

# A Review of Sexual Determination and Differentiation in Crustacean

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## Abstract

The prerequisite for monosex culture is to elucidate the molecular mechanism of sex determination and differentiation in crustaceans, as well as to explore the key genes that play a role in this process. Monosex culture technology based on crustacean economic species is of great importance in terms of genetic breeding and economic benefits of aquaculture. As a result, study into the mechanisms of sex determination and differentiation in crustaceans not only contributes to the current absence of basic theories of crustacean sexual mechanism, but also encourages technical innovation in aquaculture to increase overall economic efficiency. This study synthesizes and evaluates available research on sex determination and differentiation in crustaceans, and then provides recommendations for future research objectives and priorities in the field.

## Keywords

Crustacean, Sex Determination, Sex Differentiation

## 1. Introduction

Crustaceans are members of the arthropoda phylum, which includes a diverse range of species, many of which, such as shrimp and crabs, are vital for farming around the world and provide significant economic benefits [1] [2]. Individuals of both sexes undergo mating and reproduction processes in the common culture process, consuming large amounts of energy and affecting the growth rate [3]. Monosex culture based on crustacean sex regulation technology is therefore of great importance to improve the benefits of farming economic species in crustaceans. However, because elucidating the molecular mechanisms of sex determination and sex differentiation in crustaceans, as well as identifying the key genes involved in this process, is a precondition for monosex culture, sex-related

studies in crustaceans have attracted a lot of attention. As a result, this study summarizes available research on sex determination and differentiation in crustaceans with the objectives of providing a reference and theoretical basis for monosex culture in each crustacean species.

## 2. Sexual Determination and Differentiation in Crustacean

Animals form their sexes mainly through two processes: sex determination and sex differentiation [4]. There are two types of sex determination: genetical determination, in which the organism's genomic chromosomes determine sex, and environmental determination, in which the organism's sex is determined by environmental circumstances [5]. After sex determination, a process is termed sex differentiation occurs in which the sex traits of an embryo or larva, such as the reproductive system, move from absent to present, resulting in the acquisition of female or male sexual characteristics [4]. As described in this study, there are two types of sex determination in crustaceans: genetical determination and environmental determination.

### 2.1. Genetical Determination

In comparison to highly advanced vertebrates, crustaceans' sex determination system is rudimentary and diversified. By counting the male to female ratio of the offspring of *Penaeus monodon* after hybridization as well as triploid induction culture, Benzie *et al.* presumed that the sex determination type might be ZW/ZZ type [6]. The sex-determining type of *Cargon cataphrastus* was also presumed by the above method to be ZW/ZZ type [7]. In addition, Malecha *et al.* inferred the ZW/ZZ type of *Macrobrachium rosenbergii* from the female to male ratio of the crossed offspring of sex-reversed individuals [8].

As bioinformatics and molecular biology have developed, bioinformatics tools such as amplified fragment length polymorphism (AFLP) and genetic linkage map construction have become incredibly common in the identification of sex determination processes in crustaceans. Li *et al.* proposed that the *Marsupenaeus japonicus* chromosomal genotype would be ZW/ZZ type by using AFLP markers in a two-way pseudo-testcross strategy to construct genetic maps of this specie [9]. Zhang *et al.* Constructed genetic linkage map using AFLP and microsatellite markers, and the results suggest that the chromosomal genome of *Litopenaeus vannamei* may be of ZW/ZZ type [10]. Yu *et al.* then used genetic linkage mapping based on high-density linkage mapping to further localize sex-determining regions on chromosomes of *L. vannamei*, identifying one marker associated with females that showed female heterozygosity, bolstering the hypothesis of its ZW/ZZ sex-determining type [11]. Robinson *et al.* used the same method to identify three SNP loci significantly associated with sex in *P. monodon*, and the feminization gene *Fem-1* was also found at this location, implying that these three SNP loci are the female sex-linked loci transmitted from maternal to offspring females, further supporting its ZW/ZZ-type sex determination me-

chanism [12]. In 2015, Cui *et al.* localized sex-determining loci in the genetic map through the construction of genetic map of *Eriocheir sinensis* and consequently identified SNP loci closely linked to females and genetic linkage clusters corresponding to W and Z chromosomes, indicating that its chromosomal genome is of ZW/ZZ type of sex determination [7]. The summary of genetical determination typies mentioned in this section are shown in **Table 1**.

