

The genus *Drosophila* is characterized by a large number of sibling species showing evolutionary significance

BASHISTH N. SINGH*

Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Abstract

Mayr (1942) defined sibling species as sympatric forms which are morphologically very similar or indistinguishable, but which possess specific biological characteristics and are reproductively isolated. Another term, cryptic species has also been used for such species. However, this concept changed later. Sibling species are as similar as twins. This category does not necessarily include phylogenetic siblings as members of a superspecies. Since the term sibling species was defined by Mayr, a large number of cases of sibling species pairs/groups have been reported and thus they are widespread in the animal kingdom. However, they seem to be more common in some groups such as insects. In insects, they have been reported in diptera, lepidoptera, coleoptera, orthoptera, hymenoptera and others. Sibling species are widespread among the dipteran insects and as such are well studied because some species are important medically (mosquitoes), genetically (*Drosophila*) and cytologically (*Sciara* and *Chironomus*). The well-studied classical pairs of sibling species in *Drosophila* are: *D. pseudoobscura* and *D. persimilis*, and *D. melanogaster* and *D. simulans*. Subsequently, a number of sibling species have been added to these pairs and a large number of other sibling species pairs/groups in different species groups of the genus *Drosophila* have been reported in literature. The present review briefly summarizes the cases of sibling species pairs/groups in the genus *Drosophila* with their evolutionary significance.

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Introduction

A species is a basic unit of biological classification and a taxonomic rank variously defined under different species concepts. There are more than 20 species concepts (Mallet 2007; Singh 2012). Mayr (1969) described only three concepts of species: typological, nominalistic and biological. However, in the revised edition of his book (Mayr and Ashlock 1991), a new concept was added to this list i.e. evolutionary species concept. Of these species concepts, only one is the most widely accepted species concept i.e. biological species concept (BSC), which was mostly developed by Jordan (1905), Dobzhansky (1935) and Mayr (1940), although it has certain difficulties in its applications (Singh 2012). Mayr and Ashlock (1991) have used a number of terms pertaining to species which have been defined adequately: subspecies, biological races, superspecies, semispecies, allospecies, sibling species, cryptic species, incipient species, monotypic species and polytypic species. Mayr (1942) defined sibling species as

‘sympatric forms which are morphologically similar or indistinguishable which possess specific biological characteristics and are reproductively isolated’. Biological races were identified but later were called as sibling species. By far, the majority of the so called biological races of the literature are now acknowledged to be sibling species (Mayr 1963). For sibling species, the term cryptic species has also been used. Mayr and Ashlock (1991) stated that such very similar species are called cryptic or sibling species. However, later the term cryptic species has been used with different meanings: cryptic species for two or more species hidden under one species name, sibling species for two cryptic species that are closest relative of each other. Sibling species (aphanic species), this term was initially used as the same meaning as cryptic species, but later, authors expressed the common phylogenetic origin (Steyskal 1972). The category of sibling species does not necessarily include species which are phylogenetically siblings for each member of superspecies. The term ‘sibling species’ is arbitrarily limited to species which are as similar as twins or quintuplets (Mayr 1942). Bickford *et al.* (2007) defined sibling species as ‘cryptic sister species’ meaning two species that are the closest relative of each other

*E-mail: bashisthsingh2004@rediffmail.com, bnsingh@bhu.ac.in.

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and have not been distinguished from one another taxonomically. These two terms, sibling species and cryptic species, differ from each other. It should not be confused that sibling species are incipient or recent species.

There are a large number of reports demonstrating the frequent occurrence of sibling species in animal kingdom from protozoans to elephant. Sibling species are also known in plants (Grant 1965). These species are more frequent in invertebrates than vertebrates. Among invertebrates, sibling species are more frequent in insects. Such species are also found in sea (Knowlton 1986, 1993). Sibling species are also known to occur in prokaryotes, algae, fungi and angiosperms (Knowlton 1993). These species are well known in insects, birds, rodents, reptiles, fishes, etc. However, they are very common in insects (Mayr 1963, 1970). Sibling species are widespread in insects and are well-studied because these species are important genetically (*Drosophila*), medically (mosquitoes) and cytologically (*Sciara* and *Chironomus*). Interestingly, sibling species are known to occur in Europe that form malaria–mosquito complex (Mayr 1970). The malaria mosquito of Europe is a group of six sibling species i.e. *Anopheles maculipennis* group which has biological differences.

