Paper:
Consuegra, S. & Rodríguez López, C. (2016). Epigenetic-induced alterations in sex-ratios in response to climate change: An epigenetic trap?. *BioEssays, 38*(10), 950-958.
http://dx.doi.org/10.1002/bies.201600058

Epigenetic-induced alterations in sex-ratios in response to climate change: an epigenetic trap?

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Abstract

We hypothesize that under the predicted scenario of climate change epigenetically mediated environmental sex determination could become an epigenetic trap. Epigenetically-regulated environmental sex determination is a mechanism by which species can modulate their breeding strategies to accommodate environmental change. Growing evidence suggests that epigenetic mechanisms may play a key role in phenotypic plasticity and in the rapid adaptation of species to environmental change, through the capacity of organisms to maintain a non-genetic plastic memory of the environmental and ecological conditions experienced by their parents. However, inherited epigenetic variation could also be maladaptive, becoming an epigenetic trap. This is because environmental sex determination can alter sex ratios by increasing the survival of one of the sexes at the expense of negative fitness consequences for the other, which could lead not only to the collapse of natural populations, but also have an impact in farmed animal and plant species.

Introduction

Environmental cues are key for organisms to make decisions regarding their life history and behaviour, allowing them to anticipate environmental change and respond to it adaptively. This cue/ response correlation increases survival and reproductive success over evolutionary time, but can also become an evolutionary trap under sudden environmental change or when natural cues do not produce the expected outcomes, ultimately turning organisms' responses into maladaptive changes [1]. For example, changes in sex-ratios induced by climate change can result in common reproductive behaviours (such as competition or harrasment) becoming maladaptive, with dramatic consequences for the demographics and survival of the populations [2]. Some responses to environmental change are mediated by epigenetic mechanisms, which have the potential to regulate gene expression in the absence of underlying DNA mutations [3]. Environmentally induced epigenetic changes in response to climate change can speed up adaptation to the novel environmental or ecological conditions, but could also become epigenetic traps (i.e. epigenetic responses that arise in response to novel environmental or ecological cues and that can have negative consequences for population fitness) resulting in maladaptive phenotypes [4]. We hypothesise that epigenetically mediated changes in sex-ratios in response to environmental change (e.g. in species with environmental sex determination; ESD) could become an epigenetic trap in the long term, for example by increasing the survival of one of the sexes at the expense of negative fitness consequences for the other [5].

Human activities are amongst the main drivers of ecological change [6] and understanding how rapidly natural populations can adapt to human-mediated alterations has become essential for anticipating the consequences of change for species persistence [7]. The predicted scenario of climate change, with the associated loss of biodiversity, is probably the most alarming of the current human mediated-changes. Species can respond to climate change with variations in abundance, distribution and/or phenology (life cycle events). However, when environmental change is so rapid that individuals/species cannot migrate or acclimatise physiologically, natural populations may face extinction. Thus, the vulnerability of species to decline, genetic loss or extinction depends on their degree of exposure to change, sensitivity and adaptive capacity [8]. Some species can evolve rapidly

in response to environmental change by gene flow, selection on *de novo* mutations, or on standing genetic variation (pre-existing genetic variation) [9]. Standing variation is likely to lead to more rapid evolution in novel environments than new mutations, because beneficial alleles are immediately available and usually start at higher frequencies. In contrast, most *de novo* mutations decrease fitness and even when beneficial, new mutations initially occur only at low frequency in the population. Furthermore, traditional models of rapid evolution based solely on genetic mechanisms of inheritance [10,11] do not explain all phenotypic changes driven by rapid environmental change observed in contemporary populations, both when environment perturbations are induced by natural causes [4] or by human influences [12]. Such mismatch between genetic and phenotypic diversity can be based on the molecular diversity not being purely genetic in origin [13]. Therefore, non-genetic mechanisms of phenotypic plasticity inheritance induced by environmental change such as parental effects, cultural inheritance and epigenetic variation may also need to be considered as potential drivers of rapid evolution [3,14].

