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Interaction of Rearing Environment and Reproductive Tactic on Gene Expression Profiles in Atlantic Salmon

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Abstract

Organisms that share the same genotype can develop into divergent phenotypes, depending on environmental conditions. In Atlantic salmon, young males of the same age can be found either as sneakers or immature males that are future anadromous fish. Just as the organism-level phenotype varies between divergent male developmental trajectories, brain gene expression is expected to vary as well. We hypothesized that rearing environment can also have an important effect on gene expression in the brain and possibly interact with the reproductive tactic adopted. We tested this hypothesis by comparing brain gene expression profiles of the two male tactics in fish from the same population that were reared in either a natural stream or under laboratory conditions. We found that expression of certain genes was affected by rearing environment only, while others varied between male reproductive tactics independent of rearing environment. Finally, more than half of all genes that showed variable expression varied between the two male tactics only in one environment. Thus, in these fish, very different molecular pathways can give rise to similar macro-phenotypes depending on rearing environment. This result gives important insights into the molecular underpinnings of developmental plasticity in relationship to the environment.

Introduction

Explaining the evolution of diversity in species and forms has long been a challenging problem in biology. It has become clear that trait variation observed within and among species cannot solely be due to change in protein coding genes but must also lie at the gene regulation level and in the interactions of the genes (Carroll et al. 2001; King and Wilson 1975). For example, in many species, the same genetic makeup can develop into strikingly different morphologies or behaviors (phenotypic plasticity [West-Eberhard 2003]). This has frequently confused taxonomists, as morphological differences within species can be as significant as those across species. Developmental plasticity is not solely due to developmental noise; rather, it is often the result of evolution by natural selection, enabling organisms to exploit a wider spectrum of resources and to cope with varying conditions throughout life (Pigliucci 2001). Plasticity of behavior, such as appropriate responses to seasonal changes and to reproductive opportunities, is a crucial determinant of an animal's fitness. This relationship between behavioral, morphological, and physiological traits and the ecological context results in a complex and integrated phenotype.

Identifying the proximate mechanisms of phenotypic plasticity constitutes an essential step toward an understanding of complex traits and their evolution in general (Gibson 2002; Via et al. 1995). Much research has focused on the molecular and physiological basis of plasticity during development and in the nervous system of a few model systems (Buonomano and Merzenich 1998; Lynch 2004). Conversely, studies of the evolution of plastic phenotypes have focused almost exclusively on ultimate causes and quantitative genetics (Bradshaw 1965; de Jong 1990; Hazel et al. 1990; Ostrowski et al. 2000; Roff 1996; Scheiner 1993; Van Buskirk 2002). While the study of the ultimate causes of plasticity remains largely divorced from the analysis of its mechanistic basis, a consensus is emerging that deciphering the mechanistic basis of trait variation within species may be fundamental to the understanding of the evolution of species diversity and that it is now time to merge these two branches of research (Hofmann 2003; Robinson and Ben-Shahar 2002). Indeed, the study of proximate and ultimate causes

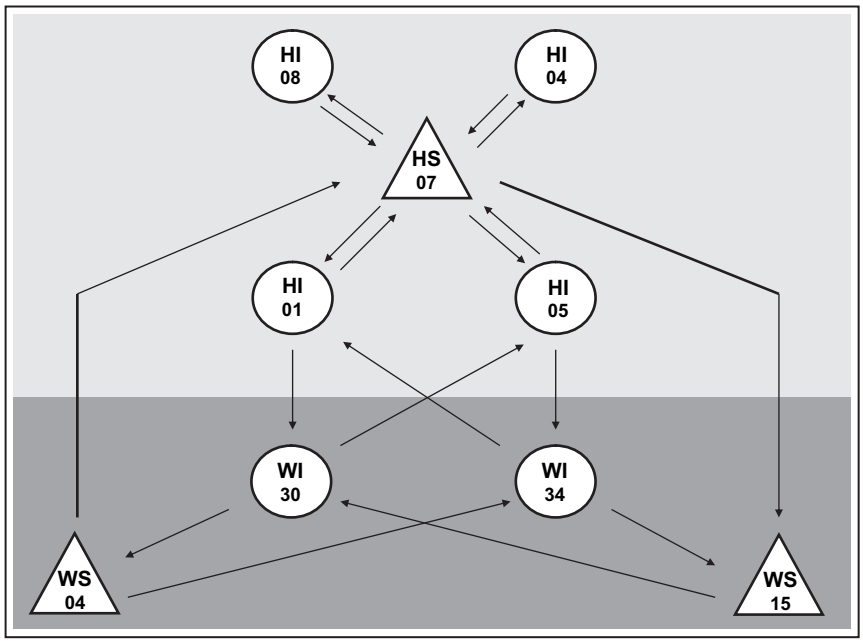

Figure 1. Hybridization design used in this experiment. Brain RNA from immature (I, circle) and mature sneaker (S, triangle) males from the same population, reared in wild environment (W, dark gray background) and hatchery-like environment (H, light gray background), were competitively hybridized according to arrows. Individuals were directly compared between phenotypes within an environment (male tactic effect) and also between environments within a phenotype (rearing environment effect). Arrowtail indicates Cy3 dye, and arrowhead indicates Cy5 dye labeling. Dye-swaps, the labeling of the same RNA sample with both dyes, were performed at least once for each fish. A total of 18 microarray slides and 36 independent labeling were used.

has recently been brought together in work on invertebrates (Abouheif and Wray 2002; Bochdanovits et al. 2003; Moczek and Nijhout 2002). Because complex traits are polygenic and gene interactions are a fundamental property of these traits, taking advantage of a genomic (multigene) approach to the study of gene expression improves the power to reveal the complex network of interrelated functional modules involved (Gracey et al. 2001; Ju et al. 2002; Koskinen et al. 2004; Oleksiak et al. 2002; Podrabsky and Somero 2004; Whitfield et al. 2003).

Atlantic salmon offer an excellent system to apply a genomic approach to the study of developmental plasticity, as males can develop very divergent reproductive phenotypes—and can do this within the same population (Aubin-Horth and Dodson 2004; Letcher and Gries 2003; Myers et al. 1986; Prévost et al. 1992; Whalen and Parrish 1999). During the first life stages in freshwater, juvenile males can either sexually mature precociously to become *sneakers* and reproduce without leaving freshwater; or they can migrate out to sea, only to return years later as large and mature *anadromous* fish to breed (Fleming 1998). Early sexual maturation of males results in gonadal growth, reduced somatic growth, changes in feeding and hormone levels, receptivity and attraction to female pheromones and adult male scent compared to immature males of the same age that will later become large anadromous males. These macroscopic changes in tactics are likely based on modifications of molecular, cellular, and physiological pathways in many tissues. The nature of the brain—in particular, as the center of the integration of environmental and endogenous cues and of control of physiology and behavior—guides initial studies to this important tissue (Hofmann 2003; Whitfield et al. 2003).

In salmon, the developmental decision that leads to divergence in male reproductive tactics is dependent on genetic and environmental factors and their interaction (Aubin-Horth and Dodson 2004; Hutchings and Myers 1994). In the present study, we analyzed expression profiles in the brains of wild-caught and laboratory-reared males (both sneaker and immature tactics) that originated from the same population. We reasoned that by comparing the neural gene expression profiles of these dichotomous phenotypes in different rearing environments, we can dissect the effects of reproductive tactic, environment, and their interactions. This way, we can determine genes that are (1) tactic-specific, independently of environment; (2) specific for a given environment, independently of tactic; and (3) regulated as a consequence of interactions between these two factors and, therefore, neither environment- nor tactic-specific.

