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Reproductive Isolating Mechanisms and Speciation

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Abstract: **Isolating mechanisms are those mechanisms or factors which alone or in combination prevent interbreeding between different species/populations and thus prevent gene flow between species which is essential for maintaining their integrity. These are genetically conditioned barriers for exchange of genes between Mendelian populations which are intrinsic to the organisms themselves. There are different types of isolating mechanisms: Geographical (spatial) isolation operating between allopatric populations/species and reproductive isolation operating between sympatric species/populations. There are different kinds of reproductive isolating mechanism operating in animal and plant species. Speciation (cladogenesis) is defined as origin of new species by splitting of pre-existing ones. During the process of speciation, the diverging populations accumulate genetic changes through the action of mutations, recombinations, natural selection, random genetic drift, migration and other evolutionary forces which lead to the origin and development of reproductive isolating mechanisms which are important and considered prerequisite for the process of speciation or cladogenesis. Thus due to the origin and development of reproductive isolating mechanisms, the new species are created and maintained as independent units. The well developed reproductive isolation prevents interbreeding and consequently gene flow between different species. In this article, different types of isolating mechanisms are defined and a few examples from *Drosophila* are given which show that origin and development of reproductive isolating mechanisms are considered prerequisite for the process of speciation. Further, the modes of speciation are also briefly summarized.**

Index Terms: **Reproductive isolating mechanisms, different types, prerequisite for speciation, modes of speciation, examples from *Drosophila.***

I. Introduction

In 1937, Dobzhansky coined the term “Isolating Mechanisms” for the first time in his book “Genetics and the Origin of Species”. His book was the basis of Modern Synthetic Theory of Evolution, the term was suggested later by Huxley in 1942. Dobzhansky provided genetical basis of evolution and in fact he integrated genetics with evolution (Singh, 2021a) . Dobzhansky remarked “Nothing in biology makes sense except in the light of evolution” and he has been considered as Darwin of twentieth century (Singh, 2012a). In 1889, Wagner suggested the theory of isolation to explain the mechanism of evolution. Even Lamarck and Darwin realized the importance of isolation in evolution because interbreeding of different populations will result in swamping of differences which they have acquired during the process of evolutionary change. It has been said that without isolation or prevention of interbreeding, evolution is not possible (Dobzhansky, 1951). Savage (1963) states “The major feature of organic evolution is the production of new adaptive types through a process of populational fragmentation and genetic divergence”. The populational fragmentation is caused by geographic (spatial) isolation. Thus isolating mechanisms are important to keep the species separate and maintain their independent status. Cook (1906) coined the term speciation. The basic process of speciation recognizes the existence of two processes: anagenesis- phyletic change in the course of time and cladogenesis or speciation-the origin of new species of organisms through splitting of preexisting ones and there are different modes of speciation. Species is a Latin word which means kind. Species is a basic unit as well as considered as basic category of biological classification which has been defined in different ways under different concepts of species (Singh 2012b). Biological species concept which was elaborated in detail by Jordan, Mayr and Dobzhansky is most widely accepted concept of species although it has certain difficulties in its applications (Mayr & Ashlok, 1991; Singh, 2012b). Under biological species concept (BSC), the species has been defined: (a) a group of potentially or actually interbreeding natural populations which are reproductively isolated from other such groups (Mayr 1940) and (b) a reproductive community of sexually and cross fertilizing individuals which share in a common gene pool (Dobzhansky, 1950). Thus, reproductive isolation is an important factor in the separation of species. Since Dobzhansky was evolutionary geneticist, he added the word gene pool to the definition of species under biological species concept. For adapting to diversity of environment, speciation is also considered as an important way. To explain the mechanism of evolution, Darwin (1859) suggested his theory of evolution in his book "On the origin of species by means of natural selection" which has two components: (i) Descent with modification- all species living and extinct descended from one or a few original forms of pre existing species and (ii) natural selection is an important causative factor of evolutionary change. Darwin also emphasized the role and importance of reproductive isolation during the process of speciation in his book with particular reference to temporal, habitat and behavioural barriers. In terms of genetics, evolution is defined as a change in genetic composition of population. As long as the population remains under Hardy-Weinberg Equilibrium which is locus specific, the frequency of particular alleles and genotypes remain constant in a population from generation to generation and evolution does not occur. Thus maintenance of genetic equilibrium is a conservative force. Evolution is modification of this equilibrium. When the population is evolving and undergoing evolutionary change, it accumulates genetic variability due to the action of various evolutionary factors such as mutation, selection, random genetic drift, migration etc. (microevolution). During the process of speciation, three factors are important: the genetic variability, isolating mechanisms and geographical component. Because of operation of these variable factors, new species are created. Thus, origin, development and perfection of reproductive isolating mechanisms are important for the creation of new species. If the population has not developed certain mechanisms of isolation, it may merge with other population or species as a consequence of removal of geographical barrier, and interbreeding with other population will lead to the swamping of the genetic differences acquired by the population. That is why it is said that for the creation and maintenance of the status of new species, origin and development of certain reproductive isolating mechanisms are essential. So very rightly it is remarked that "reproductive isolating mechanisms are prerequisite for the process of speciation or cladogenesis". It is rightly remarked that based on BSC, the question should be asked how reproductive isolating mechanisms are established instead of asking how new species evolve. Thus without isolation or prevention of interbreeding, evolution is not possible (Dobzhansky, 1951). According to Dobzhansky (1951), there are different types of isolating mechanisms which operate in animal and plant species. In this article, different types of isolating mechanisms are described briefly and a few examples are also given which provide evidence for the statement that "reproductive isolating mechanisms are considered prerequisite for speciation". In addition to this, direction of evolution based on the pattern of mating preference between closely related species of *Drosophila* in the light of different models is also summarized briefly which is of evolutionary significance. Further, a brief account of allopatric and sympatric modes of speciation.is also given.

