Sex Determination in the Fly *Megaselia scalaris*, a Model System for Primary Steps of Sex Chromosome Evolution

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ABSTRACT

The fly *Megaselia scalaris* Loew possesses three homomorphic chromosome pairs; 2 is the sex chromosome pair in two wild-type laboratory stocks of different geographic origin (designated "original" sex chromosome pair in this paper). The primary male-determining function moves at a very low rate to other chromosomes, thereby creating new *Y* chromosomes. Random amplified polymorphic DNA markers obtained by polymerase chain reaction with single decamer primers and a few available phenotypic markers were used in testcrosses to localize the sex-determining loci and to define the new sex chromosomes. Four cases are presented in which the primary male-determining function had been transferred from the original *Y* chromosome to a new locus either on one of the autosomes or on the original *X* chromosome, presumably by transposition. In these cases, the sex-determining function had moved to a different locus without an obvious cotransfer of other *Y* chromosome markers. Thus, with *Megaselia* we are afforded an experimental system to study the otherwise hypothetical primary stages of sex chromosome evolution. An initial molecular differentiation is apparent even in the new sex chromosomes. Molecular differences between the original *X* and *Y* chromosomes illustrate a slightly more advanced stage of sex chromosome evolution.

STRUCTURAL differences between *X* and *Y* or *W* and *Z* chromosomes range from grossly different (= heteromorphic sex chromosomes) to microscopically indetectable (= homomorphic sex chromosomes). Even when homomorphic, they are not identical. Spreading of simple sequence repeats in the *Y* or *W* chromosomes (*e.g.*, JONES and SINGH 1985; NANDA *et al.* 1992), invasion of transposable elements (STEINEMANN and STEINEMANN 1992) and blocks of late replication (SCHEMP and SCHMID 1981) or of heterochromatin (HAAF and SCHMID 1984; ITURRA and VELOSO 1989) are apparent forms of differentiation at the molecular level. To fulfill their function in sex determination, however, the only requirement for differentiation is the location of one or more primary sex-determining factors on the sex chromosomes in a manner that ensures the formation of male-determining and female-determining gametes by meiotic segregation of these chromosomes in the heterogametic sex.

Theories of sex chromosome evolution (BULL 1983; CHARLESWORTH 1991; JABLONKA and LAMB 1990; JONES and SINGH 1985), accordingly, assume that these chromosomes started as identical homologous chromosome pairs and that molecular and morphological differences between them evolved as consequences of their sex-determining function. The initial event of sex chromosome evolution, therefore, is the acquisition of the sex-determining function. Another requisite is a region of close linkage with the sex-determining sequence. This

may initially be the immediate vicinity of the sex-determining sequence; very often, crossover suppression extends over a larger segment or even covers the whole chromosome. The evolution of crossover suppression is thought to be promoted by sexually antagonistic genes on the sex chromosomes (RICE 1987a). As exchange is suppressed, *X* and *Y* chromosomes (*Zs* and *Ws*) evolve separately in that segment (the "differential" segment), molecular and structural differences accumulate. Under the cover of heterozygosity in the heterogametic sex, genes can become inactivated on the *Y* (or *W*) by mutation without much loss of fitness for the carrier (MULLER 1918). Since there is no repair of the damage through meiotic recombination, the process cannot turn back. It advances in the population by "MULLER's ratchet" (CHARLESWORTH 1978; FELSENSTEIN 1974) or by "genetic hitchhiking" with advantageous genes (RICE 1987b). Thus besides becoming different, *Y* (or *W*) chromosomes tend to lose their informational content—to "degenerate"—with the exception of their sex-specific functions like sex determination and/or fertility.