## 2.2. Environmental Determination

Crustaceans have a primitive sex determination system compared to vertebrates. Environmental factors like as temperature, season, light, and salinity, for example, have been demonstrated to affect the sex determination and differentiation process of several crustaceans [13].

After clutching eggs, the embryos of female *Fenneropenaeus chinensis* can produce different sex ratios of offspring males and females, depending on temperature [14]. The parent shrimp of *Gammarus duebeni* produced significantly different sex ratios of offspring groups when living in different water temperatures [15]. When the quality of the surrounding environment deteriorates in *Daphnia magna*, the response to the environment switches from sexual reproduction to parthenogenesis and the production of male individuals, followed by the regulation of male gonadal development by the sex-regulatory key gene Doublesex to ensure population survival [16].

Light and salinity factors in the crustacean culture environment can affect the sex determination and differentiation process. The ration of *G. duebeni* male-to-female can be affected by light [17], while variations in salinity can impact the gonadal development cycle of *E. sinensis* [18]. The summary of environmental determination typies mentioned in this section are shown in **Table 2**.

## 3. Sex Regulation in Crustacean

### 3.1. Sex-Regulated Organs

The androgenic gland (AG) is a male crustacean endocrine organ that regulates male sexual development and secondary sexual characteristics by the secretion of insulin-like androgenic hormone (*IAG*) [19]. In 1947, Crosin *et al.* first utilized

**Table 1.** Genetical determination typies in crustacean.

Species	Genomic sex chromosomes	References
<i>P. monodon</i>	might be ZW/ZZ	[6]
<i>C. cataphrastus</i>	might be ZW/ZZ	[7]
<i>M. rosenbergii</i>	might be ZW/ZZ	[8]
<i>M. japonicus</i>	might be ZW/ZZ	[9]
<i>L. vannamei</i>	ZW/ZZ	[10] [11]
<i>P. monodon</i>	ZW/ZZ	[12]
<i>E. sinensis</i>	ZW/ZZ	[7]

**Table 2.** Effects of environmental factors on sex differentiation of crustaceans.

Species	Environmental factors	Roles	References
<i>F. chinensis</i>	Temperature	Different Sex ratios of male and female embryos	[14]
<i>G. duebeni</i>	Temperature	Sex ratios of offspring were different	[15]
<i>D. magna</i>	Light	Ration of male-to-female	[17]
	Quality of the surrounding environment	Different Reproductive model	[16]
<i>E. sinensis</i>	Salinity	Gonadal development cycle	[16]

histological sections to identify the androgenic gland as a paragland wrapped around the lateral side of the vas deferens in the reproductive system of *Callinectes sapidus* [20]. Subsequently, Taketomi Y *et al.* injected male androgenic gland extracts into male *Procambarus clarkii* in 1990, resulting in rapid development of external male sexual features [21]. Thereafter, Sagi *et al.* removed the male androgenic gonads of *M. rosenbergii* at the stage of sex differentiation to obtain sex-reversed individuals with male to female reversal whose external sexual characteristics showed female, and then mated the sex-reversed individuals with normal males to successfully obtain all-male offspring [22]. Meanwhile, by transplanting male androgenic gonads into sexually differentiated females of *M. rosenbergii*, Rungsin *et al.* obtained reproductively competent neomales [23]. Studies in other crustaceans have shown that androgenic gland adenotomy and transplantation will lead to feminization of males and androgenesis of females, respectively, and that male reproductive pathways, such as spermatogenesis, were observed to be significantly inhibited after androgenic adenotomy in some species [19] [24] [25] [26] [27] [28]. In conclusion, the removal or transplantation of androgenic gland during the early development of isopods and decapods may result in the production of sex-reversed individuals, and androgenic gland removal may cause spermatogenic pathway blockage in males, implying that androgenic gland play an important role in sex differentiation and reproductive function in crustaceans.

