HYPOTHESIS

About females and males: continuity and discontinuity in flies

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Through the decades of relentless and dedicated studies in Drosophila melanogaster, the pathway that governs sexual development has been elucidated in great detail and has become a paradigm in understanding fundamental cell-fate decisions. However, recent phylogenetic studies show that the molecular strategy used in Drosophila deviates in some important aspects from those found in other dipteran flies and suggest that the Drosophila pathway is likely to be a derivative of a simpler and more common principle. In this essay, I will discuss the evolutionary plasticity of the sex-determining pathway based on studies in the common housefly, Musca domestica. Diversification appears to primarily arise from subtle differences in the regulation of the key switch gene transformer at the top of the pathway. On the basis of these findings I propose a new idea on how the Drosophila pathway may have evolved from a more archetypal system such as in M. domestica. In essence, the arrival of an X counting mechanism mediated by Sex-lethal to compensate for X-linked gene dose differences set the stage for an intimate coupling of the two pathways. Its precedent recruitment to the dosage compensation pathway allowed for an intervention in the regulation of transformer where it gradually and eventually' completely substituted for a need of transformer autoregulation.

Evolutionary studies on sex determination in insects

In contrast to the myriad developmental decisions that have to be taken to correctly pattern the early embryo, determination of its sexual fate seems to operate on a much less complex level. In fact the process of sex determination can be reduced to a simple binary decision between only two alternative fates, male or female. This difference in complexity, the multitude of different positional fates determined by elaborate regulatory circuitries on the one hand and the simple binary decision between male or female on the other hand, makes the latter a more shapeable play-dough for adaptive changes in the course of evolution. At least this seems to hold true in insects which—thanks to the great impact of developmental studies in *Drosophila melanogaster*—have become preferred objects for studies of the evolution of developmental mechanisms. While little or no differences in the basic body patterning mechanisms have been observed among *Drosophila* and other dipteran species, the strategies used for determining the sexual fate vary extensively. At least, at the level of the primary signal that instructs the embryo which of the two programmes to execute we find a bewildering diversity. For instance, many species make use of dominant Mendalian cues, commonly referred to as *M* when determining male development or *F* when determining the female fate. It is the absence or presence of these genetic signals which determines the sex of the embryo. In other species it is the genetic make-up of the mother that decides whether her progeny will be male (arrhenogenic) or female (thelygenic). Some species even involve environmental cues, e.g., temperature or population density, to resolve the sexual programme of the embryo. In the best-documented case, *D. melanogaster*, the embryo bases this decision on the numbers of X chromosomes present in the diploid zygote. Presence of two X chromosomes imposes female development while male development follows when only one X is present (Cline and Meyer 1996; Erickson and Quinter 2007). This rather peculiar mechanism of counting X chromosomes as a signal for determining the sex appears to be common among drosophilids but is rarely seen outside the genus *Drosophila* (Schutt and Nothiger 2000; Saccone *et al*. 2002).

Though many different types of sex-determining signals seemed to have evolved in the insect world, it has been postulated some years ago that these differences may simply reflect variations on a common theme (Nöthiger and Steinmann 1985). The authors of this postulate argued that despite

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differences in character these signals may act on a cascade of conserved genetic switches. In principle, this pathway is defined as being composed of three constituents: the primary signal, a key gene which responds to this signal in an ON/OFF manner and an executor at the end of the pathway which acts as a bifunctional switch directing either male or female differentiation. Differences in the systems can be explained by varying only the signals in the cascade but leaving the core transductional components below untouched. This led to the idea that a conserved core pathway exists in all insects and that the observed differences merely reflect subtle changes in the genetic architecture of the pathway. No experimental evidence was given for this idea at the time.

Some 10 years later Wilkins (1995) proposed that sex-determining pathways have evolved in a retrograde fashion. This idea was in part derived from the hypothesis proposed for the evolution of biochemical pathways. The enzyme at the end of a pathway must have been the first on scene to manufacture a functional end product. New enzymes were then sequentially recruited to the pathway to produce intermediates step by step in a reverse order. Projecting this idea on the pathway of sex determination, we expect the bottom-most gene, which is directly responsible for proper expression of dimorphic traits, to be the first that has arrived on the scene. The bottom level constitutes a bottleneck in the pathway restraining adaptive changes. On the contrary, the mode of how the activity of this global regulator is controlled could have been more malleable and taken different routes during evolution. So, this level may have become the starting point for divergence by sequentially adding different regulators.

It became obvious that an experimental approach to test these ideas must involve a reference system such as *D. melanogaster* and a small but representative number of other dipteran species for comparison. The reference system provides genetic and molecular information on the pathway that can be used to retrieve matching information (candidate genes) from other species. A direct comparison of the molecular and genetic data is expected to eventually disclose the extent of conservation in the pathways that determine sex.