Most heteromorphic sex chromosomes and even the examples of homomorphic sex chromosomes cited above are rather late stages of sex chromosome evolution in this context. With the fly species *Megaselia scalaris*, on the other hand, we are afforded a model system to study the earliest stages of sex chromosome evolution since the sex-determining function changes its linkage

group at a very low rate (MAINX 1964; TOKUNAGA 1958; TRAUT and WILLHOEFT 1990). *Megaselia* has three chromosome pairs, including homomorphic sex chromosomes. Chromosome translocations specified chromosome 2 as the X/Y pair in two wild-type laboratory stocks (TRAUT *et al.* 1990; WILLHOEFT and TRAUT 1990). For convenience, these chromosomes are designated "original" X and Y chromosomes in this report. From selected males five lines were established ("Except" strains) in which the original Y chromosome had been lost and primary sex determination was associated with a new chromosome, in the majority of cases probably by transposition of the male-determining function (TRAUT and WILLHOEFT 1990). In this report, sex-linked inheritance of random amplified polymorphic DNAs (RAPDs) produced by polymerase chain reaction (PCR) with single decamer primers (WELSH and MCCLELLAND 1990; WILLIAMS *et al.* 1990) and of a few available conventional genetic markers was used to identify and characterize the new sex chromosomes.

MATERIALS AND METHODS

Strains and sex chromosome nomenclature: "Wien" and "Tennessee" are wild-type laboratory stocks. Wien was derived from MAINX's (1966) cultures which were of mixed geographic origin (Italy, South America and Japan). Tennessee was isolated and taken into laboratory culture in Johnson City, Tennessee (BENNER and OSTERMEYER 1980). The original Y chromosome of the Wien stock (a 2^Y chromosome: a chromosome 2 functioning as a Y chromosome) is involved in the two translocations, *T6* and *T4*. *T6* is a reciprocal *t*(1;2^Y) translocation, and *T4* is a reciprocal *t*(2^Y;3) translocation. The translocations are viable only in the balanced condition. The two derivative chromosomes of each translocation are transmitted in the male line (JOHNSON *et al.* 1988; TRAUT *et al.* 1990). Phenotypic markers *ge* and *rb* are recessive eye color mutants. *Sgm* is a dominant mutant with variable expression and incomplete penetrance; it causes loss of an abdominal segment (TRAUT *et al.* 1994). Strains with new sex chromosomes (Except1, 10, 11, 42 and 45) were isolated by TRAUT and WILLHOEFT (1990). With the exception of *rb*, all mutant strains were derived from the Wien stock. Culture and crossbreeding conditions were as described by JOHNSON *et al.* (1988).

DNAs for PCR analysis: Males and virgin females for DNA isolation were routinely taken from the same cultures. DNA was isolated according to BLIN and STAFFORD (1976) with minor modifications.

PCR for RAPDs: RAPDs were obtained in PCR with single decamer primers (purchased from Operon Technologies Inc., Alameda California; sequences listed in Table 1). PCR conditions were modified from WILLIAMS *et al.* (1990). The 25- μ l reaction volume contained 50 mM KCl, 2.3 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 0.1 mM of each dNTP, 1 unit of Taq polymerase (Perkin-Elmer or Pharmacia), 0.4 μ M decamer primer and 40 ng genomic DNA. After an initial 5-min period at 90°, 35 reaction cycles were performed for 15 sec at 90°, 30 sec at 37° and 90 sec with an increase of 3 sec/cycle at 72°. The last cycle was followed by another 10 min at 72°. If necessary, boost reactions were set up for 15 cycles in the same volume with 1/5 of the original reaction product. PCR products were run in 1.5% agarose gels at 1.5 V/cm for about 16 hr and stained with ethidium bromide. A mix of

TABLE 1

Sequences of decamer primers used in this paper

Designation	Sequence 5' → 3'
decA1	CAGGCCCTTC
decA3	AGTCAGCCAC
decA5	AGGGGTCTTG
decA7	GAAACGGGTG
decA8	GTGACGTAGG
decA12	TCGGCGATAG
decA13	CAGCACCAC
decA14	TCTGTGCTGG
decA19	CAAACGTCGG
decB5	TGCGCCCTTC
decB6	TGCTCTGCC
decB11	GTAGACCCGT
decB15	GGAGGGTGTT
decB17	AGGAACGAG

*Hind*III + *Eco*RI double-digested λ DNA with *Hpa*II-digested pBluescriptSK+ DNA was used as a size marker set.

