Population Genetics and Biological Control of Goldspotted Oak Borer, an Invasive Pest of California Oaks[1,*]

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Abstract

California's oak woodlands are threatened by the recent introduction of goldspotted oak borer (*Agrilus auroguttatus*). This invasive wood-borer is indigenous to mountain ranges in southern Arizona where its low population densities may be due to the presence of co-evolved, host-specific natural enemies. Reuniting *A. auroguttatus* with these natural enemies can potentially provide permanent control of this pest. To initiate a classical biological control program, our research focused on investigating the genetic variation within and between populations in the native and introduced ranges in attempt to identify the geographic origin of the invasive California population. The area of origin for the invasive population was not determined conclusively, although molecular data suggests the Dragoon Mountains in Cochise County, Arizona as a potential source of this beetle, and its host-specific natural enemies. Native and introduced range surveys for natural enemies were conducted in 2012 and 2013 by deploying more than 30,000 *A. auroguttatus* sentinel. In 2012, the first known egg parasitoid was collected in Arizona and identified as a generalist *Trichogramma* sp., but in 2013, no egg parasitoids of *A. auroguttatus* were found. Despite the lack of success in explorations for host-specific egg parasitoids, classical biological control may still be the most viable long-term management option. However, additional research on the biology, life history, and potential natural enemies of this beetle is needed in order to advance its management in southern California.

Key words: classical biological control, cytochrome C oxidase I, egg parasitoids, foreign exploration, invasive species, phylogeography, wood borers

Introduction

California's oak woodlands are seriously threatened by the goldspotted oak borer (*Agrilus auroguttatus*), an invasive woodborer native to southern Arizona and perhaps northern Mexico (based on a single specimen collected in Baja California Sur in 1977) (Coleman and Seybold 2011). In 2004, this beetle was first detected in San Diego County, but was not a known pest of indigenous oaks until 2008 (Coleman and Seybold 2008). Approximately 25,000 coast live oak (*Q. agrifolia*), California black oak (*Q. kelloggii*), and canyon live oak (*Q. chrysolepis*) have been killed due to larval *A. auroguttatus* feeding in the cambial region (Coleman and others 2012a, USDA FHM 2013). This infestation continues to expand in San Diego and Riverside Counties (Coleman and Seybold 2008, Jones and others 2013). In contrast to the high

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[1] An abbreviated version of this paper was presented at the Seventh California Oak Symposium: Managing Oak Woodlands in a Dynamic World, November 3-6, 2014, Visalia, California.

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* Information presented in this paper has been previously published by the authors in peer-reviewed journals (Lopez and Hoddle 2013, Lopez and others 2014).

levels of mortality caused by *A. auroguttatus* in California, this beetle has never been considered a pest in its native range, and exhibits behavior similar to other *Agrilus* species that preferentially attack trees already in decline (Coleman and others 2012a).

Invasive woodborers are notoriously difficult to manage, especially in natural environments, because they can remain undetected until populations are too large and widespread to control using conventional methods such as pesticides or mechanical removal of infested trees (Cappaert and others 2005, Haack and others 2010, Van Driesche and others 2010). Coleman and Seybold (2008) provided the first reports on *A. auroguttatus* infestation in southern California which spanned 6447 ha and killed an estimated 17,000 trees, indicating that the introduction of this beetle likely occurred (probably via infested oak firewood) several years prior to its initial detection. Due to the widespread infestation of *A. auroguttatus* across federal, state, private, and Native American lands in southern California, classical biological control (the importation of co-evolved, host-specific natural enemies from a pest's native range) is considered to be a viable and promising long-term management strategy for suppressing damaging populations.

Classical biological control has been an effective tool for lowering populations of non-native forest pests (Hajek 1999, Roland and Embree 1995, Ryan and others 1978, Van Driesche and others 2010). While the role of host plant resistance is suspected to play an important role on *A. auroguttatus* population densities (Coleman and Seybold 2011), the rapid increase of this beetle within the oak woodlands of southern California compared to the low densities observed in Arizona may, in part, be due to an absence of co-evolved natural enemies in California. Reuniting *A. auroguttatus* with these host-specific natural enemies may potentially provide permanent control of this invasive pest.

The implementation of an effective classical biological control program for an invasive species requires the knowledge of several key components such as the pest's area of origin, natural enemies (in the native and introduced range), and life history traits. However, before 2008, none of this foundational information was available for *A. auroguttatus*. To initiate a classical biological control program for *A. auroguttatus* in southern California, our research focused on the following components: 1) investigation of genetic variation within and between populations in the native and introduced ranges in attempt to identify the geographic origin of the invasive California population, and 2) conduct native and introduced range surveys for natural enemies of this woodborer.