What is the minimal common denominator for proper execution of the sexual programme?

For quite some time it has been assumed that the gene *doublesex* (*dsx*) acts as the final and only executor of the selected sexual programme. The often depicted linearity presents a simplistic view of the pathway and is deceptive in that it neglects other branches which must be controlled in parallel to *dsx*. In *Drosophila* there is clear evidence that *dsx* is not the sole target of the signal. In fact the gene fruitless (*fru*) and presumably a number of yet unknown genes are under the same control as *dsx*. Thus it is rather a coordinated regulation of all these targets which is imperative to the proper expression of the sexual phenotype. At least in *Drosophila*, limitations of *dsx* as the only executor of the selected fate have been clearly demonstrated. For instance, forced expression of the male activity of dsx, *DsxM*, does not suffice to transform karyotypic XX *Drosophila* individuals into fertile males. Of course, the case in *Drosophila* is complicated by the fact that Y, which is absent in karyotypic females, harbours a number of genes needed in spermatogenesis. Another problem in *Drosophila* is, when a germ line with a female karyotype is placed in a completely normal testicular environment it cannot differentiate into sperm. But even if these reverted males could produce mobile sperm, they would not behave as males and would not be able to copulate. So, *dsx* alone does not suffice for a full implementation of the sexual programme. It is reasonable to assume that *dsx* in other dipteran insects is no different in this respect. We can expect that targets like *fru* are also present in other flies and have a conserved function in the proper sex assignment of the CNS (central nervous system). For instance, our current studies in the housefly strongly suggest that a *fru* homologue controls male courtship behaviour (N. Meier, S. C. Käppeli and D. Bopp, unpublished data). So, if we take for granted that *dsx* is only one of several targets of the sex-determining pathway, the question arises, what then is the minimal requirement to implement a fully functional male or female programme.

Only a gene that has the capacity to select and execute a full version of one of the alternative programmes would meet the standard of a master switch. Rather than *dsx*, its upstream regulator is a more likely candidate. In fact, the *transfomer* gene (*tra*) in *Drosophila* does control all aspects of the sexual phenotype including mating behaviour and other CNS-derived-dimorphic traits. In this respect *tra* is closer to the role of a master switch for the proper execution of the programme than its target *dsx*. This level of control may thus be widely conserved in insects. Of course, this assumption can be simply tested by searching for *tra* related genes in other insects. However, the candidate gene approach was considerably impeded by the finding that the *tra* gene ranks amongst the most rapidly evolving proteins within the family of drosophilids. Thus, the structural divergence of *tra* is high, making it particularly difficult to isolate it from distantly related species (O'Neil and Belote 1992). Exploiting its overlapping linkage to a well-conserved gene, Pane *et al*. (2002) and Saccone *et al*. (2002) succeeded in isolating a highly diverged *tra* homologue from the genome of the medfly, *Ceratitis capitata.* This gene appears to act as the master switch in sexual development of the medfly: when on, it directs all aspects of female development and, when off, normal male development follows. Furthermore, the *Ceratitis* homologue of *dsx* was identified as one of its downstream targets. Similar findings in other Tephriditae suggest the cascade *tra*→*dsx* is widely used among Acalyptratae to transmit the sex-determining signal (Lagos *et al*. 2007; Ruiz *et al*. 2007). More recently, we were able to isolate the *tra* orthologue in the Calyptratea species, *M. domestica*. Functional studies and structural analysis of mutant alleles proved that the *Musca transformer* gene (*Mdtra*) gene corre-

sponds to *F*, the key switch gene in *Musca* sex determination (Hediger *et al*. 2010). Likewise, expression studies of the *tra* orthologue in other Calyptratea species such as *Lucilia cuprina* (Concha and Scott 2009) and the tsetse fly, *Glossina morsitans* (Hediger *et al*. 2010) point to a similar switch role in sex determination.

Previous studies by our research group demonstrated that zygotic activation of *F* requires the presence of an active *F* in the female germ line (Dubendorfer and Hediger 1998). This maternal contribution seems to be responsible for the activation of the zygotic gene suggesting that *F* involves an autoregulatory function to sustain its active state. In line with this prediction, the *Mdtra* gene is regulated at the splicing level involving an autoregulatory function of its own RNA-binding product (Hediger *et al*. 2010). Also, we found that substantial amounts of female-specific transcripts of *Mdtra* are deposited into the egg during oogenesis. We propose that this maternal source of TRA activity is needed to engage the *tra* feedback loop zygotically. Once activated, this feedback mechanism guarantees a continuous production of active TRA to remember the selected female fate and, of course, to execute the corresponding programme. Male development, in contrast, results from a mere prevention of the establishment of this loop. Pane *et al*. (2002) proposed that the male dominant factor in *Ceratitis* represses the female splice mode of *tra* and thereby prevents the production of functional TRA products. As a consequence the loop can no longer be perpetuated and will finally collapse. Absence of TRA constitutes a signal for male development. What makes this idea particularly attractive is that the postulated inhibitory activity exerted by the male factor needs to be expressed only transiently, a feature often found associated with primary signals e.g. as in *Drosophila* and *Musca* (Sánchez and Nöthiger 1983; Hilfiker-Kleiner *et al*. 1993).

