17

Sex Steroids in Insects and the Role of the Endosymbiont *Wolbachia*: A New Perspective

Ilaria Negri[1] and Marco Pellecchia[2]
[1]*DIVAPRA - University of Turin*
[2]*Koiné - Consulenze Ambientali S.n.c. Parma*
Italy

1. Introduction

Sex steroids play a pivotal role in sex differentiation and sex reversal in several species of vertebrates, both with genotypic and environmental sex determination systems (Nakamura, 2010; Norris & Carr, 2006). Steroidal sex hormones can be found naturally in both sexes of vertebrates, although the proportions of hormones may differ between males and females. Feminization of males or masculinization of females can be induced by altering the levels of 'female' and 'male' hormones, respectively. Estrogens for example have a feminizing effect on gonadal differentiation in many species of fish, amphibians, reptiles, and birds (Guiguen et al., 2010; Nakamura, 2009, 2010). In humans, androgen receptor defect disorder may lead to gonadal feminization and, in its complete form, the syndrome causes sex reversal of genotypical (XY) males and a female phenotype (Oakes et al., 2008).

Vertebrate-like sex steroids occur in several groups of invertebrates including nematodes, arthropods, echinoderms, but full information on the precise action and function of sex steroids is still missing (Janer & Porte, 2007). Some intriguing data have been provided in mollusks, where an involvement of steroids in gender determination and sexual differentiation of the brain, and even in a "superfeminization syndrome", has been demonstrated (Oehlmann et al., 2006; Wang & Croll, 2004).

In insects the existence of sex hormones is under debate. Indeed sex differentiation is generally thought to be a strictly genetic process, in which each cell decides its own sexual fate autonomously, based on its sex chromosome constitution. Therefore, differentiation of primary and secondary sexual characteristics should be exclusively under the control of the genotype of each single cell (Schütt & Nöthiger, 2000; Steinmann-Zwicky et al., 1989). This hypothesis was born studying insect gynandromorphs, i.e. aberrant specimens with an intermediate feature between female and male (according to the Greek roots gyne = female, aner = male, morphe = form; Fig. 1). In the fruit fly *Drosophila melanogaster*, gynandromorphs may arise when one embryonic nucleus loses an X chromosome and the insects possess a mixture of XX (i.e. female) and X0 (i.e. male) tissues. According to Gilbert (2000), because there are no sex hormones in insects to modulate such events, each cell makes its own sexual "decision".

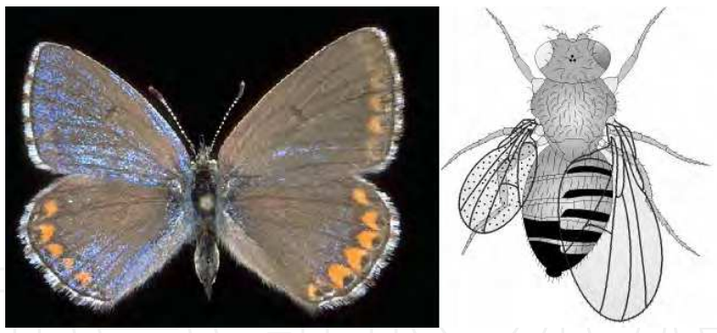


Fig. 1. Gynandromorphs, i.e. aberrant specimens made up of both female and male cells, are common in insects. On the left: *Polyommatus bellargus* (Lepidoptera, Lycaenidae) mosaic gynandromorph in which male (blue) and female (brown) features are mixed (Courtesy of R. Villa, Bologna, Italy). On the right: *D. melanogaster* bilateral gynandromorph in which one side is female and the other male (Modified from Griffiths et al., (2000). *An introduction to genetic analysis*, 7th edition, New York: W. H. Freeman).

However recent data demonstrate that in insects as in vertebrates, non-autonomous (= hormonal) sex determination controls sex dimorphism (DeFalco et al., 2008). In the germ line of the *D. melanogaster* embryo there is evidence for both autonomous and non-autonomous regulation of sexual identity, but non-autonomous signals from the soma are dominant, and germ cells establish their sexual identity as they contact the somatic gonad (Casper & Van Doren, 2009; DeFalco et al., 2008). In fact, XX (i.e. female) and XY or X0 (i.e. male) germ cells are not irrevocably committed to female or male identity, respectively (Waterbury et al., 2000).

According to these results, the presence of signals coordinating the development of a gender-specific phenotype (i.e. sex hormones) is conceivable. In his fine review, De Loof (2006) suggests that the loss of an X chromosome in *Drosophila* embryonic cells possibly makes the mutant cells react differently to a given hormonal environment and/or signals from their neighbours than XX cells.

Finally, it is noteworthy to note that, in addition to gynandromorphs, intersexes specimens do exist in insects. As previously discussed, gynandromorphism is the simultaneous presence within the same organism of genotypically and phenotypically male and female tissues rather than of masculinized or feminized tissues, as is the case with intersexes. Indeed intersexes are characterised by phenotypically male and female regions, but genetically homogeneous pattern (Laugé, 1985; White, 1973). In 1934, for example, Whiting and colleagues described individuals of the hymenopteran *Habrobracon juglandis* which were found to be genetically male but with feminized genitalia.

How can the existence of intersexes be explained, if each cell makes its own sexual decision?

2. Ecdysteroids: A role as sex hormones in insects?

De Loof (2006) proposes that ecdysteroids are the best candidates for a role as sex steroids in insects since, for example, they are involved in the appearance of sex dimorphic structures; are produced by the gonads; and induce different gender-specific physiological effects. Indeed the role of ecdysteroids is not restricted to moulting but they have a much wider effect on the insect biology, both at the larval and adult stages.

Insect moulting is induced by the steroid hormone 20-hydroxyecdysone (20E). Ecdysone pulses in the insects' hemolymph trigger moulting, and the presence or absence of juvenile hormone determines whether moults will lead to another larval stage or, through metamorphosis, to a pupa and an adult form (Gilbert at al., 2002). The 20E precursor is secreted by the prothoracic glands after their stimulation by the brain prothoracicotropic hormone (PTTH) whose release is governed by both intrinsic factors, like the body size, and extrinsic factors, like photoperiod and temperature (Gilbert at al., 2002).

Dietary cholesterol is then converted to 20E thanks to many hydroxylation reactions catalysed by cytochrome P450 enzymes of microsomal and/or mitochondrial origin, the final step being characterised by the action of a P450 monooxygenase that hydroxylates the ecdysone s.s. (E) at carbon 20.

Cytochrome P450s are encoded by the Halloween genes family, first characterised in *D. melanogaster* and then described in lots of insect species (Christiaens et al., 2010; Rewitz et al., 2007).

Once 20E is biosynthesized, it binds the heterodimeric nuclear receptor EcR/USP composed of EcR (Ecdysone Receptor) and USP (Ultraspiracle, homologous to the vertebrate retinoid-X receptor), which shares many commonalities with the human thyroid hormone receptor. Then, the EcR/USP complex activates the transcriptional processes underlying the cellular and morphogenetic moulting cascade events (Gilbert et al., 2002). In *D. melanogaster*, pulses of 20E throughout fly development have proved to regulate cell proliferation, differentiation, and programmed cell death in a highly controlled manner. During metamorphosis, for example, ecdysone is a primary regulator of apoptosis in larval tissues such as salivary glands, midgut and neural tissues which are destroyed or remodelled into an "adult" form (Mottier et al., 2004; Tsuzuki et al., 2001). The activation and execution of ecdysis (i.e. shedding of the old cuticle during embryonic and larval development) are controlled by a series of peptide hormones produced by Inka cells and neuropeptides within the central nervous system, whose expression is again under ecdysteroid control (Zitnan et al., 2007).

In adults the role played by ecdysteroids is much less explored: for example, it has been demonstrated that they control several important aspects of reproduction, including ovarian development and oogenesis (Carney & Bender, 2001; Raikhel et al., 2005; Riddifort, 1993; Swevers & Iatrou, 2003). In many insect species 20E is also directly involved in the regulation of vitellogenin biosynthesis by the female fat body, a metabolic tissue functionally analogous to the vertebrate liver, and it can also induce vitellogenin synthesis in males (Bownes et al., 1983; Bownes et al., 1996; Huybrechts & De Loof, 1977; Zhu et al., 2007). The 20E has also been shown to affect sexual behaviour, having a role in courtship initiation by males, and promoting male–male sexual attraction (Ganter et al., 2007).

De Loof (2006, 2008) suggests that ecdysteroids already served as sex hormones long before they acquired a function in moulting. In particular, 20E secreted by the follicle cells of the insect ovary could be the physiological equivalent of vertebrate estrogens, while E - the precursor of the active moulting hormone 20E - should act as a distinct hormone, being the physiological equivalent of the vertebrate testosterone (De Loof & Huybrechts, 1998; De Loof, 2006). Indeed, by using *Drosophila* larval organ culture Beckstead and colleagues (2007) demonstrate that E can regulate a set of genes that are distinct from those controlled by 20E, thus confirming that it may exert different biological (=hormonal) functions from 20E.