Dipteran species of the genus *Drosophila* have been extensively used as model systems in studies of genetics, evolution, behaviour, ecology, developmental biology, molecular biology and other branches of biology. At a global level, this genus comprises more than 1500 species with more than 150 already described in India (Singh 2015). About 500 species are known to occur in Hawaiian islands which served as interesting materials for evolutionary studies by Carson (1970, 1981, 1983) and Carson and Kaneshiro (1976). Sibling species are known to occur in Hawaiian species of *Drosophila*. In this genus, most of species complexes contain groups of sibling species (Patterson and Stone 1952). The classical pair of sibling species is *D. pseudoobscura* and *D. persimilis*, which were initially considered as races A and B of *D. pseudoobscura*. These species differ in male genitalia (Mayr 1970). Both these species occur in USA and their evolutionary genetics is well-studied by Dobzhansky (1951). Another classical pair of sibling species is *D. melanogaster* and *D. simulans*, which are cosmopolitan and domestic species (Sturtevant 1920; Coulthart and Singh 1988). Studies on sibling species have significant relevance with respect to species concepts, applied biology and speciation. *Drosophila* has been extensively employed for this purpose and a number of species pairs/groups have been studied in this respect. The present review briefly summarizes the cases of sibling species pairs/groups in the genus *Drosophila* with their evolutionary significance.

In table 1, a list of sibling species pairs/groups is provided for different species groups of *Drosophila*. The genus *Drosophila* is divided into many species groups and sibling species are known to occur in the following species groups: *melanogaster*, *obscura*, Hawaiian *Drosophila*, *willistoni*, *immigrans*, *repleta*, *mesophragmatica*, *virilis*, *melanica*, *quinaria* and *cardini*.

Table 1. List of sibling species pairs/groups in the genus *Drosophila*.

Sibling species pairs/groups	Species groups
<i>D. ananassae</i> <i>D. pallidosa</i>	<i>melanogaster</i>
<i>D. melanogaster</i> <i>D. simulans</i> <i>D. mauritiana</i> <i>D. sechellia</i> <i>D. erecta</i> <i>D. orena</i> <i>D. teissieri</i> <i>D. santomea</i> <i>D. yakuba</i>	<i>melanogaster</i>
<i>D. kikkawai</i> <i>D. leontia</i> <i>D. bocki</i>	<i>melanogaster</i>
<i>D. lini</i> <i>D. ohnishii</i> <i>D. ogumai</i>	<i>melanogaster</i>
<i>D. auraria</i> <i>D. triauraria</i> <i>D. quadraria</i> <i>D. biauraria</i> <i>D. subauraria</i>	<i>melanogaster</i>
<i>D. serrata</i> <i>D. birchii</i> <i>D. dominicana</i>	<i>melanogaster</i>
<i>D. pseudoobscura</i> <i>D. persimilis</i> <i>D. bogotana</i> <i>D. lowei</i> <i>D. miranda</i> <i>D. frolovae</i>	<i>obscura</i>
<i>D. athabasca</i> <i>D. azteca</i>	<i>obscura</i>
<i>D. bifasciata</i> <i>D. imaii</i>	<i>obscura</i>
<i>D. heedi</i> <i>D. silvestris</i>	Hawaiian <i>Drosophila</i>
<i>D. disjuncta</i> <i>D. affinisdisjuncta</i> <i>D. bostrycha</i>	Hawaiian <i>Drosophila</i>
<i>D. williston</i> <i>D. tropicalis tropicalis</i> <i>D. tropicalis cubana</i> <i>D. equinoxialis</i> <i>D. insularis</i> <i>D. parloviskiana</i> <i>D. paulistorum</i>	<i>willistoni</i>
<i>D. bocainensis</i> <i>D. parabocainensis</i> <i>D. bocainoides</i>	<i>willistoni</i>

Table 1 (contd)

Sibling species pairs/groups	Species groups
<i>D. nasuta</i> <i>D. albomicans</i>	<i>immigrans</i>
<i>D. longicornis</i> <i>D. pachua</i> <i>D. proopachua</i>	<i>repleta</i>
<i>D. aldrichi</i> <i>D. wheeleri</i>	<i>repleta</i>
<i>D. arizonensis</i> <i>D. mojavensis</i>	<i>repleta</i>
<i>D. mercatorum</i> <i>D. paranaensis</i>	<i>repleta</i>
<i>D. gouveai</i> <i>D. antonietae</i> <i>D. barborema</i> <i>D. koepferae</i> <i>D. serido</i> <i>D. seriema</i> <i>D. buzzatii</i>	<i>repleta</i>
<i>D. pavani</i> <i>D. gaucha</i>	<i>mesophragmatica</i>
<i>D. virilis</i> <i>D. novamexicana</i> <i>D. americana americana</i> <i>D. americana texana</i> <i>D. lummei</i>	<i>virilis</i> <i>virilis</i> phylad
<i>D. montana</i> <i>D. litoralis</i> <i>D. borealis</i> <i>D. flavomontanae</i> <i>D. izoana</i> <i>D. kanekoi</i>	<i>montana</i> phylad
<i>D. melanica</i> <i>D. paramelanica</i>	<i>melanica</i>
<i>D. palustris</i> <i>D. subpalastris</i>	<i>quinaria</i>
<i>D. parthenogenetica</i> <i>D. procardinoides</i>	<i>cardini</i>
<i>D. neomorpha</i> <i>D. polymorpha</i>	<i>cardini</i>
<i>D. D. bedichecki</i> <i>D. cardinoides</i>	<i>cardini</i>
<i>D. nigrodummi</i> <i>D. antillea</i>	<i>cardini</i>

