REVIEW ARTICLE

Sciara as an experimental model for studies on the evolutionary relationships between the zygotic, maternal and environmental primary signals for sexual development

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Abstract

Sex determination refers to the developmental programme that commits the embryo to either the male or the female pathway. There are a plethora of mechanisms via which gender is decided. These mechanisms can be classified into three main categories depending on the origin of the primary, genetic, sex-determination signal, which can be zygotic, maternal or environmental. In the dipteran *Sciara*, the zygotic signal is a consequence of the maternal signal, and this in turn can be a consequence of the environmental signal. This makes *Sciara* a unique experimental model for studying the evolutionary relationships between the three primary signals triggering sexual development.

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Introduction

Perpetuation by sexual reproduction is the rule within the animal kingdom. Males and females are different at the morphological, physiological, and behavioural levels. This sexual dimorphism results from the integration of two processes: sex determination and sexual differentiation. Sex determination refers to the developmental programme that commits the embryo to either the male or the female pathway. The genes underlying this programme are the sex-determination genes. Sexual differentiation refers to the expression of the sex-cytodifferentiation genes (which are controlled by the sex-determination genes), the expression of which give rise to the formation of sexually dimorphic structures that characterize the male and female adults.

The animal kingdom possesses a wealth of mechanisms via which gender is decided (Bull 1983; reviewed in Sánchez 2008). This is no more evident than among insects, among which all known types of sex-determination mechanism are represented. These mechanisms can be classified into three main categories depending on the origin of the primary, genetic, sex determination signal, which can be zygotic, maternal or environmental.

Among the zygotic signals, sex determination can be based on chromosome differences, one sex being homomorphic XX (female) and the other heteromorphic XY (male) for the sex chromosomes. Examples are *Drosophila*, tephritid flies (*Ceratitis, Bactrocera* and *Anastrepha*) and *Musca*. In *Drosophila*, the Y-chromosome plays no role in sex determination, and the instructive primary genetic signal is based on the ratio between the X and the autosomal chromosomes (recently, it has been reported that it is the number of X chromosomes rather than the X/A signal that has an instructive role in *Drosophila* sex determination (Erickson and Quintero 2007, and references therein). In the tephritids and in *Musca*, however, the Y chromosome carries the male-determining factors, though in some *Musca* populations, this factor may, instead, be located on one of the autosomes. In other cases, such as the lepidopterans, the male is the homomorphic sex (ZZ) and the female the heteromorphic sex (ZW) (the letters Z and W are used to distinguish this system from the XY system) (for a revision on the relationships between ZW and XY sex chromosome systems see Ezaz *et al*. (2006) and

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references therein). In other orders, such as Hymenoptera, the chromosomal difference between males and females is based on haploidy/diploidy, e.g., in *Apis mellifera* females are diploid and males are haploid (Cook 1993; Beye *et al*. 2003).

The blowfly *Chrysomya rufifacies* provides an example of maternal sex determination in which no heteromorphism of the sex chromosomes exists, the sex of the zygote being exclusively determined by the genotype of the mother. In this insect, two types of female exist: gynogenic females, which produce only female offspring, and androgenic females, which produce only male offspring. The gynogenic females are heterozygous for the gene *F*, which encodes a maternal factor that accumulates in the oocytes during oogenesis and which imposes female development on the zygotes derived from them. Androgenic females and males are homozygous for the recessive *f* allele, which does not produce the maternal factor (Ulleric 1975).

In most species, the sex of an individual is fixed at fertilization, for example in *Drosophila, Ceratitis, Bactrocera, Anastrepha* and *Musca*; the chromosomal constitution of the zygote being a direct consequence of the chromosomal constitution of the gametes (Bull 1983). However, in other species, such the dipteran families Cecydomyiidae (White 1973; Stuart and Hatchett 1991) and Sciaridae (DuBois 1933; Metz 1938), the chromosomal differences determining sex are brought about by the specialized behaviour of the sexual X-chromosome. A paradigmatic example is *Sciara* (figure 1). All zygotes start with the XXX constitution; the loss of either one or two X chromosomes determines whether the zygote becomes XX (female) or X0 (male) (reviewed in Sánchez 2008, and references therein).

Finally, environmental sex determination is also seen among the insects. The ratio of males to females (sex ratio) in the population of some *Sciara* species depends on the temperature at which the insects are raised (reviewed in Sánchez 2008, and references therein).