II. Kinds of Isolating Mechanisms

The isolating mechanisms are classified into different types (see Dobzhansky 1951; Savage, 1963; Mayr, 1966; Coyne & Orr, 2004; Hall & Hallgrimsson, 2008; Singh, 2014) Considering the classification of isolating mechanisms suggested by these authors, broadly there are two types of isolating mechanisms:

1. Geographical or spatial isolation- which operates between populations/species which are allopatric in geographic distribution and thus they are inhabiting in different geographical areas which are separated by different geographical barriers. As a consequence of this, males and females of different populations/species cannot mate because they cannot cross these barriers. So gene flow between them is prevented. However, it is not necessary that these populations are reproductively isolated. They may or may not show reproductive isolation. In case, they have not developed certain mechanisms of reproductive isolation, removal of geographical barrier will lead to merger of these populations and interbreeding between them will cause swamping of the differences which they have acquired during their separation.
2. Reproductive Isolation- Reproductive isolating mechanisms operate between sympatric populations/species which inhabit in the same geographical area, These mechanisms operate alone or in combinations and are genetically conditioned. Thus, gene exchange between sympatric species are prevented due to prevention of interbreeding between them. And the independent status of species or Mendelian population is maintained. Broadly, the reproductive isolating mechanisms are further classified into two types: (a) premating and postmating- which operate before mating or after mating. (b) prezygotic and post zygotic-which operate before the formation of zygotes or after the formation of zygotes.

Considering the suggestions by different evolutionists, the reproductive isolating mechanisms are divided into the following types:

i. Ecological isolation- It is caused due to ecological factors when the representatives of the populations occur in different habitats of the same geographical area.
ii. Seasonal or temporal isolation- When mating or flowering periods vary during different seasons of the year.
iii. Sexual or behavioural or ethological or psychological isolation- It is caused due to lack of mutual attraction between males and females of different species.
iv. Mechanical isolation-Basically physical non correspondence of the genitalia or floral parts are responsible for this type of isolation.
v. Gametic or gametophytic isolation-Poor viability of sexual gametes in the female genital tracts of another species is responsible for this type of isolation. Spermatozoa or pollen tubes of one species are not attracted to the female gametes of another species.
vi. Hybrid inviability- The zygotes of hybrids between two species are not viable or adaptively inferior as compared to the parental species. It causes wastage of gametes as it operates one generation later.
vii. Hybrid sterility- Interspecific hybrids fail to produce functional gametes mainly due to chromosomal and genic differences. It also causes wastage of gametes as it operates one generation later.
viii. Hybrid breakdown-In this type of isolation, F2 or backcross hybrids are either fully or partially inviable or adaptively inferior.

