Why hybrid males are sterile in *Drosophila*?

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Hybrid male sterility is one of the most rapidly evolving postzygotic reproductive barriers, which has received special attention in the study of speciation. In *Drosophila*, in majority of interspecific crosses, hybrid males are sterile while females are fertile. Why hybrid males are sterile remains a fundamental question for evolutionary biologists. A number of investigations have been carried out to understand the causes of hybrid male sterility and the results suggest that it may involve either X–Y, X-autosomes, Y-autosomes, cytoplasmic incompatibilities or a number of genes. The genetic basis of hybrid sterility remains nebulous, but it seems that it involves a large number of genes and almost all chromosomes. The only characterized speciation gene in *Drosophila* today is *Odysseus*, but no functional tests yet have been reported that support its role in hybrid sterility and its functional equivalence to the homeobox gene. To understand the mechanism of hybrid male sterility, several theories have been proposed. The three most important theories are dominance theory, faster-male theory and faster-X theory, among which the dominance theory is the most popular. Although studies on hybrid male sterility in *Drosophila* are well documented, no conclusive mechanism of sterility that is uniformly obeyed in all species is still known. During last two decades, the resurgence of interest in hybrid male sterility and the use of *Drosophila* as a model organism for such study warrant a comprehensive review on this topic to facilitate better understanding of this subject. In view of this, a brief history as well as the recent advances in the field of hybrid male sterility in *Drosophila* are documented in the present article.

Keywords: *Drosophila*, genetic interactions, hybrid male sterility, *Odysseus*.

SPECIES maintain their entity through isolating mechanisms, which act as reproductive barriers and restrict the intermingling of genomes from two different species. There are two forms of reproductive barrier: prezygotic and postzygotic. In the former, the reproductive barriers prevent formation of the zygote (such as courtship difference), while in the latter these barriers restrict the survival and reproduction of the zygote (such as hybrid inviability and sterility). Preferential sterility or inviability of hybrids of heterogametic sex is one of the most common[1] and, presumably, earliest manifestations of postzygotic reproductive isolation[2].

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In the genus *Drosophila*, interspecific hybridization between most species pairs produces sterile males and fertile females[3]. Why there is a discrimination of sex for sterility in hybrids or why hybrid males are sterile have been an intriguing question for evolutionary biologists for a long time. Also hybrid sterility has received special attention from speciation geneticists, partly because it is easier to study in backcrosses, and partly because it is considered as a milder form of postzygotic isolation, and therefore, is more likely to appear earlier in the chain of genetic events leading to speciation[4]. A number of investigations were carried out on hybrid male sterility since the pioneering works of Sturtevant[5] and Dobzhansky[6]. Several mechanisms and theories on hybrid male sterility were put forward, but none were universally manifested in all species of *Drosophila.* Moreover, the genetic basis of hybrid male sterility remains obscure[7].

In the last two decades tremendous work has been performed on speciation mainly in *Drosophila* and many workers are now seriously engaged in unravelling this mystery, which is apparent from the recent publications in this area. Recognizing the progress, some reviews were also published on reproductive isolation, speciation and Haldane's rule[7–12]. Since the investigation on hybrid male sterility is burgeoning rapidly and now attracting a number of *Drosophila* workers, an extensive review is required to update the recent advances in this field. In view of the above, we endeavour to include all major investigations on hybrid male sterility starting from pioneering works of Sturtevant[5] and Dobzhansky[6] till date.

History

The temptation to understand the putative causes of sterility dates back to Aristotle, who discussed at length the sterility of the mule[13]. However, in *Drosophila,* Sturtevant[5,14], for the first time analysed the case of hybridization and threw some light over the causes of hybrid male sterility and inviability. He found that in the cross between *D. melanogaster* and *D. simulans*, if *D. melanogaster* is the female parent, only female offspring are produced (exceptional males produced when the composition of female is XXY) and if *D. melanogaster* is the male parent, usually only male offspring are produced which are sterile (sometimes small number of female hybrids also appear in the culture). He suggested that in the offspring, only those hybrids survive who carry a *D. simulans* X-chromosome, but in the presence of *D. simulans* egg cytoplasm and a *D. melanogaster* X-

chromosome, even if a *D. simulans* X-chromosome is present, survival usually does not occur[14]. Haldane[2] accumulated sufficient data on sterility of heterogametic sex from a large number of species belonging to different animal phyla. Based on the data, he proposed the Haldane's rule: When in the F_1 offspring of two different animal races (or species) one sex is absent, rare or sterile, that sex is the heterozygous (heterogametic) one. Dobzhansky[15] and Muller[16] have suggested the possible mechanisms that underlie Haldane's rule. According to Dobzhansky, some genes that lie on the X-chromosome of one species may lie on the autosomes of other species. In hybrid females, the genotype is normal as that of the parental female and so fertility of females is maintained. Hybrid males, being hemizygous for sex-linked genes, suffer from a disturbance in the genic balance resulting into sterility and inviability. Muller[16] had added in the theory that recessive and semi-dominant sex-linked genes, which had become established in parental species will be strongly expressed in the heterozygous sex, while the corresponding genes in the autosomes of the hybrid will be suppressed by their dominant alleles.