Continuity is a female quality

Putting the pieces together from the studies performed in the housefly, the following picture emerges: *Mdtra* occupies a central position in the sex-determining pathway of the housefly. Once activated in the early zygote, its activity is maintained throughout the life cycle and guarantees that cells remember the selected female fate and differentiate accordingly by regulating downstream targets such as *Mddsx* into the female mode of expression. This female determining activity of *Mdtra* is maintained by a positive feedback mechanism. This loop serves as a cellular memory to enforce the proper execution of the female programme. The critical question arises on how the loop is initiated in the early embryo and, as a direct consequence, how the female fate is selected. I propose that the loop is continually active in the distaff side without a defined start point or end point. In principle daughters inherit the self-sustaining *Mdtra* activity from their mothers and pass it on to the next generations of females. There is no discontinuity in this lineage and thus no reassessment of the female fate is required. I base this on the assumption that eggs are predisposed to develop along to the female pathway. The mother supplies the egg with active products of *Mdtra* and other essential cofactors such as *Mdtra2* to guarantee that *Mdtra* remains expressed in the active female mode state. In this respect, female development can be regarded as a 'default setting' meaning that females have an intrinsic tendency to produce females again. It seems reasonable that females have a preference to produce females. In the extreme case, when only females are produced, parthenogenetic reproduction can be adapted as a measure to guarantee the survival of the species. A preference for male development, on the other hand, will in the extreme case lead to an evolutionary dead end. Because of the proposed intrinsic disposition for female development, I like to refer to this idea as the 'female continuity' hypothesis (figure 1).

But what does it take to bear males? I propose that male development is a product of inflicted discontinuity on the female lineage. The sole measure that needs to be taken to impose male development is to interrupt the perpetual activity of *Mdtra.* For this purpose, it suffices to prevent the establishment of the loop in the early embryo. Once the loop collapses, cells loose their female identity and become reprogrammed to resume a male fate. Thus, male development presumes an irrevocable loss of *Musca tra* activity. The intrinsic instability of an autoregulatory loop makes it a facile target for destruction. A single blow can decrease the activity needed to sustain the loop below a critical threshold from which it cannot recover. I believe that there are many ways how the loop can be irreversibly silenced. In this respect, there is not much restriction on the performance range of a male determinant. Interference at any regulatory level, e.g., transcriptional, posttranscriptional, or posttranslational may be sufficient to let the loop collapse. For instance, the efficiency by which complete male sex reversal is induced in *Musca* with single injections of *Mdtra* or *Mdtra2* dsRNA in early embryos clearly demonstrates the susceptibility of the female-determining system. It may be feasible to create an artificial *M* by introducing an inheritable inverted repeat construct that produces dsRNA of *Mdtra* or *Mdtra2*. Such a construct is expected to mimic the male determining activity of naturally occurring *M*.

The straightforward manner in which a male determiner can impinge on female development may explain the manifold occurrence of *M* factors in natural *Musca* populations (Hiroyoshi 1964; Rubini and Palenzona 1967; Wagoner 1969). There has been a long standing dispute whether these different *M* are transposed versions of the same gene to different chromosomal locations or whether these are different genes that have adopted the function of a dominant male determiner. In my viewpoint, the latter is the more attractive explanation. If a particular *M* loses its efficiency of interrupting the female cycle and causes a female-biased shift in sex ratio, it can be compensated by the emergence of a new loop-breaker. It is particularly the dominant destructive