Out of all these mechanisms of reproductive isolation, ecological, sexual, mechanical and temporal isolation are pre mating as well as prezygotic, gametic isolation is post mating as well as prezygotic and hybrid inviability, hybrid sterility and hybrid breakdown are post mating and post zygotic. In *Drosophila*, which is an important biological model, sexual isolation and hybrid sterility are extensively studied and interesting information pertaining to species phylogeny and mechanism of speciation has been obtained. There is rich species diversity in the genus *Drosophila* and the species which have been studied for genetic variability, have been found to show ample evidence for genetic diversity. At global level, there are more than 1500 species of *Drosophila* known and in India about 150 species are known so far which include both, the new species and new records. More than five hundred species have been reported from Hawaiian Islands which include picture winged *Drosophila* which have been extensively used for cytogenetical, behavioural and evolutionary studies (Singh, 2015). A number of studies on sexual isolation in the genus *Drosophila* have been conducted from the time of Patterson, Stone, Mayr, Dobzhansky, Carson, Stalker, Spieth and others (see Coyne & Orr, 2004). Extensive data have been reported concerning both inter and intra specific studies (Chatterjee & Singh, 1989). These results have often been used to discuss the phylogenetic relationship among the species. Sexual isolation may be complete or incomplete. When it is incomplete, it may be symmetrical or asymmetrical which is used for predicting the evolutionary sequence of the species/direction of the evolution among the species. Some times strains of the same species may show isolation providing evidence for incipient speciation. Based on asymmetrical mode of mating preference, two opposite models have been proposed to predict the direction of evolution between the closely related species. Kaneshiro (1976) suggested that ancestral females discriminate against the derived males. However, Watanabe and Kawanishi (1979) suggested that derived females discriminate against the ancestral males. Evidence in favour of both these models have been provided (for references see Singh, 1997). Interestingly, the Kaneshiso model is based on founder principle (allopatric mode of speciation) for Hawaiian *Drosophila* species. However, Watanabe and Kawanishi model is based on sympatric mode of speciation where natural selection is more important. Thus, it has been suggested that these models cannot be generalized for the whole of genus *Drosophila*. However, it may have a predictive value in certain specific species groups because evolution might have proceeded differently in different species groups (see Singh, 1997). It has been suggested that sexual isolation is most important class of reproductive isolating mechanisms than others in animal species and plays a key role in speciation. Similarly, hybrid sterility (post mating/post zygotic) has also been extensively studied in the genus *Drosophila* because a large number of species pairs are known to hybridize (Singh, 1994). However, there is gradation in the results of hybridization depending upon the phylogenetic relationship between the species. When adult hybrids are produced, generally males are sterile as per Haldane's rule. A number of studies have been done to identify the genes which are involved in hybrid sterility by making crosses and backcrosses and using molecular techniques. Furthermore, hybrid male sterility becomes a well defined developmental system for genetic analysis, namely, spermatogenesis. There is involvement of chromosome interactions (X x Y, X x autosomes, and Y x autosomes) resulting in hybrid sterility and this area of studies come under speciation genetics (Singh, 1994).

III. Origin of Reproductive Isolating Mechanisms

Evolutionary biologists differ in their opinion regarding the factors involved in the origin of reproductive isolating mechanisms which is prerequisite for speciation and keeping the species as separate gene pool by preventing interbreeding between the species leading to prevention of gene flow between them. Basically there are two important models to explain the origin of these mechanisms: random genetic drift and natural selection. Mayr (1966) has suumarized the views of Dobzhansky and Muller about the origin of reproductive isolating mechanisms. Dobzhansky (1940) suggested that ethological isolation evolves as an ad hoc product of natural selection.

