

The genetics of sexual behavior in *Drosophila*

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Abstract: Genes play a profound role in the regulation of *Drosophila* behavioral system, either sexual or nonsexual. Those regulating sexual behavior may lead to differences at the level of species, thus causing behavioral isolation. Courtship in *Drosophila* involves several behavioral repertoires which are controlled genetically. It involves the use of several sensory stimuli that may affect the behavioral responses of both the sexes. These sensory signals are sex specific, and the specificity is due to differential expression of the genes. In addition to the premating behavior, certain candidate genes also affect several postmating responses. Genes also play a magnificent role in the differentiation of two morphologically and behaviorally distinct sexes as well as in their brain structures, which may explain the basis of sexual dimorphism in *Drosophila*. Also, the advancement in the area of system genetics like the use of mutants and GAL4/UAS and CRISPR/Cas9 systems provides a much better way to understand the role of genes in controlling several aspects of behavior more closely.

Keywords: courtship, *Drosophila*, system genetics, sexual behavior, pheromones, sexual dimorphism

Introduction

Sex differences in behavior are prevalent among the animal kingdom. It is generally believed that behavior acquires such differences because the sensory system of males and females receives external and internal signals differently. It has also been a matter of controversy how the genes shape the different aspects of behavior and how the environment modulates this process. So to answer this question, the area of behavior genetics has come forward in order to emphasize how the genes control different behavioral repertoires via nervous system. Behavior has already attained a four-dimensional array during the course of evolution, and this was understood when several studies provide evidence about the role played by the genes during the time of development.¹

In the recent era, *Drosophila* has come up as a powerful model system for studying the neurogenetics of behavior. This is due to the presence of large number of behavioral mutants which influence several neuronal subsets that may directly or indirectly involve in executing several behavioral phenotypes. Behavior genes have a special evolutionary interest because they are potentially involved in speciation and in many forms of adaptation. Dozens of loci affecting different aspects of behavior have already been identified and cloned in *Drosophila*. Some of these genes determine variation in such ethologically complex phenotypes as the male “love song” that is produced during courtship and a locomotor sleep–wake activity cycle that are controlled by the circadian clock.² Among the four different stages of *Drosophila* life cycle, only two

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life cycle stages have been suggested to exhibit most of the behavioral responses: larvae and adults. This is due to the presence of several literatures which provide evidence regarding the behavioral activities of both the stages. However, in the genetic context, it is not easy to understand the process of behavioral analysis since the process is multifaceted and requires deeper insight into the several wide range of those genes which act at several time points in an individual's life. The easiest modes to identify the key players executing several behavioral responses are firstly to look into those developmental genes that are interacting with each other to bring out a definite behavioral phenotype and secondly to identify those neuronal subsets which are interconnected with each other to produce definite repertoires. In view of this, behavior genetic analysis has come up as an approach to identify the role played by the genes in order to give a definite behavior system.^{3,4} Two kinds of behaviors have been found in the *Drosophila*: one is the sexual and the other is the nonsexual behavior. In this review, we are more likely to discuss about genes that play direct as well as indirect roles in the sexual behavior of *Drosophila*. Sexual behavior of *Drosophila* is among the more important of these fruit flies actions both from the organism perspective and in terms of how its nervous system can be investigated. In fact, some of the first behavioral genetic experiments in the genus *Drosophila* involve courtship and mating. Therefore, the main aim of this review is to discuss the evolutionary perspective of sexual behavior and to speculate how different genes control several aspects of sexual behavior. This also provides a glimpse about the usage of different tools and techniques, which help us to understand the *Drosophila* mating system.

Courtship and pheromonal communication in *Drosophila*

Mating behavior of *Drosophila* spp. consists of species-specific fixed action patterns, which are accompanied by orientation movements. It is basically divided into premating and postmating behavior. The premating behavior usually constitutes the courtship behavior, which involves mostly the exchange of different kinds of stimuli as well as mate choice, and the postmating behavior involves the remating, sperm competition, and sperm displacement. Certain genetic approaches were undertaken to study several courtship parameters in *Drosophila*. The first approach is to simplify the complexity of behavioral phenotype into its simplest genetic constituents and use recent molecular methods to disentangle the biochemical origin of behavior. During sexual communication, there is an exchange of certain sensory signals

between the two partners, which is always in the influence of species-specific mate recognition system.⁵ Therefore, it is important to understand the key role played by the genes during courtship, and this could be investigated by identifying the function of those signals which are involved in species discrimination.⁶ In the genus *Drosophila*, courtship involves a series of fixed action patterns where a male performs a series of behavioral repertoires, for example, tapping, singing, licking, and bending his abdomen in order to make a contact with the genitalia of the female in order to copulate her. It has been predicted that these behavioral components are sexually selected and are conserved during the course of evolution and thereby found to be present among all the species of the genus *Drosophila*.⁷

During the course of sexual interplay, both the partners exchange several sensory cues which include visual, acoustic, olfactory, gustatory, and tactile signals.⁶ These signals are species specific and are important constituents of species-specific mate recognition system and thereby vary across the genus.⁸ Such kind of variations may also emphasize about the environmental restraint on the sexual communication between the two partners. For example, *Drosophila auraria* flies inhabit in a dark environment and do not prefer visual cues for their communication and hence utilize only chemical signals that are exchanged during their courtship interplay. These pheromonal signals are volatile in nature and can be detected from a short distance.⁹ Pheromones have been found to exert stimulatory as well as inhibitory effects on the *Drosophila* nervous system. On this basis, pheromones are classified as stimulatory and inhibitory pheromones. Stimulatory pheromones are localized on the proximal legs and inhibitory on the maxillary palps.^{10,11} In addition to these two pheromones, there are certain other kinds of aggression pheromones like cis-vaccenyl acetate in *Drosophila melanogaster* and *Drosophila simulans*, which show similar kinds of effects as that with the food odors and thereby attract different *Drosophila* spp. on the same area.¹²⁻¹⁴

Genes involved in premating behavior of *Drosophila*

Premating or courtship behavior of *Drosophila* involves several behavior repertoires. These behavior repertoires are supported by visual, auditory, and chemosensory cues, which thereby form an integral part of successful courtship.¹⁵⁻²⁰ These behavioral responses contribute in the assessment of the sex and receptivity of potential partners,⁶ and expressing all these components in the appropriate situation and sequence increases the likelihood of mating success. Specific

behaviors in this pattern are involved in reproductive isolation and are presumably the targets of intersexual selection, including courtship song,²¹ as well as latencies and durations of courtship and copulation in response to species-specific pheromonal differences.²² These forms of behaviors are governed by certain sets of genes that have a direct as well as indirect role in courtship behavior and were suggested to be involved in variations in mating preference like *cacophony*, *white*, *fruitless (fru)*, *Voila*, *courtless*, *desat* as well as *per*, *nonA/dissonance*, and *dissatisfaction* (Table 1). Variants of *fru* and *dsx* expressing in different regions of the brain involve in male-specific courtship pattern like wing song.^{23–29} Similarly, female-specific behaviors are also under the control of certain genes, for example, *retn*, *spinster (spn)*, and *chaste (chst)*, which regulate the behavioral response via nervous system (Table 1).^{28,30–32} Among them, *retn* and *dsx* play a profound role in the receptivity of females and courtship activity of males. *dsxM* decreases the courtship behavior

of males, whereas *retn* plays an antagonistic effect on the courtship behavior of males by influencing the development of muscle of Lawrence. Hence, *fru* and *dsx* play a combined role in regulating the function of developmental gene *retn*.³³ In addition to this, *dsx* and *fru* also have a profound effect on pre- and postcopulatory behavior via Dh44 neuron, hence regulating sperm storage and release.³⁴ Nanda and Singh³⁵ documented that the genes affecting the mate recognition system of *Drosophila* are located on the third chromosome (Table 1). Later, Gaertner et al³⁶ found strong resemblance between several genes located on 3R chromosome with the courtship pattern.

Apart from visual and acoustic communication, chemical communication also plays a profound role in the *Drosophila* mating behavior. The cuticular hydrocarbons of *Drosophila* female are under the control of female-specific *desaturase* locus (Table 1).^{37–40}

Chromosomal polymorphism also affects the sexual activity of *Drosophila*. Several studies documented the role of karyotypes in affecting the mating ability.^{41,42} Singh and Chatterjee^{43,44} found that the heterokaryotypes show higher mating propensity as compared to homokaryotypes. Later, the results of the selection experiment in different species of *Drosophila* suggest that the males are more prone toward selection.^{45,46}

Genes involved in postmating behavior of *Drosophila*

One of the most important postcopulatory behaviors of *Drosophila* is remating and sperm competition. Female remating involves several postmating responses which are categorized into short-term and long-term responses. Short-term response includes increase in the egg-laying rate assisted by seminal protein ovulin, and long-term response includes decrease in the receptivity of female assisted by sex peptide.⁴⁷ The cAMP-responsive transcriptional factor (CREB), is highly conserved among animal species. In *D. melanogaster*, one of the alternatively spliced products of *CREB* gene, dCREB-2, is a repressor of dCREB2-a. Sakai and Kidokora⁴⁸ demonstrated that overexpression of dCREB2-b in virgin females enhances their sexual receptivity and CREB may be involved in female sexual behavior (Table 1). There are several mutations which have direct as well as indirect effect on female receptivity, and among them are *yellow*, *ebony*, *hypoactive C*, *inactive*, and *apterous* (Table 1).⁴⁵ Female remating is always under polygenic control as described by Singh and Singh.⁴⁹ Several models were proposed to explain the genetic basis of sperm displacement. Prout and Bundgaard⁵⁰ provide evidence

Table 1 Role of genes/chromosomes in controlling several aspects of sexual behavior of *Drosophila*

Genes/ chromosomes	Behavioral phenotypes	References
<i>white, fru, dsx, Voila, nonA, dissatisfaction</i>	Courtship repertoires	23–29
<i>cacophony</i> and <i>dissonance</i>	Courtship song	29
<i>Per</i>	Circadian rhythm and locomotor activity	45
<i>retn, spinster, chaste, dsxF</i>	Female-specific behavior	27,28,31–33
<i>dsxM</i>	Male-specific behavior and imaginal disc development	25,28,34
Chromosome III	Mate recognition system	35
<i>Desat</i>	Pheromonal communication	30,37–40
<i>tra-2</i>	Sexual differentiation	45
Karyotypes (ST, CH, TL, PP)	Mating speed and duration of copulation	41–46
<i>CREB, yellow, ebony, hypoactive C, inactive, and apterous</i>	Female receptivity	45,48
Chromosome II	Remating speed	49–51
<i>CG1656/1652, CG17575, CG9997, Acp70A, antares, aquarius, intrepid</i>	Increase egg laying after mating and decrease receptivity	52–55
<i>HOX, Scr, vg, dpp, Distal-less, dachshund, rotund</i>	Sexual dimorphism and sex comb development in male	88–90
<i>bab1</i> and <i>bab2, ABD-B, DsxF</i>	Pigmentation	33,34,91–112

that suggests genes have a direct influence on postmating responses, but this is not the case always as many studies found no relation between chromosomal arrangements and remating rate (Table 1).⁵¹

During mating, molecular contribution of males as well as females is required. Males provide sperm as well as seminal proteins to the female. In *Drosophila*, almost all the seminal proteins are made in the male accessory gland, which thereby induce several postmating responses like increase in the egg laying, sperm storage, release, and decrease in the female receptivity.^{52–54} Studies pertaining to evolutionary rate covariation identify three male- as well as three female-expressed candidate genes that are required in eliciting postmating responses (Table 1).⁵⁵

Several studies pertaining to genetic bases of sperm competition ability suggest a close association between polymorphism of several accessory gland protein loci and the number of progeny produced by first and second male after remating.^{56–58}

Genetic basis for sexual dimorphism

Sexual dimorphism is defined as the differentiation in the reproductive traits between males and females. It can usually be explained on the basis of sex linkage and/or genotype–sex interactions (exclusive of sex linkage). The distinction between these two mechanisms is particularly difficult to assess in the case of quantitative traits that show different degrees of development in males and females. Darwin⁵⁹ explained the phenomena of sexual dimorphism in the context of intersexual selection by taking the example of peacock whose long feathers attract peahen which has comparatively short feathers. Two prerequisites must be met for the evolution of sexual dimorphism. First, the intersex genetic correlation must be less than one,^{60,61} indicating that genetic variation exists for the independent evolution of the trait in each sex. Second, the strength of natural and/or sexual selection acting on the trait in each sex must be different,⁶² resulting in a net selection differential between the sexes. The development of a trait in one sex and not the other must be the result of differential gene expression. Correlations have been found between gene expression patterns and dimorphic trait production. For example, *msxC* expression is associated with the development of the male “sword” of the sword tail fish.⁶³ Only relatively few investigations have specifically considered the evolution of sexual dimorphism, covering a wide range of study systems including the skull in primates,^{64–67} body properties of lizards,^{68,69} newts,⁷⁰ the head shape of *Chironomus* larvae,⁷¹ and lycium flowers and insects including

Drosophila. Examples of sexual dimorphism in *Drosophila* include abdominal bristle number, abdominal tip pigmentation in males and females, sex comb pattern of males, brain differentiation of male and female, body size, shape, and genital architecture.

In the genus *Drosophila*, both the sexes exhibit differences in several aspects of behavior.^{61–74} In *D. melanogaster*, 15%–70% of known genes have sexually dimorphic expression.^{75–78} In several animals, sexual dimorphism is not only restricted to reproductive organs but also present in certain morphological structures. Most animals demonstrate characteristics of sexual dimorphism for body size. Male mammals are usually larger than females. Substantial progress has been made in order to elucidate the physiological and environmental factors that are involved in the differences in body size. Carrieri et al⁷⁹ studied the effect of candidate gene mutation on the body size of *D. melanogaster* males and females. The results of their study suggest that the mutations affect the body size of the males and the females reversibly. Patterns of chromosome inversions also favor the body size dimorphism as reported by Taylor et al⁸⁰ in *Drosophila pseudoobscura*.

In addition to body size, there is another most important sexual dimorphic character, the sex comb of *Drosophila*. It is one of the most evolved structures and present in the *melanogaster*, *obscura*, *fima*, and *dentissima* species groups.^{81–85} Variations in the orientation of sex comb as well as sex comb tooth number exist among different species of *Drosophila*.^{86,87} Several studies pertaining to sex comb depict the roles of *HOX* and *Scr* genes in the development of this morphological structure. *Scr* deficiency causes reduction in the number of sex combs, and *Scr* duplication increases the number of sex comb teeth.^{88,89} These temperature-sensitive *Scr* alleles are required for sex comb development during the third instar stage.⁹⁰ Similarly, the two specific isoforms *dsxM* and *dsxF* act differently in the development of male- and female-specific structures (Table 1).^{91–96} Both the isoforms of *dsx* act antagonistically on different targets present on multiple tissues with one being a positive regulator of certain gene in one tissue while negative regulator in others and vice versa.⁹⁷ Several other genes also play a role in the development of sex comb, for example, *wg*, *dpp*, *Distal-less (DII)*, *dachshund (dac)*, *rotund (rn)*, and *bric-a-brac (bab)* (Table 1).^{97,98}

Various types of pigmentation variations within and between closely related species are observed in many insect taxa as well as in *Drosophila* spp.^{99–103} Among them, an intriguing point one would notice is the existence of numerous reports on closely related species pairs with contrasting appearances in terms of body pigmentation intensity. Those cases

include species pairs, *Drosophila ananassae*–*Drosophila pallidosa*,¹⁰⁴ *Drosophila yakuba*–*Drosophila santomea*,¹⁰⁵ *Drosophila malerkotliana malerkotliana*–*Drosophila malerkotliana pallens*,¹⁰⁶ *Drosophila pseudoananassae pseudoananassae*–*Drosophila pseudoananassae nigrens*, *Drosophila bipectinata*–*Drosophila parabipectinata*,¹⁰⁶ and *Drosophila novamexicana*–*Drosophila americana*. Actually, *D. novamexicana* is distinctively pale compared to other *Drosophila virilis* group species.¹⁰⁷ These species pairs have divergence time short enough that ability to interbreed has allowed genetic analyses and has helped narrowing down or to identify responsive genetic changes underlying the pigmentation differences.^{102,106–108} Males of this group show darkly pigmented dorsal cuticular tergites, while in females, pigmentation is restricted to posterior tergites. This differential pigmentation is regulated by the expression of two genes *bab1* and *bab2* of the *bab* locus.^{108–110} However, the function of *bab* is under the control of female-specific *ABD-B* and *DsxF* (Table 1).^{111,112}

Several other genes were also found to be responsible for the abdominal pigmentation, which are transcribed and translated only at the time of development.^{102–118} Among different *Drosophila* spp. subgroups, species and subspecies in the *ananassae* species subgroup have darkly pigmented abdominal tip, while others lack completely. In many species of the *melanogaster* group, abdominal pigmentation is sexually dimorphic with the last two or three segments completely covered by dark melanin in males and not in females. Although sex-specific pigmentation is fixed in the closest relative of *D. melanogaster*, it is highly variable in the more basal *ananassae* species subgroup where some species and subspecies have dark male abdominal pigmentation, while others lack it completely.¹¹⁹ Wing spot in certain species of *Drosophila* has put forward a very good example in order to explain the phenomenon of sexual dimorphism in this genus. The wing spot in *Drosophila* has been evolved from the regulatory network of some novel genes and is regulated at the transcriptional level by the cis-regulatory elements of yellow pigmentation gene.¹²⁰ Experiments pertaining to mating behavior in this species suggest a greater mating success of the males with black wing spot as compared to the males without spot. These studies suggest the role of wing spot during courtship and thereby provide an excellent example of the adaptive function of pigmentation and its diversification.¹²¹ Recently, Takahashi¹²² has discussed the pathway underlying the biochemical basis of pigmentation variation in different *Drosophila* spp. Yassin and Orgogozo¹²³ found that there are several new female-specific genital structures that coevolve

with male genital structures providing a very good example of sexual dimorphism at the postcopulatory level.

Tools and techniques to analyze several behavioral paradigms: an approach to system genetics

As it is already discussed, *Drosophila* is one of the best model systems to understand neurogenetics of behavior. This is due to simple and sophisticated sets of genetic tools and techniques. One of the most important and easiest modes to analyze the behavioral defects is the use of behavioral mutants. Mutants have been supported by several behavioral biologists as an approach to understand the complexity of the behavioral system. For example, a known mutant in the genus *Drosophila* is *fru*, which disrupts the male and female orientation during mating. There are two mutants like *satori* (*sat*) and *he is not interested* (*hni*) involved in homosexual courtship.^{124,125}

A recent advancement in the *Drosophila* molecular genetics system is the entry of UAS/GAL4 expression system. By this system, it is easier to identify those behavioral genes that are expressed in neuronal mass in the brain and other neuronal innervations.^{126–130} Some other advantages of this system are 1) identification of genes by making use of enhancer trap mechanism, 2) targeted knockdown of gene of interest to produce RNAi lines of that corresponding gene, and 3) screening of mutations affecting a certain known phenotype. Thus, this system helps us to provide a way to observe several behavioral phenotypes.

In the recent era, technologies like optogenetics and thermogenetics have made possible the identification of certain genes, which directly affect courtship stimuli. One such discovery in the field of thermogenetics is the use of the thermosensitive channel TrpA1 to activate neurons expressing *fru*, a major courtship regulator gene, and thereby induce most of the elementary courtship acts in a solitary male exposed to temperature increase. The TrpA1 flies are mostly activated at 29°C, and temperature-sensitive behavioral response can be easily analyzed and corresponding genes will be identified through GAL4 system.^{131,132} The area of optogenetics helps in visualizing any phenotype misexpression in *Drosophila* eyes. This utilizes the GMR-GAL4 in order to create visual defects and thereby makes easier the analysis of behavioral responses due to such type of disruptions.¹³³

More recent advancement in fly genetics is the use of synthetic nuclease technologies, especially the CRISPR/Cas9 system. Application of these new genome-editing approaches in *Drosophila* will modernize our understanding to analyze

the function of gene in the near future. Several groups have used the CRISPR/Cas9 system to induce targeted mutations in *Drosophila*^{134–139} but differed in their approach to supplying the Cas9 protein and sgRNA components of the system. Synthetic nucleases are programmable nucleases that can be directed to cleave a specified sequence in the genome and thereby provide different knockout lines of *Drosophila*. The simplicity at which it can be reprogrammed to target different sites will also enable large-scale application of this genome-wide technology, to allow more powerful reverse genetic analyses, and allow us to more easily study combinations of any type of behavior-based mutations.

Conclusion

D. melanogaster has proven to be a wonderful model system to understand the genetic basis of sexual behavior. This is due to the presence of small number of chromosomes as well as availability of several mutants that makes understanding of behavior genetics of *Drosophila* very easy. Still, there is a big question that how these genes control the sex specificity of behavior during the time of development. However, it has been observed that the behavioral genes have a wide range of functions. Therefore, it is very much necessary to understand those complex networks which have direct role with the behavioral genes. Also, the recent advancement in the areas of optogenetics and thermogenetics and the development of different tools/technologies like GAL4/UAS system has made any kind of behavior genetic analysis very easier. And the use of CRISPR/Cas9 method to generate any kind of single point mutations helps in understanding numerous aspects of behavior.

Therefore, the main aim of this review is to summarize certain aspects of behavior of *Drosophila* which are under the genetic and nervous regulation.

Acknowledgments

Financial support in the form of UGC-BSR faculty fellowship to BNS and Meritorious fellowship to AS from the UGC, New Delhi, is gratefully acknowledged. The authors also thank the Editor-in-Chief and two anonymous reviewers for their helpful comments and suggestions on the original draft of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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