Thus, the three primary signals for sex determination (zygotic, maternal and environmental) occur in *Sciara*. As it will be shown below, these signals are concatenated. The sexual development followed by the embryo depends entirely on its chromosome constitution (zygotic signal), either XX (female) or X0 (male), which is formed by the elimination of either one or two X chromosomes, respectively, from the initial XXX embryo; this elimination process being controlled by a factor (maternal signal) provided by the oocyte. Finally, in some *Sciara* species the presence of this maternal factor depends on the temperature (environmental signal) at which these species are raised. Thus, in *Sciara*, the zygotic signal is a consequence of the maternal signal, and this in turn can be a consequence of the environmental signal. This makes *Sciara* a unique experimental model for studying the evolutionary relationships between the three primary signals trigering sexual development.

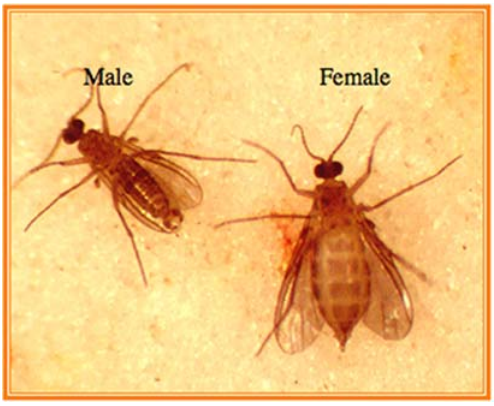

Figure 1. Photographs showing a male and a female of *Sciara ocellaris*. Courtesy of Dr M. Fernanda Ruiz.

The life cycle of *Sciara*

Figure 2 shows the chromosome behaviour of sciarid flies. The initial XXX chromosome constitution of the *Sciara* zygote is a direct consequence of the chromosomal constitution of the gametes: oocytes are X and sperm are XX, which are sister chromatids of paternal origin (Metz 1938). When the zygotic nuclei reach the egg cortex, one paternal X chromosome is eliminated in the somatic cells of embryos destined to be females (XX) (see example in figure 3) and two are eliminated in those destined to become males (X0) (DuBois 1933; Perondini *et al*. 1986; reviewed in Goday and Esteban 2001). Therefore, in the formation of the chromosomal signal in sciarids an 'imprinting' process must occur in one of the parents, which determines that the chromosomes to be eliminated are of paternal origin. Historically, the term 'imprinting' was coined to describe selective identification of paternal chromosomes in sciarids (Crouse 1960).

The pole cells, which are set apart at the fourth cleavage (DuBois 1933; Berry 1941; Perondini *et al*. 1986), do not eliminate X chromosomes at the same time as the nuclei in the somatic regions of the embryos. Elimination of one paternally derived X chromosome occurs later, at the beginning of the germ band segmentation; just one of the two paternal X chromosomes is eliminated in both male and female embryos (Berry 1941; Rieffel and Crouse 1966; Perondini and Ribeiro 1997; Perondini 1998). Consequently, germ cells are XX and will produce either oocytes or sperms depending on the sex of the gonad, whether it is female (ovary) or male (testis), respectively. Meiosis in females is orthodox, whereas in males is highly specialized (figure 2) (reviewed in Goday and Esteban 2001, references therein). During the first meiotic division, all the paternally derived chromosomes are eliminated into a cytoplasmic bud so that only the maternal-derived chromosomes remain. During the second meiotic division, the two chromatids of each autosome segregate normally, i.e., one is located into the previous cytoplasmic bud and the other chromatid will form the chromosome complement of the sperm. However, the two chromatids of the

