Trends in Endocrinology & Metabolism
October 2022, Volume 33, Issue 10, Pages 670-679
https://doi.org/10.1016/j.tem.2022.07.002
https://archimer.ifremer.fr/doc/00787/89859/

Energy as the cornerstone of environmentally driven sex allocation

Geffroy Benjamin [1, *]

[1] MARBEC, Univ Montpellier, Ifremer, IRD, CNRS, Montpellier, France

* Corresponding author : Benjamin Geffroy, email address : bgeffroy@ifremer.fr

Abstract :

In recent years, observations of distinct organisms have linked the quality of the environment experienced by a given individual and the sex it will develop. In most described cases, facing relatively harsh conditions resulted in masculinization, while thriving in favorable conditions promoted the development of an ovary. This was shown indistinctively in some species presenting a genetic sex determination (GSD), which were able to sex-reverse, and in species with an environmental sex determination (ESD) system. However, this pattern strongly depends on evolutionary constrains and is detected only when females need more energy for reproduction. Here, I describe the mechanisms involved in this environmentally driven sex allocation (EDSA), which involves two main energy pathways, lipid and carbohydrate metabolism. These pathways act through various enzymes and are not necessarily independent of the previously known transducers of environmental signals in species with ESD: calcium–redox, epigenetic, and stress regulation pathways. Overall, there is evidence of a link between energy level and the sexual fate of individuals of various species, including reptiles, fish, amphibians, insects, and nematodes. As energy pathways are evolutionarily conserved, this knowledge opens new avenues to advance our understanding of the mechanisms that allow animals to adapt their sex according to the local environment.

Highlights

► The quality of the environment directly or indirectly influences the sex of a variety of species. ► The phenotypic sex of some animals is linked to energy level at a given, key moment. ► Regulation of lipid and carbohydrate metabolism is involved in the process. ► This change in metabolism is accompanied by changes in stress hormone levels and epigenetic modifications. ► Findings in invertebrates, fish, amphibians, and reptiles open new avenues in our understanding of sex allocation in mammals.

Keywords : sex determination, lipid, glucose, stress, epigenetic, evolution

ESD and sex allocation

Environmental sex determination (ESD) has been shown to occur in about 15% of different vertebrate orders, mostly reptiles and fishes [1]. Those species that exhibit such a specificity do not possess sex chromosomes, by contrast to most mammals and birds that harbor respectively a XX/XY and a ZZ/ZW **genetic sex determination (GSD) system.** From an evolutionary point of view, ESD was shown to be favored over GSD if (i) the progeny have little or no control over the environment to which they will be exposed, (ii) the environment differentially affects the fitness of males and females, such that one sex would be better adapted to a given environment, and (iii) the environment is patchy, such that all sexes are produced [2]. This means that some individuals will develop into one sex when exposed to unfavorable conditions (i.e. a "bad" patch), while other that develop in favorable conditions (i.e. a "good" patch) will develop into the opposite sex [3]. This form of **sex allocation**, which concern the organisms itself, depends on the local environment it experiences (not the one of the parents) and can thus be termed **'Environmentally-driven sex allocation' (EDSA)**. EDSA, as defined here, thus includes different cases, ranging from species with GSD under environmental influence (**GSD + EE**), to species displaying pure ESD, in contrast to the conditional sex expression hypothesis, which has been proposed only for species with ESD [4]. The most extreme example of EDSA concerns sequential hermaphrodite species that are able to change sex during adult life depending on local (abiotic or biotic) conditions. The emergence of this strategy has been theorized many times, and involves the "size advantage hypothesis" first proposed by Ghiselin in 1969 [5]. In this context, sex change was hypothesized to occur to ensure the biggest individuals (independently of their sex) reaped the most benefits (i.e. reproductive success) [3,5–7]. This apply well for monogamous and protandrous species, where the reproductive success of the pair is limited by the size of the female, and for polygynous and protogynous species where the biggest individual (i.e. male) is able to control and mate with multiple females. Extending this reasoning, one might propose a similar theory for gonochoristic species with EDSA. Several species with EDSA exhibit a strong sexual size dimorphism, and depending on the reproductive strategy, one sex could have enhanced fitness relative to the other, by being bigger. Hence, in relatively favorable conditions for growth, the sex that gain the highest fitness by being large should be overproduced, while in relatively harsh conditions, the "less costly" sex will be produced.

