Evolved pesticide tolerance in amphibians: Predicting mechanisms based on pesticide novelty and mode of action

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ARTICLE INFO

ABSTRACT

We examined 10 wood frog populations distributed along an agricultural gradient for their tolerance to six pesticides (carbaryl, malathion, cypermethrin, permethrin, imidacloprid, and thiamethoxam) that differed in date of first registration (pesticide novelty) and mode-of-action (MOA). Our goals were to assess whether: 1) tolerance was correlated with distance to agriculture for each pesticide, 2) pesticide novelty predicted the likelihood of evolved tolerance, and 3) populations display cross-tolerance between pesticides that share and differ in MOA. Wood frog populations located close to agriculture were more tolerant to carbaryl and malathion than populations far from agriculture. Moreover, the strength of the relationship between distance to agriculture and tolerance was stronger for older pesticides compared to newer pesticides. Finally, we found evidence for cross-tolerance between carbaryl and malathion (two pesticides that share MOA). This study provides one of the most comprehensive approaches for understanding patterns of evolved tolerance in non-pest species.

1. Introduction

Populations face rapidly changing environments caused by multiple and diverse anthropogenic stressors including pesticides (Christensen et al., 2006; Gilliom, 2007; Rhind, 2009). While pesticides are vital tools in crop management and disease prevention, their persistent use to control pest populations has led to frequent observations of pest species evolving increased tolerance and cross-tolerance (Georghiou, 1990). To combat the evolution of pesticide tolerance and cross-tolerance, there is an increasing push to develop new pesticides with novel modes of action to slow the evolutionary process in pest populations (Moffat, 1993). Consequently, non-target populations are increasingly exposed to a broad range of pesticides that vary in mode of action and frequency of use (Grube et al., 2011; Newman, 2010; Ritter, 2009). Given the complex chemical milieu that non-target populations frequently encounter in nature, there is a need to explore how pesticides influence evolutionary processes in these populations (Jansen et al., 2011).

The evidence for evolved tolerance in non-target populations continues to accumulate (Bendis and Relyea, 2014; Brausch and Smith, 2009a; Cothran et al., 2013). Indeed, past studies have documented high levels of variation in pesticide tolerance among non-target populations. For example, Bridges and Semlitsch (2000) demonstrated that populations of leopard frogs (Lithobates sphenocephalus) vary in their tolerance to the insecticide carbaryl. Similarly, Cothran et al. (2013) and Hua et al. (2013a) found that populations of wood frogs (Lithobates sylvaticus) vary substantially in their tolerance to the insecticides chlorpyrifos, carbaryl, and malathion. Further, consistent with predictions of evolved tolerance, Bendis and Relyea (2014) and Cothran et al. (2013) demonstrated that populations of waterfleas (Daphnia pulex) and wood frogs living farther from agriculture were less tolerant to a single, commonly applied insecticide (i.e. chlorpyrifos) than populations living closer to agriculture. While the evidence for evolved tolerance in non-target populations continues to accumulate, our ability to provide generalizations regarding patterns of evolved tolerance in response to multiple pesticides remains limited. Here, we

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investigated whether distance to agriculture could be used as a tool to predict spatial patterns in pesticide tolerance using a suite of commonly used insecticides.

In addition to demonstrating spatial patterns of evolved tolerance, we need to increase our ability to make *a priori* predictions regarding the likelihood of evolved tolerance to different pesticides (Pimentel, 2005). Because the evolution of tolerance to pesticides requires multiple generations, the likelihood of evolved tolerance is greater for pesticides that have been used for longer periods relative to more novel pesticides (Georghiou, 1990). Thus, pesticide novelty (i.e. time since registration) might be a useful predictor about the likelihood of evolutionary responses of populations to pesticides. Toward this goal, the U.S. EPA's pesticide first registration records, which indicates when the U.S. EPA registered the first product containing a particular active ingredient (EPA, 2014), provides a well-documented record of when populations might have been first exposed to certain pesticides. Using these records, we examined whether pesticide novelty was associated with the likelihood of evolved pesticide tolerance.