Determining the area of origin for *A. auroguttatus* is a fundamental step in the search for co-evolved natural enemies that may be considered for use in a classical biological control program against this invasive pest (Lopez and others 2014). Collection records indicate that *A. auroguttatus* inhabits several mountain ranges in southern Arizona including the Chiricahua, Huachuca, Santa Catalina, and Santa Rita mountains (Coleman and Seybold 2011), and the most recent collection from the Dragoon mountains in 2011, a new locality record, suggests there are additional mountain ranges within the native range inhabited by this woodborer. These "sky island" mountain ranges are isolated from each other by the surrounding Sonoran Desert, which presents a natural dispersal barrier for *A. auroguttatus* and its natural enemies. Narrowing the geographic source of the invasive California population to a specific mountain range in Arizona would, in theory, allow the collection of host-specific natural enemies which have formed a co-evolutionary relationship with the genotype of the invasive population (Stouthamer 2008).

Natural enemy surveys throughout the introduced and native range of an invasive pest are an essential component of any classical biological control program (Goolsby and others 2003, Hoddle and others 2002, O'Neil and others 2005, Rosen and DeBach 1992, Toepfer and Kuhlmann 2004). In 2009, *A. auroguttatus* natural enemy surveys were conducted throughout the Santa Rita, Huachuca, Chiricahua, and Santa Catalina Mountain ranges, and on the Descanso Ranger District of the Cleveland National Forest in San Diego County, California (Coleman and Seybold 2011). From these initial surveys, two larval parasitoids were associated with *A. auroguttatus*, along with several generalist predators.

Calosota elongata, a gregarious, larval ectoparasitoid was discovered on late-instar larvae within pupal cells inside infested *Q. emoryi* in southeastern Arizona (Coleman and Seybold 2011), and was also collected from *A. auroguttatus* pupal cells found during destructive sampling of *Q. agrifolia* in San Diego County, California (Haavik and others 2012). Little is known about this newly described parasitoid (Gibson 2010), though the much lower parasitism rate observed in California (<1 percent) compared to that recorded in Arizona (15 percent) suggests that this species may have been transported into southern California along with the original population of *A. auroguttatus* (Haavik and others 2012). Molecular analyses could help confirm this assumption. *Atanycolus simplex*, a generalist larval ectoparasitoid, emerged from *A. auroguttatus*-infested *Q. emoryi* and *Q. agrifolia* collected in Arizona and California, respectively (Coleman and Seybold 2011). The remaining natural enemies reared from infested oak material or collected from *A. auroguttatus* life stages were generalist species not suitable as classical biological control agents due to their broad host ranges (Coleman and Seybold 2011).

While the majority of known natural enemies are either generalist parasitoids or predators of *A. auroguttatus*, the newly described *C. elongata* could be host-specific. However, an inability to rear adequate numbers of *A. auroguttatus* larvae in quarantine has precluded basic biology studies on *C. elongata*. In contrast, eggs of *A. auroguttatus* can easily be obtained in the laboratory by providing field-collected adults with water, oak leaves and coffee filter paper as an oviposition substrate (Lopez and Hoddle 2014). Due to the inability to rear, and thus determine the specificity of natural enemies attacking *A. auroguttatus* larvae and pupae, search efforts have focused on natural enemies attacking *A. auroguttatus* eggs. Since the overall structure (for example, topography, thickness, and coloration) of oak bark makes collecting naturally deposited *A. auroguttatus* eggs (typically oviposited inside cracks and crevices of bark) an arduous task, our strategy of locating egg parasitoids using sentinel egg masses on filter paper is a practical though semi-artificial alternative. From 2012 to 2013, surveys for *A. auroguttatus* egg parasitoids were conducted throughout the native (southern Arizona) and introduced (southern California) range by deploying sentinel egg masses on filter paper in an attempt to attract, collect, and identify potential egg parasitoids of this beetle.

Methods

Population genetics of A. auroguttatus

We utilized sequences of mitochondrial cytochrome oxidase (COI) and the nuclear ribosomal D2 domain of the 28S gene (28SD2) to investigate connectivity among invasive *A. auroguttatus* populations in San Diego and Riverside Counties in southern California, and native populations inhabiting several mountain ranges in southern Arizona. Specimens of *A. auroguttatus* were collected between May 2009 and November 2012 from infested oak trees in the San Jacinto Mountains and

Cleveland National Forest in southern California, and the Chiricahua, Dragoon, Huachuca, Santa Catalina, and Santa Rita Mountains in southern Arizona (table 1). From these individuals, DNA was extracted, amplified, sequenced, and analyzed as described in Lopez and others (2014).