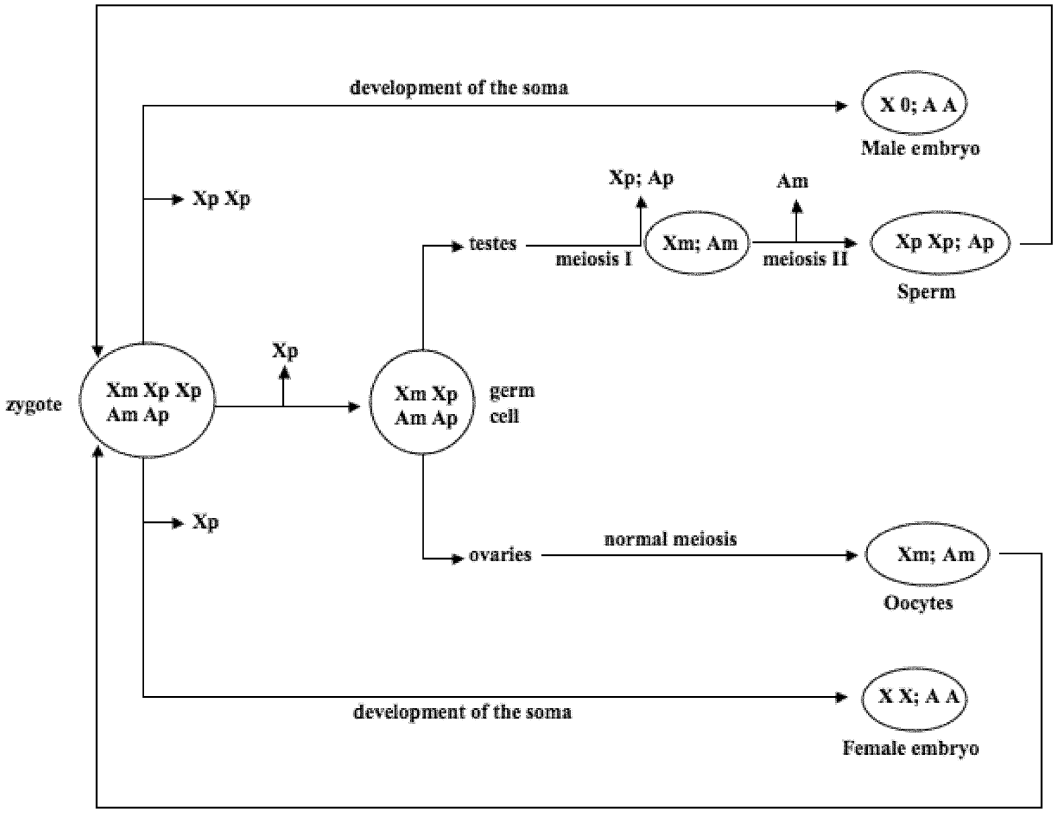

Figure 2. Diagram showing the chromosome behaviour of sciarid flies. The maternal and paternal origin of the chromosomes is denoted by m and p, respectively. X, X chromosome; A, haploid set of autosomes. Modified from Sánchez and Perondini (1999).

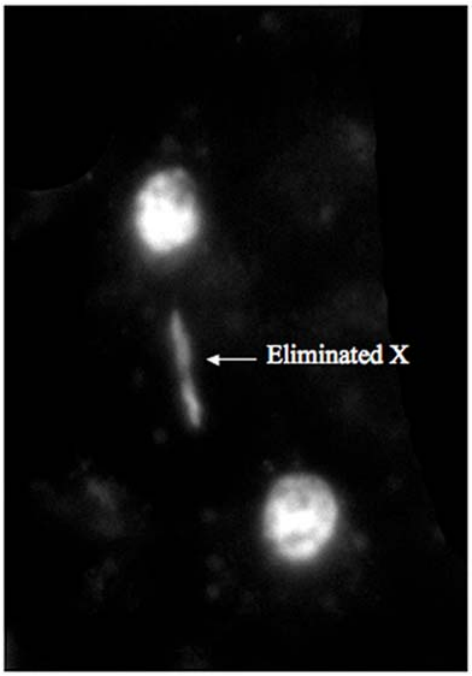

Figure 3. X chromosome elimination in an early embryo of *Sciara ocellaris*. The photograph shows telophase with the two nuclei in the poles and the eliminated X chromosome at the middle. DAPI staining. Courtesy of Dr Clara Goday.

X chromosome do not segregate and are incorporated together into the sperm. Hence, each event of meiosis in *Sciara* males renders one instead of four spermatozoids, containing a haploid set of autosomes and two X chromosomes, all of maternal origin (Gerbi 1986; Fuge 1994; Esteban *et al*. 1997).

Among the sciarids there are some species in which the females produce offspring of only one sex—the unisexual (or monogenic) species. These females are either androgenic (male producers) or gynogenic (female producers). Some species are bisexual (digenic or amphigenic), i.e., the progeny is a mixture of males and females. The sex ratio (number of males versus females) of the progeny of each female, however, is highly variable, deviating from 1:1. Nevertheless, at the level of the whole population, the sex ratio follows a normal distribution around this value (Metz 1938).

