

Chromosome inversions and linkage disequilibrium in *Drosophila*

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Chromosomal polymorphism in *Drosophila* due to paracentric inversions is common and constitutes an adaptive trait. It has been studied in a number of species and shows interspecific variation with respect to its degree and pattern. Populations of certain species may also show seasonal, geographic, latitudinal and altitudinal variations with respect to inversion frequencies. Dobzhansky¹ suggested that chromosomal polymorphism in *Drosophila* is a device to cope with the diversity of environments. Chromosome inversions provide a mechanism for maintaining heterotic systems through the suppression of crossing over. Particularly interesting in this regard is the occurrence of non-random associations (linkage disequilibrium) of linked inversions in many species of *Drosophila*. Although linkage disequilibrium between inversions was reported for the first time in *D. robusta* by Levitan², this phenomenon which is of considerable evolutionary significance, has been reported in many species of *Drosophila*. Factors causing linkage disequilibrium between inversions vary in different species and also in different chromosomes of the same species. The present review documents the cases of linkage disequilibrium between inversions, their probable causes and evolutionary significance.

Keywords: Chromosome inversions, *Drosophila*, linkage disequilibrium, polymorphism.

DROSOPHILA is no longer the queen of genetics. However, it remains probably the best material for studies on evolutionary and population genetics³. The first inversions were detected in *Drosophila* through the suppression of crossing over in inversion heterozygotes⁴. Since flies with different karyotypes produced by inversions are externally indistinguishable, many investigators, including Dobzhansky, believed inversion karyotypes to be adaptively neutral traits. This proved to be wrong. A large number of studies have been conducted on chromosomal polymorphism in numerous species of *Drosophila*, which have revealed that chromosomal polymorphism due to paracentric inversions is common in the genus *Drosophila*^{3,5-12}. It has also been demonstrated that chromosomal polymorphism in *Drosophila* is subject to natural

selection and is an adaptive character. It has been investigated in detail in a number of species such as *D. pseudoobscura*, *D. persimilis*, *D. willistoni*, *D. robusta*, *D. subobscura*, *D. melanogaster*, *D. ananassae*, *D. funebris*, *D. pavani*, *D. rubida*, *D. nasuta*, *D. flavopilosa*, *D. bipectinata*, *D. guaramunu* and others. The degree of inversion polymorphism varies in different species and also in different populations of the same species. Further, the pattern of inversion polymorphism may also vary in different species and sometimes even closely related species may show variations in their pattern of inversion polymorphism because they might have evolved different mechanisms for adjustment to their environments¹³. Observations on inversion frequencies in geographic populations of different *Drosophila* species provide the most convincing evidence for selective control of inversion frequencies. In certain species, there are geographic, seasonal, latitudinal and altitudinal variations in inversion frequencies. North-south clines in inversion frequencies have also been reported in certain cases¹⁰. Inversion polymorphism has also been studied in experimental populations maintained under laboratory conditions in certain species and persistence of inversion polymorphism in these populations could be explained by the advantage of inversion heterozygotes over corresponding homozygotes. Thus inversion polymorphism is adaptive and balanced due to higher Darwinian fitness of inversion heterozygotes, i.e. heterosis⁸. There is, however, evidence that higher fitness may be lost in heterozygotes for chromosomes with different gene arrangements coming from different geographic populations. This has been found in *D. pseudoobscura*, *D. willistoni*, *D. paulistorum* and *D. pavani*¹⁴. The explanation offered by Dobzhansky⁵ is that the gene complexes carried in the chromosomes are co-adapted or mutually adjusted through long-continued natural selection in one locality, so that inversion heterozygotes possess high adaptive value. Since heterozygotes for such foreign gene complexes are seldom found in nature, heterosis is an outcome of a historic process of adaptation to the environment. However, evidence for genetic co-adaptation is lacking in geographic populations of *D. ananassae*, since there is no breakdown of heterosis in inter-racial crosses¹⁵. It has been suggested that heterosis associated with chromosome inversions in *D. ananassae* may be simple luxuriance rather than population heterosis (genetic co-adaptation). This provides evidence against the selectional co-adaptation hypothesis¹⁶.