Ever since Waddington first proposed the term epigenotype in 1942 as the interface between genotype and phenotype [15] a plethora of interconnected mechanisms that function in complex and interactive ways has been added to our knowledge of how information is stored and utilized within the living cell [16]. Such mechanisms, which affect gene expression without altering their original sequence, are key players in eukaryotic biology by governing the use of the information encoded in DNA [3,14]. They underpin processes such as cell differentiation, development, suppression of repetitive elements, X-chromosome inactivation and parental imprinting [17]. Moreover, we are recently starting to understand how these mechanisms contribute to phenotypic variability and, critically, to individual adaptation to fluctuating environments [18]. DNA methylation is probably the most studied epigenetic mechanism. In humans, one-third of the DNA methylation differences are not associated with an underlying genetic variability, suggesting that both types of variation can arise independently [19]. Furthermore, recent studies show that variation in methylation patterns can be subject to natural selection [20]. Therefore, epigenetic variation might provide an initial step for selection during the course of evolutionary divergence [21]. Epigenetic modifications induced by the environment can also arise simultaneously in different individuals in a population [15], potentially

increasing the frequency of the adaptive phenotypes. Individual epigenetic differences have been observed, for example, in the resistance of plants to bacterial attack [22,23] and herbivory [24], in some cases involving correlated genotypic and epigenotypic responses [24] and suggesting that this environmentally induced variability is at least partially directed. Furthermore, it is now widely accepted that stress and developmental epigenetic variability can also occur in a random fashion [25] and may be the source of unexplained variability [26]. Such random variability, far from being deleterious, can increase the population resilience to stress by increasing the individuality of the components of such populations as targets for selection [27,28].

Epigenetic inheritance can enhance adaptability to environmental change

Non-genetic mechanisms of inheritance occur in all taxonomic groups for a variety of phenotypic traits [18], and epigenetic-mediated parental effects are known to influence phenotypic variation and fitness in plants and animals [29,30]. Although some epigenetic marks are known to be inherited [31], the ecological and evolutionary significance of epigenetic modifications in the adaptation of natural populations to environmental change is still unclear [32], particularly the inheritance of epigenetic changes induced by the environment [33]. For example, the role of adaptive epigenetic memory in enabling organisms to adapt to temperature change is only starting to be recognised in animals and plants [34]. Epigenetic variation can be inherited by the progeny in the form of epialleles (alternative methylation states of a gene) that regulate patterns of gene expression. This epigenetic variation should not be constrained by the limitations of genetic inheritance, and can act as a "surrogate" for genetic diversity in populations with low variability [35,36]. For example, a recent analysis of epigenetic differences between Caucasian-American, African-American, and Han Chinese-American human methylomes showed that the methylation of only 439 CpG sites were enough to explain phenotypic differences associated to their geographical origins, such as appearance, drug metabolism, response to external stimuli, sensory perception, and disease susceptibility [19]. Furthermore, epialelles can occur naturally as a consequence of genome rearrangements and can result in genetic incompatibility. This is the case in *Arabidopsis thaliana,* where a transposition event induces *de novo*

methylation of a pair of duplicated genes that affects fitness and causes genetic incompatibility that is maintained across several generations [37].

Epigenetic mutations can affect the pace and outcome of adaptation in several ways: on the one hand they can generate phenotypic variation in the absence of genetic variation [38], allowing populations to respond to environmental change even with low or no genetic diversity. Furthermore, in the absence of gene flow between populations, different environmental conditions can ultimately fix previously plastic epigenetic loci that will not be masked by genetic variability introduced by sexual reproduction [13]. In fact, certain epigenetic modifications such as hypermethylation of DNA can induce higher mutation rates of the underlying DNA backbone, which may be the basis of genetic assimilation (translation of epigenetic modifications to actual changes in the DNA sequence) as predicted by theory [18]. On the other hand, they can relax the selective constrains on genetic variation, speeding up the accumulation of genetic changes and the pace of adaptation [39]. Epigenetic changes also play a central role in the persistence and evolution of some asexual linages, particularly those that do not have mechanisms to generate genetic diversity [40]. In these species, epigenetic variation in the form of methylation [35], histone modification [41] or RNA interference creates phenotypic variation that is essential to survive environmental change [42]. However, epigenetic variation and genetic variation can also interplay in adaptive walks (i.e. the adaptation of populations suddenly placed in a new environment where they are poorly adapted, by fixing sequential *de novo* beneficial mutations) [39]. The reduced fidelity in the replication of methylated sites [43] and the ability of the epigenome to be altered by the environment, should make the methylome likely to evolve faster than the genome [31]. The influence of epigenetic mutations on adaptive walks seems to be determined by their reversibility (only those with relatively low reversibility will have an effect in adaptation) and their fitness effects [5]. Epigenetic mutations with fitness effects similar to those of genetic mutations can speed up the initial stages of adaptation but reduce the end fitness of adapted populations; in contrast epigenetic mutations with smaller effects relative to genetic mutations can slow adaptation down but increase end fitness, because in the initial stages of the adaptation process the selective pressures will be higher in genetic than epigenetic

