MAPPING CENTROMERES IN THE AXOLOTL[1]

D. L. LINDSLEY, G. FANKHAUSER, AND R. R. HUMPHREY

Biology Division, Oak Ridge National Laboratory, Department of Biology, Princeton University, and Department of Anatomy, School of Medicine, University of Buffalo

Received July 11, 1955

WHEN homologous loci separate from one another in the first meiotic division, the division is said to be reductional for those genes; if on the other hand sister loci separate, the division is said to be equational. Alternatively, these types of separation are termed prereductional and postreductional, respectively. According to most theories of meiosis, the centromere of each homologue remains undivided until the second maturation division; consequently, centromeres always separate prereductionally. Equational separation of markers can only be accomplished, therefore, if the original relation between marker and centromere is altered prior to completion of the first meiotic division. This is accomplished by exchange in the region between the marker and the centromere, the frequency of which is a function of the genetic length of this region. The relative proportions of reductional and equational first divisions, therefore, provide a measure of the distance from the marker in question to the centromere.

In organisms from which the products of meiosis are recovered singly it is not generally possible to determine whether the first division is equational or reductional. When half of or all the meiotic products are recoverable in a known order, as in Neurospora, equational and reductional separation of a marker can be scored and its location in relation to the centromere can be determined. Under special genetic conditions, centromere distance can be estimated from unordered tetrads (PERKINS 1949; WHITEHOUSE 1950; PAPAZIAN 1952). If one can contrive to recover routinely two strands from a single tetrad in one gamete, centromere location can be estimated in random gametes. This estimate is dependent on the knowledge of whether the strands carry sister or homologous centromeres or centromere regions. A case in point is the attached-X chromosome of *Drosophila melanogaster*. Caution must be exercised in concluding normal centromere distances from those measured in the special situations which allow recovery of two strands from a tetrad, since the special situation itself may alter exchange frequencies in the region under study. There is ample evidence of such altered frequencies in the case of the attached-X (BEADLE and EMERSON 1935). The distance from the locus of forked to the centromere as calculated from the rate of homozygosis of this marker in attached-X females is 5.0 units, whereas the distance from forked to bobbed, a region within but for all

[1] Work supported in part by a grant in aid from the American Cancer Society to Princeton University administered through the Committee on Growth of the National Research Council, and by the Eugene Higgins Fund of Princeton University, and under Contract No. W-7405-eng-26 for the Atomic Energy Commission. The parts of the work done at the University of Buffalo were supported there by funds of the Department of Anatomy. This project was begun while one of the authors (D. L. LINDSLEY) was a National Research Council postdoctoral fellow in the Department of Biology at Princeton University and continued through his tenure of a National Science Foundation fellowship in the Department of Zoology at the University of Missouri.

practical purposes identical in length with the region from forked to the centromere, is 9.2 units in free X females.

It is possible to recover more than a single strand from multivalents formed in polyploids and polysomics, and it might be guessed that an estimate of centromere distance could be obtained in these situations. Such an estimate is complicated, however, by altered recombination rates and irregular disjunction in multivalent associations.

A simple method is available for recovering a pair of chromatids from each tetrad in the ova of diploid axolotls and other amphibians. Oogenesis in the axolotl is arrested at metaphase of the second division until fertilization, which is effected in the cloaca of the females; the eggs are laid immediately following fertilization, and the second maturation division is completed within one hour thereafter. Refrigeration of freshly laid eggs often brings about the complete inhibition of this division, giving rise to a diploid egg and subsequently a triploid zygote (FANKHAUSER and HUMPHREY 1942). Since the two strands so recovered from each tetrad segregated together at anaphase 1, they presumably contain sister centromeres. Furthermore, the fact that the first meiotic division of a normal diploid oocyte is completed and the first polar body is separated from the oocyte prior to refrigeration eliminates as complications in this case the possibility that recombination frequencies are altered by the treatment or that any material from the first polar body is included in the egg nucleus.