The monogenic species *Sciara coprophila*

Gynogenic and androgenic females differ in the presence of a special X′ chromosome in the former: these females are X′X, whereas androgenic females are XX. The XX females produce only X0 males, which do not receive the X′ chromosome. The gynogenic females produce both gynogenic and androgenic females in a 1:1 ratio (Moses and Metz 1928;

Metz and Schmuck 1929; Metz 1938; Gerbi 1986). The system functions as if the gene encoding the factor for either female or male production is located on the X chromosome. There is an inversion in the X′ chromosome that prevents its recombination with the homologous X chromosome, thus retaining in the X′ the factor for female production (Crouse 1960).

The elimination of the paternally derived chromosome is maternally controlled. The gynogenic females produce two classes of oocytes carrying either the X′ or the X chromosome. Both contain the maternal factor that controls X chromosome elimination, thus these two types of oocytes are predetermined to eliminate one X chromosome of the two inherited from the father. The X′-oocytes and X-oocytes contribute to the production of the gynogenic and androgenic females, respectively, of the following generation. The XX androgenic females produce an unique class of X-oocytes without the maternal factor involved in X chromosome elimination; therefore these oocytes are predetermined to eliminate the two X chromosomes inherited from the father. Consequently, the X-oocytes from the androgenic females contribute to the production of X0 males of the following generation (figure 4).

The digenic species *Sciara ocellaris*

In *S. ocellaris*, the female parent determines the sex of the offspring (Liu 1968; reviewed in Sánchez and Perondini 1999, references therein), as it happens in *S. coprophila*. However, whereas in *S. coprophila* females produce only either female-producer or male-producer oocytes, in *S. ocellaris* each female generates both classes of oocytes. Thus, in this species the offspring of each female is composed of males and females, although the sex ratio varies widely among the females (Metz 1938; Davidheiser 1943; Mori *et al*. 1979). This variation depends on temperature: at 18–20°C, the sex ratio distribution, although variable, shows a median at approximately 50%, but at 24–29°C the sex ratio moves towards the production of more females. This change in sex ratio is not caused by a higher mortality among males, but by a transformation of male into females; i.e., an increase in the number of embryos that eliminate one instead of two paternal-derived X chromosomes (Nigro *et al*. 2007).Temperature-shift experiments have shown that the temperature-sensitive period for the determination of the final sex ratio is from the mid-pupa stage to the emergence of adult females (Nigro *et al*. 2007), the period during which oogenesis takes place (Berry 1941). Hence, the *S. ocellaris* females produce at distinct temperatures different ratios of oocytes that either contain or do not contain the maternal factor for X chromosome elimination. In *Sciara* females, the number of oocytes is fixed during the early larval stages and no further mitosis occurs nor any new oocytes produced in the pupal/adult stages (Berry 1941).

A strain of *S. ocellaris* has been described that carries *sepia*(s)-X chromosome (an X chromosome that carries the *sepia* mutation as a marker) that causes alterations in the sex ratio towards the production of more males, without differential mortality of female embryos (Mori *et al*. 1979). Moreover, the offspring of this strain contain as well a significant number of gynandromorphs (individuals with some portions of the body typically male X0 and others typically female XX); i.e., individuals in which some somatic nuclei eliminate only one and other nuclei eliminate both two paternal-derived X chromosomes

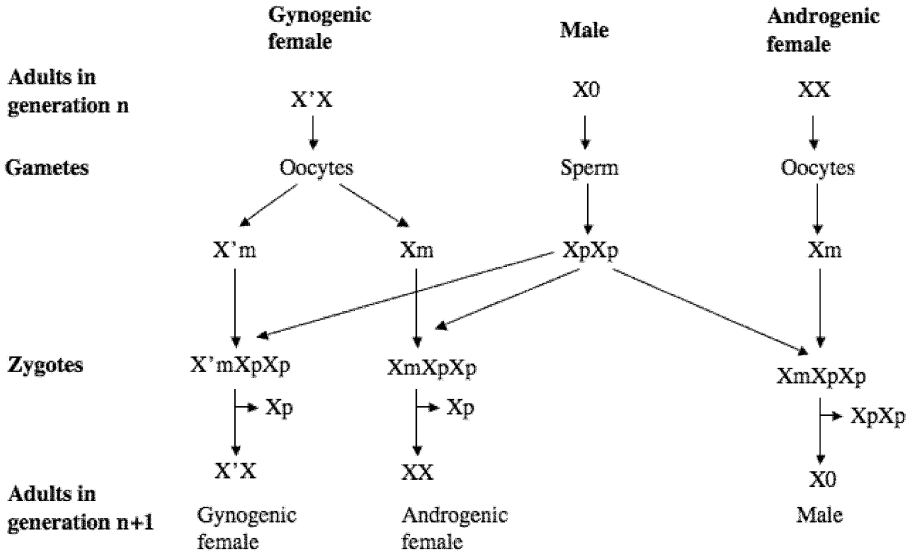

Figure 4. Diagram showing the production of gynogenic and androgenic females as well as males in *Sciara coprophila*. The maternal and paternal origin of the chromosomes is denoted by m and p, respectively. Modified from Sánchez (2008).

