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Are there fitness costs of adaptive pyrethroid resistance in the amphipod, *Hyalella azteca*?[☆]

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ABSTRACT

Pyrethroid-resistant *Hyalella azteca* with voltage-gated sodium channel mutations have been identified at multiple locations throughout California. In December 2013, *H. azteca* were collected from Mosher Slough in Stockton, CA, USA, a site with reported pyrethroid (primarily bifenthrin and cyfluthrin) sediment concentrations approximately twice the 10-d LC50 for laboratory-cultured *H. azteca*. These *H. azteca* were shipped to Southern Illinois University Carbondale and have been maintained in pyrethroid-free culture since collection. Even after 22 months in culture, resistant animals had approximately 53 times higher tolerance to permethrin than non-resistant laboratory-cultured *H. azteca*. Resistant animals held in culture also lacked the wild-type allele at the L925 locus, and had non-synonymous substitutions that resulted in either a leucine-isoleucine or leucine-valine substitution. Additionally, animals collected from the same site nearly three years later were again resistant to the pyrethroid permethrin. When resistant animals were compared to non-resistant animals, they showed lower reproductive capacity, lower upper thermal tolerance, and the data suggested greater sensitivity to, 4, 4′-dichlorodiphenyltrichloroethane (DDT), copper (II) sulfate, and sodium chloride. Further testing of the greater heat and sodium chloride sensitivity of the resistant animals showed these effects to be unrelated to clade association. Fitness costs associated with resistance to pyrethroids are well documented in pest species (including mosquitoes, peach-potato aphids, and codling moths) and we believe that *H. azteca* collected from Mosher Slough also have fitness costs associated with the developed resistance.

1. Introduction

Pyrethroids are a major class of synthetic insecticides that target the voltage-gated sodium channel (*vgsc*) of the nervous system and are highly toxic to invertebrates and fish, while having relatively low mammalian toxicity. These insecticides are used in and around homes, on pets, for the treatment of head lice and scabies, in disease vector control, and in agriculture (Rinkevich et al., 2013). Intensive use of pyrethroids for pest control has caused many instances of resistance in pest species globally; often associated with single nucleotide polymporphisms (SNPs) in the *vgsc* although other mutations are possible and have been documented (e.g. metabolic resistance mutations) (Feyereisen et al., 2015). As of 2013, more than 50 resistance-associated mutations had been noted in a variety of pest species (Rinkevich et al., 2013). These SNPs result in amino acid substitutions that cause conformational changes of the pyrethroid binding sites that inhibit pyrethroid binding and ultimately decrease pyrethroid sensitivity (Rinkevich et al., 2013). Toxicity in resistant animals appears to be associated with oxidative stress (Weston et al., 2013), similar to pyrethroid toxicity in mammals that have sodium channels insensitive to pyrethroids (Giray et al., 2001).

Most mutations found on the *vgsc* have been identified in terrestrial target species including the whitefly (*Bemisia tabaci*) (Morin et al., 2002), common housefly (*Musca domestica*) (Tomita and Scott, 1995), and peach-potato aphids (*Myzus persica*) (Foster et al., 1999). However, the use of pyrethroids has led to

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unintentional consequences to invertebrates not directly targeted by their application. Pyrethroid exposure to terrestrial non-target species includes behavioral effects to honeybees (Ingram et al., 2015) and the beneficial beetle *Platynus assimilis* (Tooming et al., 2014). Wild populations of *Drosophila melanogaster* have been shown to have pyrethroid resistance (Vais et al., 2003), despite fruit flies rarely being targeted for control with insecticides.

In 2010, Weston et al. (2013) discovered thriving populations of *H. azteca* at sites with known pyrethroid contamination. The *vgsc* genes of these animals were sequenced and resistant animals had either of two amino acid substitutions associated with pyrethroid resistance in pest species: the M918L or the L925I mutations (Weston et al., 2013). The M918L mutation has been documented in many pest species including cotton aphids (*Aphis gossypii*), diamondback moths (*Plutella xylostella*), and houseflies (*M. domestica*) (Rinkevich et al., 2013). The L925I mutation has also been reported, including in sweet potato whiteflies (*B. tabaci*) and the southern cattle tick (*Rhipicephalus microplus*) (Rinkevich et al., 2013). In addition to populations documented by Weston et al. (2013), a recent study of undeveloped or developed sites (with nearby agriculture or urban development) across California identified many pyrethroid-resistant populations of *H. azteca* (Major et al., in press). Resistance alleles were present at high frequencies only at developed sites—adding to the body of evidence that this is a human-driven phenomenon.