Chromosome assignment of RAPD markers: Commonly several different DNA fragments were amplified to a copy number which made them visible as bands in ethidium bromide stained electrophoresis gels. Among them were male-specific bands, strain-specific bands, bands polymorphic within strains, and bands common to all strains of *Megaselia* used in this study. Most informative for the purpose of this study were those chromosome-specific fragments which occurred in all or most Wien derived strains but not in the Tennessee strain. These RAPD markers were designated according to the decamer primer used, *e.g.*, decB5, and the size of the amplified DNA fragment, *e.g.*, 0.9 kb: decB5-0.9. Chromosome assignment of the markers was based on holandric inheritance typical for Y-linked sequences as opposed to the crisscross type of inheritance of X-linked sequences. Transmission to females and males of backcross F₂s from wild-type Wien, *T4* (chromosome 3 sequences linked to the original Y) and *T6* males (chromosome 1 sequences linked to the original Y) to Tennessee females according to the scheme in Table 2 was exploited to define chromosome 1, chromosome 2 and chromosome 3 markers.

RESULTS

Linkage with phenotypic markers: Sex is determined in *Megaselia* by the presence or absence of a male-determining factor, *M* (Mainx 1966). In the Wien and Tennessee laboratory stocks, *M* is located on a 2^Y chromosome in the Wien and Tennessee stocks (TRAUT *et al.* 1990; WILLHOEFT and TRAUT 1990), designated "original" Y chromosome in this paper. In Except strains, primary sex determination was taken over by other chromosomes. I designate the new sex determining loci *M1*, *M10*, *M11* and *M45* in strains Except1, Except10, Except11 and Except45, respectively. To identify the new Y chromosomes and the sex determining loci, I tested available phenotypic markers for sex linkage in a series of backcrosses. Owing, presumably, to the much lower crossover frequencies in males compared to females (ratio 1/6 to 1/>2000, depending on the loci tested) (BURISCH 1963; MAINX 1964; SPRINGER 1958), a single marker per chromosome was sufficient in the male parent to detect linkage.

TABLE 2

Scheme for assigning molecular markers to their appropriate chromosomes, using data from F_2 backcrosses

Chromosome	Wien		Tenn		Wien F_2		$T4$		$T4 F_2$		$T6$		$T6 F_2$	
	f	m	f	m	f	m	f	m	f	m	f	m	f	m
1	+	+	-	-	+	+	+	+	+	+	+	+	-	+
2 (original X)	+	+	-	-	-	-	+	+	-	-	+	+	-	-
2 (original Y)	-	+	-	±	-	+	-	+	-	+	-	+	-	+
2 (both, X and Y)	+	+	-	±	-	+	+	+	-	+	+	+	-	+
3	+	+	-	-	+	+	+	+	-	+	+	+	+	+

Markers are from wild-type Wien males and from Wien-derived $T4$ and $T6$ males. $T4$ is a balanced 2^Y-3 translocation, $T6$ a balanced $1-2^Y$ translocation. Wien, wild-type Wien; Tenn, Tennessee; Wien F_2 , Tenn ♀ × (Tenn ♀ × Wien ♂) ♂; $T4 F_2$, Tenn ♀ × (Tenn ♀ × $T4$ ♂) ♂; $T6 F_2$, Tenn ♀ × (Tenn ♀ × $T6$ ♂) ♂; f, females; m, males; +, present; -, absent.