mutations [5]. Heritable epigenetic marks that increase fitness should increase their frequency in a population, and their associated phenotypic variation in morphology, behaviour, or physiology can be transmitted to the offspring without being disrupted by recombination [44]. In fact, epigenetic divergence can be higher than genetic diversity and a stronger predictor of the strength of behavioural reproductive isolation, as has been shown in darters (a freshwater fish species), where epigenetic diversity could be influencing reproductive isolation among species [45]. Environmentally-induced epigenetic variation enables previously exposed individuals to respond more rapidly and effectively to a new stress event than those individuals not previously exposed [25]. Moreover, if inherited, it can prepare the offspring to better accommodate not only to the same conditions experienced by their parents [46], but also to other types of stress [47], facilitating the rapid response of populations to environmental change. Although there is some evidence of inheritance of environmentally-induced epigenetic marks, how common these events are still remains unclear, as does the role that they may play on the evolution of populations, given their transient nature [48].

Epigenetic responses can be maladaptive

Not all the environmentally induced epigenetic changes seem to be necessarily adaptive. In some cases, epigenetic mutations can speed up early adaptation but decrease the global fitness of the population in the long term. Some maladaptive epigenetic responses may be due to 'epigenetic traps'. In the superb starling (*Lamprotornis superbus*) which lives in unpredictable environments, levels of DNA methylation of the glucocorticoid receptor promoter, a key gene on the hypothalamic–pituitary–adrenal axis (HPA) that controls stress response, are affected by climatic conditions prior to egg laying, and can result in fitness advantage for males born after a harsh climatic season [49]. However, maternal glucocorticoid stress hormones received during embryo development can also lead to maladaptive responses if the changes they induce do not match the environment experienced by the offspring, and these maladaptive responses could be exacerbated under climate change scenarios, as with the predicted increase in extreme weather events [50]. Yet, maladaptive epigenetically-mediated phenotypic plasticity could also enhance population evolution in response to environmental change, even when the direction of the plasticity is opposite to the direction of the evolved changes [51]. For

example, in marine algae plastic phenotypic responses are good predictors for the evolution of the population in response to increasing CO_2 levels, particularly for populations inhabiting unstable environments, even if not for the direction of these changes in the long term [52]. Thus, even apparently maladaptive non-genetic responses, particularly those which are inherited, might have important evolutionary implications over longer time scales, although the extent of these implications is unknown [4]. While the genetic effects of climate change have only been studied for a few species, more is known about the physiological responses, especially for ectotherms, for which growth, reproduction and even sex determination are influenced by temperature [53]. Changes in reproductive behaviour and sex-ratios can impact population demography and ultimately viability [54], and growing evidence suggests an important role of epigenetics in the regulation of reproduction sex-ratios, both in plants and animals.

Epigenetic mechanisms can influence reproductive strategies

Increasing evidence suggests that epigenetic regulation of reproduction may be wide-spread among plants and animals at different levels (for an extensive review see [55]). Geyer and collaborators suggested that DNA methylation is key for the determination of phenotypic sexual differences and reproductive success of the human parasite *Schistosoma mansoni*, by modulating major aspects of ovary development and oviposition [56]. In some animals, epigenetic mechanisms can also affect reproduction in more indirect ways, for example the exposure of mice to endocrine-disrupting chemicals can result in epigenetic modifications that affect brain gene expression [57] and behaviour [58] in a sex-specific manner and over several generations, ultimately affecting reproductive success. In plants, epigenetic mechanisms have also been associated with the regulation of an array of traits linked to floral induction and development such as: flowering time [59], flower colour [60], sex-determination [61], floral symmetry [62], male sterility [63] and self-compatibility/self-incompatibility [64]. Epigenetic modifications associated with floral initiation and development can also occur indirectly, in pathways through which plants control stress tolerance [65]. In this way, environmental stress could potentially affect flowering by indirect effects in other traits such as reversion of male sterility under drought stress [66] or induction of pseudovivipary [67], which

