Screening for Dmrt genes from embryo to mature Macrobrachium rosenbergii prawns

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A R T I C L E   I N F O

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A B S T R A C T

The doublesex and mab-3 related transcription factor (Dmrt) gene family is known to be related to the sexual regulators doublesex of arthropods and mab-3 of annelids and to hold highly conserved functions in sexual determination and differentiation across phyla. Here, we report a study of the Dmrt gene family in the freshwater prawn Macrobrachium rosenbergii, a crustacean whose sexual differentiation has been widely researched. A wide transcriptomic screen, from the embryo to the adult M. rosenbergii, identified five novel Dmrt genes (MroDmrts) and confirmed two known MroDmrt genes. The seven MroDmrts encode proteins of 275–855 amino acids; each protein contains at least one conserved DNA-binding DM domain, which is typical of Dmrt proteins, and five proteins contained 1–4 transactivation domains (TADs). Importantly, in the embryonic, larval and post-larval stages, MroDmrt genes exhibited time-dependent expression patterns rather than sex-specific expression. In-silico screening of the expression of the MroDmrt genes in adult males revealed the enrichment of MroDmrt1b and MroDmrt1c in the androgenic gland (AG) as compared to the eyestalks. In vivo silencing of the androgenic gland insulin-like (IAG) encoding gene significantly decreased the expression of the above two Dmrt genes, while not affecting the expression of control genes, thereby suggesting the possible role of these two genes in the IAG-switch and in sex-differentiation processes.

1. Introduction

The freshwater prawn Macrobrachium rosenbergii is one of the best investigated crustacean species, with studies ranging from its aquacultural importance (Nair et al., 2006; New, 2008); through its typical social structure (Karpus and Sagi, 2000; Kuris et al., 1987) and physiology (Chen et al., 2003; Manush et al., 2004; Zhu et al., 2018), to its sexual differentiation and reproductive biology on the protein (Okumura and Hara, 2004), transcriptomic (Jung et al., 2016; Sharabi et al., 2016; Shpak et al., 2017) and genomic levels. In the last regard, a high-quality M. rosenbergii genome was recently sequenced (Levy, personal communication), revealing sex-specific chromosomal regions. This prawn is thus an ideal species for the study of the important doublesex and mab-3 related transcription factor (Dmrt) gene family; these genes are related to the sexual regulators doublesex (dsx) of arthropods and male-abnormal-3 (mab-3) of annelids and hold highly conserved functions in sexual determination and differentiation across phyla (Roth et al., 2013; Veenstra, 2016).

The Dmrt gene family encodes putative transcription factors that contain a common zinc finger DNA binding motif known as the DM domain. The transcriptional activity of such a Dmrt protein is determined by its C terminus through its transactivation domain/s (TAD/s) (Ma et al., 2014; Mapp and Ansari, 2007). Some Dmrt proteins are known to be involved in sex determination and/or sex differentiation (Raymond et al., 1999) in insects (Miller et al., 2003), nematodes and vertebrates (Kopp, 2012), but functional information regarding other gene family members and other phyla is limited. For example, the dsx gene of the insect Drosophila melanogaster was the first family member to be discovered in the sex determination cascade (Hildreth, 1965). In the nematode Caenorhabditis elegans, it has been shown that the DM domain gene male abnormal-3 (mab-3) is required for male-specific development (Hodgkin, 2002). In vertebrates, orthologs of Dmrt genes, e.g., human Dmrt1, Dmrt2, and Dmrt3, have been identified and shown to be involved in sexual development (Raymond et al., 1999). However, it is only recently that scientists have begun to study Dmrt genes in crustaceans. The first crustacean DM-domain gene to be reported was the EsDmrt-like gene — identified in the Chinese mitten crab Eriocheir sinensis — which presents a testis-specific expression pattern (Zhang and Qiu, 2010). Other Dmrt genes were subsequently reported in a variety of crustacean species; e.g., in Daphnia magna the functional Dmrt gene,

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**DmaDsx1**, was found to exhibit a sexually dimorphic expression pattern and to be responsible for male-specific trait development (Kato et al., 2011). The first heterogametic sex-linked *Dmrt* gene, *Sv-iDMY*, to be discovered in an invertebrate species was found in the Eastern spiny lobster, *Sagmariasus verreauxi* (Chandler et al., 2017). In *M. rosenbergii*, two *Dmrt* genes, designated *MroDmrt11E* and *MroDmrt99B*, have been identified (Yu et al., 2014). It has been shown that *MroDmrt11E* RNAi induced a significant decrease of the transcript of the androgenic gland (AG) insulin-like hormone encoding gene (IAG), an important regulatory gene in the male determination mechanism of the prawn, while silencing of *MroDmrt99B* had no effect on genes related to sexual development (Yu et al., 2014).