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In certain species, frequencies of inversions are higher in urban populations than in rural populations. It has been suggested that the high degree of urbanization leads to increase in the number of ecological niches and consequently to high chromosomal variability¹¹. Dobzhansky and coworkers^{17,18} found good correspondence between the mean number of heterozygous inversions and an index expressing environmental heterogeneity in natural populations of *D. willistoni*. Marginal populations showed a lower degree of chromosomal polymorphism than those at the centre of geographical distribution. This led Dobzhansky and others to suggest that chromosomal polymorphism is a device to cope with the diversity of environments. Their hypothesis has been supported by numerous observations in different species. A similar pattern has been found by Carson¹⁹ in *D. robusta*. Carson¹⁹⁻²¹ suggested that the amount of recombination is an essential adaptive factor and that the low level of polymorphism in marginal populations where homoselection predominates, gives a high potential for free recombination from which adaptive novelties can be synthesized. In geographically central populations, there is a high level of inversion polymorphism and heteroselection predominates. The marginal populations have more adaptability, whereas the central ones have more adaptedness²¹. Chromosomal polymorphism has also been studied in certain *Drosophila* species found in India, which vary in their pattern and degree of inversion polymorphism¹³.

While the entire gene pool is integrated by the selection of genes, which gives a good combination in most of the genotypes, chromosome inversion protects co-adapted polygenic complexes against dissociation. The genotype interaction can go even a degree further. Two independent inversions of the same chromosome tend to occur together due to epistatic interaction. Mutual adjustment involves the establishment of favourable linkage relation and selection of genes, which interact to maximize fitness¹⁴. Evidence for coadaptation is thus evidence for the importance of interaction in evolution²². The individual mutations that are favourable in some combinations may be unfavourable in others. Thus selective values can be assigned only to the genetic system as a whole²³. Epistatic selection and balanced polymorphism tend to produce supergenes, which are major features of evolution. Although breakdown of heterosis in interracial crosses clearly supports the theory of genetic coadaptation in *Drosophila*, non-random associations (linkage disequilibria) between inversions has been studied in various species of *Drosophila*, which are relevant to determine the extent of coadaptation as it involves gene interaction at fitness level.

Lewontin and Kojima²⁴ used the term linkage disequilibrium for non-random associations between genes or gene arrangements, since the rate of approach to random association is reduced by linkage. According to Hedrick *et al.*²³, this term is misleading as factors other than linkage may affect the rate of decay, and in their opinion it

should not be used. Even unlinked loci on different chromosomes (or loci far apart on the same chromosome) may show linkage disequilibrium²⁵. Since it concerns non-random associations of alleles at two or more loci within gametes as a phenomenon, which can be influenced by many factors other than linkage, Hedrick *et al.*²³ preferred the term gametic disequilibrium to describe this phenomenon. This is a shortened form of the term 'gametic phase disequilibrium' used by Crow and Kimura²⁶. Also linkage by itself is not sufficient to result in linkage disequilibrium. That is, alleles at linked loci are often not statistically associated so that they are not in linkage disequilibrium. However, the amount of linkage disequilibrium is generally a function of the rate of recombination²⁵. Although both terms, linkage disequilibrium and gametic disequilibrium, have been frequently used in the literature, the present author has preferred to use the term linkage disequilibrium in this review, because only those cases are cited which deal with linked inversions. When linkage disequilibrium, is present, there has been a tendency to attribute it to differential selection involving multilocus interaction. Besides selection, there are other factors such as tight linkage, genetic drift, migration, gene flow and genetic hitchhiking which can generate linkage disequilibrium²⁵. If significant linkage disequilibrium is present and is consistent between populations, it can be attributed to selection²⁷. Non-random associations (linkage disequilibrium) between linked inversions were reported for the first time in *D. robusta* by Levitan². Since then, a number of studies on linkage disequilibrium between inversions of the same chromosome have been reported in natural and laboratory populations of different species of *Drosophila*, by studying inversion polymorphism through polytene chromosomes following the usual squash preparation method using salivary glands from third instar larvae and obtaining data on various intrachromosomal associations. The present review documents the findings on linkage disequilibrium between inversions in different species of *Drosophila* and it also discusses the role of different factors causing linkage disequilibrium between inversions.

