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Different survival of chromium-exposed *Oxya chinensis* among allozyme genotypes

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Abstract

The goal of the present study was to compare the different survival of *Oxya chinensis* exposed to chromium(VI) among allozyme genotypes to gain a better understanding of the relationship between the genetic variations and environmental disturbance. This study analyzed the occurrence of genotypes in *O. chinensis* population exposed to Cr(VI). *O. chinensis* samples were collected at Yuanping, Shanxi Province, China and used in acute toxicity tests. Specimens were assigned to Cr(VI) exposure (LD_{50}: 291.0 mg/kg) for 24 h. The genetic composition of both dead and survived specimens was analyzed with horizontal starch gel electrophoresis in four enzymes (GPI, PGM, LDH, and ME). The results indicated that under Cr(VI) exposure, specimens with different genotypes had various mortalities at the four loci in laboratory conditions, and there was a genetic basis for tolerance in *O. chinensis* during acute exposure to Cr(VI).

Key words: allozyme genotype; tolerance; chromium(VI); *Oxya chinensis*
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Introduction

Chromium(Cr) exists in the forms of Cr(VI) and Cr(III), and Cr(VI) is toxic in nature (Valérie *et al*., 2003). Cr(VI) is the major stable chemical form in the environment and cause greater concern because of its toxic, carcinogenic properties (Mei *et al*., 2002). Industrial wastes and effluents which usually contained high amount of Cr were discharged randomly on soils, into canals and rivers, along road sides, and in the vicinity of industrial without any treatment. They polluted productive soils, natural water systems as well as ground water and became toxic to plants and human health (Kashem and Singh, 1999). Cr was known to affect seed germination, seedling growth, pigment content, nutrient content and enzyme activities of various crop plants (Panda *et al*., 2003). It also affects the production of reactive oxygen species, change of plasma membrane (Li *et al*., 2005b; Xie and Zhuang, 2001), loss of weight, expression of obese gene, levels of blood sugar, TC, TG, and the HDL-C concentration (Sun *et al*., 2001), and the function of non-specific immunity (Chen *et al*., 2002) of animals.

Oxya chinensis (Orthoptera: Acridoidae) is one of the most common and widespread insect in the far eastern part of Russia, China, and Japan, and Southeast Asia. It is abundant in rice paddies, in sugar cane and other gramineous plants. It has been known highly harmful pest to crops and gramineous plants. This species has received much more attention at different levels because of its extensive damage in agricultural production (Lu *et al*., 2004; Yang *et al*., 2004; Zhang *et al*., 2004; Li *et al*., 2005a).

Horizontal starch gel electrophoresis is the most commonly used approach for quantifying population genetic structure. This technique may be used to identify the resistant sensitive genotypes, the relationship between genetic diversity and tolerance, as well as how genotype and/or allele frequencies change following exposure to contaminants (Yap *et al*., 2004). Guttman (1994) demonstrated the significance of genotypic diversity in populations for both short-term resistance and long-term adaptation to a variety of environmental stressors. Some publications predicted that animals with different allozyme genotypes showed differential survival when exposed to various metals and insecticides in laboratory (Diamond *et al*., 1989; Gillespie and Guttman, 1989; Schlueter *et al*., 1995; Duan *et al*., 1997; Duan *et al*., 2000; Li *et al*., 2004a; Virgilio and Abbiati, 2004), but the impact of Cr(VI) on allozyme polymorphisms of grasshopper is currently unknown.

The main objective of the present study was to examine whether differential survival of grasshopper (*O. chinensis*) was associated with specific genotypes at selected enzyme loci during acute exposure to Cr(VI).

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1 Material and methods

1.1 Insect models

O. chinensis were collected from Yuanping (113°4′E, 38°40′N), Shanxi Province, China, in August, 2003. The samples were brought to the laboratory and acclimatized for 7 d.