(Mori *et al*. 1979; Mori and Perondini 1980). Therefore, the *sepia* strain makes more male-producer than female-producer oocytes, i.e., more oocytes that contain the maternal factor for X chromosome elimination.

Sciara matogrossensis shows both monogenic and digenic reproduction: some females behave as digenic, others as gynogenic, some others as androgenic and still others produce offspring with one predominant sex (either male or female). These offspring sex ratios being maintain in successive generations, thus suggesting that the control of offspring ratio (either elimination of one or two X chromosomes) may involve more than one locus, at least, more than one pair of alleles (Rocha and Perondini 2000).

A model for the control of differential X-chromosome elimination in *Sciara*

Spontaneous deviations and mistakes in X-chromosome elimination can occasionally occur in the sciarids (reviewed in Sánchez and Perondini 1999). Two basic types of error have been reported. Quantitative errors, involving the number of paternally-derived X chromosomes that are eliminated (i.e., some zygotic nuclei of embryos derived from oocytes predetermined to eliminate one X chromosome end up eliminating two X-paternally derived chromosomes instead). These quantitative errors produce gynandromorphs. Qualitative errors, in which the X chromosome eliminated is the one inherited from the mother rather than either of the two inherited from the father. This type of error leads to mosaic flies, either male or female, bearing a mixture of tissues with the normal chromosome set plus tissues patroclinous for the X chromosome.

Errors in X-chromosome elimination have been experimentally induced by the UV irradiation of embryos (Perondini *et al*. 1987; Guatimosin and Perondini 1994; reviewed in Sánchez and Perondini 1999). The UV doses used did not penetrate more than a few micrometers into the cytoplasm and therefore had little effect on egg survival. If irradiation occurred at the early cleavage stage, when only the cytoplasm of the insect egg was affected by the UV radiation (the nuclei remained deep in the endoplasm and were thus shielded), gynandromorphs (individuals with quantitative errors) were produced. If irradiation took place at the syncytial blastoderm stage, by then the nuclei have migrated to the periplasm and thus both the cytoplasm and the nuclei were UV targets, both types of errors, gynandromorphs and mosaics (individuals with qualitative errors) were produced. Both types of errors were reversed after photo-reactivation treatment of the UV-irradiated embryos (Guatimosin 1996).

Collectively, the results obtained from *Sciara* species allow us to conclude that the mechanism of X-chromosome elimination is similar in monogenic and digenic species: a maternal factor is produced during oogenesis, which collects in the oocyte and then governs the elimination of the X chromosome in the developing zygote.

A model has been proposed for the control of differential X-chromosome elimination in sciarid flies. This model is compatible with the basic features of the sciarid chromosomal system, some of which are outlined here (for a more detailed discussion see Sánchez and Perondini 1999). This model is based on the following assumptions: (i) a chromosomal factor (CF) bind to the paternal X chromosome causing its elimination. This factor is produced in limiting amounts and at similar concentrations in both male and female embryos. Whether CF is maternal or zygotic is irrelevant. (ii) A maternal factor (MF) determines the number of paternal X chromosomes to be eliminated. This maternal factor interacts with CF, inactivating it, so that the CF–MF complex cannot interact with the paternal X chromosome. Therefore, the number of X chromosomes eliminated depends on the amount of free CF, which in turn depends on the amount of MF. (iii) Imprinting, in relation to the identification of the X chromosome to be eliminated, occurs in the maternal chromosomes and not in the paternal counterparts. The imprinted state is manifested by the inability of the maternal X chromosome to bind CF; consequently, this chromosome cannot be eliminated.

