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Characterisation and expression of the paired box protein 7 (*Pax7*) gene in polymorphic Arctic charr (*Salvelinus alpinus*)

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Abstract

Arctic charr (*Salvelinus alpinus* L.) from Lake Thingvallavatn, Iceland occur as four distinct morphs: large benthivorous (LB), dwarf benthivorous (DB), piscivorous (PI) and planktonivorous (PL). The morphs differ with respect to body size, head morphology, growth rate, and life history. The aim of this study was to investigate the paired box protein 7 (*Pax7*) gene as a candidate for such polymorphisms due to its importance in cranio-facial, skeletal muscle, and central nervous system development. No variation in coding and intronic sequences was found between morphs. We identified 10 alternate *Pax7* isoforms with insertions/deletions: a four-residue (GNRT) deletion, a GEASS insertion truncated by the first serine residue (GEAS), and a thirteen-residue insertion (GQY**A**/**T**GPEYVYCGT). The latter insertion with a threonine (T) contains a putative casein kinase II (CK-2) phosphorylation site. *Pax7* spatial expression patterns were identical in embryos of DB-, LB-, and PL-morphs, and were similar to those described for zebrafish *Pax7c*, but a difference in temporal expression for segmentation was observed between DB and LB morphs. At the end of segmentation, novel expression was observed in the mandibular region as two bilateral domains. The potential role of multiple alternative splicing of the *Pax7* gene for the generation of different Arctic charr morphs is briefly discussed.

Keywords: Salmonid; Arctic charr; *Salvelinus alpinus*; Resource polymorphism; Morph; *Pax7*; Multiple splice variants; Evolution; Skeletal muscle; Development

1. Introduction

The salmonid species Arctic charr (*Salvelinus alpinus*) has a northern circumpolar distribution and occurs as anadromous and non-migratory populations (Johnson, 1980). Resource polymorphism is common in landlocked populations (Skúlason and Smith, 1995), and an extreme case of resource polymorphism is observed in Thingvallavatn, Iceland (64°11′N 21°08′W) where there are four distinct coexisting morphs: large benthivorous (LB), small or dwarf benthivorous (DB), piscivorous (PI) and planktonivorous (PL) (Skúlason et al., 1989). Further clustering of morphs, based on trophic morphology, segregates charr into benthic (LB/DB) and limnetic (PI/PL) morphotypes (Snorrason et al., 1994). The morphs differ with respect to behaviour and life history characteristics including somatic growth, age at sexual maturity, head morphology, colour, and maximum body size (Skúlason et al., 1989; Sandlund, 1992; Skúlason and Smith, 1995). Intraspecific phenotypic variation is thought to have several mechanisms including phenotypic plasticity, maternal effects, genetic polymorphism and genetic differentiation (Skúlason et al., 1992). Hypotheses regarding intraspecific polymorphism based on phenotypic plasticity argue that the formation of stable, alternate phenotypes represents the early stages of the speciation process (West-Eberhard, 1998; Adams and Huntingford, 2002b; West-Eberhard, 2003).

Studies of molecular genetic heterogeneity using minisatellite data in the Thingvallavatn charr, show no intra-morphotypic differences, but do show significant differences between morphs (benthic versus limnetic) compared to other Icelandic lakes (Volpe and Ferguson, 1996). Recent microsatellite data also confirm that sympatric morphs have evolved locally following

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post-glacial invasions from a postulated single charr lineage (Wilson et al., 2004).

In contrast, common-garden rearing experiments with the progeny of the four morphs from Thingvallavatn suggest genetic differences in morphology, body colour, growth, age at first sexual maturity, and foraging behaviour (Skúlason et al., 1989, 1993; Skúlason and Smith, 1995; Eiriksson et al., 1999). Inherited genetic differences have also been identified in polymorphic Arctic charr species from Loch Rannoch, Scotland (Adams and Huntingford, 2002b,a, 2004). Significant differences in head allometry between benthivorous and planktonivorous morphs has lead Adams and Huntingford (2002a,b) to propose that Loch Rannoch morphs are separate species based on Kottelat's criteria (Kottelat, 1997). The differences in head morphology in the Arctic charr morphs in Thingvallavatn are shown in Fig. 1.

The lack of genetic differentiation between the Arctic charr morphs has led to the proposal that only a few regulatory genes may be responsible for the developmental differentiation that accounts for the observed phenotypic differences between morphs (Danzmann et al., 1991). More recent findings in cichlids support this assumption, which show that rapidly speciated lineages acquire a complex regulatory mechanism of alternative splicing over a very short evolutionary period (Terai et al., 2003). In this respect, we postulated that *Pax* proteins were interesting candidates as they are key regulators of early development and also exhibit extensive alternative splicing in mammals (Chi and Epstein, 2002) and latterly the Atlantic salmon (*Salmo salar* L.) (Gotensparre et al., 2006).

The *Pax* family of proteins are highly conserved transcription factors defined by a so-called 'paired box' or paired domain (PD) of 128-amino acids. The PD, first identified in the *Drosophila paired* (*prd*) and *gooseberry* genes (Bopp et al., 1986; Frigerio et al., 1986), is a bipartite DNA-binding domain composed of two subdomains (PAI and RED) each consisting of helix-turn-helix (HTH) motifs. Further complexity in some *Pax* proteins is acquired by the addition of a conserved octapeptide (OP), and a *prd*-type homeodomain (HD) that also comprises a HTH motif. The important role of *Pax* proteins is highlighted by various gene mutations, which are linked to cancer (e.g. alveolar rhabdomyosarcoma), human disease (e.g. Waardenburg Syndrome), and physical defects (e.g. aniridia). Such profound effects confirm *Pax* proteins as 'master' controllers (Gehring, 1996; Underhill, 2000) and essential morphoregulators (Tremblay and Gruss, 1994).

In particular, we are interested in the *Pax7* gene as a possible candidate involved in the development/plasticity of polymorphic Arctic charr due to its involvement in cranio-facial, skeletal muscle, and central nervous system development (Mansouri et al., 1996), and myogenic stem cell specification (Seale et al., 2000). To date, no studies have compared gene sequences from different morphs of Arctic charr. Our *Pax7* study is the first to address this, with a detailed examination of this nuclear regulatory gene and its expression patterns in the Arctic charr morphs of Lake Thingvallavatn, Iceland.

2. Materials and methods

2.1. Collection, rearing, and embryo preparation

Arctic charr (*S. alpinus* Linnaeus, 1758) were sampled by gill-nets at various locations in Lake Thingvallavatn, Iceland. Adult fish were collected for analysis of *Pax7* and RNA content, and eggs were also stripped from ovulating females of DB-, LB-,

Fig. 1. The four morphs of Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. (a) Planktonivorous morph (PL), (b) Piscivorous morph (PI), (c) Dwarf or small benthivorous morph (DB), and (d) Large benthivorous morph (LB). The two pelagic or limnetic morphotypes (a, b) have terminal mouths, pointed snouts and shorter pectoral fins than the two benthic morphotypes (c, d). Note the blunter snouts and sub-terminal mouths of the benthic morphotypes. Scale bars represent 1 cm.