From this theoretical framework, one might expect that both early growth rate and the **condition index** would be good proxies to predict the future sex of individuals displaying EDSA. In

mammals and birds, energy of the parents is also key to understand variation in offspring sex ratio, although sex bias does not necessarily go in the same direction to that often observed for species with EDSA (Box 1). Dr Ursula Mittwoch [8] first hypothesized that "divergence in energy metabolism is at the root of the differences between the sexes". More specifically, Mittwoch proposed a central role for mitochondria in the downstream effects of SRY and SOX9 in mammals that would explain the higher metabolic rate found in the testis and in the whole male fetus compared to in female fetuses [8]. Recent studies in non-mammalian species also pinpointed a specific role for energy pathways, but that acted upstream of the classical cascade of genes involved in **sex differentiation**.

Indirect evidence for a link between energy and EDSA

In recent decades, converging lines of evidence have shown that several species with a **sex determination** system that is influenced by the environment (this includes species with strict ESD) also display sexually dimorphic growth. Yet, all species displaying **temperature sex determination (TSD)** are ectothermic, so that the temperature directly regulates the metabolism of each individual, thereby influencing their growth pattern. This is well described in fishes and reptiles [9,10], but one question remains unanswered, does sex influence the differential growth rate? or does early growth rate influence sex determination?

Some studies may provide the answer by circumventing the problem of the direct effect of temperature on growth and by manipulating energy pathways. This can be attained by focusing on the parents, as shown in the jacky dragon *Amphibolurus muricatus*, a species of reptile exhibiting TSD, where mothers fed a poor quality diet produced highly male biased clutch (60%) compared to controls (38%) kept at the same temperature [11]. This could also be performed by manipulating the density of individuals, affecting the sex of individuals of some fish species (including some with **GSD + EE**), without changing the temperature. This most often resulted in a bias of males produced at high density [12–15]. Here again, the notion of "good" and "bad" patches is essential to understand the underlying mechanisms. Fish kept - or found - at a lower density may have better access to food, resulting in a higher growth rate. In those cases, the density would only impact the early growth rate, which, in turn, would be the real factor affecting sex determination, as proposed in eels, European sea bass and lampreys [13,16,17]. In these species, females generally grow better than males, so that the "size advantage hypothesis" first proposed to explain sex change in hermaphrodite animals [18] could also explain why individuals develop as females in relatively favorable environments

(Box 1). Interestingly, in European eels *Anguilla anguilla*, future females tended to display a better condition index than future males [13]. All these studies argue for an effect of the early growth rate on the sexual fate of individuals and suggest that other mechanisms than those affecting growth, likely linked to early differences in energy allocation, would affect the sex of these environmentally sensitive species.

Recent evidence for a major role of the energy balance in determining the sexual fate of distinct organisms

In *Drosophila*, sex determination depends on the number of X chromosomes [19]. The fact of having two X chromosomes triggers the production of a functional splicing factor called Sex-lethal (Sxl) only in future phenotypic females [19]. Sxl-dependent splicing of the transformer (tra) pre-mRNA allows the production of a functional Tra protein only in females and is thus considered to be a sex determination gene [19]. A recent study showed that this protein promotes a higher level of whole body fat storage in females, whereas lack of this functional Tra in males leads to less fat storage [20]. Higher fat storage and reduced fat breakdown in females means adult females display much higher starvation resistance than males [20]. Hence, the sex determining gene is directly involved in the differential pattern of energy allocation between sexes. However, it is worth noting that genetic manipulations failed at changing the phenotypic sex of individuals: genetic tra^{ko} females mutants did not sex-reverse [20]. Experiments on wild population of *Drosophila melanogaster* manipulating density and female size did not show any sex-ratio bias in the progeny [21], though multiple exposures of mother to cold treatments did skewed the sex ratio toward males and decreased glycogen and triglyceride contents [22]. The authors suggested that daughters would be more costly to produce or failed to completely hatch in link with this energetic stress [22]. From an evolutionary perspective, being a female require the opportunity to sufficiently accumulate whole body fat (which might led them to better tolerate starving, as a consequence); if the environment do not allow the mother doing so, then it might be adaptive producing more sons in this species.