The *melanogaster* species group

As far as the number of sibling species are concerned, this is the largest species group in the genus *Drosophila*.

D. ananassae and *D. pallidosa* (*ananassaesubgroup*)

Futch (1966) studied speciation in South-Pacific populations of *D. ananassae* and found light and dark pigmented flies from the same collection from Tutuila, American Samoa. Based on chromosomal studies and sexual isolation

tests in the light and dark forms of *D. ananassae*, he suggested that the light form is second species which is closely related to *D. ananassae* (dark cosmopolitan form). The light form was given separate name as *D. pallidosa*, described as a new species by Bock and Wheeler (1972). However, they did not find any difference in male genitalia between them. Their separation as independent species was based on variation in the pattern of sex combs, number of teeth and sexual isolation in sympatric populations. Futch (1973) designated them as sibling species. Both these species are crossable in the laboratory and produce fully fertile hybrids. Thus, there is a lack of postmating reproductive isolation. Based on this, it is concluded that they are not separate species rather they are in statu nascendi (Singh and Singh 2015).

D. melanogaster, *D. simulans*, *D. mauritiana* and other species (*melanogaster subgroup*)

This group has nine sibling species: *D. melanogaster*, *D. simulans*, *D. mauritiana*, *D. sechellia*, *D. erecta*, *D. orena*, *D. teissieri*, *D. santomea* and *D. yakuba*. The classical pair of sibling species which has been extensively investigated for different kinds of genetical, evolutionary, behavioural and ecological studies is *D. melanogaster* and *D. simulans* to which other species are added from time to time. This pair is very similar morphologically and they were confused until 1919, when Sturtevant (1920) described *D. simulans* as a separate species based on the difference of external male genitalia. Both are cosmopolitan and domestic species showing high degree of sexual isolation. Hybrids are rarely produced and when produced, they are sterile (Parsons 1975). The other species which are added to this classical pair of sibling species are: (i) *D. mauritiana*, endemic to the island of Mauritius; (ii) *D. sechellia*, endemic to the Seychelles archipelago; (iii) *D. erecta*, endemic to Africa; (iv) *D. orena*, endemic to Africa; (v) *D. teissieri*, endemic to Africa; (vi) *D. santomea*, endemic to the oceanic island of Sao Tome; and (vii) *D. yakuba*, endemic to Africa.

All the species belonging to this sibling species group have been subjected to various kinds of studies in the areas such as genetics, evolution, behaviour, ecology, molecular biology, etc. (Parsons 1975; Bos and Boerema 1981; Palopoli and Wu 1994; Lohe and Roberts 2000; Lachaise *et al.* 2000; Coyne *et al.* 2002, 2004; Barbash *et al.* 2003; Tao *et al.* 2003; Moehring *et al.* 2006; David *et al.* 2007; Jeong *et al.* 2008; Ereyilmaz and Stern 2013; Manier *et al.* 2013).

D. kikkawai, *D. leontia* and *D. bocki* (*montium subgroup*)

These three sibling species belong to the *montium* subgroup. *D. kikkawai* is wide spread in Asian and Pacific areas, and is also found in South America. The other two species are confined to the Asian areas. Karyotypic variations and cytogenetic relationships among these sibling species have been discussed by Baimai (1979, 1998), Baimai *et al.* (1980) and Baimai and Chumchong (1980).

***D. lini*, *D. ohnishii* and *D. ogumai* (montium subgroup)**

D. lini, *D. ohnishii* and *D. ogumai* are sibling species (Li *et al.* 2012). *D. lini* was described as a new species by Bock and Wheeler (1972) from Taiwan and it occurs in China also. Two new species, *D. ohnishii* and *D. ogumai* were described from Myanmar (Pyinoolwin) and Myanmar (Yangon), respectively (Oguma *et al.* 1995; Zannat and Toda 2002). This is a cluster of three sibling species. Morphological differences among these three species do not go parallel with the degree of reproductive isolation (Zannat and Toda 2002). DNA barcoding and molecular phylogeny supports the monophyly of the cluster of these three sibling species (Li *et al.* 2012).

***D. auraria*, *D. triauraria* and other species (montium subgroup)**

D. auraria species complex consists of five sibling species: *D. auraria*, *D. triauraria*, *D. quadraria*, *D. biauraria* and *D. subauraria*. These species have been used for evolutionary studies (Kim *et al.* 1989; Mavragani-Tsipidou *et al.* 1992; Drosopoulou and Scouras 1995; Jian *et al.* 2002). Distribution of these species is limited to East Asia (China, Japan, Taiwan and Korea) and *D. quadraria* is only known from Taiwan (Kim *et al.* 1989). Based on the mating pattern, it has been suggested that every species of this sibling species group has been derived directly from *D. quadraria* (Kim *et al.* 1989). Phylogeny among these species has also been discussed on the basis of cytogenetic and molecular studies (Mavragani-Tsipidou *et al.* 1992; Jian *et al.* 2002).

***D. serrata*, *D. birchii* and *D. dominicana* (montium subgroup)**

These three sibling species belong to the *serrata* species group and are reproductively isolated (Ayala 1965). However, their taxonomic position was changed later. They were placed in the *montium* subgroup of *melanogaster* species group (Bock 1980). *D. serrata* and *D. birchii* occur in Australia and New Guinea, whereas *D. dominicana* occurs in Madang, New Guinea (Ayala 1965). The first two species are sympatric in certain areas. These species have been collected and studied by Dobzhansky and Mather (1961). Male genitalia are different in these species. Blows and Allan (1998) suggested that olfaction was involved in ethological isolation between *D. serrata* and *D. birchii*.

The *obscura* species group***D. pseudoobscura*, *D. persimilis* and other species (*pseudoobscura* subgroup)**

There are six sibling species in this group: *D. pseudoobscura*, *D. persimilis*, *D. bogotana*, *D. lowei*, *D. miranda* and *D. frolovae*. All these species occur in the Nearctic region of the world (Patterson and Stone 1952). The first pair of

sibling species in *Drosophila* is *D. pseudoobscura* Frolova and *D. persimilis* Dobzhansky and Epling (Patterson and Stone 1952). When originally discovered, they were called as races: *D. pseudoobscura* A and *D. pseudoobscura* B. Hybrid females are fertile but males are sterile. Later, it was found that they show differences in male genitalia, sex comb tooth number and salivary gland chromosomes. These two races were given the status of full species: *D. pseudoobscura* and *D. persimilis*. *D. pseudoobscura* has two subspecies: *D. pseudoobscura pseudoobscura* and *D. pseudoobscura bogotana*. The first one is found in western North America and the second one occurs in Bogota, Colombia (Dobzhansky *et al.* 1963). They show incomplete reproductive isolation (Prakash 1972). *D. pseudoobscura* and *D. persimilis* have been extensively used by Dobzhansky (1951) for his evolutionary studies. Among them hybrid males are completely sterile which is caused due to certain genes (Dobzhansky 1951). All the species of this group have been used for various types of evolutionary studies (Crumpacker 1973; Wang and Hey 1996; Kulathinal *et al.* 2009).

***D. athabasca* and *D. azteca* (*affinis* subgroup)**

These two sibling species were described by Strutevant and Dobzhansky (1936) from North America. *D. athabasca* has a wider geographic distribution than *D. azteca* (Miller 1958). *D. azteca* overlaps the distribution of *D. athabasca* in northern California and southern Oregon (Pascual *et al.* 2009). There are variations in taxonomic features such as male genital structures and sex comb tooth number between the two species (Solerud and Miller 1966). The size of hybrid males and females varies. Male hybrids are larger than their parents but hybrid females are of the same size as the parental species (Meer 1976). Further, all hybrids are sterile resulting from X-autosome imbalance (Patterson and Stone 1952). In this pair of sibling species, courtship and mating sounds also show variation (Chang and Miller 1978). Large random amplified polymorphic DNA (RAPD) variation was found within the species. However, more than half of the primers used in these experiments produced greater variation between *D. athabasca* and *D. azteca* than within the species (Pascual *et al.* 2009). Pascual *et al.* (1997) used allozymes, mtDNA and RAPDs to identify these species and found that all these techniques allow the classification of any individual to a particular species.

***D. bifasciata* and *D. imaii* (*obscura* subgroup)**

D. bifasciata is widespread in Japan. The yellowish form of this species was originally considered as mutant form. The mutant form has some morphological differences when compared with the original form. However, both these forms show reproductive isolation and hybrid sterility. The results of reciprocal crosses vary. Hybrid males are fully sterile but females show partial fertility. In the hybrids, the failure of pairing between homologous chromosomes has been observed and both these species have been found to be

polymorphic for several inversions. Natural hybridization has not been observed. The mutant form has been given the status of new species *D. imaii*. Thus *D. bifasciata* and *D. imaii* are sibling species (Moriwaki *et al.* 1967).

Hawaiian *Drosophila*

D. silvestris and *D. heedi*

D. heedi and *D. silvestris*, picture wing *Drosophila* from Hawaii are morphologically similar, homosequential, closely related and chromosomally monomorphic. These are sibling species (or nearly sibling) and are abundant and coexist sympatrically on the Island of Hawaii. However, *D. heedi* is slightly smaller than *D. silvestris* (Kaneshiro *et al.* 1973). These species are cytologically distinguishable only by a minor heterochromatin differences at metaphase and a puff difference in the polytene chromosomes. Both these species are ployphagous.

D. disjuncta, *D. affinisdisjuncta* and *D. bostrycha*

These three species are picture wing *Drosophila* from the islands of the Hawaiian Archipelago (Carson and Stalker 1968; Carson and Kaneshiro 1976). *D. bostrycha* occurs on Mokai and *D. disjuncta* on Maui. *D. affinisdisjuncta* was discovered later from west Maui population. They have similar morphology and are homosequential species. Detailed cytogenetic investigations confirmed the existence of three sibling species which occur allopatrically in Molokai–Maui islands (Hardy 1978; Ahearn and Baimai 1987). Baimai (1998) has suggested that the *D. disjuncta*–*D. affinisdisjuncta*–*D. bostrycha* complex is an outstanding example of allopatric speciation involving karyotypic differentiation through gain of heterochromatin.

The *willistoni* species group

D. willistoni, *D. equinoxialis*, *D. paulistorum* and other species

This group consists of six sibling species which are neotropical: *D. willistoni*, *D. paulistorum*, *D. equinoxialis*, *D. tropicalis*, *D. pavlovskiana* and *D. insularis*. All these species have overlapping geographic distribution. However, their species integrity is maintained when they are sympatric by strong reproductive isolating mechanisms (Kim and Ehrman 1999). Ayala and Powell (1972) have shown that allozyme differences can be used as species diagnostic characters. It is interesting to note that in this group subspecies as well as semispecies have been described and molecular phylogeny has also been discussed (Gleason *et al.* 1998). Courtship behaviour has also been used as reliable means of species identity (Ritchie and Gleason 1995). Phylogenetic relationship among these species has also been discussed on the basis of chromosomal segments (Rohde *et al.* 2006).

D. bocainensis, *D. parabocainensis* and *D. bocanoides*

Carson (1954) found that under the taxonomic designation of *D. bocainensis* (Pavan and Da Cunha 1947), three reproductively isolated populations exist which were called as separate species: *D. bocainensis*, *D. parabocainensis* and *D. bocanoides* from Brazil. *D. bocainensis* is the commonest and most widespread. *D. parabocainensis* is more frequent in cool climates, whereas *D. bocanoides* is found in the super humid coastal rainforests of Sao Paulo, Brazil (Salzano 1956). It has also been shown that the degree of sexual isolation and the pattern of inversion polymorphism vary in these species (Salzano 1956).

The *immigrans* species group

D. nasuta and *D. albomicans* belong to the *nasuta* subgroup of the *immigrans* species group. *D. nasuta* was described by Lamb (1914) from Seychelles Islands and *D. albomicans* was from Paroe, Formosa (Duda 1923). Both are considered as allopatric sibling species (Wilson *et al.* 1969). Although, this pair shows striking divergence in their karyotypes, neither morphological divergence nor reproductive isolation between the two have been attained. Their separate status as distinct species is due to their allopatric distribution (Ranganath 2002). Evolutionary studies have also been conducted in this pair by Chang and Ayala (1989) and Chang and Kung (2008). Hybrid lines between them have been maintained in the lab for over hundred generations which are called as cytoraces used for the study of speciation under laboratory conditions (Ranganath 2002).

The *repleta* species group

D. longicornis, *D. pachua* and *D. propachua* (*mulleri* subgroup)

This is a triad of sibling species and is an example of species concept conflict (Oliveira *et al.* 2005). *D. longicornis* was described from Austin, Texas (Patterson and Wheeler 1942). It is also widely distributed in Mexico. Subsequently, two new species: *D. pachua* and *D. propachua* were collected from Pachua, Hidalgo, which were studied cytologically. These species are found to be closely related with each other (Wasserman 1962). Electrophoretic pattern of these species show that they are closely related with each other and placed in a single cluster of species (Richardson *et al.* 1975; Richardson and Smouse 1977). These three species are sympatric in Mexico and make an interesting trio of species which have been called as sibling triad (Wasserman and Koepfer 1977). Hybridization among the three species leads to the production of hybrids. Males are sterile and females are fertile. This shows that gene flow among them is theoretically possible in nature (Wasserman and Koepfer 1977).

***D. aldrichi* and *D. wheeleri* (*mulleri* subgroup)**

This is an interesting pair of sibling species. *D. aldrichi* was described from Texas, USA (Patterson and Crow 1940) and *D. wheeleri* from Arcadia, California, USA (Patterson and Alexander 1952). Their phylogenetic relationship has been discussed on the basis of cytogenetic studies (Wasserman 1992). These species are homosequential for polytene chromosomes banding (Wasserman 1954) and male genitalia of both the species are similar which raises questions about their independent species status (Vilela 1983). However, hybrid males are sterile and thus, they are reproductively isolated (postzygotic isolation) which indicates multiple species (Patterson and Alexander 1952). In *D. aldrichi*, there is an incompatibility in interpopulation crosses which provides evidence for the existence of cryptic species. Presence of sterility in crosses between introduced population of *D. aldrichi* in Australia and a population from Mexico suggests that they may be different species (Krebs and Barker 1994). Molecular data using nuclear and mitochondrial genes also suggest that they are distinct species. However, *D. aldrichi* is a paraphyletic assemblage of two lineages as suggested earlier on the basis of reproductive isolation (Oliveira *et al.* 2008).

***D. arizonensis* and *D. mojavensis* (*mulleri* subgroup)**

D. arizonensis is found in Sonoran Desert of Arizona and Mexico. *D. mojavensis* is found in California and Arizona. Both sibling species show strong reproductive isolation (Markow 1981). Aedeagus size plays important role in this pair in mate recognition which causes reproductive isolation (Richmond 2014). Evolutionary studies involving this pair have been conducted by Vigneault and Zouros (1986), Zouros (1989) and Wasserman and Koepfer (1977). There is evidence for character displacement for sexual isolation between *D. arizonensis* and *D. mojavensis*. Further, asymmetrical male sterility in hybrids between these two species involves interactins between Y chromosome and autosomes.

***D. mercatorum* and *D. paranaensis* (*mercatorum* subgroup)**

These two sibling species have been described on the basis of chromosome structure and reproductive isolation (Patterson and Wheeler 1942; Barros 1950). Both species are morphologically similar but male genitalia are different in the two species (Vilela 1983). *D. paranaensis* occurs in Brazil, Argentina and Mexico, whereas *D. mercatorum* has a pair of subspecies which have interesting distribution pattern. The subspecies *mercatorum* occurs in California, Arizona, Louisiana, Hawaii, Mexico and Costa Rica. The other subspecies *pararepleta* occurs in limited areas in Brazil and eastern side of Andes (Patterson and Stone 1952; Carson 1965). It is interesting to observe that other species of the *repleta* group are associated with cacti. These two species are generalists which occur in diverse environments (Pereira *et al.* 1983).

***D. serido*, *D. buzzatii*, *D. koepferae* and other species (*mulleri* subgroup)**

This group consists of seven sibling species: *D. gouveai*, *D. antonietae*, *D. barborema*, *D. koepferae*, *D. serido*, *D. seriema* and *D. buzzatii*. This is a cluster of cactophilic species *D. buzzatii* which belongs to the *repleta* species group. All the species breed exclusively in decaying cactus tissue, except *D. buzzatii* (a colonizing subcosmopolitan species) which are endemic to South America (Ruiz *et al.* 1982). All these species are morphologically similar but show variation in male genitalia. Male genital structures and chromosome inversions are diagnostic characters (Ruiz and Wasserman 1993). Evolutionary history and phylogenetic relationships among these species have been examined by employing chromosome inversions, hybridization tests, male genital morphology, allozyme and mtDNA variations (Sene *et al.* 1988; Tidon-Sklorz and Sene 2001; Moraes *et al.* 2004; Sambucetti *et al.* 2005; Soto *et al.* 2007, 2010). There are two major evolutionary lineages in this cluster identified on the basis of mtDNA analysis: one consisting of *D. buzzatii* and *D. koepferae* and the other consists remaining species (Manfrin *et al.* 2001). In the first pair, studies were conducted on female remating, sperm competition, female receptivity and sexual selection which show interspecific variations (Hurtado and Hasson 2013).

The *mesophragmatica* species group***D. pavani* and *D. gaucha***

Both species belong to the *mesophragmatica* species group. These species are morphologically similar showing differences in male genitalia. *D. gaucha* was described by Jaeger and Salzano (1953) from Brazil and *D. pavani* by Brncic (1957) from Chile. These species have been used in genetic and evolutionary studies (Brncic and Koref Santibanez 1957). In certain areas of Argentina, both these species are sympatric but no case of natural hybridization has been observed. These species are characterized by reproductive isolation and cytogenetic differences which provide evidence for evolutionary divergence between them (Brncic and Koref Santibanez 1957). These species also show behavioural differences as well as variation in coadapted gene pools (Koref-Santibanez 2001; Godoy-Herrera *et al.* 2005).

The *virilis* species group

Sibling species of this species group fall into two phylads: *viris* and *montana* (Throckmorton 1982): (i) The *virilis* phylad: *D. virilis*, *D. novamexicana*, *D. americana americana*, *D. americana texana* and *D. lumnei*. (ii) The *montana* phylad: *D. montana*, *D. litoralis*, *D. borealis*, *D. flavomontanae*, *D. ezoana*, *D. kanekoi* and *D. lacicola*.

In total, there are 10 sibling species and two subspecies of *virilis* species group which fall into two phylads (Kulikov *et al.* 2004). The phylogenetic relationships among these

species have been elucidated from chromosomal comparisons, allozyme polymorphisms and morphological characters (Patterson and Stone 1952; Throckmorton 1982; Spicer 1993). This group is rich in karyotypic variations. *D. virilis* is currently cosmopolitan but originally it was found to be endemic to Asia (Patterson and Stone 1952). Three species occur in Europe, one in the Orient and six in North America (Throckmorton 1975). Orr and Coyne (1989) carried out a genetic study of postzygotic reproductive isolation among these species and found that X-chromosome has largest effect on sterility of male and female hybrids. Interestingly, the shape of male mating organs varies among these species as revealed by multivariate approach (Kulikov *et al.* 2004). Molecular phylogeny and genome evolution have also been studied in this group of sibling species and the results provide evidence for extensive rearrangements relative to the genome of *D. melanogaster* (Nurminsky *et al.* 1996).

The *melanica* species group

Although Patterson (1943) split *D. melanica* into two subspecies: *D. m. melanica* and *D. m. paramelanica* on the basis of rather minor differences in morphology, different geographical distribution and high degree of reproductive isolation in laboratory. They were called as two distinct species: *D. melanica* and *D. paramelanica* based on distinct differences in male genitalia. Both species are found in North America. This sibling pair is more successfully derived from other members of this group and belongs to the third subgroup of this group (Stalker 1966; Markow and O'Grady 2005). Hybrids of both sexes between this sibling pair are fertile (Griffen 1942). Phylogenetic relationships among different members of this group have been elucidated by Stalker (1966) based on taxonomic, cytological and genetic studies as well as geographic distribution. This group is related to the other members of *virilis-repleta* radiation (Throckmorton 1975).

The *quinaria* species group

D. palustris and *D. subpalustris* are two sibling species of this species group which is Holarctic clade containing 32 species (Markow and O'Grady 2005). Phylogenetic studies involving these sibling species have been conducted by Jaenike (1985), Jaenike *et al.* (1983) and Spicer and Jaenike (1996). This group possesses diversity of abdominal pigmentation patterns. Interestingly, the group may be undergoing adaptive radiation (Spicer and Jaenike 1996). Mycophagy was the ancestral condition within this group. These two sibling species produce viable and fertile hybrids of both sexes (Bock 1984). Molecular data showing divergence time suggest that the evolution of reproductive isolation between these two species has taken less time than other species of this group (Spicer and Jaenike 1996). There are two major clades in this species group (Markow and O'Grady 2005).

The *cardini* species group

(i) *D. parthenogenetica* and *D. procardinoides* (*cardini* subgroup). (ii) *D. neomorpha* and *D. polymorpha* (*cardini* subgroup). (iii) *D. bedichecki* and *D. cardinoides* (*cardini* subgroup). (iv) *D. nigrodumni* and *D. antillea* (*dumni* subgroup). These four pairs of sibling species belong to the *cardini* species group (for details see Markow and O'Grady 2005). So far the *cardini* species group has 16 species described, which are placed into two subgroups: *cardini* and *dumni* (Heed and Krishnamurthy 1959; Markow and O'Grady 2005). In this group, there are four pairs of sibling species, three pairs belong to the *cardini* subgroup and one pair to the *dumni* subgroup. This species group is completely neotropical in distribution and many members of the *dumni* subgroup are endemic to single Caribbean islands (Markow and O'Grady 2005). These species have been extensively used for cytogenetical and phylogenetic studies (Heed and Russell 1971; Hollocher 1998; Wilder and Hollocher 2003; Cordeiro and Toni 2014). This group is also studied because of clines in abdominal patterns found in certain members of *dumni* subgroup (Hollocher *et al.* 2000). Interestingly, the island populations showing recent divergence demonstrate rapid development of reproductive isolation and distinct patterns of abdominal pigmentation which occurred in these species (Wilder and Hollocher 2003).

Evolutionary significance

Since this concept of sibling species was given by Mayr (1942), it has been found that such species pairs/groups are more prevalent in insects. Among insects, the occurrence of sibling species in the genus *Drosophila* is of special interest because of the fact that *Drosophila* is the best biological model which has been subjected to various kinds of studies in the areas such as genetics, evolution, behaviour, molecular biology, ecology, etc. and sibling species have also been studied in detail in certain species groups beginning from the classical examples such as *D. pseudoobscura* and *D. persimilis* confined to North America as well as *D. melanogaster* and *D. simulans*, cosmopolitan and domestic species. After designating these two pairs of sibling species, a large number of sibling species pairs/groups have been described and evolutionary significance with particular reference to speciation has been discussed with convincing evidence. Basically, sibling species have importance in biology because they provide the opportunity to test the validity of morphological versus biological species concepts. These species also have practical importance in applied biology such as agricultural pest control as well as medical entomology and are of much importance in the study of cladogenesis (Mayr 1970). Although it was suggested by Mayr (1942) that these species are morphologically similar or identical, differences have been found between these species with particular reference to salivary gland chromosomes, male genitalia, sex combs and relative wing size. Closer examination

also showed morphological differences among such species. Further, such species may also show variation in physiological and ecological requirements. Survey of literature shows that the term such as biological races which were used with different meaning are now called as sibling species. Further, sibling species and cryptic species were used with the same meaning but now it has been suggested that sibling species may be two cryptic species which are closely related to each other. Speciation among sibling species takes place in the same manner as in other species. Barring few cases of autopolyploidy and parthenogenesis, allopatric (geographic) speciation is the usual process by which sibling species originate (Mayr 1970). Sibling species are good species and are not in statu nascendi (Dobzhansky and Spassky 1959). When subjected to thorough study, they may show differences in minor morphological characters. Sibling species may be genetically very similar to each other than closely related morphologically different species. However, the level of morphological similarities in sibling species is an indication not merely of genetic similarity but also of developmental homeostasis (Mayr 1970). The genus *Drosophila* is characterized by numerous cases of sibling species, which have been subjected to genetical and evolutionary studies. As far as the known cases of sibling species are concerned, in most cases these species have full status based on taxonomic characters. However, in certain cases, taxonomic differences are not found but they have been called as sibling species on the basis of sexual isolation (Futch 1973). An example of this is *ananassae*–*pallidosa* pair of sibling species. In this pair, male genitalia are similar in both species. However, postzygotic reproductive isolation is absent because hybrids are fully normal and fertile. It may not be appropriate to call these species as sibling species rather they may in statu nascendi (Dobzhansky and Spassky 1959). Although, the researchers traditionally pair/group species as sibling species on the basis of morphology, biogeography and anatomical studies, recent advances in DNA testing and molecular phylogeny have provided new insights to determine whether two or more species are really sibling species (Puillandre *et al.* 2012). An interesting pair of sibling species is *D. aldrichi* and *D. wheeleri* which belong to the *repleta* species group. In both these species, male genitalia are similar. These species are homosequential for polytene chromosome banding. These points raise question about their independent species level. However, their separate species level is supported by postmating reproductive isolation (hybrid male sterility) and molecular data (Oliveira *et al.* 2008).

Conclusion

Sibling species are of frequent occurrence in insects. Among insects, their occurrence in *Drosophila* is of special interest because it is an excellent biological model which has been extensively utilized in different areas of biology. In

this review, about 90 species of the genus *Drosophila* are listed, which belong to 11 species groups including the species found in Hawaiian islands. Numerous sibling species pairs/groups are briefly described. Detailed examinations may show variations among the species with respect to morphology, hybridization, reproductive isolation, ecology, geography, chromosomes, etc. although they are said to be morphologically similar and reproductively isolated as stated by Mayr (1942). The genus *Drosophila* is characterized by a large number of cases of sibling species which have been subjected to genetical and evolutionary studies. In most of the cases, these species have full status based on taxonomic features. However, in certain cases, taxonomic differences are lacking but they have been called sibling species on the basis of sexual isolation as *D. ananassae* and *D. pallidosa* (Futch 1973), which may be in statu nascendi (Dobzhansky and Spassky 1959). Further, DNA testing and molecular phylogeny have provided new insights to determine whether two or more sibling species are really sibling species (Oliveira *et al.* 2008; Puillandre *et al.* 2012).

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