SPATIO-TEMPORAL GENETIC VARIATION OF ATLANTIC BLUEFIN TUNAS FROM SARDINIAN AND MEDITERRANEAN TUNA TRAPS

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SUMMARY

Tuna traps of the Sardinian and Mediterranean have provided from long- to short-term series of data and samples of bluefin tuna (BFT) populations inhabiting the Mediterranean. By analyzing genetic variation in bluefin tuna trap samples, we have shown that more than one bluefin tuna population has entered and spread in the Mediterranean over the last century and that over the short period, the interannual composition of the bluefin tuna trap catches are genetically constant.

RÉSUMÉ

Les madragues thonières de Sardaigne et de la Méditerranée fournissent des jeux de données à long et à court terme ainsi que des échantillons de populations de thons rouges présents en Méditerranée. L'analyse des variations génétiques des échantillons de thons rouges provenant des madragues fait apparaître que plus d'une population de thons rouges est entrée dans la mer Méditerranée et s'y est répartie au cours du dernier siècle ; de plus, sur une courte période, la composition interannuelle des prises de thons rouges réalisées par les madragues est génétiquement stable.

RESUMEN

Las almadrabas de túnidos de Cerdeña y del Mediterráneo han proporcionado series de datos de largo a corto plazo y muestras de las poblaciones de atún rojo (BFT) que habitan en el Mediterráneo. Analizando la variación genética en las muestras de almadrabas de atún rojo, hemos demostrado que durante el último siglo más de una población de atún rojo ha entrado en el Mediterráneo y se ha extendido y que, a corto plazo, la composición interanual de las capturas de las almadrabas de atún rojo son genéticamente constantes.

KEYWORDS

Bluefin tuna, DNA, population genetics, population structure, Thunnus thynnus, trap fishing

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1. Introduction

In the eastern Atlantic and Mediterranean, traps have been represented for fishery scientists one of the major source of data to assess stock and population abundance of large pelagic fish and mainly of the Atlantic bluefin tuna (Mather *et al.*,1995; Ravier and Fromentin, 2001; Fromentin, 2009). For example, the bluefin tuna fisheries of the western and central Mediterranean traps of Sardinia, Sicily, Tunisia and Libya and those of the northeast Atlantic coasts of Portugal, Spain and Morocco traps have provided robust and continuous time series of annual catches in the last century which have been used to infer long-term and short cyclic bluefin tuna demographic fluctuations (Ravier and Fromentin, 2001) as well as their relationships with the variation of environmental factors (Ravier and Fromentin, 2004).

Besides the long-term series of fishery data (see for examples: Ravier and Fromentin, 2001; Idrissi and Abid, 2009) some 1900 traps can scientifically contribute today providing historical bluefin tuna specimens (e.g., vertebrae, caudal fin) that have been collected for several decades by scientists and fishermen. These historical collections represent unique and high-valuable scientific archives being each specimen often associated to fishery and biological data. At the beginning the last century, Massimo Sella, Director at the at the Istituto Italo-Germanico di Biologia Marina/Deutsch-Italienisches Institut für Meersbiologie of Rovigno, Italy (now Institute Centre for Marine Research, Rovinj, Croatia) collected and archived an impressive number of skeletal specimens of several large pelagic species, including the bluefin tuna, from several traps of the central western Mediterranean. In the least 20-30 years, several fishery institutions and scientists have been carried an archiving of hard tissues (e.g., scales, otoliths, spines and vertebrae) which bring or incorporate residual soft tissues and cells of the individuals. In the Mediterranean bluefin tuna, fishery scientists of the University of Cagliari have archived fin and muscle tissues of the bluefin tuna collected from the tuna traps still active in the South Sardinia (i.e., Isola Piana and Porto Paglia). All this specimens are potentially suitable to retrieve genetic composition of historical bluefin tuna populations at mitochondrial and nuclear genetic loci by applying appropriated molecular technologies (Hauser and Seeb, 2008; Nielsen and Hansen, 2008; Smith *et al*., 2011).

2. Aim

Here, we deal with a genetic analyses of historical and contemporary bluefin tuna samples collected in the Central-Western Mediterranean tuna traps in the last ca. 100 years in order to infer 1) the occurrence of more than one panmictic population inhabiting the Mediterranean Sea, 2) long-term and short-term spatiotemporal shift of bluefin tuna population structure in the Mediterranean tuna traps. The bluefin tuna specimens we have analysed are included in the historical Massimo Sella's trap archive and in the contemporary University of Cagliari's archive realized by Piero Addis. The genetic survey we have carried out on bluefin tuna trapped in the Mediterranean was robust for the number (total N = 537) and suitability of specimens collected for the genetic analysis, quality of the sampling and biological associated data, number and power of resolution of the genetic markers (one mitochondrial locus, 27 nuclear microsatellite loci) used to genotype bluefin tuna individuals.