Consider the eggs of a female axolotl of the constitution A/a, in which the second maturation division is inhibited. In the absence of exchange between the a locus and the centromere, separation is reductional and the products of the first meiotic division are AA and aa in equal numbers; following a single exchange between a and the centromere, separation is equational, and both products of the first meiotic division are of the constitution Aa. Fertilization of these diploid eggs by a-bearing sperm yields triploids of constitution AAa, aaa, and Aaa, respectively. It can be seen that one minus two times the frequency of the homozygous recessive class equals the frequency of equational separation of a.

For the present treatment, a tetrad will be defined to consist exclusively of the region between a marker and its centromere; it has been shown in the preceding paragraph that no-exchange and single-exchange tetrads yield 100% reductional separation and 100% equational separation, respectively. Tetrads of higher rank, however, yield both types in varying proportions, depending on rank. The following equation of MATHER (1935) expresses equational separation as a function of rank:

$$E_r = \tfrac{2}{3}[1 - (-\tfrac{1}{2})^r]$$

It can be seen that as r increases, E_r rapidly approaches 0.66. From equations of RIZET and ENGELMANN (1949) one can express the frequency of equational separation as a function of $\bar{r}$, the mean rank:

$$E_{\bar{r}} = \tfrac{2}{3}(1 - e^{-\frac{3}{2}\bar{r}})$$

$\bar{r}$ is in turn a function of the map distance in that the mean rank is twice the map distance, D, expressed in Morgans (M), e.g., an average of one exchange per tetrad corresponds to 50% recombination or 0.5 M. Consequently, $2D$ can be substituted

for $\bar{r}$ in the above equation to express the frequency of equational separation directly as a function of centromere distance. This expression is idealized in that it assumes independence of successive exchanges, i.e., that the frequency of tetrads with no, one, two, etc., exchanges follows a Poisson distribution.

It should be possible, by these relations, to determine centromere distances of the known genes in the axolotl from the frequency of recessives among the induced triploid progeny of heterozygous females by homozygous recessive males.

MATERIALS AND METHODS

The characters utilized in this investigation are (1) sex, (2) body color as influenced by a recessive gene, *d*, which inhibits melanoblast migration (DALTON 1950) resulting in white animals, and (3) development as influenced by a recessive gene, *f*, which causes a marked fluid imbalance during early embryonic development (see HUMPHREY 1948, 1952). All three loci are unlinked, and classification of homozygous recessives for each locus is unambiguous.

Female heterogamety (ZW) and male homogamety (ZZ) were demonstrated in the Mexican axolotl by HUMPHREY (1945). Although Z refers to the chromosome which carries the factor or factors that determine maleness, for present purposes it is convenient to think of it as being nothing more than the recessive allele of W, which can be considered as a single factor determining femaleness.

The sex of both diploid and triploid axolotls was determined in many instances by examination of the gonads of adults. In animals sacrificed in larval stages, the gonads were examined under low-power magnification at autopsy; any not then identified with certainty were studied in serial sections.

Induction of triploidy was accomplished by refrigeration of freshly laid eggs in the manner previously described by FANKHAUSER and HUMPHREY (1942). As a rule, the period of refrigeration was four to eight hours, and the temperature was maintained between 0 and 3 degrees centigrade; following this treatment the eggs were placed in water at room temperature. Larvae not requiring rearing were fixed entire in Bouin's fluid shortly after hatching. When it was necessary to rear the larva, as for identification of its sex, a part of its tail was removed shortly after hatching and similarly fixed in Bouin's. All tailtips were stained in Harris' acid hemalum and mounted entire. Chromosome number was determined from counts on mitotic figures in the epidermis of the transparent tail fin, or by study of nuclear size and number of nucleoli in interphase nuclei in those preparations from which counts were unobtainable.