3. Ecdysteroids and *Wolbachia*: Different roles and different manipulations

Wolbachia are members of the order Rickettsiales (α-Proteobacteria), a diverse group of symbionts with parasitic, mutualistic or commensal lifestyle. The genus *Wolbachia* is known to infect exclusively invertebrates, namely nematodes and arthropods, being widely spread in insects where it is estimated to occur in up to 66% of the species (Hilgenboecker et al., 2008; Werren et al., 2008). *Wolbachia* bacteria, and specifically the species *W. pipientis*, are transmitted through the germ line from the mother to the offspring and, occasionally, between individuals of phylogenetically distant species (Stouthamer et al., 1999). The trans-ovarial inheritance of *Wolbachia* in insects seems to be mediated by bacteryocite-like cells (cells specialized for harbouring endosymbionts) in the ovary of the infected mother, which degenerate thus ensuring transmission of bacteria to germ line cells and then to the progeny (Sacchi et al., 2010).

Phylogenetic studies based on 16S ribosomal sequences reveal that *Wolbachia* bacteria are divided into eight different supergroups: two are commonly found in Nematoda (mainly in filarial but also in non filarial species), whereas the other six supergroups are found primarily in Arthropoda, including insects, mites, spiders, scorpions and isopod crustaceans (Werren et al., 2008).

A unique feature shared by Arthropoda and Nematoda is the ability to replace the exoskeleton, a process known as ecdysis. This shared characteristic is thought to reflect a common ancestry, giving rise to the clade Ecdysozoa (Ewer, 2005a). Although the exoskeleton composition varies among ecdysozoans, the process of moulting itself is similar within the clade: the epidermis undergoes cell division producing a larger surface and separates from the exoskeleton. Then the epidermis secretes a new exoskeleton that remains soft until the residues of the old cuticle are shed at ecdysis. The new cuticle then expands and hardens (Ewer, 2005a, 2005b).

As previously discussed, arthropod moulting is induced by the steroid hormone 20E and a role for ecdysteroids in nematode ecdysis has also been observed. In filarial nematodes, moulting seems to be regulated by ecdysteroid-like hormones: in *Dirofilaria immitis*, for example, moulting from the third to the fourth larval stage can be induced in vitro by the 20E of insects (Wabrick et al., 1993), and orthologs of insects nuclear receptors involved in ecdysone response have been found (Crossgrove et al., 2008; Ghedin et al., 2007; Tzertzinis et al., 2010). In *Caenorhabditis elegans* these nuclear receptors are also involved in the regulation of sex determination and reproductive development (Höss & Weltje, 2007; Motola et al., 2006) and, interestingly, ecdysone has also a role in the fertility and microfilaria release in filarial worms (Barker et al., 1991).

In nematodes, *Wolbachia* is an obligate symbiont, as worms depend on bacteria for survival. Antibiotic curing of *Wolbachia* "infection" inhibits nematode fertility and development, suggesting a specific role for the symbiont in host oogenesis, embryogenesis and moulting (Arumugam et al., 2008; Casiraghi et al., 2002; Frank et al., 2010).

In arthropods the bacterium is able to manipulate the host reproduction in order to increase the number of infected females. The effects of the *Wolbachia* infection include cytoplasmic incompatibility, that is an aberrant or considerably reduced offspring production if uninfected females mate with infected males, or if the parents are infected with different *Wolbachia* strains; thelytokous parthenogenesis, in which infected virgin females produce daughters; feminization, in which infected genetic males develop as females; and male-killing, in which infected males die (Stouthamer et al., 1999; Werren et al., 2008).

Such phenotypic variability is thought to be linked to high genome plasticity of insect-borne *Wolbachia*, since all the sequenced genomes of the symbiont contain high number of repetitive sequences, including IS (insertion sequences) elements and prophage-like sequences (Iturbe-Ormaetxe & O'Neill, 2007; Wu et al., 2004).

According to us, except for cytoplasmic incompatibility that is a secondary effect of the infection, the phenotypic effects observed in arthropods might not be so different, but strictly interconnected, and possibly all ascribable to feminization.

Indeed, male killing could be just an unsuccessful "attempt" at feminization by *Wolbachia*. Male-killing is known in several insect species, where males die during embryogenesis or development. Insight into the mechanism of male killing comes from the moths *Ostrinia scapulalis* and *O. furnacalis*, where *Wolbachia* kills genetic males during the larval development. Intriguingly, a partial *Wolbachia* curing leads to the appearance of lepidopteran intersexes having exclusively male genotype (Kageyama & Traut, 2003). Accordingly, a partial feminization of genetic males does occur, while a complete feminization is incompatible with the survival of the male genotype (Kageyama & Traut, 2003; Sakamoto et al., 2007).

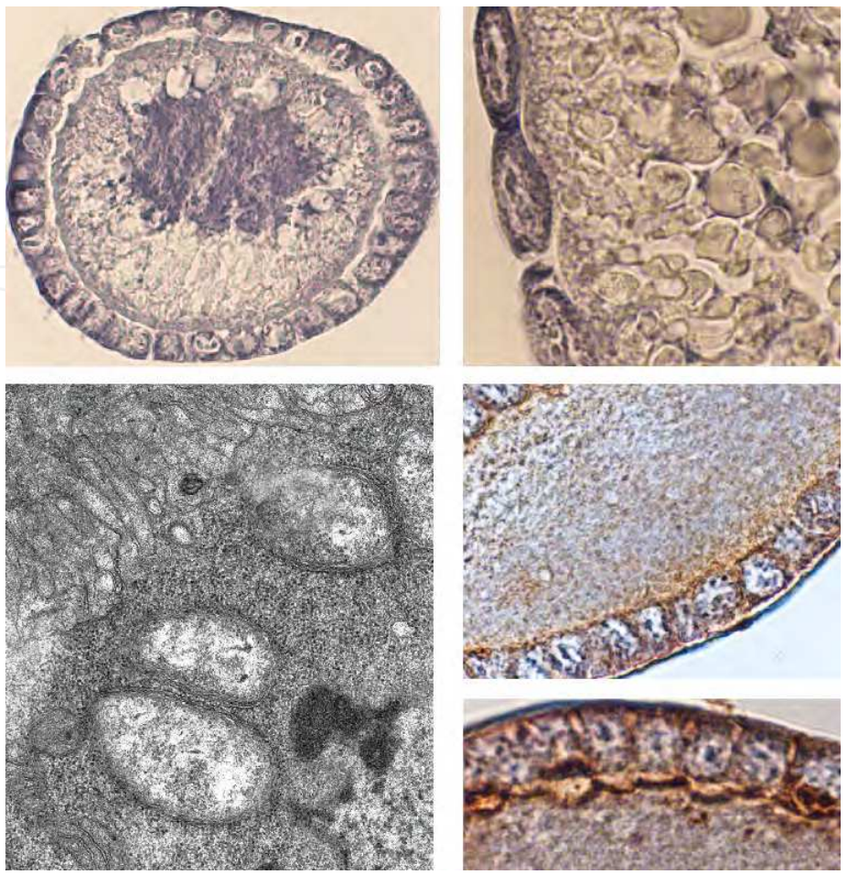
Regarding the parthenogenesis induction by *Wolbachia*, this phenomenon has been demonstrated in several haplodiploid species of mites, hymenopterans and thrips, where males naturally develop (parthenogenetically) from unfertilized haploid eggs and females from fertilized diploid eggs (Arakaki et al., 2001; Stouthamer et al., 1990; Weeks & Breeuwer, 2001). In *Wolbachia*-infected species, unfertilized eggs are subjected to a "diploidy" restoration, giving origin to (infected) females. Recently, Giorgini and colleagues (2009) observed that in the (haplodiploid) wasp *Encarsia hispida*, the symbiont *Cardinium* (which belongs to the only bacterial group known to cause similar reproductive manipulations of *Wolbachia*) doesn't induce, as expected, thelytokous parthenogenesis but feminization. In fact antibiotic treatment results in uninfected diploid male offspring, thus demonstrating that diploidy restoration is a necessary condition, but not sufficient, to elicit female development. Therefore, *Cardinium* is responsible for the feminization of the hymenopteran genetic males.

Since in studies concerning the parthenogenesis induction by *Wolbachia* no cytogenetic analyses have been performed on males produced by cured females, the hypothesis that the symbiont actually induces feminization rather than parthenogenesis may be conceivable.

As will be discussed later, feminization deals with sex determination and differentiation much more directly than the other *Wolbachia*-induced phenotypes, thus offering the opportunity to shed light on processes governing arthropod development and reproduction, and on the involvement of the endosymbiont in such processes.

On the whole, the data available in the literature suggest that the phenotypic effects induced by *Wolbachia* may be linked to differences in host physiology, and in particular to endocrine-related processes governing development and reproduction which in insects display high variability.

Interestingly, *Wolbachia* bacteria are known to localize in many hosts' steroidogenic tissues. In different insect species, the endosymbiont has been observed in the cytoplasm of the follicular cells (Gonella et al. 2011; Sacchi et al., 2010) (Fig. 2). In *Drosophila*, *Wolbachia* microinjected into the abdominal cavity has shown a tropism towards somatic stem cells that differentiate in follicular cells (Frydman et al., 2006). In insects the follicular epithelium is one of the major niches deputed to the synthesis of ecdysteroids (Swevers et al., 2005).



Upper left and right: A *Zyginidia pullula* (Hemiptera, Cicadellidae) oocyte surrounded by a single layer of follicle cells (gallocyanin–chrome alum reaction on leafhopper ovary sections). Lower left: TEM micrograph of a *Wolbachia*-infected *Z. pullula* follicle cell filled with bacteria. Lower right: Immunohistochemical reactions showing strong positivity (brown) to anti-wsp (*Wolbachia* surface protein) antibody in the leafhopper's follicular epithelium.

