

## Genetic composition of the Jadro softmouth trout following translocation into a new habitat

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**Abstract** Genetic founder effects of a historical translocation (1965; app. 12 generations ago) of endangered softmouth trout from the only remaining indigenous population to an adjacent uninhabited river were investigated.

A comparison of 15 microsatellites from contemporary samples in both the source and re-established populations indicated a very low level of genetic diversity within the two populations. Furthermore, considerable differences in estimates of variability and effective population sizes were detected between populations, but no recent bottlenecks were evident. Our data suggest that the re-established population has lost variation due to genetic drift associated with founder effects following the translocation. Accordingly, in relation to management, we suggest that the re-established population could serve as a secondary source of individuals to buffer possible extinction due to demographic events. Finally, conservation initiatives to enhance the census population size and increase levels of variability in the re-established population are proposed.

**Keywords** Translocation · Genetic diversity · Endangered population · Microsatellites · Conservation genetics

### Introduction

Establishment of new populations via translocation has become a widely employed management tool in wildlife animal conservation (reviewed by Fischer and Lindenmayer 2000). However, such populations often suffer from loss of genetic diversity, inbreeding depression and founder effects, which may limit the ability of the translocated population to adapt to the changing environment (reviewed by Mock et al. 2004). A number of studies were previously conducted investigating the genetic effects of transfer among different animal taxa (Williams et al. 2002; Mock et al. 2004; Schmitt et al. 2005) including fishes (e.g., Schönhuth et al. 2003), and genetic risks associated with poorly designed programs of animal movements have been reported (Spencer et al. 2000; Stockwell and Leberg 2002; Mock et al. 2004).

In this paper, we describe the genetic founder effects of a historical translocation of a highly endangered softmouth trout population (*Salmo obtusirostris* Heckel 1851) endemic to the five kilometre long Jadro river, Croatia (43°33' N, 16°31' E), that (based on phenotypic and genetic observations) has been recognised as an evolutionary distinctive race; for details about phylogeny, systematics and conservation status, see Sušnik et al. (in press). The population size ( $N_c$ ) of the Jadro softmouth trout including reproductives, juveniles and post-reproductives was estimated as 1150 to 1300 (Šprem et al. 2005; the estimate applies to 2005). Although stocking of brown trout of unknown origin has occurred in the past (H. Ritterman, personal communication), there are presently a very small number of stocked trout (<1%) in the Jadro river (Šprem et al. 2005).

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In 1965, 24 adult Jadro softmouth trout of an unknown sex ratio were translocated to the adjacent fishless Žrnovnica river (43°30' N, 16°32' E; K. Pažur, personal communication; see also Treer et al. 2003). Due to river-bed engineering, only the upper half (~2.5 km) of the Žrnovnica river represents a suitable trout habitat. The  $N_e$  of the Žrnovnica population was estimated as 250–380 (Šprem et al. 2005; the estimate applies to 2005). Regularly stocked rainbow trout (*Oncorhynchus mykiss*) considerably outnumber the softmouth trout in the River Žrnovnica.

Fischer and Lindenmayer (2000) suggested that prior to designing a translocation program, a broad overview and detailed studies focusing on the organism targeted for translocation should be conducted. To date, no such preliminary study has been conducted for the Jadro softmouth trout. In order to determine whether a sufficient level of genetic diversity exists in this re-established softmouth trout population to ensure its long-term survival, we evaluated changes in the genetic diversity and population composition that might have arisen as a result of the translocation.

## Material and methods

### Samples and microsatellite set

Forty softmouth trout from the Jadro river and 32 from the Žrnovnica river, mainly adults of 3+ or more, were randomly caught by electro-fishing in 2003 and 2005. DNA was extracted from fin clips using the Wizard Genomic DNA Purification Kit (Promega). Fifteen microsatellites (BFRO002, Ssa197, Str591, Str24, BFRO001, Ssosl438, Str58, Str-LDH4, One $\mu$ 2, Ssa410, Sssp2216, Ssa408, SsaD190, Omm1064, Sssp2213) were analysed. GenBank accession numbers, original references and PCR-protocols have been described elsewhere (Sušnik et al. 2007; and Lerceteau-Köhler and Weiss 2006).

### Statistical analyses

Population-specific departures from genotypic linkage disequilibrium and Hardy-Weinberg equilibrium (HWE) (10,000 permutations), allele frequency, mean number of alleles per locus ( $A$ ), allelic richness ( $AR$ ), observed ( $H_O$ ) and expected heterozygosity ( $H_E$ ),  $F_{ST}$  (calculated across 3,000 permutations) and  $F_{IS}$  were computed using FSTAT version 2.9.3 (Goudet 2001). Significance of inter-population differences in  $A$ ,  $AR$  and  $H_E$  were tested using the Mann-Whitney U test.