The AG has long been recognized as an endocrine organ unique to male crustaceans (*Charniaux-Cotton, 1954*), playing a vital role in sexual differentiation, including the development of primary and secondary sexual characteristics (Khalaila et al., 2001; Sagi and Cohen, 1990; Sagi et al., 1990). In *M. rosenbergii*, ablation of the AG at an early stage of development results in sex reversal of male to female (Sagi et al., 1990). Conversely, AG implantation into immature females inhibits vitellogenesis and results in functional sex reversal of female to male (Levy et al., 2016; Malecha et al., 1992). It has been hypothesized that the IAG induces the activity of male differentiation genes and represses genes responsible for female differentiation. Indeed, silencing of *M. rosenbergii IAG* (*Mr-IAG*) at early post-larval stages caused a full and functional sex reversal of males to neo-females (Ventura et al., 2012); thus, this process was termed the IAG-switch (Levy et al., 2016) between maleness and femaleness. However, the causative relationship between the IAG-switch and members of the *Dmrt* gene family remains to be elucidated.

In this study on *M. rosenbergii*, we report the identification of novel *Dmrt* genes, termed *MroDmrt*, together with their characterization and in-silico temporal expression in male and female embryos, larvae, and post-larvae, and in the AGs and eyestalks of male adults. The study of the *MroDmrt* genes revealed enrichment of two *MroDmrt* genes in the AG and their possible role in IAG-switch.

**2. Materials and methods**

**2.1. Identification of putative DMRT family genes**

Genes encoding putative Dmrt proteins were mined from a large array of *M. rosenbergii* transcriptomic libraries (Sharabi et al., 2016), either by a key-word-based search or by using *Daphnia magna* DMRT protein sequences (accessions: KZ507196.1, BAG12873.1, BAG12872.1, BAG12871.1 and BAJ78309.1) as a query. The search yielded several sequences, which then served as queries in BLAST searches designed to reveal the similarity between their hypothetical sequences and the homologs in the NCBI database. The complete transcripts were obtained (for all genes excluding *MroDSX* due to its low level of expression) using rapid amplification of cDNA ends (RACE), performed with the Clontech SMART RACE kit (BD Biosciences, Palo Alto, CA), according to the manufacturer’s instructions, and validated by Sanger sequencing. To obtain the deduced protein sequences, full-length cDNA of *MroDmrt*s was computationally translated using the ExPaSy Proteomics Server (http://web.expasy.org/translate/), and the most likely (i.e., the longest) frame was selected. Conserved domains were identified in the putative MroDmrt proteins using the Simple Modular Architecture Research Tool (SMART) (Schultz et al., 1998). In addition, TAD prediction was performed using the Nine Amino Acids Transactivation Domain search Tool (SMART) (Schultz et al., 1998). To further characterize the above Dmrt proteins, a few homologous proteins from crustaceans, other arthropods and vertebrates were selected for phylogenetic analysis by ClustalW alignment (Larkin et al., 2007) using the default parameters. The proteins used to construct the tree are given in Table 1. The neighbor-joining phylogenetic analysis was conducted with MEGA 7 (Kumar et al., 2016) with default parameters. Nematode *CelMAb3* served as an outgroup.

**2.2. Embryo transcriptomic library**

All-female (Levy et al., 2016) and all-male (Ventura et al., 2012) *M. rosenbergii* progenies were produced as we have described previously. Small amounts of eggs containing developing embryos were sampled using forceps from egg berried females bearing either female or male embryos on days 1, 3, 5, 11 and 17 in 3 replicates per day (a total of 30 samples). Total RNA was extracted with the EZ-RNA Total RNA Isolation Kit (Biological Industries) according to the manufacturer’s instructions. RNA samples were sequenced using Illumina Technology, yielding paired-end 100-bp reads. Subsequent bioinformatic analyses were performed at the Bioinformatics Core Facility of The National Institute for Biotechnology in the Negev (NIBN), using the NextSeq Flow workflow platform (Sklarz et al., 2017) and R/Bioconductor. To create a comprehensive reference transcriptome, we examined previous RNA-Seq datasets (Table 2) produced by our group and our collaborators as well as publicly available datasets for inclusion in the transcriptome assembly. Reads from each of the samples of the current study were aligned to the reference transcriptome and quantified. Raw read counts (average of 13.2 ± 3.3 million read pairs per sample) were submitted to DESeq2. Counts were normalized using the Variance Stabilizing Transformation (VST). A BLAST search was performed using *MroDmrt* gene sequences as a query. To find the expression of *MroDmrt*s in the embryonic stages, the matched contigs were used for clustering according to their expression patterns in terms of sex and time.