Tra has also been shown to be essential in sex determination in *Caenorhabditis elegans* and promotes the development of oocytes [23]. In this species too, fat metabolism plays a role in regulating the fate of germ cells [24]. Free fatty acids, which can be modulated by food availability, are sensed by ACS-4 in the intestine, which then triggers the repression of MPK-1/MAPK activity, inhibiting the expression of the fem-3 gene and thus promoting the

expression of TRA-1 [24]. With this mechanism, animals living in relatively good food/nutrient conditions produce oocytes, thereby promoting reproduction.

A crucial role for lipid metabolism/the energy balance was recently identified in vertebrates too. Early starvation was indeed shown to induce sex-reversal (from female to male) of the medaka *Oryzias latipas*, even though they possess a genetic sex determination system (XX/XY)[25]. The starvation period induced repression of pantothenate metabolism, which in turn, reduced fatty acid synthesis and repressed somatic masculinization by inhibiting *dmrt1* expression, a key gene for sex differentiation [25].

In the European sea bass *Dicentrarchus labrax*, which possess a polygenic sex determination system that can be influenced by the temperature, genes involved in the regulation of lipids and carbohydrates were shown to play a key role in the future sex of individuals [26]. In this species, both the MAPK and the AMP-activated protein kinase (AMPK) signaling pathways were shown to be activated only in males, while future females presented a higher response to glucose (identified by enhanced expression of *eif2b5*, *ptprn*, *epha5*, *vsnl1*, *acvr2b*, *tiam1*, *ppp3ca* and *brsk2*) and tended to have more energy in their tissues (in Joules/mg) than future males [26]. It is likely that exposure to high temperatures modulated the genes involved in metabolism regulation in individuals presenting a weak genetic propensity to become female, thereby "forcing" them to become male [26].

In various amphibians' species, the temperature can affect the sex of individuals [27]. For instance, the agile frog (*Rana dalmatina*) exhibit a Genetic Sex Determination that could be influenced by temperature effects (**GSD + TE**) [28]. Exposure of tadpoles to relatively high temperatures (i.e. 30°C) during 6 days at three different life-stages resulted in an increase in the proportion of sex-reversed genetic females [28]. Heat-induced masculinization of genetic females was accompanied by a reduction of 5% of body mass and a reduction in the number of animals presenting fat bodies [28], concordant with the hypothesis that males would need less energy than females to successfully develop, at least in this species.

In the central bearded dragon (*Pogona vitticeps*) with GSD + TE, high temperature feminizes genetic (ZZ) males [29]. Interestingly the "lipoprotein lipase activity" gene ontology process was enriched in the bipotential gonad of ZZ females compared to true genetic (ZW) females [29]. Lipoprotein lipase plays a critical role in breaking down triglycerides into fatty acids that can either be used by the body as energy or stored in fatty tissue for later use [30]. This suggests very rapid reorganization of the lipid processes in the gonad following exposure to high temperatures. Overall, both the lipid and the carbohydrate energy-sensing pathways appears to be key in our understanding of EDSA (Box 2).

The most widely described pathways converge to the energy hypothesis

In reptiles, the most recent hypothesis regarding the sensing of the temperature initiating ESD and the transduction of the signal at the gonad level involve the redox regulation and calcium signalling (CaRe). The CaRe hypothesis posit that the change in Ca2+ and the increase of reactive oxygen species (ROS) levels caused by exposure to extremes temperatures will modify the CaRe status of the cell, thereby influencing conserved epigenetic processes involved in the regulation of sex-specific genes known to be pivotal for sex determination [29,31]. Since this hypothesis involve the cells that capture the environmental signal (likely through thermosensitive transient receptor potential channels and CDC like kinase 4), it would act upstream of the epigenetic processes described for reptiles [29,32] and established as Conserved Epigenetic Regulation of Sex (CERS) model in fishes [33,34]. Yet, in fishes, the activation of the stress axis has also been proposed as a physiological transducer of the environmental signal [35–37]. The three models (CaRe, CERS and Stress) might form a complex interplay, acting on individual metabolism and thereby affecting the energy balance pathways differentially, with downstream consequences on sex (Figure 1).