One potential limitation of bottleneck tests in small populations is low marker variability. To address this possible dilemma and to ensure reliable interpretation of the results, we included two methods that employ different approaches to test recent bottleneck events:

(1) The heterozygosity excess method, implemented in the BOTTLENECK program (Piry et al. 1999) tested under the infinite allele model, the stepwise-mutation model and a two-phase model (95% single-step mutations and 5% multiple-step mutations). Distribution of allele frequency classes was also examined for a distortion from the normal L-shaped distribution. We performed all analyses across 10,000 iterations assuming mutation-drift equilibrium, and calculated significance values using the Wilcoxon signed rank test.

(2) A method based on the premise that extinction of rare alleles leads to reduction in the ratio (observed number of alleles)/(range of allele size) (Garza and Williamson 2001; MP val program:  $4N_e\mu = 0.2$ ; prop. of mutation = 0.2; average size of mutation = 4.5).

Contemporary effective population size,  $N_e$ , was estimated for the source and re-established population from genotypic data using the linkage disequilibrium method (Hill 1981), with the bias correction proposed by Waples (2006) after excluding alleles with frequency <0.02.

According to our field observation, the generation time of the species varies from two to four years, so an average value of three years was used in statistical analysis.

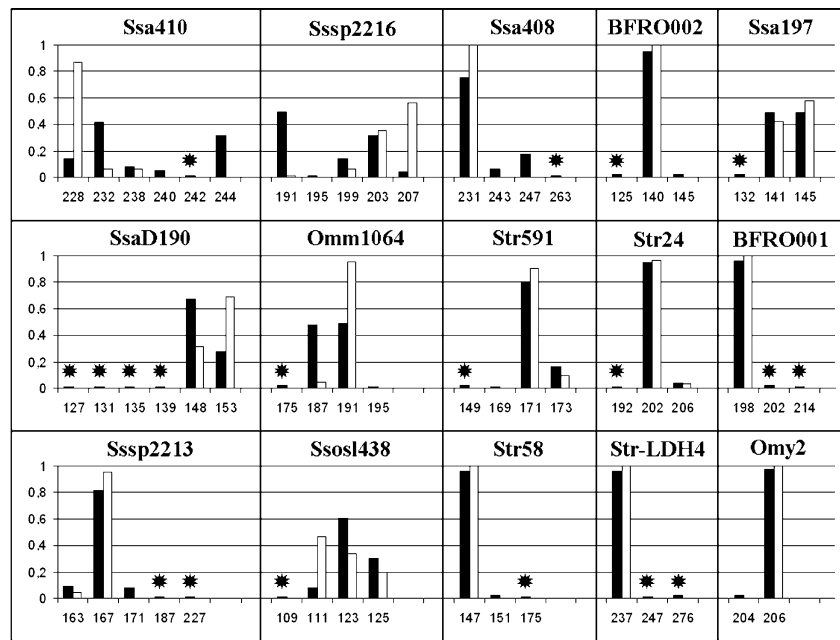
## Results

A total of 58 alleles in the Jadro population and 28 in the Žrnovnica population were identified in the study (Fig. 1). Of the 72 specimens, four from the River Jadro population were found to be introgressed with brown trout alleles at several loci and were thereby considered hybrids between the softmouth and the brown trout (see also Sušnik et al. in press). As a result, these specimens were excluded from further analysis. By contrast, no introgression of brown trout alleles was detected in the Žrnovnica population.

We found no evidence for deviations from HWE (non-significant  $F_{IS}$ ; Table 1) or linkage equilibrium (data not shown). As observed through the  $A$ ,  $AR$ ,  $H_E$  and  $H_O$  values (Table 1), an extremely low genetic diversity was observed in both populations.

The two populations were separated by large allele frequency differences ( $F_{ST} = 0.233$ ,  $P < 0.001$ ; Table 1), and Jadro had significantly more alleles per

**Fig. 1** Allele frequency distribution for softmouth trout from the Jadro river (black blocks) and Žrnovnica (white blocks) at 15 microsatellite loci. Blocks marked with asterisks represent alleles found only in hybrid animals



**Table 1** Mean number of alleles (A), mean allelic richness (AR), overall expected heterozygosity ( $H_E$ ) and ranges over all loci of these diversity indices for Jadro and Žrnovnica populations.  $F_{ST}$  and  $F_{IS}$  values are also presented. Results of Mann-

Whitney U test (Z-statistic & P-value) between the two populations for A, AR and  $H_E$  are shown as well as P-value for pairwise  $F_{ST}$

