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# Variation in genetic diversity of tree sparrow (*Passer montanus*) population in long-term environmental heavy metal polluted areas<sup>☆</sup>



POLLUTION

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#### ABSTRACT

Genetic diversity is the bedrock of evolution. The "Genetic Erosion" hypothesis posits that environmental pollution could cause reduced genetic diversity. To explore the effects of heavy metal pollution on genetic diversity in natural populations, we selected an area with more than sixty years of heavy metal contamination (Baiyin, BY) and a relatively unpolluted one (Liujiaxia, LJX), and tree sparrow (*Passer montanus*) as study models. Five tree sparrow populations were sampled in BY at sites differing in heavy metal pollution level. Lower genetic diversity based on seven microsatellite loci was observed in the five tree sparrow populations from BY compared with those from LJX. Analysis of molecular variance indicated no significant genetic differentiation between BY and LJX. However, the observed heterozygosity and allelic richness were negatively correlated to the lead and cadmium concentrations in the primary feathers of tree sparrow. Our results indicated the genetic diversity might have a negative response to long-term environmental heavy metal pollution in tree sparrow, supporting the "Genetic Erosion" hypothesis. Therefore, the findings shed lights on the possible effects of heavy metal pollution on genetic diversity of wild bird populations.

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#### 1. Introduction

Environmental pollution is becoming increasingly serious with the anthropogenic development (deFur and Foersom, 2000). Heavy metal contamination is a type of common environmental pollution, which does greatly harm to ecosystems and organisms (He et al., 2005). For example, it was reported that lead (Pb) concentration in feathers exceeding 4 mg/kg would result in sublethal effects, impaired thermoregulation, and lower chick survival in gulls (Burger and Gochfeld, 1994; Kim and Koo, 2008). Additionally, chronic heavy metal exposure could cause genetic damage (Leon et al., 2007), which originates at molecular level (Bickham et al., 2000). A previous study observed that heavy metal pollution would also result in decreased genetic diversity level known as "Genetic Erosion" hypothesis (van Straalen; Timmermans, 2002).

Genetic diversity is the basis for organisms to adapt to changing environments, and it contributes to their long-term viability (Burger and Lynch, 1995; Shannon, 1996). Nevertheless, long-term arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg) and Pb contamination could lead to the negative genetic effects on many natural populations in aquatic environment, such as intertidal crab (*Pachygrapsus marmoratus*), sandhopper (*Talitrus saltator*) and mosquitofish (*Gambusia holbrooki*) (Fratini et al., 2008; Ungherese et al., 2010; Diez-Del-Molino et al., 2018). Similarly, manganese (Mn), iron (Fe), zinc (Zn), As and Pb pollution was also reported to cause adverse influence on population genetic diversity in terrestrial animals, such as Algerian mice (*Mus spretus*) and pied flycatcher (*Ficedula hypoleuca*) (Eeva et al., 2006; Quina et al., 2019). However, the studies about genetic variation in pollution-related populations of terrestrial animals are still insufficient.

Tree sparrow (*Passer montanus*) is a human commensal passerine that is widely distributed in Eurasian continent. It is one of the most common resident bird species resting in human communities. The species is sedentary and seldom flies over a long distance, and its home range is only approximately 7600 m<sup>2</sup> (Pan and Zheng, 2003). Therefore, pollution of its habitat could have substantial effects on its breeding and survival compared to migratory birds. It was reported that heavy metals (nickel (Ni),



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cobalt (Co), As, Hg, Cd, Pb and Mn) were enriched in the muscle, liver and ventral plumage of the adult tree sparrows from a serious contaminated area (Pan et al., 2008), and environmental heavy metals (Pb and Cd) could inhibit the nestling development and increase mortality (Kamiński, 1998). It could therefore be hypothesized that heavy metal pollution has caused great environmental stress on resident populations, which may result in changes in population genetic characteristics.