TABLE 3

Test of linkage between chromosome 3 marker *ge* or chromosome 1 marker *Sgm* and the male determining locus in strains Except11, Except42 and Except45

Cross				Segregation of phenotypes				Mode of inheritance of the marker
No.	female × male	Source of males	No. of crosses	Females		Males		
				+	Mutant	+	Mutant	
1	+/+ × <i>ge/ge</i>	Except11	1	16	0	22	0	Autosomal
	<i>ge/ge</i> × +/ <i>ge</i>	F ₁	1	9	14	5	12	
2	+/+ × <i>ge/ge</i>	Except42	1	28	0	17	0	Autosomal
	<i>ge/ge</i> × +/ <i>ge</i>	F ₁	3	9	22	17	20	
3	+/+ × <i>ge/ge</i>	Except45	1	41	0	32	0	Autosomal
	<i>ge/ge</i> × +/ <i>ge</i>	F ₁	5	23	17	18	17	
4	<i>Sgm</i> /+ × + /+	Except11	7	111	46	93	17	Autosomal
	+ /+ × <i>Sgm</i> /+	F ₁	5	83	38	73	2	
5	<i>Sgm</i> /+ × + /+	Except42	7	134	48	108	52	Autosomal
	+ /+ × <i>Sgm</i> /+	F ₁	8	94	32	104	6	
6	<i>Sgm</i> /+ × + /+ <i>M45</i>	Except45	7	129	54	101	45	<i>I</i> ^x -linked
	+ /+ × <i>Sgm</i> /+ <i>M45</i>	F ₁	13	76	84	159	0	

Chromosome 3 marker *ge* was transmitted autosomally in crosses with Except11, Except42 and Except45 males (Table 3, #1–#3) indicating that the respective sex determining loci are not located on a chromosome 3. For chromosome 1 only a dominant marker with incomplete penetrance, *Sgm*, was available. Backcrosses of heterozygous *Sgm*/+ males to wild-type females showed *Sgm* to be sex-linked in crosses with Except45 males (Table 3, #6) while the marker was autosomally inherited in crosses with Except11 and Except42 males (Table 3, #4 and #5). Thus the male-determining locus *M45* in Except45 defines a new Y chromosome I^Y derived from a chromosome 1. The sex-determining loci of Except11 and Except42 are neither linked to chromosome 1 nor to chromosome 3 markers, the presumed location on chromosome 2 could not be tested in the same way due to the lack of phenotypic chromosome 2 markers (but see *Linkage with RAPD markers*).

In crosses of Except1 and Except10 males with chromosome 3 markers *ge* and *rb*, the markers were X-linked or Y-linked depending on the cis or trans configuration of the marker relative to the male determining factor (Table 4, #1 and #5 Y-linked; #2, #3, #4, #6, #7 and #8 X-linked. Note that X-linkage in Megaselia is not

associated with hemizyosity in the male). These results confirmed the earlier notion (TRAUT and WILLHOEFT 1990) that the respective new male determining loci, *M1* in Except1 and *M10* in Except10, are located on new Y chromosomes 3^Y derived from chromosome 3. This enabled me to establish lines with continuous *inter se* crosses of the type ge/ge ♀ × $ge/+$ *M1* ♂ and rb/rb ♀ × $rb/+$ *M1* ♂ from Except1 and ge/ge ♀ × $ge/+$ *M10* ♂ and rb/rb ♀ × $rb/+$ *M10* ♂ from Except10 (Table 4, #2, #4, #6, #8). In these lines, females display the mutant phenotype and males are wild-type. The marker alleles are 3^X -linked while the + alleles are located on the new 3^Y chromosomes.

Linkage of *ge* and *rb* with the male-determining loci *M1* or *M10* was not absolute: males and females with recombinant phenotypes appeared among the offspring (exceptional mutant or wild-type females or males, depending on the type of cross, see Table 4). The fraction of recombinants varied from 0.02 to 2.6% between different markers and sex-determining loci (Table 4). Testcrosses with one exceptional wild-type male offspring of the ge/ge ♀ × $ge/+$ *M1* ♂ cross, one exceptional male of the ge/ge ♀ × $ge/+$ *M10* ♂ cross and with one of two exceptional males tested from rb/rb ♀ × $rb/+$ *M10* ♂

TABLE 4

Genetic exchange between the male determining locus (*M1* of Except1, *M10* of Except10) and chromosome 3 markers *ge* or *rb*

