[3] Papazian H. P. *Genetics* **36**, 441–459(1951).
[4] Day, P. R., and R. Holliday, *Amer. Nat.*, **93**, 387–389(1959).
[5] Dobzhansky, Th., and S. Wright, *Genetics*, **26**, 23–52(1941).
[6] Stevens, W. L., *Jour. Genetics*, **43**, 301–309(1941).

---

*CYTOLOGICAL AND PHYLOGENETIC RELATIONSHIPS IN THE REPLETA GROUP OF THE GENUS DROSOPHILA**

BY MARVIN WASSERMAN

GENETICS FOUNDATION, DEPARTMENT OF ZOOLOGY, THE UNIVERSITY OF TEXAS

Communicated by J. T. Patterson, April 6, 1960

A little more than two decades ago the geneticists at The University of Texas, under the supervision of Professor J. T. Patterson, undertook an extensive study of evolution in the genus Drosophila with special emphasis on the Nearctic and Mexican forms. A wealth of information was obtained culminating in the publication of a book by the two principal researchers (Patterson and Stone[1]) in which the factors responsible for the success of this genus, presently estimated by Wheeler to contain 1500 species, were enumerated and comparisons were made to other groups of organisms. More recently under the direction of Professors W. S. Stone and M. R. Wheeler, this work has been continued and extensive collections have been made in the Neotropical Region.

Sturtevant and Dobzhansky[2] proved that paracentric inversions as seen in the salivary gland chromosomes are reliable characters for demonstrating phylogenetic relationships. We have used this method to determine affinities within the repleta group which is the largest in the genus. A series of papers (Wasserman,[3] Wasserman and Wilson,[4] and Wasserman[5]) give complete details on the cytology, genetics, and morphology of the species examined. Of the sixty-eight known species in the group, relationships have been determined for forty-six, fourteen of which will be described in Wasserman[5] and are here specified by *species A* through *species N*. As our standard gene sequence for the species group we have chosen that of *D. repleta* (Wharton[6]), a cosmopolitan species which is not known to vary cytologically. Each inversion found was labeled using two characters: the first, a number, denoting the particular chromosome involved, and the second, a letter, specifying the inversion; the inversions being named in the order of discovery. Since 103 inversions were found in the second chromosome, the alphabet was run through several times using superscripts, resulting in the last inversion being recorded as $2k^5$. There is no relationship implied among $2k$, $2k^2$, $2k^3$, etc. Centric fusions are designated by the numbers of the two chromosomes involved, followed by *F* e.g., 3–4*F*).

As a general rule few inversional differences between species were found, which simplified analysis and made this work possible. Although it is necessary to base the relationships upon independent segregating inversions, this does not detract from the accuracy of the analysis. In fact the segregation of inversions presents a clue to the process of speciation in these forms.

Phylogenetic Relationships.—The repleta group evolved in the New World, only

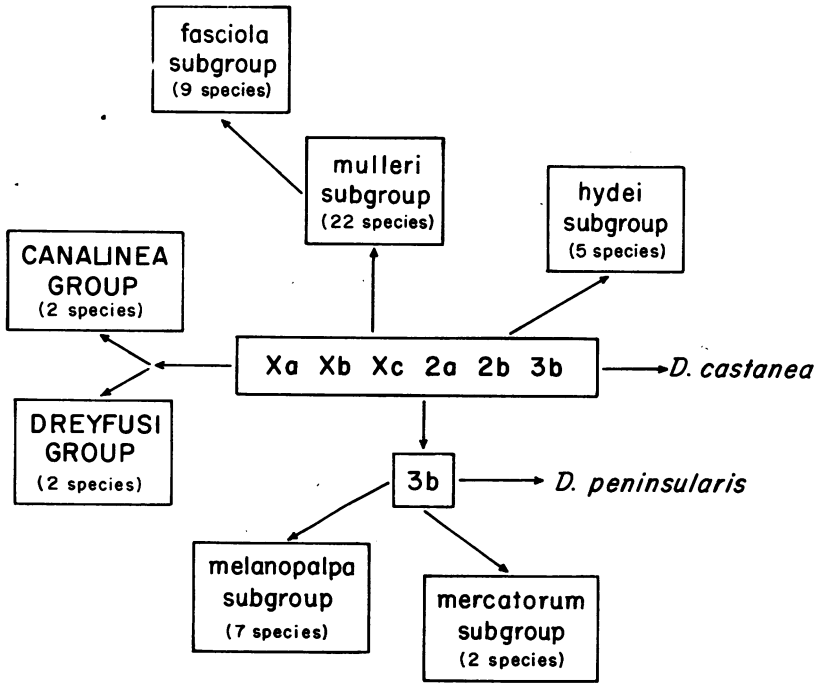

FIG. 1.—Phylogenetic relationships among 51 species in the genus Drosophila, showing the affinities among the canalinea and the dreyfusi species groups, *D. castanea*, and the five subgroups and *D. peninsularis* in the repleta group. Arrows indicate proposed direction of evolution.

six species including the cosmopolitan *D. repleta* and *D. hydei* being present elsewhere. Two of these, *D. buzzatii* and *D. mercatorum*, are found in the New World, the former also occurring in Europe, the Middle East, and Australia, and the latter having reached the Hawaiian Islands. The remaining two, *D. poecilithorax* and *D. obsoleta*, have been described from Australia. Patterson and Wheeler[7] have suggested that these last four are New World species in origin and have been transported to other regions along with the cacti with which they are closely associated.

The repleta species group has been subdivided into four subgroups: the melanopalpa, the mercatorum, the hydei, and the mulleri subgroups (Patterson and Stone[1]). These species, being predominantly desert forms, are concentrated in Mexico and Southwestern United States. Further collections in the relatively unsampled Neotropical Region led to the discovery of a forest-inhabiting subgroup, the fasciola subgroup. The four original subgroups were defined using morphological and genetic characters (see especially Wheeler[8]). Our cytological studies have confirmed the classification demonstrating the reliability of the characters used. In only one case do we differ: *D. peninsularis* had been assigned to the mulleri subgroup. Although this species is morphologically typical of this subgroup, our cytological evidence, Figure 1, demonstrates that this species should be removed from the mulleri subgroup.

Figure 1 shows the over-all relationships among the five subgroups plus *D. peninsularis* of the repleta group, *D. castanea*, and the canalinea and dreyfusi species