The first fundamental discovery regarding causes of hybrid male sterility was made by Federley[17], who found that chromosomes usually fail to form bivalents at meiosis in the sterile or semi-sterile hybrids between the moth species *Pygaera anachoreta*, *P. curtula* and *P. pigra*. He proposed that failure of pairing between chromosomes of different species leads to hybrid sterility. However, his supposition was vitiated by the occurrence of hybrids having normal chromosome pairing during meiosis in *Digitalis*[18] and other genera[19,20]. Therefore, it was proposed that disturbance in the gametogenesis in hybrids may be initiated before, during and after the meiotic chromosome pairing that leads to sterility. In the genus *Drosophila,* Dobzhansky[6] for the first time, performed backcross analysis in the hybrids of *D. pseudoobscura* and *D. persimilis* using markers on different chromosomes to study the role of different chromosomes on hybrid male sterility. He used testis size as proxy for sterility. Interestingly, he found that all chromosomes house genes involved in testis development. Since testis development is intimately related to male fertility, these genes were legitimately considered to be involved in hybrid male sterility. Further, in the backcrosses, he found that the more dissimilar the X-chromosome and autosomes, the smaller was the testis size. Males with X-chromosome of one species and all autosomes from other species have smallest testis. Sterility was supposed to involve changes either at chromosome level or at gene level or both[13]. When sterility is caused due to genetic constitution of the organism, it is called genic sterility. On the other hand, if the gene arrangement causes sterility, it is called chromosomal sterility[13]. In the forties, excellent data have been accumulated on the genetics of hybrid sterility and inviability in certain species of *Drosophila*, particularly *D. mulleri* and *D. aldrichi*[21–24]. When *D. mulleri* females were crossed to *D. aldrichi* males, they produced sterile hybrids of both sexes, but the reciprocal cross gave no offspring. Based on the results, Crow[22] suggested that *D. aldrichi* have a sex-linked gene which produces no visible effects in pure *D. aldrichi*, but acts as a dominant semilethal in the *D. mulleri* × *D. aldrichi* female hybrids.

Causes of hybrid male sterility

Role of different chromosomes and their interactions

Earlier studies on genetic analysis of hybrid male sterility revealed that all the major chromosomes carry genes involved in hybrid male sterility[6]. The cause of hybrid male sterility was attributed to X–Y interactions and interactions of these chromosomes with autosomes. The involvement of X-chromosome in hybrid male sterility has been demonstrated in the cross between *D. micromelanica* females from Texas and males from Arizona[25]. The Y-chromosome was found to play a key role in hybrid male sterility in *D. macrospina macrospina* and *D. m. limpiensis*[26] and in *D. hydei* and *D. neohydei*[27]. There are two major interactions causing hybrid male sterility: (i) X-autosome and (ii) X–Y interaction. In *D. pseudoobscura* and *D. persimilis*, the X–Y interactions have contributed maximally to hybrid male sterility[28], while in the two races of *D. pseudoobscura* (*D. p. pseudoobscura* and *D. p. bogota*), both arms of the X-chromosome and two autosomes (second and third) have been implicated in hybrid male sterility[29]. In *D. arizonensis* and *D. mojavensis* species pair, sterility may result if males carrying the cytoplasm and both sex chromosomes of *D. arizonensis* have one member of the third or fifth chromosome pair of *D. mojavensis*[30]. The 'X-autosome imbalance' hypothesis for hybrid male sterility was proposed by Muller[16], which suggests that female hybrids have an X and autosomes from each species (a balanced genotype), while males have an X from only one species (unbalanced). To test this hypothesis, Coyne[31] produced females whose both X-chromosomes were of *D. simulans* origin, with one complete haploid set of autosomes from *D. simulans* and the other from *D. mauritiana*. Contrary to the X-autosome imbalance hypothesis, these imbalanced females were fertile. From the results obtained by backcross analysis of the same pair of species, he proposed X–Y interaction as the possible cause of hybrid male sterility. It does not necessarily follow that male sterility may not be due to X-autosome imbalance, but it appears to be a reasonable inference when this observation is coupled with several known examples of nonreciprocal male hybrid sterility in which males from one interspecific cross are fertile, yet unbalanced. Therefore, to accommodate the presence of nonreciprocal hybrid sterility, the imbalance hypothesis would have to be perceived as stating that not all instances of imbalance result in sterility, but all instances of sterility are due to imbalance[32]. Turelli and Orr[33] and Orr and Turelli[34] explained Dobzhansky–Muller theory mathematically and provided a firm foundation to it. This theory was later referred to as the dominance theory.