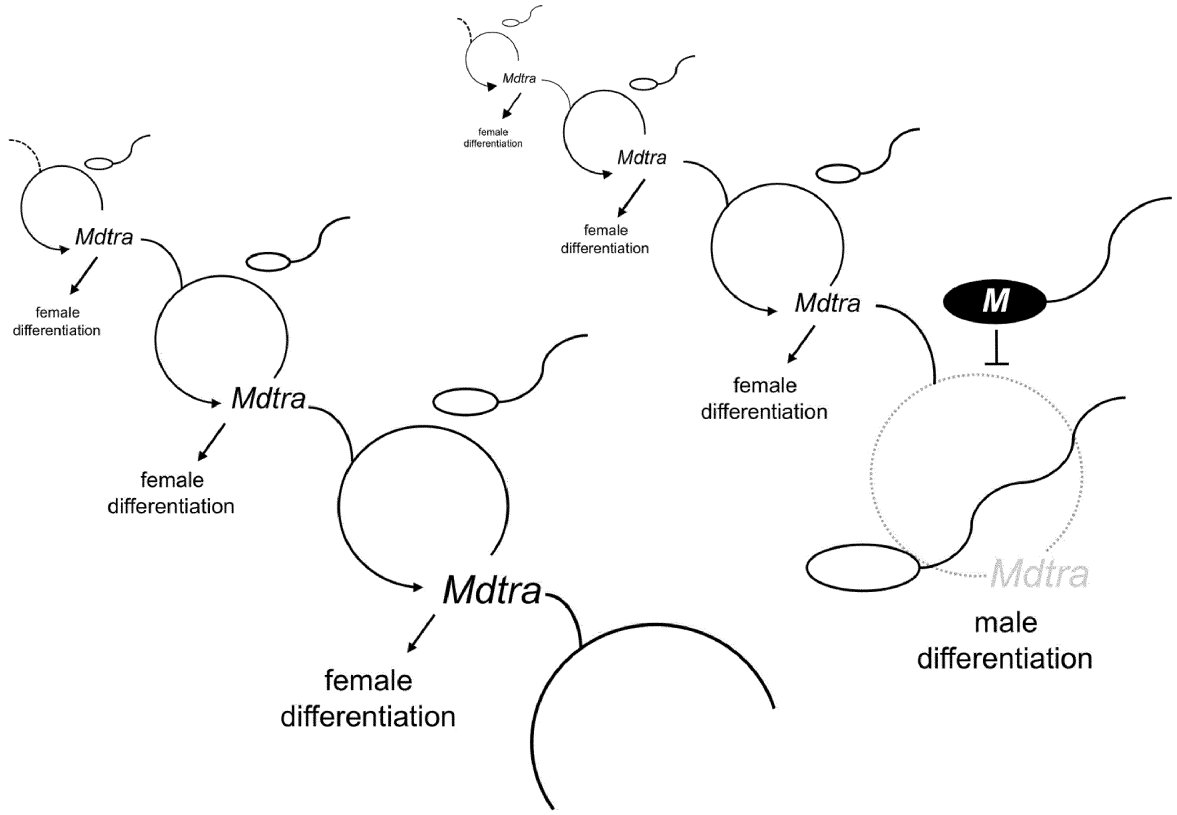

Figure 1. The 'female continuity' hypothesis. The active state of *Mdtra* (formerly *F*) is to be transmitted from mother to daughter from generation to generation in a perpetual self-sustaining cycle. Whenever the cycle is disrupted (here by a sperm transmitting *M*) it comes to a halt and male development follows.

character of *M* and the susceptibility of the female loop that facilitates the selection for a new mutation with this character. As an example, a new *M* can arise from an antimorphic mutation in an otherwise positive regulator of female expression of *Musca tra.* There may exist a number of other ways to generate dominant mutations that have the capacity to rupture the female cycle. In the end, the question whether *M* is the same gene or different genes will be solved, once the first *M* is molecularly characterized.

Musca domestica: a case study for rapid changes in the sex-determining pathway

How does this model account for the seemingly diverse sex-determining strategies observed in different *Musca* strains. The schemes are shown in figure 2. Again the basic principle is that male development follows whenever the cycle that sustains the female-determining activity of *Mdtra* is interrupted. I have discussed the dominant loop-breakers, the *M* factors that are introduced in the egg by the sperm. This mode is referred to as the standard mechanism of sex determination. In some naturally occurring strains, where *M* was found to be homozygous in males, a dominant mutation in *F* has emerged which appears to be resistant to the inhibitory activity of *M* (Rubini *et al*. 1972; McDonald *et al*. 1978). This allele, F^D, is a gain-of-function mutation in the *Musca tra* gene. The female-determining activity of this allele is not dependent on autoregulation. Thus, this allele is no longer susceptible to the loop-breaking activity of *M*. Consequently, individuals carrying this allele always develop into females irrespective of the presence of one or more *M* factors.

Another interesting sex-determining mechanism was found in the laboratory. The underlying principle resembles the maternal mode of sex determination as described in fly species such as *Chrysomya rufifacies* (Ullerich 1984). In essence, this strain consists of two types of females: those that only produce sons, and those that only produce daughters. As discussed before, zygotic activation of *Mdtra* depends on maternally contributed activities of *Mdtra* and *Mdtra2* that are supplied with the egg. If the mother fails to supply any of these essential components, the self-perpuating loop of *Mdtra* cannot be initiated in the embryo and all fertilized eggs will develop into males irrespective of whether the sperm introduces a male determiner or not. These females are termed arrhenogenic as they give rise to sons only. The laboratory strain that displays this feature carries the mutation *Ag* (*arrhenogenic*) on the first chromosome.