Fig. 2. *Wolbachia* localization in the follicular epithelium of the gonad's host.

Moreover, the endosymbiont is frequently associated to host's fat bodies, the other major niche for steroid synthesis (Kamoda et al., 2000; Thummel & Chory, 2002) (Fig. 3).
Therefore, it is conceivable that *Wolbachia* may interfere with insect reproduction and development by modulating host hormonal pathways, as it has already been shown for isopod crustaceans and will be explained in the following section.

Fig. 3. In-situ hybridization with a specific probe for *Wolbachia* 16S rRNA on *Zyginidia pullula* fat body shows positive staining (red), indicating that the tissue is filled with bacteria.

4. *Wolbachia* and the feminization of the arthropod host

In arthropods, feminization induced by *Wolbachia* was first described in isopod crustaceans (Bouchon et al., 2008; Martin et al., 1973; Rigaud et al., 1999) and, later, the phenomenon was studied in the lepidopteran species *Eurema hecabe*, *Ostrinia scapulalis* and *O. furnacalis*, and the hemipteran species *Zyginidia pullula* (Hiroki et al., 2002; Kageyama & Traut, 2003; Negri et al., 2006; Sakamoto et al., 2007).

In Crustacea, which are phylogenetically close to insects, sex differentiation and development of secondary sexual characteristics are driven by an androgenic hormone (AH), secreted by the androgenic gland (AG), whose action inhibits female differentiation (Legrand et al., 1987; Sagi & Khalaila, 2001). In fact, Crustacea are by default female and the expression of male secondary characteristics is only possible by the production of AH. Indeed, the ablation of the AG results in the degeneration from male to the female form, whereas injection with purified extracts of the AH or implantation of AGs into females results in the development of external male sexual characteristics or the complete sex reversal (Charniaux-Cotton, 1954; Sagi et al., 1997; Suzuki & Yamasaki, 1998). Therefore, it has been suggested that in Crustacea sex reversal is actually due to masculinisation of females or de-masculinisation of males (Ford, 2008).

The feminization effect induced by *Wolbachia* in isopods is thought to be linked to interactions between the bacterium and the AG differentiation process or, more probably, the AH receptors (Bouchon et al., 2008; Rigaud & Juchault, 1998). Indeed, in *Armadillidium vulgare* genetic males, AH mRNA can be detected at the beginning of male gonad differentiation, and AH may thus have an early and local action by inducing male differentiation of embryonic gonads (Negri et al., 2010). *Wolbachia* could then induce feminization (or de-masculinisation?) by targeting AH receptors, thereby inhibiting AG differentiation (Juchault & Legrand, 1985). If *Wolbachia* bacteria are experimentally inoculated in adult males, the AG become hypetrophic, but the host soon develops female genital apertures, probably because the AH receptors are no longer functional due to the infection (Martin et al., 1973; Martin et al., 1999).

In insect species, *Wolbachia* is able to feminize genetical males and, in all these cases, the existence of intersexes linked to *Wolbachia* effects has been described: in the presence of signals coordinating the development of a gender specific phenotype, intersexes might arise from a conflict between male and female sex hormones and/or receptors (Hiroki et al., 2002; Kageyama & Traut, 2003; Negri et al., 2006; Sakamoto et al., 2007).

In *E. hecabe*, feminizing *Wolbachia* acts continuously throughout the larval development to produce the female phenotype (Narita et al., 2007). As a consequence, if the bacteria act on sex differentiation rather than sex determination, sex hormone (i.e. ecdysteroid) pathways should be involved. Some clues are provided by studies on infected *E. hecabe*, where an incomplete *Wolbachia* suppression during host development, i.e. when host sex differentiation is not yet completed, leads to larval/pupal moulting defects (Narita et al., 2007). In particular, some individuals show morphological abnormalities (i.e. curled, folded or asymmetric wings), while a certain number of insects do not pupate: dissection of dead pupae reveals that many of them have actually completed adult morphogenesis but failed to escape from the pupal case (Narita et al., 2007). Interestingly, similar moulting defects may be obtained in knockdown insects using RNA interference techniques on ecdysone receptors. For example, some treated nymphs of the german cockroach *Blattella germanica* do not moult into adults, maintaining both nymphal and adult structures of ectodermal origin duplicated, whereas those nymphs that moulted into adults show characteristic

deformations in the wing extension (Cruz et al., 2006). Also *Drosophila* EcR mutants are characterised by pupal lethality: specimens rarely eclose and the pharate adults dissected from the pupal case show abnormalities (Davies et al., 2005).

Moreover, since in some lepidopteran species the ecdysteroid titer has been proven to regulate sex specific wing development (Lobbia et al., 2003), sexually intermediate traits in wing morphology observed in *E. hecabe* specimens subjected to a partial *Wolbachia* curing could also be attributed to the ecdysteroid action.

In the other lepidopteran species *Ostrinia scapulalis* and *O. furnacalis*, *Wolbachia* has the ability to feminize genetic males, but – as discussed above - a complete feminization is fatal, and genetical males die (Kageyama and Traut, 2003; Sakamoto et al., 2007). In these species male-killing occurs during the larval development while the role played by ecdysteroids is crucial. In other insects, the sex-specific killing action by *Wolbachia* occurs during embryogenesis (Dyer& Jaenike, 2004; Fialho & Stevens, 2000; Jiggins et al., 2001; Zeh et al., 2005). Embryogenesis takes place in a steroid hormone-enriched environment where steroid hormones act for the coordination of morphogenetic movements (De Loof, 2006; Kozlova & Thummel, 2003; Gaziova et al., 2004). Thus, if male-killing *Wolbachia* interacts with the host hormonal pathway involving ecdysteroids, this could interfere with the processes required for a normal development of males.

Unfortunately, little information about sex-specific action of ecdysteroids during insect embryogenesis and development is available, and it mainly concerns the effects of endocrine disrupting chemicals. For example, in the housefly *Musca domestica* and in the midge *Chironomus riparius* the sex ratio is affected by the ecdysteroid agonist bisphenol A (Izumi et al., 2008; Lee & Choi, 2007). Another ecdysteroid agonist, tebufenozide, exerts similar effects on *C. riparius* and the moth *Platynota idaeusalis* (Biddinger et al., 2006; Hahn et al., 2001). Female-biased sex ratios are also obtained after a treatment performed on the midge larvae with the ecdysteroid antagonist ethynil estradiol (Hahn et al., 2001; Lee & Choi, 2007).

According to some authors, the observed sex-specific effect could be explained by considering insect steroids as sex hormones. In particular, larval or embryo males die because they are subjected to an unsuitable, i.e. female, hormonal environment (Hahn et al., 2001).

Recent studies on the moth *Ostrinia scapulalis* are providing new data on the molecular bases of the *Wolbachia*-host interaction. Sugimoto and colleagues (2010) analysed the expression of the *doublesex* gene (*dsx*) in *Ostrinia* intersexes (= partially feminized males) generated from antibiotic treated mothers. *Doublesex* is the highly conserved gene at the bottom of the sex determination cascades in insects, and it is known to regulate the somatic sexual differentiation through the sex specific proteins DSXf (female) and DSXm (male). (Burtis & Baker, 1989). In particular, *dsx* resides at the junction of a complex network of regulatory interactions that include homeotic genes, ligand-based signal transduction cascades, and other transcriptional regulators for the differentiation of sexually dimorphic structures (Burtis, 2002; Rideout et al., 2010). In *Drosophila* males, feminization may be induced by modifying dsx expression. The ectopical expression of DSXf with the complete removal of endogenous DSXm may cause external complete feminization (Waterbury et al., 1999); even the XY (male) germ line may be feminized by ectopical expression of DSXf (Waterbury et al., 2000).

As expected, in somatic tissues of *O. scapulalis* males and females, the sex-specific isoform of DSX was found; while in the gonads the opposite sex was also weakly expressed, maybe because reproductive organs comprise also undifferentiated germ cells where both DSXf and DSXm could be expressed (Sugimoto et al., 2010). In intersex individuals originated from

Wolbachia-cured mothers, both female- and male-specific isoforms are present, suggesting that the symbiont may interfere either with the sex-specific splicing of the gene *dsx* itself or (more probably) with another upstream process involved in sex determination/differentiation (Sugimoto et al., 2010).

Male- and female-specific isoforms of DSX share a zinc finger DNA-binding domain (designated as the DM motif), which is widely conserved in the Animal Kingdom, from corals to nematodes, from arthropods to vertebrates, and characterize the *dmrt* family of genes (Erdman & Burtis, 1993; Murphy et al., 2010; Matsuda et al., 2002; Raymond et al., 1998; Smith et al., 2009; Yoshimoto et al., 2008; Zhu et al., 2000).

Despite the attention that *dmrt* factors have received, to date it has not been well elucidated how *dmrts* mediate their activities, and putative downstream targets have yet to be characterized.

In some vertebrates, such as fish, it has been demonstrated that sex steroid hormones affect *dmrt1* expression (Herpin & Schartl, 2011), thus it would be of capital interest to verify changes in *O. scapulalis dsx* expression following steroid treatments, and if feminizing *Wolbachia* may play a role in modulating *dsx* expression by interaction with hormonal pathways.