Material and Methods

Animals

We collected immature males and mature sneaker males of Atlantic salmon (*Salmo salar*) of age one year in the Sawmill River (42°30′N, 74°30′W), a tributary of the Connecticut

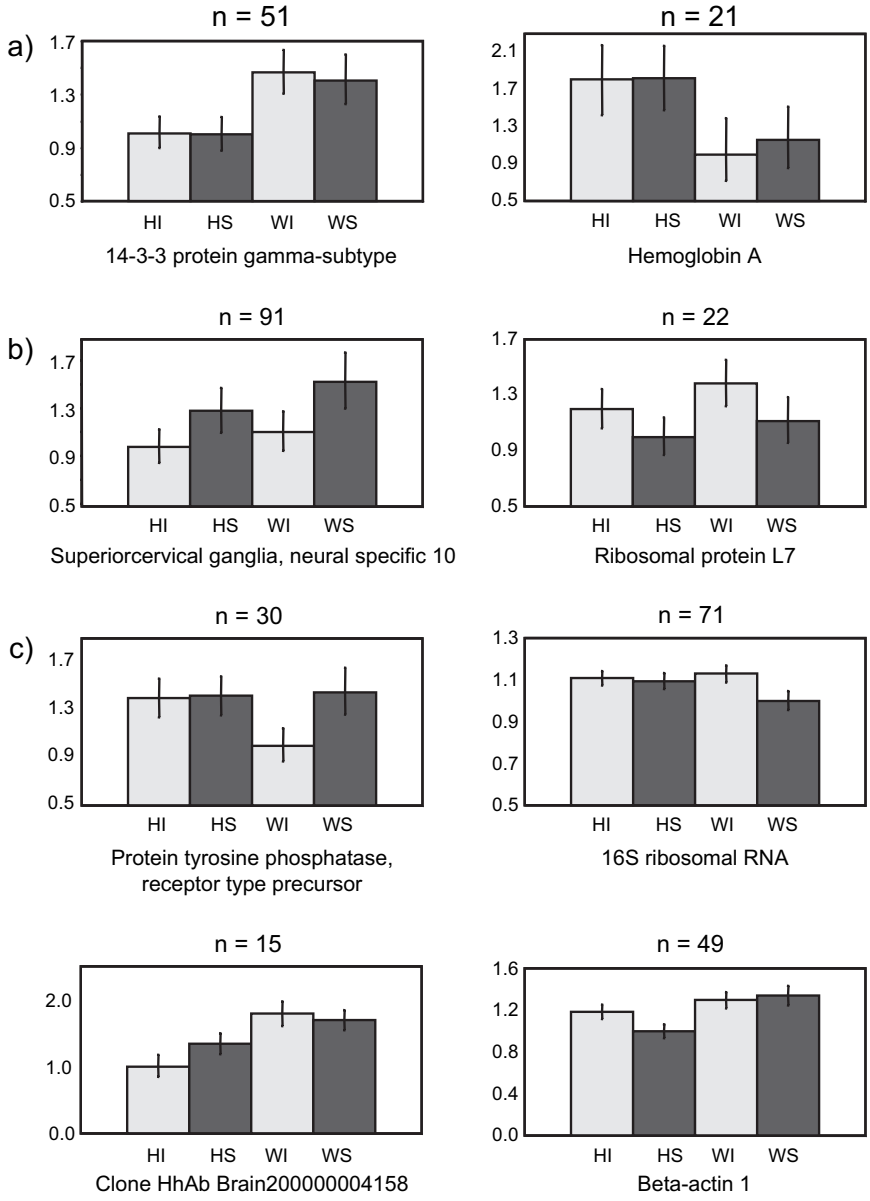

Figure 2. Examples of relative gene expression patterns observed in brains of males, reared in hatchery-like (H) or wild (W) conditions, that are immature (I) or mature sneaker (S) males from BAGEL analysis results. (A) Genes differentially expressed in the brains of males reared in wild and hatchery-like conditions, with no effect of male tactic. (B) Genes differentially expressed in the brains of sneaker males and immature (prospective anadromous) males, with no effect of rearing environment. (C) Interaction of rearing environment and male tactic.

River in western Massachusetts, United States. Laboratory-raised fish, members of the same population, were obtained from the S. O. Conte Anadromous Fish Research Center. Animals were euthanized in buffered MS-222 (100 mg l^{-1}), sexed, and had their brains dissected and immediately transferred to RNAlater storage solution (Ambion); sex was determined by dissection.

Microarray Hybridization

Total RNA was extracted from brains according to a standard Trizol protocol (Invitrogen), following tissue homogenization (Tissue Tearor, Biospec Products). The RNA was analyzed for quantity and quality on the Bioanalyzer (Agilent) and on a standard spectrophotometer (Agilent). Four μg of total RNA extracted from each brain sample were labeled according to a standard amino-allyl attachment method protocol (Renn et al. 2004). Primer was annealed in a 15.5 μl reaction with one μl of primer solution (5 μg/μl each poly dT 12–18 with 5 μg/μl random hexamer oligonucleotides) at 70°C for 10 min, followed by 10 min at 4°C. A reaction solution was prepared as followed: 5.60 μl 5X first strand buffer (Invitrogen); 0.75 μl 50× amino-allyl-dUTP/dNTP

Table 1. Genes differentially expressed in the brain of males reared in wild and hatchery-like conditions, with no effect of male tactic Gene bank accession number or clone identification (GB_acc/unique ID), TIGR contig number (TC) and annotation by sequence similarity based on TIGR gene indices for *Astatotilapia burtoni* v1.0 and BLAST analysis of the Fugu genome. Bold entries are clones that belong to a contig that show more than one pattern of expression.

GB__Acc/unique ID	TC	Annotation
Wild fish higher expression than hatchery fish ($p<0.05$)		
CN468782	TC132	Mitochondrial ATP synthase alpha-subunit, partial (50%)
CN469129	TC150	
CN472139	TC157	Solute carrier family 25 member 5 protein (SI:bZ46J2.2) (Novel ADP/ATP translocase), partial (56%)
CN470048	TC187	
CN469103	**TC193**	**Neuronal pentraxin I, partial (25%)**
CN469241	TC205	100 kDa protein {Rattus norvegicus;}, partial (23%)
CN470489	TC21	Equus caballus mitochondrial DNA complete sequence, partial (3%)
CN471321	TC242	ATP synthase beta-subunit, partial (17%)
CN468556	TC25	protein R02C2.2 [imported] - Caenorhabditis elegans {Caenorhabditis elegans;}, partial (4%)
CN469125	TC276	
CN468806	**TC29**	**ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)**
CN469141	**TC29**	**ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)**
CN470483	**TC29**	**ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)**
CN469815	TC291	Actin, cytoplasmic 2 (Beta-actin 2). {Takifugu rubripes;}, partial (43%)
CN469464	TC303	BH-Pcdh-c (Fragment), partial (12%)
CN469274	TC31	Ribosomal protein L4 (Fragment), partial (54%)
CN470087	TC31	Ribosomal protein L4 (Fragment), partial (54%)
CN470698	TC31	Ribosomal protein L4 (Fragment), partial (54%)
CN469773	TC398	
CN470637	**TC41**	**thymosin beta-4 precursor - rat (fragment) {Rattus norvegicus;}, partial (80%)**
CN468951	TC50	Neurofibromatosis 2 interacting protein, partial (83%)
CN469279	TC54	14-3-3G2 protein, partial (45%)
CN470295	TC54	14-3-3G2 protein, partial (45%)
CN471728	TC54	14-3-3G2 protein, partial (45%)
CN471793	TC54	14-3-3G2 protein, partial (45%)
CN469840	TC56	Receptor for activated protein kinase C, partial (37%)
CN468574	TC62	Poly A binding protein, cytoplasmic 1, partial (36%)
CN468730	**TC69**	**40S ribosomal protein S6. {Ictalurus punctatus;}, partial (66%)**
CN468833		
CN468926		mitochondrial ATP synthase alpha-subunit [Cyprinus carpio] (model%: 100, hit%: 96, score: 2524, %id: 95) [Euteleostomi]"
CN469057		
CN469117		
CN469192		
CN469629		
CN469801		
CN470127		
CN470515		
CN470735		Stathmin 2 (SCG10 protein) (Superior cervical ganglion-10 protein) (model%: 100, hit%: 96, score: 646, %id: 72) [Gallus gallus]"
CN470854		RNA binding motif, single stranded interacting protein 1 (model%: 100, hit%: 61, score: 763, %id: 64) [Homo sapiens]"
CN471091		
CN471106		40S ribosomal protein S9 [Ictalurus punctatus] (model%: 100, hit%: 100, score: 976, %id: 97) [Euteleostomi]"
CN471181		
CN471466		
CN471812		glutamic acid decarboxylase isoform 67 [Carassius auratus] (model%: 100, hit%: 92, score: 2556, %id: 85) [Euteleostomi]"
hh_Ab_Brain2000_000004571		
hh_Ab_Brain2000_000001451		
hh_Ab_Brain2000_000003364		
hh_Ab_Brain2000_000001626		
hh_Ab_Brain2000_000000479		
hh_Ab_Brain2000_000000678		60S RIBOSOMAL PROTEIN L3 (L4) (model%: 100, hit%: 97, score: 1918, %id: 89) [Rattus norvegicus]"
hh_Ab_Brain2000_000004575		