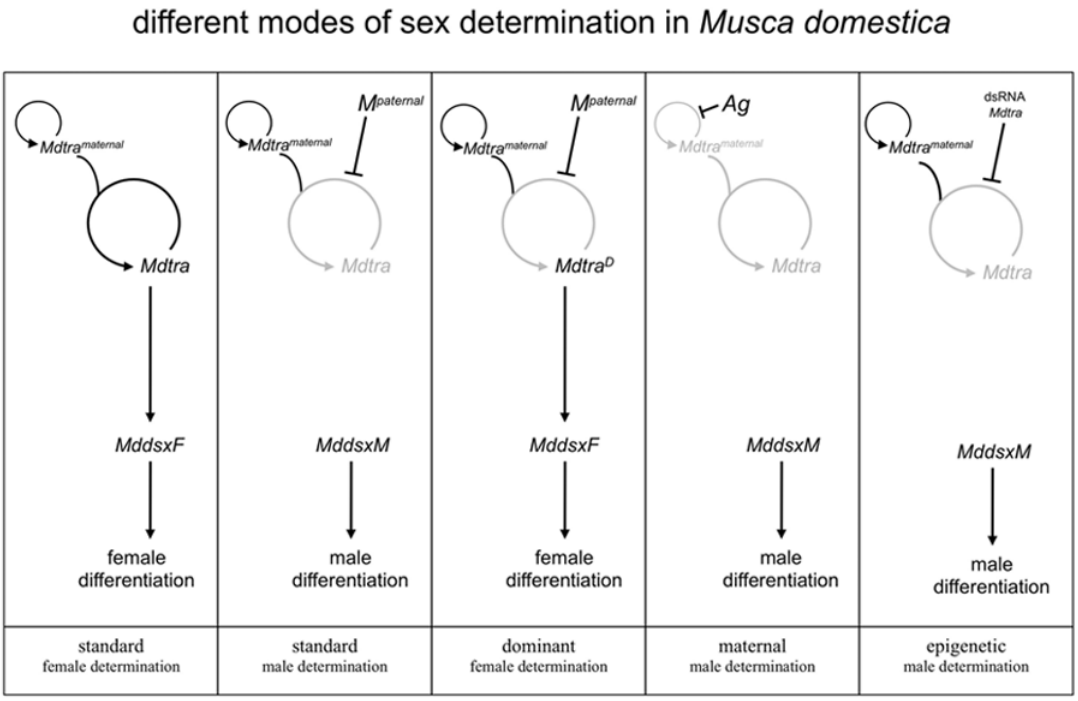

Figure 2. Schematic depiction of the different sex-determining strategies found in *Musca domestica*.

I suggest that this mutation is a specialized *M* factor which has no consequences in the soma but does so in the germ line (Vanossi Este and Rovati 1982). A female zygote can develop normally, when this mutation is introduced by the sperm. However, the loop-breaking activity of the *Ag* mutation will be active in the germ line of this female and prevent the production of maternal *Mdtra* activity. In line with this notion, we found that eggs from *Ag* mothers are devoid of maternal *Mdtra* transcripts (Hediger *et al*. 2010). It will be of particular interest to reveal the molecular nature of this *Ag* mutation. Possibly, this *Ag* mutation is a derivative of an *M*-factor that can no longer be expressed in somatic tissues. The last mode depicted in figure 2 does not naturally occur. This epigenetic mode of sex determination based on the experimental introduction of dsRNA molecules targeted against positive regulators of the loop has been explained in the previous section. Taken together, these examples in *Musca* illustrate how subtle changes in the genetic architecture of a given sex-determining pathway can lead to profound effects in its appearance. This type of microevolution in *Musca* gives support to the idea of Nöthiger and Steinmann (1985) that seemingly different strategies can arise from singular variations in an otherwise well conserved core pathway.

Why did the sex-determining pathways in *Drosophila* and *Musca* take different routes?