In decapod crustaceans, the X-organ complex of the eyestalk secretes a number of neurohormones that regulate various physiological processes, including reproduction, such as molt-inhibiting hormone (*MIH*), gonadal-inhibiting hormone (*GIH*), and crustacean hyperglycemic hormone (*CHH*) [29]. The endocrine pathway “eyestalk-androgenic gland-testis” [30] regulates sexually mature individuals in *M. rosenbergii*, in which the X-organ complex of the eyestalk negatively regulates the development and maturation of the gonad, and its released *CHH* interacts with the *IAG* secreted by the gonad. Therefore, the removal of the eyestalk in crustaceans promotes the growth and development of androgenic gonad cells, which in turn promotes the expression of *IAG* to further promote the development and maturation of the individual reproductive system

[31] [32]. Up to now, the method of accelerating crustacean sex maturation by eyestalk removal and thus cultivating parent shrimp for efficient offspring reproduction has been widely used in the industrial culture of some crustaceans and has further improved the overall economic efficiency of aquaculture.

### 3.2. Crustacean Sex Determination and Differentiation Genes

The elucidation of the molecular mechanisms of sex determination and differentiation in crustaceans, as well as the discovery of key genes that play a role in this process, are prerequisites for achieving monosex culture, and the androgenic gland hormones secreted by the crustacean androgenic gland are currently the most important key regulators of sex differentiation in crustaceans [28]; in addition to this, with the development of molecular biology techniques and high-throughput sequencing technologies nowadays, a series of homologous genes based on model organism sex determination pathways such as *Dmrt*, *Sxl*, *Tra-2*, *Fem-1*, *etc.* have been identified in crustaceans, and this section will review the information on the studies that have been conducted on these genes. The summaries of sex-related genes mentioned in this section are shown in **Table 3**.

**Table 3.** Sex-related genes and their roles in crustacean.

Gene	Species	Roles	References
IAG	<i>C. quadricarinatus</i>	The key regulator of sexual differentiation in male individuals	[33]
	<i>M. rosenbergii</i>		[34] [35]
	<i>S. paramamosain</i>		[36]
	<i>C. sapidus</i>		[37]
	<i>P. pelagicus</i>		[38]
	<i>F. chinensis</i>		[39]
	<i>L. vannamei</i>		[40]
	<i>M. japonicus</i>		[41]
	<i>P. monodon</i>		[42]
	<i>M. nipponense</i>		[43]
	<i>P. virginalis</i>		[44]
	<i>A. vulgare</i>		[45]
Sxl	<i>E. sinensis</i>	Nonsex-specific expression of genes	[47]
	<i>L. vannamei</i>	Involved in the process of spermatogenesis	[48]
	<i>C. quadricarinatus</i>	Male gonad biased expression	[49]
	<i>P. clarkii</i>	Its change was not significant after IAG RNA interference	[50]
	<i>S. verreauxi</i>	Has been identified	[51]
Tra	<i>P. monodon</i>	Developmental and gonad biased expression	[53]
	<i>F. chinensis</i>	Expression levels differ significantly during embryonic development	[54]
	<i>E. sinensis</i>	Nonsex-specific expression of genes	[55]
	<i>M. nipponense</i>	Developmental expression	[56] [57]
	<i>S. verreauxi</i>	Has been identified	[51]
	<i>P. serratus</i>	Has been identified	[58]