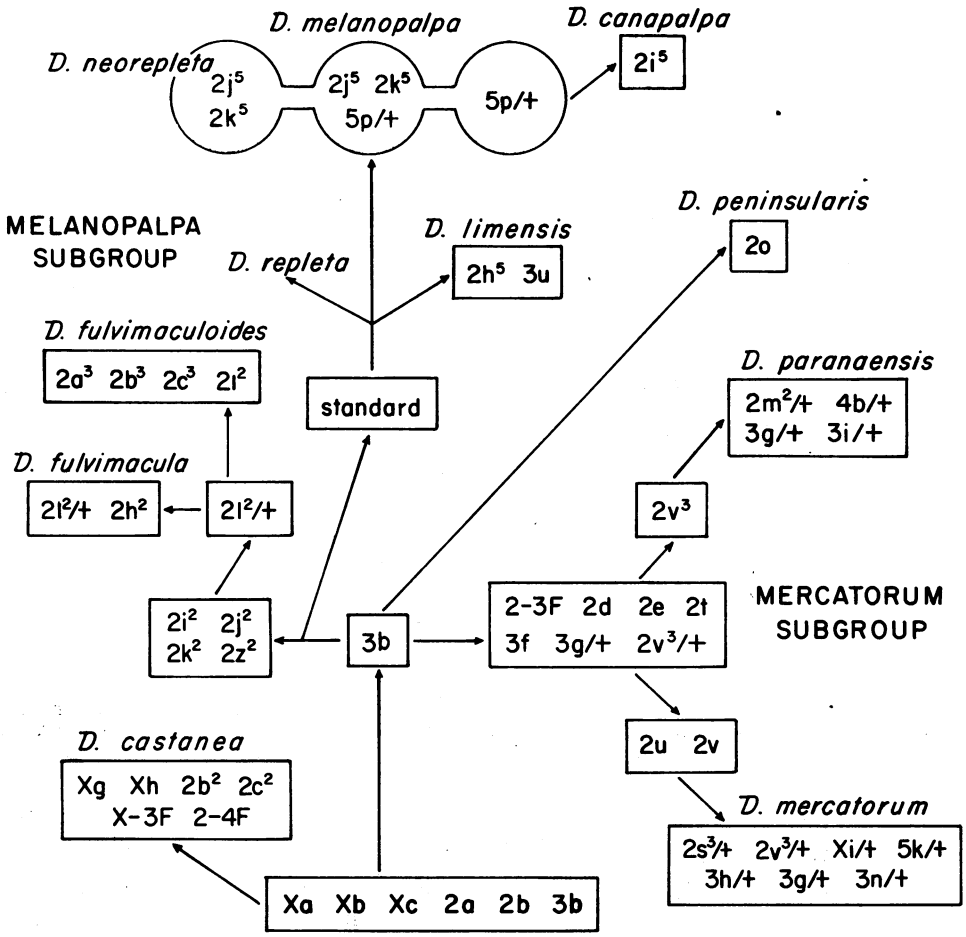

FIG. 2.—Relationships among the mercatorum and the melanopalpa subgroups and *D. peninsularis* and the nonrepleta species *D. castanea*. *D. neorepleta*, *D. canapalpa*, and *D. melanopalpa* are shown to have evolved from a single polytypic species. In this and the following figures, the standard arrangement of each species is the sum of all homozygous inversions leading out from the primitive. Heterozygous inversions which occur with the standard are indicated by the inversion shown over a plus sign.

groups—a total of 51 species. The subgroups have not diverged from a single cytological type, but rather have branched off at various points on the phylogenetic tree. Using the repleta data exclusively, it is impossible for us to determine the ancestral sequence. Inversions show relationships but not direction of evolution. The primitive karyotype is undoubtedly five pairs of rods and one pair of dots. However, any of the euchromatic sequences now present, or the intermediate forms either extinct or as yet unknown, could theoretically have been the ancestral type. The only way to determine the ancestor would be a comparison with the more primitive species groups. Although in some instances chromosome homologies can be readily determined, cytological changes in most cases are too numerous for an attempt to determine chromosome evolution between groups at this time. However, the canalinea and the dreyfusi species groups have been analyzed with a certain degree of success. These two species groups being limited to the Neotropical

Region may have evolved from the repleta group rather than being more primitive. Cytologically, they both split off together from the repleta group through the *Xa*, *Xb*, *Xc*, 2*a*, 3*b* ancestor (Fig. 1). This sequence is therefore either older than these two species groups (if the repleta group is primitive) or the primitive sequence of the repleta group (if they are primitive). *D. castanea*, a species close to the repleta group and probably a derived form, also evolved from this ancestor, Figure 1. Within the repleta group, *D. repleta* of the melanopalpa subgroup and *D. hydei*, being cosmopolitan, are probably old species. The *Xa*, *Xb*, *Xc*, 2*a*, 2*b*, 3*b* sequence is intermediate between these two species and is therefore more primitive than one if not both of their sequences. In view of the above factors, the *Xa*, *Xb*, *Xc*, 2*a*, 2*b*, 3*b* sequence will be considered the primitive gene sequence for the repleta group. Fortunately, even if this proves to be incorrect, only a few minor directional changes would be necessary in the phylogenetic trees. There would be little or no change in our discussion and conclusions.

Figures 2–6 give a more detailed cytological analysis of the relationships among the species studied. The bulk of the information is given in Wasserman.[5] A brief account will be given here.

Figure 2 shows the relationships among the melanopalpa and mercatorum subgroups, and *D. peninsularis* and *D. castanea*. The ancestor of the mercatorum subgroup was polymorphic for two arrangements, 3*g* and $2v^3$. The 3*g* arrangement has remained heterozygous in the two representatives of this species group, *D. mercatorum* and *D. paranaensis*. The $2v^3$, heterozygous in *mercatorum* has been fixed in *paranaensis*. Both species show polytypic variation. In *mercatorum* two subspecies have been described which differ markedly in the cytological structure of their populations. The populations of the Brazilian and Bolivian subspecies, *D. mercatorum pararepleta* are quite polymorphic, having both primitive and advanced sequences. The subspecies, *D. mercatorum mercatorum*, present in the South American Andes and north into the United States has very little chromosomal variability. All of these populations are homozygous for the newer 3*h* and $2v^3$ sequences. *D. paranaensis* can be divided into three major geographical populations which differ in the size and shape of the heterochromatic dot element of the metaphase chromosomes. The geographical limits of these populations are not known.

The melanopalpa subgroup has two main branches. The ancestor of the *fulvimacula* complex was heterozygous: inversion $2l^2$ has remained heterozygous in *D. fulvimacula* but has been fixed in *D. fulvimaculoides*. In the other stem, *D. neorepleta*, *D. melanopalpa*, and *D. canapalpa* share three inversions, $2j^5$, $2k^5$, and 5*p*, the latter inversion being heterozygous in *melanopalpa* and *canapalpa* (Ward and Stone[9]). A possible explanation for the sharing of the inversions by different species will be presented below.

The hydei subgroup, Figure 3, is remarkable in that only one inversion, 2*z*, has been fixed as an interspecific difference among the five species examined. *D. bifurca* and *D. nigrohydei* have as their standard the *Xa*, *Xb*, *Xc*, 2*a*, 2*b*, 3*b* sequence which we have taken as the primitive of the whole repleta group. *D. hydei*, *D. species A*, and *D. species B* are each homozygous for the 2*z* inversion. Each of these five species, is polymorphic for its own inversions. The major cytological evolution of this subgroup has been the addition of heterochromatin to the sex

