarantine were specimens from Collection #18 from Palisade, CO which were determined to be *Anagrus* sp. 1 nr. *daanei* Triapitsyn from eggs of *Erythroneura anfracta* Beamer on Virginia creeper. Both of these *Anagrus* species have

shorter ovipositors compared with *A. epos* and we speculate this might be a reason why they failed to successfully parasitize GWSS eggs.

Progress - Objective 2. Molecular Characterization of Mitochondrial and Ribosomal DNA of *A. epos* Populations.
Genetic analyses have confirmed our hypothesis that there are (at least three) cryptic species hidden within specimens that morphologically appeared to be identical *A. epos*. In addition, what was thought to be *A. daanei* in Washington appears to be a different species from the California *A. daanei*. Without genetic examination, this situation would have remained hidden. Using the *A. epos* (Minnesota) strain that is kept in culture on GWSS eggs in the Department of Entomology, UC Riverside, we first determined the DNA sequence of several indicator gene regions. We optimized PCR conditions so that other "*A. epos*" strains could be compared by amplifying and sequencing the chosen gene regions. Methods were tested and adapted from previous *Scirtothrips* research (Rugman-Jones et al. 2006) for use on *Anagrus* allowing us to extract DNA from whole wasps in such a way that after DNA extraction, the remainder of the wasp could be used for morphological studies. This allowed a direct link between the DNA characters and the morphological characters for all the individuals that have been studied.

Members of the genus *Anagrus* are very small and lack definitive species-specific morphological characters. The 28sD2 region of ribosomal RNA has been shown to be well conserved within a species but is different between species. The work here illustrates the preponderance of species that would morphologically be classified as *Anagrus epos*, but are different species (Collections 2/3 and 14/17 each different from the MN "*A. epos*" and each other) whereas Collections 15/18 and 16 morphologically resemble *A. daanei*.

Progress - Objective 3. Mating Compatibility Studies Between *A. epos* strains.
Because genetic studies clearly suggest there is a cryptic species in Mexico and one in Colorado different from the Minnesota strain of *A. epos*, as well as a species in Colorado similar to *A. daanei*, we decided mating studies would not be productive. Instead, we shipped the two Colorado species to UCR's Quarantine facility to allow parasitoids to emerge and determine if they would parasitize GWSS eggs. As mentioned above, neither appeared able to do so.
Our original plan was to conduct mating studies between closely related strains of "*A. epos*" to confirm our genetic results. However, we have run into unexpected problems rearing *A. epos* for field release and evaluation (see Objective 4 below). In addition, our genetic studies have identified what are clearly 23 different species in the "*A. epos*" complex and this, along with their collection from different areas of North America (one in Mexico, one in Colorado, both distant from Minnesota) suggests mating studies are not the highest priority at this time. We decided instead that it was most productive to determine if one or both of the Colorado species would attack and survive in GWSS eggs. For this purpose, Dr. Triapitsyn traveled to Colorado in early September 2007 (when parasitoid levels were high) to collect and ship (via permit) parasitized leafhopper eggs to UCR's Quarantine facility. In Quarantine, emerging wasps were exposed to GWSS eggs to determine if parasitization would occur (it did not).

In addition, we have shifted resources from mating studies to research on the basic biology of the Minnesota *A. epos* strain so that we can improve insectary rearing to allow greater numbers of parasitoids to be released in the field (see Objective 4).

Progress - Objective 4. Field Release and Evaluation of the "Minnesota strain" of *A. epos*.
Completing this objective has been compromised by the difficulty in rearing the Minnesota strain of *A. epos* on GWSS eggs during the winter, allowing us to build the colony to moderate numbers in spring for release. To solve this problem, Ph.D. student Rodrigo Krugner undertook a study examining other leafhopper species that might be used to rear the parasitoid (Krugner et al. 2007). Based on the results of that work, we have started to rear the aster leafhopper, *Macrosteles severini*, as an alternative leafhopper egg host.

We have spent much of the past two years studying the basic biology of the Minnesota strain of *A. epos* and working with CDFA on how to mass rear this species for field evaluation. CDFA and UCR alternated bi-weekly monitoring of endemic and released egg parasitoids at each of 6 lemon study sites, three in the coastal region and three in interior southern California.

The CDFA currently has two colonies of *A. epos*, one in Riverside County and one in Kern County. A total of 59 releases were made in 2006 and a further 10 releases have been made in 2007 in Kern, Tulare, Riverside, and Orange Counties. Over 9,000 parasitoids have been released to date but no recoveries have been made. This may be due to poor survival of *A. epos* in the field, but may also be due to the difficulty in maintaining field-collected specimens sufficiently long to allow emergence of *A. epos* from egg masses.

CONCLUSIONS
See the Abstract.

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