## Continued

	<i>M. nipponense</i>	Highly express in both ovaries and spermatocytes of mature males and females and developmental expression	[62]
	<i>S. verreauxi</i>	Male-biased expression patterns	[51]
	<i>P. serratus</i>	Mainly express in testis	[58]
<i>Dmrt</i>	<i>M. rosenbergii</i>	Expressed mainly in the testis and the expression level correlates with IAG	[59] [63] [64]
	<i>E. sinensis</i>	Express only in the testis and was highly expressed in the immature stage	[65] [67]
	<i>S. paramamosain</i>	Mainly express in testis	[66]
	<i>F. chinensis</i>	Sex-biased expression pattern in various tissues	[68]
	<i>L. vannamei</i>	Higher expression in the testis than in the ovaries	[69]
	<i>Daphnia magna</i>	Sex-biased expression	[70]
		<i>P. serratus</i>	Involved in gonad development
	<i>E. sinensis</i>	Might have a potential role in the final stages of gonad development	[73]
<i>Fem-1</i>	<i>L. vannamei</i>	Higher expression in the testis than in the ovaries	[74]
	<i>M. nipponense</i>	Highly expressed in Ovary and involved in oogenesis/vitellogenesis	[75]
	<i>S. paramamosain</i>	Identical expression in male and female gonads	[66]
	<i>P. monodon</i>	Located within the sex-determining locus region	[12]
<i>Masc</i>	<i>Artemia</i>	Involving in sex differentiation	[76]
	<i>P. serratus</i>	Mainly express in testis	[58]
<i>Sox</i>	<i>L. vannamei</i>	Higher expression in the testis than in the ovaries	[69]
	<i>M. nipponense</i>	Involved in gonadal development	[77]
	<i>S. Paramamosain</i>	Involved in gonadal development	[78] [79]
<i>BMP7</i>	<i>S. Paramamosain</i>	Involved in ovarian development	[80]
	<i>E. sinensis</i>	Involved in spermatogenesis	[81]
	<i>C. quadricarinatus</i>	Have a crucial role in developing adult female phenotypes	[82]
<i>CFSH</i>	<i>S. verreauxi</i>		[83]
	<i>C. antarcticus</i>		[84]
	<i>C. sapidus</i>		[85]
	<i>M. japonicus</i>		[86]
	<i>C. maenas</i>		[85]
<i>UCHLs</i>	<i>S. paramamosain</i>	Higher expression in the ovaries than in the testis	[87]
<i>EGFR</i>	<i>S. paramamosain</i>	Regulates the formation of male secondary sexual characteristics	[87]
<i>Sry</i>	<i>E. sinensis</i>	Potentially involved in maintaining the testis development and spermatogenesis	[88]
<i>Vasa</i>	<i>S. paramamosain</i>	Gonad-specific expression	[95]
	<i>M. rosenbergii</i>	Germ cell specific expression	[93]
<i>Vg</i>	<i>S. paramamosain</i>	Important role in yolk protein synthesis and ovarian development in female individuals	[90] [91]
<i>EcR</i>	<i>S. paramamosain</i>	A Possible Role in Promoting Ovarian Development	[89]
	<i>L. vannamei</i>	Might be related to gonadal development	[94]
<i>Ftz-1</i>	<i>E. sinensis</i>	Interact with <i>Foxl2</i> and involved in ovarian development cycle	[88] [92]
<i>Foxl2</i>	<i>E. sinensis</i>	Interact with <i>Ftz-1</i> and involved in ovarian development cycle	[88] [92]

### 3.2.1. Insulin-Like Adrogenic Gland Hormone (*IAG*)

The androgenic gland, a unique endocrine organ identified only in male crustaceans, secretes the insulin-like adrogenic gland hormone (*IAG*), which is involved in the regulation of sexual differentiation and secondary sexual characteristics [19]. In 2007, Manor *et al.* identified the first *IAG* gene, *Cq-IAG*, in a cDNA library of the androgenic gland of *Cherax quadricarinatus*, whose tissue expression and in situ hybridization (ISH) results indicated that the gene was specifically expressed in the terminal ampulla [33]. In 2009, Tomer Ventura *et al.* identified the *Mro-IAG* in a *M. rosenbergii* cDNA library and further RNA interfered with the *Mro-IAG* in mature male individuals, identifying that the regeneration of male external sexual characteristics was inhibited in interfered individuals; silenced individuals showed an atypical male growth pattern and a reproductive phenotype with stagnant spermatogenesis and spermatophore development, and an overall hypertrophic and hyperplastic gonad [34]. In 2012, Tomer Ventura *et al.* obtained *M. rosenbergii* neofemales with male reversal to females by RNA interfering with the *Mro-IAG* of male individuals in incomplete sexual differentiation, in which the appearance of secondary sexual characteristics was delayed and reduced in the interfered individuals, and the neofemales were then mated with normal males to successfully acquire all-male offspring [35]. In conclusion, *IAG* is not only involved in sex regulation, sex and gonadal development, but also in spermatogenesis in reproduction. Up to now, *IAGs* have been identified in decapod *Scylla paramamosain* [36], *C. sapidus* [37], *Portunus pelagicus* [38], *F. chinensis* [39], *L. vannamei* [40], *M. japonicus* [41], *P. monodon* [42], *M. rosenbergii* [34], *Macrobrachium nipponense* [43], and *C. quadricarinatus* [33], *Procambarus virginalis* [44], and the isopod *Armadillidium vulgare* [45].