**2.3. Spatiotemporal expression of Dmrt genes**

Sequence reads from male and female larvae, from male and female post larvae (PL) (Ventura et al., 2013) and from the AGs and eyestalks of three different male morphotypes – blue claw (BC), orange claw (OC) and small male (SM) (Sharabi et al., 2016) – were mapped to the *MroDmrt* reference sequences using the CLC Genomic Workbench7.3 (CLC Bio; default parameters). The numbers of *MroDmrt*-mapped reads per sample were normalized as described before (Sharabi et al., 2016). In addition, total RNA was extracted from the AGs, testes and ovaries using the EZ-RNA Total RNA Isolation Kit (Biological Industries, Beit Haemek, Israel) according to the manufacturer’s instructions. cDNA was synthesized in a reverse-transcribease reaction containing 1 μg of total RNA by using a qScript cDNA synthesis kit (Quanta Biosciences, Gaithersburg, MD, USA) according to the manufacturer’s instructions. Real time RT-PCR was conducted to measure *MroDmrt1a*, *MroDmrt1b*, *MroDmrt1c* and *MroDmrt11E* expression levels in ovary, testis and AG, using specific primers and probes from the Universal Probe Library (Roche) (Table 3). For normalization, *Mr-18S* (GenBank accession no.GQ131934) was used with specific primers as described in Ventura et al. (Ventura et al., 2012) and Universal Probe Library Probe 152 (Roche), with the SensiFAST Probe Hi-ROX Mix (BIOLINE). Reactions were performed with the ABI Prism 7300 Sequence Detection System (Applied Biosystems).

**2.4. Effects of Mr-IAG silencing on expression of Dmrt genes**

To further investigate possible involvement of *MroDmrt* genes in the *Mr-IAG*-switch pathway, the two *MroDmrt* genes that were found to be highly expressed in the AG (*MroDmrt1b* and *MroDmrt1c*) – compared to eyestalks – were further studied. The expression levels of those two genes were quantified by qPCR in prawns that had been injected with the entire *Mr-IAG* dsRNA (dsMr-IAG) open reading frame (ORF) (*n* = 8), as previously described by Shpak et al. (2017), and in a non-injected group (*n* = 7) as a negative control. In this experiment, small *M. rosenbergii* males (mean body weight of 11.6 g) were injected once with 5 μg of dsMr-IAG/g body weight, and then two days post-injection, the control and injected males were anesthetized in ice cold water and
dissected. Total RNA was extracted from the AGs and cDNA was synthesized as described above. To validate the silencing of Mr-IAG, its relative expression levels were measured as described in (Ventura et al., 2012) with the above-mentioned mix from BIOLINE and Universal Probe Library Probe Library Probe 144 (Roche). To quantify the relative expression of the genes designated MroiDmrt1b and MroiDmrt1c (see Section 3.1) in the above males, real time RT-PCR was done as described before. As controls, we tested the expression levels of MroiDmrt1d and Mr-actin to show the specific effects of Mr-IAG silencing, using specific primers and probes (Table 3).

3. Results

3.1. Identification of putative DMRT family genes

A search of a *M. rosenbergii* composite transcriptomic library (Sharabi et al., 2016) revealed seven transcripts putatively related to the *Dmrt* family of genes. The seven putative genes (dxx- and mab-3-related translation factors) showed high similarity in the DM domain to known *Dmrt* genes in the GenBank database. The seven *MroDmrt* genes are 825–2565 nucleotides long, with predicted ORFs encoding 275–855 amino acid (aa) translation products. Two of the above genes, Mr-IAG, respectively, were reported previously and termed, at that time, *Mr-actin* and *Mr-IAG* silencing experiment.