A number of recent studies have documented phenotypic differences (including sensitivity to contaminants and reproductive rate) between populations of *H. azteca* collected from different locations or cultured in different laboratories. These differences have been associated with varied life history characteristics, the experience level of the laboratory conducting the research, and the test protocols and conditions (Major et al., 2013; Pathammavong, 2016; Soucek et al., 2015; Thomas et al., 1997). Organisms morphologically classified as *H. azteca* have been used in toxicity studies since the mid-1980s because of their broad distribution, wide tolerance for natural environmental variables (e.g. salinity), ease of culturing in the laboratory, and relatively short maturation period (U.S. EPA, 2000). While most toxicity testing laboratories are using genetically similar *H. azteca*, wild populations in this species complex are more genetically variable than previously thought (Major et al., 2013).

While the emergence of resistance to pyrethroids may superficially demonstrate the adaptive capacity of *H. azteca* to survive exposure to anthropogenic contaminants, it is important to consider potential fitness costs associated with the developed resistance. Mutations on the *vgsc* that decrease sensitivity to pyrethroids could result in a reduction in overall fitness, because of reduced efficiency of the vgsc and metabolic costs related to the change (Zhao et al., 2000) or through "genetic hitchhiking" of less desirable traits linked to the mutations (Van Straalen and Timmermans, 2002). Fitness costs associated with mutations causing large phenotypic changes (e.g. resistance to xenobiotics) are not a new concept. Fisher (1958) developed a model of adaptation that discusses how independent selection pressures shape current, nearly ideal, phenotypes through gene coevolution. Because of the complex gene interdependence, mutations with large phenotypic effects are likely to induce deleterious effects. Several examples of fitness costs to pyrethroid-resistant populations of pest species exist. Konopka et al. (2012) showed increased developmental time, lower pupal masses, and smaller wing surface areas in the codling moth (*Cydia pomonella*). Peach-potato aphids (*M. persica*) with target-site and metabolic resistance to both pyrethroids and DDT had decreased overwintering survival, reduced alarm pheromone response (Foster et al., 1999), and increased vulnerability to parasitoids (Foster et al., 2005). Finally, potato beetle strains with organophosphate and pyrethroid resistance have decreased fertility and fecundity (Argentine et al., 1989).

The current research project had two major objectives. First, we wanted to confirm that the resistance documented in *H. azteca* populations in the U.S. was a heritable genetic change by monitoring the pyrethroid-resistant trait in long-term culture by exposing non-resistant and resistant populations to permethrin, and genotyping the L925 locus in the resistant population after 16 months of culture with no pyrethroid exposure to monitor for presence of the wild-type non-resistant allele. Second, we wanted to determine potential fitness costs of pyrethroid resistance to *H. azteca* and this was accomplished in two phases. In the first phase, cultured resistant and non-resistant *H. azteca* that belonged to two different clades were compared by measuring the reproductive output and responses to thermal stress, 4, 4′-dichlorodiphenyltrichloroethane (DDT), copper (II) sulfate, and sodium chloride. The second phase consisted of repeating the thermal stress and sodium chloride tests with both resistant and non-resistant field-collected animals from the same clade.

2. Experimental methods

2.1. Chemicals

Permethrin (98% purity; 46% *cis*, 52% *trans*) and DDT (98% purity) were obtained from ChemService (West Chester, PA, USA). Sodium chloride, sodium sulfate, trace metal grade nitric acid, and copper (II) sulfate as well as the pesticide-grade solvents hexane, acetone, and dichloromethane (DCM) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Calibration standards and electrode storage solutions for use with the chloride probe were purchased from Fisher Scientific. The surrogate standard decachlorobiphenyl (DCBP) was obtained from Supelco (Bellefonte, PA, USA).

2.2. Organisms

Four *H. azteca* populations were used in the current study; two non-resistant, and two resistant populations. The first non-resistant population was obtained from the U.S. EPA laboratory in Duluth, Minnesota, and has been cultured at Southern Illinois University Carbondale (SIUC) since 2001 (SIUC Lab). The second non-resistant population was collected from the Mojave River in southern California (35.0380, −116.3814) in November 2016 (Field Mojave). The pyrethroid-resistant populations were collected from Mosher Slough in Stockton, California (38.0325, −121.3654), where Weston et al. (2013) reported sediment pyrethroid concentrations at approximately twice the LC50 (the concentration lethal for 50% of test organisms) for laboratory-cultured *H. azteca*. Subsets of both collections from Mosher Slough were genotyped and found to have the L925I mutation known to provide pyrethroid resistance. The first collection of the resistant Mosher population (SIUC Mosher) has been maintained in pyrethroid-free culture at SIUC since December, 2013 (with one supplement of organisms from the same location in February, 2014). A later collection of the resistant Mosher population was collected in November 2016 and used for the present study within one month of collection (Field Mosher).

The organisms used in the current study have been previously identified as belonging to three species groups, based on maximum likelihood analysis of nucleotide sequences in the mitochondrial gene cytochrome *c* oxidase subunit I (COI) and the 28S nuclear ribosomal large subunit rRNA gene (*28S* rDNA) (Weston et al., 2013). The SIUC Lab belongs to Clade C, animals collected at the collection sites in Mosher Slough were primarily species D with some identified as probable species B, and the Mojave Desert have been