To understand the cause of hybrid sterility, complete pattern of interactions between all chromosomal regions, which were known to affect hybrid fertility was investigated in *D. pseudoobscura* Bogota and USA strains by Orr and Irving[35]. Their findings confirmed X-autosomal incompatibilities to be the main cause of hybrid male sterility in this species-pair. However, recently, these hybrids were reported to be weakly fertile[36]. At molecular level, analysis of hybrids of *D. pseudoobscura* and *D. persimilis* revealed that X-autosomal interactions are associated with hybrid male sterility[37]. This finding is consistent with the chromosomal analysis of hybrid male sterility by Dobzhansky[29]. The X-autosomal incompatibility is one of the several interactions (like X–Y, cytoplasmic, etc.) causing sterility in hybrid males. During analysis of hybrid sterility in *D. simulans* and *D. sechellia,* Johnson *et al.*[38] have emphasized that it is 'prudent not to emphasize X–Y interactions at the expense of other plausible models such as sex chromosome–autosome interactions', as they found that replacing Y-*simulans* with Y-*sechellia* does not lessen the sterility effect in hybrids. Further, even in closely related species, the cause of hybrid male sterility may vary[39].

The X–Y interaction was found to play important role in hybrid male sterility in other species-pairs too. Orr[28] has reanalysed hybrid sterility in *D. pseudoobscura* and *D. persimilis* species-pairs using the same backcross analysis method used by Dobzhansky[13], but taking sperm motility rather than testis size as proxy for sterility. He did so because testis size does not always reflects sterility correctly[40], which has also been recently supported by Campbell and Noor[41]. In the *D. bipectinata* species complex, significant difference has been recorded in testis size among the hybrids of four closely related species. It elicits the confounding situation when the size of the testis has been used as an indicator for sterility[42]. However, sperm motility was found to be a better proxy for sterility. Interestingly, contrary to X-autosome interactions of Dobzhansky[13], Orr[28] found X–Y interaction contributing largely to hybrid male sterility. In support of his hypothesis, he has provided the following points: (i) backcross males having equivalent X-autosomal imbalance were found to be much more fertile when they were with homospecific X- and Y-chromosomes than when they were with heterospecific X- and Y-chromosomes; (ii) in hybrid males, substitution of Y-chromosome affects male fertility in the same way as proposed in the X–Y incompatibility theory and (iii) hybrid females having an X- autosomal imbalance equivalent to F_1 males were highly fertile. Thus, the results of Orr[28] in *D. pseudoobscura* and *D. persimilis*, and also the findings of Coyne[31] in *D. simulans* and *D. mauritiana* reinforce X–Y interaction as the main cause of hybrid male sterility in *Drosophila.*

Another theory proposed by Lamnissou *et al.*[43] posits Y-autosome incompatibilities playing a major role in hybrid male sterility. They found that in *D. virilis* and *D. texana*, only one-third of F_1 males carrying the *D. texana* Y-chromosome were sterile. When fertile F_1 males were crossed with *D. virilis* females, about three-quarters of the sons were sterile. From these findings, they concluded that the Y-chromosome and at least two of the *D. virilis* autosomes were involved in hybrid male sterility. Further, if in a *D. mojavensis* male, the Y-chromosome is replaced with a Y from *D. arizonensis*, the resulting male has immotile sperm. In another experiment, one member of the fourth autosome pair of *D. mojavensis* was replaced by a *D. arizonensis* homologue. The progeny produced have fully fertile hybrid males[44–46]. The introgression and mapping experiment revealed that the sperm motility factor of *D. arizonensis* rescued fertility in male[47]. These examples confirm the role of Y-autosome interaction in hybrid male sterility in *Drosophila.*

Genes involved in sterility

It is difficult to pinpoint exactly how many genes are involved in hybrid male sterility. The minimum number of genes required for sterility should be at least two (Dobzhansky–Muller model), but the maximum number of genes involved in sterility is difficult to estimate. There are two views for the number of genes involved in sterility. The first view posits that many genes of small effect are involved in sterility[13,48–54], while the second view argues that hybrid male sterility involves few genes of major effect[55]. The first view which suggests hybrid male sterility as a polygenic trait has been divided into two variants: strong and weak[51]. According to the strong variant, the number of genes is important for sterility and the identity of genes matters little[56]. The weak variant argues that identity of genes is important because some genes cause hybrid problems while others do not[57].

Investigations based on chromosomal introgression of one species into the genetic background of another largely support the polygenic view of hybrid male sterility, but estimation of the number of genes involved in sterility varies. Coyne[58] used five markers to study the genetic basis of hybrid male sterility between *D. simulans* and *D. mauritiana*. He found that all the markers were linked to sterility, which infers that at least five genes are involved in hybrid male sterility. Palopoli and Wu[59] have estimated at least 40 loci on just the X-chromosome that are involved in hybrid male sterility. Recently, Tao and Hartl[60] and Tao *et al.*[61,62], using P-element inserts have estimated about 60 genes involved in hybrid male sterility between *D. simulans* and *D. mauritiana.* Several other studies have been carried out on genetic elements responsible for reproductive isolation in *Drosophila*[1,63,64]. The first putative hybrid male sterility gene was mapped by Coyne and Charlesworth[65] in the hybrids of *D. simulans* and *D. mauritiana* at approximately 2 cM segment of the *D. mauritiana* X-chromosome. Perez *et al.*[66] confirmed this putative gene and coined its name as *Odysseus* (*Ods*). Further investigations revealed that *Ods* has only 250 Kb of DNA[67]. Later studies showed that introgression

of only *Ods* caused sterility in hybrid males only 50% of the time, while males who carry *Ods* and a more distal region are completely sterile. It infers that at least two genes in the distal regions are required for complete hybrid sterility. At the molecular level, *Ods* was found to include only two exons which belong to a homeobox gene. Therefore, it was named *OdsH* (*Odysseus*-site homeobox gene)[68]. It arises due to gene duplication in the *Drosophila* genome and evolves at a high rate[69]. Although almost seven years have passed after the identification of *Odysseus* gene, no functional tests have yet been reported that support its role in hybrid sterility and its functional equivalence to the homeobox gene. Hence, it is still called *OdsH*[70]. The gene knockout experiment on *OdsH* revealed its normal function, i.e. modest enhancement of sperm production in young males[71]. It is highly conserved among nematodes, mice and insects. However, some evidences suggest that it is misexpresed in hybrids[72]. A genome-wide expression pattern in pure species and hybrid males revealed that a panel of genes that are primarily or exclusively expressed in males, including several involved in spermatogenesis, is disproportionately misexpressed in hybrids[73]. Under-expression of panel of transcripts (*Acyp*, *CG 5762*, *CG 14718*, *Mst 84Dc* and *Mst 98Cb*) in hybrids relative to pure species (*D. simulans* and *D. mauritiana*) is associated with infertility of hybrid males and these genes often are highly conserved[74].

Role of cytoplasm

Cytoplasm has been suspected to be involved in hybrid male sterility since a long time[29,75,76]. Orr[77] has illustrated it in the species-pair *D. pseudoobscura pseudoobscura* and *D. p. bogota*. Further, Zeng and Singh[78] have provided a scheme for testing the role of cytoplasm in hybrid male sterility, which was based on the incompatibilities of cytoplasm of one species to the nuclear genome of another species.

Tenets for hybrid male sterility

Dobzhansky[6] has suggested that a general cause of hybrid sterility is 'interactions between complementary genetic factors contributed by both parents. If the genetic constitution of one of the parental forms is SStt, and of the other is ssTT, the hybrid is SsTt. The assumption is made that the presence of the factor (or the group of factors) S alone, or of the factor T alone, permits unlimited fertility, but that the factors S and T interact in such a manner as to make sterile an organism carrying them simultaneously'. This concept and experimental approach were widely recognized and have drawn an excellent series of review articles[12,57,79–83].

Like Dobzhansky, Muller[16,84] viewed hybrid sterility as a consequence of negative interaction between 'complementary genes'. Muller[84] explained it in the following way: suppose an ancestral population has the genotype aabb, which evolves to AAbb in one daughter species and to aaBB in the other. When A and B come together in the AaBb hybrid, they may produce a harmful effect if their interaction is not fully recessive. This theory is widely recognized as dominance theory. He further elucidated that X-chromosome genes, when hemizygous in the male, are 'especially apt to meet with disharmonies of functioning' in their interactions with autosomal genes. Female hybrids have an X and autosome from each species (a balanced genotype), while male hybrids have an X from only one species (unbalanced). The X-autosomal imbalance hypothesis, although proposed by earlier workers[2,15], was explained lucidly for the first time by Muller[16] in the terms of dominance and recessivity. Dominance here refers to the dominance of an allele for fitness when it occurs on a foreign genetic background, i.e. when it occurs with its complementary partner[84]. Recently, the mathematical models proposed by Turelli and Orr [33], and Orr and Turelli[34] have given strong support to the dominance theory.

A number of hypotheses have been offered to explain sterility in hybrid males. Investigations in different species-pairs revealed that sterility is not invariably caused by incompatibilities between X and Y-chromosomes[38,77,78,85,86], meiotic drive[87–91], a disruption of dosage compensation[92], synergistic interactions between conspecific genes[47] or species-specific translocations between the X-chromosome and autosomes[79] . Each of these mechanisms may work well in different species, but evidence strongly suggests that none accounts for the ubiquity of sterility in hybrid males. With time, most hypotheses were falsified and now only three hypotheses remain viable[11]. They are dominance theory, faster male theory and faster X-theory. More evidences support the dominance and faster-male theories than the faster-X theory. The first question against dominance theory was raised by Coyne[31], who used attached X stock of *D. simulans* in the crosses *D. simulans* × *D. mauritiana* and *D. simulans* × *D. sechellia*. The hybrid females produced by attached X-chromosome were unbalanced for X-autosomes and hence should be sterile as in case of hybrid males obeying dominance theory but he found them to be fertile. Later on, it was found that different sets of loci cause male vs. female sterility and were evolved at different rates. So there is no guarantee that both types of sterility appeared at the same evolutionary rate, which does not support the interpretation made by Coyne[31].

The faster male theory states that hybrid male sterility genes afflicting male hybrids evolve at a faster rate than those afflicting female hybrids. To corroborate this, Wu and Davis[81] and Wu *et al.*[57] suggested that two factors might cause such faster male evolution: (i) spermatogenesis might be an inherently sensitive process that is easily perturbed in hybrids. Thus, even if the male-expressed genes may evolve at the same rate as female-specific genes, they cause problem in males at a higher rate. (ii) sexual selection might have caused faster evolution of genes expressed in males, as there is good evidence that male genitalia are the most rapidly

evolving of all the morphological characters in insects. Moreover, proteins from the male reproductive tract diverge between *Drosophila* species faster than proteins from most other tissues, which may cause incompatibilities between males more rapidly than those between females. Although faster male theory is well manifested in heterogametic males, it cannot explain sterility in homogametic males as in case of birds and Lepidoptera.

The faster-X theory has been proposed by Charlesworth *et al.*[87]. They speculated that X-linked genes evolve faster than autosomal genes. This theory differs from the dominance and faster-male theory in the way that it alone cannot explain the sterility in hybrid males. If genes affecting males and females evolve at the same rate and act additively in hybrids, then male and female hybrids are equally fit regardless of the rate of evolution on the X. However, if the speciation genes are partially recessive in hybrids and are concentrated on the X, then heterogametic hybrids will suffer disproportionately. Thus, the faster-X theory needs support of the dominance theory.

Although, faster-male and faster-X theories explain the sterility in hybrid males, the most popular theory is dominance theory which can explain hybrid sterility even in those hybrids where males are homogametic.

Conclusion

During the last decade, the increasing prominence of work on reproductive isolation concentrating mainly on hybrid male sterility has answered some questions regarding causes of sterility in *Drosophila*. The reason behind sterility may be X-autosome, X–Y, Y-autosome, cytoplasmic incompatibilities or involvement of one gene (*OdsH*) or different genes. Now it is well established that different species behave differently for hybrid male sterility and no single reason for sterility is known that is uniformly manifested in all species of *Drosophila*. The molecular dissection of sterility revealed that the number of genes involved in sterility varies in different species. However, only one gene (*OdsH*) may cause hybrid male sterility in *D. simulans* and *D. mauritiana*. This is the first speciation gene discovered and characterized till date. However, many questions are still to be answered such as what are the speciation genes in other species of *Drosophila?* How do these speciation genes cause sterility? Is there any single mechanism of hybrid sterility present that is universally manifested in all species of *Drosophila?* Although polygenic approach to hybrid male sterility has answered some of the questions regarding sterility, focused pursuits on the mechanism of sterility may clarify majority of questions regarding hybrid male sterility in *Drosophila*.

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