Table 1. Continued

GB__Acc/unique ID	TC	Annotation
Hatchery fish higher expression than wild fish ($p<0.05$)		
CN468735	TC315	alpha hemoglobin A {Seriola quinqueradiata;}, complete
CN469270	**TC330**	**annexin 11a, isoform 2 {Danio rerio;}, partial (33%)**
CN470168	TC4	
CN470326	TC5	AgCP7447 (Fragment), partial (6%)
CN472032	TC5	AgCP7447 (Fragment), partial (6%)
CN471965	TC65	Ras-related protein Rab-1A. {Lymnaea stagnalis;}, partial (60%)
CN469159	**TC9**	**Cytochrome b [Astatotilapia burtoni]**
CN470480	**TC9**	**Cytochrome b [Astatotilapia burtoni]**
CN471698	**TC9**	**Cytochrome b [Astatotilapia burtoni]**
CN468583		RNA polymerase II elongation factor SIII, p15 subunit (model%: 100, hit%: 55, score: 321, %id: 100) [Homo sapiens]"
CN468953		Ensembl_locations(Chr-bp):5-111675696 2310020H20Rik protein (model%: 100, hit%: 75, score: 586, %id: 88) [Mus musculus]"
CN469604		haemoglobin beta-chain [Merlangius merlangus] (model%: 100, hit%: 73, score: 445, %id: 75) [Euteleostomi]"
CN469886		
CN470638		heat shock protein 90-alpha [Danio rerio] (model%: 100, hit%: 94, score: 3097, %id: 87) [Danio rerio]"
CN471263		heat shock protein 90-beta [Danio rerio] (model%: 100, hit%: 100, score: 3308, %id: 89) [Danio rerio]"
CN471317		Laminin beta-1 chain precursor (model%: 100, hit%: 95, score: 6199, %id: 64) [Homo sapiens]"
hh_Ab_Brain2000_000003749		
hh_Ab_Brain2000_000001208		
hh_Nb_HarvardCol_000005748		Nebr DMY F1R1
hh_Ab_Brain2000_000004754		
hh_Ab_StanfordCol_000005730		Green opsin

mix (2.5 mM each dATP, dCTP, dGTP, 1.5 mM dTTP [Invitrogen], and 10 mM amino-allyl dUTP [Sigma]); 2.8 µl 0.1 M DTT, 2.8 µl of DEPC H2O, and 2 µl (200U/µl) of SuperScript II (Invitrogen) reverse transcription enzyme. After addition of the reaction solution to the primer and RNA mix, it was incubated at 42°C for 2 h. RNA left in solution after reverse transcription was then hydrolyzed; and the reverse transcription reaction was stopped by adding 10 µl of 1N NAOH and 10 µl of 0.5 M EDTA and then placing the solution at 65°C for 7 min. The reaction was neutralized with 25 µl of 1 M HEPES pH 7.5 (GIBCO BRL). The cDNA was then repeatedly rinsed and concentrated on a YM-30 filter (Millipore). The dye-coupling reaction required adding 1.5 µl of 1M sodium bicarbonate pH 9.0 and the appropriate Cy3 or Cy5 CyDye Post-labeling reactive dye pack (Amersham) and then placing it for 1 h at room temperature in the dark. The labeled cDNA was then purified using a Qiaquick column standard protocol (Qiagen), and two samples were then combined and concentrated to 50 µl on a YM 30 filter. Hybridization buffer consisted of adding 6 µl 20x SSC (Gibco), 3 µl poly (dA) poly(dT) (Sigma), 0.96 µl 1M HEPES, and 0.6 0.1M DTT (Invitrogen). After filtering on a 0.45 micron filter, 0.9 µl 10% SDS previously warmed to 37°C was added; and the labeled cDNA was denatured by placing at 100°C for 2 min. Twenty µl of probe sample were then immediately added onto each of the two replicates of the microarray on the same slide (Renn et al. 2004; NCBI GEO platform GPL928) and hybridized under a cover slip (Corning) overnight in the dark at 65°C in a humidified chamber (Telechem) submerged in a water bath. The microarray used was constructed from a brain-specific cDNA library from *Astatotilapia burtoni* (Cichlidae). Previous analysis has shown this array platform to give suitable results with RNA derived from Atlantic salmon (Renn et al. 2004). Clone sequences are available from NCBI Genbank (accession numbers CN468542–CN472211; dbEST_Id 22642169–22645838) and contig information from TIGR gene indices (http://www.tigr.org/tdb/tgi/). Excess probe was washed at room temperature in a 400 ml wash solution consisting of 12 ml 20× SSC, 1ml 10% SDS, 4 ml 0.1M DTT, 383 ml Milli-Q Water; this was followed by a wash containing 395 ml Milli-Q Water, 1 ml 20× SSC, and 4 ml 0.1M DTT. Slides were then immediately centrifuged to dry before scanning. Arrays were scanned with an Axon 4000B scanner (Axon Instruments) using Genepix 5.0 software (Axon Instruments). Spots were examined individually and flagged as "bad" if irregularities occurred.

Two to four fish (biological) replicates were assayed per phenotype (see Figure 1, exceptions noted). RNA from each fish was labeled independently three to ten times (technical replicates, including dye-swaps), such that an individual of a given group (sneaker male, immature male, wild-caught, laboratory-raised) was directly compared to individuals of other phenotypes (see Figure 1), without the need for

Table 2. Genes differentially expressed in the brain of sneaker males and immature (future anadromous) males, with no effect of rearing environment

Gene bank accession number or clone identification (GB_acc/unique ID), TIGR contig number (TC) and annotation by sequence similarity based on TIGR gene indices for *Astatotilapia burtoni* v1.0 and BLAST analysis of the Fugu genome. Bold entries are clones that belong to a contig that show more than one pattern of expression.