The question arises as to whether it is necessary to assume the existence of two factors, MF and CF, instead of only one, MF, as proposed by Saint-Phalle and Sullivan (1996). In their one-factor model, it is assumed that the maternal factor promotes, rather than prevents, the segregation of the paternal-derived X chromosome. This maternal factor would directly interact with the paternal-derived chromosome to promote separation of its sister chromatids, whereas neither the maternal-derived X chromosome, nor the autosomes need this separation factor for normal segregation. This implies that imprinting, in relation to the identification of the X chromosome to be eliminated, occurs in the paternal-derived X chromosome. The imprinted state in this case is manifested by the requirement of this chromosome to bind the separation factor. This model does not, however, explain the existence of mosaic sciarids in the UV irradiation experiments.

Why is *Sciara* a good experimental model?

Summarizing, in *Sciara* sex determination follows the XX/X0 mechanism: XX zygotes will develop as females and X0 zygotes as males. Yet the final chromosome constitution of the zygote, which stars as a XXX zygote, depends on a maternal factor contributed by the oocyte. This maternal factor, controlling the number of X chromosomes that are eliminated (either one or two), acts as the primary genetic signal defining the sexual development that the zygote will follow. This implies the existence of a concatenation of two primary, genetic signals: the maternal signal preceding and determining the final zygotic one. This occurs in the monogenic species, such as *S. coprophila*, where the amount of maternal product deposited in the oocytes is genetically determined and then independent of environmental conditions.

But in other digenic species, such as *S. ocellaris*, the amount of maternal factor depends on the temperature at which the insects are raised. Hence, in these species the environmental signal (temperature) determines the amount of maternal product (maternal signal) placed in the oocytes, which in turn determines the number of X chromosomes to be eliminated in the zygote and then its final chromosome constitution (zygotic signal) that determines its sexual development. Consequently, *Sciara* is unique in that it offers the possibility of studying the evolutionary relationships between the three main primary signals triggering sexual development. Furthermore, the comparative study between the monogenic and the digenic species will allow exploring the cues underlying the reciprocal changes between environmental and genetic systems of determining gender.

Imprinting, chromosome elimination and sex determination in insects

Sex determination in *Sciara* depends on two combined processes. One refers to the elimination of one or two X chromosomes and is controlled by a maternal factor. The other refers to the fact that the eliminated chromosomes are always those derived from the father so that an imprinting process occurs. The UV-irradiation results are compatible with the proposal that imprinting takes place during oogenesis and affect the maternal chromosomes, thus preventing their elimination in the future embryo. These processes are not specific to dipteran sciarid insects.

There exist other insect species where the chromosomal differences determining sex are brought about by the specialized behaviour of chromosomes during the first stages of embryonic development. This is the case of the coccids (scale insects, order Homoptera). Sex determination in primitive coccids is usually decided by the XX (female)–X0 (male) sex chromosome mechanism. However in some groups, the differential elimination (diaspidid coccids) or heterochromatization (lecanoid coccids) of all the paternal chromosomes gives rise to functional diploid or haploid zygotes, which develop into females or males respectively, so that there exits as well an imprinting process in these insects (White 1973).

The sex ratio of the offspring of some female coccids can fluctuate widely and is subject to environmental influence (Nelson-Rees 1960). Ageing the females before allowing them to mate alters the sex ratio in favour of males—a phenomenon not caused by any differential increase in the mortality of female zygotes. Rather, this is explained by changes in sexual dichronism (i.e., the deposition of male and female embryos at different times during oviposition), the pattern of which can be altered by maternal ageing (James 1937, 1938; Brown and Bennett 1957; Nelson-Rees 1960). Some parthenogenetic coccids are known to produce male or female embryos depending upon whether or not the heterochromatization of one chromosomal set occurs. Nevertheless, the proportion of male to female embryos is very low (about 5% males and 95% females are produced); abnormal or degenerating embryos are often observed, which are almost certainly males. Since heterochromatization naturally occurs in these coccids, no prior passage of the chromosomes through spermatogenesis is required (Nur 1963). Together, all these evidence suggests that the genome of the mother determines the heterochromatization (lecanoid) or elimination (diaspidid) of the inherited paternal chromosomes in coccid embryos. In addition, both lecanoid and diaspidid mechanisms have an associated imprinting process to distinguish between the maternal and paternal chromosomes. This chromosome behaviour is similar to that seen with respect to the elimination of the paternal X chromosome in sciarid flies. It has been proposed that the model for the control of differential X-chromosome elimination in the sciarids mentioned above can be applied to the heterochromatization or elimination of paternal chromosomes in coccids. According to this model, heterochromatization or elimination is controlled by a maternal factor, with the maternal-derived chromosomes imprinted so that they do not suffer either fate (for details see Sánchez 2008).