Populations adapted to different environments develop different polygenic complexes. When they hybridize, ill adapted genotypes are produced. Natural selection acts to develop the barriers to gene flow between these populations whose hybridization causes reproductive wastage when two allopatric populations become sympatric. In this way, the production of hybrids with lowered fitness is either avoided or reduced. On the other hand, Muller (1942) did not agree with Dobzhansky's model and suggested that reproductive isolating mechanisms might arise as a result of random genetic drift or an accidental by-product of genetic divergence in geographically isolated populations as they adapt to different environmental conditions. Such genetically divergent populations may show ethological differences. Carson (1971) is of the opinion that ethological or behavioural isolation may originate allopatrically as a by-product when gene pool undergoes major reorganization. When populations become sympatric, isolation will be strengthened. This view is also supported by the results of Powell (1978) who found strong assortative mating in the populations which were passed through successive bottlenecks in population size. These opinions give support to the role of random genetic drift. Evidences in favour of both the theories: Dobzhansky's natural selection theory and Muller's random genetic drift theory have been presented.

IV. Examples supporting the statement "Reproductive isolating mechanisms are considered as prerequisite for speciation"

There are a number of examples which show that reproductive isolating mechanisms are considered as prerequisite for speciation. It has been rightly remarked that according to the biological species concept suggested by Mayr and Dobzhansky, the question "how new species evolve", can be substituted by a more answerable question "how reproductive isolating mechanisms are established between the populations". A few examples from *Drosophila* are given which demonstrate that reproductive isolating mechanisms are prerequisite for the process of speciation:

A. Hawaiian Species of Drosophila

There is unique opportunity available for evolutionary studies in *Drosophila* species found on Hawaiian Islands. More than five hundred species are found on these islands and out of which, there are about hundred species which have black patches on their wings (picture winged *Drosophila* species). These species have been extensively used for evolutionary studies (see Carson, 1973; Kaneshiro, 1976). Speciation of these Hawaiian species has been explained by the founder principle of Mayr (1942) which is based on random genetic drift (with a narrow population bottleneck). According to the suggestion of Carson (1973), a few individuals (in extreme cases a single gravid female) migrated to a new island and initiated a new colony which evolved into a new species. Kaneshiro (1976) has explained the direction of evolution among certain picture winged Hawaiian species (*D. differens*-------*D. planitibia*--------*D. silvestris*) based on asymmetrical mode of mating preference and ancestral females discriminate against derived males. During this process, due to the founder effect, the new population becomes genetically different from the original population which causes speciation. The ethological isolation developed during the process of evolutionary divergence leads to speciation. Thus origin and development of reproductive isolation is pre-requisite for speciation.

B. A unique pair of sibling species :D. ananassae and D. pallidosa

D. ananasae and *D. pallidosa* are considered as a unique pair of sibling species because their separation is just based on sexual isolation in natural populations where they are sympatric in distribution. Why they are called unique pair of sibling species because they have identical male genitalia which is an important taxonomic character and they are crossable in the lab producing normal and fertile hybrids of both sexes and thus lacking post zygotic reproductive isolation. This is a very good example of sexual isolation causing speciation. *D. ananassae* is a cosmopolitan and domestic species but *D. pallidosa* is endemic to certain South Pacific Islands. It has been suggested that *D. ananassae* is ancestral species but *D. pallidosa* is derived one. There is sufficient evidence that their separation is a recent event and *D. pallidosa* is in *statu nascendi* (Singh & Singh, 2017; Singh, 2021b,c)