GB__Acc/unique ID	TC	Annotation
Genes up regulated in sneakers in both environments		
CN468875	TC119	Superiorcervical ganglia, neural specific 10, partial (72%)
CN471202	TC119	Superiorcervical ganglia, neural specific 10, partial (72%)
CN468695	TC139	Na+/K+ ATPase beta subunit isoform 2, partial (56%)
CN470584	TC162	orf2 [Batrachocottus baicalensis] (model%: 99, hit%: 53, score: 542, %id: 58) [Euteleostomi]"
CN470869	TC162	orf2 [Batrachocottus baicalensis] (model%: 99, hit%: 53, score: 542, %id: 58) [Euteleostomi]"
CN472120	TC218	"IPI:IPI00015442.4|ENSEMBL:ENSP00000252759 Tax_Id=9606 (model%: 100, hit%: 97, score: 2290, %id: 72) [Homo sapiens]"
CN470174	TC3	
CN470375	TC345	Sorting nexin 10, partial (13%)
CN471334	**TC40**	**arbp-prov protein {Xenopus laevis;}, complete**
CN468861		
CN469026		neuroligin 3 [Rattus norvegicus] (model%: 97, hit%: 77, score: 2705, %id: 78) [Rattus norvegicus]"
CN469064		
CN469223		Procholecystokinin precursor (CCK) (model%: 100, hit%: 100, score: 505, %id: 74) [Euteleostomi]"
CN469249		
CN469278		
CN469382		
CN469383		N-acetylglucosaminyltransferase V (model%: 100, hit%: 99, score: 2364, %id: 62) [Mus musculus]"
CN469561		
CN469693		
CN469783		
CN469963		
CN470026		integrin beta 5 subunit precursor protein [Bos taurus] (model%: 98, hit%: 96, score: 2979, %id: 69) [Bos taurus]"
CN470098		CDNA: FLJ21016 fis, clone CAE05735 (model%: 53, hit%: 96, score: 1845, %id: 92) [Homo sapiens]"
CN470109		glioma amplified on chromosome 1 protein (leucine-rich) (model%: 100, hit%: 80, score: 1530, %id: 52) [Homo sapiens]"
CN470241		
CN470453		
CN470467		
CN470468		
CN470517		
CN470572		
CN470591		
CN470649		Drosophila seven-up homolog/mammalian ARP-1 homolog [Danio rerio] (model%: 100, hit%: 81, score: 1697, %id: 92) [Danio rerio]"
CN470652		
CN470729		ATPase, Na+/K+ transporting, beta 2a polypeptide; [Danio rerio] (model%: 100, hit%: 27, score: 346, %id: 78) [Danio rerio]"
CN470764		
CN470768		semaphorin 3aa; semaphorin 1a [Danio rerio] (model%: 99, hit%: 80, score: 2956, %id: 79) [Danio rerio]"
CN470775		Neuroglycan C (model%: 100, hit%: 11, score: 252, %id: 75) [Homo sapiens]"
CN470791		
CN470837		
CN470900		Histone acetyltransferase MORF beta (model%: 100, hit%: 100, score: 4896, %id: 50) [Homo sapiens]"
CN470921		
CN470933		
CN470944		

Table 2. Continued

GB__Acc/unique ID	TC	Annotation
CN471040		
CN471136		
CN471209		
CN471265		
CN471295		Beta-soluble NSF attachment protein (model%: 100, hit%: 100, score: 1158, %id: 79) [Homo sapiens]"
CN471296		
CN471341		
CN471576		
CN471596		
CN471609		
CN471621		
CN471658		non-LTR retrotransposable element~partially supported by GENSCAN [Oryzias latipes] (model%: 100, hit%: 23, score: 568, %id: 51) [Euteleostomi]"
CN471683		
CN471734		KIAA0881 protein (model%: 100, hit%: 29, score: 886, %id: 64) [Homo sapiens]"
CN471794		
CN471802		
CN471813		
CN471848		similar to solute carrier family 4, sodium bicarbonate cotransporter-like, member 10 (model%: 100, hit%: 91, score: 3080, %id: 60) [Homo sapiens]"
CN471911		
CN471952		
CN472029		
CN472058		
CN472065		
CN472102		
CN472136		
hh_Ab_Brain2000_000005589		
hh_Ab_Brain2000_000002934		
hh_Ab_StanfordCol_000005681		p IR 6
hh_Ab_Brain2000_000000687		
hh_Ab_Brain2000_000004724		
hh_Ab_Brain2000_000002103		
hh_Ab_Brain2000_000005172		
hh_Ab_Brain2000_000005203		
hh_Ab_Brain2000_000005276		
hh_Ab_Brain2000_000004750		
hh_Ab_Brain2000_000000677		
hh_Ab_Brain2000_000002512		
hh_Ab_Brain2000_000000775		
hh_Ab_Brain2000_000003328		
hh_Ab_Brain2000_000004610		
hh_Ab_Brain2000_000005130		
hh_Ab_Brain2000_000001270		
hh_Ab_Brain2000_000004515		
hh_Ab_Brain2000_000002303		
hh_Ab_Brain2000_000005292		
hh_Ab_Brain2000_000005437		
hh_Ab_Brain2000_000005479		
hh_Ab_Brain2000_000005214		
Genes up-regulated in immature males in both environments ($p<0.05$)		
CN469309	TC120	ribosomal protein L7a [imported] - Takifugu rubripes {Takifugu rubripes;}, partial (70%)
CN471420	TC18	Elongation factor 1a, partial (45%)
CN470690	TC195	ywhae-prov protein {Xenopus laevis;}, partial (86%)
CN470973	TC23	Myelin basic protein (model%: 99, hit%: 45, score: 330, %id: 52) [Homo sapiens]"
CN471248	TC23	
CN469364	TC29	ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)
CN469463	**TC40**	**arbp-prov protein {Xenopus laevis;}, complete**
CN468922	**TC69**	**40S ribosomal protein S6. {Ictalurus punctatus;}, partial (66%)**
CN469397	**TC69**	**40S ribosomal protein S6. {Ictalurus punctatus;}, partial (66%)**

Table 2. Continued

GB__Acc/unique ID	TC	Annotation
CN469472	TC87	Ribosomal protein L7 (Fragment), partial (70%)
CN469407		
CN469441		
CN471187		
CN471206		Hypothetical protein KIAA0286 (Fragment) (model%: 99, hit%: 82, score: 1048, %id: 54) [Homo sapiens]"
CN471490		Hypothetical 32.8 kDa protein (Fragment) (model%: 100, hit%: 99, score: 7551, %id: 80) [Mus musculus]"
hh_Ab_Brain2000_000004573		
hh_Ab_Brain2000_000004065		
hh_Ab_StanfordCol_000005727		unknown #1
hh_Ab_StanfordCol_000005671		unknown #3
hh_Ab_Brain2000_000002098		
hh_Ab_Brain2000_000005537		
hh_Ab_Brain2000_000004731		

a reference sample (Churchill 2002; Townsend 2003). Using biological and technical replicates including dye-swaps has been shown to increase substantially the reliability of microarray results (Liang et al. 2003). Eighteen microarrays were used to compare thirty-six independent labeling reactions.