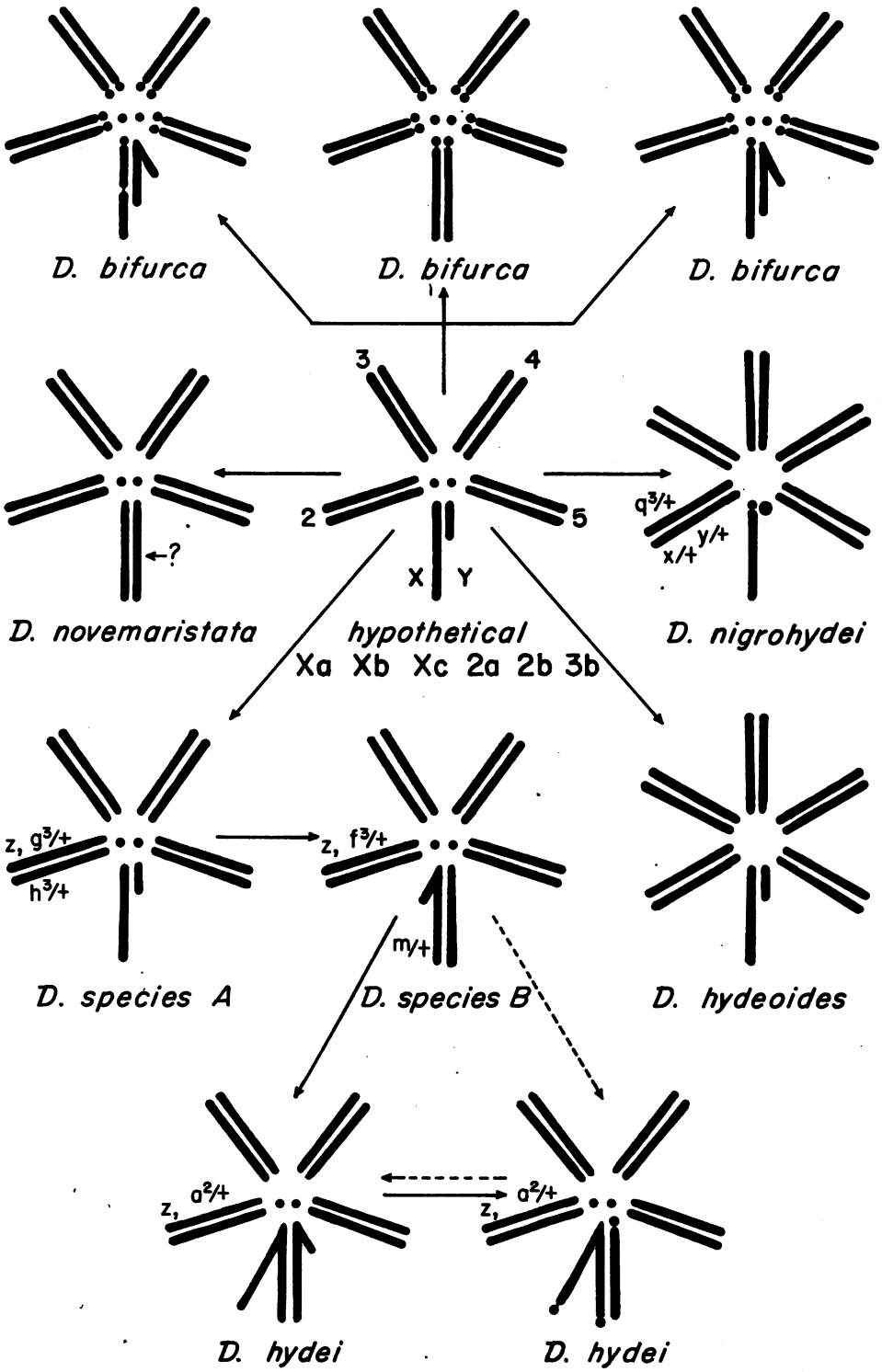

FIG. 3.—Metaphase karyotypes of seven species in the hydei subgroup. All inversions, except *Xm* found in *D. species B*, occur in the second chromosome. The 2*z* sequence is the only inversion fixed among the five species examined in this study (see text).

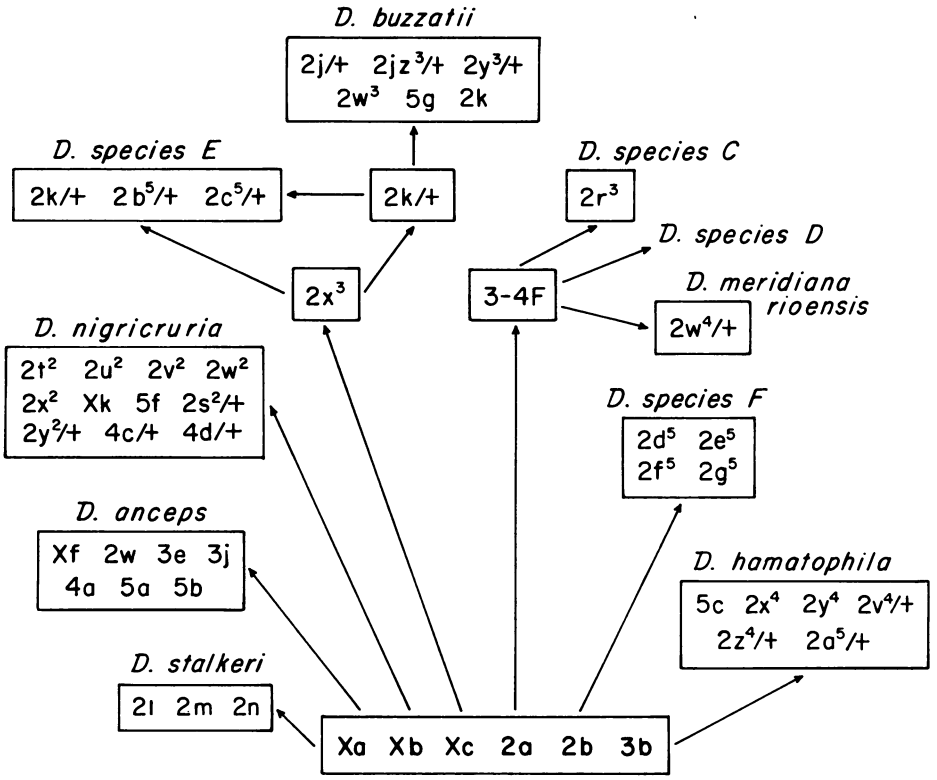

FIG. 4.—Cytological relationships among ten species in the mulleri subgroup.

chromosomes and the dots. These five species plus *D. hydeoides* (Wharton[10]) and *D. novemaristata* (Dobzhansky and Pavan[11]) show specificity in their metaphase karyotypes (Fig. 3). The metaphase evidence indicates a step-wise addition of heterochromatin from *D. species A* to *D. species B to hydei*, the three species which are homozygous for 2*z*. We assume that this indicates a phyletic line because although heterochromatic differences in karyotype are generally not reliable characters, in this situation they parallel and supplement the morphological and genetic information which shows that *hydei* and *species B* are very closely related specialized members of this subgroup. In view of the interspecific differences mentioned above it is worth noting that intraspecific variation in metaphase chromosomes have been found in *bifurca* (Wharton[12] and Ward[13]) and *hydei*.

Figure 4 shows the relationships among ten members of the mulleri subgroup. The *meridiana* complex with its three species, *D. meridiana*, *D. species C*, and *D. species D*, has as its basic sequence the primitive type and is indistinguishable from the standard found in *bifurca* and *nigrohydei* of the hydei subgroup. All of the *meridiana* forms differ from the primitive in having a fusion between the 3rd and 4th chromosomes, indicated 3–4*F* in Figure 4. The subspecies *D. meridiana meridiana* is reported to lack this fusion (Wharton[12]) and therefore to have the primitive chromosome type. Previously polymorphism in *meridiana* was unknown; seven strains (six localities) of *m. rioensis* (our data) and 21 strains (ten localities) of *m.*