New insights into the mechanisms underlying the bacterium-host interaction have been provided by studies on the leafhopper *Z. pullula*. In this hemipteran species, *Wolbachia*-infected genetic males develop into intersexes with a female phenotype, which retain secondary male features in the ano-genital zone (Negri et al., 2006) (Fig. 4).

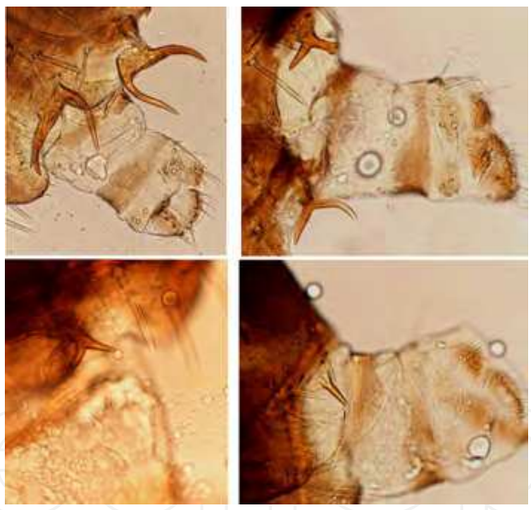


Fig. 4. *Zyginidia pullula* males feminized by *Wolbachia* maintain typical male structures (the so-called upper pygofer appendages) localized in the last abdominal segments. These forked chitinous structures are completely absent in normal females. In feminized males they appear well developed (upper left), or not completely developed but reduced to a stump (lower right).

Leafhopper feminized males are vital and even active reproductively. In laboratory rearing, couplings are often observed (Fig. 5), meaning that these individuals have a feminine 'sex appeal', and progeny is occasionally obtained (Negri et al., 2006). In addition to feminized males with ovaries ("intersex females"), some rare intersexes bear male gonads ("intersex males") (Negri et al., 2009a) (Fig. 5). Interestingly, "intersex males" possess a *Wolbachia* density approximately four orders of magnitude lower than "intersex females" (Negri et al., 2009a).

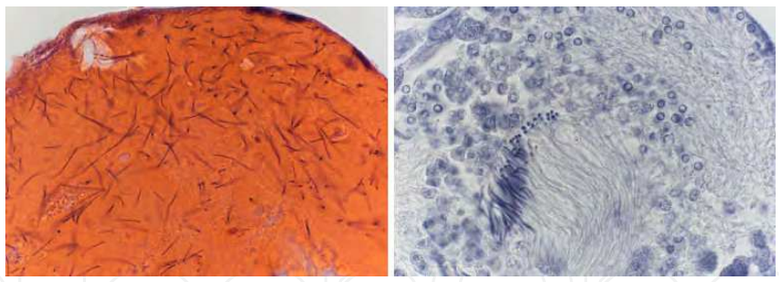


Fig. 5. On the left: a spermatheca of an intersex female of *Zyginidia pullula* full of sperms after after mating (haematoxylin/eosin stain on leafhopper gonad sections). On the right: testis of an intersex male showing different stages of spermatogenesis (gallocyanin–chrome alum reaction on leafhopper gonad sections).

Recent data demonstrate that *Wolbachia* infection is able to modulate the leafhopper's genomic imprinting through cytosine methylation of the host DNA (Negri et al., 2009a, 2009b).

Genomic imprinting is a phenomenon whereby a gene, or a region of a chromosome, is reversibly modified so that it retains a sort of "memory" of its own genetic history. The term imprinting, originally coined referring to a complex behaviour of the X chromosome in the dipteran insect *Sciara coprophila* (Crouse, 1960), indicates a situation in which the activity of the imprinted genes or chromosomes is determined by the sex of the parent that transmits them, and the altered expression is limited to the somatic tissue of the progeny, whereas the germ line is not permanently altered (Surani, 1998). Epigenetic changes are based on molecular mechanisms including methylation of cytosines, remodelling of chromatin structure through histone chemical modifications and RNA interference. These molecular processes can activate, reduce or completely disable the activity of genes.

Methylation of cytosine residues in the DNA is currently one of the most studied epigenetic mechanisms (Bender, 2004). This robust but reversible marking of genomic DNA is catalyzed by a conserved family of enzymes called DNA methyltransferases (DNMTs), which have been extensively studied in mammals, plants and fungi (Goll & Bestor, 2005).

Until now, the genomic imprinting has been found in vertebrates (Martin & McGowan 1995; Sharman 1971; Surani 1998) and invertebrates, including lots of insect species (Rewieved in Lyko & Maleszka, 2011). In particular, in the hymenopteran wasp *Nasonia vitripennis* and in the coccid *Planococcus citri* imprinting is related to sex determination (Beukeboom et al. 2007; Field et al. 2004); in *P. citri* it has been clearly assessed that DNA methylation is deeply involved in the establishment of the differential sex-specific genomic imprinting.

At a molecular level, in the hemipteran *Z. pullula* the occurrence of sex specific differences in the methylation pattern was observed (Negri et al., 2009a). Surprisingly, Random Amplification of Polymorphic DNA (RAPD) PCRs showed that *Wolbachia*-infected "intersex females" possess the same imprinting pattern of uninfected females (Negri et al., 2009a, 2009b). These data demonstrate that the infection disrupts the male imprinting thus influencing the expression of genes involved in sex differentiation and development. In addition, the alteration occurs only if the bacterium exceeds a density threshold, as "intersex males" maintain a male genome—methylation pattern (Negri et al., 2009a). Methylation-sensitive RAPD analyses were also carried out on gonads (testes and ovaries), confirming the occurrence of a sex-specific methylation of the genome, and strengthening the results obtained with somatic tissues in *Wolbachia*-infected specimens (Negri et al., 2009b). This

suggests that *Wolbachia* is not only able to induce a feminization of genetic males, but may also cause the inheritance of female imprinting in gonads of feminized males. This is particularly intriguing since in gonads the parental imprinting is generally erased and re-established on the basis of the parent sex, and clearly indicates that feminized males act as true females establishing a female genomic imprinting in their genome. On the whole data demonstrate that Wolbachia may be considered an 'environmental' factor that promotes heritable epigenetic changes in the host gene expression: the epigenetic effects of Wolbachia symbiosis are manifested as a 'maternal effect', in which infection of the mother alters the offspring phenotype.

5. Possible interplay between steroid signalling and epigenetic pathways

5.1 Role of sex steroids in mammal sex differentiation

In humans the male-determining gene *Sry* on the male-specific Y chromosome is known to promote sexual development by inducing the bipotential gonads of the embryo to form testes. Then, the differentiated gonad produces the male sex steroid (i.e. testosterone) which activates gene transcription via androgen and estrogen receptors, thus driving the masculinisation processes of the whole body (Anway et al., 2005; Chang et al., 2006). In particular, sex hormone synthesis induces not only the sexual differentiation of the reproductive system, but also the sexual differentiation of the brain. This is known to occur in a carefully defined critical period, where a brief hormone exposure permanently organizes the brain sex differences (Dohier, 1998; Gabory et al., 2009; McCarthy et al., 2009). Indeed, gonadal hormones defeminize and masculinize the male brain, while a lack of gonadal steroids allows for feminization in the female. In rodents, for example, treatments with steroids during the critical period leads to a defeminized and masculinized neural phenotype, while blocking aromatization of testosterone to estradiol or antagonizing estrogen receptor binding inhibits a correct brain organization in males (Barraclough, 1961; Baum, 1979; Vreeburg et al., 1977).

The mechanisms exerted by sex hormones are strictly linked to the epigenetic machinery. For example, gonadal hormones are able to induce sex differences in DNA methylation, methyl-binding proteins and chromatin modifications necessary for a correct sexual differentiation of the brain (Nugent & McCarthy, 2011).

The role for steroids in modulating epigenetic changes is attracting the growing interest of many researchers. In particular, the field of endocrine disruption is shedding new light on the discipline of basic reproductive neuro-endocrinology, through studies on how early life exposures to endocrine-disrupting chemicals may alter gene expression via epigenetic mechanisms, including DNA methylation and histone acetylation/methylation. Importantly, these effects may be transmitted to future generations if the germ line is affected via trans-generational, epigenetic actions.

Recent evidence shows for example that androgen and estrogen receptors interact with histone modifying enzymes (Tsai et al., 2009). Measuring levels of acetylation and methylation of histones in neonatal mouse brains, Tsai and colleagues (2009) found that H3 histone modification is sexually dimorphic in some areas of the neonatal brain, and prenatal testosterone interacts with H3 acetylation to reverse this dimorphism.

