Chromosoma (Berl.) 53, 1—24 (1975)

Intraspecific Karyotypic Differentiation in the Australian Phasmatid *Didymuria violescens* (Leach)

I. The Chromosome Races and Their Structural and Evolutionary Relationships*

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Abstract. The phasmatid species *Didymuria violescens* comprises ten distinct chromosome races parapatrically distributed such that adjacent races meet in narrow zones of overlap. The interracial karyotypic variation is remarkable and involves both diploid number differences (in the range 26–40) and differences in the sex-chromosome mechanism. Karyotypic comparisons and analyses of the meiotic pairing relationships in interracial hybrids have shown that the differences derive in large part from a series of centric fusion events and X-autosome fusions, which together contribute to the reduction in chromosome number within the species. The origin and development of the current racial pattern can best be interpreted in terms of the stasipatric hypothesis of White.

Introduction

The two classical models of speciation, allopatry and sympatry, do not link chromosomal differentiation with the speciation process and fail to account for the widespread occurrence of structural rearrangement differences between related species. An alternative chromosomal model of speciation has been proposed by White (1968) on the basis of his studies of the racial variation in certain morabine grasshoppers. Intraspecific differentiation of this kind, involving fixed cytological differences between geographically distinct forms, has been reported in many species of rodents, insects, bats and lizards (*cf.* White, 1973 for examples). The evolutionary significance of such situations depends largely upon the nature of the rearrangement differences between races. Where reduced fertility of the chromosome heterozygotes engenders a measure of genetic isolation between the two structurally homozygous forms, the interracial chromosomal barriers may subsequently become selectively reinforced by additional reproductive isolating mechanisms. In other words, the establishment of chromosomal rearrangement differences at the racial level could very well be a first step in speciation.

One remarkable case of possible incipient speciation is provided by the karyotypic differentiation in an endemic Australian phasmatid, *Didymuria violescens* (Leach). Within its total distribution, the species is differentiated into ten chromosomally (but not morphologically) distinct forms which are referred to here as chromosome races. The chromosomal variation within *Didymuria violescens* is extreme, the

* This paper is affectionately dedicated to Professor Spencer Smith-White on the occasion of his 66th birthday.

races differing widely in diploid chromosome number, in chromosome morphology and in sex-chromosome mechanism. This paper describes the geography and cytology of the ten races and interprets the observed chromosomal differences in terms of a sequence of rearrangement events, using information from the karyotypic comparisons and the cytological behaviour of chromosome hybrids.

Material and Methods

Material for this study was drawn from a sample in excess of 1,700 insects collected from forested areas within an area of approximately 145,000 sq.mi. in the south-eastern region of Australia. The specimens have been lodged in the Australian National Insect Collection (C.S.I.R.O., Canberra), together with cytological information and complete collecting and locality data. Field collections were made during the spring and summer months, November to March, when nymphal and adult stages are available. Repeated collections in the same areas over several years always yielded individuals of the same chromosomal constitutions. In some highland areas, the population density in consecutive summers alternated between extremes, due to the predominantly two-year life cycle of populations in these areas. Coastal populations of *Didymuria* usually have a one-year life cycle.

The experimental hybridizations were carried out using laboratory-reared virgin females and either laboratory-reared or field collected males of various chromosomal types. Eggs from the usually successful matings were collected and held in moistened sand with the appropriate alternation of summer and winter temperatures for the incubation period of two years. Normal embryogenesis in *Didymuria violescens* involves one or two diapause stages which determine whether hatching occurs in the first or second (or more rarely third) spring after oviposition (Hadlington and Shipp, 1961; Shipp, 1963). The hatching time of the hybrid eggs was also affected by the relative time of the oviposition period, as well as by the constitutions of the parents. Hatchlings were reared to maturity (2–3 months) on a diet of *Eucalyptus andreana* and then either used in further matings or sacrificed for cytological analysis. The gonads were dissected from live adult animals (field specimens and laboratory hybrids), fixed for 18–24 hours in ethanol-acetic acid (3:1), and stored in absolute alcohol at −10°C. In females, dissection and fixation were preceded by a treatment of 3–5 hours with 0.05 ml of a 0.05% solution of Colcemid (desacetylmethylcolchicine, CIBA) in saline, injected into the abdomen by a hypodermic syringe. Squash preparations of testis material and the terminal ovarioles of ovarian tissue were made in aceto-orcein.

Karyotypes were studied by taking chromosome measurements of Colcemid treated female mitotic metaphases, using samples of ten or more cells per race, selected so as to allow for variation within and between individuals. The long and short arms of each chromosome of the complement were measured on a photographic enlargement to within approximately 5%, and relative arm lengths expressed as a percentage of the total chromosome length of the cell. Scatter diagrams were constructed for each cell by plotting the measured long arm against the short arm of each individual chromosome for all chromosomes of the complement. Groups of morphologically similar chromosomes were defined by the clusters of points which consistently fell within the same areas for all diagrams of a particular racial karyotype. Karyotypic comparisons were performed with the aid of these scatter diagrams, those of any two races being superimposed to demonstrate the probable identity or lack of identity of two particular chromosomes or chromosome groups. The karyotypic measurements of the ten complements, the scatter diagrams and comparisons are given in detail in Craddock (1971), and only relevant parts of the data are presented here.

Results

1. The Racial Pattern in Didymuria

The phasmatid species *Didymuria violescens* is widely distributed in south-eastern Australia in forest and near-forest habitats, where it feeds on numerous species of the genus *Eucalyptus*. It is most abundant in highland forests of the

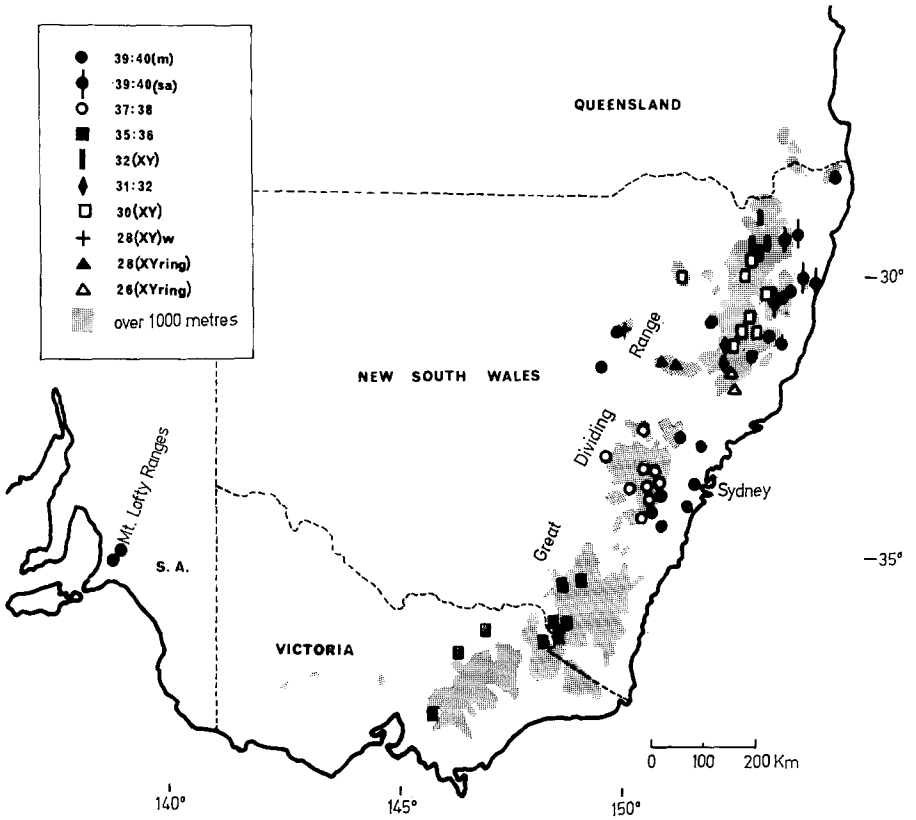


Fig. 1. Distributions of the ten chromosome races of *Didymuria violescens* in south-eastern Australia

Great Dividing Range and other mountain ranges to the west, but it also occurs in forested areas of the coastal region and the western slopes. Populations are typically sparse, although plague proportions have sometimes been attained in certain areas (Readshaw, 1965). *Didymuria* is a sedentary insect. The adult female has very small wings and cannot fly at all, and the adult male is capable of only limited flight.

The ten chromosome races are recognized primarily by the diploid number in male and female individuals and by the male sex-chromosome form. The notation used to designate each race reflects these parameters (*cf.* Table 1). By way of example, the 39:40(m) race is one in which males have 39 chromosomes (X0) and females have 40 chromosomes (XX). The (m) indicates a metacentric X-chromosome, as opposed to a subacrocentric X (sa). The 32(XY) race is one with 32 chromosomes in both males and females, males being XY and females XX. The "w" of the 28(XY)w race refers to the geographic source of this 28-chromosome form, *i.e.* the Warrumbungle Ranges.