Table 1—Genetic variation in populations of *A. auroguttatus* collected in Arizona and California, assessed as number of haplotypes, and haplotype diversity

Locality	County	State	GPS coordinates	No. of individ-uals	No. of haplo-types	Haplotype diversity
Chiricahua	Cochise	AZ	31°50′N 109°17W	20	6	0.763
Dragoons	Cochise	AZ	31°53′N 109°59′W	23	6	0.684
Huachuca	Cochise	AZ	31°24′N 110°18′W	25	12	0.917
Santa Catalina	Pima	AZ	32°26′N 110°47′W	10	3	0.733
Santa Rita	Santa Cruz	AZ	31°43′N 110°52′W	69	9	0.712
Cleveland National Forest	San Diego	CA	33°18′N 116°48′W	115	15	0.859
San Jacinto	Riverside	CA	33°44′N 116°42′W	24	3	0.359

Surveying for A. auroguttatus natural enemies in Arizona and California

Field surveys for egg parasitoids were conducted in the native and introduced range of *A. auroguttatus*. In 2012, *A. auroguttatus* sentinel eggs were deployed at two oak forest field sites in Arizona and California (table 2). In 2013, a total of eight field sites, six in Arizona and two in California, were selected from counties where this beetle had been previously collected (Coleman and Seybold 2011) (table 2). At each site, six and 10 infested oaks (in 2012, and 2013, respectively) were selected for deployment of *A. auroguttatus* sentinel eggs to survey for potential natural enemies targeting this life stage. Trees were considered infested if symptoms described in Hishinuma and others (2011) such as larval galleries and exit holes were present. At each selected tree, sentinel eggs were deployed into specified treatments which were individually suspended on infested trees. The deployment of *A. auroguttatus* eggs at each site was conducted over an eight week period during July to September 2012, and June to August 2013. Sentinel egg masses were prepared, deployed, retrieved, reared, and examined as described in Lopez and Hoddle (2013).

Table 2—Geographic information for field sites and number of sentinel eggs deployed during *A. auroguttatus* natural enemies surveys in 2012 and 2013

Site name	Locality	County	State	GPS coordinates	No. of eggs deployed (2012)	No. of eggs deployed (2013)
Gardner Canyon	Santa Rita Mtns	Pima	AZ	31°43’N/110°43’W	7428	2272
Carr Canyon	Huachuca Mtns	Cochise	AZ	31°26’N/110°17’W	-	2159
Middle March Pass	Dragoon Mtns	Cochise	AZ	31°51’N/109°57’W	-	2277
Cochise Stronghold	Dragoon Mtns	Cochise	AZ	31°55’N/109°58’W	-	2720
Turkey Creek	Chiricahua Mtns	Cochise	AZ	31°51’N/109°20’W	-	2701
Pinery Canyon	Chiricahua Mtns	Cochise	AZ	31°57’N/109°18’W	-	2575
Heise Park	William Heise County Park	San Diego	CA	33°02’N/116°35’W	5676	1658
Pine Creek Trailhead	Cleveland National Forest	San Diego	CA	32°50’N/116°32’W	-	1746

Results and discussion

Population genetics of A. auroguttatus

A section of the COI gene from 286 *A. auroguttatus* individuals (147 from Arizona and 139 from California) was sequenced in an attempt to delineate the area of origin of the invasive California population, and better understand the genetic variation within and between sampled populations. Haplotype distribution, abundance, and diversity across sample locations are shown in table 1. Additionally, identical sequences of 28SD2 from 23 specimens sampled from the Chiricahua, Huachuca, Santa Catalina, and Santa Rita Mountains in Arizona, and from the Cleveland National Forest, and San Jacinto Mountains in California indicated that the individuals collected were all one species, and not the congener *A. coxalis* or perhaps another ‘unknown’ cryptic species morphologically indistinguishable from *A. auroguttatus* or *A. coxalis* (Coleman and others 2012b).

Among the COI sequences, a total of 39 haplotypes were identified which shows high variability in the COI gene region of this species. However, the geographic distribution of these haplotypes revealed little genetic overlap between the native and introduced regions since only two haplotypes were shared between these areas (37 of 39 haplotypes sampled across California (n = 14) and Arizona (n = 23) populations were distinct to either region). The most common haplotype was shared by 82 specimens and found in every sample location except the Santa Catalina Mountains, Arizona. The second overlapping haplotype was shared only between individuals collected from the Cleveland National Forest in California (n = 4) and the Dragoon Mountains in Arizona (n = 1), highlighting the Dragoon Mountains as a possible source of *A. auroguttatus* in southern California. However, it seems likely that we have not yet sampled the full range of variation in this species (table 1), and larger samples including collections across additional unsampled mountain ranges in Arizona and northern Mexico could help to further pinpoint the geographic source of the California population of *A. auroguttatus*.