ultimately influence the induction of dioecy from hermaphrodites. In fact, it has been hypothesized that dioecy and sex chromosomes originated, both in plants [68] and animals [69], from ancestral diploid hermaphrodites through the occurrence of a differentially methylated region near to a sex-controlling loci between a pair of otherwise genetically identical autosomes. Hypermethylation would then suppress the transcription of loci controlling gamete production, ultimately transforming hermaphrodites into males or females. Therefore, environmental changes could potentially modify the methylation patterns around sex-related loci ultimately determining the sex of individuals.

While plant biologists have extensively studied the evolutionary implications of asexual propagation and self-fertilization, very little is known on how these reproductive pathways affect metazoan adaptation and evolution [70]. Asexual lineages could be seen as having a limited capacity to cope with fluctuations in the environment, because they lack the ability to generate genetic diversity through recombination [71]. Similarly, the progeny of self-fertilising species can have reduced fitness as a consequence of inbreeding depression [72], and it has even been suggested that selfing should be an evolutionary dead end [73], a suggestion that remains contentious. However, based on the strategy of the fungal parasite *Verticilium dahlia*, Seidl and Thomma proposed that for animals that do not have conventional sexual reproduction, genomic rearrangements could be a driver for adaptation [74]. These rearrangements could be potentially regulated, at least in part, by epigenetics, i.e. methylation and/or histone modifications [74]. For species without a specific mechanism to generate genetic variation, epigenetics could represent a short-term acting mechanism that creates phenotypic diversity among genetically identical individuals [32], which can result in fitness differences between individuals and ultimately be the substrate of selection [39], allowing asexual and/or selfing lineages to face environmental change [42].

Mixed-mating species (species that alternate self-fertilisation and outcrossing) seem to balance the main genetic consequences of selfing (i.e. accumulation of deleterious mutations, inbreeding depression and loss of heterozygosity) with the relative advantages of outcrossing, such as greater offspring plasticity and genetic variability [75]. Selfing rates are variable in natural populations and are also influenced by non-genetic and ecological factors, such as mate availability

[76], although the regulatory mechanisms remains unclear [77]. The recently discovered relationship between inbreeding depression and DNA methylation suggests that epigenetic modulation may influence the magnitude of environment-dependent inbreeding depression [78,79], contributing to the variability of selfing rates observed in nature (Figure 1). Morran *et al*. [80] used obligate selfing or outcrossing mutants of *Caenorhabditis elegans* to demonstrate that while selfing populations declined in fitness over generations under stressful environments, outcrossing populations displayed no evidence of decline or even increased in fitness. This is similar to the situation observed in the mangrove killifish, where outcrossed offspring displayed higher heterozygosity and lower parasite loads than their selfed counterparts [81]. Interestingly, in this species, sex determination and ultimately mating strategy are associated with epigenetic modifications induced by environmental conditions. This plastic regulation of sex determination allows a mixed-breeding strategy, alternating self-fertilization and outcrossing, that allows the species to reproduce when mate availability is low while maintaining acceptable levels of genetic variability [82]. Taken collectively, these examples highlight how environmentally induced epigenetic changes can affect the reproductive strategies of both plant and animal species, and the potentially very profound effect of climate change on populations, through the alteration of sex ratios and mating strategies.

Does ESD have an epigenetic basis?

For some plant and animal species, the population sex ratio often varies as a consequence of environmental factors such as temperature [83], sexual selection [84], or human selective exploitation [85]. Sex adjustment can be an adaptive response to environmental change. For example, in polygynous species with sexual dimorphism such as ungulates, the Trivers–Willard model predicts that mothers with more resources should bias maternal investment towards sons [86], by adjusting the sex ratios at birth and/or providing differential maternal care of sons and daughters in order to increase their reproductive success [87]. However, the amount of adaptive sex ratio adjustment can be constrained by the mechanism of sex determination [86]. An array of different genetic sex determining mechanisms have been conserved over the course of vertebrate evolution [88]. Although sex determination is genetically determined in most species, ESD seems to be phylogenetically