Analysis

Gene Expression Level

Raw data (after flags filtering and removal of spots with intensities lower than the local background intensity, plus two standard deviation of this background intensity) was imported into R software v1.9 (R Development Core Team 2004) and normalized using the *Linear Models for Microarray Data* package (LIMMA v1.6.5 [Smyth et al. 2003]). Background-subtracted mean intensities (using the minimum method) were normalized using within-array loess normalization. Ratios of intensities were used in a bayesian analysis of gene expression levels (BAGEL v3.6 [Townsend and Hartl 2002]). Out of the 4,574 cDNA spots representing fish genes on the array, a certain number could not be reliably analyzed because of low hybridization quality for these genes, most probably due to sequence divergence (Renn et al. 2004). Therefore, 3,888 ESTs were used in the gene expression level analysis. This bayesian analysis takes advantage of additional information obtained from transitive comparisons of individuals when determining probability of differential expression among groups (Churchill 2002; Townsend 2003; Townsend and Hartl 2002). Annotation of the contigs formed by ESTs and singletons was based on TIGR gene indices *for A. burtoni* v1.0 (Quackenbush et al. 2000) (http://www.tigr.org/tdb/tgi/) and BLAST analysis using the Fugu genome (http://fugu.hgmp.mrc.ac.uk/).

Similarity of Gene Expression Profiles Among Individual Males

A clustering analysis of gene expression patterns of each individual was performed using the *heatmap* function of the *stats* package (R software v1.9 [R Development Core Team 2004]) to determine similarity across brains of each male phenotype. Hierarchical clustering of males' transcription profiles was based on the dissimilarity between expression levels for a given gene using the "average link" agglomeration method. Euclidian distance—which integrates effects of amplitude of ratios, as well as direction (correlation) in patterns—was used to calculate the dissimilarity matrix. Genes whose expression levels were significantly affected by tactic and rearing environment were used for clustering.

Results

In the analysis, 3,888 ESTs (85% of spots on the array) were included, confirming the utility of heterologous hybridization (i.e., hybridizing RNA samples to an array constructed for a different species) when sufficient replication is used, as in the present study (for a systematic analysis, see Renn et al. 2004). Overall, 10.5% (n=409) of genes surveyed showed differential expression, depending on rearing environments and/or male tactics ($P < .05$).

Rearing Environment and Male Tactic Independent Effects

Some gene expression profiles varied between rearing environments, with no difference in transcription between the brains of sneaker males and immature males sharing the same environment (Figure 2a). These "rearing environment effect" genes (n=72) included haemoglobin, several ribosomal proteins, ATP synthase, 14–3–3G2 protein, and heat-shock proteins (see supplementary Table 1 for complete list). A more complicated picture emerged for some other genes (n=6): for example, one clone representing pentraxin (TIGR contig TC193) was upregulated in wild fish (both sneakers and immature males), compared with laboratory-reared fish; however, for other clones belonging to this contig, immature males showed higher expression than sneakers in the laboratory only. Similarly, depending on the clone, the TIGR contig TC330 was upregulated in laboratory-reared fish with

Table 3. Genes differentially expressed in the brain between male tactics only in wild fish
Gene bank accession number or clone identification (GB_acc/unique ID), TIGR contig number (TC) and annotation by sequence similarity based on TIGR gene indices for *Astatotilapia burtoni* v1.0 and BLAST analysis of the Fugu genome. Bold entries are clones that belong to a contig that show more than one pattern of expression.

GB__Acc/unique ID	TC	Annotation
Male type effect only in wild fish, sneaker males higher		
CN470275	TC269	homologue to MGC75936 protein, partial (21%)
CN469005	**TC298**	**kainate receptor beta chain precursor - goldfish {Carassius auratus;}, partial (26%)**
CN470164	TC337	Tumor necrosis factor ligand superfamily member 6 (FAS antigen ligand)(CD95L protein). {Macaca fascicularis;Macaca mulatta;Macaca nemestrina;}, partial (8%)
CN468779	TC34	ATPase, Na+/K+ transporting, alpha 1a.1 polypeptide, partial (24%)
CN471503	TC34	ATPase, Na+/K+ transporting, alpha 1a.1 polypeptide, partial (24%)
CN472048	TC35	Na+/K+ ATPase alpha subunit isoform 1 (Na+/K+ ATPase alpha 1B1 subunit), partial (21%)
CN469285	TC43	
CN470569	**TC8**	**Gadus morhua complete mitochondrial DNA sequence, partial (6%)**
CN468991		faciogenital dysplasia [Danio rerio] (model%: 100, hit%: 43, score: 1187, %id: 79) [Danio rerio]"
CN469100		
CN469165		
CN469215		
CN469276		KIAA1157 protein (model%: 100, hit%: 82, score: 1648, %id: 71) [Homo sapiens]"
CN469368		Ensembl_locations(Chr-bp):14-11581532 4933425A18Rik protein (model%: 100, hit%: 91, score: 1537, %id: 68) [Mus musculus]"
CN469400		Sodium/potassium-transporting ATPase alpha-1 chain precursor (Sodium pump 1) (Na+/K+ ATPase 1) (model%: 99, hit%: 80, score: 3966, %id: 92) [Euteleostomi]"
CN469724		BOVIN 2-OXOISOVALERATE DEHYDROGENASE ALPHA SUBUNIT, MITOCHONDRIAL PRECURSOR (model%: 100, hit%: 88, score: 1827, %id: 82) [Bos taurus]"
CN469875		Ensembl_locations(Chr-bp):7-87114099 (model%: 100, hit%: 91, score: 1708, %id: 64) [Mus musculus]"
CN469990		splicing factor 3b, subunit 1, 155kD (model%: 100, hit%: 99, score: 6318, %id: 93) [Homo sapiens]"
CN470072		Ensembl_locations(Chr-bp):9-14246485 Sestrin 3 (model%: 98, hit%: 76, score: 764, %id: 44) [Mus musculus]"
CN470248		RAS-RELATED PROTEIN RAB-8B (model%: 100, hit%: 100, score: 938, %id: 89) [Rattus norvegicus]"
CN470363		CG13472 protein (RE01471p) (model%: 100, hit%: 6, score: 126, %id: 47) [Drosophila melanogaster]"
CN470451		
CN470792		
CN470957		
CN471509		Ensembl_locations(Chr-bp):8-33755234 (model%: 100, hit%: 90, score: 5143, %id: 83) [Mus musculus]"
CN471531		
CN471687		KIAA1771 protein (Fragment) (model%: 100, hit%: 89, score: 5387, %id: 89) [Homo sapiens]"
CN471783		protein tyrosine phosphatase, receptor type, D, isoform 2 precursor (model%: 99, hit%: 84, score: 5935, %id: 69) [Homo sapiens]"
CN471942		B-cell lymphoma/leukaemia 11A extra long form (model%: 100, hit%: 95, score: 2556, %id: 63) [Homo sapiens]"
CN471987		
hh_Ab_Brain2000_000003770		
hh_Ab_Brain2000_000004568		
hh_Ab_Brain2000_000001511		iron-sulfur protein precursor [Bos taurus] (model%: 100, hit%: 76, score: 856, %id: 77) [Bos taurus]"
hh_Ab_Brain2000_000003948		
hh_Ab_Brain2000_000001055		
hh_Ab_Brain2000_000005453		
hh_Ab_Brain2000_000003372		

Table 3. Continued

GB__Acc/unique ID	TC	Annotation
hh_Ab_Brain2000_000005366 hh_Ab_Brain2000_000004964 hh_Ab_Brain2000_000001788		
Male type effect only in wild fish, immature males higher		
CN468741	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN468781	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN468809	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN468982	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469061	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469104	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469219	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469358	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469659	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469705	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469715	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469812	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469828	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469873	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469918	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470005	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470235	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470257	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)