RESULTS

The results of the experiments described are summarized in tables 1 to 3. These tables show the distribution of phenotypes among the treated and untreated progeny of WZ, *Dd*, and *Ff* females respectively when crossed with homozygous recessive males. The sum of the chi squares of the six diploid samples calculated on an expectation of a 1:1 ratio is 4.74, corresponding to a probability of 0.45; thus there is no significant deviation from the expected 1:1 ratio among the diploid progeny. Among the triploid progeny of WZ females, the proportion of males is 0.156; this figure

corresponds to 0.688 equational separation of the sex locus. The frequency of homozygosis for *d* is 0.153, corresponding to 0.694 equational separation. The previously observed absence of linkage between sex and *d* is also observed in these crosses; among the triploids which were fixed at a stage in which sex could be determined, the sex ratios found in the white (26♀ : 4♂) and the dark animals (90♀ : 17♂) did not differ. The frequency of homozygosis for *f* is 0.138, corresponding to 0.724 equational separation.

DISCUSSION

The observed frequency of equational separation, *E*, for each of the three genes studied in the axolotl is approximately 0.66. It is evident from the equations presented in the introductory portion of this paper that the observed values of *E* agree very well with the theoretical limiting value of *E* which is approached as the number of exchanges, and thus the map distances between the gene and the centromere becomes large. It may be concluded, therefore, that the three loci utilized in the present study are located toward the ends of chromosome arms. GALL (personal communication) has observed in lampbrush chromosome bivalents dissected from oocytes of *Ambystoma tigrinum*, a species closely related to the Mexican axolotl, that the mean distance between chiasmata tends to be constant regardless of chromosome length. This internodal distance is such that the shortest chromosome, ca. 270 μ, averages three chiasmata per bivalent (five bivalents observed) and the four longest chromosomes, 900–1000 μ, average 7.6 chiasmata per bivalent (five bivalents observed). In a triploid axolotl, he also observed up to eight or nine

TABLE 1

Sex of triploid and diploid progeny from WZ ♀ × ZZ ♂ matings

Spawning	Treated eggs				Untreated eggs	
	Triploid		Diploid		Diploid	
	WWZ or WZZ ♀♀	ZZZ ♂♂	WZ ♀♀	ZZ ♂♂	WZ ♀♀	ZZ ♂♂
65	19	7	2	0	5	7
69	5	3	5	4	7	5
99	6	3	—	—	—	—
103	20	0	7	6	2	4
106	2	0	7	6	20	26
108	8	0	4	1	27	30
109	4	0	3	3	27	19
635	72	15	1	—	8	8
671	11	1	—	2	—	—
676	33	5	3	1	21	10
682	20	3	—	—	—	—
Total	200	37	32	23	117	109
Frequency of males	0.156		0.418		0.482	

TABLE 2

Color of triploid and diploid progeny from Dd ♀ × dd ♂ *matings*

Spawning	Treated eggs				Untreated eggs	
	Triploid		Diploid		Diploid	
	DDd or *Ddd* dark	*ddd* white	*Dd* dark	*dd* white	*Dd* dark	*dd* white
103	20	3	13	11	113	137
635	118	25	12	18	117	128
671	14	1	16	17	100	84
676	44	8	17	14	114	108
719	8	0	19	12	92	53
Total	204	37	77	72	536	510
Frequency of white	0.153		0.483		0.487	

TABLE 3

Phenotype of triploid and diploid progeny from Ff ♀ × ff ♂ *matings*

Spawning	Treated eggs				Untreated eggs	
	Triploid		Diploid		Diploid	
	FFf or *Fff* normal	*fff* lethal	*Ff* normal	*ff* lethal	*Ff* normal	*ff* lethal
692	46	9	11	7	95	84
725	12	3	37	22	22	30
738	47	7	10	5	—	—
763	120	17	15	22	65	55
Total	225	36	73	56	182	169
Frequency of lethal	0.138		0.434		0.482	

chiasmata per bivalent (trivalents were also observed). If the chiasmata described by GALL (1954) for lampbrush chromosomes are actually correlated with exchange, as he suspects, the conclusion that the three genes studied are considerably remote from the centromere is quite plausible. It is noteworthy that this conclusion adds support to the idea that the sex-determining region of the sex chromosome is relatively limited and may constitute a single locus, rather than a large proportion of the chromosome.