Maternal factors and imprinting have been also invoked to explain sex determination in the hymenopteran insects, such as *Nasonnia vitripennis*, which belong to the parasitoid wasp group Chalcidoidea. Gender in these insects is regulated by haploidy/diploidy: males are haploid and develop from unfertilized eggs, whereas females develop from fertilized eggs. A new model has been put forward to explain sex determination in these parasitoid hymenopterans (Beukeboom *et al*. 2007). The maternal effect genomic imprinting sex determination (MEGISD) model proposes that the sex of the zygote depends on the activity of the *zygotic sex determiner* (*zsd*) gene, whose function determines female development. A *maternal effect* gene (*msd*) causes imprinting of the *zsd* gene during oogenesis so that the female-inherited *zsd* allele is not active in the zygote. Consequently, haploid zygotes develop as males because they carry the imprinted *zsd* allele inherited from the mother. Diploid zygotes develop as females because the paternally inherited *zsd* is not imprinted and then becomes expressed.

Acknowledgments

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References

Berry R. O. 1941 Chromosome behavior in the germ cells and development of the gonads in *Sciara ocellaris. J. Morphol.* **68**, 547–583.

Beukeboom L. W., Kamping A. and 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**, 371–378.

Beye M., Hasselmann M., Kim Fondrk M., Page Rejr and Omholt S. S. 2003 The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**, 419–429.

Brown S. W. and Bennett F. D. 1957 On sex determination in the diaspine scale *Pseudaulacaspis pentagona* (Targ.) (Coccoidea). *Genetics* **42**, 510–523.

Bull J. J. 1983 *Evolution of sex determining mechanisms.* The Benjamin/Cummings Publishing Company, Menlo Park, USA.

Cook J. J. 1993 Sex determination in the hymenoptera: a review of models and evidence. *Heredity* **71**, 421–435.

Crouse H. V. 1960 The nature of the influence of X-translocations on sex of progeny in *Sciara coprophila. Chromosoma* **11**, 146–166.

Davidheiser B. 1943 Inheritance of the X chromosome in exceptional males of *Sciara ocellaris* (Diptera). *Genetics* **28**, 193–199.

De Saint-Phalle B. and Sullivan W. 1996 Incomplete sister chromatid separation is the mechanism of programmed chromosome elimination during early *Sciara coprophila* embryogenesis. *Development* **122**, 3775–3784.

DuBois A. M. 1933 Chromosome behavior during cleaveage in the eggs of *Sciara coprophila* (Diptera) in relation to the problem of sex determination. *Z. Zellforsch. Mickrosk. Anat.* **19**, 595–614.

Erickson J. W. and Quintero J. J. 2007 Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in *Drosophila. PloS Biol.* **5**, e332.

Esteban R., Campos C. C., Perondini A. L. P. and Goday C. 1997 Role of microtubules and microtubule organizing centers on meiotic chromosome elimination in *Sciara ocellaris. J. Cell Sci.* **9**, 610–629.

Ezaz T., Stiglec R., Veyrunes F. and Marshall-Graves J. A. 2006 Relationships between vertebrate ZW and XY sex chromosome systems. *Curr. Biol.* **16**, R736–R743.

Fuge H. 1994 Unorthodox male meiosis in *Trichosia pubescens* (Sciaridae): chromosome elimination involves polar organelles degeneration and monocentric spindles in first and second division. *J. Cell Sci.* **96**, 188–201.

Gerbi S. A. 1986 Unusual chromosome movements in sciarid flies. In *Germ line–soma differentiation* (ed. W. Hennig), pp. 71–104. Springer, Berlin.

Goday C. and Esteban R. 2001 Chromosome elimination in sciarid flies. *BioEssays* **23**, 242–250.

Guatimosin V. M. B. and Perondini A. L. P. 1994 Lethal effects of far UV on preblastoderm embryos of *Sciara ocellaris* (Diptera, Sciaridae). *Braz. J. Genet.* **17**, 25–34.

Guatimosin V. M. B. 1996 *Efeitos da radiaçao ultavioleta no processao de eliminaçao do cromossomos X nos núcleos somáticuos de diptero Sciara ocellaris (Diptera: Sciaridae).* Ph.D. thesis, Universidade de Sao Paulo, Sao Paulo, Brazil.