Table 3. Continued

GB__Acc/unique ID	TC	Annotation
CN470507	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470547	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470630	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470734	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN470945	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471157	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471216	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471231	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471301	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471358	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471426	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471432	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471438	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471585	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471620	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471855	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN471931	TC1	12S ribosomal RNA, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA, partial sequence; mitochondrial genes for mitochondrial products, partial (29%)
CN469790	TC110	
CN469052	TC144	Lin-7-A, complete
CN468760	TC2	complete mitochondrial DNA sequence, partial (8%)
CN469166	TC2	complete mitochondrial DNA sequence, partial (8%)
CN469961	TC2	complete mitochondrial DNA sequence, partial (8%)
CN470094	TC2	complete mitochondrial DNA sequence, partial (8%)
CN470493	TC2	complete mitochondrial DNA sequence, partial (8%)
CN471764	TC201	

Table 3. Continued

GB__Acc/unique ID	TC	Annotation
CN468698	TC22	Myelin basic protein (model%: 99, hit%: 45, score: 330, %id: 52) [Homo sapiens]"
CN469387	TC265	
CN468961	**TC298**	**kainate receptor beta chain precursor - goldfish {Carassius auratus;}, partial (26%)**
CN470431	TC3	
CN470752	TC3	
CN471682	TC3	
CN470160	TC30	complete mitochondrial DNA sequence, partial (4%)
CN471495	TC30	complete mitochondrial DNA sequence, partial (4%)
CN468617	TC312	Zfr protein, partial (16%)
CN470577	TC346	Ornithine decarboxylase antizyme, short isoform (ODC-Az-S). {Danio rerio;}, partial (73%)
CN468904	TC371	protein kinase, cAMP-dependent, type I-alpha regulatory chain - pig {Sus scrofa domestica;}, partial (28%)
CN469269	**TC41**	**thymosin beta-4 precursor - rat (fragment) {Rattus norvegicus;}, partial (80%)**
CN468770	TC51	Creatine kinase, brain, partial (35%)
CN470960	TC59	complete mitochondrial DNA sequence, partial (3%)
CN470324	TC73	Glutathione S-transferase, partial (88%)
CN471075	TC76	gene HMG-T2 protein - rainbow trout {Oncorhynchus mykiss;}, partial (95%)
CN470350	TC81	
CN472127	**TC9**	**Cytochrome b [Astatotilapia burtoni]**
CN468631		SDC-SIGN2 type III isoform (model%: 100, hit%: 29, score: 172, %id: 43) [Homo sapiens]"
CN468649		
CN469091		
CN469092		
CN469286		claudin 12 isoform fc27c11 [Danio rerio] (model%: 100, hit%: 99, score: 1239, %id: 68) [Danio rerio]"
CN469551		
CN469888		agCP4371 [Anopheles gambiae str. PEST] (model%: 93, hit%: 25, score: 160, %id: 52) [Anopheles gambiae str. PEST]"
CN470846		"IPI:IPI00105058.1|ENSEMBL:ENSP00000298017 Tax_Id=9606 (model%: 100, hit%: 39, score: 274, %id: 78) [Homo sapiens]"
CN470925		
CN471120		calmodulin [Oryzias latipes] (model%: 100, hit%: 94, score: 663, %id: 100) [Euteleostomi]"
CN471140		
CN471167		
CN471259		
CN471357		
CN471371		
CN471408		
CN471412		Hypothetical protein KIAA1223 (Fragment) (model%: 60, hit%: 100, score: 3203, %id: 82) [Homo sapiens]"
CN471526		Ensembl_locations(Chr-bp):17-6024841 (model%: 100, hit%: 36, score: 154, %id: 60) [Mus musculus]"
CN471598		
CN471702		Ensembl_locations(Chr-bp):None Similar to hypothetical protein FLJ10008 (model%: 70, hit%: 100, score: 1545, %id: 43) [Mus musculus]"
CN471737		
CN471781		Ensembl_locations(Chr-bp):6-113953617 Hypothetical 56.4 kDa protein (model%: 100, hit%: 80, score: 1240, %id: 58) [Mus musculus]"
CN471976		
CN472009		
hh_Ab_Brain2000_000000514		
hh_Ab_Brain2000_000000335		
hh_Ab_Brain2000_000004752		
hh_Ab_Brain2000_000000586		
hh_Ab_Brain2000_000000044		
hh_Ab_Brain2000_000005539		

Table 3. Continued

GB__Acc/unique ID	TC	Annotation
hh_Ab_Brain2000_000004729		
hh_Ab_Brain2000_000004661		
hh_Ab_Brain2000_000000504		
hh_Ab_Brain2000_000005557		
hh_Ab_Brain2000_000000732		
hh_Ab_Brain2000_000002742		
hh_Ab_Brain2000_000002726		

a phenotype effect detectable only in the laboratory. Finally, TIGR contig TC69 was found to be overexpressed not only in wild fish versus hatchery fish, but also in immature males in both environments (supplementary Table 1, bold entries).

For 113 genes, expression varied only with the male tactic, without a significant effect of the environment, such that sneakers in both environments exhibited upregulation or downregulation for these genes (Figure 2b). Examples include Na^+/K^+ ATPase β subunit isoform 2, stathmin 2 (superior cervical ganglion-10 protein), sorting nexin 10, N-acetylglucosaminyltransferase V, semaphorin, neuroligin 3, and ribosomal proteins (see supplementary Table 2 for complete list). Only one gene showed an effect of environment overlaid on the tactic effect: elongation factor 1-alpha was overexpressed in immature males compared to sneaker males, and it also showed a significantly higher expression in wild immature fish compared with those from the laboratory fish.

Interaction of Rearing Environment and Male Tactic

As hypothesized, we found considerable interaction effects of tactic and environment on gene expression (n=225 genes; Figure 2c). More than half of all the genes showing differential expression had an expression pattern of divergence between sneaker males and immature males that was found only in one environment. For instance, a male tactic effect on gene expression was found only in wild salmon for 138 ESTs (supplementary Table 3), while 78 EST showed an effect of male tactic solely in fish reared in laboratory settings (supplementary Table 4). Reassuringly, genes showing opposite patterns of expression in a tactic between environments were rare (n=8; supplementary Table 5), even at $P < .05$, and are most likely false positives in one or both comparisons.

Because we performed heterologous hybridizations to a non-salmon fish array platform, we examined the level of concordance shown by spots belonging to the same contigs (and likely the same genes). As supplementary Tables 3 and 4 show, many ESTs from the same TIGR contig were upregulated concordantly. This is an important result, as it validates the utility of this array platform for heterologous hybridizations with salmon RNA (see also Figure 5 in Renn et al. 2004).

Similarity of Gene Expression Profiles Among Individual Males

Hierarchical clustering showed that similarity in transcription profiles among the brains of each individual male was both related to the reproductive tactic and the environment the fish was reared in (Figure 3). Importantly, wild sneaker males clustered together separately from all other fish. Among immature males, those obtained from the wild also clustered together.

Discussion

In the present study, we used a microarray-based approach to examine how rearing environment affects neural expression profiles that underlie the dramatic divergence between distinct male reproductive tactics of the same population. We showed that environment and reproductive tactic, as well as tactic-by-environment effects, give rise to specific gene expression patterns in the brain of male Atlantic salmon.

Rearing Environment and Male Tactic Independent Effects

We found an effect of rearing environment on gene expression such that neural expression profiles of laboratory and wild fish differed independent of male tactic. Both laboratory and wild-caught fish were derived from the same population. This result implies that the environmental cues and surrounding conditions (e.g., population density, feeding resources, and temperature and light regimes) can lead to profound differences in the molecular makeup of the brains of animals whose macro-phenotypes nevertheless are considered to be the same. It is important to note that no genes known for their role in reproductive maturation showed a rearing environment effect.