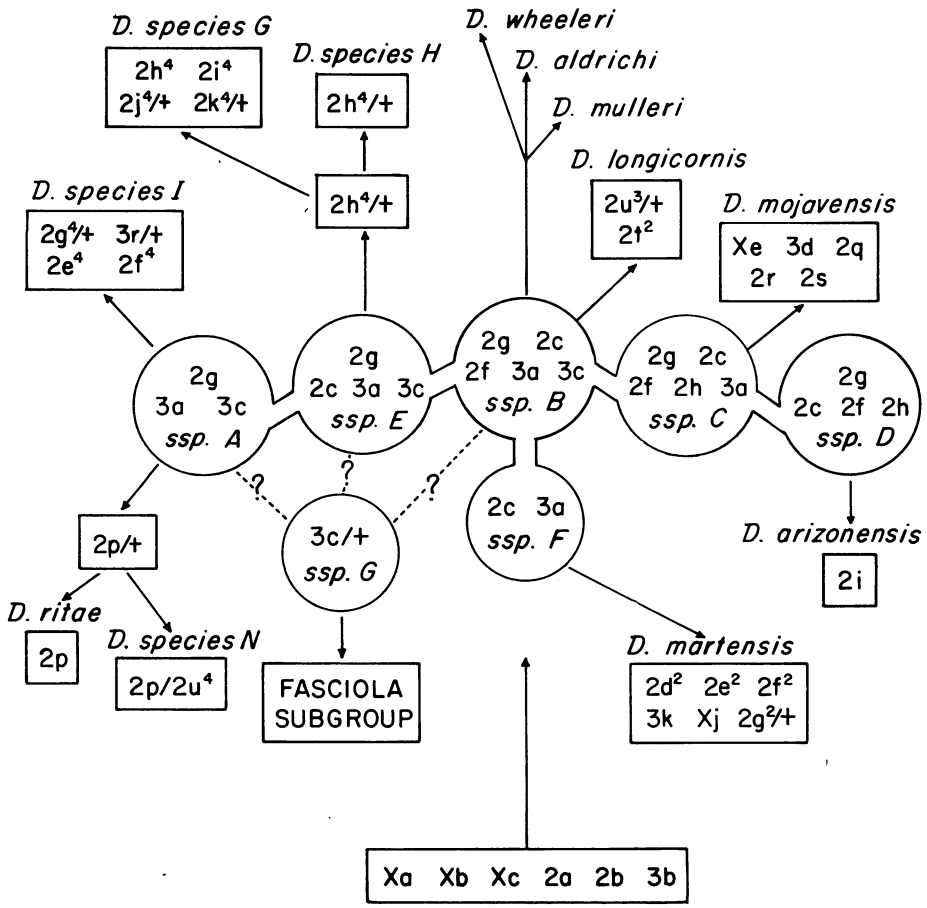

FIG. 5.—Phylogenetic relationships among 12 species in the *mulleri* complex and the ancestor of the fasciola subgroup, which are shown to have evolved from a large, cytologically polytypic species. The point of separation of the fasciola subgroup is unknown.

meridiana (Wharters[14]) were each homozygous for the same sequence. One locality was recently found to be polymorphic for $2w^4$.

A pair of morphologically very dissimilar species, *D. buzzatii* and *D. species E*, proved to be cytologically related in a very interesting manner (Fig. 4). The standard sequence of *D. species E* is the primitive plus $2x^3$. In our strain this standard is present along with a chromosome with $2k$, $2b^5$, and $2c^5$. In *buzzatii*, the $2x^3$ and $2k$ sequences have been fixed as have the inversions $2w^3$ and $5g$. *D. buzzatii* is polymorphic for additional inversions, one of which, $2j$, has been found in Lebanon, Australia, and South America.

Figure 5 gives the evolutionary picture of 12 species in the mulleri subgroup. These species have been combined into the *mulleri* complex because six inversions have segregated out among these species in such a way that a simple evolutionary history of divergence of populations is not possible. The same phenomenon is reported above in the *neorepleta-melanopalpa-canapalpa* trio of species. We will discuss this phenomenon at length below. One of the populations, *G*, gave rise to the fasciola subgroup whose phylogeny is detailed in Figure 6.

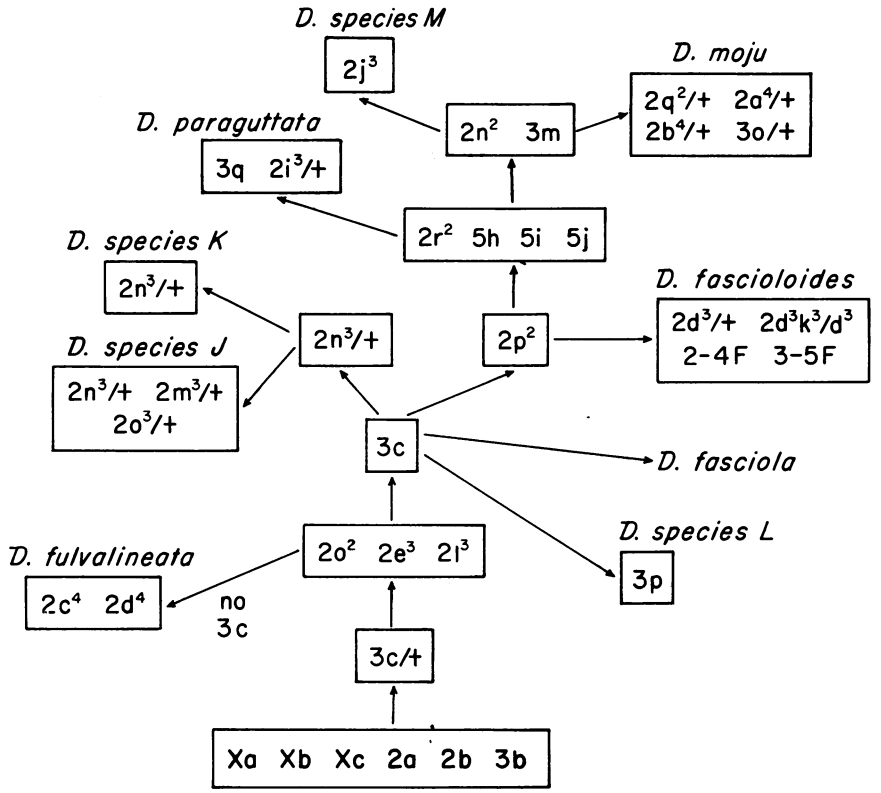

FIG. 6.—Cytological evolution within the fasciola subgroup.

The fasciola subgroup, nine species, arose as a branch from the mulleri complex with which it shares one inversion, 3*c*. The original population must have maintained heterozygosity for 3*c* during the period in which the $2o^2$, $2e^3$, and $2l^3$ arrangements were fixed. One species, *D. fulvalineata*, arose from a population which either lacked or lost the 3*c*. In the others the 3*c* became homozygous. Of interest are the two forms, *D. species J* and *D. species K* which seem to be good species although our limited sampling indicates that they may be allopatric. Both species have the same basic sequence and have maintained polymorphism for the same inversion, $2n^3$.

General Considerations.—The cytological data are direct information relating to the evolution of the repleta group. The over-all cytological picture demonstrates the changes that have taken place through time as the species evolved. Speciation has played a major role in this evolution, as the repleta group is the largest in the genus. It is desirable to attempt to determine why this is so and what role the inversions have played.

Patterson and Stone[1] discussed the cytological evolution of the genus *Drosophila*. More recently, Stone[15] and Stone *et al.*[16] reviewed the evolution of the virilis group, comparing it with the repleta group and many other species groups in the genus. The virilis group is the only other large species group where extensive phylogenetic relationships have been obtained. We might add a few details not available at that time. There have been 144 inversions involved in the evolution of the repleta

group as compared to 92 in the virilis group, Table 1. Both are underestimations even in the strains examined due to the difficulties in working with the *X* chromosome of both the virilis group, where Stone *et al.*[16] estimated there were at least another 20 homozygous inversions, and the fasciola subgroup in which about six homozygous unrecorded inversions occurred. The total number, therefore, should be approximately 150 inversions among 46 species in the repleta group compared to 112 among 9 species in the virilis group.

An examination of the specific cytological composition of the repleta species shows that with one exception (the *D. mulleri-D. aldrichi-D. wheeleri* trio of species) each of the species examined was cytologically unique either for gene arrangements, chromosome polymorphism or metaphase karyotype. Except for a few instances where it seemed applicable, the metaphase karyotype has been omitted from this paper. Only 21 of the 46 species were found to be variable in their gene sequences. Part of this is undoubtedly due to insufficient sampling of the species. Among the homozygous species we have sampled only one locality for 11 species: *D. neorepleta*,

TABLE 1

DISTRIBUTION AND CLASSIFICATION OF INVERSIONS FOUND IN THE VIRILIS AND REPLETA SPECIES GROUPS

Column	1	2 Homo-zygous	3 Homo-zygous	4 Hetero-zygous + Homo-zygous	5 Hetero-zygous	6 Hetero-zygous	7
	No. of Species	Inter-specific	Shared		Inter-specific	Intra-specific	Total
Melanopalpa subgroup	7	12	2	1	1	0	16
Mercatorum subgroup	2	6	0	1	1	8	16
D. peninsularis	1	1	0	0	0	0	1
Hydei subgroup	5	1	0	0	0	8	9
Mulleri subgroup	22	43	6†	3	0	20	72
Fasciola subgroup	9	15	1†	0	1	9	25
Inter-subgroup	..	5‡	0	0	0	0	5
Total	46	83 (6)*	8	5	3	45	144 (150)
Virilis group	9	35 (20)*	0	8§	1§	49	92 (112)

* Additional inversions estimated.
† Inversion 3c included within both mulleri and fasciola subgroups.
‡ Inversions *Xa*, *Xb*, *Xc*, 2*a*, 2*b*.
§ Inversion 4*h* included in both categories (see Stone *et al.*[16]).

D. limensis, *D. fulvalineata*, *D. anceps*, *D. stalkeri*, *D. mojavensis*, *D. wheeleri*, *D. species C*, *D. species D*, *D. species F* and *D. species L;* two localities for *D. ritae* and *D. species M;* three localities for *D. fasciola* and *D. fulvimaculoides;* four for *D. aldrichi;* five for *D. arizonensis;* seven for *D. mulleri;* eight for *D. repleta;* and 18 for *D. peninsularis*. Although many of these species will undoubtedly prove to be polymorphic, *peninsularis*, *repleta*, *mulleri* and *aldrichi*, which were sampled from practically their whole known ranges, are most probably truly monomorphic cytologically.

Among the species known to be polymorphic, there is an asymmetric distribution toward a minimum number of sequences present: 11 species have only one heterozygous inversion; six species have two; five species have three; three species have four; and one species has seven heterozygous inversions. Several of these species have been sampled quite extensively. Table 1 shows an average of about two unique arrangements fixed (col. 2) and one unique inversion heterozygous (col. 6) per repleta species. Columns 3 to 5 list inversions which cannot be easily

categorized in that they may be homozygous but shared by several species (col. 3), or heterozygous in one or more species and homozygous in others (col. 4), or heterozygous in more than one species (col. 5). These data, although only approximations, emphasize the fact that the cytological evolution involving paracentric inversions has been extremely conservative in relation to the rate of speciation in this group.

The average number of inversions per species in the virilis group is higher than that of the repleta group. In Table 1, this is exaggerated since one species, *D. montana* contributes a majority of the heterozygous inversions (29) included in column 6. According to Stone *et al.*[16] one of the major reasons for the inability to obtain comparable information for other species groups is the presence of a large number of homozygous interspecific differences. Intermediate forms have not been found and probably do not exist. Since many of the species in these groups are highly polymorphic (Stone[15]), much of the interspecific differences may be attributed to inversion fixation resulting from the replacement of old polymorphic systems by newer ones as the species evolves through time in a changing environment.

We cannot relate inversions with time. Old sequences have remained polymorphic in some forms long after they and newer sequences have become homozygous in other species. For example, the $2l^2$ sequence which must have been heterozygous in the ancestral population, is still heterozygous in *D. fulvimacula*. This sequence and also the newer $2a^3$ which overlaps it, have become homozygous in *D. fulvimaculoides*. Several examples have been enumerated where ancestral polymorphism has been maintained in more than one of the derived species. In view of this it appears highly significant that by the use of only the 144 detectable paracentric inversions only three species are indistinguishable among the 46 species studied. Paracentric inversions must have been important in the specialization of isolated populations and contributed toward species formation in the repleta group. Other cytological characters such as chromosome fusions and the addition and/or deletions of heterochromatin as seen in the metaphase chromosomes allow us to make more reliable specific identification. These undoubtedly were also important in speciation in the group. Only one pericentric inversion has been reported in this species group. Wharton found a strain of *bifurca* which was polymorphic for a pericentric inversion in the *X* chromosome (Ward[13]).

An examination of our data shows that the inversions are not randomly distributed throughout the euchromatin: the *X* has a minimum of nine inversions; chromosome 2 has 103 inversions; chromosome 3 has 18; chromosome 4 has 4; and chromosome 5 has 10. The overabundance of second chromosome inversions does not necessarily imply that this chromosome is more labile, although it might be, but rather that inversions in this chromosome probably have been more successful.

Discussion.—Stone *et al.*[17] and Wagner and Mitchell[18] discussed the various aspects of genic interaction and concluded that gene action operates through the control of metabolic pathways. Since these pathways are complex interlocking sequences, a gene although controlling only one enzyme, may affect many products. Several pathways may be present leading to the formation of a product with the result that many genes can influence a single character. Each allele has an effect in development. One might conclude that the individual allele (or larger segment of

the chromosome) has no definable adaptive value *per se*. Its fitness is determined by both the genetic and the external environment.

Populations of crossbreeding individuals with a past history of inbreeding would be expected to be homozygous for a majority of their genes, genetic variation being due to (1) a load of deleterious mutants, (2) adaptively neutral alleles, if any exist, (3) adaptive polymorphism in a heterogeneous environment, and (4) transient polymorphism where a superior allele is in the process of replacing an inferior allele. Dobzhansky[19] considered these populations as being of the classical type. In contrast he defined as balanced populations those where "the adaptive norm is an array of genotypes heterozygous for more or less numerous gene alleles, gene complexes, and chromosomal structures. Homozygotes . . . . . occur in normal outbred populations only in a minority of individuals, making these individuals more or less inferior to the norm of fitness." A past history of a large, genetically diverse population would result in the development of heterozygote superiority since selection has historically acted upon heterozygous alleles in a heterozygous background. These two concepts are not meant to be mutually exclusive. Outbred heterozygous populations will have certain classical characteristics. In inbred populations heterotic loci have been found. Even if these prove to be caused by closely linked nonallelic interaction rather than true overdominance (Mather[20]), these segments of the genotype are not acting in a true classical manner as defined by Dobzhansky.

Reproductive processes in sexual organisms shuffle the chromosomes, releasing variability for each generation. One of the sources of this variability is recombination within the chromosome. The operation of the heterozygous inversion in *Drosophila* is to prevent recombination of the alleles close to and within the inverted segment. Recent experiments by Spassky *et al.*,[21] Spiess,[22] and Dobzhansky *et al.*,[23] which are reviewed by Spiess,[24] indicate that recombination releases a large amount of genetic variability. Free recombination in species which are normally polymorphic for inversions produced the greatest amount of variability. Since there is no evidence that the total recombination frequency was altered, this increase was most probably due to the breakup of the coadaptive gene complexes which the heterozygous condition protects. Good chromosomes were used to start the experiments and a general reduction in viability was obtained. The conservative nature of the heterozygous *Drosophila* inversion (i.e., prevention of recombination among coadaptive loci) may account, in part, for the preservation of polymorphism since the offspring of inversion heterozygous females inherit unrecombined selected coadapted sequences, whereas many of the offspring of adaptive structurally homozygous but genetically heterozygous females are recombinants and therefore usually less fit.

North-south clines, altitudinal clines and seasonal frequency changes in inversions indicate that some arrangements may be adapted to general climatic conditions. Gene sequences may become more strictly specialized in relation to such factors as food, etc. (da Cunha[25]). This is not necessarily an absolute one sequence-one niche specialization in that there is still a considerable amount of genic variability within each coadapted sequence. The heterozygous population may be able to utilize several habitats (da Cunha *et al.*[26]). The stress should be placed on the environment limiting the genetic variability of the population, as done by Stone *et al.*[16]: "Regions with many varied ecological niches have these filled in time by

living systems, sometimes by several species and sometimes by one with great adaptive capacities which may depend upon genetic polymorphism, whereas regions with serious ecological restrictions must impose similar restrictions on the variability of the genotypes."

Investigations of intrapopulation inversion polymorphism by the use of population cages demonstrate that the heterozygous individuals are superior to the homozygotes (Dobzhansky[27]). In experiments where chromosomes from different localities are introduced into the cage, one inversion usually replaces the other. However, in some instances, a new heterosis originates within the population cage (Dobzhansky and Levene[28]). These experiments indicate that in a polymorphic population there will be selection for those alleles which are not only coadaptive but also work well with the alleles in the other sequence, resulting in heterosis. Chromosomes taken from a single population have already been coadapted. Those from different populations may or may not be able to develop a new heterosis before fixation.

Nonrandom fluctuations in selective forces such as seasonal variations which are longer than the generation time of the flies result in fluctuations in genic and inversional frequency. In large populations selective pressures may not be strong enough to eliminate a gene sequence before it becomes adaptive again (Dempster[29]). The process of fixation may be slow if the heterozygote approaches the homozygote in fitness and/or the conservative nature of the heterozygous inversion in preventing recombination is important. However an isolated population, if it is small and fluctuations are present (Crow[30]) or if the environment remains constant for a long period of time (Lewontin[31]), will become homozygous.

The importance of coadapted complexes in polymorphism is demonstrated in mimetic butterflies. In these organisms, Batesian mimicry is controlled by a series of very closely linked loci, between which recombination is relatively rare (Sheppard[32] and Clark and Sheppard[33]). The heterozygotes may be heterotic, but the origin, evolution, and maintenance of polymorphism is certainly due to the mimicry itself, and not to the heterosis (Fisher[34]).

The origin of coadapted complexes may occur sympatrically in one area. If several habitats are open to the organism, a system such as inversions would allow for a more efficient exploitation of the niches. Once a specialized genotype is developed in the heterogeneous area, it may be able to spread to adjacent homogeneous areas. The environment through selection may prevent the other genotypes from swamping these adaptive homozygotes. A good example is found in *D. mercatorum*. In this species polymorphism for primitive and advanced chromosomes occurs in the lowlands of Brazil and Bolivia. This species is homozygous for advanced sequences in the Andes and further north into the United States. It is the author's opinion that although gene flow may be limited some probably still exists, but migration through the Andes is limited to one type of second chromosome and two types of the third. The situation in *D. moju* appears to be identical to that of *D. mercatorum* but is not well-documented.

Semi-isolated populations occurring in a limited environment may be able to adapt to the particular habitat present. This allopatric specialization, if it is associated with a recombinant suppressor such as an inversion, would be better able to resist gene flow from other populations since the coadaptive genotype is not

readily destroyed through recombination. This allopatric inversional differentiation may have two results: genotypes developed in marginal areas might be able to reinvade the richer area and add to or produce polymorphism (Stone *et al.*[16]); or if the migrants in both directions are ill-adapted, speciation may result. A limited but constant immigration of ill-adapted genotypes which will not survive, but which will cause a drain on the reproductive potential of those flies with which they mate, may lead to the development of sexual isolation (Koopman[35]). In this way allotropic differences whether due to different polymorphic systems (Wallace[36]) or different homozygous inversions may lead to speciation.

Carson[37] has suggested that inversion polymorphism is maintained due to the homeostatic nature of the heterozygote. Lerner[38] demonstrated that the forced homozygosity for many loci through artificial breeding techniques used in agriculture and in the laboratory can cause a breakdown in vigor, fecundity, etc. Apparently, a certain minimum amount of heterozygosity is necessary. The maintenance of heterozygosity for inversions in *Drosophila* laboratory stocks which seems to be a common occurrence in many different species, may be the method by which heterozygosity for a number of loci is preserved while many other loci not as easily protected become homozygous through drift. Of particular interest are the experiments of Robertson[39] where he demonstrated that the addition of a new chromosome, apparently irrespective of the chromosome, into a highly inbred line may often eliminate much of the inbreeding effects. However, we assume that a wild population usually has sufficient genetic variability independent of the inversion system to allow a superior homozygote to replace a polymorphic system without inbreeding degeneration.

A very important factor is the effect of the gene sequence upon the rest of the genotype and vice versa. The fact that the incorporation (or exclusion) of a gene sequence into a population is determined by the genotype already in existence is obvious. Nor does this interaction have to be between overlapping inversions. Patterson and Stone,[1] in their section on the virilis group, presented a great deal of information to show that each species and often each strain is a balanced, integrated gene pool. The presence of some interspecific fertility allowed them to demonstrate genetic integration at all levels. Allelic, intrachromosomal, interchromosomal, and cytoplasmic-chromosomal interactions were all found within the group. Very little is known about chromosomal interaction in the repleta group. Of great interest are the population cage experiments of Mettler[40] on *mojavensis* and *arizonensis*. He demonstrated that two opposing forces were operating in the cages, heterosis which tended to maintain both types of chromosomes, and an interaction between the *X* and third chromosomes where individuals homozygous for one chromosome of one species and also homozygous for the other chromosome of the other species were very infrequent. The occurrence of heterosis in the hybrid is remarkable, considering that these are two homozygous species so far as we know.

Speciation in the Repleta Group.—The primary mechanism of speciation is geographical isolation. Given complete isolation, populations will diverge in time. Other factors such as differences and/or changes in the environment and population size will affect the rate of divergence. Given incomplete isolation, these factors coupled with the rate of migration will determine whether or not semi-isolated

populations can diverge to the extent that new species are formed. Although it is almost universally true that each *Drosophila* species is unique cytologically, there are several exceptions: *D. mulleri*, *D. aldrichi*, and *D. wheeleri* are cytologically identical. The following four species pairs, *mercatorum* and *paranaensis; melanopalpa* and *canapalpa;* and *species J* and *species K* in the repleta group, and *D. montana* and *D. lacicola* in the virilis group are polymorphic for the same inversions but have other unique cytological characteristics.

Cytological differentiation resulting in homozygous differences between species might have had its origin in several different ways. Identical polymorphism in different isolated populations or species may be replaced by new polymorphism of overlapping inversions resulting in homozygous differences:

	Population I	Population II
Time *a*	A//standard	A//standard
Time *b*	A//AB	A//AD
Time *c*	AB//ABC	AD//ADE

Populations I and II if examined at time *c* differ by two homozygous inversions, yet neither population was ever homozygous. There is, of course, no way to prove that this has ever happened, but phyletic lines in the repleta group often show such a sequence of overlapping inversions.

Random processes and different selective forces can cause fixation or polymorphism of different genotypes in different localities. In a semi-isolated population the inversion, having arisen only once, may become adapted to the particular local habitat and may not be able to spread into adjacent areas in which it is ill-adapted. Under proper conditions, allopatric cytological differentiation may lead to speciation. In the repleta group there is evidence that polytypic ancestral species have given rise to *neorepleta*, *melanopalpa*, and *canapalpa*, and also to the *mulleri* complex. The evidence on the origin of the *mulleri* complex follows.

Twelve species have been placed in the *mulleri* complex because they share a common inversion pool. The evolution of these forms, as determined by their inversions, is not one of simple geographical isolation and divergence; rather there has been a segregation of six basic inversions, 2*c*, 2*f*, 2*g*, 2*h*, 3*a*, and 3*c* (Table 2). Five of these are independent inversions whereas the sixth, 2*h*, includes 2*c* and over-

TABLE 2

DISTRIBUTION OF THE SIX INVERSIONS SEGREGATED AMONG THE 12 SPECIES IN THE MULLERI COMPLEX AND THE ANCESTOR OF THE FASCIOLA SUBGROUP: + INDICATING PRESENCE; − INDICATING ABSENCE

	Inversions					
Species	2*g*	2*c*	2*f*	2*h*	3*a*	3*c*
D. species N	+	−	−	−	+	+
D. ritae	+	−	−	−	+	+
D. species I	+	−	−	−	+	+
D. species G	+	+	−	−	+	+
D. species H	+	+	−	−	+	+
D. mulleri	+	+	+	−	+	+
D. aldrichi	+	+	+	−	+	+
D. wheeleri	+	+	+	−	+	+
D. longicornis	+	+	+	−	+	+
D. mojavensis	+	+	+	+	+	−
D. arizonensis	+	+	+	+	−	−
D. martensis	−	+	−	−	+	−
Fasciola subgroup	−	−	−	−	−	+/−

laps 2*f*, the latter always being present whenever 2*h* occurs. Each of these six inversions are fixed homozygous in those species where they are present. Nine of the twelve species have other unique gene sequences; three, *mulleri*, *aldrichi*, and *wheeleri*, are homozygous for identical sequences.

The most probable explanation for this type of distribution of characters is the occurrence of an ancestral species composed of geographically semi-isolated populations differing in their inversion content as shown diagrammatically in Figure 5. These populations diverged in time and formed full species. The geographical areas within the general distribution of the ancestral species differed as to environmental factors and therefore selective pressure, resulting in a variety of localized adaptive peaks. In this situation the population structure and dynamics is of utmost importance. This species must have been a desert-inhabiting form except for one forest population (all of the present-day derivatives are desert species except the fasciola subgroup) whose population structure was not one large continuous gene pool but rather a mosaic of many small semi-isolated populations. Furthermore, these populations would not have had the same mutations in the same sequence to choose between. There must have been gene exchange between neighboring populations but geographical isolation and selective forces were effective in allowing populations to adapt to local conditions. At least part of this adaptation was through the development of inversional differences, which, once established, allowed the population to better cope with ill-adapted migrants. The result would be a number of major populations, or subspecies (Fig. 5) each composed of a number of small populations. A locally adaptive inversion would be incorporated at the point of origin, and spread to neighboring subspecies if the habitat were suitable. If the neighboring subspecies rejected the inversion because the habitat was not available or the foreign sequence could not integrate with the gene pool of the population, the migration of the inversion would be stopped near the border of the two subspecies with the result that more distant populations would never get a chance to test this sequence. As local adaptation through cytological differentiation became perfected, the subspecies became more and more genetically isolated and speciation resulted. Further speciation of the seven species into the present-day 12 species plus the fasciola subgroup could have been of the more usual type of isolation and divergence of characters including the incorporation of unique inversions (Fig. 5). The six inversions are each present homozygous in those species in which they occur. It is possible that the populations were polymorphic for these inversions and homozygosity was the result of newer balanced polymorphism by overlapping inversions replacing the older system (as discussed above). Also, the presence of a pre-existing polymorphism with an inversion whose breakage points overlap the sequence in question may have prevented this sequence from entering the population. These factors can explain part but not all of the inversion picture, there being no cytological reason for most of the species-inversion relationship (see Wasserman[5] for details). For example, no other inversions are known in the *mulleri-aldrichi-wheeleri* trio of species which are homozygous for 2*g*, 2*c*, 2*f*, 3*a*, and 3*c*. Chromosomal interaction of the type described in the virilis group and between *mojavensis* and *arizonensis* probably was important, but our data give no information concerning this. We cannot eliminate the possibility that these inversions have been fixed by fluctuations in population

size. These species, as do many other Drosophilae, show marked fluctuations in population number (Patterson[41]). However, I prefer to explain the presence or absence of inversions on the basis of fitness rather than drift.

Speciation in this complex did not necessarily take place at the periphery of the ancestral population. It seems unlikely that the homozygosity of the six inversions is a result of these species having arisen from an unknown or extinct polymorphic species by either having been small peripheral populations or chance migrants starting new populations since the *mulleri-aldrichi-wheeleri* homozygous ancestor (subspecies B) is presumed to have been centrally located (Fig. 5). Nor is there any evidence of radiation of species out from the center of the population. The picture of speciation presented here in the *mulleri* complex is the simplest and most probable explanation for the unusual sharing of characters among closely related species where convergent evolution must be ruled out. We were fortunate to have six characters segregating into seven types among 12 species and another subgroup. This type of speciation if it resulted in fewer species and involved fewer characters would not be recognized as having occurred.

In conclusion, the *mulleri* complex shows every indication of having arisen from a species occupying a fairly large diverse area. The most important characteristic of this species was that it was composed of many semi-isolated populations being similar to the ideal species of Wright. Geographical isolation coupled with the origin of polytypic inversional differences allowed populations to adapt to local conditions. As coadaptive complexes were being perfected the effect of migration was lessened, inversional differences having contributed to the development of isolation. Individual populations may have been small or affected by seasonal fluctuations in numbers of individuals.

Summary.—The repleta group is the largest species group in the genus *Drosophila*. Cytotaxonomic studies have demonstrated phylogenetic relationships among 46 species within the group. The vast majority of the species are cytologically unique, there being only three species which cannot be distinguished cytologically. However, there has been a minimum amount of inversional evolution (150 inversions) considering the number of species involved.

Our information concerns inversions in the repleta group and therefore their importance has been stressed. It is proposed that the primary selective advantage of an inversion is to allow for the development of coadapted gene complexes which are specialized for the exploitation of part of the available environment. The distribution of the inversions both geographically and among the various species is interpreted as being the result of selective forces rather than random processes (except for the original occurrence of the unique event), because an inversion consists of a large block of coadapted genes whose fitness is considered basic to the population.

The possible role of allopatric cytological differentiation in speciation is discussed. It should be emphasized that most of the interspecific differences are expected to be among the loci which are independent from gene rearrangements, especially in the repleta group where so few inversions have survived. Random processes probably have been very important in fixing allelic differences in the various populations and have contributed to the rapid rate of speciation in this group. Also, I have tended to equate structural homozygosity with ecological

specialization and cytological polymorphism with ecological plasticity. This seems to be generally true, although there are exceptions such as the cytologically monomorphic species, *D. virilis* and *D. repleta*, which are world-wide general scavengers and are genetically heterogeneous, having localized differentiated populations (Patterson and Stone[1]), and also the ecologically limited laboratory strains which have maintained their cytological polymorphism in spite of, or possibly due to the fact that they are subjected to drift.