Drosophila robusta

Levitan² was first to report the occurrence of non-random associations (linkage disequilibria) between linked inversions in natural populations of *D. robusta*. Levitan²⁸ suggested that linkage disequilibrium between linked inversions is caused by two main factors, either alone or in combination: (i) suppression of crossing over between linked inversions, and (ii) natural selection acting against certain recombinant arrangements. It has been demonstrated by Levitan and co-workers²⁸⁻³³ that linked inversions in *D. robusta* are associated non-randomly due to natural selection favouring linkages between interacting genes, which are not part of the allelic blocks. The frequencies

of coupling combinations are in excess of the numbers expected if the arrangements on two arms of the same chromosome are independent, the repulsion combinations being deficient²⁸. In certain natural populations of this species, Prakash³³ observed an excess of repulsion combinations and a deficiency of coupling combinations. Linkage disequilibrium between inversions of the same chromosome has also been documented in recent studies by Levitan and co-workers³⁴⁻³⁶. There is a significant role in the arrangements of the left arm of the third chromosome in the adaptation of this species to altitude, which suggests further that interaction of linked arrangements is involved in these adaptations³⁴. In *D. robusta*, linkage disequilibrium is a long-standing hallmark of adaptation in natural populations rather than a short-term consequence of low recombination rate and low decay of linkage in non-equilibrium frequencies. Further, most selectively neutral explanations such as low recombination rate, genetic drift, gene flow among structured populations and long-term population fluctuations are discarded³⁵. Frequencies of cis and trans forms of double heterozygotes and their linkage combinations vary in different populations. Presence and degree of linkage disequilibrium also vary in different populations³⁵. Levitan³⁶ also presented evidence for directional changes in the frequencies of gene arrangement combinations in the X chromosome in natural populations of *D. robusta*, which was interpreted as being due to natural selection. Thus natural selection is the most important factor for maintenance of linkage disequilibrium between inversions in *D. robusta*.

Drosophila pavani

Brcic³⁷ studied inversion polymorphism in *D. pavani* and found non-random associations between inversions in the left and right arms of the fourth chromosome. He found linkage disequilibrium between gene arrangements in both natural populations as well as laboratory stocks. Usually there was overabundance of the coupling combinations and a scarcity of repulsion combinations of these gene orders. These findings have been explained by suggesting that the main factor in the origin and maintenance of non-random associations between chromosome arrangements is natural selection acting against certain combinations, because distance between inversions in the fourth chromosome covers more than 34% of its total length and all possible combinations between these arrangements have been observed in different frequencies in natural populations of *D. pavani*. The role of natural selection is further strengthened by the fact that the association is stronger in laboratory populations than natural populations³⁷.

Drosophila subobscura

Sperlich and Feuerbach-Mravlag³⁸ observed almost complete linkage disequilibrium between inversions of an

autosome and X chromosome of *D. subobscura*. Linkage disequilibrium between inversions of an autosome is due to complete suppression of crossing over in the region between them. However, linkage disequilibrium between inversions of the sex chromosome may be due to epistatic interaction. In the sex chromosome, recombination occurs between inversions which show linkage disequilibrium. Thus the situation in *D. subobscura* is interesting, because within the same species two chromosomes behave differently with regard to linkage disequilibrium. In one chromosome, suppression of crossing over is the main cause of linkage disequilibrium, but in the other chromosome epistatic interaction is responsible for linkage disequilibrium. It may be that the two cases demonstrate two different stages of gene interaction in evolution. Sex chromosome arrangements may represent a very early stage whereas autosomal arrangements may be considered as the end-point of the development. Inversions are suppressors of recombination and their incorporation into the gene pool of a population may depend on interacting genes. Further, supergenes may arise, including groups of interacting genes with no recombination between them³⁸.

Drosophila guaramunu

Levitan and Salzano³⁹ reported extreme non-random association between two independent inversions of the fourth chromosome of *D. guaramunu* in different Brazilian localities. Interestingly, individuals homozygous for one arrangement and heterozygous for another were rare in the populations. But over 98% of the larvae were either doubly homozygous or heterozygous at both inversion loci. According to Levitan and Salzano³⁹, the most probable explanation is differential selection for chromosomes with certain combinations of arrangements, coupling combinations being favoured and repulsion combinations selected against.