widespread, occurring in plants, worms, nematodes, fish, and reptiles [89]. In plants, fish, amphibians and reptiles such mechanisms can range from purely genetic through to purely environmental, with combinations in between [90]. Temperature is the most common sex determining environmental variable in animal species with environmental sex determination [91]. For some of those, the temperature experienced during development can determine the sex of the offspring (temperature-dependent sex determination or TSD), and can be an adaptive response when the environment differentially affects males and females, according to the Charnov-Bull model [92]. In the Atlantic silverside (*Menidia menidia*), early-hatchlings grow larger before breeding and therefore offspring produced early in the breeding season would have a fitness advantage if they become female. In contrast, the offspring maximize their relative fitness by becoming males when they are produced late in the breeding season, for which temperature provides a cue [93]. A similar situation is found in the short lived lizard *Amphibolurus muricatus*, for which it was experimentally demonstrated that incubation temperature differentially affected males and females in terms of reproductive success, the fitness of each sex being maximized at its preferred temperature [94].

TSD has evolved independently multiple times in fish and reptiles [95]. In fish, small changes in temperature (1-2 °C) can significantly alter the sex ratio from 1:1 (males:females) up to 3:1 both in freshwater and marine species. As the rate of temperature change is expected to be higher in the ocean than on land. Given that ocean-water temperatures predicted to rise by 1.5 °C or even more over this century, the consequences of climate change could be particularly severe for marine species with temperature-mediated sex-determination [83]. For example, the synergistic effects of overfishing and climate change in sex ratios can result in range shifts and exacerbate fisheries declines. This decline could be particularly striking in species with sexual dimorphism [96]. Furthermore, the ability to control fish sex ratios is also critical for some aquaculture species (e.g. tilapia, bluegill, turbot), as very often one of the sexes displays better performance in captivity than the other. Moreover, monosexual populations are favoured since they avoid problems associated with unwanted reproduction [97]. Although in a number of commercial species the techniques to produce only one

sex are fairly developed and are widely applied [98,99], this is not the case for species that have not yet reached large scale production [99].

An association between warm incubation temperatures and female-biased sex ratios has also been widely reported in sea turtles [100]. Green, hawksbill and leatherback turtles typically produce between 15% and 36% of males. It is predicted that such sex ratios could decrease to 0.4%-2.4% by 2030 [101] and that only sites with intact natural vegetation, which could provide enough shade, will be able to offer conditions suitable for male hatchling [102]. Climate change can also exacerbate the effect of endocrine disrupting chemicals (EDCs) on the sex-ratios of species with TSD, potentially resulting in transgenerational epigenetic effects [58]. In zebrafish, clotrimazole, an antifungal chemical that inhibits the enzyme CYP19 key for estrogen synthesis, has been shown to have a synergic effect with high temperature that results in male skewed sex ratios [103], which are predicted to lower population growth, an effect that may be more pronounced on inbred than outbred populations [103].

The recent discovery of an epigenetic role in the regulation of a sex-determination related gene expression in sea-bass [104] represented a breakthrough for understanding TSD, and since then epigenetic regulation of sex-determination has been suggested for other fish and reptile species [82,105,106]. Navarro-Martin *et al*. [104] showed that the exposure of sea-bass embryos to high temperature resulted in male-based sex ratios, probably mediated by an increase in the DNA methylation at the aromatase (*CYP19a*) promoter region. A similar mechanism was found in the red-eared slider turtle, a species which displays temperature-dependent sex determination. In these turtles lower methylation levels were observed in CpG sites located in putative binding sites on the 5'-flanking region of the gonadal aromatase at the temperature where females are produced compared to the temperatures that favour males [105], suggesting that a temperature-dependent epigenetic mechanism could be acting in both cases. In the half-smooth tongue sole, a fish species with GSD and TSD, the sex determination gene pathway is also the target of DNA methylation regulation during sexual reversal in response to temperature [106]. In this sense, our own work suggests that temperature-related variation in the sex-ratio of the self-fertilising fish *Kryptolebias marmoratus* is

related to changes in methylation patterns between males and hermaphrodites. We examined methylation in the brain of males and hermaphrodite fish incubated at different temperature as embryos and found that methylation of at least two genes (*cyp19a*, *sox9a*) could be involved in modulating *K. marmoratus* sex-ratios in response to environmental change during embryo development [82]. As for other fish species, we found that the variation in proportion of males occurring with temperature change seems to be the result of the influence of environmental and genetic factors in sex regulation [83]. The epigenetic influence of increasing male ratios could be an adaptive trait for this species, as males can outcross with hermaphrodites, hence increasing the genetic diversity of offspring, and resulting in lower parasite loads compared to selfed offspring [81,107]. However, it is important to emphasise that although the observed epigenetic changes could result from a direct environment/locus interaction, they could also be a consequence of the locus activation.