Baiyin (BY) is one of the nonferrous metal smelting processing and chemical bases in China since 1950s, where chronic heavy metal (Cu, Zn, Pb and Cd) pollution is a most serious environmental issue (Nan et al., 1999). Although BY turned into a resourceexhausted city 10 years ago, the Cd concentration in soil (2.01-22.97 mg/kg) exceeds the threshold of environmental quality standard (GB 15618 - 2018) (Ai et al., 2018). Cd concentration of spring wheat grains (0.35–1.15 mg/kg) in BY was also higher than the upper limits of food safety standards (GB 2762 - 2017), and the consumption of grains by local residents could result in adverse effects on their health (Liu et al., 2017). In addition, the male Bufo raddei from BY showed a relatively lower body condition and higher sperm abnormality rate (Guo et al., 2018). Previous study about tree sparrows showed that the heavy metals (Cu, Zn, Pb and Cd) in BY were biomagnified through the food chain (Ai et al., 2019), suggesting that the species has a higher risk of exposure to environmental heavy metals. And the eggshell of tree sparrows in BY was significantly thinner and darker compared with those from relatively unpolluted areas (Ding et al., 2019). Whether the level of genetic diversity in tree sparrow populations has been affected by long-term environmental heavy metal pollution in BY is worthy of study.

Microsatellites, a set of simple repeat sequences of 1- to 6-base core sequences, are interspersed in the genome and widely used in population genetic studies given their high resolving power (Duran et al., 2009; Somme et al., 2012). These markers are evolved neutrally, which make them a good set to indicate the characteristics of the effective population size. It is predictable that the reduced genetic diversity level should be observed based on "Genetic Erosion" hypothesis if the environmental pollution would lead to the decrease of the population size in a timescale. Therefore, to better understand the effects of environmental heavy metals on the genetic diversity of the wild population, we evaluated the genetic diversity of heavy metals using primary feathers of tree sparrow from BY and a relatively unpolluted area (Liujiaxia, LJX).

#### 2. Materials and methods

#### 2.1. Ethics statement

Samples were collected with the permission (ethical permission number: 2017038) of the Committee on the Ethics of Animal Experiments of School of Life Sciences of Lanzhou University (Supplementary Material 1).

#### 2.2. Study sites and sample procedures

Our previous studies demonstrated that heavy metals (Cu, Zn, Pb and Cd) were mainly found in substrate sludge which originated from the local mining areas, flowed into the Yellow River and contaminated soils and crops along the Dongdagou drainage in BY (Wang et al., 2012; Liu et al., 2016; Ai et al., 2018). According to the heavy metal pollution levels, five populations were selected from five sampling sites along the Dongdagou drainage. As a control, one population of tree sparrow from the Weijiachuan (WJC) village in

LJX (E103°10′ to E103°24′, N35°54′ to N36°01′) was selected, which is a relatively unpolluted site (Ai et al., 2018; Ai et al., 2019) and approximately 110 km away from BY (E103°33′ to E105°34, N35°33′ to N37°38′) (Fig. 1). In a small geographical scale, no obvious geographical barrier was found among these study populations.

Tree sparrows were trapped using mist nets in June 2018. At the six sample sites, 188 adult tree sparrows in total were captured and the blood  $(10-20 \ \mu$ l) was sampled using blood taking needles from the brachial vein of each individual. Blood samples were immediately stored in 1.5 ml centrifuge tubes with absolute ethanol, then delivered to the lab and stored at -80 °C freezer. The sex of adult tree sparrows was shown in Table S1 (Supplementary Material 2), and the sex ratio from every sampling site was around 1. Primary feathers as a kind of reliable and nonlethal materials were collected to analyze the concentrations of Cu, Zn, Cd and Pb (Goede and Bruin, 1986; Martinez et al., 2012; Varela et al., 2016). All individuals were ringed with uniquely numbered metal bands and released after sample collection.