In the United States, there are over 600 different active ingredients that are currently registered for pesticide use (EPA, 2010). Given the diversity of chemicals that populations could potentially face, pesticide tolerance would be particularly beneficial if it also conferred cross-tolerance to other chemicals (i.e. cross-tolerance). Theory and past empirical work predicts that populations with pesticide tolerance should be similarly tolerant to pesticides that share a similar mode of action (Hua et al., 2013a; Newman, 2010). For instance, Hua et al. (2013a) demonstrated that populations of tadpoles that are tolerant to the acetylcholine esterase (AChE)-inhibiting insecticide carbaryl are also cross-tolerant to other AChE-inhibiting insecticides (malathion and chlorpyrifos). In contrast, predictions of cross-tolerance are less straightforward across pesticides that differ in mode of action. One perspective suggests that tolerance between insecticides with different modes of action should be negatively related because adaptations to a particular pesticide can lead to energetic costs inhibiting cross-tolerance to pesticides with different modes of action (Kanga et al., 1997; Rivero et al., 2011). Alternatively, tolerance between pesticides that differ in mode of action should be positively related if tolerance is achieved via a shared detoxification mechanism. For example, Brausch and Smith (2009a, 2009b) demonstrate that cross-tolerance in fairy shrimp between methyl parathion (AChE-inhibiting insecticide), Cyfluthrin (Na^+ inhibitor), and DDT (interferes with Cl^- channel function) was due to an increase in metabolizing enzyme production of cytochrome P450s and hydrolases, which are involved in detoxification of contaminants. If cross-tolerance to multiple pesticides is indicative of shared mechanisms for achieving tolerance, investigating patterns of cross-tolerance among insecticides that share and differ in mode of action can elucidate potential mechanisms of pesticides tolerance in populations (Georghiou, 1990; Kanga et al., 1997; Nkya et al., 2014).

We investigated patterns of pesticide tolerance in 10 wood frog populations that varied in their distance to agriculture. We selected six common insecticides (carbaryl, malathion, cypermethrin, permethrin, imidacloprid, and thiamethoxam) that varied in their novelty (i.e. date of first registration) and mode of action. Our objectives were to explore: (1) the evidence for evolved tolerance to different insecticides (i.e. a negative relationship between population tolerance and distance to agriculture), (2) whether patterns of evolved tolerance are related to the novelty of insecticides, and (3) whether evolution has resulted in cross-tolerance between insecticides that share and differ in mode of action.

2. Methods

2.1. Model system

Aquatic systems provide an excellent model for studying the evolutionary response of populations to pesticides (De Meester et al., 2005). They have well defined boundaries, are widely distributed worldwide including within agricultural systems, and are exposed to a diversity of pesticides (Declerck et al., 2006; EPA, 2014; Gilliom, 2007). Our focal species for examining pesticide tolerance was the wood frog (*L. sylvaticus*). Wood frog populations vary in their naïve tolerance to the insecticides chlorpyrifos and carbaryl, with populations living far from agriculture having higher tolerance compared to populations close to agriculture (Cothran et al., 2013; Hua et al., 2015). Further, wood frogs are explosive breeders, which facilitates the collection of many individuals across multiple populations at a similar age, mass, and developmental stage (Cothran et al., 2013; Hua et al., 2013a; Relyea, 2005).

2.2. Animal collection and husbandry

We collected wood frogs from 10 populations located in western Pennsylvania, USA (Table A2). Because the genetic neighborhood for amphibians is generally within ~1 km of the breeding pond (Berven and Grudzien, 1990; Semlitsch, 2000, 1998), we chose wood frog populations that were separated by at least 4 km to minimize genetic relatedness. Early-stage embryos (i.e. egg clutches, Gosner stage 3−7; Gosner 1960) were collected from each population within a 7-d period and reared separately by population (Table A2). To control for the effects of developmental stage and size, which can affect sensitivity to pesticides, we manipulated temperature to standardize hatching time (Cothran et al., 2013; Hua et al., 2013a). Initially, all wood frog clutches were raised outdoors in 100-L pools filled with ~90 L of aged well water (air temperature ranged from 1 °C to 21 °C). On 13 April, clutches collected before 7 April were chilled in a walk-in cooler to 1.6 °C to slow development while clutches collected after 7 April remained in 100-L pools where they experienced warmer outdoor conditions (air temperature ranged from 11 °C to 26 °C). These temperatures are well within the range that natural wood frog embryos encounter (Frisbie et al., 2000). After 34 h, embryonic development of clutches collected after 7 April equaled those collected before 7 April and they were moved back into their outdoor pools. The embryos from all 10 populations hatched (Gosner stage 20) within a 20-h period on 21 April and reached the larval stage (Gosner stage 25) on 5 May. From each population, we haphazardly selected 300 tadpoles for inclusion in the experiment. The tadpoles were transferred indoors, held in 14-L plastic containers filled with 10 L of UV filtered water (150 tadpoles/plastic container), and fed rabbit chow *ad libitum*.

2.3. Pesticide background

We chose to work with six pesticides that vary in their mode of action and first registration date (EPA, 2014). We selected two acetylcholine esterase (AChE) inhibitors (carbaryl and malathion), two pyrethroid Na^+ channel disruptors (cypermethrin and permethrin), and two neonicotinoid acetylcholine disruptors (imidacloprid and thiamethoxam; Table A1). All pesticides are used in agricultural and residential settings (Fossen, 2006; Grube et al., 2011; Main et al., 2014).

2.4. Experimental design

On 6 May, we conducted a time-to-death (TTD) assay, which is an established toxicological measure of relative tolerance, to assess