CHROMOSOMAL POLYMORPHISM IN TWO SPECIES OF MYCODROSOPHILA¹

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DIFFERENCES in gene order can be demonstrated in the Diptera by the accompanying variation in polytene chromosome banding patterns. Polymorphism of the salivary gland chromosomes in *Drosophila* has been carefully exploited to measure intra- and interspecific structural variability and subsequently to define evolutionary relationships by the analysis of overlapping paracentric inversions, a method developed by STURTEVANT and DOBZHANSKY (1936; DOBZHANSKY and STURTEVANT 1938). The following species groups in *Drosophila* have been studied in this manner: *D. obscura* (DOBZHANSKY and EPLING 1944), *D. virilis* (STONE *et al.* 1960; STONE 1962), *D. repleta* (WASSERMAN 1960, 1962, 1963), *D. melanica* (SALKER 1960, 1961, 1964a, 1964b, 1965, 1966), *D. tripunctata* (KASTRITSIS 1966), and four subgroups of picture winged Hawaiian *Drosophila* (CARLSON and STALKER 1968). In addition, four distinct types of the closely related genus, *Samoaia*, have been examined (ELLISON 1968).

The genus *Mycodrosophila* is believed to be derived from the genus *Drosophila* at the level of the subgenus *Drosophila* (THROCKMORTON 1962, 1966), of which all but *D. obscura* of the above named *Drosophila* species groups are members. As presently understood the genus includes about 40 described species, mainly tropical, of world-wide distribution. The investigation to be reported was undertaken to determine whether the pattern of chromosomal evolution in two Nearctic representatives, described as *Mycodrosophila stalker*i and *M. claytonae* by WHEELER and TAKADA (1963) is similar to that found in *Drosophila*. *M. stalker*i has been found in Ontario, Ohio, Florida, and the south-central United States. *M. claytonae* has a wider distribution, from southeastern Canada and eastern United States west to the eastern ranges of the Rocky Mountains. The two species do not hybridize in the laboratory. Their salivary gland chromosomes have been mapped and homologized where possible to permit the analysis of their structural differences.

MATERIALS AND METHODS

Flies were collected in the wild and raised at $25 \pm 1^\circ\text{C}$ on corn meal-agar-corn syrup medium supplemented with live yeast. In *M. claytonae* the length of one generation in the laboratory is 24 days, in *M. stalker*i it is 22 days. Females of both species rarely oviposit before mating. Salivary gland chromosome squashes were prepared from late third instar larvae which had been

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reared in shell vials on standard medium overlaid with thick yeast paste. The glands were fixed briefly in 60% acetic acid, stained in lactic-acetic-orcein and squashed on siliconized glass slides.

The Navasota, Texas strain in each species was found to be structurally homozygous in all major chromosome arms and was designated, therefore, as the standard for chromosome banding sequence. Salivary gland chromosome squashes from homozygous standard larvae were photographed, and prints most representative for a particular chromosome region were selected for the chromosome map composite which was then rephotographed with the contrast held down to avoid losing valuable detail. The chromosomes of the two species were homologized by a photographic comparison method developed by STALKER (1965).

Chromosomal polymorphism within each species was analyzed by mating approximately 10 males per strain, individually, to two or three homozygous standard, virgin females. Analysis of up to seven salivary gland chromosome smears was considered adequate to determine the structural constitution of the male parent autosomes. A single female larva was sufficient to indicate the sequence in the paternal *X* chromosome. The standard gene arrangement was designated as + in each species. Heterozygous inversions found in the same chromosome arm of each species and the proposed rearrangements intermediate to them were given letters from a single set of symbols; the intraspecific inversions were assigned capital letters and the interspecific inversions lower case letters.