Speciation in the *mulleri* complex is discussed. Cytological evolution in this complex is unusual in some ways but this has resulted in a better understanding of the methods by which speciation has occurred. Geographical isolation and population structure are of great importance, the evidence indicating that the ancestral species of this complex was composed of many small semi-isolated populations similar to that proposed by Wright as being ideal for evolution. Allopatric cytological differentiation was an adaptation to local conditions and also aided in reducing the effect of ill-adapted migrants. The result was an acceleration of differentiation and speciation.

I am indebted to Dr. Wilson S. Stone for suggesting the problem and for his continuous interest and encouragement. Many thanks are due to Dr. Marshall R. Wheeler for his help in taxonomic problems and to Drs. William B. Heed and Hampton L. Carson, who, together with Dr. Marshall R. Wheeler, collected most of the material. This manuscript was read by Drs. Wilson S. Stone, Harrison Stalker, and Lynn Throckmorton, whose suggestions helped clarify certain points but who should not be held responsible for any mistakes in fact or interpretation as presented here.

* This work was supported by grants from the Rockefeller Foundation, the U.S. Public Health Service, the National Science Foundation, and by a contract with the Atomic Energy Commission.

[1] Patterson, J. T., and W. S. Stone, *Evolution in the Genus Drosophila* (New York: The Macmillan Co., 1952).

[2] Sturtevant, A. H., and Th. Dobzhansky, these PROCEEDINGS, **22**, 448 (1936).

[3] Wasserman, Marvin, Univ. Texas Pub., No. 5422, 130 (1954).

[4] Wasserman, Marvin, and Florence D. Wilson, Univ. Texas Pub., No. 5721, 132 (1957).

[5] Wasserman, Marvin, to be published in Univ. Texas Pub. (1960).

[6] Wharton, Linda T., Univ. Texas Pub., No. 4228, 23 (1942).

[7] Patterson, J. T., and Marshall R. Wheeler, Univ. Texas Pub., No. 4920, 207 (1949).

[8] Wheeler, Marshall R., Univ. Texas Pub., No. 4920, 157 (1949).

[9] Ward, Calvin L., and Wilson S. Stone, Univ. Texas Pub., No. 5204, 119 (1952).

[10] Wharton, Linda T., Univ. Texas Pub., No. 4445, 175 (1944).

[11] Dobzhansky, Th., and C. Pavan, *Bol. Fac. Fil. Cien. Letras, S. Paulo*, No. 36, 7 (1943).

[12] Wharton, Linda T., Univ. Texas Pub., No. 4313, 282 (1943).

[13] Ward, Calvin L., Univ. Texas Pub., No. 4920, 70 (1949).

[14] Warters, Mary, Univ. Texas Pub., No. 4445, 129 (1944).

[15] Stone, Wilson S., *Cold Spring Harbor Symposia on Quant. Biol.*, **20**, 256 (1955).

[16] Stone, Wilson S., William C. Guest, and Florence D. Wilson, these PROCEEDINGS, **46**, 350 (1960).

[17] Stone, Wilson S., and Mary L. Alexander, and Frances E. Clayton, Univ. Texas Pub., No. 5422, 272 (1954).

[18] Wagner, Robert P., and Herschel K. Mitchell, *Genetics and Metabolism* (New York: John Wiley and Sons, 1955).

[19] Dobzhansky, Th., *Cold Spring Harbor Symposia on Quant. Biol.*, **20**, 1 (1955).

[20] Mather, Kenneth, *Cold Spring Harbor Symposia on Quant. Biol.*, **20**, 158 (1955).

[21] Spassky, B., N. Spassky, H. Levene, and Th. Dobzhansky, *Genetics*, **43**, 844 (1958).
[22] Spiess, Eliot B., *Genetics*, **44**, 43 (1959).
[23] Dobzhansky, Th., H. Levene, B. Spassky, and N. Spassky, *Genetics*, **44**, 75 (1959).
[24] Spiess, Eliot B., *Cold Spring Harbor Symposia on Quant. Biol.*, **23**, 239 (1958).
[25] Da Cunha, A. Brito, *Evolution*, **5**, 395 (1951).
[26] Da Cunha, A. Brito, Th. Dobzhansky, O. Pavlovsky, and B. Spassky, *Evolution*, **8**, 389 (1959).
[27] Dobzhansky, Th., *Genetics*, **33**, 588 (1948).
[28] Dobzhansky, Th. and H. Levene, *Amer. Nat.*, **85**, 247 (1951).
[29] Dempster, Everett R., *Cold Spring Harbor Symposia on Quant. Biol.*, **20**, 25 (1955).
[30] Crow, James F., *Cold Spring Harbor Symposia on Quant. Biol.*, **20**, 54 (1955).
[31] Lewontin, R. C., *Evolution*, **7**, 494 (1958).
[32] Sheppard, P. M., *Cold Spring Harbor Symposia on Quant. Biol.*, **24**, 131 (1959).
[33] Clarke, C. A., and P. M. Sheppard, *Genetics*, **44**, 1347 (1959).
[34] Fisher, R. A., *Genetical Theory of Natural Selection* (New York: Dover Publ. 1958).
[35] Koopman, Karl F., *Evolution*, **4**, 135 (1949).
[36] Wallace, Bruce, *Cold Spring Harbor Symposia on Quant. Biol.*, **24**, 193 (1959).
[37] Carson, Hampton L., *Cold Spring Harbor Symposia on Quant. Biol.*, **24**, 87 (1959).
[38] Lerner, I. Michael, *Genetic Homeostasis* (New York: John Wiley and Sons, 1954).
[39] Robertson, Forbes W., *J. Gen.* **52**, 494 (1954).
[40] Mettler, Lawrence E., Univ. Texas Pub., No. 5721, 157 (1957).
[41] Patterson, J. T., Univ. Texas Pub., No. 4313, 7 (1943).

---

*A QUANTITATIVE FORMULATION OF SYLVESTER'S LAW OF INERTIA, II**

BY A. M. OSTROWSKI

UNIVERSITY OF BASEL, SWITZERLAND

Communicated by Joseph O. Hirschfelder, March 31, 1960

13. In the first part of this paper, which appeared in May 1959,† we generalized Sylvester's law of inertia in the following way. Let H be an Hermitian matrix of order n with eigenvalues λ_ν ordered increasingly and, for a square matrix S,

$$K = S^*HS, \tag{21}$$

the "transformed" Hermitian matrix with eigenvalues Λ_ν ordered increasingly. Then, if p_1 and p_n denote the smallest and greatest eigenvalues of the nonnegative Hermitian matrix S^*S, we have

$$\Lambda_\nu = \theta_\nu\lambda_\nu, \qquad p_1 \leq \theta_\nu \leq p_n(\nu = 1, \ldots, n), \tag{22}$$

and obviously equation (22) remains true if λ_ν and Λ_ν are ordered *decreasingly*.

14. In what follows, we denote for an arbitrary Hermitian matrix A by π_A and ν_A, respectively, the numbers of positive and negative eigenvalues of A. Then we have in particular under the above conditions the relations

$$\pi_K \leq \pi_H, \qquad \nu_K \leq \nu_H, \tag{23}$$

generalizing Sylvester's law of inertia to the case of *singular transformations*.

15. In Part I of this paper, S was explicitly assumed as real. However, all statements and proofs given in Part I remain valid without change in the case of a nonreal square matrix S.