3. Materials and methods

3.1 The historical Massimo Sella's trap archive and the historical genetic analyses.

The "Massimo Sella" archive was collected by Massimo Sella at the Istituto Italo- Germanico di Biologia Marina/Deutsch-Italienisches Institut für Meersbiologie of Rovigno, Italy (now Institute Centre for Marine Research, Rovinj, Croatia). The Massimo Sella archive now at the Laboratory of Marine Biology and Fisheries, University of Bologna, Fano, Italy, includes more than 6,000 individual skeletal specimens (dried caudal vertebrae and fins; **Figure 1**) of juvenile and adult fish of Mediterranean large pelagic species (e.g., *T. thynnus*, *Thunnus alalunga*, *Euthynnus alletteratus*, *Sarda sarda*, *Xiphias gladius*) caught in Italian, Spanish, and North African tuna traps of the western and central Mediterranean traps.

Using genomic DNA extraction and PCR amplification procedures developed specifically for the historical specimens, we have estimated genetic variation in three historical bluefin tuna samples collected by M. Sella in the traps and trap-like gear classes from South Tyrrhenian (HSTY, Pizzo and Ganzirri, 1911, N = 39, age class 2), Adriatic Sea (HADR, Istria, 1926-1927; N = 69, age classes 2-4) and Lybian coasts (HLYB, Sliten, 1911-1926, N = 111, age classes 4-12) at a mitochondrial DNA marker (e.g. the nucleotide sequence of a 178bp-control region fragment, CR) and at 8 nuclear, potentially neutral, microsatellite loci (Riccioni *et al*., 2010).

3.2 The contemporary Sardinian trap time series and the contemporary genetic analyses.

From 2005 to 2009, 269 bluefin tunas were collected in the tuna trap of Isola Piana (Isola di San Pietro, year classes 2-24). In 2007, 19 bluefin tunas were also collected in the trap of Porto Paglia (South Sardinia, year classes 2-16). A total of 120 bluefin tuna individuals were phenotypically analysed and recorded for the patched or not-patched external body surface (**Figure 2**). A finclip tissue specimen was collected and stored in ethanol 80%. Standard genomic DNA extraction and PCR amplification procedures were used to estimate the genetic variation at (i) the CR marker (see above), (ii) 11 nuclear, potentially neutral, microsatellite loci (including the 8 loci also scored in the historical bluefin tuna specimens), and (iii) 16 nuclear, potentially under-selection, EST-linked microsatellite loci (Ferrara et al. 2010).

3.3 Data analyses

Multiple approaches have been scheduled for population differentiation analyses among samples in both spatial and temporal scales (by sites, namely traps and by time, namely collecting years and year classes), as this strategy is crucial for population identification in large pelagic and highly migratory species. Genetic variation at the molecular markers was disentangled by commonly used descriptive statistics (PCA, DAPC and MDS) using specific packages of the R software. Population genetic statistical tests implemented in the up to date versions of population genetic software improved to deal with large datasets and to increase the resolution of analysis. We used F-statistics tests implemented in software as Genepop, Genetix, FSTAT, Arlequin and the Bayesian MCMC clustering approaches implemented in the software Structure (v 2.3.3) and BAPS. We have also applied the Analysis of Molecular Variance (AMOVA) across all data sets by grouping individuals according to sampling years, year classes, patched-non patched phenotypes and sex and testing such groupings against the hypothesis of panmixia. Conceptual and methodological details of all statistical analyses and tests were available upon request to the contributing and/or first authors.

4. Results

4.1 Genetic variation in historical Mediterranean traps

In the historical bluefin tuna samples collected from the tuna traps of the south Tyrrhenian (HSTY), Adriatic (HADR) and Libyan coasts (HLYB) neither the descriptive analysis at all markers nor the mtDNA-based F-statistics provided evidence of significant differentiation (overall mtDNA F_{st} = -0.0034, P = 0.654). On the contrary, the F-statistic analysis based on the 8 nuclear, potentially neutral, microsatellite loci provided evidence of significant genetic differentiation among historical bluefin tuna samples (HLYB-HSTY F_{st} = 0.071; HLYB-HADR F_{st} = 0.066; HSTY-HADR = 0.020; all values with P < 0.0001). The great genetic divergence of the HLYB sample from those collected from the northernmost traps and trap-like gear classes was more apparent from the Bayesian clustering of individual genotypes (**Figure 3**). In addition, the genetic composition of the HLYB sample was unique with respect to that of any other contemporary bluefin tuna sample of the Mediterranean (data not shown).

The comparison of the HSTY and HADR genetic variation with that of contemporary bluefin tuna samples (2003-2007, N = 112) collected in the same areas using different gears (i.e. purse seines and long lines) was lower but still significant (HSTY-CSTY F_{st} = 0.016; HADR-CADR F_{st} = 0.017; both values with P < 0.0001).