### Table 1

<table>
<thead>
<tr>
<th>Genus and species</th>
<th>Protein</th>
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<tr>
<td>M. rosenbergii</td>
<td>doublesex and mab-3-related translation factor 99B, AHI47025.1 and doublesex and mab-3-related translation factor 11E, AHI47024.1</td>
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<td>Aedes aegypti</td>
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<td>Apis mellifera</td>
<td>doublesex isoform M, NP_001104725.1</td>
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<td>Caenorhabditis elegans</td>
<td>protein male abnormal 3, NP_001102464</td>
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<td>Danio rerio</td>
<td>doublesex- and mab-3-related translation factor 1 isoform 1, NP.991191 2, doublesex- and mab-3-related translation factor 2, NP.571027 1, doublesex- and mab-3-related translation factor 3a, NP.00105779 2, doublesex- and mab-3-related translation factor 5, AUA58258 1</td>
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<td>Daphnia magna</td>
<td>doublesex-mab related 93B, BAG12872, doublesex-Mab related 98B, BAG12873 1, doublesex-mab related 11E, BAG12871, doublesex1-alpha BAJ78307 1, doublesex2 BAJ78309 1</td>
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<td>Drosophila melanogaster</td>
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<td>F. meerlethi</td>
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<td>Eriocheir sinensis</td>
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<td>Sagmariasus verreauxi</td>
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<td>Takifugu rubripes</td>
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### Table 2

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<th>Dataset name or SRA accession</th>
<th>Description</th>
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<tr>
<td>Current study and <em>M. rosenbergii</em> composite transcriptome</td>
<td>Embryos, larval and post larval (whole body), hepatopancreas, muscle, testis, eyestalk, claw, stomach and androgenic gland. Ben-Gurion University line <em>M. rosenbergii</em></td>
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<td>SRX092198</td>
<td>Gill, hepatopancreas and muscle tissues in <em>M. rosenbergii</em></td>
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<td>SRX097638</td>
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<td>Prawn antennal gland</td>
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### Table 3

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<th>Primer used for qPCR quantifying the expression levels of <em>MroDmrt</em> genes and <em>Mr-actin</em> in the Mr-IAG silencing experiment.</th>
<th>F primer</th>
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<th>Probe</th>
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<td>MroiDmrt1a</td>
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<td>MroiDmrt1LE</td>
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<td>Mr-actin</td>
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<td>gnnagctctgctgatccag</td>
<td>104</td>
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</table>
(Chandler et al., 2017), and were therefore named according to the degree of their similarity, MroiDmrt1a-d (accession numbers MK468649, MK468650, MK468651 and MK468652), respectively. The remaining gene was termed MroDSX (accession number MK468653) due to its high similarity to known decapod DSX genes (FcDSX and SveDSX) (Chandler et al., 2016; Li et al., 2018).

The cDNA and predicted protein sequences of the five newly described Dmrt genes are shown in the Supplementary material (Fig. S1a-e). Examination of the deduced amino acid sequences of all the putative Dmrt candidate genes revealed the conserved domains that characterize the Dmrt proteins reported to date. Specifically, each putative Dmrt included one or two DM domains. In addition, five of the predicted Dmrt proteins contained 1–4 TAD prediction domains (Fig. 1).

The phylogenetic analysis confirmed that MroDmrt proteins do indeed cluster with similar Dmrt proteins from other species and even from remote taxa (Fig. 2).

### 3.2. Embryonic expression patterns

The expression of MroDmrt reads was studied in males and females at five embryonic stages. MroDmrt genes did not show any sex-specific expression pattern, but some of the genes were found to exhibit time-dependent expression patterns along embryonic development. These patterns were found to be similar in males and females, as may be seen in Fig. 3 for four representative genes. The expression of those genes was low at early embryonic stages and started to rise in later stages. For example, MroiDmrt1b started to rise on day 3, MroiDmrt11E started to rise on day 5, whereas MroiDmrt99B and MroiDmrt1c started to rise on day 11.

### 3.3. Larval and post-larval expression patterns

To study gene expression patterns during early developmental stages, the numbers of DMRT-reads per million (RPKM) per sample were mined from transcriptomic libraries of male larvae, female larvae, male PLs, and female PLs (Ventura et al., 2013) (Fig. 4). MroiDmrt1c and MroiDmrt1d had high RPKMs in the larval stage compared to the other putative Dmrt genes. The reads of MroiDmrt1c and MroiDmrt1d were even higher in the PL stage, in both males and females. For MroiDmrt1c there were about 120 RPKMs in the larval stage, and more than twofold that number in PLs of both genders. MroiDmrt1d exhibited 60–90 RPKMs in the larval stage, and more than fourfold that number in PLs of both genders. The remaining five Dmrt genes exhibited basal to low RPKMs (between 0 and 26) in both larval and PL stages.