Following a stressful situation, the release of glucocorticoids can modulate glycogen metabolism to mobilize energy [38]. In that case, being stressed would be the first cause of changing the energy balance, later influencing the phenotypic sex via the proposed mechanisms (Box 2). When social cues (e.g. the density of individuals) influence food availability [13] or when the social hierarchy causes stress in some individuals [36], this would result in the same phenomenon as previously described (Figure 1). The change in light [39], photoperiod [40], oxidative stress [41] or pH [42,43] could also result in stress at the individual/cellular level as demonstrated in various fish species, with similar downstream consequences (Figure 1). Regarding temperature, the increased production of cortisol following a warming episode might simply transduce an increase in metabolism [44], which would then affect available energy and hence, sex (Figure 1). In reptiles, the link between corticosteroid and sex is not as evident as in fishes, since contradicting patterns were found: some studies detected a sex ratio bias toward males, other toward females and some studies did not detect any effects on sex ratio [35]. As highlighted above, most results converge to the CaRe and CERS hypotheses. Indeed, temperature has been shown to modulate methylation patterns in the promoters of key genes involved in sex differentiation in reptiles [45], but also in fishes [33]. Similarly, parental exposure to toxic substances or to a high density of individuals were found to affect the sex

ratio of subsequent generations through epigenetic mechanisms in fish [46,47]. Can temperature/toxins/crowding modulate metabolism at the level of the organism, and in turn, affect methylation/acetylation signal? This remains an open question, although recent findings in invertebrates may offer new avenues of investigation.

In *Caenorhabditis elegans* exposed to a period of acute starvation, the AMPK signaling pathway was shown to plays a crucial role in blocking modifications of the chromatin landscape, which was followed by inhibition of genes in the germ-line precursors during these unfavorable conditions [48]. However, in mutants (AMPK/aak-1/2) that did not display the AMPK signaling, critical chromatin modifications appeared in the primordial germ cells (PGCs) of emergent starved larvae that correlated with lower reproductive fitness for both the stress-exposed generation and subsequent generations that had not experienced the initial starvation event [48]. More recently, in another nematode: *Auanema freiburgensis*, phenotypic sex itself was shown to be inherited intergenerationally through that same energy sensing signal [49]. In this species, the first environmental trigger of sex determination is crowding of individuals that causes feeding stress at both the individual and cellular levels. High population density led to the production of hermaphrodite offspring that are more resistant to starvation and can reproduce by self-fertilization once the surrounding conditions improve ("good patch"). Activation of AMPK and of the insulin pathway in mothers induced the production of hermaphrodites, as did the maternal inhibition of the intracellular nutrient sensor mechanistic target of rapamycin complex 1 (mTORC1) [49] that stimulates anabolism when nutrients are abundant. It is likely that, in turn, these energy sensing pathways regulate the chromatin status in the maternal germline [49], as demonstrated for AMPK, which activates the transcription of specific genes through histone H2B phosphorylation [50]. Interestingly, the pattern of DNA methylation of genes involved in the regulation of lipids (i.e. diacylglycerol kinase delta, *dgkd*) and carbohydrate (i.e. insulin-like peptide receptor, *insr*) metabolisms, was the best predictor of sex in the oyster *Crassostrea gigas* [51]. More specifically, hypomethylation on the promoters of these genes resulted in their higher expression in females than in male gonads [51]. Overall, it seems possible that environmental factors first modulate the metabolism, which, in turn, impacts epigenetic mechanisms at the level of genes involved in sex determination and sex differentiation (Figure 1).