Population	A	AR	$H_E$	$F_{ST}$	$F_{IS}$
Jadro	2.846 (2–5)	2.756 (1.778–4.991)	0.3383 (0.0274–0.6933)	0.2327	0.036 (ns)
Žrnovnica	2.000 (1–4)	1.990 (1.000–3.875)	0.2108 (0.0000–0.6307)		
Z-statistic	-2.105	-1.423	-1.310	0.00033	–
P value	0.035	0.155	0.190		

locus (2.85 vs 2.00;  $P < 0.05$ ; Table 1). Žrnovnica also had 28% lower allelic richness and 38% lower expected heterozygosity, but these differences were not statistically significant (Table 1). No significant heterozygosity excess was detected in either population and when considering the mode-shift indicator, the alleles of both populations were of normal L-shaped distribution. The Garza-Williamson M ratio was also well above critical ( $M_c$ ) values for both populations ( $M = 1.83$  and  $M_c = 0.75$  for the Žrnovnica;  $M = 1.45$  and  $M_c = 0.77$  for the Jadro). We thus found no genetic evidence in contemporary samples of a recent bottleneck.

Estimates of contemporary  $N_e$  (and the 95% confidence intervals; Waples 2006) were 16.0 (2.2–74.0) for the re-established Žrnovnica, and infinity (48.0-infinity) for the source population Jadro.

**Discussion**

Inter-population genetic differences reflected in a significantly lower A value in the re-established

population and a high  $F_{ST}$ , point to significant differentiation between the two populations of softmouth trout. Together with the much lower (albeit non-significant) values of AR and  $H_E$  in Žrnovnica, these results suggest that habitat translocation considerably reduced genetic diversity in the re-established population. This observation is also in agreement with heterozygosity reduction (up to 50% and more) detected at most loci in the re-established population, however, with notable exception of few loci, where a higher  $H_E$  was observed compared to the one of the source population. This high variance across loci might have contributed to poor statistical support of differentiation based on average  $H_E$  values (Table 1).

Despite the somewhat higher genetic diversity detected in the source population, the levels of  $H_E$  and A in both populations are typical of the microsatellite variation observed for small and threatened populations (Frankham et al. 2004) that have suffered reductions in population size, and should be considered extremely low compared to related non-endangered trout taxa of larger population sizes ( $H_E$  from 0.5 to

0.7; cf. Jug et al. 2005). The recent bottlenecks however, were not detected within the two populations, despite the fact that the number of generations after the translocation (12) fell within the interval (0.25–2.5 times  $2N_e$ ; Cornuet and Luikart 1996) having the highest power at detecting the bottleneck in Žrnovnica population. It is plausible that low genetic diversity in Jadro population could be a consequence of much older reduction(s) in population size that fall out of our tested time frame. Thus, it is most likely that the loss of genetic diversity in the re-established population is due to genetic drift associated with the founder effect following the translocation. This is clearly reflected in a change of frequency distribution in several microsatellite alleles which appear at rather high frequencies in the source population but have been lost, or found at extremely low frequencies, in the re-established one (e.g., Ssa410-224, Sssp2216-191; Fig. 1).

Assuming that the effective population sizes are frequently about one order of magnitude lower than  $N_c$  (Frankham et al. 2004) or even lower in fishes (e.g., Chinook salmon:  $N_e/N_c = 0.04$ ; Bartley et al. 1992), an  $N_e$  of 30–12 can be calculated from the census size,  $N_c \sim 300$ , in the re-established population. Our estimate of contemporary  $N_e$  for the introduced population is toward the low end of this range. The point estimate of contemporary  $N_e$  for Jadro was infinity, but the confidence interval is so wide that we cannot be sure the effective size is larger than about 50. Because no gene flow currently occurs between the two populations, a continuous decline of genetic diversity resulting from genetic drift along with inbreeding can depress population fitness and increase extinction risk of the re-established population. However, the re-established population could serve as a secondary source of individuals to buffer the possible extinction of the Jadro softmouth trout due to demographic events, and for this reason, its conservation value should be taken into account and a conservation plan should be established where its effective size can be maintained at a sufficient level over the long term. It has already been demonstrated that genetically similar immigrants can positively effect a genetic rescue of small, inbred, at-risk population by alleviating inbreeding depression and boosting fitness (for review see Tallmon et al. 2004). Accordingly supplemental introductions from the Jadro population into the Žrnovnica river might play an important role in the evolution of this small population. Together with habitat protection and along with other more specific conservation measures such as ban on fishing and eradication of the unnatural competitors

(e.g., rainbow trout), they could represent an effective conservation tool for the Jadro softmouth trout.

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