Crosses			Segregation of phenotypes				Fraction of recombinants	
No.	Female × male	No. of crosses	Females		Males		%	90% confidence
			+	Mutant	+	Mutant		
1	<i>ge/ge</i> × <i>+/ge M1</i>	5	76	1	1	74	1.5	1.3–1.746
2	<i>ge/ge</i> × <i>ge/+ M1</i>	260	119	6353	4404	46		
3	<i>rb/rb</i> × <i>rb/+ M1</i>	16	0	563	607	0		
4	<i>rb/rb</i> × <i>rb/+ M1</i>	50	1	1207	1647	0	0.02	0.00–0.12
5	<i>ge/ge</i> × <i>+/ge M10</i>	46	1060	25	4	682		
6	<i>ge/ge</i> × <i>ge/+ M10</i>	100	85	4468	3395	56		
7	<i>rb/rb</i> × <i>rb/+ M10</i>	14	4	314	465	25	2.6	2.2–3.0
8	<i>rb/rb</i> × <i>rb/+ M10</i>	50	2	1328	1904	74		

Crosses #1, 3, 5 and 7 are backcrosses of F_1 hybrid males; #2, 4, 6 and 8 are from continuous lines with *inter se* crosses. Results of the two crosses of each pair of markers were homogeneous and, therefore, pooled (last two columns). The difference between *ge-M1* and *ge-M10* is statistically not significant (χ^2 test, $p > 0.05$), all others are significant ($p < 0.01$).

showed 3^X -linked inheritance of the previously 3^Y -linked marker. This indicates that a considerable fraction of the recombinant phenotype classes resulted from crossover events and not from transpositions of the sex-determining factor. I use the fractions of recombinants from Table 4 as genetic map distances and infer the order (*rb*, *M1*)-*ge* for the 3^Y chromosome of Except1 and *rb-ge-M10* for the 3^Y chromosome of Except10 (Figure 1). The maps show that *M1* and *M10*—though both on chromosome 3—are different loci.

Linkage with RAPD markers: Chromosome-specific RAPD markers were selected and defined by testcrosses with translocation strains as detailed in MATERIALS AND METHODS. They were used for further characterization of the new Except sex chromosomes. The respective functional Y chromosomes, new or original ones, were identified by holandric Y-linked inheritance of chromosome-specific RAPD markers in testcrosses set up with Tennessee females.

Five chromosome 1 markers used in this way map *M45* of Except45 to a 1^Y chromosome (Table 5). Similarly, three chromosome 3 markers map *M1* of Except1 to a 3^Y chromosome. Three chromosome 2 markers of the original X and one that labels the original X and the Y locate *M11* in Except11 to the original X. In Except11, therefore, one of the original X chromosomes has become the new Y chromosome 2^Y .

In the wild-type Wien strain, six male specific fragments from the original Y chromosome were identified by comparing male and female DNA lanes of the Wien strain. As could be expected, these markers showed holandric inheritance in the Tennessee ♀ × (Tennessee ♀ × Wien ♂) ♂ backcross. With one exception, *decA19-1.9*, original Y chromosomes from the Tennessee strain

FIGURE 1.—Most likely order of *rb*, *ge* and the male determining factor *M1* or *M10* on the 3^Y chromosome in Except1 and Except10, respectively. The order of *rb* and *M1* in Except1 is arbitrary. Genetic distances are uncorrected genetic exchange frequencies from Table 4.

contained the same fragments as those from the Wien strain.