RESULTS

The metaphase chromosomes from brain ganglion cells in both *M. stalker*i and *M. claytonae* consist of two pairs of V's, a pair of rods, and a pair of dots in females. The arms of the V-shaped *X* chromosome are similar in length to those of the autosomes and approximately twice the length of those of the heterochromatic V-shaped *Y* chromosome. Accordingly, the five arms of the salivary gland chromosomes are designated as XL and XR, 2, and 3L and 3R. The dot chromosome was not easily identified and is not included in this study. The metaphase configuration is consistent with derivation from the ancestral *Drosophila* arrangement of five pairs of rods and a pair of dots. Any cytologically observable variation from the primitive configuration must arise through a fusion, translocation, or pericentric inversion. The reversion of any of these events is extremely unlikely since a free centromere would be required for a fused, metacentric (V) chromosome to reopen into two rods and the chance for breaks occurring that would return a translocated or inverted chromosome to the ancestral condition is very low. To arrive at the condition in *M. stalker*i and *M. claytonae* requires the fusion of two autosomal rods to give the autosomal V and an *X*-autosomal fusion for the V-shaped *X* chromosome. The homologous autosome apparently did not fuse with the *Y* chromosome since both arms of the *X* chromosome in males are unpaired in salivary gland preparations. There is no evidence of an unpaired rod in metaphase configurations and presumably this unfused autosome was lost. Loss of an autosomal rod in this manner has been recorded in several *Drosophila* species (WHARTON 1943; WASSERMAN 1963). It is possible that a pericentric inversion arose in the originally rod-shaped *Y* chromosome to give the small V which had no difficulty in surviving since crossing over does not normally take place in males of higher Diptera. A J-shaped *Y* chromosome has also been reported in *M. stalker*i (WHEELER and TAKADA 1963).

Chromosomal polymorphism: Chromosome 3 in *M. stalker*i has one simple inversion in the left arm and two nonoverlapping inversions in the right arm

TABLE 1

Chromosomal polymorphism in M. stalker

Source	Strain No.	Chromosome*	
		3L	3R
Navasota, Texas	S1	+	+
	S5	+	+
	S6	+	+
Fayetteville, Arkansas	S2	+	A
	S3	+, A	A
	S4	+	+, A
Lake County, Florida	S7	+, A	+, B
Farmington, Missouri	wild male	+, A	A

* Chromosomes X and 2 are monomorphic for the standard gene arrangement in all strains.

(Table 1) in addition to the standard arrangement. In *M. claytonae* all chromosome arms except XR showed inversion heterozygosity for a total of 11 arrangements plus standard (Table 2). The XL arm has one simple inversion and chromosome 2 has three overlapping and included inversions. Chromosome 3 has five overlapping and included inversions in the left arm and two inversions, one included within the limits of the other, in the right arm. In the left arm, the complex arrangement E(FG)I appears to be composed of four inversions of which inversion E was completely identified, inversion I was identified as to proximal limit only, and the remaining two inversions (F and G) could not be identified as to end points. Possibly the complex may be an inverted EFG chromosome because of the nature of some nonrandom chromosome relationships which will be presented later.

TABLE 2

Chromosomal polymorphism in M. claytonae

Source	Strain No.	Chromosomes*			
		XL	2	3L	3R
Navasota, Texas	C1	+	+	+	+
Fayetteville, Arkansas	C2	+	+, D	E, EF, EFG	E, EF
	C4	+	+	EF, E(FG)I, EH	+, E, EF
Farmington, Missouri	C5	+	+, CD	E, EF, E(FG)I, EH	+, E, EF
Jefferson Co., Missouri	C6	+, A	+, CD, DE	EF, E(FG)I, EH	+, E
Lake Co., Florida	C7	A	+, D	+, E, EH	+

* The right arm of the X chromosome was monomorphic for standard gene sequence in all strains.

Chromosomal homologies: Interspecific chromosomal comparisons based on the photographic maps and additional photographic records allow the establishment of homology for a short distal segment of XR, more extensive homology for XL, and nearly complete homology for the three autosomal arms (Figures 1 and 2). Blocks of unbroken gene sequences common to the two species are found in transposed and/or inverted order in the chromosomes of one species relative to their homologs in the other species. In chromosome 2, for example (Figure 1), region 1-2 at the distal end of *M. stalker*i is found in the same order near the centromere in *M. claytonae*. On the other hand, region 7-8 near the centromere in *M. stalker*i lies near the distal end and in inverse order in *M. claytonae*. Small sections containing a few bands could not be satisfactorily homologized. Inability to identify such areas could be due to adjacent inversion breaks producing a section too short to recognize, or to mutation induced change of the banding pattern (LEVINE and VAN VALEN 1962). The reverse situation, where transposition or transformation causes mistaken homology is unlikely for segments of at least ten bands, the length of the shortest homologized section in the study.