### 3.2.2. Sex-Lethel (*Sxl*)

Sex-lethel (*Sxl*) is a key component of the sex determination cascade in *Drosophila*, which produces various spliceosomes in response to sex chromosomal signals and regulates sex-specific shearing of downstream *Tra*, *Dsx* [46]. *EsSxl* was found to be expressed in both male and female tissues of *E. sinensis*, and its embryonic expression was identified to be insignificant from the blastula stage to the egg-nauplius stage, but it increased significantly from the nauplius stage to the original zoea stage, suggesting that *EsSxl* may play a role in embryonic development [47]. *Sxl* tissue expression was similar to *EsSxl* in *L. vannamei*. All six splice variants of *PvanSxl* were expressed in fertilized eggs during embryonic development, but *PvanSxl2* and *PvanSxl4* were expressed at the highest levels up to blastocoele; *PvanSxl3* expression increased in the gastrula stage, and *PvanSxl1*, *PvanSxl3*, *PvanSxl6* expression decreased in the mysis stage. Furthermore, *PvanSxl* ISH results revealed that it was involved in both male and female gametogenesis and gonadal development [48]. Among the four splice variants of *Sxl* identified and obtained in *C. quadricarinatus*, *CqSxl3* is tissue-specific, with higher expression in the testis than in the ovaries; *CqSxl1* and *CqSxl4* are widely

expressed in a variety of tissues, and *CqSxl2* is less expressed in the shrimp, while transcript levels of *CqSxl1/3/4* slowly increase with embryonic development [49]. Up to now, in addition to the *Sxl* homologs identified in *E. sinensis* [47], *L. vannamei* [48], and *C. quadricarinatus* [49], *Sxl* homologs have been identified in *P. clarkii* [50], *Sagmariasus verreauxi* [51]. In decapods, it has been reported that *Sxl* is not sex-specific and that it is involved in sex differentiation, embryonic development, and gonadal development.

### 3.2.3. Transform-2 (*Tra-2*)

In *Drosophila*, Transform-2 (*Tra-2*) is involved in the sex determination cascade, where it controls sex-specific splicing of *Dsx* pre-mRNA production, as well as sex differentiation and development [52]. Leelatanawit *et al.* identified the *PmTra-2* sequence in crustaceans by constructing a cDNA library of *P. monodon* testis, but tissue expression revealed that *PmTra-2* was insignificant expressed in ovaries and testis and was a nonsex-specific gene [53]. In *F. chinensis*, *Tra-2c* has three spliced mRNA transcripts, *FcTra-2a*, *FcTra-2b* and *FcTra-2c*, and the sequence analysis revealed that the three splice variants are encoded by the same genomic locus and are generated by alternative splicing of the pre-mRNA [54]. Among which, *FcTra-2a* and *FcTra-2b* were not significantly differentially expressed in the male and female gonads of the shrimp while the expression of *FcTra-2c* was significantly higher in the ovaries than in the testis [54]. In addition, the expression of *FcTra-2c* was detected to be significantly increased at the mysis stage during the early stages of shrimp embryonic development, thus *FcTra-2* may be involved in the sex determination of females [54]. Luo *et al.* identified four *Tra-2* splice variants from *E. sinensis*, termed *Estra-2a*, *Estra-2b*, *Estra-2* and *Estra-2d*, and the sequence analysis revealed that the four splice variants are encoded by the same genomic locus and are generated by alternative splicing of the pre-mRNA [55]. In addition, four splice variants of *Estra-2* were all highly expressed in the fertilized egg, and in the 2 - 4 cell and blastula stages compared with larval stages, suggesting their maternal origin in early embryonic developmental stages, and tissue expression showed that *Estra-2a* was expressed at higher levels in male somatic tissues than in other shrimp tissues; *Estra-2b* and *Estra-2d* were both showed small sex differences in gonads; *Estra-2c* was expressed at higher levels in the ovary, suggesting that *Estra-2c* is involved in sexual differentiation of the crab [55]. In addition, *Tra-2* plays a role in the sex differentiation and embryonic development of *M. nipponense* [56] [57]. In summary, in crustaceans, *Tra-2* is associated with sex determination and differentiation, and embryonic development processes. Up to now, *Tra-2* genes have been identified and obtained in *P. monodon* [53], *F. chinensis* [54], *M. nipponense* [56], *E. sinensis* [55], *S. verreauxi* [51], and *Palaemon serratus* [58].