Prereduction of centromeres at metaphase 1 is an implicit assumption in most considerations of the genetic consequences of meiosis, although there are few organisms in which there is genetic evidence in favor of such a contention. In this context, the presently observed values of E are interpreted as recovery of sister centromeres accompanied by recovery of a random pair of loci. It should be pointed

out, however, that random pairwise separation of centromeres from the bivalent would result in the recovery of a random pair of loci regardless of the position of the gene on the chromosome. The simplest way to rule out such behavior as the explanation of the current observations is to find a gene that separates equationally with some frequency less than 0.66; only three genes are presently available for testing in the axolotl, however. It might be considered that the sex locus of any amphibian in which triploidy can be induced as described and in which the female is heterogametic might provide evidence on the point in question. It is known that *Triturus viridescens* fulfills the former of these requirements (FRANKHAUSER and GRIFFITHS 1939), and there is reason to suspect that it also fulfills the latter; unfortunately, however, diploid ratios in laboratory populations of this species are so variable that sex ratios of triploids would not be a reliable index of equational separation. At present, therefore, it is not possible to distinguish between random first meiotic separation of chromatids at a particular locus caused by frequent exchange between the locus and the centromere and that caused by random separation of centromeres from the bivalent.

SUMMARY

By refrigeration of freshly laid eggs of the Mexican axolotl (*Ambystoma mexicanum*), it is possible to inhibit the second meiotic division to produce diploid ova. Reductional separation of *A* from *a* in a female of constitution *A/a* yields diploid ova of constitutions *AA* and *aa* in equal numbers; equational separation yields *Aa* ova. Fertilization of these ova with *a*-bearing sperm results in triploid zygotes of constitutions *AAa*, *aaa*, and *Aaa*, respectively. The frequency of equational separation of three different markers determined from the proportion of homozygous recessive triploids among the refrigerated progeny of heterozygous females by homozygous recessive males was approximately 0.66. On the basis of the known relation existing between the frequency of equational separation and the map distance between the centromere and a linked marker, it is concluded that the three genes studied are located toward the ends of chromosome arms. An alternative interpretation of the data is that in the axolotl centromeres separate in random pairs from the bivalent at the first maturation division.

LITERATURE CITED

BEADLE, G. W., and S. EMERSON, 1935 Further studies of crossing over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **20:** 196–206.

DALTON, H. C., 1950 Inhibition of chromoblast migration as a factor in the development of genetic differences in pigmentation in white and black axolotls. J. Exp. Zool. **115:** 151–173.

FANKHAUSER, G., and R. B. GRIFFITHS, 1939 Induction of triploidy and haploidy in the newt, *Triturus viridescens*, by cold treatment of unsegmented eggs. Proc. Nat. Acad. Sci. U. S. **25:** 233–238.

FANKHAUSER, G., and R. R. HUMPHREY, 1942 Induction of triploidy and haploidy in axolotl eggs by cold treatment. Biol. Bull. **83:** 367–374.

GALL, J. G., 1954 Lampbrush chromosomes from oocyte nuclei of the newt. J. Morphol. **94:** 283–352.

HUMPHREY, R. R., 1945 Sex determination in ambystomid salamanders: a study of the progeny of females experimentally converted into males. Am. J. Anat. **76:** 33–66.

1948 A lethal fluid imbalance in the Mexican axolotl inherited as a simple Mendelian recessive. J. Heredity **39:** 255–261.

1952 Modification of a lethal trait in the Mexican axolotl through influence of its genes upon the developing ovum. Anat. Record **112:** 416–417 (Abstr.).

MATHER, K., 1935 Reductional and equational separation of chromosomes in bivalents and multivalents. J. Genet. **30:** 53–78.

PAPAZIAN, H. P., 1952 The analysis of tetrad data. Genetics **37:** 175–188.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

RIZET, G., and C. ENGELMANN, 1949 Contribution à l'étude génétique d'un Ascomycète tétrasporé: *Podospora anserina* (Ces.) Rehm. Rev. cytol. et Biol. Végétales **11:** 201–304.

WHITEHOUSE, H. L. K., 1950 Mapping chromosome centromeres by the analysis of unordered tetrads. Nature **165:** 893.