a.) *Chromosome 2:* The standard maps for chromosome 2 in Figure 1 show five numbered sections which differ in linear order between the two species. The *M. stalker*i standard arrangement (+) can be derived from that of *M. claytonae* by a minimum of two inversions identified in *M. claytonae* (C,D) and two additional postulated inversions (a,b). Inversion D (regions 9-10, 3-4) in the *M. claytonae* standard (+) chromosome results in the D chromosome arrangement, 4-3, 10-7, 5-6, 1-2, 11-12. Inversion C (regions 10-9, 8-7, 5-6, 1-2) in this chromosome gives the CD chromosome arrangement, 4-1, 6-5, 7-12. Inversions a (regions 4-3, 2-1) and b (region 6-5) complete the transition to the *M. stalker*i standard sequence, 1-12. Since it is not clear whether the interspecific (fixed) inversions a and b overlap, their order cannot be determined. Inversion D in *M. claytonae* represents a branching point in the phylogeny which is outlined in Figure 3.

b.) *Chromosome arm 3L:* At least four inversions are required to transpose the *M. claytonae* standard sequence to that of *M. stalker*i (Figure 2). Inversion E (regions 7-8, 2-1) in *M. claytonae* 3L-+ (standard) produces the E chromosome arm: 1-2, 8-7, 3-4, 10-9, 6-5, 11-12. Inversion d (regions 10-9, 6-5) followed by inversions c (regions 3-4, 5-6) and b (regions 8-7, 6-5, 4-3) results in the *M. stalker*i standard sequence, 1-12. Inversions d and E do not overlap and thus they may have arisen in reverse order from that presented here; however, no d rearrangements of *M. claytonae* 3L-+ were observed in this study whereas 3L-E has been identified in *M. claytonae* samples from Arkansas, Florida, and Farmington, Missouri (Table 2). Thus the sequence d-E-*M. claytonae* + appears most reasonable. The order of interspecific (fixed) inversions b and c is unknown

FIGURE 1.—Homologies between *M. stalker*i (S) and *M. claytonae* (C) in the X and second chromosomes. The numbered sections designate homologous chromosome regions. Inversion break-points are indicated by arrows; capital letters designate intraspecific inversions, lower case letters the interspecific inversions. The centromere is at the right.

FIGURE 2.—Homologies between *M. stalker* and *M. claytonae* in the left and right arms of the third chromosome. See Figure 1 for explanation.

FIGURE 3.—Intra- and interspecific phylogeny of inversion arrangements. *a.* Chromosome 2. *b.* Left arm of chromosome 3. *c.* Right arm of chromosome 3. *d.* X chromosome. The two-headed arrows indicate that the direction of evolution is not known. The length of the arrow is for diagrammatic purposes only and bears no relation to length of time between origin of each arrangement. The position of arrangement E(FG)I is speculative.

since they do not overlap. The only *M. stalker* inversion identified in this arm, A, extends the phylogeny beyond standard in that species. In *M. claytonae* the E arrangement represents a branching point in the phylogenetic sequence for four additional inversions. Inversion H overlaps and always occurs with E. Inversion F included within E (their distal limits appear to coincide) always occurs with E but never with H which it overlaps. Inversion G always occurs with EF which it overlaps, giving the EFG complex. Inversion I is always found in a complex with E and two unidentified inversions, possibly F and G. The evolutionary sequence is shown in Figure 3.