### 3.2.4. Doublesex and Mab-3 Related Transcription (*Dmrt*)

The *Dmrt* family is involved in sex determination and sexual differentiation in insects, nematodes, and vertebrates [59], and Doublesex (*Dsx*), a member of the



*Dmrt* family, is also a member of the bottom of the sex determination cascade in *Drosophila*, where sex-specific splicing produced by regulation of *Tra* and *Tra-2* determines female and male differentiation, respectively [60]. It has been reported that the *Dmrt* family has a highly conserved structure and function in sex determination and differentiation [59] [61].

Up to now, *Dmrt* have been identified in *M. nipponense* [62], *S. verreauxi* [51], *P. serratus* [58], *M. rosenbergii* [59] [63] [64], *E. sinensis* [65], *S. paramosain* [66]. In *E. sinensis*, *EsDmrt-like* was detected only in the testis and was highly expressed in the immature stage, which is similar to the vertebrate *DMRT1* gene expression pattern, and *EsDmrt-like* mRNA was localized in the supporting cells around the germinal tubules and developing germ cells such as spermatogonia, spermatocytes, and spermatocytes and not expressed in spermatozoa, the above results suggest an important role of *EsDmrt-like* in the development/differentiation of the male crab testis [65], as evidenced by further *EsDmrt-like* RNA interference results [67]. The *MniDmrt11E* identified in *M. nipponense* was highly expressed in both ovaries and spermatocytes of mature males and females, and was mainly localized in ovarian oocytes and spermatocytes of spermatocytes; during embryonic development, the expression level of *MniDMRT11E* was higher at the oogenesis stage than at other stages; at different stages of ovarian development, the expression of *MniDMRT11E* gradually increased from stage I to III and decreased to the lowest at the end of stage IV; after further RNA interference, the expression of *VG* significantly decreased and the expression of *IAG* significantly increased in mature individuals, the above results indicated that *MniDMRT11E* is associated with embryonic development, sexual maturation and gonadal development [62]. Furthermore, RNA silencing *MroDmrt11E* in *M. rosenbergii* resulted in a significant decrease in *IAG* expression, suggesting that *IAG* is positively regulated by *MroDmrt11E*; additional research has shown putative *Dmrt* binding sites in the promoter region of the male sex differentiation effector gene *IAG* [64].

Doublesex (*Dsx*) have been identified in *M. rosenbergii* [59], *F. chinensis* [68], *L. vannamei* [69], and *Daphnia magna* [70]. Sex-biased expression of *Dsx* was associated to male sex development in *Daphnia magna*, indicating that *Dsx* may have a conservation role in sex determination in crustacean species [70]. *LvDsx* expression was reported to be higher in the testis than in the ovaries in *L. vannamei* [69]. The ablation of the eyestalk in *M. rosenbergii* significantly increased *Dsx* expression levels in the testis and adrogenic gland [59]. In *F. chinensis*, *FcDsx* showed a sex-biased expression pattern in various tissues, and its expression level increased with developmental stage. One possible *Dsx* binding site was identified in the *FcIAG* promoter region, and knocking down *FcDsx* reduced *FcIAG* expression, suggesting that *FcDsx* may be an upstream regulator of *FcIAG* [68].

In summary, *Dmrt* is an important gene in crustacean sex differentiation, as well as gonad and embryonic development. Second, gene structure and func-

tional studies have shown that the crustacean *Dmrt* interacts with *IAG*, which is an essential gene for sex regulation in crustaceans. However, there are yet insufficiently defined mechanisms of action to investigate the regulatory pathways of this cascade, necessitating further experimental investigation.