### 3.4. Adult expression patterns

In adult males, the expression levels of the above MroDMRT genes were studied in an AG transcriptomic library that included the three male morphotypes—BC, OC and SM (Fig. 5a). MroiDmrt1b and MroiDmrt1c were found to be highly expressed in the AG. To study the specificity of this phenomenon to the AG, the expression of MroiDmrt1b and MroiDmrt1c in AGs was compared to that in eyestalks for the three male morphotypes. MroiDmrt1b and MroiDmrt1c were found to be significantly specifically enriched in the AG (Fig. 5b-c). The results for the other five genes, which were not specifically enriched in the AG as compared to the eyestalk, are given in the Supplementary material (Fig. S2a, d-g). In addition, further comparisons of MroiDmrt1a, MroiDmrt1b,
MroDmrt1c and MroDmrt11E expression levels among ovary, testis and AG revealed different patterns (Fig. 6). MroDmrt1a was significantly different among the tissues with the highest expression in the AG and the lowest in the ovary. MroDmrt1b was highly expressed in the AG and testis and significantly lower in the ovary while contrary, MroDmrt1c was highly expressed in the ovary and significantly lower in the AG and testis. MroDmrt11E was significantly higher in the testis compared to the ovary.

3.5. Mr-IAG silencing effects Dmrt gene expression

The two MroDmrt genes that were found to be enriched in the AG were further studied with the aim to reveal a possible relationship with the IAG-switch pathway. Specific silencing of Mr-IAG through administration of dsMr-IAG showed a significant decrease in Mr-IAG expression (P < 0.05), with a silencing efficiency of 99.8% (Fig. 7a). The silencing of Mr-IAG significantly decreased the expression levels of MrroiDmrt1b and MrroiDmrt1c, i.e., by 95.4 and 71.9% in the IAG silenced group compared to the control, respectively (P < 0.05) (Fig. 7b&c). Mr-IAG silencing did not significantly affect the expression of the control genes, Mr-actin and MrroiDmrt1d (Fig. 7d&e).

4. Discussion

The identification and characterization of sex-specific genes are essential steps to providing insight into the molecular pathways of sex determination and differentiation in crustaceans. In this study, we...
undertook a developmentally wide screening for Dmrt gene family members of transcriptomic libraries from embryo to adult M. rosenbergii. Our screen confirmed the presence of two known Dmrt family members, MroDmrt11E and MroDmrt99B (Yu et al., 2014), and identified five additional novel Dmrt genes in the prawn. The new transcripts were named according to their phylogenetic homology to the known Dmrt proteins; MroDmrt1a, MroDmrt1b, MroDmrt1c, MroDmrt1d, and MroDSX, encoded by 1623, 876, 2421, 2564, and 825 bp mRNA, respectively. MroDmrt proteins show a high similarity to known Dmrt proteins in the conserved DM domain, which consists of cysteines and histidines, forming two zinc finger binding sites that are essential for DNA binding (Zhu et al., 2000). MroDmrt1a and MroDmrt1b are predicted to contain one TAD, and MroDmrt1c, MroDmrt1d, and MroDmrt11E are predicted to contain two, three and four TADs, respectively, where the TAD is a conserved domain in most known transcriptionally active Dmrets (Chandler et al., 2017), thus supporting a possible function of MroDmrt genes as transcription factors.

The Dmrt gene family has retained a conserved role in the sexual development of metazoans across evolution. For example, it is believed that the testis-specific Dmrt gene designated Edmrt-like plays an important role in the reproductive system, particularly in testicular development, in the crab E. sinensis, since RNAi silencing of Edmrt-like reduced testicular size and blocked spermatogenesis (Ma et al., 2016). Also important for male differentiation, the SviDMY gene, the first heterogametic sex-specific Dmrt to be identified in an invertebrate species (Chandler et al., 2017), was suggested to play a dominant role over Svi-dDMY. In the absence of Svi-dDMY, is Svi-dMrt1 predicted to promote female development (Chandler et al., 2017). In another crustacean, Daphnia magna, three Dmrt genes (DMRT11E, DMRT793B and DMRT99B) were expressed dimorphically, with DMRT11E and DMRT99B expression levels being higher in the ovary than in the testis, and DMRT793B being expressed only in the testis (Kato et al., 2008). Unlike the above-mentioned cases, the MroDmrt genes found in the present study did not show any sex-specific expression pattern in either male or female embryos, but they were found to exhibit time-dependent expression patterns along embryonic development. However, in mature prawns, four MroDmrt genes exhibited distinct sexual dimorphic expression pattern among the gonads and the AG. These patterns might shed light on the roles of MroDmrt in gonads development. MroDmrt1b was found to be expressed only in the testis and the AG and not in the ovary, whereas MroDmrt1a was high in the testis and the AG compared to the ovary, suggesting possible role of these DM genes in gonad development in males depending on gene expression level. This phenomenon was also demonstrated in birds, in which, DMRT1 is expressed higher in males than females, specifically in the gonads of chicken embryos, before to and during gonadal differentiation (Smith et al., 2009; Smith and Sinclair, 2004). MroDmrt11E was highly expressed in the testis and the AG compared to the ovary, confirming previous results (Yu et al., 2014). MroDmrt1c, unlike the above genes, was highly expressed in the ovary. However, it was expressed also in the testis and the AG, suggesting its possible role in both male and female gonadal development.