Conclusion

In most of the examples presented here, energy shortage during the period of sex determination or sex differentiation invariably resulted in masculinization, while enhanced access to food (and energy storage) resulted in feminization. Based on this literature review, I present a model in which the sexual phenotype results from a low level of available energy at a key developmental stage in species with GSD, GSD + EE and ESD (Figure 2). In the examples described above, adult females also store more fat than males. This pattern has been shown numerous times in fish [52] and insects [53], but also in mammals [54], including humans (the difference between the sexes is about 10%) [55,56]. From an adaptive point of view, a likely explanation involves the need for higher levels of energy for females to successfully reproduce, since the females of species that produce many propagules (insects, amphibians, fish and reptiles) obviously need more lipids to include in their eggs, than males need for sperm. For instance, a review conducted on 656 species estimated that the cost of developing eggs was an order of magnitude 3.5 higher than the cost of producing sperm [57]. Hence, to correctly interpret and predict the likelihood of the EDSA strategy, one should necessarily take the life-history trait of the species into account: If adult females need more energy than adult males to successfully reproduce, then a "good" patch will result in the production of more females. The opposite may hold true for males in some species presenting EDSA, such as protogynous fishes and type-2 TSD species: crocodilians and snapping turtles. In these type-2 TSD species, medium temperature (supposedly optimal for growth) led to higher production of males compared to extremes temperatures (supposedly stressful) that favor females. In these examples of protogynous fishes and type-2 TSD reptiles, males would need more energy because they exhibit strong dominance hierarchies [58,59], as exemplified for mammals (Box 1). But detailed mechanistic examples regarding the specific link between environment and energy balance are still lacking in these species (see outstanding questions below).

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Glossary

Condition index: Describe the proportion of muscle and fat relative to skeletal size. It is often viewed as a proxy of available energy.

Environmentally-driven Sex Allocation (EDSA): When the phenotypic sex depends on environmental conditions, independently of the sex determination system. It is adjusted to local conditions by the organism itself.

Environmental sex determination (ESD): sex is determined by external abiotic factors (such as temperature, water pH, oxygen), or biotic factors (e.g. density of conspecifics).

Genetic sex determination (GSD): sex is determined by the presence or absence of genes that are generally located on sex chromosomes.

Genetic sex determination with Environmental Effects (GSD+EE): Species that present a genetic sex determination that can be influenced by the environment (this includes, but is not limited to, temperature).

Genetic sex determination with Temperature Effects (GSD+TE): Species that present a genetic sex determination that can be influenced by external temperature

Sex allocation: the allocation of resources to male versus female reproduction in sexual species

Sex determination: the process by which a sexually reproducing organism initiates differentiation as a male or female.

Sex differentiation: the process by which the development of an undifferentiated primordial gonad into testes or ovaries takes place.

Temperature sex determination (TSD): Species that present a sex determination system that is only dependent upon external temperature.

Text boxes

Box 1: Where the Trivers-Willard hypothesis (TWH) meet, and differs from, EDSA

Sex allocation has been described and applied to both gonochoristic and hermaphrodite species [60]. In gonochoristic species, the focus has mainly been on mammals and birds, where the mother is able to adjust the sex of the progeny [61], particularly since the seminal study of Trivers and Willard [62], who predicted that in good conditions, mothers should produce more sons than daughters. This theory has been verified many times since then, in both birds and mammals [61,63], though many studies also failed to support it [63]. It is noteworthy that males are also able to adjust their offspring's sex ratio through different mechanisms [64]. In all cases, sex allocation strongly depends on environmental conditions experienced by the mother (most likely) but also by the father: this is the main difference with species with EDSA that directly adjust their sex to the local environment. Interestingly, energy is also the core of the TWH

where the condition index of the parents is essential to explain evolutionary constraints on sex allocation. In this case, more sons are produced in good conditions, but this is the results of different evolutionary pressures than those exposed for EDSA:

- 1. Physiological constraints: The primary sex ratio in mammals and birds is controlled by the parents, and once the sex fixed in these species with sex chromosomes, the environment could not affect the sex of the progeny, contrarily to species with EDSA. This possibly occurred because the environment during sex differentiation is much more stable in mammals (intra-uterine) and birds (mother incubate eggs), compared to most reptiles and fishes.
- 2. Life history traits: In mammals' species where the TWH has been supported, a relatively strong son will be able to monopolize many females, and will thus have enhanced fitness, relative to a large daughter. This is because many mammals' species are polygynous, and we could thus make a parallel with protogynous hermaphrodites' fishes, where a given female become the dominant male, thereby gaining access to the harem. This highlight that EDSA could also favor masculinization if the male gain higher relative fitness than the female by being bigger.

Box 2: What are the energy pathways?

Two main energy-balance pathways have emerged as crucial links between the environmental triggers and the sex of individuals, the lipid and the carbohydrate energy-sensing pathways (Figure I). Regarding lipids, activation of the protein kinase A (PKA) promotes fat breakdown (lipolysis) of triglycerides within lipid droplets [65]. When metabolic fuels are not sufficient to meet energy demands, a lipolytic cascade involving PKA is activated and results in the breakdown of energy stored in the form of triglycerides in free fatty acids and glycerol produced through the activation of the hormone-sensitive lipase (HSL) and the adipose triglyceride lipase (ATGL) (Figure I). The master metabolic regulator AMP-activated protein kinase (AMPK) is also traditionally considered to be an enzyme that mediates metabolic adjustment during starvation. Indeed, caloric restriction activates the AMPK pathways to maintain energy homeostasis by stimulating the ATGL [66,67]. But interestingly AMPK is also activated by certain drugs and xenobiotics, most of which act by inhibiting mitochondrial function [66]. When food is abundant and energy expenditure low, this prolonged positive energy balance leads adipocytes to take up circulating fatty acids, resulting in an increase in body fat mass [20].

The major pathway of free fatty acid uptake is mediated by lipoprotein lipase (LPL), an enzyme that hydrolyzes meal-derived triglycerides to produce fatty acids that can either be used or stored (Figure I). Overall, all the pathways that favor the catabolism of lipids (AMPK, PKA and LPL) are likely stimulated by environmental factors in future males, while those promoting anabolism of lipids (e.g. Panthothenate) are hypothesized to be stimulated in future females (Figure I). Regarding glucose, AMPK also plays a crucial role in controlling the mechanistic target of rapamycin complex 1 (mTORC1), which, in turn acts on glycolysis (Figure I). Glycolysis, which ultimately results in lipogenesis, is also under the control of insulin and its receptor (INSR). Interestingly, INSR is part of the sex determination gene ontology of the mouse, and is involved in testis differentiation [26,68], and activation of the insulin pathway also promotes the development of male cells in *Auanema freiburgensis* [49]. Conversely, overexpression of *insr* was rather found in the ovary of the oyster *Crassostrea gigas* [51].

Figure I: Key proteins, enzymes and vitamins (in blue) of energy-signaling pathways (in orange) are involved in the sexual fate of organisms displaying EDSA. The arrows in the dashed squares depict up-regulation or down-regulation in males and females. Black arrows indicate activation, red lines repression.