Drosophila ananassae

D. ananassae is a cosmopolitan and domestic species. Three cosmopolitan inversions (AL-2L, DE-3L and ET-3R) are common in natural populations and often persist in laboratory populations due to heterotic buffering associated with these inversions^{40,41}. DE and ET inversions occur in the opposite arms of the third chromosome. The distance between them is nearly 25% of the chromosome length. Recombination between these two inversions when heterozygous, has been studied cytologically⁴²⁻⁴⁵. From the results reported by Singh and co-workers, it is evident that the rate of recombination between heterozygous inversions is low in spite of long chromosome distance between them and is controlled by background karyotype and background genotype^{44,45}. There is extreme linkage between DE and ET inversions due to suppression of

crossing over⁴⁶. Linkage disequilibrium between these two inversions has been observed in certain laboratory strains, which appears to be due to epistatic interaction and suppression of crossing over^{47,48}. Although these two inversions show non-random association in laboratory strains, no linkage disequilibrium has been observed in natural populations^{49,50}. To know the cause of linkage disequilibrium between delta and eta inversions in laboratory strains of *D. ananassae*, Singh and Singh^{49,50} collected flies from natural populations and obtained data on the frequencies of inversions. The same data were analysed to know the associations between different karyotypes in 3L and 3R in natural populations. From the flies collected from natural localities, mass culture stocks and isofemale lines were established in the laboratory and maintained for 10–15 generations. Mass culture stocks and isofemale lines were analysed chromosomally and data on the associations between 3L and 3R karyotypes were obtained. The main findings of this study were: (i) inversions were associated randomly in natural populations and mass culture stocks, and (ii) the same inversions showed linkage disequilibrium in several isofemale lines. The random associations between inversions in natural populations and mass culture laboratory populations as contrasted to laboratory populations established from single females suggest that random genetic drift is the cause of linkage disequilibrium in isofemale lines. The tight linkage between these two inversions as evidenced by the results of recombination studies support the notion that linkage disequilibrium is caused by genetic drift^{49,50}.

Drosophila bipectinata

Singh and Das⁵¹ observed linkage disequilibrium between two independent inversions of the second chromosome of *D. bipectinata* in three laboratory stocks, and these inversions are widespread in natural populations. They have also suggested that linkage disequilibrium is maintained by epistatic gene interaction based on significant excess of doubly heterozygous and doubly homozygous larvae in all the stocks analysed. On the other hand, other combinations were less than expected. These inversions also occur in non-random associations in natural populations of *D. bipectinata*, which has been explained by suggesting epistatic interaction between both inversions⁵².

Drosophila melanogaster

Singh and Das⁵³ tested associations between linked inversions of the second and third chromosomes in Indian natural populations of *D. melanogaster* and found that these inversions are associated randomly. However, non-random associations of linked inversions have been reported to occur in natural populations of *D. melanogaster* from Korea, Australia and Tunisia⁵⁴.

Drosophila nasuta

Kumar and Gupta^{55,56} reported an extreme linkage disequilibrium between two inversions of the third chromosome in laboratory stocks and natural populations of *D. nasuta*. According to them, natural selection influencing recombination rates in several ways and intrachromosomal epistasis between the two inversions are the main factors for maintenance of linkage disequilibrium in *D. nasuta*.

Drosophila rubida

Data by Mather^{57,58} on inversion polymorphism in two New Guinean natural populations of *D. rubida* were analysed by Levitan and Fukatami⁵⁴ to test associations between linked arrangements in the second and third chromosomes. Their analysis has shown that linked gene arrangements in *D. rubida* occur in non-random associations because in many cases there was significant deviation from randomness, which seems to support the conclusion that they have an adaptive basis rather than being so many instances of genetic drift⁵⁴.

Drosophila lutescens

Levitan and Fukatami⁵⁴ observed linkage disequilibrium between gene arrangements in the second and third chromosomes of *D. lutescens* in Japanese populations. Their results also support the hypothesis that these associations are adaptive in nature, because certain combinations have superior selective values by virtue of interaction of linked genes⁵⁴.

Drosophila sulfurigaster neonasuta

Shyamla *et al.*⁵⁹ detected a high degree of non-random associations between two inversions of the second chromosome in natural populations of *D. s. neonasuta*. They have also shown that the extent of linkage disequilibrium was similar in all the populations analysed, as Lewontin²⁷ had stated that the loci involved were under natural selection.

