

## Behavioural reproductive isolation and speciation in *Drosophila*

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The origin of premating reproductive isolation continues to help elucidate the process of speciation and is the central event in the evolution of biological species. Therefore, during the process of species formation the diverging populations must acquire some means of reproductive isolation so that the genes from one gene pool are prevented from dispersing freely into a foreign gene pool. In the genus *Drosophila*, the phenomenon of behavioural reproductive isolation, which is an important type of premating (prezygotic) reproductive isolating mechanisms, has been extensively studied and interesting data have been documented. In many cases incomplete sexual isolation has been observed and the pattern and degree of isolation within and between the species have often been used to elucidate the phylogenetic relationships. The present review documents an overview of speciation mediated through behavioural incompatibility in different species groups of *Drosophila* with particular reference to the models proposed on the basis of one-sided ethological isolation to predict the direction of evolution. This study is crucial for understanding the mechanism of speciation through behavioural incompatibility and also for an understanding of speciation genetics in future prospects.

[Nanda P and Singh BN 2012 Behavioural reproductive isolation and speciation in *Drosophila*. *J. Biosci.* 37 359–374] DOI 10.1007/s12038-012-9193-7

### 1. Introduction

In the origin and maintenance of races and species, isolation is an indispensable factor, and its role and importance has been recognized for a long time. Without isolation, evolution is impossible (Dobzhansky 1951). The role of isolation in evolution was stressed by Wagner in 1889 and in a modern form by Jordon in 1905. Races and species differ from each other in many genes and gene combinations. If these genetically distinct populations interbreed, these systems will breakdown and the differences between them will be swamped. The gene pool of every species is discretely isolated from that of any other (Dobzhansky 1970; Mayr 1970). Dobzhansky (1937) coined the term ‘isolating mechanisms’ for genetically conditioned barriers that prevent gene flow between Mendelian populations. These mechanisms are characterized as premating, postmating-prezygotic and post-zygotic barriers (Coyne and Orr 2004). Behavioural isolation (also called as ethological or sexual isolation) is included in the category of premating barriers that include all differences which lead to the lack of cross-attraction between opposite

sexes of different species, preventing them from initiating courtship or copulation (Coyne and Orr 2004). The origin of sexual isolation is the central event in the evolution of biological species and plays a key role in maintaining biological diversity. It is common in nature and plays a central role in maintaining genetic and phenotypic differences between sibling species (Dobzhansky 1937; Mayr 1963; Coyne and Orr 2004).

Species differences in characters involved in behavioural isolation are candidates for factors that cause speciation. Studying incipient sexual isolation and speciation is an important pursuit in evolutionary biology. Since Darwin’s publication of *The Origin of Species* in 1859, the study of speciation has generated much enthusiasm and passionate debate. Interest in this subject continues to grow, as evidence by an exponential rise in citations of speciation studies over the last three decades (Sobel *et al.* 2009). During the process of speciation the diverging populations must acquire some means of isolation so that the genes from one gene pool are prevented from dispersing freely into a foreign gene pool.

**Keywords.** Behavioural reproductive isolation; *Drosophila*; isolating barriers; models of asymmetry; speciation

Speciation may often require the evolution of different forms of reproductive isolation (Mayr 1963; Lowry *et al.* 2008) in which the strength of each barrier varies in light of its relative contribution to total isolation. The coherence between speciation and reproductive isolation remains a successful legacy from this period, although increasingly more emphasis is currently being placed on populational aspects of the speciation process (Howard and Berlocher 1998; Sobel *et al.* 2009). Premating reproductive isolation is an early-acting isolating mechanism that arises due to behavioural incompatibility and has more potential to impede gene flow because barriers that act later can only stop the gene flow that remains after earlier barriers. Premating isolation is a potential cause of rapid speciation particularly in sympatric populations (Coyne and Orr 1989). Sexual or ethological isolation is a premating barrier to gene exchange in which the opposite sexes of different populations fail to mate due to behavioural incompatibility. Sexual isolation may also evolve due to direct selection on actual mate preference rather than selection on ecological *per se* (Servedio 2001).

In many taxa, individual species show species-specific stereotypical sexual behaviours before mating (Ewing 1983; Choe and Crespi 1997). This view is supported by meta-analysis revealing that selection on traits involved in mating is on average two times stronger than selection acting on traits that influence survival and fecundity (Kingsolver and Pfennig 2007; Hoskin and Higgie 2010). The evolutionary forces that can cause behavioural isolation are those that alter mating signals and preferences, including direct selection both on signalling traits that increase attractiveness and on preference traits that improve mate acquisition (for a review, see Panhuis *et al.* 2001; Coyne and Orr 2004). The present review documents an overview of speciation mediated through premating barriers in different species of *Drosophila* with particular reference to the models proposed on the basis of one-sided ethological isolation to predict the direction of evolution.

## 2. Reproductive isolation and speciation

Studies of reproductive isolation continue to help elucidate the process of speciation. Species differences in characters involved in reproductive isolation are candidates for factors that cause speciation. The Marie Curie speciation network (2012) presented different mechanisms and genetic basis of speciation and the relationship between speciation and diversity. Mate choice underlying sexual isolation depends on mating signals and associated preferences for the signals referred to as 'mating traits' and any divergence of mating traits among populations may initiate sexual isolation (Endler 1989; Andersson 1994; Coyne and Orr 2004). Heisler *et al.* (1987) defined 'mate choice' as the pattern of

mating which arises because of these mating preferences. Paterson (1980) proposed that every species possesses its own distinct specific mate recognition system that controls the exchange of sensory information sent and received by both sexual partners during courtship. The differentiation of the mate recognition system is one of the major steps that leads to speciation in animals, but little is understood about the evolution of mate recognition systems, because of its complexity (Ishii *et al.* 2001). Divergence in mating preferences among populations is considered as important source of reproductive isolation. Mating preferences may diverge between populations as a result of genetic drift or because of divergent natural selection between environments (Schluter 2001; Rundle *et al.* 2005). Behavioural traits that are involved in a wide range of mating cues and preferences fall under the generalized category of sex traits. Such characters, often species- or genus-specific, can be quite divergent between species. Mate recognition systems consist of secondary sexual characteristics in one sex (usually males) associated with a preference for the trait(s) in the other sex (usually females) (Servedio and Saetre 2003). These systems have been considered as important species attributes that act as cohesive forces (Paterson 1980). Paterson (1978) argued that the evolution of a specific mate recognition system, composed of multiple 'coadapted stages', is essential to intersexual signalling and is likely to be maintained by strong stabilizing selection. In *Drosophila*, the diversity of the mating behaviour in various species and basic similarity between some species emphasize that mating behaviour has gone through evolutionary changes. Ruedi and Hughes (2008) studied variation in mating behaviour of *D. melanogaster* and emphasized the genetics of courtship behaviour and various mechanisms underlying sexual selection. This helps to understand the evolution of the genus. Variation of different signals by which two sexes exchange and their predominance during mating can contribute to the appearance of the premating isolation (Butlin and Ritchie 1994). If behavioural divergence results in reduced gene flow, then increasing genetic differentiation among populations may help preserve incipient reproductive isolation, and if selection is strong enough, reproductive isolation can persist in sympatry despite low levels of interpopulational gene flow (Feder 1998; Gomulkiewicz *et al.* 1999; Berlocher and Feder 2002; Massie and Markow 2005). Species may overlap with a mosaic of species across its geographic range. Species interactions causing selection on mating traits play an important role in generating species divergence. Hoskin and Higgie (2010) said that reproductive character displacement among populations may be generated by a variety of species interactions beyond reinforcement and these interactions are responsible for mating trait divergence and speciation. Selection against hybrids between closely related taxa results in enhanced premating isolation (i.e. reinforcement) between

the taxa in the areas of overlap (Dobzhansky 1951; Servedio and Noor 2003). Reinforcing selection operates on the mate recognition system of a species through selection on preferences and display traits. It is as an important component of speciation that is strengthened by natural selection and also strengthens prezygotic isolation between closely related taxa (Dobzhansky 1940; Noor 1995; Servedio and Noor 2003; Coyne and Orr 2004). Yukilevich (2012) emphasized the role of reinforcement due to enhanced prezygotic isolation among sympatric species, and the findings suggest that reinforcement plays a key role in *Drosophila* speciation. Divergence of such mating signals and recognition systems can lead to speciation (Coyne and Orr 2004). Therefore, identifying species-specific characters involved in sexual behaviour is important in the study of sexual isolation and, ultimately, speciation. Takahashi and Ting (2004) studied the genetic basis of sexual isolation in *D. melanogaster* and emphasized on evolution and maintenance of behavioural polymorphism in nature. It was suggested that speciation would be harder to achieve in general if reproductive isolation is based on a large number of loci that may potentially generate many intermediate phenotypes (Takahashi and Ting 2004). The phenomenon of sexual isolation has been extensively studied and found to be widespread (Ehrman and Parsons 1980; Henderson and Lambert 1982; Speith and Ringo 1983; Dodd 1989; Hollocher *et al.* 1997a; Gleason and Ritchie 1998; Carracedo *et al.* 2000; Korol *et al.* 2000; Ishii *et al.* 2001; Ting *et al.* 2001; Haerty *et al.* 2005; Etges *et al.* 2006; Castrezana and Markow 2008; Etges and Tripodi 2008; Huttunen *et al.* 2008; Schug *et al.* 2008; Yukilevich and True 2008; Jennings and Etges 2010; Singh 2010a).

Since the speciation process takes many generations, it has to be studied by comparison of many snapshots of divergent and partially isolated population pairs. These populations will eventually evolve into completely isolated species nevertheless, they may be used to determine the genetic architecture of reproductive isolation and to dissect the contributions of different traits to the overall reduction of gene flow. The process of speciation that begins with genomically localized barriers to gene exchange is associated with loci for local adaptation and this barrier then spreads until reproductive isolation influences the whole genome (Butlin 2010). However, in some species groups, the phenomenon of reproductive isolation has given rise to different races or subspecies that has been discussed.

### 2.1 *Drosophila melanogaster* species group

Earlier studies suggested that *D. melanogaster* mates randomly across its range (Henderson and Lambert 1982), but recent evidences indicate that some African populations in and near Zimbabwe ('Z-type') have evolved incipient sexual

isolation from 'cosmopolitan' ('M-type') populations (Wu *et al.* 1995; Hollocher *et al.* 1997a; Greenberg *et al.* 2003). Females from both Zimbabwe and cosmopolitan strains tend to mate preferentially with their own 'Z-type' or 'M-type' males, respectively, with some Zimbabwe strains showing very strong mating preferences (Wu *et al.* 1995; Hollocher *et al.* 1997b). There is substantial divergence in nuclear genes (Begun and Aquadro 1993), microsatellite loci (Kauer *et al.* 2002; Kauer and Schlotterer 2004), chromosomal inversions (Aulard *et al.* 2002) and various phenotypic traits including body size, pigmentation, cuticular hydrocarbon composition and wing beat frequency, that are all known to be under genetic control (Colegrave *et al.* 2000; Rouault *et al.* 2001; Takahashi *et al.* 2001). This system suggests that the evolution of reproductive isolation in recently widespread and human commensal species is possible.

This species also segregates for mating preferences in other parts of the world, such as the case of 'microhabitat' sexual isolation in the West African Brazzaville populations (Capy *et al.* 2000; Haerty *et al.* 2005). Evidence for non-random mating in *D. melanogaster* laboratory populations derived from closely adjacent ecologically contrasting slopes of Evolution Canyon was found (Korol *et al.* 2000; Singh *et al.* 2005). It was suggested that the inter-slope microclimatic differentiation causes strong differential selection for stress tolerance, which in turn promote behavioural adaptations facilitating reduced gene exchange. Moreover, it has recently been discovered that Caribbean and West African populations mate randomly with each other, but show partial sexual isolation against US cosmopolitan flies and against Zimbabwe flies (Yukilevich and True 2008).

Sexual isolation among three sibling species, *D. melanogaster*, *D. simulans* and *D. mauritiana*, was studied (Carracedo *et al.* 2000). *D. melanogaster* and *D. simulans* are cosmopolitan species largely associated with human habitats, whereas *D. mauritiana* is only found in the Island of Mauritius (Tsacas and David 1974). Phylogenetic studies based on banding pattern homology of polytene chromosomes, DNA sequence and hybrid sterility showed that *D. simulans* and *D. mauritiana* are more closely related to each other than to *D. melanogaster* (Lemeunier and Ashburner 1976; Cariou 1987; Kliman and Hey 1993). Sexual isolation among the three species was studied and asymmetrical mating preferences were observed. *D. mauritiana* males mate with both *D. melanogaster* and *D. simulans* females, and females of *D. mauritiana* discriminate strongly against males of these two species, and *D. simulans* males mate with *D. melanogaster* females but the reciprocal cross is difficult. (Watanabe and Kawanishi 1979; Carracedo and Casares 1985; Coyne 1989). Carracedo *et al.* (2000) suggested that species accumulate prezygotic isolation genes during allopatric speciation and genetic systems involved

in sexual isolation are species-pair specific, i.e. species use different cues to discriminate against other species.

*D. santomea* and *D. yakuba*, two sister species of the *melanogaster* species group, show substantial reproductive isolation, hybrid male sterility, habitat isolation based on temperature tolerance and preference and some forms of postmating-prezygotic isolation (for references, see Matute and Coyne 2009). Matute and Coyne (2009) described several new intrinsic barriers that do not depend on species' ecology, i.e. faster depletion of sperm, reduced egg number and reduced egg hatchability in interspecific as compared to intraspecific matings, which suggests that these species diverged recently and are separated by different isolating barriers that act both before and after mating. Molecular data suggest that *D. yakuba* and *D. santomea* began diverging about 400,000 years ago (Llopart *et al.* 2005a). It is likely that *D. santomea* evolved from a common ancestor with *D. yakuba* that colonized the island at that time, and that the present contact between *D. santomea* and *D. yakuba* reflects a secondary colonization by *D. yakuba* from the African mainland, possibly during the last 500 years when Portuguese colonists converted coastal rainforest into plantation (Llopart *et al.* 2005b).

## 2.2 *Drosophila obscura* species group

Species of the *D. obscura* group are inhabitants of temperate forests throughout the Holarctic region and temperate-like habitats in the Afrotropical, Neotropical and Oriental regions. Reproductive isolation between *D. sinobscura* and *D. hubeiensis*, two allopatric species of the *D. obscura* group, was studied and asymmetrical mating preferences were observed (Watabe and Aoki 2000). The *D. pseudoobscura* species subgroup comprises two *D. pseudoobscura* (*D. ps.*) subspecies (*D. ps. pseudoobscura* and *D. ps. bogotana*) and two closely related species (*D. persimilis* and *D. miranda*). The *D. pseudoobscura* subspecies are geographically isolated, share chromosomal arrangements and represent the earliest stages of species divergence (Ayala and Dobzhansky 1974). Both *D. pseudoobscura* subspecies differ from *D. persimilis* by fixed chromosomal inversion frequencies on three of their major chromosomal arms and F<sub>1</sub> hybrid males from crosses between these species are sterile (though females are fertile). In contrast, *D. miranda* is an outgroup species that cannot produce any fertile hybrids with *D. pseudoobscura* or *D. persimilis* (Dobzhansky 1937). The relationship of *D. ps. pseudoobscura*, *D. ps. bogotana*, *D. persimilis* and *D. miranda* are well established by DNA sequences, chromosomal inversions and reproductive isolation (Dobzhansky and Powell 1975; Powell 1997). Nucleotide divergence between the hybridizing species *D. ps. pseudoobscura* and *D. persimilis* is high within and near the three chromosomal inversions (Machado *et al.*

2007a; Noor *et al.* 2007), which are linked to factors conferring hybrid sterility, mating discrimination and other barriers to gene flow (Noor *et al.* 2001; Brown *et al.* 2004). Kulathinal *et al.* (2009) studied genetics of speciation in *D. ps. pseudoobscura* and *D. persimilis* and suggested that autosomal gene exchange between these species occurred since the split of the subspecies, likely within the last 200,000 years. Kulathinal *et al.* (2009) concluded that chromosomal rearrangements have been vital to the ongoing persistence of these species despite recent hybridization.

## 2.3 *Drosophila repleta* species group

*D. mojavensis* and *D. arizonae*, two cactophilic members of the *mulleri* complex of the *repleta* species group, show strong, yet incomplete, pre- and postzygotic isolation in the laboratory (Reed and Markow 2004). Although no evidence for hybridization between *D. mojavensis* and *D. arizonae* has been found in nature (Counterman and Noor 2006; Machado *et al.* 2007b), hybrids can be formed in the laboratory with incomplete postzygotic isolation. Furthermore, hybrid males with *D. arizonae* mothers are sterile whereas those with *D. mojavensis* mothers differ in sterility depending on the origin of the *D. mojavensis* used in the cross (Ruiz *et al.* 1990; Reed and Markow 2004). Jennings and Etges (2010) studied premating sexual isolation between *D. mojavensis* and *D. arizonae* and showed that experimental designs (chamber size, host plant presence and rearing substrates) had significant effects on levels of premating isolation between these two species. Cytological evidence suggests that *D. mojavensis* originated in Baja California and was derived from an ancestral population of *D. arizonae*-like ancestor on the mainland (Ruiz *et al.* 1990). These derived mainland populations of *D. mojavensis*, therefore, subsequently colonized southern California, northwestern Mexico and Arizona from Baja California by switching host plants. *D. mojavensis* is considered to be in the initial stages of divergence, as there is significant reproductive isolation between populations (Zouros and D'Entremont 1980; Etges 1992). Populations of *D. mojavensis* were examined using a variety of molecular markers (allozymes, mitochondrial DNA and nuclear DNA including microsatellites) to study population genetic structure and incipient speciation in this species (Hocutt 2000; Ross and Markow 2006; Machado *et al.* 2007b; Reed *et al.* 2007). Based on analyses of allozyme data and previously published data on behavioural, ecological, morphological and reproductive differences, Hocutt (2000) suggested the existence of four subspecies of *D. mojavensis*, i.e. *D. m. mojavensis*, *D. m. baja*, *D. m. sonorensis* and *D. m. wrightleyi*. Support for the subspecies assignments proposed by Hocutt (2000) has been provided by several molecular studies that examined variation in mtDNA (Reed *et al.* 2007), nuclear DNA (Ross and Markow

2006), multiple nuclear loci (Machado *et al.* 2007b) and the glutathione S-transferase D1 (*Gst D1*) gene (Matzkin 2008) in a number of populations of *D. mojavensis* sampled from each of the four main geographic areas.

#### 2.4 *Drosophila willistoni* species group

*D. paulistorum* is one of the six sibling species that constitute the *D. willistoni* group which comprises *D. willistoni*, *D. equinoxialis*, *D. tropicalis*, *D. insularis*, *D. pavlovskiana* and *D. paulistorum*. *D. paulistorum* complex is a cluster of species *in statu nascendi*, a borderline case of incomplete speciation (Dobzhansky and Spassky 1959) that comprises six races or subspecies, i.e. Centro-American, Amazonian, Andean-South Brazilian, Orinocan, Guianan and Transitional. The genetic basis of reproductive isolation has been shown in *D. paulistorum* and genetic factors are distributed all over the chromosomes (Ehrman 1961). However, variation in degree of sexual isolation within *D. paulistorum* is considered as a classical example of character displacement (Ehrman 1965). Gleason and Ritchie (1998) studied reproductive isolation in *D. willistoni* species complex and found that both types of isolation (pre-mating and post-mating) increase with genetic distance and post-mating isolation is more strongly correlated with genetic divergence. Song divergence is not correlated with genetic divergence but evolve more quickly within this species group, probably as a result of sexual selection.

#### 2.5 *Drosophila ananassae* subgroup

*D. ananassae* belongs to the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group. It is a cosmopolitan and domestic species. It occupies a unique status among the *Drosophila* species due to certain peculiarities in its genetic behaviour. The most unusual feature of this species is spontaneous male meiotic recombination in appreciable frequency (Singh 2010b). It possesses high degree of inversion polymorphism, but only three paracentric inversions, namely, subterminal alpha (2L), terminal delta (3L) and basal eta (3R), are found to be coextensive with the species (Singh 1996).

In *D. ananassae*, mate discrimination varies considerably throughout the species range, being higher among the populations outside the ancestral Indonesian range and the highest in South Pacific. Results suggest that colonization and genetic differentiation affect the evolutionary origin of mate discrimination (Schug *et al.* 2008). Pair-wise  $F_{ST}$  values showed that Indian populations of *D. ananassae* exhibit strong genetic differentiation, display population sub-structuring and exist as semi-isolated populations (Singh and Singh 2010). In *D. ananassae*, the degree of sexual

isolation is stronger in isofemale lines than in natural populations and may involve genetic bottlenecks (Singh and Chatterjee 1985). It has been demonstrated that there is instability of mate recognition system in *D. ananassae* (Singh and Chatterjee 1985; Nanda and Singh 2011a, b, c).

Sexual isolation, maintained by strong mating preferences has been reported in the light and dark forms of *D. ananassae* in laboratory stocks (Futch 1966). These forms were found to be sibling species (*D. ananassae* and *D. pallidosa*) of the *ananassae* complex which show strong sexual isolation (Futch 1973; Doi *et al.* 2001; Sawamura *et al.* 2006; Vishalakshi and Singh 2006). In spite of their sympatric distribution, post-mating reproductive barriers such as hybrid sterility or hybrid inviability do not exist between them (Futch 1966; Bock and Wheeler 1972). Analyses of Y-chromosomal and mitochondrial haplotypes, shared chromosomal arrangements, pre-mating isolation and hybrid male sterility suggested that these taxa represented a recent evolutionary radiation and experienced substantial gene flow that explained the existence of *D. parapallidosa* (Matsuda *et al.* 2009). New taxa that are closely related to *D. ananassae* in the *ananassae* species cluster have been identified whose species affiliations are unclear. These strains, viz. *D. parapallidosa*, *D. pallidosa*-like, *D. pallidosa*-like Wau and *D. papuensis*-like, are similar to *D. ananassae* and *D. pallidosa*, but they are partially reproductively isolated from these species and have distinct chromosome arrangements.

The *bipectinata* species complex is a group of four morphologically similar species, namely, *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae*. The latter two species have two allopatric subspecies: *D. m. malerkotliana* and *D. m. pallid*, and *D. p. nigrens* and *D. p. pseudoananassae*. Kopp and Frank (2005) described two allopatric subspecies of *D. bipectinata*, namely, Pacific and Asian, that show a continuum of reproductive isolation. Crosses between strains of the same subspecies produce fully fertile offspring and each subspecies harbours extensive variation for the degree of reproductive isolation from the other subspecies. The two subspecies show little or no evidence of genetic differentiation at three chromosomal loci, suggesting that they diverged very recently or continue to experience significant level of gene flow. However, Matsuda *et al.* (2005) have reported three subspecies of *D. bipectinata*, namely, *bipectinata* (from Southeast Asia), *szentivani* and *pacificiae* (South Pacific Ocean), which are reproductively isolated from each other and produce sterile  $F_1$  males. Singh *et al.* (1981) tested sexual isolation among three species of the *bipectinata* complex. The results reveal that there is preferential mating between *D. bipectinata* and *D. malerkotliana* in both directions. The females of both the species show sexual discrimination against males of other species. Thus, neither of the two species can be

considered as ancestral or as derived. However, there is a difference in the degree of sexual isolation in reciprocal crosses. Similar results were found in crosses between *D. parabiepectinata* and *D. malerkotliana*. *D. biepectinata* and *D. parabiepectinata* when subjected to sexual isolation test show strong sexual isolation owing to preferential mating in one direction only, suggesting that *D. biepectinata* has given rise to *D. parabiepectinata* (Singh *et al.* 1981). Intraspecific sexual isolation was examined among wild strains of *D. biepectinata*, *D. malerkotliana*, *D. parabiepectinata* and *D. pseudoananassae*, and the results provide evidence for incipient sexual isolation within *D. biepectinata*, *D. malerkotliana* and *D. parabiepectinata* due to genetic divergence (Singh and Chatterjee 1991, 1992).

### 2.6 Hawaiian *Drosophila planitibia* subgroup

The Hawaiian *Drosophila* represents 20% of the species in the genus *Drosophila*, despite the fact that the Hawaiian Islands have such a small land area (Carson 1982). This fly diversity resulted from immigration from older to younger islands, with each new species forming allopatrically in habitat similar to that occupied by the ancestral species (Carson 1982). The three best studied members of the genus in Hawaii, *D. planitibia*, *D. silvestris* and *D. heteroneura*, inhabit cloud forests on the flanks of volcanoes on Maui (*D. planitibia*) and Hawaii (*D. silvestris* and *D. heteroneura*). Kaneshiro (1976, 1980) studied sexual isolation by using male-choice technique among four recently evolved species, i.e. *D. planitibia*, *D. silvestris*, *D. heteroneura* and *D. differens* of the *planitibia* group, and proposed that behavioural isolation is often asymmetrical, with females of the ancestral species being unlikely to mate with males of the derived species. Their results also suggested that sexual selection could lead to speciation after founder event. Ahearn *et al.* (1974) studied sexual isolation among three species of *Drosophila* i.e. *D. heteroneura*, *D. silvestris* and *D. planitibia*. *D. heteroneura* and *D. silvestris* are sympatric species while *D. planitibia* is allopatric species. Ethological isolation was found between the sympatric species and not between the allopatric ones. However, *D. planitibia* females discriminated against *D. heteroneura* males. Male hybrids are sterile in allopatric crosses but fertile in sympatric ones. These investigators concluded that premating isolation between closely related species originated as an *ad hoc* product of natural selection while postmating isolation is an incidental result of genetic divergence. Boake (2005) reviewed different models that are relevant in understanding of sexual selection and speciation in Hawaiian *Drosophila*.

### 2.7 *Drosophila virilis* species group

*D. virilis* species group represents an important species group for studying the genetics of reproductive isolation (Patterson and Stone 1952). This group is divided into two subgroups: *virilis* and *montana*. The *virilis* subgroup consists of five closely related taxa: *D. virilis*, *D. lummei*, *D. novamexicana*, *D. americana americana* and *D. americana texana*. Hilton and Hey (1996) studied DNA sequence variation at the *period* locus in the *D. virilis* species group. Their results suggested that there was no evidence of divergence between *D. a. americana* and *D. a. texana* and also suggested that *D. novamexicana* consists of two distinct groups (Nova-A and Nova-B) each with low population size and no gene flow between them, suggesting that two groups could have arisen independently, and these characteristics suggested that *D. novamexicana* arose once and then split into two groups. Sweigart (2010) studied phenotypic and genetic basis of postmating-prezygotic isolation between two closely related species of *Drosophila*, i.e. *D. virilis* and *D. americana*. Results showed that there is strong barrier to interspecific fertilization, resulting in 99% reduction in progeny production and genetic interaction among maternal and paternal alleles at few loci that prevents the fertilization of *D. virilis* females by *D. americana* males. It was also suggested that male-female coevolution within *D. americana* may have driven postmating-prezygotic isolation between species. Patterson *et al.* (1942) described reproductive isolation due to gamete mortality in reciprocal crosses between *D. virilis* and *D. americana*. In later studies, these investigators discovered that very few eggs from interspecific crosses become fertilized and speculated that sperm become 'immobilized in the reproductive tract of the alien female' (Patterson and Stone 1952). Consistent with the evolution of these interspecific barriers, male and female reproductive tract proteins have been shown to evolve rapidly in the *D. virilis* species group (Civetta and Singh 1995; Haerty *et al.* 2007). Nickel (2007) analysed the male courtship behaviour of closely related species of the *virilis* phylad when paired with conspecific or heterospecific females and found that *Drosophila virilis* males do not prefer to mate with closely related heterospecific females and members of the *D. virilis* group show variation in the female cuticular hydrocarbon profiles that causes males of the *virilis* phylad in species recognition. Saggia and Civetta (2010) studied male-female interactions and postmating-prezygotic isolation and found that *D. virilis* females from different geographic locations mate with *D. novamexicana* males in which egg laying is normal, but fertilization rates are severely reduced, despite normal rates of sperm transfer. This reduction in fertilization is probably due to lower retention of heterospecific sperm in female storage organs

1–2 days after copulation. A recent study has shown that when *D. novamexicana* females are exposed to *D. virilis* males for up to 2 weeks, only 14% of females produce progeny. Moreover, *D. virilis* males are able to recognize heterospecific females at the first stage of courtship (tapping), indicating strong premating isolation (Nickel and Civetta 2009). For the reciprocal cross between *D. virilis* females and *D. novamexicana* males, there is evidence of strong postzygotic isolation with 7% hybrid male fertility (Orr and Coyne 1989). *D. virilis* females mated with *D. lummei* males produce 95% fertile hybrid males, but 47% of the hybridizations die before becoming pupae, and 25% fail to emerge from their case (Lumme and Heikkinen 1990).

In the *D. virilis* group of species, interpulse interval does not play an important role in species recognition or in female choice (Hoikkala and Lumme 1987). Song simulation studies have shown that in *D. virilis*, females are able to recognise some species-specific characters in male song, and that there is variation among females in their preferences (Isoherranen *et al.* 1999). Hoikkala and Lumme (1987) have also shown that the species specificity of the courtship song in several species of the *D. virilis* group is mainly caused by X-chromosomal genes.

Several species of the *D. virilis* phylad show strong premating isolation (Stalker 1942; Coyne and Orr 1989). It was suggested that levels of premating isolation among members of the phylad could be a direct consequence of gradual divergence or reinforcement after speciation. Other pairs of closely related species show quantitative and/or qualitative differences in CHC blend and associated premating isolation (Doi *et al.* 1996).

*D. montana* is an excellent model system for studying the early stages of speciation. Jennings *et al.* (2011) studied pre- and postmating barriers between flies from European (Finnish) and North American (Canadian) populations of *D. montana*. The results suggested that there is evidence for reproductive isolation in allopatric populations of *D. montana* populations and emphasized the importance of experimental design in the study of premating isolation between recently diverged taxa. Postmating barriers may be due to postcopulatory-prezygotic mechanisms and *D. montana* populations seem to be evolving through multiple barriers to gene flow in allopatry (Jennings *et al.* 2011). Saarikettu *et al.* (2005) showed that male song (especially the interpulse interval) plays an important role in sexual isolation between sympatric species: *D. montana* and *D. lummei*. Liimatainen and Hoikkala (1998) also showed that song plays an important role in species recognition, and results suggested that hybrids cannot be produced between *D. montana* females and *D. lummei* males in the laboratory because of high discrimination exercised by the females (Hoikkala 1988).

## 2.8 *Drosophila nasuta-albomicans* complex

*D. nasuta-albomicans* complex belongs to the *nasuta* subgroup of the *immigrans* species group. The *nasuta* subgroup represents an interesting cluster of morphologically nearly identical members with different degrees of reproductive isolation (Ranganath 2002). *D. nasuta* and *D. albomicans* are representative examples of evolutionary diversification within this subgroup, and despite striking divergence in their karyotypes, neither morphological divergence nor reproductive isolation between the two species have been achieved (Ranganath 2002). Ramachandra and Ranganath (1987, 1994) studied sexual isolation between *D. n. nasuta* and *D. n. albomicans* and also among the parental races and newly evolved cytoraces. Their results have shown behavioural asymmetry which has been discussed in the light of different models to predict the evolutionary sequence. Bachtrog (2006) studied speciation history of the *D. nasuta* complex and reported nucleotide variability for five X-linked and two mtDNA loci in eight taxa from *nasuta* with deeper sampling from *D. albomicans* and its sister species *D. nasuta*. The results suggested that phylogenetic relationship among these species varies among different genomic regions and levels of genetic differentiation, suggesting that this species group diversified only about 1 million year ago and *D. albomicans* share nucleotide polymorphism with few fixed differences (for references, see Bachtrog 2006).

## 3. Origin of sexual isolation

The evolution of reproductive isolation involves studying the process before it has reached completion. Natural populations that occupy different environments may show divergence in traits involved in assortative mating (Noor 1995; Tregenza *et al.* 2000; Tregenza 2002; Nosil *et al.* 2002, 2007). These examples have helped to elucidate the geographical, ecological and historical context of early stages of speciation. Recent evidences suggest that incipient sexual isolation may occur multiple times in various parts of the species range, especially among geographically widespread species (Rundle *et al.* 2000; Nosil *et al.* 2002; Tregenza 2002; Boughman *et al.* 2005). It thus becomes important to understand the similarities and differences between multiple cases of sexual isolation within species, i.e. how much genetic and phenotypic differentiations accompany each case of incipient sexual isolation and what is the genetic and phenotypic basis of assortative mating in each case (Panhuis *et al.* 2001; Hendry 2001; Boughman *et al.* 2005; Nosil *et al.* 2005; Hendry *et al.* 2007; Jennings *et al.* 2011). If multiple cases of sexual isolation showing unique

genetic and phenotypic bases, it would indicate that species segregate different types of variation associated with assortative mating in nature. On the other hand, if different cases of sexual isolation share common phenotypes and occupy similar environments, it would imply that there might be adaptive constraints on the conditions and the type of variation underlying these processes (Schluter and Nagel 1995; Rundle and Schluter 2004). Sexual isolation at initial stages of evolutionary divergence may be important in sympatric species formations (Coyne and Orr 2004; Dieckmann and Doebeli 1999; Michel *et al.* 2010) about which different mechanisms were proposed (Kondrashov and Kondrashov 1999; Gavrillets and Waxman 2002). Premating isolation is a potential cause of rapid speciation, particularly in sympatric populations (Coyne and Orr 1989). To explain how the reproductive isolating mechanisms originate between populations, different theories have been proposed. The evolution of premating isolation caused by divergent mating signals and preferences is an important component of speciation in many taxa (Coyne and Orr 2004). To explain how the reproductive isolating mechanisms originate between populations, different theories have been proposed. According to Muller (1942), reproductive barriers appear as a side effect of genetic divergence because populations adapted to different environments acquire genetic differences that lead to reproductive isolation. Dobzhansky (1940) gave more importance to natural selection acting on appropriate genetic variation when the allopatric populations that have an incipient isolation become sympatric. Carson (1971) emphasized the role of genetic drift through founder effect in the origin of reproductive isolation which serve as a barrier to gene flow. Carson's 'flush-crash' speciation theory (1975) has been supported by the work of Powell (1978), who observed strong assortative mating developed between cage populations of *D. pseudoobscura* that were passed through successive bottlenecks in population size.

Experimental tests of founder-flush theory have found significant levels of premating isolation in bottlenecked populations of *D. pseudoobscura* (Powell 1978; Galiana *et al.* 1993), *D. mercatorum* (Templeton 1981a), *D. simulans* (Ringo *et al.* 1986) and *D. ananassae* (Nanda and Singh 2011c), whereas Rundle *et al.* (1998) have found no significant cases of reproductive isolation in a bottlenecked population of *D. melanogaster*. Dodd and Powell (1985) have found that only 1 of 11 cases of prezygotic isolation was significant even after 8 years. Similarly, Moya *et al.* (1995) and Meffert *et al.* (1999) have reported the loss of assortative mating in bottlenecked populations through time.

Although natural selection may often play an important role in the initiation of divergence in mate recognition

between speciating populations (Turelli *et al.* 2001; Nosil *et al.* 2007), sexual selection is likely to contribute to the generation of further divergence in many instances (Schluter 2001). Although there have been many attempts to determine if divergent natural selection results in the evolution of mate recognition and subsequently sexual isolation (Rice and Hostert 1993), there is little experimental evidence for how sexual selection, particularly the interaction between natural and sexual selection, affects the evolution of mate recognition. Servedio and Saetre (2003) found linkage between genes affecting postzygotic and prezygotic isolation leading to a positive feedback loop in which both are strengthened. These investigators also found that genes causing hybrid incompatibility would hitchhike along with those improving premating isolation, leading to stronger hybrid incompatibility and there may be coevolution of postzygotic and prezygotic barriers that would be enhanced by the sex linkage of genes affecting mate recognition and hybrid viability (Servedio and Saetre 2003). The evolutionary process of 'reinforcement', often suggested as an important component of speciation, involves the strengthening of prezygotic isolation between closely related taxa by natural selection in response to maladaptive hybridization (Noor 1995; Servedio and Noor 2003; Coyne and Orr 2004).

#### 4. Genetics of premating isolation

What type of genetic changes bring about speciation is one of the most basic questions in biology. By applying genomic techniques to the analysis of laboratory crosses and natural populations has helped to determine the genetic basis of barriers to gene flow that create new species. Although new methodologies have not changed the concept of speciation, they have accelerated the pace of data collection by facilitating the compilation of case studies, advances in genetic techniques concerning the relative frequency and the importance of different processes that cause speciation (see review by Noor and Feder 2006).

The genetic architecture underlying reproductively isolating traits may have substantial impact on the likelihood and pace of speciation (see review by Arbuthnott 2009). Reproductive isolation, responsible for speciation, is likely to involve complex, coevolved, polygenic traits [leading to a 'type I' genetic architecture in the terminology of Templeton (1981a, b), i.e. numerous genes of small effect]. Most empirical studies of reproductive isolation, especially of sexual isolation, have found polygenic effects (Hollocher *et al.* 1997b; Ritchie and Philips 1998; Ting *et al.* 2001); however, a few large effect genes have been identified for both postmating (Barbash *et al.* 2003; Presgraves *et al.* 2003) and premating isolation (Greenberg *et al.* 2003). Recent models have suggested that divergence among populations and consequent speciation may be more likely when traits are

controlled by few loci (Arnegard and Kondrashov 2004; Gavrillets and Vose 2007; Hayashi *et al.* 2007), and that speciation may also occur more rapidly under such genetic architecture (Gavrillets and Vose 2007).

Identification of genes involved in reproductive isolation are important to investigate links between stages of speciation process. Qvarnström and Bailey (2009) reviewed the role of sex-linked genes at different stages of speciation and concluded that sex-linked genes coding for sexual isolation traits causes hybrid sterility. Ecological divergence often coupled with evolution of sexual isolation to avoid the homogenizing effect of gene exchange mainly occurs in sympatric populations or when populations come into secondary contact before they have become completely reproductively isolated. The genetic basis of mate recognition was studied by Civetta and Cantor (2003) between *D. simulans* and *D. sechellia* and their results suggested that majority of quantitative trait loci (QTL) responsible for both male mating behaviour and pheromone concentration are located on the third chromosome. One QTL found on the third chromosome showed variation in time needed to start courtship and copulation as well as time spent courting (Civetta and Cantor 2003). Moehring *et al.* (2006) mapped QTL contributing to sexual isolation between the sibling species *D. santomea* and *D. yakuba* and found three QTL affecting discrimination of *D. santomea* females against *D. yakuba* males.

In *D. ananassae*, heterosis was found to be associated with alpha inversion and male mating ability as heterokaryotypic males were superior in mating activity than homokaryotypic ones. Thus, inversion polymorphism may have a partial behavioural basis and males are more subject to intra-sexual selection than females (Singh and Chatterjee 1986, 1988). Inversions may facilitate the accumulation of alleles that contribute to reproductive isolation between populations connected by gene flow. This is particularly true for Bateson-Dobzhansky-Muller (BDM) incompatibilities, as otherwise the ancestral, compatible genotypes would be favoured (Noor *et al.* 2001). Analytical models of parapatric speciation confirm that BDM incompatibilities are more likely to accumulate at species boundaries in the presence of inversions than in the presence of genetic barriers that do not reduce recombination (Navarro and Barton 2003). Behavioural isolation has been found between two karyotypically different homozygous strains of *D. ananassae* derived from same geographic location, which shows that chromosome arrangements may affect mate recognition system in *D. ananassae* (Nanda and Singh 2010b). These inversions may promote sympatric or parapatric speciation by creating associations between alleles under divergent natural selection and those that cause assortative mating (Trickett and Butlin 1994; Butlin 2005). Also, if the inversion (or incompatibilities associated with it) causes some degree of postzygotic isolation, then selection may favour alleles that

increase the strength of prezygotic reproductive barriers (i.e. reinforcement), but only where populations are in contact (Servedio and Noor 2003; Butlin 2005).

Doi *et al.* (2001) mapped genes contributing to the female discrimination behaviour and showed significant effects of second and third chromosomes in *D. ananassae* and *D. pallidosa* that leads to sexual isolation. Yamada *et al.* (2002a, b), in their extensive studies of sexual isolation between two sibling and sympatric species *D. ananassae* and *D. pallidosa*, recorded and analysed male courtship songs and observed species specificity in the courtship song parameters that was the basis of sexual isolation between these two species. It was suggested that these parameters play a role in mate recognition and enforces sexual isolation. Multiple regression analysis by using interspecific mosaic genome lines of *D. ananassae* and *D. pallidosa* indicated highly significant effects on 2L for female mating willingness with *D. ananassae* males and on XL, 2L and 3R for that with *D. pallidosa* males (Sawamura *et al.* 2008).

Gleason *et al.* (2005) mapped genes affecting cuticular hydrocarbons that differ between *D. simulans* and *D. sechellia* leading to sexual isolation. Etges *et al.* (2007) studied genetic basis of incipient speciation among diverging populations of *D. mojavensis* and suggested that the genetic basis of traits that are directly associated with host use and fitness and those determining mate choice cause genetic divergence and reproductive isolation among geographically isolated populations of *D. mojavensis*

## 5. Asymmetry in behavioural isolation

It is often observed that when two closely related taxa exhibit premating isolation, the pattern is not symmetrical. The degree of sexual isolation and the mode of mating preference are often used to indicate the phylogenetic relationship between the species and also their evolutionary sequence (Singh 1997). Some studies have indicated that there is no correlation between asymmetrical mate choice and polarity of mating behaviour (Wasserman and Koepfer 1980; Markow 1981; Moodie 1982; Koepfer 1991), except that Kaneshiro (1976) and Watanabe and Kawanishi (1979) proposed the opposite. One states that ancestral females prefer to mate with conspecific males (Kaneshiro 1976), and the other proposes that derived females prefer to mate with conspecific males (Watanabe and Kawanishi 1979). These two hypotheses may refer to different sets of *Drosophila* species. Kaneshiro (1976) observed asymmetrical mate choice in Hawaiian *Drosophila*, with the polarity of mating behaviour being inferred according to the ages of the islands. Since the islands were isolated by the ocean, speciation was promoted by the existence of this geographical barrier. Kaneshiro concluded that ancestral females prefer conspecific males, but derived females do not. Interestingly, Watanabe and

Kawanishi (1979) found that in species evolved with overlapping ranges, derived females prefer conspecific males, but ancestral females do not. The speciation mechanism of species with an effective geographical barrier might differ from that of species lacking such a barrier. The former might have involved losing some courtship elements through genetic drift or adaptation to a different environment (Kaneshiro 1976), which explains the rejection of a derived male by an ancestral female. In the latter case, without an effective geographical barrier, if derived females reject original males, then the derived population might have a higher chance of differentiating from the original population. This might be related to Watanabe and Kawanishi's model. These derived males may have evolved new male performances instead of having lost some courtship elements.

However, Markow (1981) suggested that the relationships proposed in these models are not general concomitant of the evolutionary process. The relationship may depend on ecological and evolutionary history of a group. As evolutionary events may occur differently in different groups, a generalized model predicting the evolutionary sequence cannot be proposed (Markow 1981).

## 6. Conclusion

Reproductive isolating mechanisms are essential in the process of cladogenesis. Reproductive isolation, the reduction of gene flow between populations due to intrinsic features of organisms, plays a primary role in maintaining biological diversity. One of the major goals of speciation research is to identify the relative contributions of relevant isolating barriers between recently diverged species. This requires estimating the strength of all potential barriers, evaluating the time course for the evolution of barrier strengths and determining how each barrier contributes to the total isolation. In terms of the order of appearance in the life history, premating barriers that first come into play are of particular significance as subsequent barriers can only stop the gene flow that remains after the effect of earlier-acting barriers. In this regard, sexual isolation is often the first potential barrier to operate, and thus must be considered in any comprehensive study. Failure to include early-acting barriers may lead to overemphasis on the importance ascribed to barriers such as gametic isolation and intrinsic genetic incompatibilities that can only operate in sympatry.

Various species pairs amenable to genetic analyses are known in the genus *Drosophila* that has enhanced our knowledge in the field of speciation. Further, a large number of closely allied or representative species, now inhabiting open and continuous areas, were originally formed in parts formerly isolated, or that varieties became in fact isolated from haunting different stations, disliking each other and breeding at different times so as not to cross (Stauffer

1975). Thus, the origin of reproductive isolating mechanisms is an important event in the process of speciation that is evident from the work done in *Drosophila* (Singh 2010a). The foremost architect of synthetic theory of evolution and leading evolutionary geneticist of his time, Theodosius Dobzhansky (1973) remarked that 'Nothing in biology makes sense except in the light of evolution'.

In many cases, species arisen by genetic divergence and subsequent reproductive isolation of geographically separated and differentially adapted races and subspecies enrich our knowledge on speciation genetics. In many species groups genomes have been sequenced (Clark *et al.* 2007), and so genomic approaches may help in identifying the molecular genetic and neurophysiological changes responsible for the evolution of mating behaviour and sexual isolation in the genus *Drosophila*. The evolution of female mate mechanism favouring males of their kind is considered a crucial step in the early stages of speciation. Female mate choice is the product of the interplay between neurological and physiological processes, which in turn are regulated by gene expression patterns during courtship and mating. Therefore, the genomics of mate choice and identification of genes underlying assortative mating is an important future prospective for understanding of speciation genetics.

## Acknowledgements

The financial support from the UGC, New Delhi, in the form of a Major Research Project to BNS and a Junior Research Fellowship from UGC (NET) to PN is gratefully acknowledged. We also thank the two anonymous reviewers for their helpful comments on the original draft of the manuscript, and Ms Parul Banerjee for her valuable inputs during the revision of the manuscript.

## References

- Ahearn JN, Carson HL, Dobzhansky T and Kaneshiro KY 1974 Ethological isolation among three species of the *planitibia* subgroup of Hawaiian *Drosophila*. *Proc. Natl. Acad. Sci. USA* **71** 901–903
- Andersson MB 1994 *Sexual selection* (Princeton, New Jersey: Princeton University Press)
- Arbuthnott D 2009 The genetic architecture of insect courtship behavior and premating isolation. *Heredity* **103** 15–22
- Arnegard ME and Kondrashov AS 2004 Sympatric speciation by sexual selection alone is unlikely. *Evolution* **58** 222–237
- Aulard S, David JR and Lemeunier F 2002 Chromosomal inversion polymorphism in Afrotropical populations of *Drosophila melanogaster*. *Genet. Res.* **79** 49–63
- Ayala FJ and Dobzhansky T 1974 A new subspecies of *Drosophila pseudoobscura*. *Pan-Pacif. Entomol.* **50** 211–219
- Bachtrog D 2006 The speciation history of the *Drosophila nasuta* complex. *Genet. Res.* **88** 13–26

- Barbash DA, Siino DF, Tarone AM and Roote J 2003 A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **100** 5302–5307
- Begun D and Aquadro C 1993 African and North American populations of *Drosophila melanogaster* are very different at the DNA level. *Nature* **365** 548–550
- Berlacher SH and Feder JL 2002 Sympatric speciation in phytophagous insects: moving beyond controversy? *Annu. Rev. Entomol.* **47** 773–815
- Boake CRB 2005 Sexual selection and speciation in Hawaiian *Drosophila*. *Behav. Genet.* **35** 297–303
- Bock IR and Wheeler MR 1972 The *Drosophila melanogaster* species group. *Stud. Genet. VII Univ. Tex Publ.* **7213** 1–102
- Boughman JW, Rundle HD and Schluter D 2005 Parallel evolution of sexual isolation in sticklebacks. *Evolution* **59** 361–373
- Brown KM, Burk LM, Henagan LM and Noor MAF 2004 A test of the chromosomal arrangement model of speciation in *Drosophila pseudoobscura*. *Evolution* **58** 1856–1860
- Butlin RK 2005 Recombination and speciation. *Mol. Ecol.* **14** 2621–2635
- Butlin RK 2010 Population genomics and speciation. *Genetica* **138** 409–418
- Butlin RK and Ritchie MG 1994 Behavior and speciation; in *Behavior and evolution* (eds PJB Slater and TR Halliday (Cambridge: Cambridge University Press) pp 43–79
- Capy P, Veuille M, Paillette M, Jallon JM, Vouidibio J and David JR 2000 Sexual isolation of genetically differentiated sympatric populations of *Drosophila melanogaster* in Brazzaville, Congo: the first step towards speciation? *Heredity* **84** 468–475
- Cariou ML 1987 Biochemical phylogeny of the eight species in the *Drosophila melanogaster* subgroup, including *D. sechellia* and *D. orena*. *Genet. Res.* **50** 181–184
- Carracedo MC and Casares P 1985 Intrapopuational genetic variation in the hybridization between *Drosophila melanogaster* females and *D. simulans* males. *Experientia* **41** 106–108
- Carracedo MC, Suarez C and Casares P 2000 Sexual isolation between *Drosophila melanogaster*, *D. simulans* and *D. mauritiana*: sex and species specific discrimination. *Genetica* **108** 155–162
- Carson HL 1971 Speciation and founder principle. *Stadler Genet. Symp.* **3** 51–70
- Carson HL 1975 Genetics of speciation. *Am. Nat.* **109** 83–92
- Carson HL 1982 Evolution of *Drosophila* on the newer Hawaiian volcanoes. *Heredity* **48** 3–25
- Castrejana SJ and Markow TA 2008 Sexual isolation and mating propensity among allopatric *Drosophila mettleri* populations. *Behav. Genet.* **38** 437–445
- Choe JC and Crespi BJ 1997 *The evolution of mating systems in insects and arachnids* (Cambridge: Cambridge University Press)
- Civetta A and Cantor EJF 2003 The genetics of mating recognition between *Drosophila simulans* and *D. sechellia*. *Genet. Res.* **82** 117–126
- Civetta A and Singh RS 1995 High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* groups species. *J. Mol. Evol.* **41** 1085–1095
- Clark AG, Eisen MB, Smith DR, Bergman CM, Oliver B, et al. 2007. Evolution of genes and genomes on the *Drosophila* phylogeny. *Nature* **450** 203–218
- Colegrave N, Hollocher H, Hinton K and Ritchie MG 2000 The courtship song of African *Drosophila melanogaster*. *J. Evol. Biol.* **13** 143–150
- Counterman BA and Noor MAF 2006 Multilocus test for introgression between cactophilic *Drosophila mojavensis* and *D. arizonae*. *Am. Nat.* **168** 682–696
- Coyne JA 1989 Genetics of sexual isolation between two sibling species, *Drosophila simulans* and *Drosophila mauritiana*. *Proc. Natl. Acad. Sci. USA* **86** 5464–5468
- Coyne JA and Orr HA 1989 Patterns of speciation in *Drosophila*. *Evolution* **43** 362–381
- Coyne JA and Orr HA 2004 *Speciation* (Sunderland, Massachusetts: Sinauer and Associates)
- Darwin CR 1859 *The origin of species* 6th edition (London: John Murray)
- Dieckmann U and Doebeli M 1999 On the origin of species by sympatric speciation. *Nature* **400** 354–357
- Dobzhansky T 1937 *Genetics and the origin of species* 1st edition (New York: Columbia University Press)
- Dobzhansky T 1940 Speciation as a stage in evolutionary divergence. *Am. Nat.* **74** 312–321
- Dobzhansky T 1951 *Genetics and the origin of species* 3rd edition (New York: Columbia University Press)
- Dobzhansky T 1970 *The genetics of the evolutionary processes* (New York: Columbia University Press)
- Dobzhansky T 1973 Nothing in biology makes sense except in the light of evolution. *Am. Biol. Teach.* **35** 125–129
- Dobzhansky T and Powell JR 1975 *Drosophila pseudoobscura* and its American Relatives, *Drosophila persimilis* and *Drosophila miranda*; in *Invertebrates of genetic interest* (ed) RC King (New York: Plenum Press) pp 537–587
- Dobzhansky T and Spassky B 1959 *Drosophila paulistorum*, a cluster of species *in statu nascendi*. *Proc. Natl. Acad. Sci. USA* **45** 419–428
- Dodd DMB 1989 Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. *Evolution* **43** 1308–1311
- Dodd DMB and Powell JR 1985 Founder-flush speciation: an update of experimental results with *Drosophila*. *Evolution* **39** 1388–1392
- Doi M, Tomaru M, Matsubayashi H, Yamanoi K and Oguma Y 1996 Genetic analysis of *Drosophila virilis* sex pheromone: Genetic mapping of the locus producing Z-(11)-pentacosene. *Genet. Res.* **68** 17–21
- Doi M, Matsuda M, Tomaru M, Matsubayashi H and Oguma Y 2001 A locus for Female discrimination behavior causing sexual isolation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98** 6714–6719
- Ehrman L 1961 The genetics of sexual isolation in *Drosophila paulistorum*. *Genetics* **46** 1025–1038
- Ehrman L 1965 Direct observation of sexual isolation between allopatric and between sympatric strains of different *Drosophila paulistorum* races. *Evolution* **19** 459–464
- Ehrman L and Parsons PA 1980 Sexual isolation among widely distributed populations of *Drosophila immigrans*. *Behav. Genet.* **10** 401–407
- Endler JA 1989 Conceptual and other problems in speciation; in *Speciation and its consequences* (eds) D Otte and JA Endler

- (Sunderland, Massachusetts: Sinauer and Associates) pp 625–648
- Etges WJ 1992 Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. *Evolution* **46** 1945–1950
- Etges WJ and Tripodi AD 2008 Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. VIII. Mating success mediated by epicuticular hydrocarbons within and between isolated populations. *J. Evol. Biol.* **21** 1641–1652
- Etges WJ, Over KF, Oliviera CC and Ritchie MG 2006 Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*. *Anim. Behav.* **71** 1205–1214
- Etges WJ, Oliviera CC, Gragg E, Ortiz-Barrientos D, Noor MAF and Ritchie MG 2007 Genetics of incipient speciation in *Drosophila mojavensis*. I. Male courtship song, mating success, and Genotype x Environment interactions. *Evolution* **61** 1106–1119
- Ewing AW 1983 Functional aspects of *Drosophila* courtship. *Biol. Rev.* **58** 275–292
- Feder JL 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? in *Endless forms: Species and speciation* (eds) DJ Howard and SH Berlocher (New York: Oxford University Press) pp 130–144
- Futch DG 1966 A study of speciation in South Pacific populations of *Drosophila ananassae*. *Univ. Tex. Publ.* **6615** 79–120
- Futch DG 1973 On the ethological differentiation of *Drosophila ananassae* and *Drosophila pallidosa* in Samoa. *Evolution* **27** 299–312
- Galiana A, Moya A and Ayala FJ 1993 Founder-flush speciation in *Drosophila pseudoobscura*: a large scale experiment. *Evolution* **47** 432–444
- Gavrilets S and Vose A 2007 Case studies and mathematical models of ecological speciation. II. Palms on an oceanic island. *Mol. Ecol.* **16** 2910–2921
- Gavrilets S and Waxman D 2002 Sympatric speciation by sexual conflict. *Proc. Natl. Acad. Sci. USA* **99** 10533–10538
- Gleason JM and Ritchie MG 1998 Evolution of courtship song and reproductive isolation in the *Drosophila willistoni* species complex: Do sexual signals diverge most quickly? *Evolution* **52** 1493–1500
- Gleason JM, Jallon JM, Rouault JD and Ritchie MG 2005 Quantitative trait loci for cuticular hydrocarbons associated with sexual isolation between *Drosophila simulans* and *D. sechellia*. *Genetics* **171** 1789–1798
- Gomulkiewicz R, Holt RD and Barfield M 1999 The effect of density dependence and immigration on local adaptation and niche evolution in black-hole sink environment. *Theor. Popul. Biol.* **55** 283–296
- Greenberg, AJ, Moran JR, Coyne JA and Wu CI 2003 Ecological adaptation during incipient speciation revealed by precise gene replacement. *Science* **302** 1754–1757
- Haerty W, Lesbats M and Capy P 2005 Pre-reproductive isolation as a consequence of allopatric differentiation between populations of *Drosophila melanogaster*. *Mol. Ecol.* **14** 3801–3807
- Haerty W, Jagadeeshan S, Kulathinal RJ, Wong A, Ravi Ram K *et al.* 2007 Evolution in the fast lane: rapidly evolving sex-related genes in *Drosophila*. *Genetics* **177** 1321–1335
- Hayashi TI, Vose M and Gavrilets S 2007 Genetic differentiation by sexual conflict. *Evolution* **61** 516–529
- Heisler IL, Andersson MB, Arnold ST, Boake CRB, Borgia G, Hausfater G, Kirkpatrick M, *et al.* 1987 The evolution of mating preferences and sexually selected traits; in *Sexual selection: Testing the alternatives* (eds) JW Bradbury and MB Andersson (New York: John Wiley and Sons) pp 96–118
- Henderson NR and Lambert DM 1982 No significant deviation from random mating of worldwide populations of *Drosophila melanogaster*. *Nature* **300** 437–440
- Hendry AP 2001 Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. *Genetica* **112–113** 515–534
- Hendry AP, Nosil P and Rieseberg LH 2007 The speed of ecological speciation. *Funct. Ecol.* **21** 455–464
- Hilton H and Hey J 1996 DNA sequence variation at the *period* locus reveals the history of species and speciation events in the *Drosophila virilis* group. *Genetics* **144** 1015–1025
- Hocutt GD 2000 Reinforcement of premating barriers to reproduction between *Drosophila arizonae* and *Drosophila mojavensis*. Dissertation, University of Arizona, Tempe, AZ
- Hoikkala A 1988 The importance of different courtship stimuli in the mating behaviour of European species of the *Drosophila virilis* group. *Ann. Zool. Fennici* **25** 257–263
- Hoikkala A and Lumme J 1987 The genetic basis of evolution of the male courtship sounds in the *Drosophila virilis* group. *Evolution* **41** 827–845
- Hollocher H, Ting CT, Wu ML and Wu CI 1997a Incipient speciation by sexual isolation in *Drosophila melanogaster*: extensive genetic divergence without reinforcement. *Genetics* **147** 1191–1121
- Hollocher H, Ting CT, Wu ML and Wu CI 1997b Incipient speciation by sexual isolation in *Drosophila melanogaster*: variation in mating preference and correlation between sexes. *Evolution* **51** 1175–1181
- Hoskin CJ and Higgie M 2010 Speciation via species interactions: the divergence of mating traits within species. *Ecol. Lett.* **13** 409–420
- Howard DJ and Berlocher SH 1998 *Endless Forms: Species and Speciation* (New York: Oxford University Press)
- Huttunen S, Aspi J, Schlotterer C, Routtu J and Hoikkala A 2008 Variation in male courtship song traits in *Drosophila virilis*: The effects of selection and drift on song divergence at the intraspecific level. *Behav. Genet.* **38** 82–92
- Ishii K, Hirai Y, Katagiri C and Kimura MT 2001 Sexual isolation and cuticular hydrocarbons in *Drosophila elegans*. *Heredity* **87** 392–399
- Isoherranen E, Aspi J, Hoikkala A 1999 Variation and consistency of female preferences for simulated courtship songs in *Drosophila virilis*. *Anim. Behav.* **57** 619–625
- Jennings JH and Etges WJ 2010 Species hybrids in the laboratory but not in nature: A reanalysis of premating isolation between *Drosophila arizonae* and *D. mojavensis*. *Evolution* **64** 587–598
- Jennings JH, Mazzie D, Ritchie MG and Hoikkala A 2011 Sexual and postmating reproductive isolation between allopatric *Drosophila montana* suggest speciation potential. *BMC Evol. Biol.* **11** 68

- Jordan K 1905 Der Gesetzes zwischen geographischer und nicht-geographischer variation. *Zeits. Wiss. Zool.* **83** 151–210
- Kaneshiro KY 1976 Ethological isolation and phylogeny in the *planitibia* subgroup of Hawaiian *Drosophila*. *Evolution* **30** 740–745
- Kaneshiro KY 1980 Sexual isolation, speciation, and the direction of evolution. *Evolution* **34** 437–444
- Kauer M and Schlotterer C 2004 An analysis of genetic differentiation among assortatively mating *Drosophila melanogaster* in Zimbabwe. *J. Evol. Biol.* **17** 493–500
- Kauer M, Zangerl B, Dieringer D and Schlotterer C 2002 Chromosomal patterns of microsatellite variability contrast sharply in African and non-African populations of *Drosophila melanogaster*. *Genetics* **160** 247–256
- Kingsolver JG and Pfennig DW 2007 Patterns and power of phenotypic selection in nature. *Bioscience* **57** 561–572
- Kliman RM and Hey J 1993 DNA sequence variation at the period locus within and among species of the *Drosophila melanogaster* complex. *Genetics* **133** 375–387
- Koepfer HR 1991 Asymmetrical mating patterns between geographic strains of *Drosophila mercatorum*: a test of the Kaneshiro hypothesis. *Evolution* **45** 455–458
- Kondrashov AS and Kondrashov FA 1999 Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400** 351–354
- Kopp A and Frank AK 2005 Speciation in progress? A continuum of reproductive isolation in *Drosophila bipectinata*. *Genetica* **125** 55–68
- Korol A, Rashkovetsky E, Iliadi K, Michalak P, Ronin Y and Nevo E 2000 Nonrandom mating in *Drosophila melanogaster* laboratory populations derived from closely adjacent ecologically contrasting slopes at 'Evolution Canyon'. *Proc. Natl. Acad. Sci. USA* **97** 12637–12642
- Kulathinal RJ, Stevison LS and Noor MAF 2009 The genomics of speciation in *Drosophila*: Diversity, Divergence and Introgression estimated using low-coverage genome sequencing. *PLoS Genet.* **5** e1000550
- Lemeunier F and Ashburner M 1976 Relationships within the *Drosophila melanogaster* subgroup of the genus *Drosophila* (Sophophora). II. Phylogenetic relationships between six species based upon polytene chromosome banding sequences. *Proc. R. Soc. Lond. B.* **193** 275–294
- Liimatainen JO and Hoikkala A 1998 Interactions of the males and females of three sympatric *Drosophila virilis*-group species, *D. montana*, *D. littoralis*, and *D. lummei* (Diptera: Drosophilidae) in intra- and interspecific courtships in the wild and in the laboratory. *J. Insect Behav.* **11** 399–417
- Llopart A, Lachaise D and Coyne JA 2005a Multilocus analysis of introgression between two sympatric sister species of *Drosophila*: *Drosophila yakuba* and *D. santomea*. *Genetics* **171** 197–210
- Llopart A, Lachaise D and Coyne JA 2005b An anomalous hybrid zone in *Drosophila*. *Evolution* **59** 2602–260
- Lowry DB, Modliszewski JL, Wright KM, Wu CA and Willis JH 2008 The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philos. Trans. R. Soc. Lond. B.* **363** 3009–3021
- Lumme J and Heikkinen E 1990 Viability of first and second generation hybrids of *Drosophila virilis* and *Drosophila lummei*. *Heredity* **65** 435–447
- Machado CA, Haselkorn TS and Noor MAF 2007a Evaluation of the genomic extent of effects of fixed inversion differences in intraspecific variation and interspecific gene flow *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **175** 1289–1306
- Machado CA, Matzkin LM, Reed LK and Markow TA 2007b Multilocus nuclear sequences reveal intra- and interspecific relationships among chromosomally polymorphic species of cactophilic *Drosophila*. *Mol. Ecol.* **16** 3009–3024
- Markow TA 1981 Mating preferences are not predictive of the direction of evolution in *Drosophila*. *Science* **213** 1405–1407
- Massie KR and Markow TA 2005 Sympatry, allopatry and sexual isolation between *Drosophila mojavnensis* and *D. arizonae*. *Hereditas* **142** 51–55
- Matsuda M, Tomimura Y and Tobari YN 2005 Reproductive isolation among biogeographical populations of *Drosophila bipectinata* Duda (Diptera, Drosophilidae) with recognition of three subspecies. *Genetica* **125** 69–78
- Matsuda M, Ng CS, Doi M, Kopp A and Tobari YN 2009 Evolution in the *Drosophila ananassae* species subgroup. *Fly* **3** 157–169
- Matute DR and Coyne JA 2009 Intrinsic reproductive isolation between two sister species of *Drosophila*. *Evolution* **64** 903–920
- Matzkin LM 2008 The molecular basis of host adaptation in cactophilic *Drosophila*: molecular evolution of a glutathione S-transferase gene (*GstD1*) in *Drosophila mojavnensis*. *Genetics* **178** 1073–1083
- Mayr E 1963 *Animal species and evolution* (Cambridge, Massachusetts: Belknap Press)
- Mayr E 1970 *Population, species and evolution* (Cambridge: Harvard University Press)
- Meffert LM, Regan JL and Brown BW 1999 Convergent evolution of the mating behavior of founder-flush populations of the housefly. *J. Evol. Biol.* **12** 859–868
- Michel AP, Sim S, Powell T, Nosil P and Feder JL 2010 Widespread genomic divergence during sympatric speciation. *Proc. Natl. Acad. Sci. USA* **107** 9724–9729
- Moehring AJ, Llopart A, Elwyn S, Coyne JA and Mackay TFC 2006 The genetic basis of prezygotic reproductive isolation between *Drosophila santomea* and *D. yakuba* due to mating preference. *Genetics* **173** 215–223
- Moodie GEE 1982 Why asymmetric mating preference may not show the direction of evolution. *Evolution* **36** 1096–1097
- Moya A, Galiana A and Ayala FJ 1995 Founder-flush speciation theory: failure of experimental corroboration. *Proc. Natl. Acad. Sci. USA* **92** 3983–3986
- Muller HJ 1942 Isolating mechanisms, evolution and temperature. *Biol. Symp.* **6** 71–125
- Nanda P and Singh BN 2011a Evidence for incipient sexual isolation within *Drosophila ananassae*. *Zool. Stud.* **50** 577–587
- Nanda P and Singh BN 2011b Effect of chromosome arrangements on mate recognition system leading to behavioral isolation in *Drosophila ananassae*. *Genetica* **139** 273–279
- Nanda P and Singh BN 2011c Origin of sexual isolation in *Drosophila ananassae* due to founder effects. *Genetica* **139** 779–787

- Navarro A and Barton NH 2003 Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. *Evolution* **57** 447–459
- Nickel D 2007 You're not my kind of girl: The genetics of pre-mating isolation in the *Drosophila virilis* group. PhD thesis, Department of Biology, University of Winnipeg
- Nickel D and Civetta A 2009 An X chromosome effect responsible for asymmetric reproductive isolation between male *Drosophila virilis* and heterospecific females. *Genome* **52** 49–56
- Noor MAF 1995 Speciation driven by natural selection in *Drosophila*. *Nature* **375** 674–675
- Noor MAF and Feder JL 2006 Speciation genetics: evolving approaches. *Nature* **7** 851–861
- Noor MAF, Garfield DA, Schaffer SW and Machado CA 2007 Divergence between the *Drosophila pseudoobscura* and *D. persimilis* Genome sequences in relation to chromosomal inversions. *Genetics* **177** 1417–1428
- Noor MAF, Grams KL, Bertucci LA and Reinland J 2001 Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci. USA* **98** 12084–12088
- Nosil P, Crespi BJ and Sandoval CP 2002 Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature* **417** 441–443
- Nosil P, Crespi BJ, Gries R and Gries G 2007 Natural selection and divergence in mate preference during speciation. *Genetica* **129** 309–327
- Nosil P, Vines T and Funk DJ 2005 Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59** 705–719
- Orr HA and Coyne JA 1989 The genetics of postzygotic isolation in the *Drosophila virilis* group. *Genetics* **121** 527–537
- Panhuis TM, Butlin RK, Zuk M and Tregenza T 2001 Sexual selection and speciation. *Trends Ecol. Evol.* **16** 364–371
- Paterson HE 1978 More evidence against speciation by reinforcement. *S. Afr. J. Sci.* **74** 369–371
- Paterson HE 1980 A comment on 'mate recognition systems'. *Evolution* **34** 330–331
- Patterson JT, Stone WS and Griffin RK 1942 Genetic and cytological analysis of the *virilis* species group. *Univ. Texas Publ.* **4228** 162–200
- Patterson JT and Stone WS 1952 *Evolution in the genus Drosophila* (New York: Macmillan Company)
- Powell JR 1978 The founder-flush speciation theory: an experimental approach. *Evolution* **32** 465–474
- Powell JR 1997 *Progress and prospects in evolutionary biology: The Drosophila model* (New York: Oxford University Press)
- Presgraves DC, Balagopalan L, Abmayr SM and Orr HA 2003 Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature* **423** 715–719
- Qvarnström A and Bailey RI 2009 Speciation through evolution of sex-linked genes. *Heredity* **102** 4–15
- Ramachandra NB and Ranganath HA 1987 Mating preference between *Drosophila nasuta nasuta* and *D. n. albomicans*. *Indian J. Exp. Biol.* **25** 55–57
- Ramachandra NB and Ranganath HA 1994 Pattern of sexual isolation between parental races (*Drosophila nasuta nasuta* and *D. n. albomicans*) and the newly evolved races (Cytorace I and II). *Indian J. Exp. Biol.* **32** 98–102
- Ranganath HA 2002 Evolutionary biology of *Drosophila nasuta* and *D. albomicans*. *Proc. Indian Nat. Sci. Acad.* **B68** 255–272
- Reed LK and Markow TA 2004 Early events in speciation: polymorphism for hybrid male sterility in *Drosophila mojavensis*. *Proc. Natl. Acad. Sci. USA* **101** 9009–9012
- Reed LK, Nyboer M and Markow TA 2007 Evolutionary relationships of *Drosophila mojavensis* geographic host races and their sister species *Drosophila arizonae*. *Mol. Ecol.* **16** 1007–1022
- Rice WR and Hostert EE 1993 Laboratory experiments on speciation: What have we learned in forty years. *Evolution* **47** 1637–1653
- Ringo J, Barton K and Dowse H 1986 The effect of genetic drift on mating propensity, courtship behavior, and postmating fitness in *Drosophila simulans*. *Behavior* **97** 226–233
- Ritchie MG, SDF Philips. 1998. The genetics of sexual isolation; in *Endless forms: Species and speciation* (eds) DJ Howard and SH Berlocher (New York: Oxford University Press) pp 291–308
- Ross CL and Markow TA 2006 Microsatellite variation among diverging populations of *Drosophila mojavensis*. *J. Evol. Biol.* **19** 1691–1700
- Rouault J, Capy P and Jallon JM 2001 Variations of male cuticular hydrocarbons with geoclimatic variables: An adaptive mechanism in *Drosophila melanogaster*? *Genetica* **110** 117–120
- Ruedi EA and Hughes KA 2008 Natural genetic variation in complex mating behaviors of male *Drosophila melanogaster*. *Behav. Genet.* **38** 424–436
- Ruiz A, Heed WB and Wasserman M 1990 Evolution of the *mojavensis* cluster of cactophilic *Drosophila* with descriptions of two new species. *J. Hered.* **81** 30–42
- Rundle HD and Schluter D 2004 Natural selection and ecological speciation in sticklebacks; in *Adaptive speciation* (eds) U Dieckmann, M Doebeli, JAJ Metz and D Tautz (International Institute for Applied Systems Analysis: Cambridge University Press) pp 192–209
- Rundle HD, Mooers AO and Whitlock MC 1998 Single founder-flush events and the evolution of reproductive isolation. *Evolution* **52** 1850–1855
- Rundle HD, Chenoweth SF, Doughty P and Blows MW 2005 Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.* **3** e68
- Rundle HD, Nagel L, Boughman JW and Schluter D 2000 Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287** 306–308
- Saarikettu M, Liimatainen JO and Hoikkala A 2005 The role of male courtship song in species recognition in *Drosophila montana*. *Behav. Genet.* **35** 257–263
- Sagga N and Civetta A 2010 Male-female interactions and the evolution of postmating prezygotic reproductive isolation among species of the *virilis* subgroup. *Int. J. Evol. Biol.* **2011** 1–11
- Sawamura K, Tomimura Y, Sato H, Yamada H, Matsuda M and Oguma Y 2006 Establishing interspecific mosaic genome lines between *Drosophila ananassae* and *D. pallidosa* by means of parthenogenesis. *Genet. Res.* **88** 1–11
- Sawamura K, Zhi H, Setoguchi K, Yamada H, Miyo T, Matsuda M et al. 2008 Genetic analysis of female mating recognition between *Drosophila ananassae* and *Drosophila pallidosa*: application of interspecific mosaic genome lines. *Genetica* **133** 179–185

- Schluter D 2001 Ecology and the origin of species. *Trends Ecol. Evol.* **16** 372–380
- Schluter D and Nagel L 1995 Parallel speciation by natural selection. *Am. Nat.* **146** 292–301
- Schug MD, Baines JF, Amada K, Mohanty S, Das A, Grath S *et al.* 2008 Evolution of mating isolation between populations of *Drosophila ananassae*. *Mol. Ecol.* **17** 2706–2721
- Servedio MR 2001 Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibility. *Evolution* **55** 1909–1920
- Servedio MR and Noor MAF 2003 The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* **34** 339–364
- Servedio MR and Saetre GP 2003 Speciation as a positive feedback loop between postzygotic and prezygotic barriers to gene flow. *Proc. R. Soc. Lond. B.* **270** 1473–1479
- Singh BN 1996 Population and behavior genetics of *Drosophila ananassae*. *Genetica* **97** 321–332
- Singh BN 1997 Mode of mating preference and the direction of evolution in *Drosophila*. *Indian J. Exp. Biol.* **35** 111–119
- Singh BN 2010a The origin of reproductive isolating mechanisms is an important event in the process of speciation: Evidences from *Drosophila*; in *Nature at work: Ongoing saga of evolution* (ed) VP Sharma (New Delhi, India: Springer Private Limited) pp 159–173
- Singh BN 2010b *Drosophila ananassae*: A good model species for genetical, behavioral and evolutionary studies. *Indian J. Exp. Biol.* **48** 333–345
- Singh BN and Chatterjee S 1985 Symmetrical and asymmetrical sexual isolation among laboratory strains of *Drosophila ananassae*. *Can. J. Genet. Cytol.* **27** 405–409
- Singh BN and Chatterjee S 1986 Mating ability of homo- and heterokaryotypes of *Drosophila ananassae* from natural populations. *Heredity* **57** 75–78
- Singh BN and Chatterjee S 1988 Parallelism between male mating propensity and chromosome arrangement frequency in natural populations of *Drosophila ananassae*. *Heredity* **60** 269–272
- Singh BN and Chatterjee S 1991 Evidence for incipient sexual isolation within *Drosophila bipectinata*. *Evol. Biol.* **5** 105–113
- Singh BN and Chatterjee S 1992 Intraspecific sexual isolation in *Drosophila*. *Indian J. Exp. Biol.* **30** 260–263
- Singh BN, Dwivedi YN and Gupta JP 1981 Sexual isolation among three species of the *Drosophila bipectinata* species complex. *Indian J. Exp. Biol.* **19** 898–900
- Singh P and Singh BN 2010 Population genetics of *Drosophila ananassae*: Evidence for population sub-structuring at the level of inversion polymorphism in Indian natural populations. *Int. J. Biol.* **2** 19–28
- Singh SR, Rashkovetsky E, Iliadi K, Nevo E and Korol A 2005 Assortative mating in *Drosophila* adapted to a microsite ecological gradient. *Behav. Genet.* **35** 753–764
- Sobel JM, Chen GF, Watt LR and Schemske DW 2009 The biology of speciation. *Evolution* **64** 295–315
- Speith HT and Ringo JM 1983 Mating Behavior and sexual isolation in *Drosophila*; in *The genetics and biology of Drosophila* (eds) MA Ashburner, HL Carson and JN Thompson Jr (New York: Academic Press) Vol 3c, pp 223–284
- Stalker HD 1942 Sexual isolation in the species complex *Drosophila virilis*. *Genetics* **27** 238–257
- Stauffer RC 1975 *Charles Darwin's natural selection, being the second part of his big species book written from 1856–1858* (New York: Cambridge University Press)
- Sweigart AL 2010 The genetics of postmating, prezygotic reproductive isolation between *Drosophila virilis* and *D. americana*. *Genetics* **184** 401–410
- Takahashi A and Ting CT 2004 Genetic basis of sexual isolation in *Drosophila melanogaster*. *Genetica* **120** 273–284
- Takahashi A, Tsaur SC, Coyne JA and Wu CI 2001 The nucleotide changes governing cuticular hydrocarbon variation and their evolution in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98** 3920–3925
- Templeton AR 1981a The theory of speciation via the founder principle. *Genetics* **94** 1038–1101
- Templeton AR 1981b Mechanisms of speciation – a population genetic approach. *Annu. Rev. Ecol. Syst.* **12** 23–48
- The Marie Curie Speciation Network 2012 What do we need to know about speciation. *Trends Ecol. Evol.* **27** 27–39
- Ting CT A Takahashi and CI Wu 2001 Incipient speciation by sexual isolation in *Drosophila*: concurrent evolution at multiple loci. *Proc. Natl. Acad. Sci. USA* **98** 6709–6713
- Tregenza T 2002 Divergence and reproductive isolation in the early stages of speciation. *Genetica* **116** 291–300
- Tregenza T, Pritchard VL and Butlin RK 2000 The origins of premating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*. *Evolution* **54** 1687–1698
- Trickett AJ and Butlin RK 1994 Recombination suppressors and the evolution of new species. *Heredity* **73** 339–345
- Tsacas L and David JR 1974 *Drosophila mauritiana* n. sp. du groupe *melanogaster* de l'île Maurice. *Bull. Soc. Entomol. Fr.* **79** 42–44
- Turelli M, Barton NH and Coyne JA 2001 Theory and speciation. *Trends Ecol. Evol.* **16** 330–343
- Vishalakshi C and Singh BN 2006 Sexual isolation between two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa*. *Curr. Sci.* **90** 1003–1006
- Wagner M 1889 *Die Entstehung der Arten durch raumliche Sonderung* (Basel: Beno Schwalbe)
- Wasserman M and Koepfer HR 1980 Does asymmetrical mating preference show the direction of evolution? *Evolution* **34** 1116–1126
- Watabe H and Aoki H 2000 Reproductive isolation between *Drosophila sinobscura* and *D. hubeiensis*, closely related species of *obscura* group (Diptera: Drosophilidae). *J. Hokkaido Univ. Ed.* **51** 83–86
- Watanabe TK and Kawanishi M 1979 Mating preference and the direction of evolution in *Drosophila*. *Science* **205** 906–907
- Wu CI, Hollocher H, Begun DJ, Aquadro CF, Xu Y and Wu ML 1995 Sexual isolation in *D. melanogaster*: a possible case of incipient speciation. *Proc. Natl. Acad. Sci. USA* **92** 2519–2523
- Yamada H, Sakai T, Tomimura M, Doi M, Matsuda M and Oguma Y 2002a Search for species specific mating signal in courtship songs of sympatric sibling species, *Drosophila ananassae* and *D. pallidosa*. *Genes Genet. Syst.* **77** 97–106

- Yamada H, Matsuda M and Oguma Y 2002b Genetics of sexual isolation based on courtship song between two sympatric species, *Drosophila ananassae* and *D. pallidosa*. *Genetica* **116** 225–237
- Yukilevich R 2012 Asymmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. doi:[10.1111/j.1558-5646.2011.01534.x](https://doi.org/10.1111/j.1558-5646.2011.01534.x)
- Yukilevich R and True JR 2008 Incipient sexual isolation among cosmopolitan *Drosophila melanogaster* populations. *Evolution* **62** 2112–2121
- Zouros E and d'Entremont CJ 1980 Sexual isolation among populations of *Drosophila mojavensis*: response to pressure from a related species. *Evolution* **34** 421–430

*MS received 20 September 2011; accepted 17 January 2012*

Corresponding editor: AMITABH JOSHI