4.2 Genetic variation in contemporary Sardinian traps

A great variation in size and age of the bluefin tuna individuals was observed among the five collecting years. Individuals collected in 2005 and 2006 were significantly older and larger than those collected in later years, even if in the 2009 few individuals of age class > 13 were collected. Sex ratios of the samples collected in the years 2005, 2006 and 2009 were not significantly skewed. The sex gender of all individuals collected in 2007 and 2008 was not assessed. Among the 120 individuals analysed for external body appearance, the frequency of the patched phenotype predominated over the not patched phenotype (79 against 31 individuals). The patched phenotype (**Figure 2**) is more frequent in the old year classes than in the young year classes. According to this pattern, local fishermen hypothesized that annual catches of the Sardinian tuna traps are composed by two types of bluefin tuna with different migratory behaviour (i.e. the migrant and the locally resident tunas).

Of the 288 bluefin tuna individuals in the two Sardinian traps, 245 individuals were analyses at the CR variation, 286 at the 11 neutral microsatellite loci and 288 at the 16 EST-linked microsatellite loci.

The CR analysis revealed the introgression of the *T. alalunga* and *T. orientalis* mtDNAs in the gene pool of the Mediterranean *T. thynnus* being found CR sequence assigned to these species in 4 and 3 bluefin tuna individuals, respectively. Among the remaining 238 individuals with a *T. thynnus* mtDNA, the CR marker was not able to detect significant genetic differences among the 5 collecting years (pairwise F_{st}s < 0.006, not significant). Limiting the analysis to the more frequent age classes (from the class 4 to 13), all the pairwise F_{st} values were not significant after applying the Bonferroni correction from multiple table. The lack of genetic differentiation among samples at this marker was also apparent by the AMOVA in which any significant grouping was detected.

The genetic variation analysis of 286 bluefin tuna individuals at the 11 neutral microsatellites revealed a certain degree of differentiation of the sample collected in the Porto Paglia tuna trap in 2007 with respect to the Isola Piana samples collected in the same year and in 2006 (F_{st} = 0.012 and 0.008, respectively). However, both F_{st} values became not significant after the Bonferroni correction. Testing the genetic homogeneity across the most frequent age classes at these loci, it resulted that all classes were not differentiated with the exception of the age class 13 whose pairwise F_{st} values with the classes 7 and 11 (0.017 and 0.015, respectively) remained significant after the Bonferroni correction (P < 0.001). The Bayesian analysis of the individual genotypes revealed any clustering of samples, neither across sampling years or age classes. As in the mtDNA analysis, any of the groupings tested in the AMOVA based on neutral microsatellite loci was significant.

The analysis carried out on all collected individuals (N = 288) at the 16 EST-linked, potentially under-selection, microsatellite loci revealed genetic heterogeneity among sampling years with several pairwise F_{st} values > 0.01. All these F_{st} values referred to comparisons involving the samples collected in 2007 at the two tuna traps of Isola Piana and Porto Paglia. However, only the pairwise F_{st} value observed in the comparison between samples collected in the tuna trap of Isola Piana in 2007 and 2009 remained significant after the Bonferroni correction (F_{st} = 0.018, P < 0.0001). Such differentiation of bluefin tuna individuals among sampling years was also confirmed by the significant AMOVA F_{st} value (0.005, P = 0.001). No significant clustering of individual genotypes was observed in the Bayesian analysis and no evidence of significant genetic differentiation were obtained either comparing the genetic structure of the most frequent year classes, the phenotypic classes or the sex classes.

Slight and not significant variation of the genetic diversity estimators (i.e. allelic richness, observed and unbiased expected heterozygosities) between phenotypic and sex classes were observed at both type of microsatellite markers.

5. Discussion and conclusions

According to the results we have obtained on the genetic variation in the bluefin tuna samples collected in the Mediterranean tuna traps, the following issues can be inferred

5.1 Population genetic and ecological issues from the historically trapped bluefin tunas

1) At the beginning of the last century, genetically differentiated groups of bluefin tunas were collected in the central and western Mediterranean tuna traps. Among them, those collected in the Libyan tuna trap of Sliten by Massimo Sella exhibited a great genetic divergence at the neutral microsatellite loci with respect historical bluefin tunas collected in the south Tyrrhenian tuna traps of Ganzirri and Pizzo and in the north Adriatic Istrian traps.
2) The genetic analysis of historical samples is strongly limited by the availability of bluefin tuna samples and results might be biased by methodological errors in the genotyping process, this pattern of genetic structuring detected in the historical tuna trapped bluefin tunas is coherent with the contemporary pattern of population genetic structuring of bluefin tuna within the Mediterranean (Carlsson *et al.*, 2004; Riccioni *et al*., 2010).
3) Recent evidence of a correlation between genetic variation of contemporary bluefin tunas and the variation of environmental parameters in the Mediterranean speaks in favour of a latitudinal (from south to north) pattern of genetic structuring of bluefin tuna (Riccioni *et al*. unpublished data) beside an already ascertained longitudinal (from west to east) genetic break (Carlsson *et al*., 2004, 2007; Reeb, 2010). The finding of deep genetic structuring of historical bluefin tuna collected in the North African tuna trap with respect to those collected in the northernmost traps of Messina strait and Istria might be coherent of the environmental structuring of bluefin tuna in the Mediterranean.

4) The unique genetic composition of the HLYB sample with respect to those estimated in all other historical and contemporary bluefin tuna samples seems to indicate that some spatiotemporal shifts of bluefin tuna population structure and dynamics might have occurred in the Mediterranean. The well-known disappearance of bluefin tunas in the Black and Marmara Seas occurred since the 1960s and the famous "Brazilian episode" (Fromentin and Powers, 2005) might corroborate this issue and the general scenario of a more complex demography on the Mediterranean bluefin tunas.

5.2 Population genetic and ecological issues from the contemporarily trapped bluefin tunas

5) Little evidence of significant genetic differences was obtained in the bluefin tuna trapped in the still active Sardinian tuna traps of Isola Piana and Porto Paglia. Such differences, detected at both types of microsatellite markers (i.e. neutral and potentially under selection loci) are low in number and correlated significantly neither with the age classes nor with the phenotypic classes. Such issue of short-term genetic homogeneity in the bluefin tuna collected in the Isola Piana trap speaks in favour of an interannual stability of the bluefin tuna population exploited by this trap.

6) The fact that some of these genetic differences were detected in the comparisons involving the Porto Paglia could speak in favour of different bluefin tuna groups/populations exploited by the two Sardinian traps. However, this inference seems fully unreliable because the close proximity of these traps and the simultaneous catches.

5.3 General issues from the genetic analysis of Mediterranean trapped bluefin tunas

7) Our genetic data and hose present the literature clearly indicated that more than one bluefin tuna panmictic population are exploited in the Mediterranean.

8) The spatio-temporal shift in the Mediterranean bluefin tuna population structure have been occurred and to figure out such dynamics more robust sampling design and high-performance markers for population genetic studies are required.

9) Mitochondrial DNA markers are not sensitive at all to population structuring in the bluefin tuna (Vinas *et al*., 2011) and neutral or potentially under-selection microsatellite markers have only limited power of resolution at this small geographic scale. New concept of markers such as the under-selection Single Nucleotide Polymorphisms today easily obtained and scored by the Next Generation Sequencing technologies are required to solve the bluefin tuna population genetic structure within the Mediterranean (Milano *et al*., 2011).

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Figure 1. Example of the bluefin tuna skeletal specimens archived in the Massimo Sella: caudal fins of juvenile bluefin tuna and associated fishery data.

Figure 2. Patchy and non patchy morphotypes observed in the external body surface of the bluefin tuna individuals collected at the Isola Piana and Porto Paglia traps in Sardinia. The total weight of each individual is reported in the bottom-right corner.

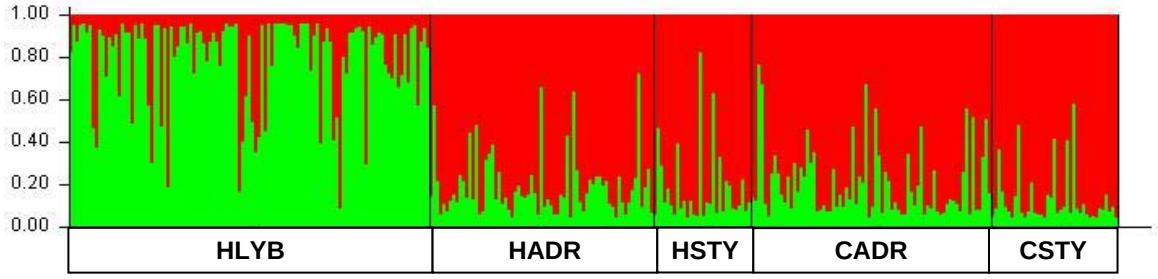

Figure 3. Bar plot of the Bayesian clustering analysis of the genotypes at the 8 neutral microsatellite loci of the historical individual bluefin tunas collected in the tuna traps of the central western Mediterranean by Massimo Sella (HLYB: Sliten trap, Libyan coasts; HADR: Istrian traps, Adriatic Sea; HSTY: south Tyrrhenian traps of Pizzo and Messina strait). Contemporary bluefin tuna samples collected from the Adriatic Sea and the south Tyrrhenian (CADR and CSTY) by purse seine and longline are also included for comparison.