When comparing the pathway of *Musca* with that of *Drosophila* some fundamental differences are striking. The most important is that the role of the key female determiner in *Drosophila* is not *tra* but *Sxl.* In this species, the tasks of interpreting the signal and memorizing the sexual state have been delegated to a gene located upstream of *tra.* Though *tra* has been left as a mere mediator of *Sxl's* jurisdiction, it still exerts the role of a global executor of the female programme in *Drosophila*. The recruitment of *Sxl* to the sex-determining pathway is believed to be an evolutionarily recent event that occurred after the divergence of Acalyptratae and Calyptratae (Schutt and Nothiger 2000). Nonetheless, *Sxl* is present and well conserved in structure in all insect species examined so far (Traut *et al*. 2006). But in none of these does it meet the basic premise for a master switch, namely the expression of sex-specific activities. In fact, *Sxl's* role of a key female determiner has so far only been validated in few *Drosophila* species: *D. melanogaster*, *D. virilis* and *D. subobscura* (Bopp *et al*. 1996; Penalva *et al*. 1996) (M. Hediger and D. Bopp, unpublished data).

A more straightforward strategy seems to be based on deployment of maternal *tra* products into the embryo to activate zygotic *tra* and engage the female promoting loop. This mode of operandi has been suggested for *tra* regulation in several species of Tephritidae and Calypatratae (Pane *et al*. 2002; Ruiz *et al*. 2007; Concha and Scott 2009; Hediger *et al*. 2010). These findings led to the proposal that sex determination in the common ancestors of Acalyptratae and Calyptratae may have operated on the basis of an autoregulatory *tra* gene which is activated by its maternal activity. This system may even represent an archetypal mechanism common to most dipteran insects. It is thus possible

that the counting system in *Drosophila* originated from a maternal based system. Thus, dominant loop breakers such as *M* may have existed in the archetypal *Drosophila* and functioned as the primary signal. Maybe remnants of *M* factors are still contained in the present day genome of *Drosophila*. This will be interesting to investigate, once the molecular nature of *M* is known.

This raises the question of why *Sxl* has been recruited as an upstream regulator of *tra* in the *Drosophila* lineage. The important clue to understanding this particularity is its intimate connectivity with another essential process, namely dosage compensation. In *Drosophila* the X : A counting system with *Sxl* as its key mediator adjusts the transcriptional activity of X-linked genes according to the relative content of X present in the cell. If the X content is high (XX), the activity of *Sxl* is needed to keep X-linked transcription at a basal rate. In cells with a low content (XY), *Sxl* remains off and the transcription rate of X-linked genes increases by two-fold (Cline 1993; Lucchesi *et al*. 2005). It is conceivable that this compensating mechanism evolved independently of the control of sexual differentiation and that *Sxl* was first recruited as a regulator of dosage compensation. This is in fact an important assumption to explain why sex determination followed a different route in *Drosophila*, compared to other dipterans. There are examples of systems where sex determination and dosage compensation originated from unrelated pathways. For instance, in mammals these mechanisms must have evolved separately, as no interdependence between the two pathways has been observed so far (reviewed in Payer and Lee 2008). Thus, the two processes do not necessarily need to be interconnected. In *Drosophila*, it seems plausible that the mechanism that counts the relative numbers of X chromosomes to sets of autosomes primarily served the purpose of compensating for differences in X-linked gene dose. Its use as a primary signal in sex determination may have been a secondary adaptation. I propose that *Sxl*'s dosage compensatory function preceded its sex-determining function. As a matter of fact, it was this particular assignment to dosage compensation which made *Sxl* predestined to become later a key player in sex determination.

If we take a closer look at how *tra* is regulated in the housefly, we find that it is in principle based on differential splicing of *tra* pre-mRNA. In absence of female-specific TRA products, *tra* will be spliced such that the resulting messages will include additional sequences (stage I in figure 3). These sequences introduce stop signals and thereby interrupt the ORF. Presence of active TRA, on the other hand, will be in association with TRA2 bind to specific sites on the nascent transcript and prevent the inclusion of these translation-terminating sequences (blue box in figure 3). This modus operandi based on a positive feedback loop guarantees a continuous production of active TRA. Here the integrity of the interaction of TRA with its nascent RNA is central to female development. Any disturbances at this level will affect the stability of the cycle. For instance, if the strength of the interaction is weakened by changes in the binding sites or in the TRA protein, the loop will become corrupted and more susceptible to interference. As a result, cases of intersexuality and male-biased-sex ratios may become increasingly more prevailing. This repercussion may have developed into a serious problem in the *Drosophila* lineage. To counteract the cumulative loss of fertile females, a compensatory mechanism was required to detach the expression of TRA from an exclusive dependence on its autocatalytic function. To achieve this two attributes are mandatory. First, this compensatory mechanism must involve an established splicing regulator given that regulation of *tra* operates at this level, namely skipping the translation–terminating sequences (blue box in figure 3). Second, its activity must be restricted to females. This is when *Sxl* came into the play. It fulfilled both requirements. As an established RNA binding protein it had the ability to regulate RNA processing and, secondly, as a key control element in dosage compensation it was only active in females. As these qualities were assumed before, no prior selection was needed to accommodate *Sxl* for a new sex-specific function. Also, it was not yet necessary that *Sxl* fully adopted the sex-specific regulation of *tra*. Rather *Sxl* gradually relieved *tra* from upholding the productive mode of *tra* splicing in females. The products of both genes suppressed the inclusion of translation–terminating sequences into the mature *tra* messengers, but they acted independently by binding to different non-overlapping sites. The emergence of SXL binding sites near the regulated splice sites was of course a premise for a productive interaction. But in a first stage, these sites may have been suboptimal and *Sxl* was merely assisting *tra* in preventing the unproductive mode of *tra* splicing (stage II in figure 3).