1.2 Acute toxicity experiment

$K_2Cr_2O_7$ was dissolved in triple-distilled water and the concentration of the solution was 291.0 mg/kg Cr(VI) (LD_{50}), with which the insects were injected (4 μL, ip (Intraperitoneal Injection)) at 2 to 3 abdominal segments. After being injected for 24 h, the insects were separated into two groups as live and dead individuals. Then, they were immediately stored at –80°C for electrophoresis.

1.3 Allozyme electropresis

Starch gel (12.5%) was prepared with the mixture of soluble starch, potato starch (Sigma S-5651), and refined starch (self-prepared) at a ratio of 2:1:1 (*W*:*W*:*W*). The gel buffers were Na_2HPO_4-NaH_2PO_4 and the concentration ratio of electrode to gel buffer was 9:1.

Four enzymes, GPI (glucose-6-phoshate isomerase, EC 5.3.1.9), PGM (phosphoglu-comutase, EC 5.4.2.2), LDH (lactate dehydrogenase, EC 1.1.1.27), and ME (malic enzyme, EC 1.1.1.40) were identified for their polymorphism.

The femur muscle tissue was removed, and homogenized in 20 μL double distilled water on an ice pan. Filter papers (3 mm × 9 mm) were used as wicks for loading samples. The size of the gel mold was 235 mm × 135 mm × 8 mm. Electrophoresis was conducted at 4°C with constant voltage (11 V/cm).

After electrophoresis, the gel was sliced horizontally into two pieces, and each was used to stain for one enzyme activity using a substrate. Allozyme staining was followed according to the standard methods (Wang, 1998) with modifications, in zymogram scoring, alleles were identified from anode to cathode by labeling the fastest migrating allele as “A”, the second fastest allele as “B”, and so on.

1.4 Data analysis

The software BIOSYS-II (Swofford and Selander, 1981) was used to calculate allele frequency, goodness-of-fit for Hard-Weinberg equilibrium, the percentage of polymorphic loci (*P*), the mean number of alleles per locus (*A*), and mean heterozygosity (*H*).

The genotype numbers which dead or survival lower than five was omitted at polymorphic loci when data were analyzed.

2 Results

2.1 Mortalities of the individuals with different genotypes

The mortality was 54.74% (179/327) at the end of the test exposure. Staining of the four enzymes revealed four loci. Under the present experimental conditions, two enzymes, GPI and PGM, presented different sub-bands. From the electrophoresis zymogram, the stained enzymes migrated from cathode to anode. Among the four enzyme loci, two alleles were in LDH and GPI, and three were in PGM and ME. The different selective effects on the genotypes were observed at four loci (Table 1). The mortality among the individuals varied depending on genotype, and they were 37.8% (LDH-AB), 45.7% (LDH-AA), and 79.5% (LDH-BB); 40.0% (GPI-BB), 59.3% (GPI-AB), and 60.7% (GPI-AA); 48.2% (PGM-BC), 50.0% (PGM-AA), 52.3% (PGM-BB), 57.1% (PGM-CC), and 77.4% (PGM-AB); 25.0% (ME-BB), 50.0% (ME-BC), 55.8% (ME-CC), 57.1% (ME-AC), 57.8% (ME-AA), and 75.0% (ME-AB), respectively. The maximum mortality was 79.5% at LDH-BB, 60.7% at GPI-AA, 77.4% at PGM-AB, 75.0% at ME-AB. The X^2 (H-W) test showed the significant genotypic effects on the surviving and dead groups in the following genotype pairs, LDH-AA vs. LDH-BB, LDH-AB vs. LDH-BB; GPI-AA vs. GPI-BB, GPI-BB vs. GPI-AB; PGM-AB vs. PGM-BB, PGM-AB vs. PGM-BC; ME-AA vs. ME-BB, ME-AB vs. ME-BB, ME-AC vs. ME-BB, ME-BB vs. ME-CC.