c.) Chromosome arm 3R: The minimum number of inversion steps separating the standard arrangements in each species requires three overlapping inversions (Figure 2). Interspecific inversion d (regions 10-9, 3-4, 6-5) in *M. claytonae* 3R-+ (standard) followed by a second postulated inversion, c (regions 8-7, 5-6, 4-3), results in the B chromosome arm in *M. stalker*, 1-4, 6-5, 7-12. Inversion of region 6-5, the B inversion in *M. stalker*, concludes the transition to *M. stalker*

standard arrangement, 1-12. More distantly related sequences include A in *M. stalker*i and E and EF in *M. claytonae*. Inversion F is included within the limits of E so that an evolutionary sequence, *M. claytonae* +—F—EF is possible with the E arrangement subsequently arising from a double exchange within the limits of E and external to those of F. However, no F arrangements have been identified while the E and EF chromosomes are present in the Arkansas and Missouri samples (Table 2); therefore the sequence +—E—EF is likely. (A similar argument applies to the A—+—B sequence in *M. stalker*i). The complete phylogenetic sequence is given in Figure 3.

d.) *X chromosome*: In the left arm of the *X* chromosome at least three fixed (interspecific) inversions are required to transpose regions 3-4 and 5-6 in *M. stalker*i to the position they occupy in *M. claytonae* (Figure 1). Since the chromosome could only be homologized in part, this suggests that considerably more than three inversions were involved. Clearly *M. claytonae* + is more closely related to *M. stalker*i + than *M. claytonae* A. The evolutionary sequence is outlined in Figure 3. Little can be said concerning homology in XR.

Nonrandom associations: Three types of nonrandom association between left and right arm arrangements in chromosome 3 were observed during the analysis for structural polymorphism in *M. claytonae*. The same pattern of nonrandomness exists in each of four strains which are polymorphic in both arms (Table 3). Inversion 3L-EH associates with 3R+, 3L-E either alone or in the inversion, complex 3L-EF associates with 3R-E, and either 3L-EFG or 3L-E(FG)I is found with 3R-EF.

On the basis of the third association the 3L-E(EF)I complex appears to be an inversion of the EFG arrangement. The only exception to these pairings is the

TABLE 3

Nonrandom associations of inversions in chromosome 3 of M. claytonae

Source	Progenitors	Age of strain when sampled	Association 3L · 3R	Observed frequency	P
Arkansas C2	1 wild ♀	12 months	E, EF · E EFG · EF	14 8	<.005
Arkansas C4	1 wild ♀	24 months	EH · + EF · E E(FG)I · EF	4 1 3	
Farmington, Missouri	1 virgin pair	9 months	EH · + E, EF · E E(FG)I · EF	1 10 11	<.005
Jefferson Co., Missouri	1 wild pair	10 months	EH · + EF · E E(FG)I · +*	6 13 1	
					<.005

* Crossover type

single E(FG)I · + chromosome scored in the Jefferson County, Missouri strain which indicates crossing over has occurred in the structurally homozygous section separating the two inversion complexes. This interval is shorter than those separating the proximal limits of rearrangement in the other two associations and it is reasonable to assume that recombination is also possible in these longer sections of uninverted chromosome. Where two pairs of linked loci are known to be separable by crossing over, their zygotic frequencies could be expected to behave as though independent in an equilibrium population with random mating (LI 1955). Assuming that recombination is possible between all of these left and right arm arrangements and that such arrangements are distributed randomly in each laboratory strain, the differences in the frequencies of the observed combinations are highly significant in the three populations to which a contingency test could be applied (Table 3). Nonrandom associations of linked inversions have been studied in *Drosophila* and *Chironomus* (review, LEVITAN 1958; LEVITAN and SALZANO 1959; STALKER 1961; BRNCIC 1961). The deviation from independence by linked inversions known to be recombinable is believed to be due either to the recent origin of a particular association, or to selective elimination of non-adaptive recombinants as they arise. The four *M. claytonae* strains represent a limited sample of the Arkansas and Missouri populations; however, it is interesting to note that the same pattern of nonrandomness prevails in each.

DISCUSSION

M. claytonae and *M. stalker*i differ by at least 24 inversions, eleven of which are known to be heterozygous in the former species and three in the latter one. The difference in polymorphism may reflect the relative ages of these two species. Related species in *Drosophila* are rarely heterozygous for the same inversion, suggesting that most new species are monomorphic or lose any shared inversions (STALKER 1966). It is likely that *M. stalker*i which has far less inversion polymorphism than *M. claytonae* is the more recently evolved species. This is supported by their distribution, the range of *M. claytonae* overlapping and extending far beyond that of *M. stalker*i.