The distributions of the chromosome races are parapatric (Smith, 1955) and the racial pattern in *Didymuria* is constant over successive seasons (Fig. 1). Extensive collections over a broad region in almost every possible accessible Eu-

calypt forest or semi-forest habitat have significantly extended the previously known distribution and probably ranged very close to the actual geographic and ecological limits for the species. It is not anticipated that future collecting will reveal many more chromosome races. Each race occupies a defined geographic area, and in some instances, this corresponds to a particular biogeographic zone. In general, the two 39:40 races are coastal or lowland forms, whereas the remaining races are montane. More specifically, the distribution of the 35:36 race covers the entire southern highlands of New South Wales and Victoria, whereas the 37:38 race is found in the central eastern highlands of New South Wales, another discrete region of the main tableland. The 28(XY)w race appears to be confined to the Warrumbungles, an outlying range of the Eastern Highlands; the 28 (XYring) race is restricted to the Liverpool Ranges, a western spur of the Great Dividing Range; and the 26(XYring) race occurs only on the Barrington Tops-Gloucester Tops plateau at the southern end of the northern highland block.

The northern section of the species range is the most dissected chromosomally, being composed of a mosaic of chromosome races, many of which are contiguous with two or more other races. In addition to those already mentioned, five other races are present in the north. The 32(XY), 30(XY) and 31:32 races occupy the northern tableland region in succession from north to south. Of these, the 30(XY) race is the most widely distributed, and further, the most ecologically variable of the montane races. It occupies large and diverse areas of the main Range, and also occurs in the Nandewar Range further to the west.

The 39:40(m) race is the most widely distributed of all, occupying the northern, eastern and western limits of the range of *Didymuria*. Not unexpectedly, it shows the broadest range of ecological tolerance. It is found in all kinds of habitats varying from dry savannah forests of the western slopes and plains to extremely moist mountain forests (up to 5,000 ft.), encompassing the total range of moisture conditions tolerated by the species. The 39:40(m) race is the only race with a distribution which is definitely known to be disjunct. The discontinuities arise both from geographic barriers and from local replacement by other chromosomal forms, as for example where the coastal distribution of the 39:40(m) race is segmented into northern and southern regions by the occurrence of the 39:40(sa) race in part of the coastal strip. The population of the 39:40(m) race in the Mt. Lofty Ranges in South Australia is an apparent geographic isolate, currently separated from other populations further to the east by the arid Murravian Gulf in south-western Victoria (Crocker and Wood, 1947; David, 1950). The populations to the east and the west of the northern highlands may not be altogether discontinuous, perhaps being connected by populations of 39:40(m) constitution which might occur in the lower mountain gaps. The observed racial distributions of *Didymuria* are strongly suggestive of a pattern in which the lower-numbered montane races have islandic distributions within a sea of populations of the 39:40(m) constitution. This geographic pattern is important in considerations of the evolutionary relationships of the races and interpretations of their mode of origin and divergence.

2. The Cytology of the Races

The distinctive cytological characteristics of each of the ten chromosome races of *Didymuria* are summarized in Table 1. These include the morphology

Table 1. Cytological characteristics of the ten chromosome races of *Didymuria violescens*

Race	Male meiosis					Female karyotypes			
	Male sex chromosomes	Mean no. of Xta/cell	Mean no. of Xta/bivalent	Terminalization coefficient[a]	Recombination index[b]	No. of chromosome pairs: Group I	II	III	N.F. (haploid)
39:40(m)	X0, meta. X	19.56±0.06	1.029±0.003	0.663	39.6	1	9	10	30
39:40(sa)	X0, subacro. X	19.56±0.09	1.029±0.005	0.744	39.6	—	7	13	27
37:38	X0, meta. X	19.18±0.05	1.066±0.018	0.716	38.2	2	7	10	28
35:36	X0, meta. X	19.16±0.07	1.127±0.017	0.764	37.2	3	7	8	28
31:32	X0, meta. X	16.43±0.07	1.096±0.006	0.879	32.4	5	5	6	26
32(XY)	XY, primary	17.13±0.07	1.070±0.005	0.796	33.1	4	6	6	26
30(XY)	XY, primary	16.42±0.06	1.095±0.013	0.831	31.4	5	5	5	25
28(XY)w	XY, primary	17.51±0.08	1.251±0.009	0.552	31.5	5	2	7	21
28(XYring)	XY, secondary, unequal ring	17.83±0.10	1.274±0.012	0.904	31.8	5	5	4	24
26(XYring)	XY, secondary, unequal ring	17.06±0.08	1.312±0.010	0.859	30.1	5	5	3	23

[a] Terminalization coefficients were computed as the proportion of terminal to total chiasmata at Metaphase I.

[b] The recombination index for each race was computed as the sum of the haploid number including the X-chromosome plus the mean total chiasma frequency per male metaphase cell. Because of the occurrence of XO and XY races, these recombination estimates are not exactly comparable.

of the karyotype, diploid numbers and sex-chromosome mechanism, as well as chiasma characteristics. Fig. 2 shows the male meiotic chromosomes of each race at metaphase I. Cells of the respective races have from 19 to 12 autosomal bivalents, together with a single unpaired heterochromatic X-chromosome, or alternatively, a sex bivalent. *Didymuria violescens* is remarkable in that it exhibits three different sex-chromosome mechanisms (Craddock, 1970). An X0(♂):XX(♀) system is found in the five races with higher chromosome numbers, *i.e.* the 39:40 (m), 39:40(sa), 37:38, 35:36 and 31:32 races. In four of these, the X-chromosome is a large metacentric, the primitive form of the X in phasmatids (Hughes-Schrader, 1959), but in the 39:40(sa) race, the X is a long subacrocentric chromosome with markedly subequal arms (see also Fig. 3b). Two additional kinds of sex-chromosome system are found amongst the five lower numbered races—a primary neo-XY(♂):neo-XX(♀) system in the 32(XY), 30(XY) and 28(XY)w races, and a secondary neo-XY(♂):neo-XX(♀) system in the 28(XYring) and 26(XYring) races. Both of these neo-XY systems are derived types which incorporate some autosomal material in addition to the primitive X-chromosome. In the primary XY system, the short acrocentric neo-Y chromosome pairs during meiosis with a short terminal region on one arm of the neo-X to give rise to a J-shaped sex bivalent (Fig. 2f, g, h). In the secondary neo-XY system, the neo-Y is a short submetacentric chromosome, both arms of which undergo meiotic pairing and chiasma formation with the terminal regions of the two arms of the neo-X to form a highly unequal ring bivalent (Fig. 2i, j).

Chiasma frequency estimates for males of the various races were obtained from first meiotic metaphases (50 cells per individual and several individuals per race, *cf.* Craddock, 1971 for complete data), since in this material earlier prophase stages are very rare and cytologically unfavourable. Due to the extensive chiasma terminalization at metaphase I (Table 1 and Fig. 2), these data probably underestimate the actual frequencies at early diplotene, and hence the recombination potential within the species. The chiasma distribution in the 28(XY)w race differs from that of all other races (Table 1, also comp. Fig. 2h and i), but there is at present no explanation for this difference. Decrease in chromosome number in the species is accompanied by a pronounced decrease in mean recombination index (Darlington, 1958), together with a slight reduction in mean number of chiasmata per cell (Table 1). This reduction in total chiasma frequency is not an automatic result of reduction in the number of bivalents, since the mean number of chiasmata per bivalent is not constant for all the races; it shows a slight increase with decreasing chromosome number (Table 1), which is probably correlated with the increase in mean chromosome length (*cf.* Fig. 3).

Karyotypes: Fig. 3 presents the haploid karyotypes of the ten races, prepared from representative mitotic metaphases of females (*cf.* Methods). In *Didymuria*, the chromosomes in diploid cells show a very close gradation in size, with many chromosomes being similar or apparently identical in arm lengths, and except in a few instances where identification was absolutely certain, no attempt was made to distinguish individual pairs. The errors which can arise from this practice have been discussed elsewhere (Patau, 1960; Essad *et al.*, 1966). Rather, the chromosomes were treated as groups, even although there was often a large and clearly recognizable difference between the longest and shortest chromosome of a particular group. Within all the racial karyotypes, three major groupings of chromosomes can be distinguished. These are classified as Group I chromosomes, the long metacentrics, Group II chromosomes, the medium to small metacentrics and submetacentrics, and Group III chromosomes, which include all subacrocentric and acrocentric chromosomes. The karyotypes in Fig. 3 are arranged according to this classification with one exception: the long subacrocentric X-chromosome of the 39:40(sa) race which is a member of Group III has been placed in Group I for comparison with the X of the 39:40(m) race. The numbers of chromosome pairs in each Group, together with the haploid number of chromosome arms (N.F.) of each karyotype are presented in Table 1. The N.F. ("nombre fondamentale" of Matthey, 1945) is readily estimated, chromosomes belonging to Groups I and II being scored as having two chromosome arms and those of Group III as having one chromosome arm.