2.2 Comparative allozyme analysis of *O. chinensis* exposes to Cr(VI)

The allele frequencies of LDH, GPI, ME, and PGM are included in Table 2. In the present experimental condition, the genotype frequencies at ME and PGM loci were significantly deviated from Hardy-Weinberg expectation, while GPI was in Hardy-Weinberg expectation. The heterozygote deficiency was showed at the LDH, ME, and PGM loci ($F > 0$).

2.3 Population genetic background of *O. chinensis*

Table 3 predicates that there was polymorphism at four allozyme loci as shown in the mean number of alleles per locus (A: 2.5), the mean of observed heterozygosities (H_o: 0.276–0.324), and the mean of Hardy-Weinberg expected heterozygosities (H_e: 0.435–0.478). In addition, the mean of observed heterozygosities was lower than their Hardy-Weinberg expected heterozygosities.

Table 1 Mortalities of the individuals with different genotypes at four polymorphic loci (LDH, GPI, PGM, and ME) of *O. chinensis* injected with Cr(VI)

Genotype	Mortalities			
	LDH	GPI	PGM	ME
AA	0.457 (153) a	0.607 (28) a	0.500 (12)	0.578 (116) a
AB	0.378 (74) ab	0.593 (123) ac	0.774 (31) a	0.750 (28) a
AC	–	–	–	0.571 (49) a
BB	0.795 (39) c	0.400 (138) b	0.523 (205) b	0.250 (40) b
BC	–	–	0.482 (27) b	0.500 (16) ab
CC	–	–	0.571 (35)	0.558 (43) a

The number of sampling was shown in parentheses; the same letter after data within a column represents no significant different at 95% probability level. “–”: without this allele.

Table 2 Allele frequency, chi-square tests for Hardy-Weinberg expectations of genotype frequencies, heterozygosity (*H*) and fixation index (*F*) in alive, dead and initial samples of *O. chinensis* injected with Cr(VI)

Locus	Sample	*N*	A	B	C	H-W	*H*	*F*
LDH	Alive	137	0.774	0.226	–	0.276	0.336	0.041
	Dead	129	0.651	0.349	–	35.764**	0.217	0.522
	Initial	266	0.714	0.286	–	27.337**	0.278	0.318
GPI	Alive	144	0.25	0.75	–	0.87	0.347	0.074
	Dead	145	0.369	0.631	–	0.876	0.503	–0.081
	Initial	289	0.31	0.69	–	0.012	0.426	0.005
ME	Alive	134	0.47	0.28	0.25	96.327**	0.296	0.579
	Dead	158	0.579	0.155	0.266	42.201**	0.361	0.367
	Initial	292	0.529	0.212	0.259	140.260**	0.318	0.476
PGM	Alive	139	0.068	0.773	0.158	107.553**	0.151	0.594
	Dead	171	0.105	0.74	0.155	98.103**	0.216	0.482
	Initial	310	0.089	0.755	0.156	183.983**	0.187	0.53

N: individual number of the specimens at each locus; A, B, and C: allele; H-W: H-W expectations of genotype frequencies (*$P < 0.05$, **$P < 0.01$); *H*: direct count of heterozygosity. "–": without this allele.

Table 3 Genetic variability at four polymorphic loci in alive, dead and initial samples of *O. chinensis* injected with Cr(VI)

Sample	Mean sample size per locus	Mean number of alleles per locus	Mean heterozygosity	
			H_o	H_e*
Alive	138.5	2.5	0.276	0.435
	(2.1)	(0.3)	(0.045)	(0.069)
Dead	150.8	2.5	0.324	0.478
	(9.0)	(0.3)	(0.069)	(0.033)
Initial	289.3	2.5	0.302	0.461
	(9.0)	(0.3)	(0.071)	(0.050)

Standard errors are in parentheses. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95. H_o: observed heterozygosity; H_e: Hardy-Weinberg heterozygosity.
*Unbiased estimate (Nei, 1978).

3 Discussion

In the present study, grasshoppers exposed to Cr(VI) showed sufficient polymorphism and heterozygosity at LDH, GPI, PGM, and ME loci (Table 1). Table 1 predictes that the tolerance of grasshoppers to Cr(VI) was different at LDH, GPI, PGM, and ME genotypes loci, which identified an association in *O. chinensis* between LDH, GPI, PGM, and ME allozyme genotypes and differential survival related to Cr(VI) (Diamond *et al*., 1989; Gillespie and Guttman, 1989; Schlueter *et al*., 1995; Harper-arable *et al*., 2004). Among the genotypes, the mortalities of the LDH-BB, GPI-AA, PGM-AB, and ME-AB genotypes were higher than others exposed to Cr(VI), thereby it was more difficult for the individuals with LDH-BB, GPI-AA, PGM-AB, and ME-AB genotypes to survive than other genotypes. The mortalities of the LDH-AB, GPI-BB, PGM-BC, and ME-BB were lowest in each locus for the four enzymes demonstrated that *O. chinensis* with LDH-AB, GPI-BB, PGM-BC, and ME-BB genotypes were more tolerant to Cr(VI) than other genotypes, therefore, the individuals with these genotypes were possible to survive in the environment with Cr(VI) pollution. This showed that the LDH-AB, GPI-BB, PGM-BC, and ME-BB genotypes possibly played important roles to protect *O. chinensis* from Cr(VI) (Harper-arable *et al*., 2004). Specific resistant alleles of any population would become pronounced if organisms were continually exposed to a pollutant (Yap *et al*., 2004). Meanwhile, the LDH-BB, GPI-AA, PGM-AB, and ME-AB genotypes of *O. chinensis* might reduce if grasshoppers exposure to Cr(VI) intensively or successively. The mortalities of heterozygous individuals were lower than that of homozygous individuals at LDH locus, which indicated that heterozygous individuals at LDH allozyme loci had lower metabolic requirements and higher energy in severe metabolic process including to tolerance the stress of Cr(VI) exposure (Harper-arable *et al*., 2004; Virgilio and Abbiati, 2004). Considering the GPI, PGM, and ME enzyme, the tolerance to Cr(VI) of homozygous individuals was higher than that of heterozygous individuals. Under the experimental conditions, the tolerance to Cr(VI) stress in *O. chinensis* was not related to the levels of heterozygosity. Conversely, exposure to Cr(VI) resulted in higher survivorship of *O. chinensis* owing specific allozyme genotypes. Results were in agreement with other studies which showed that the tolerance in species, such as *Hyalella azteca*, *Pimephales promelas*, *Gambusia affinis*, *G. holbrooki*, and *Hediste diversicolor*, was not associated with heterozygosity levels, but with specific genotypes (Virgilio and Abbiati, 2004).

The toxicity of Cr(VI) to *O. chinensis* might result in: (1) reducing the size of *O. chinensis* population, and causing the loss of a random number of alleles and the reduction of genetic variability of heterozygosity or homozygosity; (2) causing different mortality rates for the various genotypes, pressing the abundance of the less resistant ones, and changing the genotypic frequencies in *O. chinensis* population involved (Cimmaruta *et al*., 2003).

It was demonstrated that ME and PGM allozyme genotype frequencies deviated remarkably from Hardy-Weinberg expectations (Table 2), which might be related to gene flow, inbreeding, null alleles, nonrandom mating, parthenogenesis, population subdivision, natural selection, bottleneck effects, higher frequency of alleles, and heterozygote deficiency (Hong and Ando, 1998; Lewis *et al*., 2001; Li *et al*., 2003, 2004b). The above factors could affect the conditions for the populations to fit Hardy-Weinberg equilibrium. Therefore, the heterozygote deficiency was found at the ME, and PGM loci (Table 2) consistent with the above conclusion. This result had also been observed in grasshopper exposure to insecticides (Li

et al., 2004a).

In our study, the survival under Cr(VI) stress was associated with allozymes genotypes. This suggested that the genotype-tolerance responses at LDH, GPI, PGM, and ME loci could be used as markers of genetic responses to Cr(VI) in *O. chinensis*. This had also been done in other organisms (Benton *et al*., 2002).

4 Conclusions

The present study showed that Cr(VI) exposure to *O. chinensis* resulted in differential survivorship of individuals with LDH, GPI, PGM, and ME different genotypes in *O. chinensis* under experimental conditions. This suggested that there was a genetic basis for tolerance to acute concentrations of contaminants, and the possession of these alleles and genotypes could allow *O. chinensis* to survive longer in a Cr(VI)-contaminated environment. The *O. chinensis* population was not in Hardy-Weinberg equilibrium at LDH, ME, and PGM loci, and showed a deficit of heterozygotes at these three loci, which could be affected by some factors, including Cr(VI). Changes in genetic structure could be suggested as an early indicator for contaminant-induced damage to a population.

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References

Benton M J, Malott M L, Trybula J, Dean D M, Guttman S I, 2002. Genetic effects of mercury contamination on aquatic snail populations: allozyme genotypes and DNA strand breakage. *Environmental Toxicology and Chemistry*, 21(3): 584–589.

Chen J Z, Qu J, Hu G D, 2002. Effect of Cr on non-specific immunity of *Cyprinus carpio* linn. *Journal of Zhejiang Ocean University (Natural Science)*, 21(1): 13–15, 29.

Cimmaruta R, Scialanca F, Luccioli F, Nascetti G, 2003. Genetic siversity and environmental stress in Italian populations of the cyprinodont fish *Aphanius fasciatus*. *Oceanologica Acta*, 26: 101–110.

Diamond S A, Newman M C, Mulvey M, Philip M D, Douglas M, 1989. Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard), during acute exposure to inorganic mercury. *Environmental Toxicology and Chemistry*, 8: 613–622.

Duan Y, Guttman S I, Oris J T, 1997. Genetic differentiation among laboratory populatios of *Hyalella azteca*: implications for toxicology. *Environmental Toxicology and Chemistry*, 16(4): 691–695.

Duan Y, Guttman S I, Oris J T, 2000. Genotype and toxicity relationships among *Hyalella azteca*: I. Acute exposure to metals or low pH. *Environmental Toxicology and Chemistry*, 19(5): 1414–1421.

Gillespie R B, Guttman S I, 1989. Effects of contaminants on the frequencies of allozymes in populations of the central stoneroller. *Environmental Toxicology and Chemistry*, 8: 309–317.

Guttman S I, 1994. Population genetic structure and ecotoxicology. *Environmental Health Perspectives*, 102 (Suppl.12): 97–100.

Harper-arable R M, Wirth E F, Fulton M H, Scott G I, Ross P E, 2004. Protective effects of allozyme genotype during chemical exposure in the grass shrimp, *Palaemonetes pugio*. *Aquatic Toxicology*, 70: 41–54.

Hong Z D, Ando Y, 1998. Parthenogenesis in three species of genus *Oxya*. *Japanese Journal of Applied Entomology and Zoology*, 42(2): 65–69.

Kashem M D A, Singh B R, 1999. Heavy metal contamination of soil and vegetation in the vicinity of industries in Bangladesh. *Water, Air, and Soil Pollution*, 115: 347–361.

Lewis S S, Klerks P L, Leberg P L, 2001. Relationship between allozyme genotype and sensitivity to stressors in the western mosquitofish *Gambusia affinis* detected for elevated temperature but not mercury. *Aquatic Toxicology*, 52: 205–216.

Li C X, Duan Y H, Zheng X Y, Ma E B, 2003. Genetic studies on eight populations of eight locust species from Shanxi Province, China. *Acta Genetica Sinica*, 30(2): 119–127.

Li C L, Duan Y H, Lu F P, Guo Y P, Ma E B, 2004a. Lethal responses of allozyme genotypes of *Oxya chinensis* twoards cyhalothrin. *Journal of Agro-Environment Science*, 23(3): 444–447.

Li C X, Ma E B, Zheng X Y, Guo Y P, 2004b. Genetic structure of four geographic populations of *Locusta migratoria manilensis* in China. *Acta Entomologica Sinica*, 47(1): 73–79.

Li L J, Liu X M, Guo Y P, Ma E B, 2005a. Activity of the enzymes of the antioxidative system in cadmium-treatment *Oxya chinensis* (Orthoptera: Acridoidae). *Environmental Toxicology and Pharmacology*, 20: 412–416.

Li L J, Zhang F, Liu X M, Guo Y P, Ma E B, 2005b. Oxidative stress related enzymes in response to Cr(VI) toxicity in *Oxya chinensis* (Orthoptera: Acridoidae). *Journal of Environmental Sciences*, 17(5): 823–826.

Lu F P, Li C L, Duan Y H, Guo Y P, Ma E B, 2004. Impact of malathion on population genetic structure of *Oxya chinensis*. *Hereditas*, 26(5): 663–668.

Mei B, Jeffrey D P, Ronald J N, 2002. Assessment of Cr tolerance and accumulation in selected plant species. *Plant and Soil*, 247: 223–231.

Panda S K, Chaudhury I, Khan M H, 2003. Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. *Biologia Plantarum*, 46(2): 289–294.

Schlueter M A, Guttman S I, Oris J T, Bailer A J, 1995. Survival of copper-exposed juvenile fathead minnows (*Pimephales promelas*) differs among allozyme genotypes. *Environmental Toxicology and Chemistry*, 14(10): 1727–1734.

Sun C H, Zhang W, Wang S R, Liu Q M, Zhang Y, Song D F, 2001. Effect of Cr on the expression of obese gene and its relationships with blood sugar and lipida. *Acta Nutrimenta Sinica*, 23(4): 346–349.

Swofford D L, Selander RB, 1981. BIOSYS-I: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity*, 72: 419–426.

Virgilio M, Abbiati M, 2004. Allozyme genotypes and tolerance to copper stress in *Hediste diversicolor* (Polychaeta: Nereididae). *Marine Pollution Bulletin*, 49: 978–985.

Valérie D, Rémy B, Phlippe L, Rémy G, 2003. Utilisation

of supernatants of pure cultures of streptomyces thermocarboxydus NH50 to reduce Cr toxicity and mobility in contaminated soils. *Water, Air, and Soil Pollution: Focus*, 3: 153–160.

Wang Z R, 1998. Plant Allozyme Analysis. Beijing: Science Press. 95–108.

Xie Y, Xiong Z Z, 2001. Cr(VI)-induced production of reactive oxygen species, change of plasma membrane potential and dissipation of mitochondria membrane potential in Chinese hamster lung cell cultures. *Biomedical and Environmental Sciences*, 14: 199–206.

Yang M, Wu H, Guo Y, Ma E B, 2004. Characterization and comparison of general esterases from two field populations of the grasshopper *Oxya chinensis* (Thunberg) (Orthoptera: Acridoidea). *Acta Entomologica Sinica*, 47(5): 579–585.

Yap C K, Tan S G, Ismail A, Omar H, 2004. Allozyme polymorphisms and heavy metals levels in the green-lipped mussel *Perna viridis* (Linnaeus) collected from contaminated and uncontaminated sites in Malaysia. *Environment International*, 30: 39–46.

Zhang J Z, Ren L, Guo Y P, Ma E B, 2004. Genetic relationships among give populations of *Oxya chinensis* in Shanxi Province and adjacent region based on RAPD. *Acta Genetica Sinica*, 31(2): 159–165.