C. Drosophila bipectinata species complex

The *Drosophila bipectinata* species complex belongs to the *ananassae* subgroup of the *melanogaster* species group. There are four species in this complex: *D. bipectinata, D. parabipectinata, D. malerkotliana* and *D. pseudoannanassae.* They are sympatric over part of their distribution but do not hybridize in nature. Females of all the four species are morphologically similar but males can be identified with the help of abdominal colouration and their pattern of sex combs. They are sympatric and phylogenetically closely related. All the four species hybridize in the laboratory but the degree of crossability varies in different crosses. Hybrid males are sterile but females are fertile. In nature they have separate gene pools and their separation is maintained by strong preferential mating between males and females of the same species. It has been suggested that they have not diverged very long back in the evolutionary history. Based on chromosomal studies and the pattern of sexual isolation in this complex, the phylogenetic relationship has been suggested that *D. pseudoananassae*, *D. malerkotliana* and a common population ancestral to *D. bipectinata* and *D. parabipectinata* were suggested to be derived from a common ancestral population. Further, it has also been suggested that *D. bipectinata*, *D. parabipectinata* and *D.*

malerkotliana share a close phylogeny. However, these three species are distantly related to *D. pseudoananassae*. Based on asymmetrical mating pattern, it has been suggested that *D.parabipectinata* is derived from *D. bipectinata* (Singh & Banerjee, 2016). Thus as far as speciation is concerned, both isolating mechanisms are important: sexual isolation (pre-mating) and hybrid sterility (post mating) in this complex.

D. Laboratory experiments of disruptive selection in D. melanogaster

A very interesting experiment was conducted by Thoday and Gibson (1962) in *D. melanogaster* for disruptive selection on sternopleural bristle phenotypes for 12 generations. Two different lines for bristle numbers were maintained: high and low lines. The results demonstrated that the disruptive selection led to the divergence of bristle numbers in high and low lines. Interestingly, the two lines showed strong ethological isolation between them. Thus, from the view point of speciation, such results are important and significant because in natural population the disruptive selection plays an important role as genes underlying disruptively selected traits and assortative matings are restrictive (Nanda & Singh, 2011). This provides very good example for sympatric speciation. These are certain examples from *Drosophila* which show that reproductive isolating mechanisms are prerequisite for the process of speciation.

E. Powell (1978) has discussed founder-flush speciation theory based on experimental evidence in *Drosophila pseudoobscura*. He maintained different populations of *D. pseudoobscura* in population cages in laboratory which were passed through flush-crash cycles. At each crash, the bottleneck population was small and genetic drift was strong. In certain populations, some degree of reproductive isolation could evolve rapidly following flush-crash cycles. This lends support to Carson theory of speciation through fonder effect (Carson, 1971, 1973, 1975). Thus because of founder effect (random genetic drift) reproductive isolation is initiated which leads to creation of new species providing evidence for the statement that reproductive isolation is prerequisite for the process of cladogenesis (true speciation).

The key element during the process of speciation (cladogenesis) is the origin and development of reproductive isolating mechanisms that restrict or prevent gene flow between Mendelian

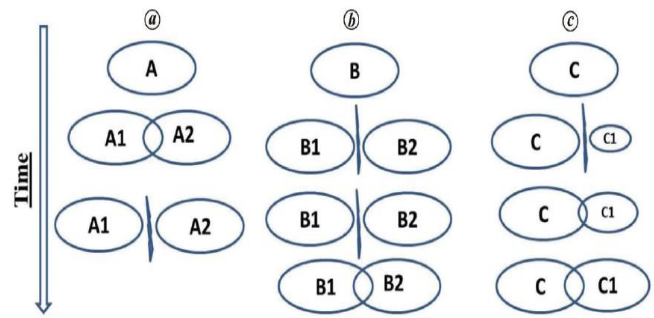

Figure 1. Schematic representation of (a) Sympatric, (b) Allopatric without a narrow population bottleneck and (c) Allopatric with a narrow population bottleneck (peripatric/founder effect) modes of speciation (modified from Bush, 1975 and Savage, 1963). The geographical component and development and perfection of reproductive isolating mechanisms are important for the origin of species (Singh B.N. 2021d Current Science 121:56-60).

