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POPULATION GENETICS OF *TAPES DECUSSATUS* IN THE LAGOA DE SANTO CRISTO, SÃO JORGE - PRELIMINARY RESULTS

T. BACKELJAU[1], C. BRITO[2], A. RODRIGUES[2], B. MORTON [3], R. VERHAGEN[4], T. WILLEMS [4]& B. WINNEPENNIX [5]

[1] Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1040 Brussels, BELGIUM.
[2] Universidade dos Açores, Dept. de Biologia, PT- 9500 Ponta Delgada (Azores), PORTUGAL.
[3] The Swire Marine Laboratory, University of Hong Kong, Cape d'Aguilar, Shek O, HONG KONG
[4] University of Antwerp (RUCA), Dept. Biology, Groenenborgarlaan 171, B-2020 Antwerp, BELGIUM
[5] University of Antwerp (UIA), Dept. Biochemistry, Universiteitsplein 1, B-2610 Antwerp, BELGIUM

INTRODUCTION

The Lagoa de Santo Cristo in São Jorge is the only place in the Azores where the venerid bivalve *Tapes decussatus* (Linnaeus, 1758) forms a well established and stable population. This is in itself already a remarkable situation, for the geographically nearest populations of this species live, as far as we know, along the Atlantic coasts of Portugal and Morocco. Thus the Azorean stock is separated from its neighbors by an oceanic gap of more than 1500Km. Another important characteristic of the Azorean *Tapes* population is the constant human pressure under which it survives. Indeed, *Tapes* is considered as a popular food item for human consumption and it has therefore been intensively fished in the Lagoa de Santo Cristo, even though in the last years several measures have been taken to regulate the *Tapes* fisheries.

Currently, it seems likely that the *Tapes* population in the Lagoa de Santo Cristo was deliberately introduced by man some 30 to 40 years ago. Yet, this does not *a priori* exclude the possibility of larval transport via oceanic currents. Such transport, however, is only possible if the duration of the planctonic larval stage of *Tapes decussatus* is sufficiently long to permit transportation over large distances, which according to our current knowledge does not seems to be the case. On the other hand, if the population was introduced by man it is not clear whether this was a single unique event or whether there were additional subsequent introductions.

Whatever scenario is involved, one would expect that the *Tapes* population of the Lagoa de Santo Cristo may show a decrease of genetic variation through reduced effective population size, in breeding, founder effects and limited (or no) gene flow with respect to neighboring stocks.

Against this background one may wonder how this population maintains itself and how does it cope with the genetic consequences of being a remote, presumably strongly isolated founder population, living under a constant human pressure. In order to study these problems we initiated a comparative population genetic survey of allozyme variation in *Tapes* from the Lagoa de Santo Cristo and "adjacent" regions (French Mediterranean, Atlantic coasts of Morocco, Portugal, Spain and France). In this report we present the preliminary observations on the organ distribution and variation of different enzymes selected for further genetic analysis. We stress that we have deliberately avoided to present a literature survey as this will be included forthcoming, in more extensive and detailed publications about the population genetics of Azorean *Tapes*.

MATERIAL AND METHODS

A first sample of about 150 individuals (all size classes) of *Tapes Decussatus* was collected in the Lagoa de Santo Cristo during the São Jorge e Topo expedition (1992). A second sample of about 80 individuals was taken during the Faial expedition (1993). A first sample for comparative purposes was collected in the Thau Lagoon, French Mediterranean (August, 1993). All specimens were killed and transported in liquid nitrogen. In the laboratory they were stored at -80°C until preparation for genetic analysis by means of vertical polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing (IEF).

Each individual was dissected on ice to remove four organs: gills, digestive gland, foot muscle and adductor muscles. Each organ was then separately weighted and homogenized in a 20% (W/V) aqueous sucrose solution (5 µl sucrose solution per mg tissue). Crude homogenates were subsequently centrifuged for 45 minutes at +/- 27 000 g (15 000 rpm) and 4 °C. The clear supernates were separated from the pellet and divided over several fractions for application on the PAGE gels.

PAGE was basically performed as described by Backeljau & Warmoes (1992). Three buffer systems were used: (1) Tris/glycine pH=9.0 in the tray and Tris/HCl pH=9.0 in the gels (TG buffer); (2) Tris/Citric acid pH=8.0 in both trays and gels (TC buffer); (3) Tris/Borate/EDTA pH=8.9 in both trays and gels (TBE buffer). After PAGE, enzymes were stained according to standard procedures.

