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Short communication

Host expansion modifies activity of phosphatases in a legume store pest *Acanthoscelides obtectus* (Say)

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ABSTRACT

Bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae, Bruchinae) is a cosmopolitan pest of legume stored products. Storages with various legume seeds can facilitate shifts of the weevil from its primary host, common bean (*Phaseolus vulgaris* L.), to other legume species and enable host expansion, i.e. broadening of the range of acceptable plant-hosts. In the first generation of host shift, survival of an insect depends on ability to adjust its physiology to altered content of nutrients and secondary metabolites in novel host. On a long-term scale, physiological adaptations to a new host can comprise modifications both in level and plasticity of physiological traits. Changes in activity of phosphatases play an essential role in this process due to their involvement in diverse functions. This study investigated alterations in activity of total acid, lysosomal acid and alkaline phosphatases using laboratory populations of *A. obtectus* which were maintained either on the optimal host (common bean) or on the suboptimal host (chickpea, *Cicer arietinum* L.) for 28 years. To determine short-term (plastic) effects, subsets of individuals from each population were exposed to the alternative host for one generation. Our results revealed that one-generation shift to chickpea significantly increased phosphatases' activity reflecting immediate plastic response to nutritional/allelochemical stress where these enzymes might be involved in defense mechanisms. On the other hand, both level and plasticity of phosphatases' activities significantly declined as a long-term response to *Cicer*-based diet suggesting that selection on chickpea favored resistance mechanisms that were less costly than phosphatases. Considering diverse roles of phosphatases we suggest that such modifications could be crucial for expanding host range and might have implications on efficiency of chemical and botanical insecticides.

1. Introduction

Bean weevil, *Acanthoscelides obtectus* (Say), is a storage pest of common white beans but it also successfully develops on other seeds from the legume family. Storages may contain several different legume species at the same time which is an essential condition if *A. obtectus* is about to expand the range of suitable plant-hosts. Uncontrolled insect infestations could result in up to 30% loss of the total yield (Cardona, 1989).

At the initial phase of host expansion, survival of organisms on new host depends on adaptive phenotypic plasticity, that is, on the ability of individuals to receive novel environmental signals and accordingly change the patterns of individual development, which will result in different, functionally appropriate phenotypes (Ghalambor et al., 2007). If associated with fitness increase, novel phenotypic variability induced by environmental changes can trigger new courses of evolution (Fitzpatrick, 2012). This scenario has been recognized as a major process during host expansion in insects (Agosta, 2006). For *A. obtectus*, it was shown that larval development within a novel legume seed provokes plastic response in various physiological, morphological, behavioral and life-history characteristics (Savković et al., 2012; Stojković et al., 2012, 2014). The capacity to adjust physiology to novel nutritional/allelochemical environments allows an insect to obtain adequate amounts of

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resources and energy, which inevitably influences survival.

Alkaline and acid phosphatases, which catalyze reactions of transphosporylation and hydrolisis of ortophosphate monoesters, are recognized as crucial for various physiological functions. Alkaline phosphatases are involved in digestion/absorption of nutrients and detoxification of xenobiotics in intestines (Silva et al., 1999; Yi and Adams, 2001; Shekari et al., 2008; El-Ebiarie, 2012), epithelial transport of fluids in Malpighian tubules (Cabrero et al., 2004), synthesis of dopamine (Rauschenbach et al., 2007) and production of cuticle and other hardened or sclerotized structures (Bourtzis et al., 1991; Funk, 2001). The activity of acid phosphatase is also related to cytolysis during metamorphosis (Goncu and Parlak, 2011) and metabolism of carbohydrates and lipids (Jedrzejas, 2000; Coleman and Lee, 2004). Both phosphatases have roles in oogenesis and spermatogenesis (Zahia et al., 2009; da Cruz-Landim et al., 2013; Oliveira et al., 2013). Many studies have shown that activities of phosphatases exhibit plastic responses to changing food environment (Xiao-Zhen and Ying-Hong, 2007; Basiouny et al., 2010; Yan et al., 2011).

In this study we compared alkaline and acid phosphatases' activity in *A. obtectus* adults on seeds of a primary host - common bean (*Phaseolus vulgaris* L.) and a suboptimal host - chickpea (*Cicer arietinum* L.). These legume species differ in concentration and structure of insecticidal proteins and secondary metabolites (Rembold et al., 1989; Pratt et al., 1990; Ishimoto and Kitamura, 1992; Katre et al., 2005; Qureshi et al., 2006; Cronk et al., 2006; Hao et al., 2009; Lu et al., 2010). We analyzed patterns of long-term physiological divergence on common bean and chickpea as well as the short-term plastic responses of phosphatases after one-generation of reciprocal host shift.

2. Materials and methods

Experimental populations of *A. obtectus* used in this research were raised and kept in dark incubators at 30 °C on common bean and chickpea seeds. Seeds were previously frozen to avoid any infestation. No food or water was offered to adults.