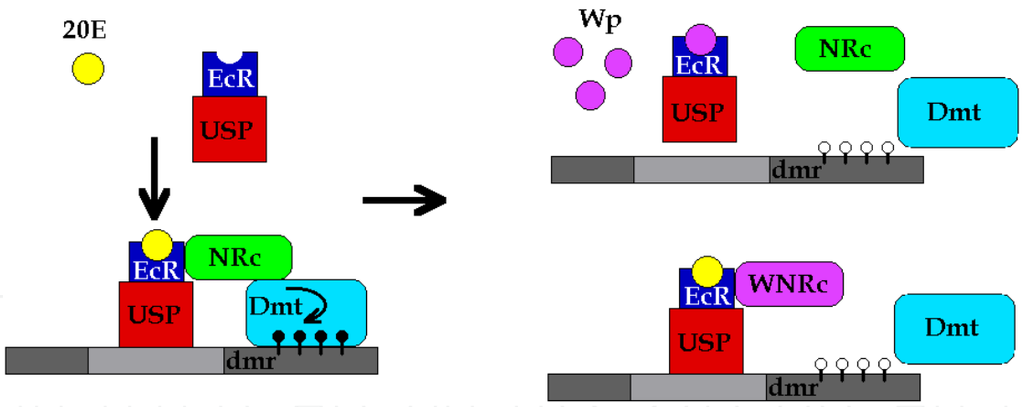
In another study, tamoxifen - a selective estrogen receptor modulator - has been shown to interfere with imprinting at the specific locus Insulin-like growth factor 2/H19 in rat spermatozoa (Pathak et al., 2010). Since imprint at this locus is acquired during

spermatogenesis in the male germ line, a role for estrogen signalling in the methylation dynamics of the testis is hypothesized. In particular it has been hypothesized that tamoxifen could exert an epigenetic action by directly affecting DNA methylation in the male germ cells. The observed reduction in sperm DNA methylation suggests imprinting error in the male germ-line mediated by defective estrogen signalling (Pathak et al., 2009; Pathak et al., 2010). Hence decipher interaction between estrogen signalling and DNA methylation pathways is of primary importance.

5.2 The *Wolbachia*-host interaction: A new perspective

The model proposed in Fig. 6 tries to explain a possible *Wolbachia*/host interaction involving host hormonal signalling and epigenetic regulation. In view of the absence of genes codifying for typical eukaryotic DNA methyltransferases in the sequenced genomes of *Wolbachia* strains isolated from *D. melanogaster* and the nematode *B. Malayi* (Foster et al., 2005; Wu et al., 2004), we cannot exclude that the bacterium encodes for some proteins interfering with ecdysteroids signalling pathway thus modulating the expression of the host DNMTs and/or histone modifying enzymes.

Hormone signalling orchestration is done by nuclear receptors, and over the past decade it has become increasingly clear that the recruitment of co-regulatory proteins to nuclear receptors is required for hormone-mediated transcriptional and biological activities. Many nuclear receptor co-regulators are key epigenetic regulators and utilize enzymatic activities to epigenetically modify the DNA and chromatin, through DNA methylation and histone acetylation/methylation (Hsia et al., 2010 Mahajan & Samuels, 2000; Rosenfeld et al., 2006).



20E = 20-hydroxyecdysone; EcR = Ecdysone receptor; USP = Ultraspiracle; NRc = Nuclear Receptor co-regulator; Dmt = DNA-methyltransferase; dmr = Differentially metylated regions; Wp = *Wolbachia* product; WNRc = *Wolbachia* Nuclear Receptor co-regulator.
Filled lollypops and open lollypops indicate methylated and unmethylated CpGs, respectively.

Fig. 6. Model illustrating the possible interplay between ecdysone signaling and epigenetic regulation. For simplicity, among epigenetic mechanisms, only DNA methylation is considered.

In particular, as proposed in Fig. 6, once 20E is biosynthesized, it binds the nuclear receptor EcR which heterodimerizes with USP. Then, the EcR/USP complex binds DNA constitutively and complexes with nuclear receptors co-regulators, thus catalyzing DNA methyltransferases (and/or histone modifying enzymes) which results in a proper DNA

methylation. In *Wolbachia*-infected insects, alterations of the methylation patterns may be due to hypothetical *Wolbachia* products (directly binding the nuclear-receptor or functioning as/or interfering with nuclear receptor co-regulators) that could inhibit EcR binding to DNA or DNA-methyltransferases (and/or histone modifying enzymes) recruitment, respectively.
Accordingly, studies on *Wolbachia*-host interactions should give great attention for example to selective nuclear receptor modulators; substances with an antagonist action on the ecdysone nuclear receptor; or co-regulators of nuclear receptors, in view of their emerging role in integrating transcriptional co-regulation with epigenetic regulation (Rosenfeld et al., 2006; Kato et al., 2011). This could eventually clarify the nature of this fascinating microbial symbiosis and the extraordinary effects on the host sexual development and reproduction.

6. Conclusion

An interaction between *Wolbachia* and host hormonal signalling pathways involving ecdysteroids may suggest the mechanistic way the bacterium uses for manipulating the host sexual behaviour and reproduction. Thus, the various phenotypic effects induced by the symbiont may be due to differences in the host physiology, considering that endocrine-related processes governing host development and reproduction display an enormous variability.
Recent data demonstrate a role of the symbiont in inducing epigenetic trans-generational changes in the host: by establishing intimate relationships with germ-line cells, epigenetic effects of *Wolbachia* symbiosis are manifested as a 'maternal effect', in which infection of the mother modulates the offspring phenotype. Indeed the *Wolbachia* infection is known to disrupt male imprinting, corresponding to changes in the genomic methylation pattern and in the host sexual phenotype towards females.
These observations raise a key question: what is the molecular basis of such an interaction? Some fascinating clues are provided by the recent demonstrations of interplay between hormone signalling and epigenetic pathways.
The mechanisms exerted by hormones are strictly linked to the epigenetic machinery, where steroids promote sex differences in DNA methylation, methyl-binding proteins and chromatin modifications, even if some epigenetic sex differences can also be directly attributed to the sex chromosomes. According to recent studies, selective nuclear receptor modulators and co-regulators of nuclear receptors are key factors in inducing epigenetic changes via DNA methylation and histone chemical modifications. These complex interactions influence the transcriptional output of many gene networks: the disruption of their normal function or expression by environmental factors can contribute to a vast spectrum of physiological abnormalities and disorders.
Hence, we propose a new perspective supporting a role of the symbiont *Wolbachia* as an "environmental factor" experienced by a mother that promotes heritable epigenetic changes by interaction with hormonal signalling pathways. Although further efforts are needed to fully clarify the genetic and molecular bases of such an interaction, new work hypotheses have been now offered for the study of the mechanisms (yet largely unknown) used by symbionts to dialogue with their hosts. Likewise, the *Wolbachia*-host interaction could become an emerging model system for the study of hormone signalling orchestration by nuclear receptors, and for shedding light on the role of nuclear receptor coregulators in integrating transcriptional coregulation with epigenetic regulation.

7. References

Anway, M.D., Cupp, A.S., Uzumcu, M. & Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility, *Science* 308:1466– 1469.

Arakaki, N., Miyoshi, T. & Noda, H. (2001). *Wolbachia* mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: Insecta), *Proc. R. Soc. Lond. B* 268: 1011–1016.

Arumugam, S., Pfarr, K.M. & Hoerauf, A. (2008). Infection of the intermediate mite host with *Wolbachia*-depleted *Litomosoides sigmodontis* microfilariae: impaired L1 to L3 development and subsequent sex-ratio distortion in adult worms, *Int J Parasitol* 38: 981-7.

Barker, G.C., Mercer, J.G., Rees, H.H. & Howells, R.E. (1991). The effect of ecdysteroids on the microfilarial production of *Brugia pahangi* and the control of meiotic reinitiation in the oocytes of *Dirofilaria immitis*, *Parasitol Res* 77: 65–71.

Barraclough, C.A. (1961). Production of anovulatory, sterile rats by single injections of testosterone propionate, *Endocrinology* 1: 62–67.

Baum, M.J. (1979). Differentiation of coital behaviour in mammals: a comparative analysis, *Neurosci Biobehav Rev* 4: 265–284.

Beckstead, R.B., Lam, G. & Thummel, C.S. (2007). Specific transcriptional responses to juvenile hormone and ecdysone in *Drosophila*, *Insect Biochemistry and Molecular Biology* 37: 570–578.

Bender, J. (2004). DNA methylation and epigenetics, *Annu Rev Plant Biol* 55: 41-68.

Beukeboom, L.W., Kamping, A. & van de Zande, L. (2007). Sex determination in the haplodiploid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea): a critical consideration of models and evidence, *Sem Cell Dev Biol* 18: 371-8.

Biddinger, D., Hull, L., Huang, H., McPheron, B. & Loyer, M. (2006). Sublethal effects of chronic exposure to tebufenozide on the development, survival and reproduction of the tufted apple bud moth (Lepidoptera: Tortricidae), *J Econ Entomol* 99: 834-42.

Bouchon, D., Cordaux, R. & Grève, P. (2008). *Feminizing Wolbachia* and evolution of sex determination in isopods, In: *Insect symbiosis*, K. Bourtzis & T.A. Miller (Eds), 273-94, Boca Raton FL., Taylor and Francis Group.

Bownes, M., Blair, M., Kozma, R. & Dempster, M. (1983). 20E stimulates tissue-specific yolk-protein gene transcription in both male and female *Drosophila, J. Embryol. Exp. Morphol.* 78: 249–263.

Bownes, M., Ronaldson, E. & Mauchline, D. (1996). 20-Hydroxyecdysone, but not juvenile hormone, regulation of yolk protein gene Expression can be mapped to cis-acting DNA sequences, *Developmental Biology* 173: 475–489.

Burtis, K.C. & Baker, B.S. (1989). *Drosophila* doublesex gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides, *Cell* 56 (6): 997-1010.