With the exception of inversion XL-A in *M. claytonae*, polymorphism is known only in the autosomal arms of the two species. The difficulty encountered in homologizing the standard sequences in the X chromosome suggests, however, that both arms have undergone considerable reorganization in their evolution. If mutation induced change of the banding pattern is responsible for the changes, it must be restricted to the X chromosome in these species because the autosomes are clearly homologous for over 95% of the salivary gland chromosome length. Deletions and other gross changes in the chromosome associated with lethality would not have been transmitted through the males and thus would not be expected to survive to influence the change in banding sequence. A more likely explanation is that the ancestral X chromosome has undergone a large number of inversions in each species which subsequently became fixed during their evolution. In the left arm considerably more than three inversions are required to

explain the divergence in arrangement between those regions which could be homologized. Possibly *M. stalker* and *M. claytonae* are distantly related members in the genus and other species more closely related to both of them carry some of the intermediate *X* chromosome arrangements. The situation resembles that found in 22 closely related species of Hawaiian *Drosophila* (CARSON, CLAYTON and STALKER 1967) where all 26 inversions identified in the one-armed *X* chromosome are fixed (interspecific) while in 42 autosomal inversions, 31 are fixed and 10 are heterozygous (intraspecific).

The evidence for the nonrandom inversion associations in *M. claytonae* laboratory strains is more interesting if it is a reflection of such systems in natural populations. BRNCIC (1961) found a direct relationship between the number of generations a stock was carried in the laboratory and the strength of the association between left and right arm inversion arrangements in chromosome IV of *D. pavani*. The four *M. claytonae* strains, from 9–24 months old at the time of sampling (Table 3), represent a limited sample of their respective natural populations; nevertheless it is possible to infer the conditions in nature from the Farmington, Missouri strain which was established by one virgin pair nine months before inversion analysis. The four founder chromosomes in this strain must have consisted of the E, EH, EF, and E(E_G)I left arm arrangements and included the +, E, and EF right arm arrangements (Table 2) giving at least 12 different possible combinations. The probability for getting four chromosomes containing all seven arrangements, assuming they assort independently in nature and neglecting the probability of the presence of other arrangements, is $6/64 \cdot 2/9 = 2\%$. Granted this fortuitous event and assuming the associations arose in the laboratory, it is unlikely that three associations found here should be the same nonrandom combinations found among the other three strains unless some system of genetic interaction was initiated in the parent population from which the Arkansas and Missouri populations were derived.

A parallel between inversion association and inversion origin becomes obvious when one compares the phylogeny of the left and right arms of chromosome 3. All five chromosome types found in the three associations, i.e. EH+, E·E and EF·E, and EFG·EF and E(FG)I·EF, are noncrossover types (assuming that the E(FG)I arrangement is an inversion of 3L-EFG) and thus the left-right combinations agree with the phylogenetic sequences (Figure 4).

The fact that each step in the phylogeny is involved suggests that some of the associations are very old. Nothing is known about selection pressures and recombination rates in these strains; however, the nonrandom pattern of these chromosomes implies selection for some element(s) common to each strain early in their history.

FIGURE 4.—Correspondence between inversion origin and inversion association in the third chromosome of *M. claytonae*.

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SUMMARY

The pattern of chromosomal evolution in two species of *Mycodrosophila* was analyzed by a determination of homology between their salivary gland chromosomes and by a survey of structural heterozygosity in population samples. Homologization was nearly complete in the three autosomal arms and partial in the two arms of the *X* chromosome. Eleven inversions are heterozygous in *M. claytonae*, and three in *M. stalker*; at least ten differences are fixed between these two species. Thirteen of the fourteen heterozygous inversions are located in the autosomes. The inability to homologize the greater part of the *X* chromosome may be due to the fixation of a number of inversions. Three nonrandom inversion associations between arrangements in the left and right arms of chromosome 3 of *M. claytonae* are observed in samples from the Missouri and Arkansas populations. The patterns of these associations is related to their phylogenetic origin, suggesting that some of them are quite old.

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