In this study we used two laboratory populations of *A. obtectus* that were reared on two different host seeds. The first population originated approximately 28 years ago from a population established by combining three local populations that had been collected from storages in Belgrade, Serbia. This population, reared on common bean seeds, was named '*Phaseolus*' or P population and for each generation around 5000 individuals was transferred into glass jar with uninfected seeds and contributed in the formation of the next generation. After four generations of laboratory maintenance on bean seeds, approximately 1000 randomly chosen individuals from the P population were transferred to the novel host, chickpea, and a new population was created (hereafter referred to as '*Cicer*' or C population).

Prior to the biochemical analyses of the activity of phosphatase enzymes, samples of *A. obtectus* from both populations were reared for one generation on the alternative plant host species. In this way four experimental groups were created – P individuals developed on chickpeas (PC group), C individuals developed on bean seeds (CP), and two groups, PP and CC, in which individuals from P and C populations developed on their common plant-host, i.e., bean and chickpea, respectively. Analyses of PP and CC experimental groups served to reveal long-term divergence in enzymes' activity between the two populations maintained on different plant-hosts, whereas short-term shift (i.e., PC and CP experimental groups) enabled investigation of patterns of plastic responses of phosphatases to changes in host availability.

To measure phosphatase activity, newly eclosed (virgin) female and male adults were homogenized separately in cold 0.9% NaCl saline (w:w 1:10) and sonicated. For each experimental group (PP, PC, CP and CC) and for each sex within a group, five homogenates containing 20 individuals were prepared. To remove debris the homogenates were first centrifuged at 500 × g for 15 min and then the supernatant was centrifuged at 16,000 × g for 30 min at 4 °C. Final supernatants were collected and used for determination of activities of acid (ACP, EC3.1.3.2) and alkaline phosphatases (ALP, EC3.1.3.1). ACP activity was determined by the method of Nemec and Socha (1988), while for ALP analysis the method of Terra et al. (1979) was used.

Procedures were modified to achieve optimal conditions for *A. obtectus* phosphatases. Besides homogenate, reaction mixture for ACP contained 0.1 M citric buffer (pH 4.2), 5 mM substrate p-nitrophenyl phosphate (p-NPP) and 5 mM $MgCl_2$; for lysosomal ACP, a specific inhibitor NaF (25 mM) was added. Reaction mixture for ALP contained, along with homogenate, 0.1 M Tris/HCl buffer (pH 7.8), 5 mM p-NPP and 5 mM $MgCl_2$. Controls lacked homogenates and were assayed as blanks. After 30 min of incubation for ACP and 60 min of incubation for ALP, reaction was stopped with 0.5 M NaOH. The assays were carried out at 30 °C and production of p-nitrophenyl from p-NPP was measured as a change in absorbance at 405 nm. One enzyme unit (U) hydrolyzes 1 μmol of the substrate per minute. Specific phosphatase activities were calculated by dividing the activity (U) by the amount of proteins (mg). Protein concentration in the homogenates was estimated by the method of Bradford (1976) using BSA as the standard.

The effects of the long-term and short-term host shift and their interaction on the variance of activity of total acid (ACP_t), lysosomal acid (ACP_l) and alkaline phosphatase (ALP) in females and males were tested using two-way ANOVA (SAS Institute, 2010) with long- and short-term hosts as fixed factors. Prior to the analysis, data on ACP_t, ACP_l and ALP activities were log transformed, following an examination of the normality and homogeneity of variance. Also, a t-test was used for estimation of significance of responses to one-generation host shift.

3. Results and discussion

Analyses of phosphatase responses to novel plant-hosts may be crucial for understanding mechanisms that facilitate host expansion in insects. Phosphatases are essential for many physiological processes that enable survival, development, growth and reproduction of insects in heterogeneous environments. In the first phase of host expansion, when depletion of preferable seeds force larvae to develop within unsuitable chickpea seeds, changes in enzyme activity reflect immediate plastic response that potentially contribute to better matching with nutritional/allelochemical stress and consequently ensure adult survival. If bean seeds are no longer available in storages, subsequent insect generations will be imposed to new selection pressures which may lead to novel adaptive physiological patterns on chickpea. Finally, after a number of generations, secondary contact with common bean could induce the reverse shift and novel plastic responses to once optimal host.

Our results clearly demonstrate that activities of all phosphatases were elevated in P *A. obtectus*, especially in males, after one generation of novel nutritional experience (PP vs. PC comparisons; Fig. 1B, D and F). Considering their roles in dopamine synthesis, detoxification, excretion, metabolism of carbohydrates and providing phosphate ions for ATP synthesis, increased phosphatase activity might be important for *A. obtectus* defense against chickpea secondary metabolites and insecticidal proteins. Similar response has been revealed in other insects exposed to non-host-plant extracts (Zibaee et al., 2011; Amirmohammadi et al., 2013; Kaur et al., 2013; Ghoneim et al., 2014). That such physiological plastic response to short-term food stress could be adaptive was indicated