

Possible molecular genetic mechanisms of resistance in populations of the Colorado potato beetle

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Abstract. Expansion of the Colorado potato beetle (CPB) in the Eurasia is continuing. At the same time, there is an increase in the level of insecticide resistance in populations of CPB in Russia. Regular detection of individuals resistant to diagnostic doses of insecticides during the last 10 years shows an increase of their prevalence in local populations in Bashkortostan. Genetic base of insecticide resistance in the Colorado potato beetle populations contains both mutations in the genes of target receptors or membrane channels, as well as changes in expression of these and many other genes. Role of the diapause proteins capable to bind xenobiotics and withdraw them from metabolism is discussed.

Expansion of Colorado potato beetle (CPB) and consolidation of its populations continues on the Eurasian continent. Northern and eastern borders of its current distribution in Russia are far beyond those predicted at the end of the last century [1]. At the same time, resistance of this pest to commonly used chemical insecticides is increasing. Regular detection of specimens resistant to diagnostic doses of formulations during the last years shows a substantial increase of their prevalence in local populations in Bashkortostan (Fig.1).

A rise in resistance levels is reflected in constant increase of the toxicity indices, such as a 3-fold change for Aktellik (organophosphate compounds, OPC), 8-fold for Bancol (bensultap, nereistoxins), 10-fold for Regent (phenylpyrazoles), 16-fold for Decis (pyrethroids), and 40-fold for Actara (neonicotinoids).

More than 10 years ago, we have reported on a mosaic structure of multiple insecticide resistance in the CPB populations of Bashkortostan. Simultaneously, we have performed a toxicological analysis and investigated mechanisms of the establishment and spread of resistance in the CPB populations. In particular, mutations in genes *ache*, *ldvssc1*, and *rdl* were found confirming the resistance to OPC, pyrethroids and phenylpyrazoles in the local pest populations. However, presence of these mutations is not sufficient to explain establishment and further spread of insecticide resistance. Historically, insecticide resistance mechanisms were associated with mutations in genes encoding receptors, membrane channels, and detoxifying enzymes. In XXI century, however, these views are being replaced by a novel knowledge of the CPB genome expression changes caused by a strong human activity-driven impact and climatic conditions accompanying the CPB settling in new areas. These changes underlie adaptivity of species and are facilitated by

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preadaptation associated with food specialization in the CPB. Major host plants of this species belong to Solanaceae and contain toxic glycoalkaloids in high concentrations, driving the CPB to form a wide range of adaptations, considered as a factor of nonspecific tolerance. Enhancement of detoxification systems facilitated by the adaptation to the host plants is directly related to intensive expression of large quantity of genes encoding detoxifying enzymes, such as cytochrome P450 and glutathione-S-transferases [2, 3]. Induction of a set of these genes by different insecticides has been also demonstrated. In addition, novel molecular technologies such as RNAi-silencing revealed a significant contribution of expression of genes coding for carboxylesterases and cuticular proteins to the insecticide resistance [4, 5]. These findings provide new insights into evaluation of the role of genetic systems that control CPB diapause under conditions unfavorable for reproduction and resettlement.

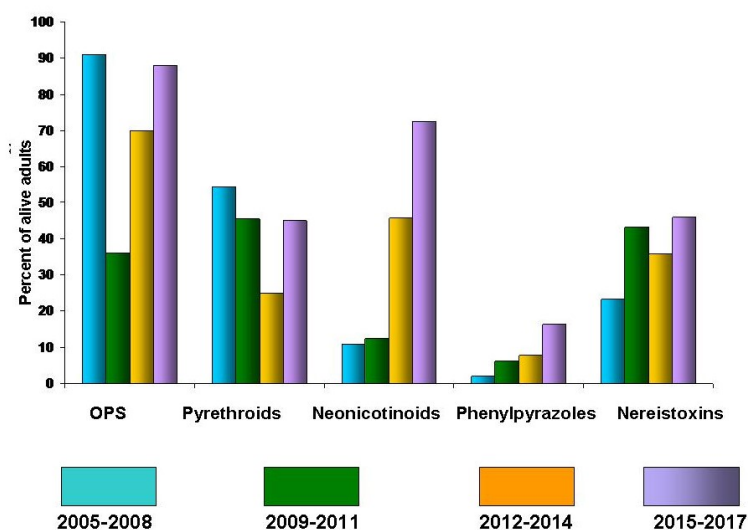


Fig. 1. Temporal dynamics of changes in proportions of insects resistant to insecticides in Colorado potato beetle populations in Bashkortostan. Y-axis – percent of adults surviving after treatment with insecticides at diagnostic concentration.

Review of molecular and toxicological data on resistance in the CPB populations reveals a disagreement between frequencies of detected mutations and resistant phenotypes, with phenotypes frequency exceeding mutation frequency. We hypothesized that in the beetles surviving a treatment by a high dose of insecticide, the diapause-associated genes are expressed differently compared to the susceptible (naïve) individuals. For several years, we have been monitoring the transition of the CPB diapause stages and expression of diapause-associated and resistance-associated genes under laboratory conditions.

Expression study of genes *ache* and *rdl* responsible for OPC and phenylpyrazoles resistance has shown an increased transcription in the fat body, muscles and gonads of the CPB adults that survived after the insecticide treatment [6]. Observed selection of resistant individuals has been probably connected to the ability of a part of the CPB population to upregulate expression of these genes, as well as a large cluster of genes taking part in diapause initiation [7], metabolism control, transport and deposition of essential compounds and xenobiotics entering the insect's organism (fig.2).

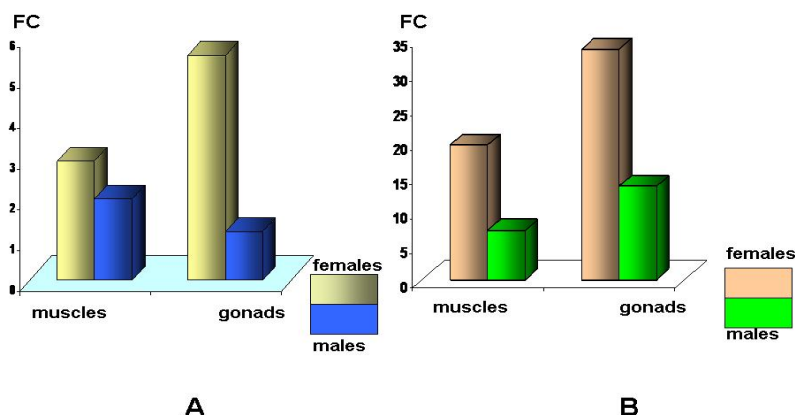


Fig. 2. Expression of diapause-associated genes in the CPB adults that survived after insecticide treatment, estimated at the stage of the diapause. A – gene of ecdysone receptor EcR; B – gene of reserve protein, DP1. Y-axis: FC – fold change, ratio of mRNA content in insecticide-treated to untreated adults significant under $p \leq 0.05$.

The directional selection was confirmed by stimulation of gene expression by sublethal doses of insecticides [6] and by hormetic effect of lethal doses in resistant beetles [8]. Repeated treatments by insecticides support the idea that selection of resistant individuals and anthropogenic factors become the main cause of genetic fixation of resistance in CPB populations.

Until now, the genetic system of signalling which triggers adult superpause remains unknown. This phenomenon allows the species maintaining the reproducing populations loci with diverse levels of resistance to different pesticides. All these data indicate the necessity of toxicological analysis and studies of molecular mechanisms as the basis for more detailed forecasts of resistance development.

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