Even though no sex-specific expression pattern was found for MroDmrt genes in embryos, the silencing of a sex-related gene, the IAG, significantly reduced the expression of two genes, MroDmrt1b and MroDmrt1c, thereby suggesting their possible roles in the IAG-switch and the sex-differentiation mechanism. In light of the findings of a recent RNAi-targeting study (Yu et al., 2014), it should be stressed that these two genes have predicted TADs: in that study it was shown that RNAi-targeting of MroDmrt99B, which has no predicted TAD, had no effect on several male reproduction-related genes, while MroDmrt11E RNAi induced a significant decrease of the transcript of IAG. These findings suggest possible transcriptional activity of the TAD in MroDmrt11E. However, although previous studies have shown that Dmrt genes are regulating the IAG in some species (Li et al., 2018; Wang et al., 2019; Yu et al., 2014), in the present study we suggested that MroDmrt1b and MroDmrt1c are correlated with the Mr-IAG system but it is hard to conclude whether the relationship is up or downstream. Our efforts did not succeed in achieving loss of function of Dmrt genes through RNAi, and previous studies claiming successful loss of function are reporting conflicting results in which in M. rosenbergii (Yu et al., 2014) and Fenneropenaeus chinensis (Li et al., 2018) a reduction of expression of IAG was reported following Dmrt silencing while contrary to this study, in Macrobrachium nipponense (Wang et al., 2019) the opposite was shown. Thus, we cannot offer a clear conclusion regarding the regulatory role of Dmrt genes with respect to IAG expression.

Previous studies have indicated that Dmrt genes also belong to a family of important developmental regulators, providing evidence that this gene family has evolved functionally in relation to developmental pathways that are distinct from of sex-determination or sex-differentiation pathways (Hong et al., 2007). One example of such a non-sex related gene is terra, which encodes a putative zinc-finger (DM domain) protein in the zebrafish and is specifically expressed in the prismatic mesoderm and developing somite. It has been proposed that terra is a conserved somite-specific factor that mediates very early events of vertebrate somitogenesis (Meng et al., 1999). Many Dmrt genes that are expressed in the developing gonad appear to share similar functional characteristics. However, in non-gonadal tissues, Dmrt factors appear to have the ability to regulate a broad range of developmental processes (Hong et al., 2007), to which only limited attention has been paid to date. The time-dependent expression pattern of MroDmrt genes along the embryo, larval, post-larval and adult stages might suggest possible involvement of these proteins in somitogenesis rather than in reproductive development.

In light of our findings, it may be suggested that Dmrt genes play regulatory roles in both sexual and somatic development: On the one hand, in M. rosenbergii the expression pattern of Dmrt genes was similar in male and female embryos, while on the other hand, some of the genes show different expression patterns among gonads in mature males and females. In addition, silencing of IAG, a pivotal gene in sexual development, caused a significant decrease in the expression of two Dmrt genes. Further studies are thus needed to determine the precise function of Dmrt genes in the developing reproductive system and other tissues.

![Fig. 6. Relative quantification of MroDmrt1a, MroDmrt1b, MroDmrt1c and MroDmrt11E in the gonads and in the AG from five females and six males. Different letters on the bars indicate significant differences (P < 0.05; post hoc Tukey’s test) after natural log transformation of the data as to fit proper statistical analysis.](image-url)
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.06.009.

References


Fig. 7. Levels of transcripts in the androgenic gland following in vivo Mr-IAG dsRNA injection into adult male prawns. Levels of (a) Mr-IAG transcripts; (b&c) MroiDmrt1b and MroiDmrt1c transcripts, respectively, and (d&e) control genes, Mroidmrt1d and Mr-actin, respectively. Asterisk indicates significant difference (P < 0.05; Mann Whitney U test).