populations. As suggested by White (1978), theoretically there are three sets of variables involved in the process of speciation: genetic mechanisms generating genetic variability, reproductive isolating mechanism (prezygotic and postzygotic) and geographic component which ranges from complete (strict allopatry) to absent (strict sympatry). These variables provide basis for different models of speciation. Mayr (1957) suggested 12 different models of speciation. However, only two are important and considered significant: polyploidy and geographic speciation. On the other hand, White (1978) suggested seven models of speciation. Primarily, these models are based on geographic component but secondarily, genic or chromosomal mechanisms may also play role. Considering all these models of speciation, two are most important and significant: allopatric and sympatric models of speciation. Sympatric mode of speciation may be defined as the origin of new species characterized by reproductive isolation within the dispersal area of parental species (Bush, 1975). This mode of speciation involves instantaneous appearance of reproductive isolation between the segments of the same population and the new species originates in the same geographical area. If both the populations remain in the same geographical area, they are recognized as sympatric species. If one or both the populations move away from the original habitat, they are called as allopatric species (Singh, 2012b). In the allopatric mode of speciation, there are two types: without a narrow population bottleneck (dichopatric or dumbbell model) and with a narrow population bottleneck (peripatric//bottleneck effect//fonder effect). In the dichopatric model, there is no role of random genetic drift. However, in the peripatric model, there is role of random genetic drift because new population may start from a few individuals and in extreme cases a single gravid female. Thus, due to bottleneck effect/flush-crash cycles/fonder effect, the random genetic drift plays an important role in speciation (Carson, 1971). The schematic representation of sympatric and allopatric modes of speciation is depicted in Figure 1. A large number of examples demonstrating the occurrence of speciation in different cases through sympatric and allopatric modes are described by Bush (1975), White (1978), and Singh (2012b, 2021d).

CONCLUSION

Isolating mechanisms are important for maintaining the integrity of species which is basic biological unit. Their importance was recognized even by Lamarck, Darwin and Wagner. It has been said that without isolation evolution is not possible. There are different types of isolating mechanisms which operate alone or in combination to maintain the integrity of species. Reproductive isolating mechanisms operating between sympatric species/populations play crucial role to prevent interbreeding between populations so that exchange of genes do not occur between them and their integrity is maintained. As a consequence of this, the genetic differences which they have acquired in the long run of time is maintained. When the populations are evolving and accumulating genetic changes due to microevolutionary processes, it leads to the origin, development and perfection of isolating mechanisms. If they do not develop certain mechanisms of reproductive isolation, the removal of barrier will lead to merging of the populations causing swamping of genetic differences which they have acquired during the process of microevolutionary changes. That is why it is said that origin, development and perfection of reproductive isolating mechanisms are prerequisite for speciation. It is rightly remarked that under biological species concept the question of how new species originate should be replaced by more answerable question of how reproductive isolating mechanisms are established. The examples described in this article clearly substantiate the statement that reproductive isolating mechanisms are prerequisite for speciation. A brief description of two modes of speciation, allopapatrc and sympatric is also given. Examples are given from *Drosophila* which is an important biological model used extensively in different kinds of studies such as genetics, evolution, behavior, ecology, molecular biology etc.(Singh, 2014, 2015).

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REFERENCES

Bush, G. L. (1975). Modes of animal speciation. *Annual Review of Ecology and Systamatics* 6, 339–364.

Carson, H.L. (1971). Speciation and the founder principle. *Stadler Genetics Symposium*,3, 51- 70.

Carson, H.L. (1973). Ancient chromosomal polymorphism in Hawaiian *Drosophila*. *Nature*, 241, 200-202.

Carson, H.L.(1975). Genetics of speciation. *American Naturalist*, 74, 312-321.

Chatterjee, S. & Singh, B.N. (1989). Sexual isolation in *Drosophila*. *Indian Review of Life Sciences*,9, 101-135.

Cook, D. F. (1906). Evolution without isolation. *American Naturalist*, 42, 727-731.

Coyne, J.A. & Orr, H.A.(2004). *Speciation.* Sinauer Associates Inc. Sunderland, Massachusetts.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection.* John Murray, London.

Dobzhansky, Th.(1937 & 1951), *Genetics and the Origin of Species*. Columbia University Press, New York,

Dobzhansky, Th. (1940). Speciation as a stage in evolutionary divergence. *American Naturalist*, 74, 312-321.

Dobzhansky, Th. (1950).Mendelian poplations and their evolution. *American. Naturalist*, 84, 401-418.

Hall,B. K. &, Hallgrimsson, B. (2008). *Strickberger's Evolution,* Jones and Bartlett, Massachusetts..

Huxley, J. (1942). *Evolution, the Modern Synthesis.* Allen and Unwin, London,

Kaneshiro, K. Y. (1976). Ethological isolation and phylogeny in the *planitibia* subgroup of Hawaiian *Drosophila*. *Evolution*, 30,740–745.

Mayr, E. (1940).Speciation phenomena in birds. *American Naturalist*, 74, 249-278.

Mayr, E. (1942). *Systematics and the Origin of Species*. Columbia University Press, New York.

Mayr, E. (1957). Die denkmoglichenFormen der Artentstehung. *Revue Suisse deZoologie*, 64, 219-235.

Mayr, E.(1966). *Animal Species and Evolution.* The Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

Mayr, E. and Ashlok, P.D.(1991)., *Principle of Systematic Zoology*. McGraw Hill International Edition, Singapore..

Muller, H.J.(1942). Isolating mechanisms, evolution and temperature. *Biological Symposium* , 6**,** 71-125.

Nanda, P. & Singh, B.N., (2011).Disruptive selection for sternopleural bristle phenotypes in *Drosophila ananassae. Genome*, 54**,** 845- 851.

Powell, J.R. (1978).The founder flush speciation theory: an experimental approach. *Evolution*, 32, 465-474.

Savage, J.M.(1963). *Evolution*. Holt, Rinehart and Winston, Inc. New York.

Singh, B.N. (1994). Hybrid sterility and its genetic basis in *Drosophila*. *Indian Review of Life Sciences*,14, 3-20.

Singh, B.N. (1997). Mode of mating preference and the direction of evolution in *Drosophila*. *Indian Journal of Experimental Biology*,35, 111-119.

Singh, B. N. (2012a). Darwin of the 20th century-Mayr or Dobzhansky? *Current Science*,103, 125.

Singh, B. N. (2012b).Concepts of species and modes of speciation. *Current Science*,103**,**784-790.

Singh, B.N.(2014). Reproductive isolating mechanisms: prerequisite for speciation. *Journal of Experimental Zoology, India*, 17, 23-31.

Singh,B.N. (2015).Species and genetic diversity in the genus *Drosophila* inhabiting the Indian subcontinent. *Journal of Genetics*, 94, 351361.

Singh, B.N. (2021a). How Theodosius Dobzhansky integrated genetics with evolution, *Current Science***,** 121,201-204,

Singh, B. N. (2021b). *Drosophila ananassae* Doleschall and *Drosophila pallidosa* Bock and Wheeler: a unique pair of sibling species. *Current Science*, 120,1282-83.

Singh, B.N. (2021c). Phylogenetic relationship between two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa.Current Science*,121, 873-75.

Singh, B.N. (2021d). Allopatric and sympatric modes of speciation; examples from *Drosophila*. *Current Science*, 121, 56-60.

Singh, B.N. & Banerjee, P. (2016). Population genetical, behavioural and evolutionary studies in the *Drosophila bipectinata* species complex. *Proceedings of Indian National Science Academy*, 82, 99-115

Singh, B.N. & Singh, R. (2017). *Drosophila pallidosa*-whether a separate species or a light form of *D. ananassae. Journal of Genetics*, 97, 1039-46.

Thoday, J.M. & Gibson, J.B. (1962). Isolation by disruptive selection. *Nature*, 193, 1164- 1166.

Wagner, M. (1889), In Die Enstechung der Arten Durch RaumlicheSonderang. Beno Schwalbe, Basel.

Watanabe, T. K. & Kawanishi, M. (1979).Mating preference and the direction of evolution in *Drosophila. Science*, 205, 906–907.

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