RESULTS

Hitherto we screened the following 26 enzyme systems: Glutamate piruvate transaminase (GPT), Nucleosid phosphorilase (NP), Aconitase (ACON), Aldehyde dehydrogenase (ALDH), Lactate dehydrogenase (LDH), α-glycerophosphate dehydrogenase (GPD), α-amylase (AMY), Phosphoglucomutase (PGM), Superoxide dismutase (SOD), Isocitrate dehydrogenase (IDH), Aspartate aminotransferase (AAT), Mannose phosphate isomerase (MPI), Glucose-6-phosphate isomerase (GPI), Esterases (EST), NADH-Diaphorase (DIA), Octopin dehydrogenase (ODH), Strombine dehydrogenase (STDH), Glucose-6-phosphate dehydrogenase (G6PD), Fumarase (FUM), 6-Phosphogluconate dehydrogenase (PGD), Hexokinase (HK), Malate dehydrogenase (MDH), Malic enzyme (ME), Xanthine dehydrogenase (XDH), Leucyl-alanine aminopeptidase (PEP) and Leucyl-aminopeptidase (LAP).

Eight of these enzymes showed no or inconsistent, reactivity (NP, LDH, α-GPD, α-AMY, STDH, G6PD, FUM and LAP) in the PAGE conditions employed. For the other enzymes we described the patterns here below in anticipation of a more formal genetic analysis, which will be published elsewhere (and for which many more specimens have been screened).

GPT: appears as single, relatively diffuse bands with highest activity in the digestive gland, even though the enzyme is clearly active in the four tissues investigated.

ACON: appears as single, rather weak bands; only present in the adductor muscles.

ALDH: yields strong, single bands; only present in the digestive gland.

PGM: reveals a more complex profile consisting of a relatively strong, single band at the anodal gel side and a more diffuse double band in the middle of the gel. The anodal band is present in all tissues, The middle double band is only visible in the gills and in the digestive gland.

SOD: appears as relatively weak, diffuse bands in the middle of the gel. These bands are present in all tissues. In the gills, however, a second SOD band is expressed near the cathodal gel side.

IDH: yields single, well-defined bands, which are only present in the digestive gland.

GOT: reveals strong and well-delimited bands; is highly active in all tissues, at least when resolved with the TBE buffer, for neither the TC buffer, nor the TG buffer, produced any GOT activity.

MPI: is relatively poorly resolved as diffuse anodal bands which are only visible in foot muscle homogenates.

GPI: The resolution of this enzyme is still difficult and inconsistent between experiments. Further work will be needed to overcome this problem.

EST: we use α-naphtylacetate as a substrate to resolve this enzyme system. The obtained multiple band profiles are complex. With the TC buffer we observed a clear difference between tissues: both in the gill and digestive gland homogenates yield highly reactive bands in the middle of the gel, whereas the muscle homogenates only reveal a relatively weak two band pattern at the anodal gel side. These anodal bands are not recovered with the TBE buffer, which neither resolves the EST profiles of gills. However, the TBE buffer yields a much better resolution of the digestive gland esterases than the TC buffer. The TG buffer only results in some smears.

DIA: appears as a strong band system in the digestive gland and as a weak single band (with a slightly more anodal position) in the adductor muscle. Neither the gills, nor the foot muscle show activity for this enzyme.

ODH: shows very clear, well-defined, single bands in the adductor muscles. The three other tissues reveal no ODH activity.

PGD: yields very strong, single bands in all the tissues, even though the degree of enzyme activity varies considerably between different individuals.

HK: reveals enigmatic, but interesting profiles. Activity is only seen in the gills and foot muscles. In the former tissue we observed in one individual three-banded pattern indicative of a heterozygote condition for a dimeric enzyme, whereas in the foot muscle of the same animal only one single band was expressed, corresponding to the most cathodal band of the heterozygote phenotype of the gills. This phenomenon needs further confirmation by running both samples next to each other and by screening more specimens.

MDH: appears as a well-defined, strong band, present in all tissues. Anodally of this clear band there is a second, more weak band, which also occurs in all tissues.

ME: yields variable bands in all tissues, even though the activity is strongest in the adductor muscle. The Band show slight positional variations, which makes them less convenient for further genetic analysis.

XDH: Shows activity in all tissues, but the strongest reaction is seen in the digestive gland. The obtained

bands are rather ill-delimited and shows slight positional variations, which makes them less appropriate for genetic analysis.

PEP: appears as a two-banded enzyme system, which by analogy with other molluscs, probably represents two *loci*. The enzyme is highly active in the gills and foot muscle, and less active in the digestive gland and adductor muscle.

DISCUSSION

These preliminary results clearly demonstrate the importance of separating and analyzing different tissues. The observed tissue distribution of the different enzyme activities undoubtedly reflects metabolic specializations related to the function of the tissue involved (e. g. the presence of ODH and ACON in the adductor muscles). Yet, this topic will be treated in more detail elsewhere.

As this initial survey is mainly intended to determine which enzymes (and which PAGE conditions) are suitable for further genetic analysis, it is still much too early to present any population genetic interpretation. Yet, from our current data we already selected following enzymes for routine analysis: ALDH, PGM, IDH, GOT, EST, DIA, ODH, PGD, HK and MDH, while GPT, ACON, SOD, MPI, GPI, ME, XDH and PEP will need some further testing before they can really relied upon.

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