### 3.2.5. Feminization-1 (*Fem-1*)

The feminization-1 (*Fem-1*) gene is characterized by one of the most common protein-protein interaction motifs, ankyrin repeat motifs, displays many expression patterns in vertebrates and invertebrates, and plays an essential role in the sex-determination/differentiation pathway in *Caenorhabditis elegans* [71]. In the model species *C. elegans*, a system analogous to the *Drosophila* sex regulatory cascade occurs, in which *Fem-1*, together with *Fem-2* and *Fem-3*, plays a crucial part in the sex determination cascade by downregulating *Tra-1* expression in nematodes [71] [72]. Up to now, *Fem-1* has been identified in *P. serratus* [58], *E. sinensis* [73], *L. vannamei* [74], *M. nipponense* [75], *S. paramamosain* [66], *P. monodon* [12]. In *M. nipponense*, *Mnfem-1* is highly expressed in both unfertilized eggs and cleavage stage and thereafter dropped to a low level from blastula to zoea during embryogenesis, indicating that the *Mnfem-1* in early embryos is maternally inherited [75]. In *L. vannamei*, natural antisense transcript regulation leads to the expression of *Fem-1* in the female gonads, resulting in the expression of *Fem-1* in the female gonads, thereby allowing sexual differentiation in female individuals, and in addition, *LvFem-1* expression in spermatogonia suggests that it is also required for male gonad differentiation [74]. Tissue expression pattern of *Fem-1* in *E. sinensis* revealed persistently high levels of *Fem-1* expression in the spermatophore, ovary, hepatopancreas and muscle suggesting its potential role in the final stages of gonad development [73]. Based on the high expression of *Fem-1* in early embryonic development of crustaceans, Song *et al.* and Ma *et al.* suggested that *Fem-1* may be maternally inherited in decapods [73] [75]. In summary, the *Fem-1* gene may be involved in the process of sex and gonadal differentiation in crustaceans, but the exact process and mechanism of its role need further experimental investigation.

### 3.2.6. Other Sex Determination and Differentiation-Related Genes

In recent years with the high throughput sequencing technology and the construction of cDNA libraries in crustaceans, many other sex-related genes have been identified in addition to the homologous genes found in crustaceans based on the inclusion of model organism sex regulatory pathways. In the crustacean *Artemia*, silencing of *ArMasc* by RNA interference resulted in a significant upregulation of its male-to-female ratio, suggesting that *ArMasc* is involved in the sex determination process [76]; the *Sox* family is involved in male gonad development and reproduction in crustaceans [58] [69] [77] [78] [79]; *BMP7* is involved in the gonad development process [80] [81]; *CFSH* regulates part of decapod sex development [82] [83] [84] [85] [86]; ubiquitin C-terminal hydrolases (*UCHLs*) regulate ovarian development as well as *EGFR* regulates the for-

mation of male secondary sexual characteristics [87]. In addition, with the development of sequencing technology and molecular biology, more sex-related genes have been successfully mined, such as *Vasa*, *Foxl2*, Vitellogenin (*Vg*), Ecdysone Receptor (*EcR*), *Sry*, *Ftz-1*, etc. [88]-[95], however, there is still a lack of research on the functional mechanisms of genes in sex determination, differentiation and gonadal development.

#### 4. Conclusions

Many species of crustaceans have a promising future in aquaculture, and with the development of science and economy, academic problems in the field of aquaculture have attracted much attention with the rapid development of aquaculture industry in China, among which monosex culture has attracted much attention because of its advantages in immunization and farming practice. The prerequisite for monosex culture is to elucidate the molecular mechanisms of sex determination and differentiation in crustaceans and to identify the key genes that play a role in this process. However, up to now, only Sagi *et al.* have successfully achieved monosex reproduction in *M. rosenbergii* and disclosed the technique of preparing all-male shrimp, and no research has been done to achieve sex reversal in other economic species such as *L. vannamei*, therefore needs to refine the existing sex theory and to elucidate the patterns of sex determination and differentiation in crustacean species as a whole through more researches [19] [28].

To date, the mechanisms of sex determination and differentiation have been initially elucidated in model organisms such as *Bombyx mori* and *C. elegans*, while studies on sex determination in crustaceans are relatively backward, most notably: The genome sequences of most crustacean species have not yet been elucidated, and therefore the bioinformatic function prediction around gene sequences is difficult and the prediction accuracy is low. On the other hand, it needs to elucidate the genome sequence of each crustacean species through bioinformatics to facilitate the mining of key genes for sex determination and differentiation.

#### Conflicts of Interest

The author declares no conflict of interest.

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