All markers of the original Y were missing in Except1, Except11 and Except45, confirming that the original Y chromosome had been lost in these lines while sex determination was taken over by a different locus on a different chromosome. In Except42, however, two markers of the original Y chromosome, *decA19-1.9* and *decB17-0.5*, remained male-specific while the other four had been lost. This confirms previous results with RFLP markers (TRAUT and WILLHOEFT 1990) that a segment of the original Y has been retained in the new Y chromosome of Except42. Holandric inheritance of *decB5-0.9* and *decB15-1.1* defines the other part of the new Y chromosome as derived from the original X (Figure 2, Table 5). A third original X marker, *decB17-1.8*, is not included in the new 2^Y chromosome; it maps presumably to the segment which is derived from the original Y. Thus the new Y chromosome in Except42 is a 2^Y

TABLE 5
Test of linkage between chromosome-specific RAPD markers of the Wien strain and the sex locus

RAPD marker	Wien		Tenn		Wien F ₂		E1		E1 F ₂		E11		E11 F ₂		E42		E42 F ₂		E45		E45 F ₂	
	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m
Chromosome 1																						
decA3-2.2	+	+	-	-	-	-	+	+	w	w	+	+	w	w	+	+	w	w	+	+	-	+
decA8-0.7	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
decA13-0.5	+	+	w	w	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	w	+
decB6-2.1	+	+	-	-	w	w	+	+	+	+	+	+	+	+	+	+	w	w	+	+	-	+
decB17-0.9	+	+	-	-	w	w	+	+	+	+	+	+	+	+	+	+	w	w	+	+	-	+
Chromosome 2 (original X and Y)																						
decA7-0.9	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
Chromosome 2 (original X)																						
decB5-0.9	+	+	-	-	-	-	+	+	w	w	+	+	-	+	+	+	-	+	+	+	-	-
decB15-1.1	+	+	-	-	-	-	+	+	w	w	+	+	-	+	+	+	-	+	+	+	w	w
decB17-1.8	+	+	-	-	-	-	+	+	w	w	+	+	-	+	+	+	-	-	+	+	w	w
Chromosome 2 (original Y)																						
decA3-1.7	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
decA14-2.2	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-
decA19-1.9	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	w	w	-	-
decB6-1.5	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
decB11-0.7	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
decB17-0.5	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
Chromosome 3																						
decA1-1.6	+	+	-	-	ND	+	+	-	+	+	+	+	w	w	+	+	w	w	+	+	w	w
decA12-1.1	+	+	-	-	+	+	+	+	+	+	+	+	w	w	+	+	w	w	+	+	w	w
decB11-1.5	+	+	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Wien-derived strains were testcrossed to Tennessee females. Wien, Wien wild-type strain; Tenn, Tennessee; Wien F₂, Tenn ♀ × (Tenn ♀ × Wien ♂) ♂; E1, Except1; E1 F₂, Tenn ♀ × (Tenn ♀ × E1 ♂) ♂; E11, Except11; E11 F₂, Tenn ♀ × (Tenn ♀ × E11 ♂) ♂; E42, Except42; E42 F₂, Tenn ♀ × (Tenn ♀ × E42 ♂) ♂; E45, Except45; E45 F₂, Tenn ♀ × (Tenn ♀ × E45 ♂) ♂; f, females; m, males; +, present; -, absent; w, weak band, this may be either the same fragment or a different one of the same mobility; ND, not determined.

FIGURE 2.—Transmission of the chromosome 2 (original X) marker decB5-0.9 to various backcross F₂s. PCR products on genomic DNAs with decamer primer decB5 from various genomic DNAs were separated by gel electrophoresis. The informative band is indicated by arrows, all other bands are not informative either because they are Tennessee specific bands or polymorphic among Wien-derived strains. For abbreviations, see Table 5.

chromosome composed of original X and original Y segments.

Molecular differentiation of a new sex chromosome:

In one substrain of Except1, the new Y chromosome, 3^Y, could be distinguished from the new X chromosome, 3^X. DecA8-1.3 was present in 3^Y and absent in 3^X (Figure 3b). In another substrain, both, 3^Y and 3^X, carried the marker (Figure 3a). DecA8-1.3 was present in 3^Y chromosomes of Except1 and polymorphic in the 3^X chromosomes of Except1. Although decA8-1.3 is obviously a

FIGURE 3.—3^Y-linkage of decA8-1.3 in Except1. (a) Substrain with the marker in both, 3^X and 3^Y. (b) Substrain of Except1 with the marker only in 3^Y. For abbreviations see Table 5.

chromosome 3 marker, it had previously not been mapped to chromosome 3 because the polymorphism among the Wien-derived translocation strains inhibited mapping.