Transitions between mating systems also occur in plants [108], although the idea that sex determination is fixed and genetically determined is prevalent in the current literature. Possibly the most extreme cases of mating system change are the induction of pseudovivipary and sex reversion. Pseudovivipary, which is more prevalent in perennial grasses, has been described as a functional form of environmentally induced flower/inflorescence reversion (i.e. switch from floral development to vegetative development) that leads to the formation of leafy shoots or bulbils [109]. Transitions between pseudovivipary and sexual reproduction have been observed in both directions in experimental set ups and in natural populations [110]. While flower reversion has been traditionally explained by genetic causes, Wang *et al.* [111] showed that in rice pseudovivipary is, at least partly, regulated by an epigenetic mutation. Moreover, Chiurugwi *et al.* identified a link between unfavourable environmental conditions for flowering and the appearance of *Festuca vivipara* (pseudoviviparous form) from genetically similar *Festuca ovina* (Flowering form) [67]. The lack of genetic differentiation between both forms in wild populations suggests that the induction of pseudovivipary could be of epigenetic nature and regulated by environmental conditions. In fact, pseudovivipary has been previously seen as insurance of successful reproduction under extreme

environments, such as high precipitation and humidity, strongly seasonal climates, high altitudes and latitudes, late-thawing habitats, or arid/semi-arid areas [109].

While still a matter of debate, environmentally labile sex determination (sex reversion) in plants is considered to be the result of the interaction between individual hereditary potencies and environmental factors. Plausible reasons for sex reversion in plants are a decrease in pollinator or mate availability [112], pollination intensity [113] and a female survival strategy under suboptimal environments (injury, disease and physico/chemical characteristics of the environment (e.g., light intensity, day length, temperature, soil fertility, relative availability of soil moisture and air chemistry) to avoid bearing the cost of fruit production [109]. Such changes have been observed in over 50 species (25 families) reported to have sex chromosomes. In some of these species, sex reversion seems to be mediated by epigenetic modifications (DNA methylation and histone acetylation) of specific regions of the sex chromosomes (reviewed in [114]). For example, in the white campion (*Silene latifolia*) genome hypomethylation induced hermaphrodites from genetically male plants [115]. In melon, the transition from male to female flowers in gynoecious lines (with only female or hermaphrodite flowers) takes place through epigenetic changes in the promoter of a transcription factor (*CmWIP1*), that leads to carpel abortion and results in unisexual male flowers [61]. This change seems to occur naturally through the insertion of a transposon and it has been deemed to be key for environmental adaptation and evolution, because it affects such a critical trait for reproduction.

Epigenetically mediated changes in sex-ratios could be maladaptive under climate change

Given the effect that environmental conditions seem to have on the epigenetic make-up and ultimately on mating systems and sex determination of certain species, it seems plausible that at least in some animals and plants, ESD is an epigenetically regulated adaptive trait that contributes to the adaptability and evolvability of populations under environmental stress. However, environmentally induced sex-ratio imbalances can be problematic when reproduction requires both sexes, as lack of suitable mates can negatively impact population growth and viability [54,83], an effect that may be more pronounced on inbred than outbred populations [103]. The fact that the epigenetic signatures of sperm (but not oocyte) are maintained in the embryos of zebrafish [116], suggests that the methylation

profiles of environmentally induced males can be inherited by their offspring without the need for subsequent temperature changes. Even if there is still scarce evidence of whether such temperature-dependent epigenetic changes affecting sex determination have an impact on a single generation or can be inherited, epigenetic variability can, nonetheless, contribute to adaptive changes over thousands of generations [5]. Furthermore, sex imbalances can become even more extreme due to the synergistic effect of ESD with sex-specific fitness payoffs shown previously both in plants [117] and animals [118]. Potentially, under the predicted global warming conditions, some dioecious tree species sex ratios would be male skewed not only by epigenetically regulated sex reversions but also by an enhanced risk of female mortality [117], which could lead to the collapse of entire populations due to the lack of available mates (Figure 2). Similarly, in animal species with environmental sex determination the alteration of sex-ratios in response to climate change could impact on population-level reproduction, challenging their long-term survival [83], an effect that can be, at least in part, mediated by epigenetic modifications in response to temperature changes [104]. Such scenarios would lend support to the model predictions by Kronholm and Collins [5], where epigenetic mutations can speed up early adaptation (i.e. higher survivability of males) but could also result in lower final fitness (collapse of population due to lack of females) in the long term, when their contribution to fitness is high.