Little genetic overlap was also observed between *A. auroguttatus* populations within the native range. Out of the 23 distinct haplotypes identified from Arizona collections, 17 were unique to either the Chiricahua (n = 4), Dragoon (n = 1), Huachuca (n = 7), Santa Catalina (n = 3), or Santa Rita (n = 2) Mountains, supporting the idea that populations of *A. auroguttatus* from these "sky islands" in southern Arizona are genetically isolated from one another. The lack of gene flow between native populations of *A. auroguttatus* revealed by our data justifies the use of population genetics to focus the search for co-evolved natural enemies of this woodborer. Consequently, future surveys for *A. auroguttatus* natural enemies should focus on unsurveyed mountain ranges in southern Arizona and northern Mexico, and the Dragoon Mountains in southern Arizona, as a potential source for classical biological control agents.

Surveying for A. auroguttatus natural enemies in Arizona and California

During the natural enemy surveys conducted in 2012 and 2013, more than 30,000 *A. auroguttatus* sentinel eggs were deployed through the native and introduced range of this insect. In 2012, the first known egg parasitoid of *A. auroguttatus* was collected at very low levels (approximately 0.1 percent) from sentinel eggs deployed in the Santa Rita Mountains, Arizona, and was identified as *Trichogramma* sp. Investigation into the identity of this species (using ITS2 sequences) found a previous collection record of this parasitoid from Lepidoptera eggs collected in Riverside County, California (Richard Stouthamer, unpublished data), indicating that this species is likely a generalist that opportunistically parasitized the sentinel eggs.

In 2013, no egg parasitoids of *A. auroguttatus* were found in the native or introduced range of this woodborer despite the increase in field sites surveyed and number of eggs deployed. Considering the absence of parasitism from >18,000 *A. auroguttatus* eggs deployed throughout infested sites in southern Arizona and California in 2013, and the rarity of parasitism recorded from >12,000 eggs deployed in similar treatments in 2012 (Lopez and Hoddle 2013), it may be reasonable to conclude that host-specific parasitoids of this beetle may not exist within the areas sampled. In addition, the low number of *Trichogramma* sp. collected from 2012 surveys suggests that parasitism from generalist egg parasitoids does not significantly contribute to *A. auroguttatus* egg mortality. However, inadequate surveying techniques, and an insufficient search range or duration could have led to the detection of very few egg parasitoids during our concentrated survey for this particular guild of *A. auroguttatus* natural enemies.

Future directions for *A. auroguttatus* biological control

The recent introduction of *A. auroguttatus* has resulted in the mortality of tens of thousands of mature red oaks (section *Lobatae*) in southern California, which is drastically changing the composition of these important and unique oak woodland communities (Allen-Diaz and others 2007, Coleman and others 2012a). The difficulty in managing this wood-boring pest in both urban and natural environments has underscored the importance of developing a classical biological control program. Despite the lack of success in explorations for host-specific egg parasitoids, classical biological control may still be the most viable long-term management option. However, additional research on the general biology, life history, and potential natural enemies is needed in order to continue making advances in this control program. Research investigating the life history of *A. auroguttatus* within its native range is necessary to increase understanding on the phenology of this insect, which may potentially lead to improved methods for natural enemy surveys, especially detection of egg parasitoids. Lastly, the ability to rear and maintain all life stages of *A. auroguttatus* in the laboratory is needed to dramatically improve the potential success of a classical biological control program as this will increase the number of potential host stages that can be examined from natural enemy surveys, and ultimately will affect the ability to rear natural enemies for study in quarantine and eventual potential release into infested regions in southern California.

Acknowledgments

We thank Mike Lewis, William Joseph, Allison Bistline, and Ruth Amrich (University of California, Riverside). We also thank Leah S. Bauer (USDA Forest Service-Northern Research Station), Laurel Haavik (Great Lakes Forestry Centre, Sault Ste Marie, Canada), Andrew Graves (USDA Forest Service-Forest Health Protection, Region 3), and Michael Jones (USDA Forest Service-Forest Health Protection, Region 5). We are very grateful to San Diego County Parks and Recreation. Funding for this study was supported, in part, by two agreements between the USDA Forest Service and the UC Riverside Department of Entomology: Cooperative Agreement # 09-CA-11420004-357 and Joint Venture Agreement # 10-JV-11272172-059.

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