DISCUSSION

Sex-determining loci in *Megaselia*: The primary sex-determining signal in the best known fly *Drosophila* is the X/A ratio, the balance between X chromosomal numerator and autosomal denominator elements (BRIDGES 1925; CLINE 1988). By contrast in *Megaselia*, presence or absence of a single male-determining factor decides upon male or female development of an embryo. In this

paper, the chromosomal location of the male-determining factor was identified in five lines in which the original *Y* chromosome had been lost (Except lines) (TRAUT and WILLHOEFT 1990). In one line, Except42, the original sex-determining locus, *M*, was preserved within a larger original *Y* segment; the new *Y* chromosome owes its existence to a recombination event between the original *X* and *Y* chromosome. In the remaining four lines, Except1, 10, 11 and 45, primary sex determination was taken over by different new loci, thereby creating completely new *Y* chromosomes. The sex-determining locus *M45* in Except45 is located on a 1^Y chromosome; *M11* in Except11 maps to a new 2^Y chromosome which was derived from an original *X* chromosome. In Except1 and Except10 the sex-determining loci, *M1* and *M10*, are both located on 3^Y chromosomes.

M1 and *M10* though each on a chromosome 3^Y are different loci according to their map positions relative to two phenotypic markers. Similarly different maps could have resulted from only one sex-determining locus but an inversion of a chromosome segment which comprises the two markers. This is, however, improbable since both 3^Y chromosomes were derived from the same source chromosome as reported earlier (TRAUT and WILLHOEFT 1990).

MAINX (1966) had mapped new sex-determining loci in *Megaselia* in two cases each to the same end distal of a group of two markers of his linkage groups *II* and *III* (presumably representing chromosomes *I* and *3* of this study). He interpreted the result by assuming translocations of a terminal sex-determining chromosome segment to the same end of the target chromosomes. As all of MAINX's markers have been lost, it is impossible to compare his maps with the maps in this report. It is clear, however, that the positions of the new sex-determining loci are either not always terminal or not always at the same chromosome end. Although the sequences have not yet been identified, transposition of the primary sex-determining factor *M* into various chromosomal sites is the most plausible mechanism (WILLHOEFT and TRAUT 1990). *M1*, *M10*, *M11* and *M45* are not just non-allelic primary sex-determining factors but new locations of *M*. Frequency of transpositions and size of transposable elements [for review, see BINGHAM and ZACHAR (1989)] are compatible with the observed data.

Megaselia is probably not unique in this respect. In different species of the dipteran genera *Chironomus* (MARTIN and LEE 1988) and *Simulium* (FERADAY *et al.* 1989) the sex-determining function maps to various nonhomologous chromosomes and chromosome arms. The resemblance with *Megaselia* is even stronger in species like *Chironomus tentans* (BEERMANN 1955) and *Culex tritaeniorhynchus* (BAKER and SAKAI 1976) where nonhomologous *Y* chromosomes coexist within the same species. Although actual jumps have not been observed, transposition of a male determining factor is a

likely cause. Different sex-deciding loci in the housefly, *Musca domestica*, on the other hand, are qualitatively different and, therefore, presumably originated from non-homologous genes (DÜBENDORFER *et al.* 1992).

The sex-determining region of the original *Y* chromosome: The new *Y* chromosome in Except42 consists of original *X* and original *Y* chromosomal segments. Two of six RAPD markers (this study) and two of seven RFLP markers (TRAUT and WILLHOEFT 1990) of the original *Y* chromosome are retained in this new *Y* chromosome. Of the three original *X* markers, two map to the *X* chromosomal portion and one to *Y* chromosomal portion. Provided the molecular markers are distributed randomly over the *Y* chromosome, roughly 1/3 of the new *Y* chromosome is derived from the original *Y* chromosome, including the original sex-determining locus, *M*. With *in situ* hybridization, one of the conserved markers was mapped to a site near the telomere of the short arm of chromosome 2 (U. WILLHOEFT, personal communication). Thus the retained original *Y* chromosomal segment is the terminal segment of $2p$. By this, the position of *M* is narrowed in to the p terminal third of the original *Y* chromosome.