The dynamics of sex ratios in farmed organisms is particularly important for agriculture, fisheries management, aquaculture and ultimately for food security. Understanding and controlling reproduction is essential in agriculture and aquaculture, in order to synchronise growth and maturation between sexes [97], as well as to maximise yield. In many farmed species sex selection is applied when one sex performs better than the other in culture [119], to avoid unwanted reproduction or simply in dioecious plants species because ultimately only females plants bear fruits. Environmentally driven changes in population sex ratio are particularly problematic in small populations (such as those of farmed species), where variation in reproductive success can have important consequences for the loss of genetic diversity and fixation of deleterious alleles [120]. Understanding the mechanisms underlying the role of the environment in sex-determination is particularly timely given the potential

effects of climate change on sex ratios, and its synergistic effects on range shifts. It is therefore critical to generate theoretical and empirical work to further understand the implications of environmentally induced epigenetic variation in the generation of epigenetic traps under the predicted scenario of climate change.

Conclusions and outlook

Given the overriding influence of sex-ratios and phenology for population persistence, epigenetic mechanisms of adaptation could be particularly relevant in view of the fast pace of climate change. Labile regulation of sexual expression can enhance an individual's genetic contribution to the next generation under unfavourable conditions. Epigenetic mutations can reduce selection pressures on genetic variation, while it is acting on the epigenetic variation [39], hence facilitating the survival of novel genotypes. In addition, epigenetic mutations may have a greater contribution in evolutionary response to many simultaneous environmental challenges [5], as predicted during global warming. However, under the current climate change scenario, such environmentally induced sex imbalances could become an epigenetic trap in the long term. Currently, most evidence of inheritance of environmentally induced epigenetic changes is based on laboratory studies, which lack relevant ecological conditions. In order to understand the importance of epigenetic inheritance on phenotypic plasticity and its adaptive value, long term empirical studies in the wild are needed to provide an environmental and ecological context. Only by studying animals in their natural environment will it be possible to test for the adaptive or maladaptive consequences of epigenetic polymorphisms. To test for the stability and functional relevance of epigenetic modifications, it would be particularly relevant to compare the offspring of the same parents during consecutive years, under variable environmental conditions, analysing the relation between the epigenome and the genome. This can be particularly challenging in natural populations, and claims for the establishment of new models for epigenetic research. Organisms which can alternate clonal and sexual reproduction such as *Daphnia* or *Arabidopsis*, where natural and laboratory populations are available, have already proved useful for this type of research. Furthermore, the mangrove killifish (*K. marmoratus*) is emerging as a promising

new model for vertebrates, because of its mixed-mating system strategy that results in natural populations with genetically very uniform lines living under variable environmental conditions [82].

Acknowledgements

Carlos Garcia de Leaniz and Tamsyn Uren-Webster provided very valuable discussion and comments.

Figure legends

Figure 1. The influence of environmental heterogeneity and fluctuating inbreeding depression on the evolution of mixed-mating (alternance between self-fertilisation and outcrossing). The space where complete selfing, mixed mating, and outcrossing are evolutionarily stable is defined by the mean and the variance of the inbreeding depression distribution, which can in turn be modified by the variable selective pressures resulting from environmental heterogeneity. The relationship between inbreeding depression and DNA methylation suggests that epigenetic modulation may influence the magnitude of environment-dependent inbreeding depression contributing to the polymorphism of selfing rates.

Figure 2. Theoretical effect on short/long term population survivability of an epigenetic trap induced by increased temperatures affecting sex ratios. Green arrows show effects supported with experimental data. Red arrows show predicted effects without experimental data available to our knowledge. (Figure adapted by authors from Tognetti, 2012 ; Kronholm and Collins, 2015 and O'Dea et al, 2016).

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