Drosophila euronotus

Stalker⁶⁰ studied inversion polymorphism in a natural population of *D. euronotus* from North America and found non-random associations between the sets of inversions in the second chromosome capable of recombination. He demonstrated six different types of such non-random associations. In one instance coupling phase predominates in the north, repulsion phase predominates in the middle zone and in the far south, the coupling phase predominates again. Non-random associations of inversions

in nature form models of polygenic balance as it has been found in *D. euronotus*, which is characterized by internally balanced inversion systems⁶⁰.

Drosophila silvestris

Craddock and Johnson⁶¹ found strong linkage disequilibrium between certain adjacent but nonoverlapping sequences of the fourth chromosome of *D. silvestris*, a Hawaiian *Drosophila* species. Further, the degree of linkage disequilibrium between the fourth chromosome inversions appears to vary in different populations. The third chromosome displays no disequilibrium in six of the eight populations in which both third chromosome inversions are polymorphic⁶¹.

In addition to the above-mentioned species of *Drosophila*, non-random associations between linked inversions have been reported in several other species such as *D. americana*, *D. bifasciata*, *D. gasici*, *D. konkoo*, *D. mediopunctata*, *D. n. albomicans*, *D. nigromaculata*, *D. paramelanica*, and *D. pseudoobscura* and others (see Levitan and Fukatami⁵⁴).

Conclusion

The phenomenon of linkage disequilibrium between inversions has been reported in many species of *Drosophila*, which are characterized by considerable degree of inversion polymorphism. It occurs in both natural as well as laboratory populations. However, the factors which cause non-random associations between linked inversions may vary and different species present different pictures regarding the maintenance. According to Levitan²⁸, two main causes, alone or in combination, could explain linkage disequilibrium between inversions: (i) suppression of crossing over between the arrangements and (ii) natural selection acting against certain recombinant arrangements. Levitan²⁸ has emphasized the role of natural selection in maintaining the associations based on his work in *D. robusta*, since complete suppression of crossing over probably never occurs as long as there is uninverted area between the arrangements. Levitan²⁸ has also postulated that natural selection would be acting at various stages in the history of the associations in two ways: (i) to favour mechanisms which reduce recombination, and (ii) to counter any recombination that occurs. The third probable action of natural selection in relation to association is regulating the frequencies of karyotypes because population recombination rate depends not only on the cross-over frequency in individuals of certain karyotypes, but also on the frequency of karyotypes in the population.

Levitan and Fukatami⁵⁴ have explained linkage disequilibrium between inversions by suggesting that (i) various combinations have equal selective values but significant departure from randomness stems from genetic

drift or we have accidentally sampled a stage in the historical processes leading to equilibrium or (ii) adaptive nature of associations by virtue of interaction of linked gene arrangements. In several species, the role of natural selection which involves interaction between widely separated loci has been demonstrated. In *D. subobscura*, two cases of linkage disequilibrium have been studied in detail³⁸. Linkage disequilibrium between two inversions of an autosome is caused due to lack of crossing over, while between independent inversions of the sex chromosome it is due to epistatic interaction. On the basis of their findings in *D. subobscura*, Sperlich and Feuerbach-Mravlag³⁸ have suggested that selection may favour linkages between interacting genes and the chromosomes may be considered as functional and selectional units. Kumar and Gupta^{55,56} have supported this argument of Sperlich and Feuerbach-Mravlag³⁸, on the basis of their findings of linkage disequilibrium between inversions in natural populations and laboratory stocks of *D. nasuta*.

In *D. ananassae*, there is strong suppression of recombination between heterozygous inversions^{42-45,62}. Linked inversions are associated randomly in natural populations and mass culture laboratory populations established from naturally impregnated females. However, the same two inversions of the third chromosome show linkage disequilibrium in isofemale laboratory stocks, which has been explained by suggesting the role of genetic drift (founder effect). The tight linkage between these two inversions as evidenced by recombination studies enhances the chance of genetic drift, because the strains were initiated from single females^{49,50}. Thus, it is evident that linkage disequilibrium between independent inversions of the same chromosome is common in *Drosophila*, but factors responsible for maintaining this phenomenon may vary in different species. It is hoped that further studies will be undertaken in future to obtain new data to understand this interesting phenomenon, which is of considerable evolutionary significance.

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