The establishment and differentiation of new sex chromosomes: Results presented in this paper illustrate three primary steps of sex chromosome evolution, envisaged theoretically (see Introduction): (1) the acquisition of the sex determining function by a former autosome or *X* chromosome, (2) the beginning of a molecular differentiation between *X* and *Y* and (3) the spreading of crossover suppression between *X* and *Y*.

In general models, acquisition of the sex-determining function by a former autosome pair has been proposed to occur with transition from polygenic to "monogenic" sex determination (RICE 1986) or when populations turn from monoecy to dioecy (CHARLESWORTH 1978). Transfer of the sex-determining function to a new chromosome as in *Megaselia* is a third mode and it is one which can be addressed experimentally in this species.

The transfer of the primary male-determining function to another chromosome starts the differentiation of new *X* and *Y* chromosomes. By that transfer, one copy of an original autosome is singled out to become a new *Y* chromosome while all homologous chromosomes in that line automatically are the new *X* chromosomes (Figure 4). The one chromosome that happens to become a new *Y* chromosome is propagated and transmitted in a clonal fashion in the male lineage of descendants, all male offspring receiving a copy of the new *Y* chromosome (Figure 4, *Y*). The recombination rate in male *Megaselia* is very low and the region of non-recombination (negligible recombination) between *X* and *Y* around the sex determining locus must be accordingly large. In that region, therefore, all new *Y* chromosomes of a specific male lineage are the same variant.

FIGURE 4.—Evolution of X and Y chromosomes. Y chromosome evolution starts from a single chromosome, polymorphisms may arise subsequently by mutation. All Y chromosomes—at least their differential segments—in the male lineage are clonal descendants of that chromosome. The chromosomes homologous to the new Y in that line become the new X chromosomes. They start as a set of chromosomes with inherent polymorphisms, they recombine and segregate in a Mendelian fashion.

In the new X chromosomes in contrast, as multiple copies exist from the start, preexisting polymorphisms like that of RAPD marker decA8-1.3 may be further transmitted or else one of the variants become fixed (Figure 4, X). Thus from the start, besides the location of *M*, Y chromosomes differ statistically from X chromosomes.

Most of the original Y markers, RFLPs (WILLHOEFT and TRAUT 1990) and RAPDs (this report), are present in both independently established laboratory stocks, Wien and Tennessee. This indicates that their Y chromosomes are not only both 2^Y chromosomes but are in a lineage from the same transposition event. The same chromosome has served as a functional Y chromosome since it was established in a common ancestor. Therefore, the “original” X and Y chromosomes are older than the Xs and Ys from recent transposition events, and quite in accordance with theory (see Introduction), they present a more advanced stage of sex chromosome differentiation, visible in RFLPs and RAPDs. They are, however, still a homomorphic chromosome pair and at least one gene, *sh* (the only one that could be investigated), was active on the original Y (JOHNSON *et al.* 1988).

While crossover rates are generally low in male *Megaselia*, the tight linkage with molecular markers implies that crossover suppression in the original Y was extended over a region covering *M* and the Y specific markers. An exceptional crossover in that region was the event by which the 2^Y chromosome of Except42 was brought about.

Transmission of that chromosome in the male line of ancestors was not interrupted by transposition of the male-determining function, at least since the time when the source populations of the two Y chromosomes separated. Competition experiments with *Megaselia* in population cages showed that the favored type of sex chromosomes depends on environmental conditions (SPRINGER 1967). Therefore, the conservation of a com-

mon Y chromosome in the Wien and Tennessee source populations may be due to selection pressure for a favorable allele tightly linked to the male-determining function.

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