We do not know whether laboratory conditions can be considered extreme or "unnatural" with respect to their molecular consequences on the brain. However, we can hypothesize that similar large-scale differences may arise after transplanting wild fish of the same genetic population into different rivers (or different locations along the same river). Our results therefore have implications for the study of gene expression variation among populations and their interpretation as examples of local adaptations. Indeed, our findings suggest that gene expression profiles can vary significantly as the result of environmental variation only, even with similar genetic background, suggesting that experimental manipulations such as reciprocal transplants and common garden experiments would be necessary to partition the genetic and environmental components underlying variation in gene expression and to determine the relative importance of these factors (Falconer and Mackay 1996).

Table 4. Genes differentially expressed in the brain between male tactics only in laboratory fish
Gene bank accession number or clone identification (GB_acc/unique ID), TIGR contig number (TC) and annotation by sequence similarity based on TIGR gene indices for *Astatotilapia burtoni* v1.0 and BLAST analysis of the Fugu genome. Bold entries are clones that belong to a contig that show more than one pattern of expression.

GB__Acc/unique ID	TC	Annotation
Male type effect only in hatchery fish, sneaker males higher		
CN471431	TC206	
CN471553	TC68	14-3-3 protein (Fragment), partial (7%)
CN470757	**TC8**	**complete mitochondrial DNA sequence, partial (6%)**
CN471456	TC98	SI:dZ105L16.15 (Novel TC1-like transposase) (SI:dZ173M20.15) (Novel transposase), partial (44%)
CN468767		Gamma-aminobutyric-acid receptor beta-3 subunit precursor (GABA(A) receptor) (model%: 100, hit%: 94, score: 2034, %id: 86) [Gallus gallus]"
CN469626		Noelin precursor (Neuronal olfactomedin-related ER localized protein) (Olfactomedin 1) (Pancortin) (model%: 100, hit%: 100, score: 2208, %id: 85) [Gallus gallus]"
CN470479		Kinase-like protein (model%: 100, hit%: 95, score: 2475, %id: 64) [Homo sapiens]"
CN470885		
CN471036		
CN471551		
CN472036		
hh_Nb_HarvardCol_000005861		Sox 9b
hh_Ab_Brain2000_000004671		
hh_Ab_Brain2000_000004741		
hh_Ab_Brain2000_000005170		
hh_Ab_Brain2000_000003769		
hh_Ab_Brain2000_000000961		
hh_Ab_HarvardCol_000005745		Astatotilapia burtoni GABA
Male type effect only in hatchery fish, immature males higher		
CN469235	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN469932	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN470091	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN470181	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN470478	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN470767	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN470859	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN471184	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN472199	TC12	Actin, cytoplasmic 1 (Beta-actin 1). {Oreochromis mossambicus; Takifugu rubripes;}, complete
CN469203	TC155	Ribosomal protein L13a (Fragment), partial (84%)
CN470379	TC155	Ribosomal protein L13a (Fragment), partial (84%)
CN472190	TC179	Ribosomal protein L5a, partial (32%)
CN468743	TC18	Elongation factor 1a, partial (45%)
CN469360	TC18	Elongation factor 1a, partial (45%)
CN471679	TC18	Elongation factor 1a, partial (45%)
CN471488	TC185	40S ribosomal protein S4 (Fragment), partial (47%)
CN472093	**TC193**	**Neuronal pentraxin I, partial (25%)**
CN469574	TC236	lactate dehydrogenase B {Fundulus heteroclitus;}, partial (37%)
CN471294	TC259	similar to GB|AAH44073.1|28277250|BC044073 MGC52653 protein {Xenopus laevis;}, partial (14%)
CN469643	TC28	Beta tubulin, partial (74%)

Table 4. Continued

GB__Acc/unique ID	TC	Annotation
CN470742	TC28	Beta tubulin, partial (74%)
CN471441	TC28	Beta tubulin, partial (74%)
CN468696	**TC29**	**ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)**
CN471280	**TC29**	**ribosomal protein L3, cytosolic - human {Homo sapiens;}, partial (46%)**
CN469126	**TC330**	**annexin 11a, isoform 2 {Danio rerio;}, partial (33%)**
CN469736	TC347	Elongation factor 1a, partial (31%)
CN470419	TC373	EBNA1 binding protein 2, partial (18%)
CN470906	TC382	Ceruloplasmin, partial (10%)
CN470158	**TC40**	**arbp-prov protein {Xenopus laevis;}, complete**
CN469813	TC45	40S ribosomal protein S2 (Fragment), partial (88%)
CN468689	TC48	Beta tubulin, partial (37%)
CN470132	TC53	Myelin proteolipid protein (PLP) (Lipophilin) (DM20). {Oncorhynchus mykiss;}, partial (51%)
CN471170	TC53	Myelin proteolipid protein (PLP) (Lipophilin) (DM20). {Oncorhynchus mykiss;}, partial (51%)
CN468665	**TC9**	**Cytochrome b [Astatotilapia burtoni]**
CN469799	TC94	40S ribosomal protein S5 (Fragment), partial (72%)
CN469000		Actin, cytoplasmic 1 (Beta-actin 1) (model%: 100, hit%: 100, score: 1961, %id: 100) [Euteleostomi]"
CN469304		putative 40S ribosomal protein 20S protein [Oncorhynchus mykiss] (model%: 99, hit%: 99, score: 592, %id: 99) [Euteleostomi]"
CN469371		t-complex polypeptide 1 [Danio rerio] (model%: 100, hit%: 95, score: 2498, %id: 92) [Danio rerio]"
CN469565		putative ribosomal protein L14 [Takifugu rubripes] (model%: 99, hit%: 99, score: 704, %id: 100) [Euteleostomi]"
CN469719		60S ribosomal protein L8 (model%: 100, hit%: 100, score: 1320, %id: 96) [Homo sapiens]"
CN469952		stromal cell derived factor receptor 1; glycoprotein 55; glycoprotein 65 [Rattus norvegicus] (model%: 99, hit%: 86, score: 1278, %id: 69) [Rattus norvegicus]"
CN470093		Elongation factor 1-alpha 1 (model%: 100, hit%: 100, score: 2267, %id: 94) [Homo sapiens]"
CN470187		
CN470544		ribosomal protein L6 [Ictalurus punctatus] (model%: 95, hit%: 93, score: 1119, %id: 86) [Euteleostomi]"
CN470587		
CN470799		Actin, cytoplasmic 1 (Beta-actin 1) (model%: 100, hit%: 100, score: 1961, %id: 100) [Euteleostomi]"
CN471336		
CN471347		proopiomelanocortin [Acanthopagrus latus] (model%: 100, hit%: 100, score: 776, %id: 66) [Euteleostomi]"
CN471387		
CN471824		Thymopoietin, isoforms beta/gamma (model%: 100, hit%: 11, score: 112, %id: 50) [Homo sapiens]"
CN471863		elongation factor 1-alpha [Danio rerio] (model%: 99, hit%: 99, score: 2187, %id: 90) [Danio rerio]"
CN472099		
hh_Ab_Brain2000_000005192		
hh_Ab_Brain2000_000005264		
hh_Ab_StanfordCol_000005677		GnRH1
hh_Ab_Brain2000_000002474		iron-sulfur protein precursor [Bos taurus] (model%: 100, hit%: 76, score: 856, %id: 77) [Bos taurus]"
hh_Ab_Brain2000_000002267		
hh_Ab_Brain2000_000004629		
hh_Ab_Brain2000_000004498		
hh_Ab_Brain2000_000003777		

Table 5. Opposite patterns of gene expression between rearing environments
Gene bank accession number or clone identification (GB_acc/unique ID), TIGR contig number (TC) and annotation by sequence similarity based on TIGR gene indices for *Astatotilapia burtoni* v1.0 and BLAST analysis of the Fugu genome.