Quantitative comparisons among the X-chromosomes of the various races have been made in order to help define the relationships between the various kinds of sex-chromosome mechanism found within the species. Table 2 presents data on the mean relative arm lengths and arm ratios of the X-chromosomes of all races, calculated from the karyotype measurements given in detail in Craddock (1971). In some cases, identification of the X was certain, as for example in the two 39:40 karyotypes (Fig. 3a and b), in which the X is the longest chromosome of both complements; significantly, in the case of the 39:40(m) karyotype, the X is the sole Group I metacentric. In such cases the variances of arm lengths were calculated using paired observations from each cell, since the variation

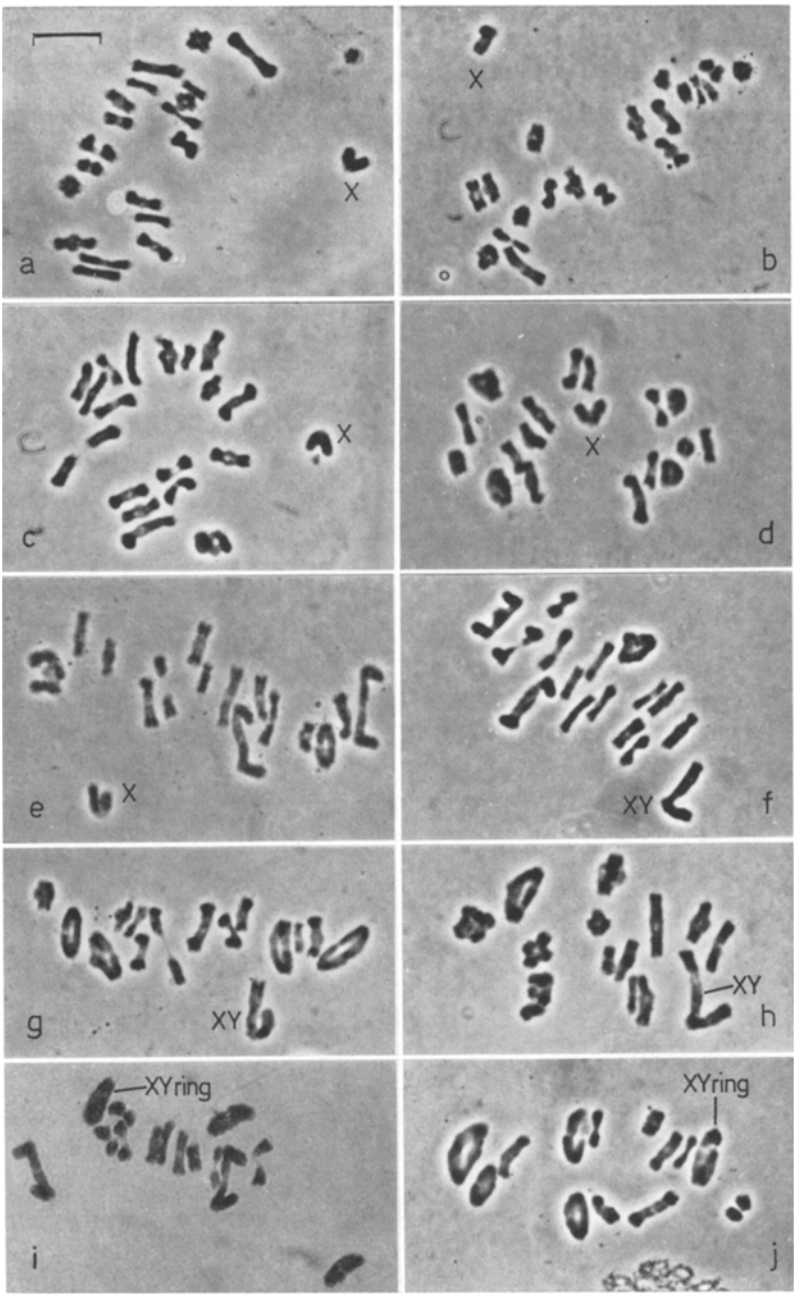


Fig. 2a—j. First metaphase of meiosis in males of the ten races of *Didymuria violescens*. a–e, the X0 races; f–h, the primary XY races; i, j, the secondary XY races. The X-chromosomes and XY bivalents are indicated. The bar (a) corresponds to a length of 10 μm. Fig. 2a. The 39:40(m) race. Note metacentric X. Fig. 2b. The 39:40(sa) race. Note subacrocentric X. Fig. 2c. The 37:38 race. Fig. 2d. The 35:36 race. Fig. 2e. The 31:32 race. Fig. 2f. The 32(XY) race. The XY is a large J-shaped bivalent. Fig. 2g. The 30(XY) race. Fig. 2h. The 28(XY)w race. Note the several nonterminalized chiasmata. Fig. 2i. The 28(XYring) race. The XY is a large unequal ring bivalent. Fig. 2j. The 26(XYring) race

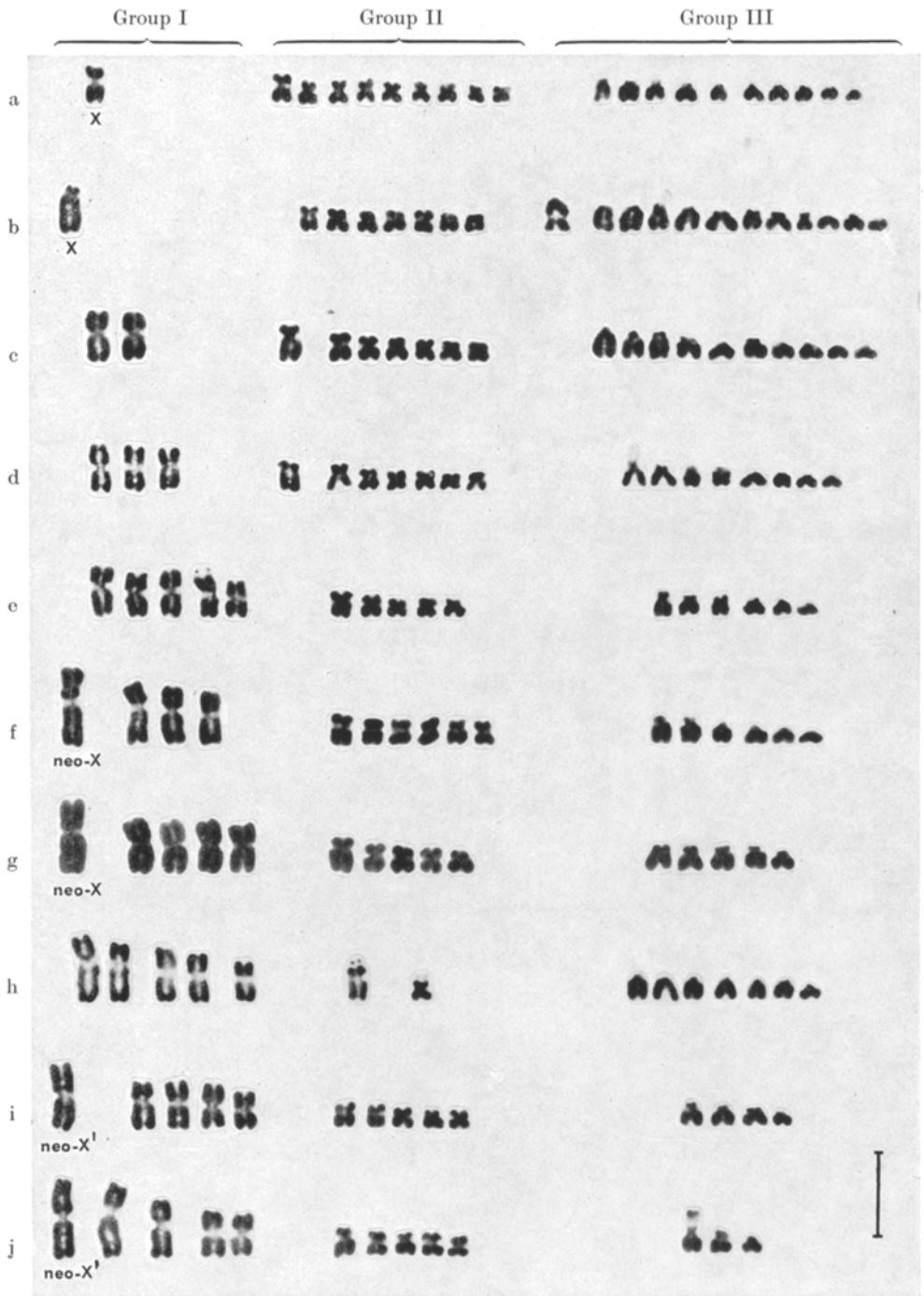


Fig. 3. Haploid karyotypes of the ten chromosome races of *Didymuria violescens*, prepared from Colcemid-treated female mitotic metaphases. The Groups I, II and III represent the long metacentrics, the medium to small metacentrics, and the subacrocentric and acrocentric chromosomes respectively. In b, the 39:40(sa) karyotype, the subacrocentric X-chromosome from Group III is placed in Group I for comparison with the X of the 39:40(m) race. The bar corresponds to a length of 10 μm. The order of the karyotypes a—j corresponds to the order of races in Fig. 2a—j and in Tables 1 and 2

Table 2. Mean values of the X-chromosomes of the races

Race	Chromosome	Mean length			Arm ratio L/S
		Long arm	Short arm	Total	
39:40(m)	Pair 1	2.59±0.05	2.15±0.08	4.73±0.10	1.21
39:40(sa)	Pair 1	3.91±0.06	0.93±0.03	4.84±0.08	4.21
37:38	One of pairs 1 and 2	(2.82±0.04)	(2.32±0.03)	(5.14±0.06)	1.22
35:36	One of pairs 1–3	(2.66±0.03)	(2.32±0.03)	(4.98±0.05)	1.14
31:32	One of pairs 1–5	(2.71±0.04)	(2.27±0.02)	(4.98±0.06)	1.19
32(XY)	Pair 1	4.05±0.06	3.03±0.10	7.08±0.15	1.34
30(XY)	Pair 1	4.08±0.10	3.00±0.10	7.08±0.10	1.36
28(XY)w	One of pairs 1 and 2	(4.13±0.03)	(2.57±0.05)	(6.70±0.08)	1.61
28(XYring)	Pair 1	4.02±0.05	3.78±0.09	7.80±0.14	1.06
26(XYring)	Pair 1	4.10±0.09	3.83±0.09	7.93±0.17	1.07

between the two homologues of a cell was substantially less than that between different cells. In other cases, the X-chromosome could only be identified as one pair of a group of several pairs of chromosomes (*e.g.* the X of the 37:38, 35:36 and 31:32 races which is one of the two or more pairs of Group I metacentrics). In these instances, only approximate arm lengths could be estimated, and in Table 2, these values are given in parentheses. Two things are immediately apparent from the data. The X-chromosome in the XY races is considerably longer than that in the X0 races. Furthermore, the total length of the secondary neo-X exceeds that of the primary neo-X. Clearly, the sex-chromosome in *Didymuria* has undergone a number of structural rearrangements in the course of evolution of the various chromosome races.

3. Comparisons between Racial Karyotypes

Karyotypic comparisons sometimes permit useful inferences about chromosome rearrangements, although, of course, morphologically identical chromosomes in two different races need not be genetically identical. Thus the relationships indicated by this method should be considered provisional until substantiated by demonstrations of homology. Moreover, where there is a difference in chromosome number, two interpretations of the kind of chromosome rearrangement are possible, depending on which is the parental and which the derived form. In the case of *Didymuria*, there are several firm pieces of evidence (*cf.* Discussion) which strongly suggest that the lower chromosome numbers are derived. Thus we can define most of the rearrangements relating the various chromosome races. The following comparisons between complete karyotypes will be preceded by a more detailed comparison of the X-chromosomes of the ten races, in order to distinguish changes in the sex-chromosome system from purely autosomal changes.

X-Chromosome Comparisons: The differences between long arms, between short arms and between the total lengths of the various X-chromosomes have been compared by means of the t-test (Table 3). The X-chromosomes of the two 39:40 races show a highly significant difference in the lengths of both long and

Table 3. Comparison of the mean arm lengths of X-chromosomes. t_L = t value for difference between mean lengths of long arms: t_S = that for short arms: t_T = that for total length. *0.01<P<0.05, **0.001<P<0.01, ***P<0.001

Races compared	t_L	t_S	t_T	d.f.
39:40 (m) and 39:40 (sa)	16.77***	12.26***	0.81	10
39:40 (m) and 32 (XY)	18.76***	6.69***	13.20***	12
39:40 (m) and 30 (XY)	13.38***	6.80***	13.05***	12
39:40 (m) and 28 (XY)w	23.06***	3.60**	12.33***	33
32 (XY) and 30 (XY)	0.23	0.17	0	12
30 (XY) and 28 (XY)w	0.64	3.61**	2.25*	33
30 (XY) and 28 (XYring)	0.55	5.94***	3.46**	11
28 (XYring) and 26 (XYring)	0.72	0.34	0.53	14

short arms, but no difference in total length, suggesting the occurrence of an asymmetrical pericentric inversion. The X-chromosomes of the remaining X0 races were not compared statistically since none can be precisely identified, but all are apparently similar in arm lengths, and probably structurally identical to the metacentric X of the 39:40(m) race.

The neo-X chromosomes of the three primary XY races [32(XY), 30(XY) and 28(XY)w], when compared with the X of the 39:40(m) race, are found to be significantly greater in the lengths of both arms. Comparisons between these three races suggest that the neo-X chromosomes of the 32(XY) and 30(XY) races are identical, whereas that of the 28(XY)w race has probably been separately derived. Lack of precision in the identification of the neo-X of the 28(XY)w race somewhat reduces the dependability of this comparison.

If the origin of the neo-XY mechanisms in these three races involved a simple unequal interchange between the X and an acrocentric autosome, then one arm of the X-chromosome should remain unchanged in length. Tables 2 and 3 show that only one such identity is possible—that involving the short arm of the 28 (XY)w and the long arm of the 39:40(m) X-chromosomes. Thus the neo-X of the 28(XY)w race could have been derived by the translocation of a sizeable acrocentric autosome onto the shorter arm of the primitive X. The simplest derivation of the X-chromosomes of the 32(XY) and 30(XY) races requires that there was a pericentric inversion in the X in addition to an X-autosome translocation.

The secondary 28(XYring) and 26(XYring) races have neo-X chromosomes which are very similar in the lengths of both arms. These X-chromosomes are the longest of all the races. Compared with the primary neo-X of the 30(XY) race, the increase in length is solely due to a change in the short arm. Thus the secondary ring neo-XY system may have originated directly from the primary neo-XY system *via* a second X-autosome translocation.

Comparisons between the X0 Karyotypes: The five X0 races have the highest diploid numbers and the greatest numbers of small subacrocentric chromosomes, but the fewest long metacentrics. Comparisons between the races with a metacentric X show that the 39:40(m), 37:38, 35:36 and 31:32 female karyotypes have respectively one, two, three and five pairs of Group I chromosomes (Fig. 3 and Table 1), of which one pair in each karyotype represents the X. The sequential

increase in the number of long metacentric autosomes with the decrease in diploid number is matched by a concomitant decrease in the small Group III autosomes, although the correspondence is not precise. This suggests that this series of races has been derived *via* a succession of "centric fusion" events, whereby two short one-armed chromosomes undergo an unequal reciprocal translocation which, with the loss of one centromere, gives rise to a longer two-armed chromosome. Such rearrangements do not involve any change in arm number, which should therefore remain constant between races, provided that no other rearrangement differences exist. Only the 37:38 and 35:36 races show the same N.F. (28) and furthermore, an identical complement of Group II autosomes (*cf.* Fig. 3c, d), suggesting that these two races may differ by only a single centric fusion.

The variation in arm numbers between the other X0 karyotypes can be attributed to the presence of other structural differences, specifically pericentric inversions, in addition to the major fusion differences responsible for the change in diploid number. The earlier comparisons between the two 39:40 races demonstrated such an inversion difference between their X-chromosomes (*cf.* Tables 2 and 3), but this is not the only karyotypic difference. The numbers of both the Group II and Group III autosomes differ, contributing further to the difference in N.F., and at least two autosomal inversion differences must be assumed. One of these must involve chromosome 9 in the 39:40(sa) race, which as a very large acrocentric with a marked secondary constriction (*cf.* Fig. 3b) is distinct from any chromosome of the 39:40(m) race.

Numerous differences exist between the karyotypes of the 37:38 race and each of the two 39:40 races, and these differences involve all three chromosome groups. For example, in Group II of the 37:38 karyotype, there is an autosome (number 3) which has no apparent homologue in either of the 40-chromosome karyotypes. This chromosome may perhaps be related *via* a pericentric inversion to chromosome 9 of the 39:40(sa) race, since the two chromosomes have mean total lengths of 3.65 ± 0.06 and 3.73 ± 0.09 respectively, which are not significantly different ($t_{11} = 0.78$). Chromosome 9 of the 39:40(sa) race may in turn be related by a pericentric inversion difference to one of the larger Group II chromosomes of the 39:40(m) race, but this cannot be shown quantitatively. Additional differences exist such that the 37:38 karyotype differs from both 39:40 karyotypes by at least three pericentric inversions and one centric fusion. This makes it unlikely that the 37:38 race is a direct derivative of either 39:40 race, by contrast to the simple relationship between the 37:38 and 35:36 races.

In the transition from the 35:36 race to the 31:32 race, again there must be other rearrangements in addition to the two centric fusions, to account for the difference in arm number and in the group II complements of these two karyotypes.

Comparisons between the XY Karyotypes: Amongst the primary XY races, the 32(XY) and 30(XY) races have comparable neo-X chromosomes, as established earlier (*cf.* Tables 2 and 3). Further, this neo-X can be related to the primitive X of the X0 races *via* an X-autosome translocation and a pericentric inversion. This difference in sex-chromosome mechanism appears to account completely for the karyotypic differences between the 31:32 and 30(XY) races, and the observed difference in N.F.

The 32(XY) and 30(XY) races have different numbers of autosomes in Groups I, II and III of their karyotypes, but only one centric fusion and one pericentric inversion would be required to account for these differences.

It has already been indicated that the secondary neo-XY sex-chromosome system could be related to the primary neo-XY system of the 32(XY) and 30(XY) races. Furthermore, the autosomes of Groups I and II of the 30(XY) and 28 (XYring) races appear to be morphologically identical. The differences between these female karyotypes can be simply accounted for by a single translocation between an acrocentric autosome and the 30(XY) neo-X chromosome. The formation of the ring XY bivalent in male meiosis does however, require a further change.

The two XY ring races have sex-chromosomes and Group II autosomes which are virtually identical. Chromosome 2 of the 26(XYring) karyotype is distinctly longer than any Group I autosome of the 28(XYring) karyotype, suggesting a translocation of an acrocentric chromosome onto a group I metacentric. The reduction in the number of Group III chromosomes from four to three, and the reduction in N.F. from 24 to 23, supports this interpretation.

Only the 28(XY)w race cannot be related *via* simple rearrangement differences to the other races of *Didymuria*. Effective karyotypic comparisons are difficult since the 28(XY)w race shows little similarity with any of the other karyotypes. Having only two Group II and seven Group III chromosomes, it has the lowest N.F. (21) of any race. Chromosomes 3 and 4 of its karyotype might correspond with some metacentric Group I chromosomes of other races, but chromosomes 1 and 2, including the X-chromosome, appear to be unique (Craddock, 1971).

4. Interracial Hybridization Data

The chromosomal homologies between the various races of *Didymuria* have been established more precisely *via* a study of the meiotic pairing relationships in male hybrids of several interracial combinations. Identification of the major structural differences has been facilitated by the high level of synapsis generally found in the chromosome hybrids of *Didymuria*. Most of the cytological data have been obtained from laboratory hybrids produced by experimental hybridizations between some of the available races. A few hybrids collected from field zones of contact between geographically adjacent races have also been used in this analysis (iv and vi in Table 4). Such field hybrids were interpreted as F_1 individuals (rather than as backcross or other hybrid derivatives) and their parentage was deduced from a knowledge of the geographic distributions of the chromosome races and the presence of particular races within and adjacent to the area of collection.

The chromosome hybrids were characterized by the presence of trivalent associations at first meiotic metaphase (Fig. 4). These usually consisted of one large metacentric chromosome and two small acrocentric chromosomes which formed chiasmate associations with each of the arms of the metacentric, producing a large V-shaped configuration when the trivalent oriented disjunctionally as was most often the case. Each such trivalent corresponded to one centric fusion difference between the parental races. The forms of other trivalents indicated the

Table 4. Summary of cytological data on F_1 interracial hybrid males

	Racial cross	2n $F_1♂$	Most frequent M_I configuration	Major structural differences between parental races
(i)	40(m)♀ × 35♂ 36♀ × 39(m)♂	37	$2_{III}+15_{II}+X$	2 centric fusions
(ii)	32♀ × 35♂	33	$2_{III}+13_{II}+X$	2 centric fusions
(iii)	32♀ × 39(m)♂ 40(m)♀ × 31♂	35	$4_{III}+11_{II}+X$ and $3_{III}+11_{II}+1_{ub}{}^{a}+1_{I}+X$	4 centric fusions
(iv)	28♀ × 26(XYring)♂ 26♀ × 28(XYring)♂	27	$1_{III}+11_{II}+$XYring	1 autosomal translocation
(v)	32♀ × 30(XY)♂	31	$15_{II}+X$	1 X-autosome translocation
(vi)	30♀ × 26(XYring)♂ 26♀ × 30(XY)♂	28	$2_{III}+11_{II}$ $2_{III}+11_{II}$	1 autosomal translocation, an X-autosome translocation and neo-Y fusion
(vii)	32♀ × 26(XYring)♂ (XO race)	29	$2_{III}+11_{II}+X$	neo-Y fusion and 1 autosomal translocation (+ X-rearrangements given by reciprocal cross)
(viii)	40(m)♀ × 26(XYring)♂	33	— (meiotic pairing irregular)	(5 autosomal translocations, 2 X-autosome translocations and 1 neo-Y fusion)

[a] ub = Unequal bivalent.

translocation of an acrocentric chromosome onto a large metacentric (Fig. 4e, g, h, i), or alternatively, the occurrence of pericentric inversions in one or both of the short chromosomes in addition to the centric fusion rearrangement. The hybrid data and inferences drawn from each of the interracial combinations studied are commented upon in the following and summarized in Table 4. Complete descriptions of the hybrids are presented elsewhere (Craddock, 1971).

Hybrids between Races with the Same Sex-Chromosome Mechanism: Four interracial combinations in this category (i-iv, Table 4) were studied, three in which the parental races were both X0 types and one in which the parental races both possessed the XY ring sex mechanism. These hybrids provided information on some of the autosomal rearrangement differences between chromosome races. The most frequently observed MI configuration usually corresponded to the maximum homologous pairing association, except in the case of the 39:40(m)/31:32 hybrids (iii, Table 4), which showed substantially more asynapsis and a greater variety of metaphase associations (150 classes amongst 844 cells from 14 males) than most other hybrids. The maximum pairing association of $4_{III}+11_{II}+X$ (Fig. 4c) occurred in 11.4% of cells and was less abundant than the association $3_{III}+11_{II}+1_{ub}+1_{I}+X$ (Fig. 4d) which occurred in 18.6% of cells. Despite the degree of asynapsis, there is clear meiotic evidence for four translocation dif-

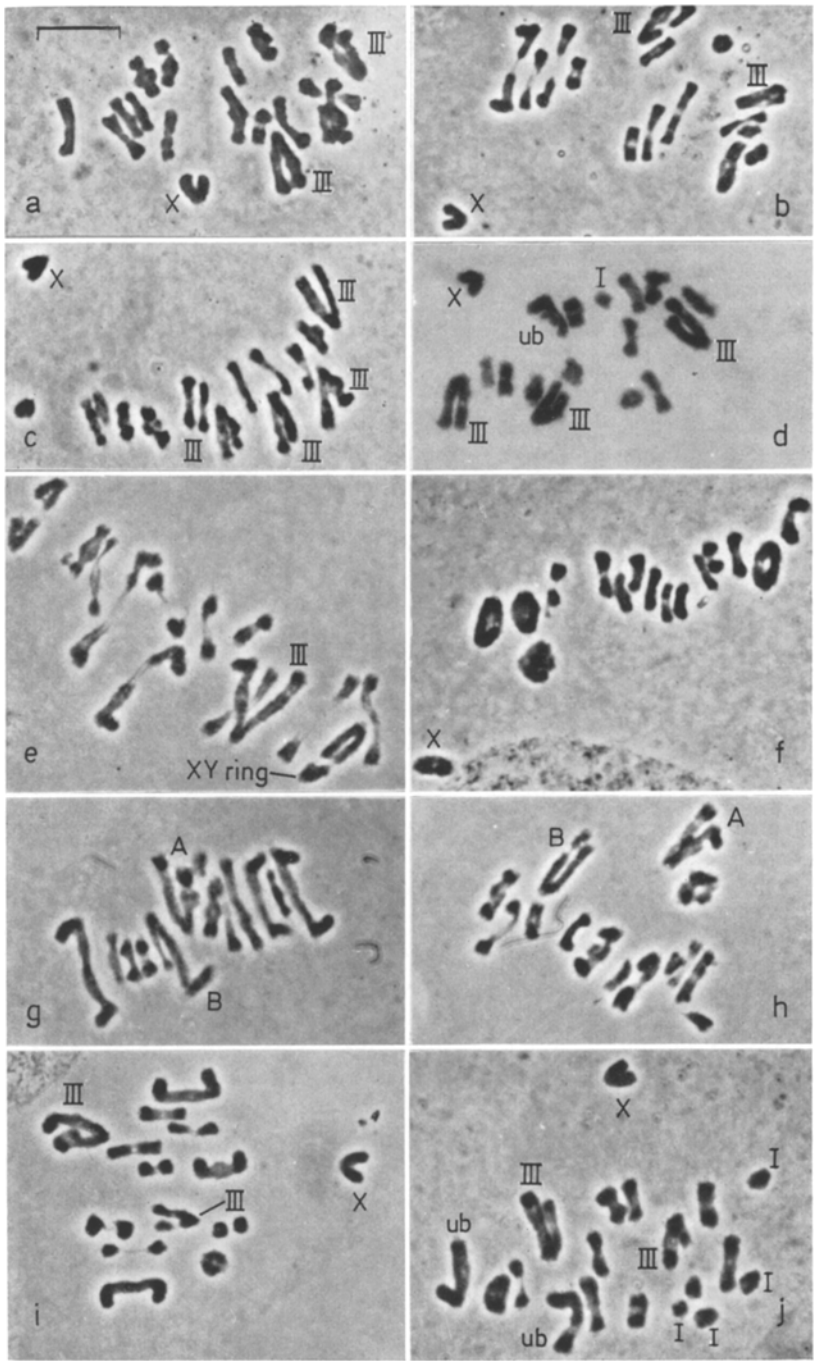
ferences of the centric fusion type between the 39:40(m) and 31:32 races. The result is consolidated by the data from combinations (i) and (ii) of Table 4 (Fig. 4a and b) which involve the intermediate 35:36 race. For all these three combinations between X0 races, there is also suggestive evidence of pericentric inversion differences between parental races involving some of the autosomes implicated in fusions. The remaining autosomes lack additional rearrangements for the most part, and show normal bivalent pairing in the interracial hybrids. Hybrids of combination (iv) in Table 4 show that the autosomal translocation which differentiates the 26(XYring) race from the 28(XYring) race involves a large metacentric chromosome and an acrocentric chromosome (Fig. 4e).

Hybrids between Races with Different Sex-Chromosome Mechanisms: The karyotypic data suggested that the three basic kinds of sex mechanism in *Didymuria* are structurally related. The hybridization data from pairs of races which differ in their sex mechanism (v-viii, Table 4) demonstrate the interracial homologies of each segment of the various sex-chromosomes and confirm that the neo-XY mechanisms do indeed include autosomal material homologous to that present in X0 karyotypes. Further, the hybrid data prove that the secondary neo-XY mechanism is simply related to one of the primary neo-XY mechanisms from which it probably evolved, rather than having been independently derived from the X0 mechanism. The genetic homologies of the primary XY mechanism in the 28(XY)w race have not been demonstrated, since this race has not yet been involved in the interracial hybridizations.

The F_1 males from the 31:32/30(XY) combination (v, Table 4) are unusual in that they cannot be distinguished cytologically as hybrids. Reciprocal male hybrids have chromosomal constitutions completely equivalent to those of the two parental races. The only identifiable structural difference between races is the single X-autosome translocation related to the derivation of the neo-XY sex-chromosome system from the X0 system. It is noteworthy that the translocated autosome shows completely normal pairing, both when the neo-Y chromosome of the 30(XY) male associates with an autosome of the 32-chromosome karyotype (Fig. 4f) and in the reciprocal hybrid (field data) when an autosome from the 31(X0) male associates with the homologous region translocated onto the neo-X of the 30-chromosome female of the 30(XY) race.

Reciprocal male hybrids between the 30(XY) and 26(XYring) races have similar meiotic configurations of $2_{III} + 11_{II}$ (vi, Table 4), but they differ in the form of the trivalent involving the sex-chromosomes. The autosomal trivalents

Fig. 4a—j. Meiotic associations at first metaphase in F_1 hybrid males of various racial combinations. The trivalents (III), unequal bivalents (ub), univalents (I), X-chromosomes and XY bivalents are indicated. The bar (a) corresponds to a length of 10 μm. Fig. 4a. F_1 male of a cross 40(m)♀×35♂. Fig. 4b. F_1 male of a cross 32(XO race)♀×35♂. Fig. 4c. F_1 male of a cross 40(m)♀×31♂. Fig. 4d. A second male of the cross 40(m)♀×31♂ with partial asynapsis of one trivalent to an unequal bivalent and a univalent. Fig. 4e. F_1 male hybrid between the 28(XYring) and 26(XYring) races. Fig. 4f. F_1 male of a cross 32(XO race)♀×30(XY)♂ with a constitution similar to males of the 31:32 race. Fig. 4g. F_1 male of a mating 30♀×26(XYring)♂. A is an autosomal trivalent. B is the sex trivalent, composed of a primary neo-X, metacentric neo-Y and an autosome. Fig. 4h. F_1 male of the reciprocal mating 26♀×30



(XY)♂. The autosomal trivalent (A) is the same as in Fig. 4g. The sex trivalent (B) consists of a large secondary neo-X and two acrocentric chromosomes. Fig. 4i. F_1 male of a cross 32(X0 race)♀×26(XYring)♂. Fig. 4j. F_1 male of a cross 40(m)♀×26(XYring)♂, showing partial asynapsis and a configuration of $2_{III}+9_{II}+2_{ub}+4_{I}+X$

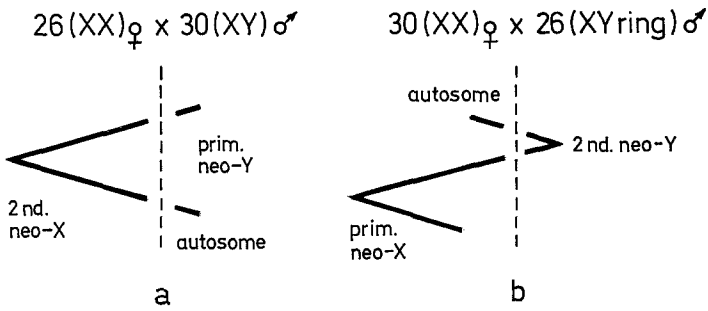


Fig. 5. The conformation of the resultant sex trivalents in reciprocal F_1 hybrids between the 30(XY) and 26(XYring) races

of the two hybrids are identical (A in Fig. 4g, and A in Fig. 4h) and furthermore, equivalent to that in the 28(XYring)/26(XYring) hybrid (Fig. 4e). They represent the same translocation difference. In the case of the mating 30♀×26(XYring)♂, the sex trivalent (B in Fig. 4g) involves a small metacentric, the neo-Y chromosome from the 26(XYring) male parent, one arm of which pairs with a short acrocentric autosome and the other with one arm of a large metacentric, the primary neo-X chromosome derived from the 30-chromosome female parent. The sex trivalent in the reciprocal hybrid (B in Fig. 4h) consists of a very large metacentric chromosome, the secondary neo-X from the 26-chromosome female parent, paired with two short acrocentric chromosomes, one an autosome and the other the primary neo-Y donated by the 30(XY) male parent. The structure of these two sex trivalents is diagrammed in Fig. 5. These pairing relationships would only obtain if the secondary neo-XY mechanism had been directly derived from the primary neo-XY mechanism.

The two hybrids between XO and secondary XY races (vii and viii in Table 4) both confirm the autosomal origin of the two segments of the small metacentric neo-Y of the XY ring system and identify the fusion involved in its derivation (*cf.* the very small trivalents in Fig. 4i and j). The primitive X-chromosomes, derived from the respective female parents (XO races), of course have no pairing affinities with the neo-Y or any other chromosome. Crosses in the reciprocal direction (XO male parent) would clearly demonstrate the origins of the secondary neo-X chromosome, but these have not yet been carried out. The autosomal homologies of the two terminal regions of the secondary neo-X can, however, be inferred from (a) the pairing of these regions with the autosomally derived secondary neo-Y in the XY ring bivalent, and (b) the form of the sex trivalent in one of the previous hybrids (*cf.* Fig. 4h) involving the intermediate primary neo-XY mechanism.

The rearrangement differences between the two extreme chromosome races of *Didymuria* were not clearly demonstrated by their interracial hybrid (viii, Table 4), because the meiotic pairing in all males of this combination was very much disturbed by the occurrence of considerable asynapsis and the formation of irregular higher multiples, probably involving nonhomologous associations. Some of the meiotic configurations observed in this hybrid are however, consistent

with expectations deduced from hybrids between chromosomally more similar races. It can be inferred that the 39:40(m) and 26(XYring) races differ by five autosomal translocations plus the several rearrangements involved in the derivation of the secondary neo-XY ring mechanism from the XO mechanism.

Discussion

1. Analysis of the Chromosomal Differentiation in Didymuria

The ten diverse racial karyotypes found in the species *Didymuria violescens* have been shown to be closely related. The differences are due to structural rearrangement of the genetic material of the species, without major loss or addition of chromosomal material. Preliminary comparisons between races have demonstrated that 39- and 31-chromosome males for example, show no significant difference in their DNA content (Craddock, 1971), although they differ by eight centromeres. The chromosomal material necessarily lost in the course of the several structural changes involved must therefore have been minimal.

The Probable Direction of Evolution: It is postulated that the primitive chromosomal constitution of *Didymuria* was close to that of the present 39:40(m) race, with the same diploid chromosome number and a similar XO(♂):XX(♀) sex-chromosome mechanism, the X-chromosome being of the metacentric form. This sex mechanism occurs in most members of the Order Phasmatodea and was considered primitive by Hughes-Schrader (1959). The thesis is further supported by several kinds of geographic evidence—the extensive distribution of the 39:40(m) race, its ecological latitude between the extremes of altitude and moisture regimes tolerated by the species, and the occurrence of apparently relict isolates of this race, such as that in the Mt. Lofty Ranges near Adelaide. The populations in the region west of the Great Dividing Range in central N.S.W. may also be isolates, although they may be connected with the eastern coastal populations via the Hunter Valley-Cassilis Gap between the northern and central highland blocks (*cf.* Fig. 1).

The direction of evolution in the species is thus inferred to be towards a decrease in chromosome number. All the currently existing races need not have been derived in a single sequence of reduction in number from 40 to 26; there may have been several or branched lines of descent and some few changes may have been in the reverse direction.

The significance of the evolutionary decrease in chromosome number in this species may depend on the limitation on the amount of recombination which it achieves (*cf.* Table 1). A reduction in the recombination level results in less variability and so permits a closer adaptation to a particular habitat. The trend towards apparent ecological specialization in the species, together with the decrease in morphological variation in lower-numbered races (Craddock, 1971) may be correlated with the restrictions on recombination imposed by the chromosome number differences.

Types of Structural Change: The numerous rearrangements involved in the cytological differentiation of *Didymuria* have included some resulting in a change in chromosome number and others causing only a difference in karyotype mor-

phology (mainly pericentric inversions). The decrease in number from the higher- to the lower-numbered races has been accompanied by a reduction in the number of chromosome arms or N.F., resulting from changes of both types. The rearrangements responsible for the decrease in chromosome number have been of three kinds—centric fusions between acrocentric autosomes, other translocations between autosomes, and translocations between autosomes and sex-chromosomes.

Apparent "centric fusion" in *Didymuria* must always have been due to unequal reciprocal translocation between acrocentric chromosomes (Darlington, 1937; Tobgy, 1943), since none of the chromosomes of *Didymuria* appear to have truly terminal centromeres (*cf.* Fig. 3). Such centric fusions are the major rearrangement events involved in the derivation of the numerically descending series of XO races, as indicated by the karyotypic comparisons and the hybrid data. For example, the 39:40(m) and 35:36 races which differ karyotypically by two long metacentric pairs form a hybrid with two trivalents, each composed of a long metacentric paired with two short autosomes (Fig. 4a).

Some of the autosomes involved in this series of fusions display additional pericentric inversion differences. Hence the lack of constancy in the N.F., except between the 37:38 and 35:36 races (Table 1). It is not implied that pericentric inversions occurred in direct association with particular fusion events. Rather, the ancestral acrocentric chromosomes in the higher-numbered race could have been inverted to metacentric or submetacentric chromosomes a long time after the fusion event, to give rise to the observed karyotypic differentiation between present day races.

Autosomal translocations involving the transfer of the long arm of an acrocentric chromosome terminally onto one arm of a metacentric or submetacentric chromosome result in reduction in arm number as well as in centromere and chromosome number. This kind of change could have been involved in the derivation of the 26(XYring) race from the 28(XYring) race. The karyotypic data and the conformation of the single large autosomal trivalent in their interracial hybrid (Fig. 4e) together suggest that chromosome pair 2 of the 26(XYring) karyotype was derived by the fusion of an acrocentric autosome with a Group I metacentric chromosome in the 28(XYring) race.

This kind of fusion offers an alternative explanation for some of the instances where differences between karyotypes have been inferred to result from pericentric inversion and centric fusion.

Autosome—sex-chromosome interchanges differ from autosome-autosome interchanges in that heterozygosity for the rearrangement is maintained in the male sex. These changes are discussed in more detail in the following section.

Evolution in the Sex-Chromosome System: It is postulated that the different sex-chromosome systems of *Didymuria* are directly related by means of structural rearrangements involving the X-chromosome. On the assumption that the 39:40 (m) race is primitive not only in number, but also in sex-chromosome form and mechanism, changes in the X-chromosome have involved both pericentric inversions and interchanges with autosomes, the latter creating neo-XY systems. It has been shown (Tables 2 and 3) that the X-chromosome of the 39:40(sa) race has similar total length to that of the 39:40(m) race, and may differ from it by only a single pericentric inversion.

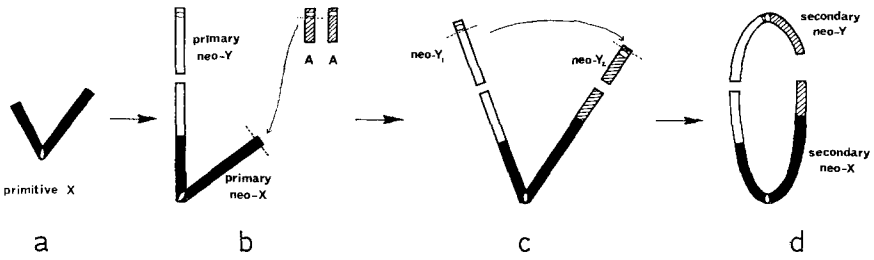


Fig. 6a—d. Postulated origin of the secondary ring neo-XY system in *Didymuria* from the primitive X of the XO(♂):XX(♀) mechanism *via* two autosomal translocations onto the X and a subsequent centric fusion event. (a) to (b): X-autosome translocation; (b) to (c): translocation of autosome onto neo-X short arm; (c) to (d): fusion between the two neo-Y chromosomes

The derivations of the primary neo-XY system in the 32(XY), 30(XY) and 28(XY)w races must have involved unequal translocations between the long arm of an acrocentric autosome and one arm of the X. The data on arm lengths of X-chromosomes suggest that the system has originated at least twice. The neo-X of the 28(XY)w race requires only a single X-autosome translocation. The X-chromosomes of the 32(XY) and 30(XY) races, which appear to have a common origin, can be simply derived *via* a pericentric inversion together with an X-autosome translocation. Evidence for the translocation is provided by the hybrid between the 31:32 and 30(XY) races (hybrid (v) in Table 4).

In the two races with a ring XY system, the 28(XYring) and 26(XYring) races, the neo-X chromosomes are closely similar in arm lengths. It is assumed that they are monophyletic—the ring neo-XY system has been derived but once in the history of *Didymuria*. The novel nature of the mechanism, and the proximity of the geographic distributions of the two races both conform to this hypothesis.

It is argued that the ring neo-XY system originated from a primary neo-XY system rather than directly from the primitive XO system. The latter origin would require the simultaneous establishment of three structural rearrangements, which is highly improbable. The data of Tables 2 and 3 show that the difference in length of the X-chromosomes of the 30(XY) and 28(XYring) races is due solely to their short arms. The neo-X of the latter race has almost certainly been derived by the translocation of a small acrocentric autosome onto the short arm of the neo-X of the 30(XY) race. Support for this view is given by the occurrence of a secondary constriction in one arm of both X-chromosomes, and by the geographical proximity of the two races. The strongest proof is provided by the form of the sex trivalents in hybrids between races possessing this primary neo-XY system *vs.* the ring XY system (hybrid (vi) in Table 4, *cf.* also hybrids (vii) and (viii)).

A possible sequence for the origin of the neo-XY ring system is diagrammed in Fig. 6. A first translocation with an autosome gives the primary XY system. A second X-autosome translocation gives an XY_1Y_2 system. This kind of system is a necessary step in the derivation of the ring mechanism, although it is unknown

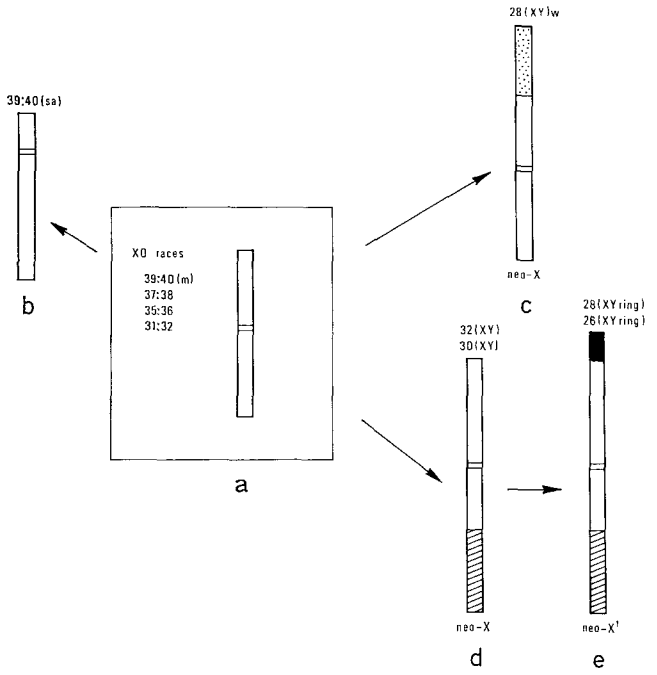


Fig. 7a—e. Postulated sequence of X-chromosome rearrangements in *Didymuria violescens*, demonstrating the interrelationships between the sex-chromosome mechanisms of the ten races. (a) to (b): pericentric inversion; (a) to (c): translocation of autosome onto short arm of primitive X; (a) to (d) X-autosome translocation + pericentric inversion; (d) to (e): translocation of second autosome onto short arm of neo-X

in phasmatids. Fusion of the two acrocentric neo-Y chromosomes gives the metacentric neo-Y and the distinctive XY ring bivalent. Such a fusion would be selectively favoured if pairing and segregation behaviour of the sex-trivalent were irregular enough to lead to lowered male fertility. In the XY ring bivalent, by contrast, disjunction would be regular even if only one of the pairing regions was effectively paired.

The sex-chromosomes of *Didymuria violescens* have thus been involved in a number of structural rearrangements in the course of the evolutionary differentiation of the species. The relationships are illustrated in Fig. 7. At least one, and probably two pericentric inversions in the X, and at least three X-autosome translocations have occurred.

2. Mode of Origin of the Racial Pattern

The present geographic differentiation of the species *Didymuria violescens* into a number of chromosomally homozygous races is inferred to have originated *via* the sequential origin, fixation, establishment and spread of a series of structural

rearrangements of the genome. Chromosome mutations are presumably unique events, and thus, in a sense, each of the present chromosome races must be derived from a structural change in a single individual. The problem is how such new rearrangements succeed in becoming established. The major classes of rearrangement in *Didymuria*, centric fusions and X-autosome fusions, both lead to reduced fertility of the chromosome heterozygote. Since new arrangements arise initially in the heterozygous condition, and are present mainly as heterozygotes (and at low frequency) in the transient polymorphic stage following their origin, selection should theoretically lead to their elimination. Despite the extremely low probability of initial fixation of such translocation rearrangements (Wright, 1941), it is obvious that such fixations have occurred at least ten times and perhaps up to fifteen times in the evolutionary history of *Didymuria*. These fixations must have occurred largely as a result of random drift. A certain degree of isolation is a prerequisite, and such isolation may be provided by a variety of population structures involving some subdivision into small local populations. It does not seem absolutely necessary that the isolation be complete, nor does the local isolate need to be on a periphery, although this may sometimes be the case. Species whose population structure conforms to the "island model" of Wright (1940a, b) provide sufficient isolation for the occurrence of extensive genetic and conceivably chromosomal differentiation between the local demes of the total population. This population structure would seem to apply to the *viatica* group of wingless grasshoppers (White *et al.*, 1967) and to pocket gophers of the family *Geomyidae* (Thaeler, 1968; Patton and Dingman, 1968, 1970), both of which show extensive chromosomal differentiation. *Didymuria violescens*, which has sparse but probably mostly continuous populations, low powers of dispersal and sedentary habits, appears to provide the requisite conditions for Wright's "isolation by distance" model (Wright, 1946), in which the total population is effectively subdivided into neighbourhoods as a consequence of the very limited dispersal. The striking variations in density known to occur within the range of *Didymuria* would increase the differentiating effects of isolation by distance (Wright, 1946). It is notable that *Didymuria*, in common with other phasmatids, possesses an array of features in addition to its population structure which significantly enhance the chances of initial establishment of new chromosomal rearrangements (Craddock, 1971, 1972). Furthermore, rearrangements of the centric fusion and X-autosome fusion types have been shown to possess an innate advantage (in *Didymuria* and in some other organisms which meet certain conditions) which would substantially increase their chance of fixation, compared with rearrangements of other kinds (Craddock, 1971).

In general, only chromosome rearrangements which possess some adaptive advantages, be it even a frequency advantage, will be successful and spread further. Such rearrangements may spread allopatrically into unoccupied territory, as during the re-expansion of a species distribution, following restriction to refugia during unfavourable environmental conditions—the situation suggested for the evolution of the three chromosome races of *Perognathus penicillatus* (Patton, 1969).

Alternatively, rearrangements may spread into occupied territory, displacing an existing chromosomal type by the gradual movement of a narrow hybrid

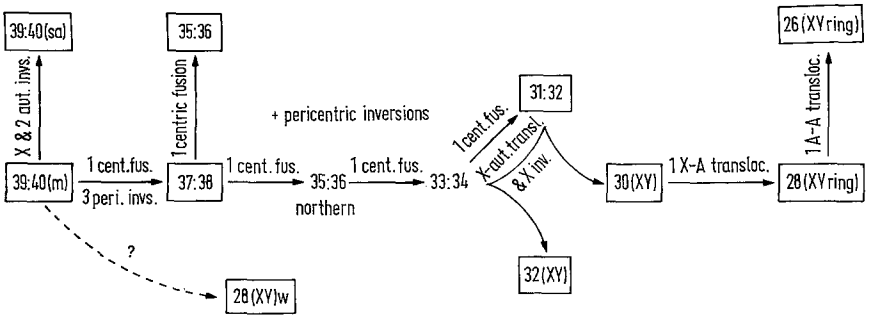


Fig. 8. Scheme of the relationships between the chromosome races of *Didymuria violescens*, indicating the major chromosomal rearrangement events at each step in the proposed evolutionary sequence. The two without boxes represent intermediate forms which are now extinct

zone or "tension zone" (Key, 1968) in the direction of the less adapted form until an equilibrium point is reached at which the two chromosomal homozygotes are of equal fitness. This is the essence of the stasipatric process propounded by White (White *et al.*, 1967; White, 1968) and favoured to explain the evolution of the racial pattern in *Didymuria*. The support for this view will be presented elsewhere, together with detailed interpretations of the geographic sequence of chromosomal events in the differentiation of this species.

In outline, it is envisaged that the original range of *Didymuria* was not substantially less than at present, and that the total distribution was originally occupied by populations of a uniform 39:40 chromosomal type. Then the lower-numbered races displaced the primitive type from parts of its original range *via* the stasipatric process, rather than originating by expansion into major areas previously unsuitable for the species. The cytological and geographic relationships between the present day races of *Didymuria* suggest an evolutionary sequence as summarized in Fig. 8.

At least two chromosomal forms (not in boxes) must be postulated in addition to the existing races, and an unknown number of intermediate forms may have been involved in the differentiation of the 28(XY)w race. Because of doubt as to the origin and chromosomal homologies of this race, it is not possible to specify all the rearrangements which must have been fixed in *Didymuria*. A minimum estimate involves (a) in autosomes, at least five and perhaps up to ten centric fusions, one unequal reciprocal translocation and at least four pericentric inversions, and (b) in sex-chromosomes, three X-autosome translocations, two X-chromosome pericentric inversions and one centric fusion between neo-Y chromosomes. By any standards, this represents a vast array of structural changes in the history of one species, and the resulting mosaic pattern of chromosome races is indeed remarkable. The dynamics of the present situation in terms of the reproductive relationships between races will be considered in a following paper of this series.

Acknowledgements: The results reported here were part of a Ph.D. thesis presented to the University of Sydney which provided laboratory facilities for the project, vehicles and financial assistance for much of the field work and support via a Teaching Fellowship. Mr. P. Hadlington and other officers of the Forestry Commissions of N.S.W. and Victoria, Dr. J.L. Readshaw of the C.S.I.R.O. and fellow graduate students all assisted in obtaining specimens for the study. Dr. K.H.L. Key, Division of Entomology, C.S.I.R.O., confirmed the taxonomic identity of the material. My special gratitude goes to Professor S. Smith-White for his inspiration and encouragement throughout the investigation.

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Received April 29–June 21, 1975 / Accepted by H. Bauer
Ready for press June 30, 1975