For some time, thus, female determination may have actually operated on two signals: (i) absence of *M*; and (ii) high dose of X chromosomes. A recent report by Siera and Cline (2008) unveils a novel and unanticipated link between *tra* and *Sxl* in *Drosophila*, namely the existence of a reverse interdependence in which not only *Sxl* regulates *tra* but *tra* also regulates the activity of *Sxl*, although at a much weaker level. The authors propose that this regulatory back talk is a vestige of the evolutionary transition between *tra* and *Sxl*. They argue that this functional redundancy in positive autoregulation may have facilitated the transition from *tra* to *Sxl* to become the master switch. In the context of events described above, it seems plausible that, in the process of *Sxl* becoming a relevant regulator of *tra, Sxl* itself may have become a target of *tra*. By these means a positive feedback circuit was established to uphold female expression of both genes. Eventually, regulation by *Sxl* must have become more dominant and by and large taken over splicing regulation of *tra*. The reasons for this could be various. *Sxl* may have turned out to be more effective and robust in performing this task. Consequently, *tra's* direct contribution to its processing gradually diminished (stage III in figure 3). Eventually, this led to a complete substitution from a former purely autocatalytic mode of reg-

ulation to the full implementation of *Sxl* as the sole regulator of the female splice mode (stage IV in figure 3). It seems reasonable that there was no want to carry on a system operating on two signals, if one sufficed.

But there may be another important consideration for why the X:A→Sxl module took over the government of sexual differentiation. As mentioned earlier, the germ line in *Drosophila* is confronted with a specific problem. While in some insect species, such as *Musca*, the sexual fate of the germ line is solely dictated by the sex of the surrounding soma, this does not hold for *Drosophila.* For the formation of functional gametes, the karyotype of the germ line must be compatible with the sexual phenotype of the gonadal soma. For instance, only XX germ cells, but not XY, develop into eggs in an ovarian environment, and vice versa only XY germ cells, but not XX, become mobile sperm in testes. In view of this restraint, it is imperative to reconcile the sexual phenotype with the sex karyotype of the individual to guarantee the production of functional gametes. This condition is exactly what the X:A→Sxl module provides, namely an intimate coupling of the sexual fate of individual with the karyotype of its germ line.

In conclusion, the reason why *Drosophila* diverged from *Musca* in controlling sex determination is possibly an adaptive measure to adjust for different requirements that were impinged on the system. An essential difference between *Musca* and *Drosophila* was the emergence of a new rapidly degenerating Y in the *Drosophila* lineage. Here, the imbalance of gene doses of the Y and the corresponding X had two important consequences: a need for dosage compensation in the soma and for reconciliation of the germline karyotype and the sexual phenotype of the soma. These needs are absent in *Musca* given that the X and Y, with the exception of *M*, are genetically equivalent. Nonetheless, *Drosophila* had to cope with these problems and choose first to install an X dosage compensatory mechanism based on X counting mediated by *Sxl*. It is likely that *Sxl* already at this time involved a feedback mechanism to sustain its activity, once the transient X : A signal was assessed. In a next step, the control of this process converged with that of sex determination. Thereby, the rank of a key switch and memory of the selected state was passed from *tra* to *Sxl*. As a result, the autocatalytic cycle of *tra* and the *M* became obsolete and eventually disappeared. The co-ordinated control of two pathways under the command of one signal (the X : A) presents the current state of affairs in *Drosophila*. Figure 4 describes these important steps in the direction that *Drosophila* took since it diverted from a common ancestor.