GB__Acc/unique ID	TC	Annotation
Opposite patterns of gene expression between environments, sneaker higher in wild, immature in hatchery		
CN468740	TC280	
CN469324		Phosphoserine aminotransferase (model%: 100, hit%: 82, score: 1226, %id: 73) [Homo sapiens]"
CN470928		
CN471411		CDNA FLJ14563 fis, clone NT2RM4000215, weakly similar to MAK16 protein (model%: 100, hit%: 56, score: 807, %id: 88) [Homo sapiens]"
hh_Ab_Brain2000_000001431		
hh_Ab_Brain2000_000003661		iron-sulfur protein precursor [Bos taurus] (model%: 100, hit%: 76, score: 856, %id: 77) [Bos taurus]"
Opposite patterns of gene expression between environments, Immature higher in wild, Sneaker in hatchery		
CN469889	TC13	NADH dehydrogenase subunit 2 [Astatotilapia burtoni]
CN470701		

Organisms (or at least their brains) reared in different environments appear to implement the same reproductive tactics (e.g., sneaker) at least in part by different molecular mechanisms. This remarkable ability may allow organisms to integrate, at the molecular and physiological level, variations in external as well as internal cues and to "canalize" them into one or a few macro-phenotypes. In other words, there is more than one way to "make" a sneaker brain.

While we are not yet able to assign a biological function to all genes whose activities differ between laboratory and wild fish, some intriguing interpretations are already possible. For example, extraretinal opsins were upregulated in the brains of laboratory-reared fish, possibly indicating a different regulation of photoperiod entrainment (Alvarez-Viejo et al. 2004; Kojima et al. 2000). Similarly, HSP-90 and other chaperones upregulated in laboratory fish independent of male tactic may indicate increased growth and/or a stress response. In fish, HSP-90 supports various components of the cytoskeleton and of steroid hormone receptors (Basu et al. 2002). Gobies acclimatized to summer temperature had higher levels of HSP-90 in the brain (as well as a higher induction threshold) than fish acclimatized to winter temperature (Dietz and Somero 1992). HSP-70 were significantly raised in the brains of goldfish that were reared in the presence of a predator, an effect that is likely mediated by circulating cortisol levels (Kagawa and Mugiya 2002). Also, increased HSP expression shuts down protein synthesis (Rose et al. 1989), which is consistent with our finding that several ribosomal proteins were among the genes underexpressed in the brains of laboratory fish, potentially indicating lower levels of protein synthesis activity.

Another laboratory-specific gene encodes a laminin (beta-1 chain) precursor, a major component of basement membranes that has numerous biological activities (Meiners and Mercado 2003), including promotion of cell adhesion, migration, growth, and differentiation (e.g., neurite outgrowth). Interestingly, the DMY gene, which has been implicated in sex differentiation of males (Matsuda 2003; Winkler et al. 2004), is also upregulated in the brains of laboratory-reared fish, although its function here can only be guessed. Another gene upregulated in laboratory fish is Ras-related protein Rab-1A, which belongs to a family of small GTP-binding proteins relevant in regulating intracellular vesicle trafficking (Wright and Harding 2004).

Additionally, a number of genes involved in basic metabolic processes were upregulated in the brains of wild animals, which may indicate increased metabolic demands in a natural stream, compared with controlled laboratory conditions. One example is ATP synthase, which is of crucial importance in ATP production. Glutamic acid decarboxylase (GAD67), a GABA-synthesizing enzyme, is also up in wild fish, which may be related to differences in the water temperatures (Fraser et al. 2002).

We found that expression of some genes was affected by male reproductive tactic independent of the environment the fish were reared in, such that core sneaker genes could be determined. For example, superior cervical ganglia neural-specific protein (stathmin), a small regulatory protein integrating diverse intracellular signaling pathways involved in the control of cell proliferation and differentiation (Curmi et al. 1999), was overexpressed in sneakers in two spots from the same contig annotated as this gene.

Interaction of Rearing Environment and Male Tactic

More than half of all genes that showed variable expression exhibited an interacting effect of male tactic and rearing environment, such that differences between the two male tactics was found in only one environment. In this context, it is interesting that the number of genes found to be upregulated in sneakers in only one environment is 39 (wild) + 18 (laboratory) = 57, while the number of genes found upregulated in immature males in only one environment is 57 (wild) + 45 (laboratory) = 102 (i.e., almost twice as high). Whether this result means that neural transcription profiles in sneakers are less susceptible to a variable environment (or that immature males are more susceptible), though intriguing as a hypothesis, cannot yet be decided.

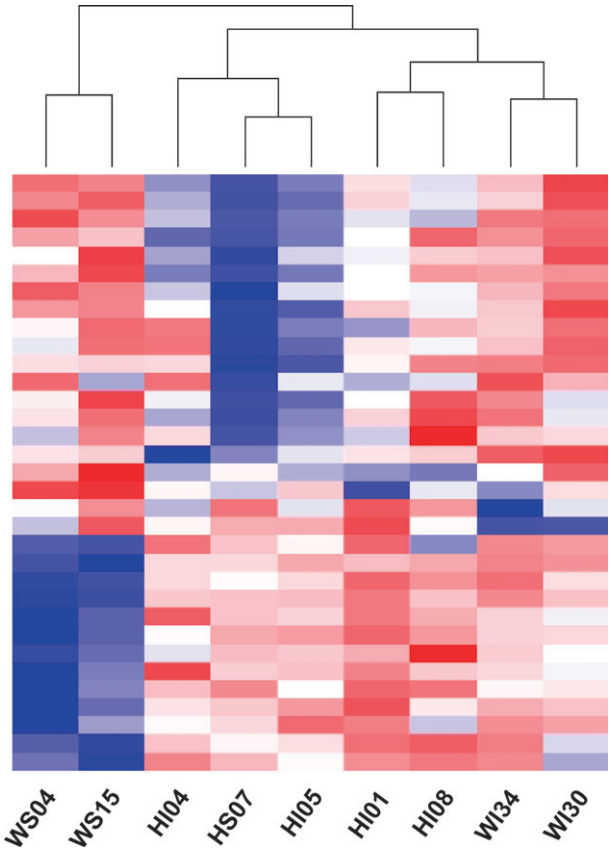

Figure 3. Hierarchical clustering of individual males based on gene expression profiles similarity. Distance (similarity) matrix based on Euclidian distance (see Methods).

Similarity of Gene Expression Profiles Among Individual Males

We used a clustering analysis to determine if gene expression profiles were more similar between individuals raised in the same environment regardless of tactic or between the same male tactics in different environments. It is notable that the environmental effect seems as important as the tactic adopted. Laboratory fish clustered together independent of tactic, while wild fish tended to cluster by tactic rather than together. This may be indicative of the homogenization of gene expression in fish raised in controlled (and more stable) conditions and of the wider variation between male tactics when faced with larger range or more realistic ecological factors. The way laboratory fish clustered together may also suggest that the numerous molecular and biochemical modules, which are integrated into a reproductive tactic, are triggered by various (environmental) cues. Thus, when only some cues are present, only certain pathways become activated in the brain.

Conclusion

There is a growing consensus that knowledge of the proximate mechanisms underlying organismal diversity is necessary for a thorough understanding of the evolution of complex phenotypes. The work on Atlantic salmon presented here highlights the extent of transcriptional plasticity in the face of environmental variation and the surprising insights that can be derived from a genomic dissection of phenotypic plasticity. We expect our results to be broadly applicable to other systems and to facilitate the integration of knowledge on molecular and cellular pathways with data on physiological, behavioral, and ecological processes toward an understanding of organismal plasticity in the natural environment.

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