Burtis, K.C. (2002). Doublesex in the Middle, *Science* 29 (5584): 1135-1136.

Carney, G.E. & Bender, M. (2000). The *Drosophila* ecdysone receptor (EcR) gene is required maternally for normal oogenesis, *Genetics* 154: 1203–1211.

Casiraghi, M., McCall, J.W., Simoncini, L., Kramer, L.H., Sacchi, L., Genchi, C., Werren, J.H. & Bandi, C. (2002). Tetracycline treatment and sex-ratio distortion: a role for *Wolbachia* in the moulting of filarial nematodes?, *Int J Parasitol* 32: 1457-68.

Casper, A.L. & Van Doren, M. (2009). The establishment of sexual identity in the *Drosophila* germline, *Development* 136 (22): 3821-30.

Chang, H.-S., Anway, M.D., Rekow, S.S. & Skinner M.K. (2006). Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination, *Endocrinology* 147(12): 5524–5541.

Charniaux-Cotton, H. (1954). Decouverte chez un Crustace Amphipode (*Orchestia gammarella*) d'une glande endocrine responsable de la differenciations des caracteres sexuels primaires et secondaires males, C.*R. Acad. Sci. Paris* 239 : 780–782.

Christiaens, O., Iga, M., Velarde, R. A., Rougé, P. &. Smagghe, G. (2010). Halloween genes and nuclear receptors in ecdysteroid biosynthesis and signalling in the pea aphid, *Insect Molecular Biology* 19 (2): 187–200.

Crossgrove, K., Maina, C.V., Robinson-Rechavi, M. & Lochner, M.C. (2008). Orthologues of the *Drosophila melanogaster* E75 molting control gene in the filarial parasites *Brugia malayi* and *Dirofilaria immitis*, *Mol Biochem Parasitol* 157: 92-7.

Crouse, H.V. (1960). The controlling element in sex chromosome behaviour in *Sciara*, *Genetics* 45: 1429-1443.

Cruz ,J., Mané Padròs, D., Belleés, X. & Martin, D. (2006). Functions of the ecdysone receptor isoform-A in the hemimetabolous insect *Blattella germanica* revealed by systemic RNAi in vivo, *Dev Biol* 297: 158-71.

Davis, M.B., Carney, G.E., Robertson, A.E. & Bender, M. (2005). Phenotypic analysis of EcR-A mutants suggests that EcR isoforms have unique functions during *Drosophila* development, *Dev Biol* 282: 385-96.

De Loof, A. & Huybrechts, R. (1998). '"Insects Do Not Have Sex Hormones'': a myth?, *General and Comparative Endocrinology* 111: 245–60.

De Loof, A. (2006). Ecdysteroids: the overlooked sex steroids of insect? Males: the black box, *Insect Science* 13: 325-38.

De Loof, A. (2008). Ecdysteroids, juvenile hormone and insect neuropeptides: recent successes and remaining major challenges, *Gen Comp Endocrinol.* 155(1): 3-13.

DeFalco, T., Camara, N., Le Bras, S. & Van Doren, M. (2008). Non-autonomous sex determination controls sexually dimorphic development of the *Drosophila* gonad, *Dev Cell* 14: 275-86.

Dohier, K.D. (1998). Influence of hormones and hormone antagonists on sexual differentiation of the brain, *Arch Toxicol* 9: 131–141.

Dyer, K.A. & Jaenike, J. (2004). Evolutionarily stable infection by a male-killing endosymbiont in *Drosophila innubila*: molecular evidence from the host and parasite genomes, *Genetics* 168: 1443–1455.

Erdman, S.E. & Burtis, K.C. (1993). The *Drosophila* doublesex proteins share a novel zinc finger related DNA binding domain, *EMBO J* 12: 527–535.

Ewer, J. (2005a). How the ecdysozoan changed its coat, *PLoS Biology* 3: 1696-9.

Ewer, J. (2005b). Behavioral actions of neuropeptides in invertebrates: insights from *Drosophila*, *Hormones and Behavior* 48: 418 – 429.

Fialho, R.F. & Stevens, L. (2000). Male-killing *Wolbachia* in a flour beetle, *Proc. R. Soc. Lond. B* 267: 1469–1473.

Field, L.M., Lyko, F., Mandrioli, M. & Prantera, G. (2004). DNA methylation in insects, *Insect Mol Biol* 13: 109- 15.

Ford, A.T. (2008). Can you feminise a crustacean? *Aquatic Toxicology*, 88: 316–321.

Foster J., et al. (2005). The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode, *PLoS Biol* 3: e121.

Frank, K., Frank, K. & Heald, R.D. (2010). The emerging role of *Wolbachia* species in heartworm disease, *Compend Contin Educ Vet.* 32(4): E1-5.

Frydman H. M., Li J.M., Robson D.N. & Wieschaus, E. (2006). Somatic stem cell niche tropism in *Wolbachia*, *Nature* 441: 509-512.

Gabory, A., Attig, L. & Junien, C. (2009). Sexual dimorphism in environmental epigenetic programming, *Molecular and Cellular Endocrinology* 304: 8–18.

Ganter, G.K., Walton, K.L., Merriman, J.O., Salmon, M.V., Brooks, K.M., Maddula, S. & Kravitz, E.A. (2007). Increased male male courtship in ecdysone receptor deficient adult flies, *Behav Genet* 37:507-12.

Gaziova, I., Bonnette, P.C., Henrich, V.C. & Jindra, M. (2004). Cell-autonomous roles of the ecdysoneless gene in *Drosophila* development and oogenesis, *Development* 131: 2715-25.

Ghedin, E., et al. (2007). Draft genome of the filarial nematode parasite *Brugia malayi*, *Science* 317: 1756- 60.

Gilbert L.I., Rybczynski, R. & Warren, J.T. (2002). Control and biochemical nature of the ecdysteroidogenic pathway, *Annu. Rev. Entomol.* 47: 883–916.

Gilbert, S.F. (2000). *Developmental Biology* (6th edition), Sinauer Associates, Sunderland (MA).

Giorgini, M., Monti, M.M., Caprio, E., Stouthamer, R. & Hunter, M.S. (2009). Feminization and the collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*, Heredity 102(4): 365-71.

Goll, M.G. & Bestor, T.H. (2005). Eukaryotic cytosine methyltransferases, *Annu. Rev. Biochem.* 74: 481–514.

Gonella, E., Negri, I., Marzorati, M., Mandrioli, M., Sacchi, L., Pajoro, M., Crotti, E., Rizzi, A., Clementi, E., Tedeschi, R., Bandi, C., Alma, A. & Daffonchio, D. (2011). Bacterial endosymbiont localization in *Hyalesthes obsoletus*, the insect vector of Bois Noir in *Vitis vinifera*, *Applied and Environmental Microbiology*, 77(4):1423-35.

Guiguen, Y., Fostier A., Piferrer, F. & Chang, C.F. (2010). Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish, *Gen Comp Endocrinol.* 165(3): 352-66.

Hahn, T., Liess, M. & Schulz, R. (2001). Effects of the hormone mimetic insecticide tebufenozide on *Chironomus riparius* larvae in two different exposure setups, *Ecotox Environ Safe* 49: 171-8.

Herpin, A. & Schartl, M. (2011). Dmrt1 genes at the crossroad: a widespread and central class of sexual development factors in fish, *FEBS J.* 278(7): 1010-9.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. & Werren, J. H. (2008). How many species are infected with *Wolbachia*? — a statistical analysis of current data, *FEMS Microbiol. Lett.* 281: 215–220.

Hiroki, M., Kato, Y., Kamito, T. & Miura, K. (2002). Feminizationof genetic males by a simbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Pieridae), *Naturwissenschaften* 89: 67-70.

Höss, S. & Weltje, L. (2007). Endocrine disruption in nematodes: effects and mechanisms, *Ecotoxicology* 16: 15-28.

Hsia, E.Y., Goodson, M.L., Zou, J.X., Privalsky, M.L. & Chen, H.W. (2010). Nuclear receptor coregulators as a new paradigm for therapeutic targeting, *Adv Drug Deliv Rev*. 62 (13): 1227-37.

Huybrechts, R. & De Loof, A. (1977). Induction of vitellogenin synthesis in male *Sarcophaga bullata* by ecdysterone, *Journalof Insect Physiology* 23: 1359-1362.

Iturbe-Ormaetxe, I. & O'Neill, S. (2007). *Wolbachia*-host interactions: connecting phenotype to genotype, *CurrOpin Microbiol* 10:1-4.

Izumi, N., Yanagibori, R., Shigeno, S. & Sajiki, J. (2008). Effects of bisphenol A on the development, growth and sex ratio of the housefly *Musca domestica*, *Environ Toxicol Chem* 27: 1343-53.

Janer, G. & Porte, C. (2007). Sex steroids and potential mechanisms of non-genomic endocrine disruption in invertebrates, *Ecotoxicology* 16:145–160.

Jiggins, F.M., Hurst, G.D., Schulenburg, J. H. & Majerus, M. E. (2001). Two male-killing *Wolbachia* strains coexist within a population of the butterfly *Acraea encedon*, *Heredity* 86: 161–166.

Juchault, P. & Legrand, J.J. (1985). Mechanism of the refractory state of androgen hormone in *Armadillidium vulgare* Latr. (crustacean, isopod, oniscoid) harboring a feminizing bacteria, *Gen Comp Endocrinol* 60: 463-7.