James H. C. 1937 Sex ratios and the status of the male in Pseudococcinae (hem. Coccidae). *Bull. Ent. Res.* **28**, 429–461.

James H. C. 1938 The effect of the humidity of the environment on sex ratios from over-aged ova of *Peudococcus citri* (Risso). *Roy. Ent. Soc. London, Proc. Ser. A Gen. Ent.* **13**, 73–79.

Liu P. Y. 1968 *Estudo biologico de cultura de Bradysi tritici (Diptera, Sciaridae) parasitada por gregarina.* M.S. Dissertation, Departamento de Biología, Instituto de Biociencias, Universidade de Sao Paulo, Sao Paulo, Brazil.

Metz C. W. and Schmuck M. L. 1929 Unisexual progenies and the sex chromosomes mechanism in *Sciara. Proc. Natl. Acad. Sci. USA* **15**, 863–866.

Metz C. W. 1938 Chromosome behavior, inheritance and sex determination in *Sciara. Am. Nat.* **72**, 485–520.

Mori L., Dessen E. M. and Perondini A. L. P. 1979 A gene that modifies the sex ratio in a bisexual strain of *Sciara ocellaris. Heredity* **42**, 353–357.

Mori L. and Perondini A. L. P. 1980 Errors in the elimination of X chromosomes in *Sciara ocellaris. Genetics* **94**, 663–673.

Moses M. S. and Metz C. W. 1928 Evidence that the female is responsible for the sex ratio in *Sciara* (Diptera). *Proc. Natl. Acad. Sci. USA* **14**, 928–930.

Nelson-Rees W. A. 1960 A study of sex predetermination in the mealy bug *Planococcus citri* (Risso.). *J. Exp. Zool.* **144**, 111–137.

Nigro R. G., Campos C. C. and Perondini A. L. P. 2007 Temperature and the progeny sex-ratio in *Sciara ocellaris* (Diptera: Sciaridae). *Genet. Mol. Biol.* **30**, 152–158.

Nur U. 1963 Meiotic parthenogenesis and heterochromatization in a soft scale, *Pulvinaria hydrangeae* (Coccoidea: Homoptera). *Chromosoma* **14**, 123–139.

Perondini A. L. P., Gutzeit H. O. and Mori L. 1986 Nuclear division and migration during early embryogenesis of *Bradysia tritici Coquillet* (syn. *Sciara ocellaris*) (Diptera: Sciaridae). *Int. J. Insect Morphol. Embryol.* **15**, 155–163.

Perondini A. L. P., Gutzeit H. O. and Sander K. 1987 Double abdomen induction by UV in *Bradysia tritici* (sy. *Sciara ocellaris*, Sciaridae): sensitive stages and conditions for photoreversal. *Roux's Arch. Dev. Biol.* **196**, 268–272.

Perondini A. L. P. and Ribeiro A. F. 1997 Chromosome elimination in germ cells of Sciara embryos: involvement of the nuclear envelope. *Invertebr. Repr. Dev.* **21**, 20–30.

Perondini A. L. P. 1998 Elimination of X chromosomes. In *Genome analysis in eukaryotes: developmental and evolutionary aspects* (ed. R. N. Chatterjee and L. Sánchez), pp. 38–55. Narosa, New Delhi, India, and Springer, Berlin.

Rieffel S. and Crouse H. 1966 The elimination and differentiation of chromosomes in the germ line of *Sciara. Chromosoma* **8**, 120–165.

Rocha L. S. and Perondini A. L. P. 2000 Analysis of the sex ratio in *Bradysia matogrossensis* (Diptera, Sciaridae). *Genet. Mol. Biol.* **23**, 97–103.

Sánchez L. and Perondini A. L. P. 1999 Sex determination in sciarid flies: a model for the control of differential X-chromosome elimination. *J. Theor. Biol.* **197**, 247–259.

Sánchez L. 2008 Sex-determining mechanisms in insects. *Int. J. Dev. Biol.* **52**, 837–856.

Stuart J. J. and Hatchett J. H. 1991 Genetics of sex determination in the Hessian fly, *Mayetiola destructor. J. Hered.* **82**, 43–52.

Ullerich F. H. 1975 Identification of the genetic sex chromosomes in the monogenic blowfly *Chrysomya rufifacies* (Caliphoridae, Diptera). *Chromosoma* **50**, 393–419.

White M. D. J. 1973 *Animal cytology and evolution*, 3rd edition. Cambridge University Press, Cambridge, UK.

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