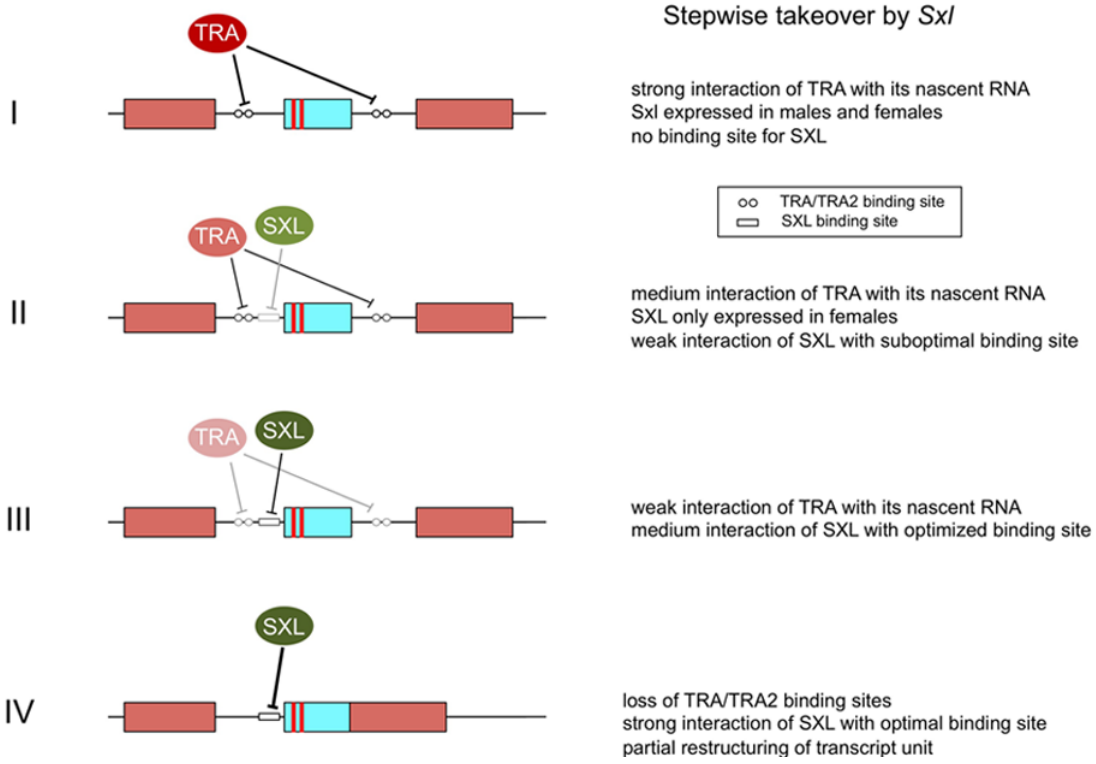

Figure 3. Gradual substitution of TRA by SXL as the main regulator of the productive female splice mode of nascent *tra* transcripts. Simplified scheme of sex-specific regulation: the blue box with red bars represents the translation–terminating exon that is included in the male message but skipped in the female message. The red bars indicate the positions of the in frame translational stop signals.

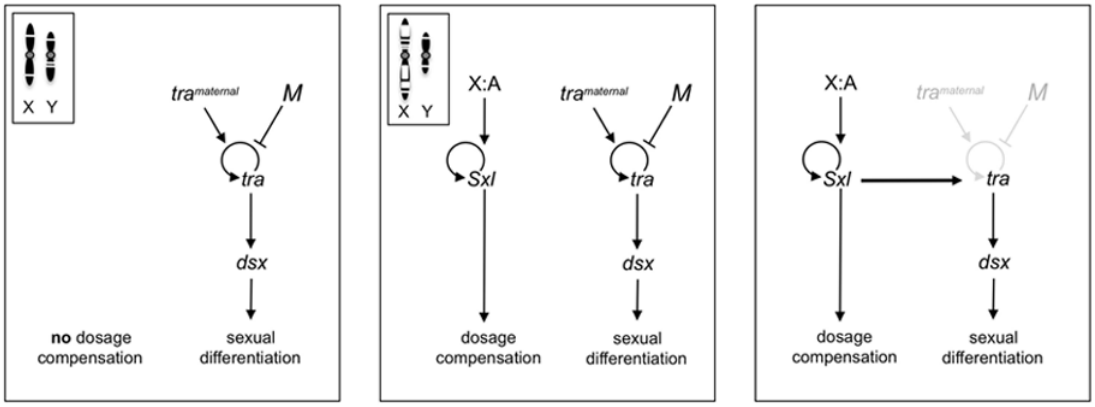

Figure 4. Evolution of the *Drosophila* pathway from left to right. The left panel depicts the situation before the *Musca* and *Drosophila* lineages diverted. At this stage the gonosomes were of low genic content and genetically equivalent. They eventually got lost in the *Drosophila* lineage. The middle panel marks the onset of dosage compensation as a consequence of the emergence of a new degenerating proto Y. The X : A counting system and its mediator *Sxl* were acquired to control X-linked gene activity. In the right panel, depicting the current state, *Sxl* feeds into the sexual pathway to co-ordinate the control of these two sex-specific processes. *M* and the autocatalytic cycle of *tra* became redundant and eventually vanished.

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