Kageyama, D. & Traut, W. (2003). Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*, *Proc R Soc B* 271: 251-8.

Kamoda, S., Masui, S., Ishikawa, H. & Sasaki, T. (2000). *Wolbachia* infection and cytoplasmic incompatibility in the cricket *Teleogryllus taiwanemma*, *J Exp Biol* 203: 2503-9.

Kato, S., Yokoyama, A. & Fujiki, R. (2011). Nuclear receptor coregulators merge transcriptional coregulation with epigenetic regulation, *Trends in Biochemical Sciences* 36 (5): 272-281.

Kozlova, T. & Thummel, C.S. (2003). Essential roles for ecdysone signaling during *Drosophila* mid-embryonic development, *Science* 301:1911-4.

Laugé, G. (1985). Sex determination: Genetic and epigenetic factors, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 1, Embryogenesis and Reproduction, G. A. Kerkut & L. I. Gilbert (Eds), 295-318, Pergamon Press, Oxford.

Lee, S.-B. & Choi, J. (2007). Effects of bisphenol A and ethynyl estradiol exposure on enzyme activities, growth and development in the fourth instar larvae of *Chironomus riparius* (Diptera, Chironomidae), *Ecotoxicology and Environmental Safety* 68 (1): 84-90.

Legrand, J.J., Legrand-Hamelin, E. & Juchault, P. (1987). Sex determination in Crustacea, *Biol Rev* 62: 439-70.

Lobbia, S., Niitsu, S. & Fujiwara, H. (2003). Female-specific wing degeneration caused by ecdysteroid in the Tussock Moth, *Orgyia recens*: hormonal and developmental regulation of sexual dimorphism, *J Insect Sci* 3: 1-7.

Lyko, F. & Maleszka, R. (2011). Insects as innovative models for functional studies of DNA methylation, *Trends in Genetics* 27(4): 127-31.

Mahajan, M.A. & Samuels, H.H. (2000). A new family of nuclear receptor coregulators that integrate nuclear receptor signaling through CREB-binding protein, *Molecular and Cellular Biology* 20 (14): 5048-5063.

Martin, C.C. & McGowan, R. (1995). Genotype-specific modifiers of transgene methylation and expression in the zebrafish, *Danio rerio*, *Genet. Res.* 65: 21-28.

Martin, G., Juchault, P. & Legrand, J.J. (1973). Mise en évidence d'un micro-organisme intracytoplasmique symbiotede l'oniscoïde *Armadillidium vulgare* Latreille dont la présence accompagne l'intersexualité ou la féminisation totale des mâles génétiques de la lignée thélygène, *C R Acad Sc Paris* 276: 2313-6.

Martin, G., Sorokine, O., Moniatte, M., Bulet, P., Hetru, C. & Van Dorsselaer, A. (1999). The structure of a glycosylated protein hormone responsible for sex determination in the isopod, *Armadillidium vulgare*, *Eur J Biochem* 262: 727-36.

Matsuda M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S. & Sakaizumi, M. (2002). DMY is a Y-specific DM-domain gene required for male development in the medaka fish, *Nature* 417: 559–563.

McCarthy, M.M., Auger, A.P., Bale, T.L., De Vries, G.J., Dunn, G.A., Forger, N.G., Murray, E.K., Nugent, B.M., Schwarz, J.M. & Wilson, M.E. (2009). The epigenetics of sex differences in the brain, *The Journal of Neuroscience* 29(41):12815–12823.

Motola, D.L., Cummins, C.L., Rottiers, V., Sharma, K.K., Li, T.T., Li, Y., Suino-Powell, K., Xu, H.E., Auchus, R.J., Antebi, A. & Mangelsdorf, D.J. (2006). Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans, Cell* 124: 1209-23.

Mottier, V. , Siaussat, D., Bozzolan, F., Auzoux-Bordenave, S., Porcheron, P. & Debernard, S. (2004). The 20-hydroxyecdysone-induced cellular arrest in G2 phase is preceded by an inhibition of cyclin expression, *Insect Biochemistry and Molecular Biology* 34: 51–60.

Murphy, M.W., Sarver, A.L., Rice, D., Hatzi, K., Ye, K., Melnick, A., Heckert, Zarkower, D. & Bardwell, V.J. (2010). Genome-wide analysis of DNA binding and transcriptional regulation by the mammalian Doublesex homolog DMRT1 in the juvenile testis, *Proc Natl Acad Sci USA* 107 (30): 13360–13365.

Nakamura, M. J. (2010). The Mechanism of Sex Determination in Vertebrates—Are Sex Steroids the Key-Factor? *Exp. Zool.* 313: 381–398.

Nakamura, M.J. (2009). Sex determination in amphibians, *Semin Cell Dev Biol.* 20(3): 271-82.

Narita, S., Kageyama, D., Nomura, M. & Fukatsu, T. (2007). Unexpected mechanism of symbiont-induced reversal of insect sex: feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development, *Appl Environ Microbiol.* 73(13): 4332–4341.

Negri, I., Franchini, A., Gonella, E., Daffonchio, D., Mazzoglio, P.J., Mandrioli, M. & Alma, A. (2009a). Unravelling the *Wolbachia* evolutionary role: the reprogramming of the host genomic imprinting, *Proc R Soc B* 276: 2485-91.

Negri, I., Mazzoglio, P.J., Franchini, A., Mandrioli, M. & Alma, A. (2009b). Male or female? The epigenetic conflict between a feminizing bacterium and its insect host, *Communicative & Integrative Biology* 2 (6): 1-2.

Negri, I., Pellecchia, M., Grève, P., Daffonchio, D., Bandi, C. & and Alma, A. (2010). Sex and stripping: The key to the intimate relationship between *Wolbachia* and host? *Communicative & Integrative Biology* 3:2, 1-6.

Negri, I., Pellecchia, M., Mazzoglio, P.J., Patetta, A. & Alma, A. (2006). Feminizing *Wolbachia* in *Zyginidia pullula* (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system, *Proc R Soc B* 273: 2409-16.

Norris, D.O. & Carr, J.A. (2006). *Endocrine disruption: biological basis for health effects in wildlife and humans,* Oxford University Press, Oxford.

Nugent, B.M. & McCarthy, M.M. (2011). Epigenetic underpinnings of developmental sex differences in the brain, *Neuroendocrinology* 93(3): 150-8.

Oakes, M.B, Eyvazzadeh, A.D., Quint, E. & Smith, Y.R. (2008). Complete androgen insensitivity syndrome--a review, *J Pediatr Adolesc Gynecol.* 21(6): 305-10.

Oehlmann, J., Schulte-Oehlmann, U., Bachmann, J., Oetken, M., Lutz, I., Kloas, W. & Ternes, T.A. (2007). Bisphenol A induces superfeminization in the Ramshorn Snail *Marisa cornuarietis* (Gastropoda: Prosobranchia) at environmentally relevant concentrations, *Environmental Health Perspectives* 114 (1): 127-133.

Pathak, S., D'Souza, R., Ankolkar, M., Gaonkar, R. & Balasinor, N.H. (2010). Potential role of estrogen in regulation of the Insulin-like growth factor2–H19 locus in the rat testis, *Mol Cell Endocrinol*. 314(1): 110-7.

Pathak, S., Kedia-Mokashi, N., Saxena, M., D'Souza, R., Maitra, A., Parte, P., Gill-Sharma, M. & Balasinor, N.H. (2009). Effect of tamoxifen treatment on global and Insulin-like growth factor 2-H19 locus specific DNA methylation in rat spermatozoa and its association with embryo loss, *Fertil. Steril.* 91: 2253–2263.

Raikhel, A.S., Brown, M. & Belles, X. (2005). Hormonal control of reproductive processes, In: C*omprehensive Insect Physiology, Biochemistry, Pharmacology and Molecular Biology*, Vol. 3, Endocrinology, L. Gilbert, S. Gill & K. Iatrou (Eds), 433-491, Elsevier Press.

Raymond, C.S., Shamu, C.E., Shen, M.M., Seifert, K.J., Hirsch, B., Hodgkin, J. & Zarkower, D. (1998). Evidence for evolutionary conservation of sex-determining genes, *Nature* 391: 691–695.

Rewitz, K. F., O'Connor, M.B. & Gilbert, L. I. (2007). Molecular evolution of the insect Halloween family of cytochromeP450s: phylogeny, gene organization and functional conservation, *Insect Biochemistry and Molecular Biology*, 37: 741–753.

Riddiford, LM. (1993). Hormones and *Drosophila* development, In: *The Development of Drosophila melanogaster*, M. Bate & A.M. Arias (Eds), 899–939, Cold Spring Harbor Laboratory Press

Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie S. & Goodwin, S.F. (2010). Control of sexual differentiation and behavior by the doublesex gene in *Drosophila melanogaster, Nature Neuroscience* 13: 458–466.

Rigaud, T. & Juchault, P. (1998). Sterile intersexuality in an isopod induced by the interaction between a bacterium (*Wolbachia*) and the environment, *Can J Zool* 76: 493-9.

Rigaud, T., Moreau, J. & Juchault, P. (1999). *Wolbachia* infection in the terrestrial isopod *Oniscus asellus*: sex ratio distortion and effect on fecundity, *Heredity* 83: 469-75.