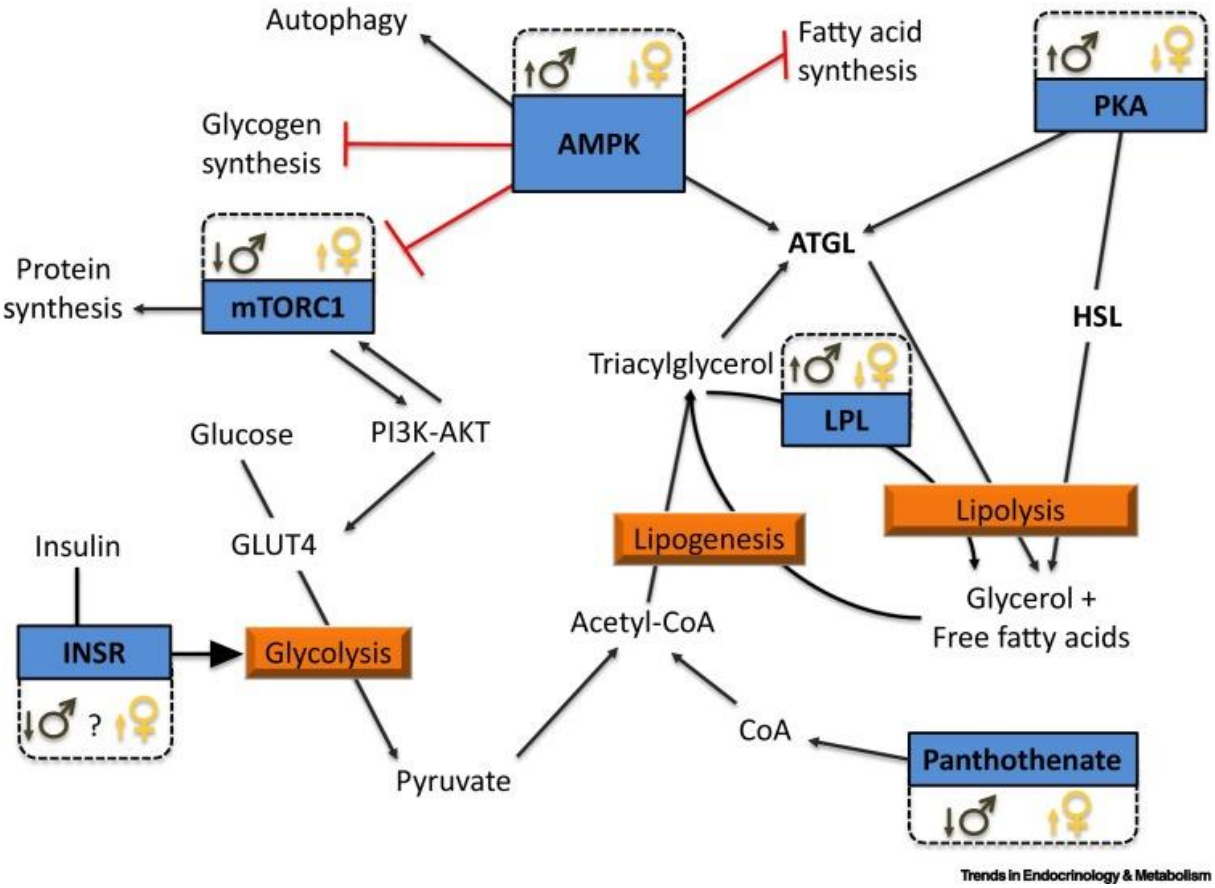

Figure 1) **How environmental factors modulate the energy balance through activation of the stress axis and changes in epigenetic gene regulation.** Biotic factors, inter-individual relationship and competition for food, lead to an increase (blue arrows) in stress hormones. Abiotic factors like water acidity (low pH, maroon arrow); a long-term change in the light regime (e.g. photoperiod, grey arrow) as well as a short term change in temperature (high or low), all led to increased production of stress hormones (i.e. cortisol and corticosterone) and reduced the individual's energy level, with downstream effects on sex. Similarly, long term exposure to high temperature could increase glucocorticoid production, through an increase in metabolism (red arrows) with similar effects. Both a change in temperature (red arrows) and exposure to xenobiotics (purple arrow) modify epigenetic processes that later affect sex (solid black arrow). The possibility that metabolism and energy pathways directly modulate (dashed black arrows) patterns of methylation and acetylation of key genes involved in sex determination or differentiation is highlighted in the text (see "The most widely described pathways converge to the energy hypothesis" part). Numbers in brackets indicate the corresponding reference in the text. ROS: Reactive oxygen species.

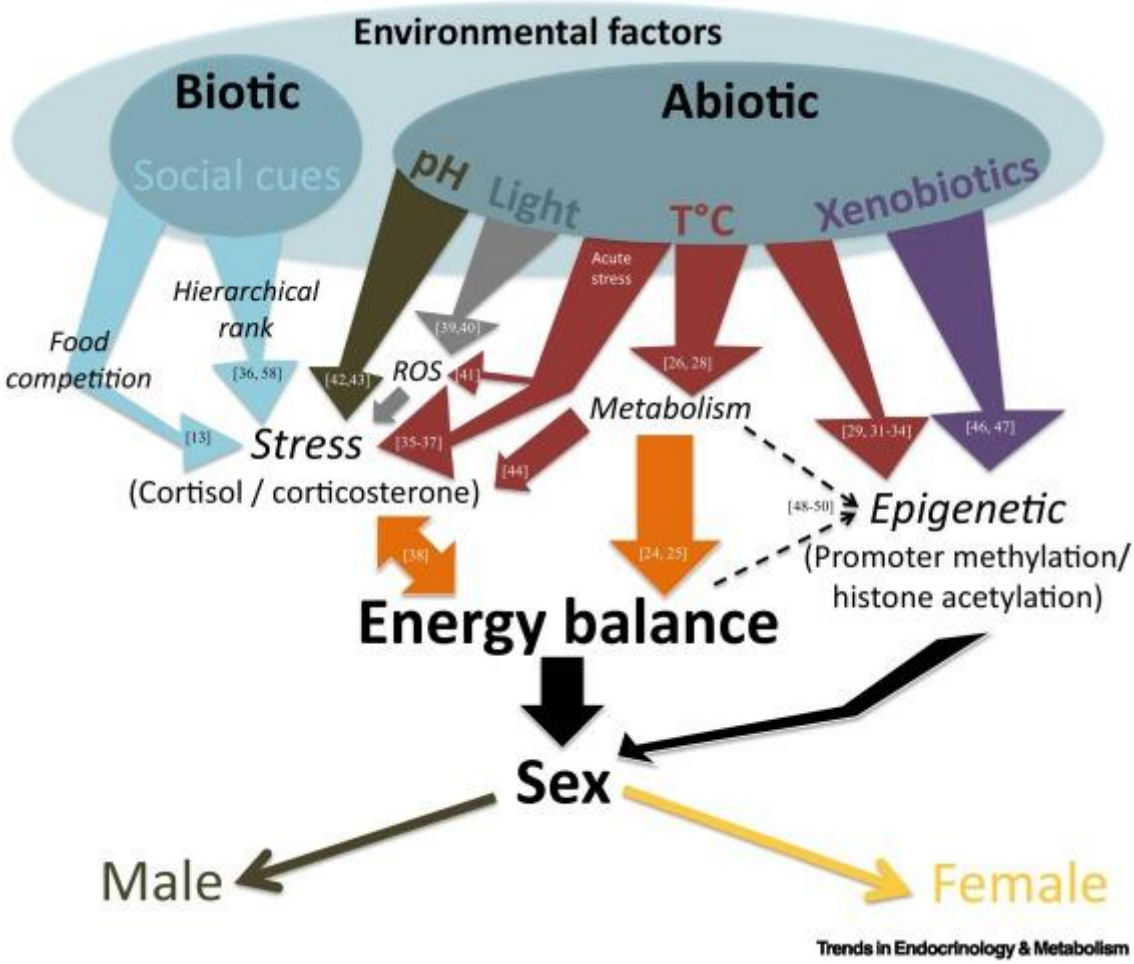

Figure 2) **Energy content during development affects the phenotypic sex of several species: a model**. Energy content could be indicated by, for instance, the condition index, the quantity of Joules/mg of tissue or the quantity of lipid accumulated. SDet: Sex determination period; Sdif: Sex differentiation period. For GSD species, the solid vertical purple line indicates that sex determination occurs at a precise, defined moment: conception, while dashed lines indicates that sex determination occurs during a more or less extended period in ESD and GSD + EE species. Similarly, dashed light blue vertical lines indicates the period of

sex differentiation that could varies between individuals of the same species. In species with a polygenic sex determination system, the specific gene combination is hypothesized to affect energy allocation differentially between individuals, which in turn, affects their sex.