#### 2.3. DNA extractions and multiplex PCR

DNA was extracted from blood samples using the QIAGEN DNeasy Blood & Tissue Kit. Seven microsatellite loci in two multiplex systems were selected based on previous publications (Table S2, Supplementary Material 2). Multiplex PCRs were performed in a total volume of 10  $\mu$ l including 1x HotStarTaq buffer, 3.0 mM Mg<sup>2+</sup>, 0.3 mM dNTP, 0.15  $\mu$ M of Primer Mix, 1 U HotStarTaq polymerase (Qiagen Inc.) and 1  $\mu$ l template DNA. The following temperature profile was used for PCR. Multiplex I: 95 °C for 2 min; 11 cycles of 94 °C for 20 s, 64 °C - 0.5 °C per cycle for 40 s, 68 °C for 2 min; 60 °C for 1 h, 4 °C indefinitely; Multiplex II: 95 °C for 2 min; 24 cycles of 94 °C for 20 s, 58 °C for 30 s, 68 °C for 2 min; 60 °C for 1 h, 4 °C indefinitely. The PCR products were analyzed using an ABI PRISM 3730xl genetic analyzer with GeneScanTM-500 Liz Size Standard (Applied Biosystems). GeneMapper 5.0 (Applied Biosystems) was used to evaluate and score original results.

#### 2.4. Heavy metal accumulation assessment

To remove external contamination, acetone solution (1 M) and deionized water were used to wash the primary feathers. Subsequently, all the primary feather samples were dried at 60 °C for 12 h in an oven. The dried samples were weighed using an analytical balance (Sartorius BSA224S, Germany) and digested using an intelligent microwave digestion instrument (PreeKem TOPEX+; PreeKem Scientific Instruments Co., Ltd., Shanghai, China). The microwaved samples were diluted by deionized water and stored in a 4 °C refrigerator for further utilization. The analyses of heavy metal concentrations (Cu, Zn, Cd and Pb) of all digested solutions were performed using a flame atom absorption spectrophotometer (FAAS, Analytik Jena, ZEEnit 700P, Germany). The reference material of Biology (GBW 10052, GSB - 30) was used to ensure the quality of experiments. The results showed that the relative standard deviation (RSD) was less than 5%, and the recovery percentages varied from 90% to 110%.

#### 2.5. Data analysis

The presence of null alleles was assessed with Microchecker ver. 2.2 (Oosterhout et al., 2004). FSTAT ver. 2.9.3 was applied to calculate the indices of genetic diversity within population, such as observed heterozygosity (Ho), expected heterozygosity (He), within population inbreeding coefficient ( $F_{IS}$ ) and allelic richness (Ar) (Goudet, 1995).

To estimate the conformance to Hardy-Weinberg expectations



Fig. 1. Map of the study areas and sampling sites. GYY, MQ, SH, LZ, YX and WJC represent Guanyinya village (E104°15′29.0640″, N36°30′04.9167″), Minqin village (E104°18′47.8493″, N36°28′38.1722″), Shuanghe village (E104°21′23.0545″, N36°27′25.5600″), Liangzhuang village (E104°23′22.2040″, N36°25′59.2220″), Yongxing village (E104°24′00.5329″, N36°27′41.7516″) and Weijiachuan village (E103°15′40.2173″, N35°56′41.5949″) respectively. The number of samples is detailed in the map of each sampling site.

(HWE) in all microsatellite loci, the exact probability test of Guo and Thompson (Sun, 1992) was used in GENEPOP 4 (Markov chain parameters for all tests: dememorization number = 10 000, number of batches = 20, iteration per batch = 5000) (Rousset, 2008). Meanwhile, the linkage disequilibrium between pairs of loci was assessed in GENEPOP 4. Sequential Bonferroni corrections (Rice, 1989) were performed for all statistics. A matrix of pairwise  $F_{ST}$ values was calculated using Arlequin 3.0, and the significance of these values was computed by a permutation test with 1000 random permutations. The nonparametric mantel test as implemented in GenAlEX ver. 6.502 (Peakall and Smouse, 2006) was applied to assess correlation between genetic distance and geographic distance (isolation-by-distance). The shortest distance between populations was used as the geographical distance. To assess the partition of genetic variation at three hierarchical levels, including within populations, among groups and among



**Fig. 2.** Concentrations of heavy metals in primary feathers. A, B, C and D represent the comparison of mean concentration of zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) in feathers of tree sparrows from the six sampling sites. Labels containing different lowercase letters such as 'a' and 'b' indicate significant differences between sites (p < 0.05) while same lowercase letters contained in labels mean no significant difference between sites such as 'a' and 'ab' (p > 0.05). The error bar represents standard deviation. For sampling sites and sample numbers, see Fig. 1.

#### Table 1

Test of Hardy-Weinberg equilibrium (HWE) at seven microsatellite loci across the six tree sparrow populations.

S	ite		Pdou3	Ctc105	Pamo17	ZC02	Pdo10	Pdou5	Pamo3
В	BY	GYY	0.1363	0.2598	0.0103*	0.3238	0.0026**	0.7178	0.5226
		MQ	0.0079*	0.2055	0.7005	0.8315	0.0000**	0.5293	0.2561
		SH	0.4217	0.4827	0.4139	0.8277	0.0182*	0.3162	0.8584
		LZ	0.5753	0.4693	0.2986	0.8206	0.5465	0.2991	0.2431
		YX	0.9071	0.7262	0.9984	0.0560	0.0057**	0.3908	0.6480
L	JX	WJC	0.5516	0.1851	0.8192	0.7509	0.0004**	0.7686	0.1521

\*p < 0.05, \*\*p < 0.00714 following sequential Bonferroni.

populations within groups, analysis of molecular variance (AMOVA) was performed using Arlequin 3.0 (Excoffier, 2005). The significance was computed using a permutation test with 10 000 random permutations.

Spearman's rank correlation coefficients between the indices of genetic diversity and the concentrations of heavy metals in primary feathers of tree sparrow were calculated. Statistical comparisons and correlation analysis were performed using the SPSS 22.0 statistical package program (IBM SPSS Inc., USA). A significance level of p < 0.05 was chosen for the statistical tests. Scatter charts and bar graphs were generated using Originpro 9.0 (OriginLab, USA). Graphs were plotted using ArcGIS 10.3 (Esri, Inc.).

#### 3. Results

#### 3.1. Heavy metal accumulation in tree sparrows

Significant differences were observed for Pb and Cd concentrations in tree sparrows between the GYY, MQ, SH and LZ populations from BY and the WJC population from LJX. However, no significant difference in Cd and Pb concentrations was record between YX population from BY and WJC population from LJX. At BY, high concentrations of Cd and Pb were detected in MQ and GYY populations. However, given the greatest distance from the Dongdagou drainage, the YX population exhibited the lowest Cd and Pb concentrations. No significant difference in Cu and Zn concentrations was detected among all the six sample populations (Fig. 2). Compared to the WJC population in LJX, the five BY populations

#### Table 2

Average genetic diversity indices (±SD) of tree sparrow within populations at the seven microsatellite loci. For sampling sites and sample size, see (Table S7, Supplementary Material 2).

Site		Но	Не	Ar	F <sub>IS</sub>
BY	GYY	$0.7662 \pm 0.1522$	$0.8239 \pm 0.0801$	10.8756 ± 2.8774	0.071 ± 0.139
	MQ	$0.7512 \pm 0.1365$	$0.8254 \pm 0.0780$	11.2293 ± 3.2011	$0.091 \pm 0.158$
	SH	$0.7980 \pm 0.1428$	$0.8290 \pm 0.0789$	11.7143 ± 4.1115	$0.038 \pm 0.125$
	LZ	$0.8190 \pm 0.0504$	$0.8207 \pm 0.0746$	$11.7603 \pm 3.8312$	$0.002 \pm 0.098$
	YX	$0.8182 \pm 0.1545$	$0.8378 \pm 0.0833$	$11.7829 \pm 4.7766$	$0.024 \pm 0.140$
LJX	WJC	$0.8482 \pm 0.1556$	$0.8196 \pm 0.0909$	$12.0289 \pm 4.6954$	$-0.036 \pm 0.131$

Ho: observed heterozygosity, He: expected heterozygosity, Ar: allele richness and  $F_{IS}$ : within population inbreeding coefficient.

#### Table 3

Pairwise genetic differentiation ( $F_{ST}$ ) between populations (values in bold indicate significant values at p < 0.05). For sampling sites and sample size, see Fig. 1.

	GYY	MQ	SH	LZ	YX	WJC
GYY	0.00000					
MQ	0.00428	0.00000				
SH	0.00992	0.00687	0.00000			
LZ	0.00127	0.00390	0.00690	0.00000		
YX	0.00146	0.00451	0.00227	-0.00197	0.00000	
WJC	0.00899	0.01075	0.00613	0.00501	0.00679	0.00000



**Fig. 3.** The relationship between genetic distance  $(F_{ST}/(1-F_{ST}))$  in tree sparrows and geographical distance (km) for all pairs of sampling sites.

except YX population showed significant increased accumulation of Cd and Pb and similar levels of Cu and Zn accumulation in the primary feathers of tree sparrows.

#### 3.2. Genetic diversity

Several loci deviated from HWE on each locus per population at p < 0.05. However, significant deviations from HWE were observed at locus Pdo10 in GYY, MQ, YX and WJC populations after sequential Bonferroni correction (Table 1). No significant linkage disequilibrium between pairs of loci was observed (Table S3, Supplementary Material 2). Microchecker analyses suggested that only locus Pdo10 showed evidence for null alleles in GYY, MQ, SH, YX and WJC populations. The estimated frequencies for null alleles at locus Pdo10 in GYY, MQ, SH, YX and WJC populations were 0.1254, 0.1999, 0.0998, 0.1359 and 0.0886, respectively (Table S4, Supplementary Material 2). Therefore, the presence of null alleles was responsible for these significant deviations from HWE. However, the locus pdo10 was retained in this study because similar results were obtained regardless of whether the locus was present or removed.

Seven microsatellite loci were polymorphic in all samples with *Ar* ranging from 10.8756 for GYY population to 12.0289 for WJC

population. *Ho* varied from 0.7512 for MQ population to 0.8482 for WJC population. The range of *He* within populations was from 0.8196 for WJC population to 0.8378 for YX population. The five populations of BY showed lower genetic diversity (*Ho* and *Ar*) compared to the WJC population in LJX. However, there was no significant difference between the MQ and WJC populations for *Ho* (Mann-Whitney *U* test, p = 0.097). No significant difference was found between the GYY and WJC population s for *Ar* (Mann-Whitney *U* test, p = 0.805). Only the WJC population exhibited a negative *F*<sub>IS</sub> value (Table 2).

#### 3.3. Population differentiation

Significant differentiation was found in 4 out of 15 pairwise comparisons between populations (p < 0.05). The largest pairwise  $F_{ST}$  (0.01039) was detected between MQ and WJC. The relatively unpolluted site, WJC, was significantly different from MQ, GYY and YX (Table 3). No significant differentiation was observed among the five populations (GYY, MQ, SH, LZ and YX) of BY except pairwise  $F_{ST}$  between GYY and SH. The Mantel test revealed that no significant correlation between genetic distances ( $F_{ST}/(1 - F_{ST})$ ) and geographical distances was found (r = 0.4972, p = 0.14) (Fig. 3).

AMOVA showed no significant genetic variation among populations within groups (Fsc = 0.38655, p = 0.05297). Moreover, the genetic variation components between groups were low and not significant (Fct = 0.36444, p = 0.14792) (Table 4).

## 3.4. The relationship between heavy metal accumulation and genetic diversity

Among the four heavy metals, Cd and Pb concentrations in the primary feathers of tree sparrows showed a significant negative correlation with *Ho* ( $r_s = -0.886$ , df = 5, p = 0.019;  $r_s = -0.829$ , df = 5, p = 0.042). Similarly, we found significant negative correlations between Cd and Pb concentrations and *Ar* ( $r_s = -0.829$ , df = 5, p = 0.042;  $r_s = -0.886$ , df = 5, p = 0.019). As the concentrations of Pb and Cd increased, the *Ho* and *Ar* decreased. However, there was no significant correlation between *Ho* and the concentrations of Cu and Zn ( $r_s = -0.543$ , df = 5, p = 0.266;  $r_s = -0.543$ , df = 5, p = 0.266). No significant correlations between *Ar* within populations and Cu and Zn concentrations were observed either ( $r_s = -0.657$ , df = 5, p = 0.156;  $r_s = -0.657$ , df = 5, p = 0.156) (Fig. 4).

#### 4. Discussion

Tree sparrows from contaminated areas (GYY, MQ, SH, LZ and YX) showed different accumulations of four heavy metals in primary feathers. In particular, the concentrations of Cd and Pb from MQ and GYY populations were relatively higher than those from other populations (Fig. 2). These findings are consistent with the detection of heavy metals in soils at these sites (Table S5, Supplementary Material 2) because of the biomagnification of four heavy metals through the food chain in tree sparrows (Ai et al., 2019). Cd and Pb concentrations in primary feathers of tree sparrows from MQ and GYY populations (Fig. 2) exceed those from the industrial

Table 4

Analysis of molecular variance (AMOVA) for 188 individuals sampled from the six tree sparrow populations. The two groups of tree sparrow are from BY (GYY, MQ, SH, LZ, YX) and LJX (WJC).

Source of variance	df	Sum of squares	Variance components	Percentage variation	р
Among groups Among populations within groups Within populations Total	1 4 370	4.737 14.375 1069.838 1088.949	0.01062 0.01126 2.89145 2.91333	0.36444 0.38655 99.24901	0.14792 0.05297



**Fig. 4.** Relationship between concentration of four heavy metals in feathers of tree sparrow and genetic diversity of its population expressed as observed heterozygosity *Ho* (A, B, C, D), and as allele richness *Ar* (a, b, c, d). The Spearman rank-order correlation coefficient test (r<sub>s</sub>) value, degrees of freedom (df) and probability (*p*) are given for each metal.

area of Beijing (Cd:  $0.28 \pm 0.24$  mg/kg, Pb:  $15.06 \pm 12.04$  mg/kg) (Pan et al., 2008) and the Pb concentration at the mining area of Mudanjiang (Pb:  $3.63 \pm 1.14$  mg/kg) (Lv et al., 2008), suggesting that the primary feathers could represent an effective sentinel for the detection of heavy metal accumulation in tree sparrows. Some studies showed that heavy metal exposure could change the contents of amino acids in chicken pectoral muscles (Qu et al., 2020)

and delay the time of fledging in great tit (*Parus major*) (Janssens et al., 2003). However, genetic diversity measurements have been rarely used to assess eco-health risk. Our results showed that the genetic diversity of tree sparrow populations decreased as the concentrations of Pb and Cd increased, which could provide a reference for probing the effects of environmental pollution on genetic diversity of bird populations.

Compared to the relatively unpolluted one (WJC), genetic diversity (Ho and Ar) in tree sparrow populations from the five contaminated areas (GYY, MQ, SH, LZ, YX) was lower. In particular, the lowest Ho (0.7521) was observed in the MQ population and the lowest Ar (10.8756) was detected in the GYY population. All populations from BY showed positive  $F_{IS}$  values, indicating that the heterozygosity is relatively deficient in contaminated areas. Moreover, Cd and Pb concentrations in the primary feathers of tree sparrow showed significant negative correlations with genetic diversity (Ho and Ar). Therefore, the genetic diversity of tree sparrow populations is low in long-term heavy metal polluted areas, supporting the "Genetic Erosion" hypothesis which mainly consists of two explanations. On one hand, the environmental pollution could reduce the effective population size, consequently raising the degree of inbreeding. As the level of inbreeding increased, the genetic diversity of tree sparrow populations decreased (Bickham et al., 2000). On the other hand, pollutant tolerant genotypes would be emerged subjected to environmental pollution, which also probably decreased genetic diversity of populations (Quina et al., 2019; Oziolor et al., 2019). Moreover, the reduction in genetic diversity due to heavy metal accumulation has also been reported in other studies. The genetic variability was negatively correlated to Hg concentration in sandhopper tissues (Ungherese et al., 2010). Similarly, significant negative relationships were found between the measured genetic diversity and the accumulation of Cd, Co, and Cu in the tissues of giant tiger prawn (Penaeus monodon) (Rumisha et al., 2017).

Environmental pollution could theoretically reduce the genetic diversity of populations or lead to localized population extinction (van Straalen and Timmermans, 2002). However, other factors such as elevation, climatic factors, food availability, geographical barriers, etc., may be also influential for the determinants of genetic diversity. Then, we collected the elevation and climatic factors of BY and LJX as shown in Table S6 (Supplementary Material 2). The results showed the precipitation, temperature and sunshine time of BY and LJX in the last 20 years and their elevation are similar. And no obvious geographical barrier was found among these localities in a small geographic scale. In addition, our previous studies found the food sources of tree sparrows from the two areas are similar too (Ai et al., 2019). Taking all these factors account, we propose that long-term heavy metal pollution should be the important influence on genetic diversity of tree sparrow populations.

The polluted areas were detected as barriers to some natural populations, such as fish populations (Diez-Del-Molino et al., 2018). However, our research indicated that most of the genetic variation was due to differences among individuals within populations, while no significant genetic difference was noted between the tree sparrow populations from the polluted area (BY) and that from the relatively unpolluted area (LIX). Mantel test results showed that the genetic differentiation between populations is not significantly correlated with geographical distance, rejecting the isolation-bydistance hypothesis. These data reflected that heavy metals (Pb and Cd) have limited influences on the genetic differentiation of tree sparrow populations. There are several explanations accounting for these observations. On one hand, it is difficult to detect the anthropogenic barrier effect for species with large effective population sizes (Gauffre et al., 2008). The possibility of detecting genetic differences between populations was reduced due to the relatively large population size of tree sparrows in our study sites. On the other hand, extensive gene flow between the populations might weaken the impact of environmental heavy metals on genetic structure (Muller et al., 2007; Giska et al., 2015). Moreover, some species, such as brown trout (Salmo trutta), may have innate genetic plasticity to adapt to elevated levels of heavy metals (Durrant et al., 2011). As higher vertebrates, tree sparrows could potentially have a good genetic plasticity (Qu et al., 2019). Significant genetic differences were found in 4 out of 15 pairwise comparisons between populations. MQ and GYY populations from the Pb and Cd contaminated seriously sites in BY were significantly different compared with the WJC population. In particular, the largest pairwise  $F_{ST}$  (0.01039) was found between MQ and WJC populations. These findings suggest that heavy metals may be responsible for weak genetic differences between the tree sparrow populations from seriously polluted (MQ and GYY) areas and that from a relatively unpolluted area (WJC).

#### 5. Conclusion

The genetic differentiation between tree sparrow populations from polluted areas and that from a relatively unpolluted area was low, suggesting that heavy metals have a limited influence on the genetic structure of tree sparrow populations. However, our research revealed that heterozygosity is relatively deficient at contaminated areas. A significant negative relationship was observed between the measured genetic diversity (Ho and Ar) and the concentrations of heavy metals (Pb and Cd) in tree sparrow populations, supporting the "Genetic Erosion" hypothesis. Therefore, the present study may contribute to a better understanding of adaptation and population genetic changes of the tree sparrow in an area polluted with heavy metals for decades. Although genetic changes at some contaminated areas are not significant, it is necessary to strengthen pollution control measures to protect populations from genetic diversity loss, as it is essential for species' evolution and adaptation.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### **CRediT authorship contribution statement**

Wenzhi Yang: Methodology, Software, Writing - original draft, Formal analysis. Jian Ding: Validation, Investigation. Shengnan Wang: Investigation. Ying Yang: Resources. Gang Song: Funding acquisition. Yingmei Zhang: Conceptualization, Supervision, Funding acquisition.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114396.

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