Rosenfeld, M.G., Lunyak, V.V. & Glass, C.K. (2006). Sensors and signals: a coactivator/ corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response, *Genes & Development* 20: 1405–1428.

Sacchi, L., Genchi, M., Clementi, E., Negri, I., Alma, A., Ohler, S., Sassera, D., Bourtzis, K. & Bandi, C. (2010). Bacteriocyte-like cells harbour *Wolbachia* in the ovary of *Drosophila melanogaster* (Insecta, Diptera) and *Zyginidia pullula* (Insecta, Hemiptera), *Tissue Cell.* 42(5): 328-33.

Sagi, A. & Khalaila, I. (2001). The crustacean androgen: a hormone in an isopod and androgenic activity in decapods, *Amer. Zool.* 41: 477–484.

Sagi, A., Snir, E. & Khalaila, I. (1997). Sexual differentiation in decapod crustaceans: role of the androgenic gland, *Invertebr. Reprod. Dev.* 31: 55–61.

Sakamoto, H., Kageyama, D., Hoshizaki, S. & Yshikawa, Y. (2007). Sex specific death in the Asian corn borer moth (*Ostrinia furnacalis*) infected by *Wolbachia* occurs across larval development, *Genome* 50:645-52.

Schütt, C. & Nöthiger, R. (2000). Structure, function and evolution of sex-determining systems in Dipteran insects, *Development* 127: 667-77.

Sharman, G.B. (1971). Late DNA replication in the paternally derived X chromosome of female kangaroos, *Nature* 230: 231-232.

Smith, C.A., Roeszler, K.N., Ohnesorg, T., Cummins, D.M., Farlie, P.G., Doran, T.J. & Sinclair, A.H. (2009). The avian Z-linked gene DMRT1 is required for male sex determination in the chicken, *Nature* 461: 267–271.

Steinmann-Zwicky, M., Schmid, H. & Nöthiger, R. (1989). Cell-autonomous and inductive signals can determine the sex of the germ line of *Drosophila* by regulating the gene Sxl, *Cell* 57(1): 157-66.

Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction, *Annu. Rev. Microbiol.* 53: 71–102.

Stouthamer, R., Luck, R. F. & Hamilton, W. D. (1990). Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex, *Proc. Natl Acad. Sci. USA* 87, 2424–2427.

Sugimoto, T.N., Fujii, T., Kayukawa, T., Sakamoto, H. & Ishikawa, Y. (2010). Expression of a *doublesex* homologue is altered in sexual mosaics of *Ostrinia scapulalis* moths infected with *Wolbachia, Insect Biochem Mol Biol.* 40(12): 847-54.

Surani, M.A. (1998). Imprinting and the initiation of gene silencing in the germ line, *Cell* 93: 309-312.

Suzuki, S. & Yamasaki, Y. (1998). Sex reversal by implantations of ethanol-treated androgenic glands of female isopods, *Armadillidium vulgare* (Malacostraca, Crustacea), *General and Comparative Endocrinology* 111: 367–375.

Swevers, L. & Iatrou, K. (2003). The ecdysone regulatory cascade and ovarian development in lepidopteran insects: insights from the silkmoth paradigm, *Insect Biochemistry and Molecular Biology* 33: 1285–1297.

Swevers, L., Raikhel, A.S., Sappington, T.W., Shirk, P. & Iatrou, K. (2005). Vitellogenesis and post-vitellogenic maturation of the insect ovarian follicle. In: *Comprehensive Molecular Insect Science,* vol.1, Reproduction and Development, L.I. Gilbert, K. Iatrou & S.S. Gill (Eds), 87-156, Elsevier Ltd., Oxford, UK.

Thummel , C.S. & Cory, J. (2002). Steroid signaling in plants and insects — common themes, different pathways, *Genes and Development* 16 (24): 3113–3129.

Tsai, H.-W., Grant, P.A. & and Rissman, E.F. (2009). Sex differences in histone modifications in the neonatal mouse brain, *Epigenetics* 4(1): 47–53.

Tsuzuki, S., Iwami, M. & Sakurai, S. (2001). Ecdysteroid-inducible genes in the programmed cell death during insect metamorphosis, *Insect Biochemistry and Molecular Biology* 31: 321–331.

Tzertzinis, G., Egaña, A.L., Palli, S.R., Robinson-Rechavi, M., Gissendanner, C.R., Liu, C., Unnasch, T.R. & Maina, C.V. (2010). Molecular evidence for a functional ecdysone signaling system in *Brugia malayi*, *PLoS Negl Trop Dis*. 4(3): e625.

Vreeburg, J., van der Vaart, P.D.M. & Van Der Schoot, P. (1977). Prevention of central defeminization but not masculinization in male rats by inhibition neonatally of oestrogen biosynthesis, *J Endocrinol* 74: 375-382.

Wang, C. & Croll, R.P. (2004). Effects of sex steroids on gonadal development and gender determination in the sea scallop, *Placopecten magellanicus*, *Aquaculture* 238: 483–498.

Warbrick, E.V., Barker, G.C., Rees, H.H. & Howells, R.E. (1993). The effect of invertebrate hormones and potential hormone inhibitors on the third larval moult of the filarial nematode, *Dirofilaria immitis*, in vitro, *Parasitology* 107: 459-63.

Waterbury, J. A., Horabin, J. I., Bopp, D. & Schedl, P. (2000). Sex determination in the *Drosophila* germline is dictated by the sexual identity of the surrounding soma, *Genetics* 155: 1741–1756.

Waterbury, J. A., Jackson, L. L. & Schedl, P. (1999). Analysis of the doublesex female protein in *Drosophila melanogaster*: role in sexual differentiation and behavior and dependence on intersex, *Genetics* 152: 1653–1667.

Weeks, A. R. & Breeuwer, J. A. (2001). *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites, *Proc. R. Soc. Lond. B* 268, 2245–2251.

Werren, J.H., Baldo, L. & Clark, M.E. (2008). *Wolbachia*: master manipulators of invertebrate biology, *Nat RevMicrobiol* 6: 741-51.

White, M.J.D. (1973). *Animal cytology and Evolution* (Third Edition), Cambridge University Press, Cambridge.

Whiting, P.W., Greb, R.J. & Speicher, B.R. (1934). A new type of sex-intergrade, *Biol. Bull.* 66: 152-165.

Wu, M. et al. (2004). Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements, *PLoS Biol.* 2: 327–341.

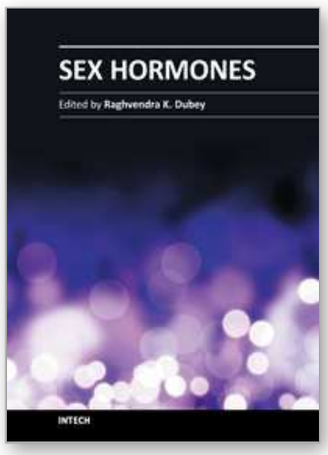
Yoshimoto, S., Okada, E., Umemoto, H., Tamura, K., Uno, Y., Nishida-Umehara, C., Matsuda, Y., Takamatsu, N., Shiba, T. & Ito, M. (2008). A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*, *Proc Natl Acad Sci USA* 105: 2469–2474.

Zeh, D. W., Zeh, J. A. & Bonilla, M. M. (2005). *Wolbachia*, sex ratio bias and apparent male killing in the harlequin beetle riding pseudoscorpion, *Heredity* 95: 41–49.

Zhu, J., Chen, L. & Raikhel, A.S. (2007). Distinct roles of Broad isoforms in regulation of the 20-hydroxyecdysone effector gene, Vitellogenin, in the mosquito *Aedes aegypti*, *Mol Cell Endocrinol.* 267(1-2): 97–105.

Zhu, L., Wilken, J., Phillips, N.B., Narendra, U., Chan, G., Stratton, S.M., Kent, S.B. & Weiss, M.A. (2000). Sexual dimorphism in diverse metazoans is regulated by a novel class of intertwined zinc fingers, *Genes Dev* 14: 1750–1764.

Zitnan, D., Kim, Y.-J., Zitnanova, I., Roller, L. & Adams, M.E. (2007). Complex steroid–peptide–receptor cascade controls insect ecdysis, *General and Comparative Endocrinology* 153: 88–96.



Sex Hormones

Edited by Prof. Raghvendra Dubey

ISBN 978-953-307-856-4
Hard cover, 430 pages
Publisher InTech
Published online 08, February, 2012
Published in print edition February, 2012

Sex Hormones not only regulate reproductive function, but they also play a prominent role in the biology and physiology of several organs/tissues and in the pathophysiology of several diseases. During the last two decades, the information on the mechanisms of action of sex hormones, such as estrogens and androgens, has rapidly evolved from the conventional nuclear receptor dependent mechanisms to include additional non-nuclear, non-genomic and receptor-independent mechanisms. This highlights the need to update the current knowledge on sex hormones and their mode of action. Increasing evidence that exogenous/epigenetic factors can influence sex hormone production and action highlights the need to update our knowledge on the mechanisms involved. This book provides a systematic and updated overview of the male/female sex-hormones and their impact in the biology and physiology of various organs. Additionally, the book discusses their positive and negative association with the pathophysiology of various diseases (e.g. osteoporosis, cardiovascular-disease, hypogonadism, reproduction, cancer) and their therapeutic potential.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen