program package [J. Devereux, P. Haberli, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984)].
12. A. J. Courey *et al.*, *Cell* **59**, 827 (1989).
13. J. F. X. Diffley and B. Stillman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7864 (1991).
14. A yeast chromosome filter (Clontech) was screened with a radiolabeled probe made from the 0.6-kb Eco RI fragment.
15. Genotypes of yeast strains used in this study are the following: AA295, *MATa/MATα ura3-52/ura3-52 ade2/ade2 +/trp1-1 lys2Δ201/lys2Δ201 his3-200/his3-200 leu2-3,112/leu2-3,112;* SB1, *MATa/MATα ixr1-1::LEU2/+ ura3-52/ura3-52 ade2/ade2 +/trp1-1 lys2Δ201/lys2Δ201 his3-200/his3-200 leu2-3,112/leu2-3, 112;* SB2, *MATa ixr1-1::LEU2 ura3-52 ade2 trp1-1 lys2Δ201 his3-200;* and SB3, *MATa ura3-52 ade2 trp1-1 lys2Δ201 his3-200 leu2-3,112.*
16. Media were prepared and sporulation and tetrad dissections carried out as in (*17*). Yeast transformations were performed by the lithium acetate method [H. Ito, Y. Fudada, K. Murata, A. Kimura, *J. Bacteriol.* **153**, 163 (1983)]. To obtain strain SB1, strain AA295 [H. K. Rudolph *et al.*, *Cell* **58**, 133 (1989)] was transformed to leucine prototrophy with a disrupted copy of the *IXR1* gene. We disrupted the *IXR1* gene by replacing by Eco RV–Pst I insert present in the 3.0-kb Sac I fragment carrying the *IXR1* gene with a DNA fragment containing a copy of the *LEU2* gene. Southern blot analysis confirmed the *IXR1* gene deletion. Strains SB2 and SB3 were grown from spores from dissected tetrads.
17. F. Sherman, *Methods Enzymol.* **194**, 3 (1991).
18. Yeast extracts were prepared from cells grown to mid-logarithmic phase. The pelleted cells were resuspended in 3.5 volumes of lysis buffer [50 mM tris HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 10 mM potassium acetate, 2 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride] and lysed in a French pressure cell at ~15,000 psi. An equal volume of extraction buffer [lysis buffer + 0.8 M $(NH_4)_2SO_4$] was added to the lysate and the mixture rocked gently for 15 min. After centrifugation at 35,000 rpm for 30 min in a Sorvall 45Ti rotor, the supernatant was dialyzed against several changes of storage buffer [20 mM tris-HCl (pH 7.5), 100 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride] and flash frozen in liquid nitrogen. During the extraction, all materials and samples were kept at 4°C.
19. S. J. Brown, P. J. Kellet, S. J. Lippard, unpublished data.
20. Cells grown to saturation in synthetic complete media were treated for 4 hours at 30°C with 10 μM *cis-* or *trans*-DDP and then harvested. We prepared DNA as in (*21*). Platinum concentrations were determined by flameless atomic absorption spectroscopy with a Varian AA1475 instrument equipped with a GTA95 graphite furnace. Concentrations of DNA were measured by ultraviolet spectroscopy.
21. P. Philippsen, A. Stotz, C. Scherf, *Methods Enzymol.* **194**, 169 (1991).
22. S. E. Sherman and S. J. Lippard, *Chem. Rev.* **87**, 1153 (1987).
23. P. M. Pil and S. J. Lippard, *Science* **256**, 234 (1992).
24. E. N. Hughes, B. N. Engelsberg, P. C. Billings, *J. Biol. Chem.* **267**, 13520 (1992).
25. D. E. Szymkowski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10772 (1992).
26. Z. Wang *et al.*, *Biochemistry* **31**, 3694 (1992).
27. S. L. Kelley and M. Rozencweig, *Eur. J. Cancer Clin. Oncol.* **25**, 1135 (1989).
28. We thank J. M. Essigmann, G. R. Fink, and P. O. Ljungdahl for assistance and advice and R. A. Young for the λgt11 yeast expression library. Supported by the National Cancer Institute and by NIH postdoctoral fellowships to S.J.B. and P.J.K.

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A Detailed Genetic Map for the X Chromosome of the Malaria Vector, *Anopheles gambiae*

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***Anopheles gambiae*, the primary vector of human malaria in Africa, is responsible for approximately a million deaths per year, mostly of children. Despite its significance in disease transmission, this mosquito has not been studied extensively by genetic or molecular techniques. To facilitate studies on this vector, a genetic map has been developed that covers the X chromosome at an average resolution of 2 centimorgans. This map has been integrated with the chromosome banding pattern and used to localize a recessive, sex-linked mutation (*white eye*) to within 1 centimorgan of flanking markers.**

We describe the development of a detailed genetic map of the principal malaria vector, *Anopheles gambiae*, using microsatellite markers (*1*). Microsatellites are tandem arrays of simple sequence repeats and have the advantage of being abundant, widely dispersed in the genomes of humans and other higher organisms, extensively polymorphic, and easily assayed by the polymerase chain reaction (PCR) (*1*). Despite the difficulties in maintaining inbred strains and performing crosses in anopheline mosquitoes, the advantages of microsatellites greatly facilitated construction of a map consisting of 24 sex-linked microsatellite markers. This map is anchored to the *white eye* (*w*) mutation (*2*) and is usable with any of the *An. gambiae* strains examined to date (*3*).

To generate the map, we isolated clones containing dinucleotide repeats from libraries of total adult mosquito genomic DNA (*4*) or chromosomal division-specific DNA pools (*5*) by screening with oligonucleotide probes: $(GT)_{15}$, or more rarely $(CA)_{15}$ or $(GA)_{15}$. Tandem arrays of 5 to 48 repeats were revealed by sequencing in 125 $(GT)_{15}$-positive clones, some of which contained interrupted arrays. For selected clones, pairs of primers, usually 20 nucleotides in length and based on unique sequences flanking the repeat array, were designed, synthesized, and used to amplify by PCR the corresponding genomic DNA fragment (*6*) from individual mosquitoes of five *An. gambiae* strains (*3*). The sizes of the amplified fragments were evaluated by electrophoresis in a sequencing gel. Markers encompassing as few as 6 dinucleotide repeats were found to be polymorphic between strains, the size difference ranging from 1 to more than 30 repeats. The inter-strain polymorphism between two strains, Suakoko and WE, was on the order of 85%, and within-strain polymorphism was also observed for many markers.

We constructed the genetic map and simultaneously mapped the *w* mutation by performing crosses involving the WE strain (homozygous for *w*) (*3*) and the Suakoko strain (homozygous for w^+, the dominant allele for the wild-type dark purple eye color). These strains are largely homosequential, that is, they do not seem to differ in terms of gross chromosomal inversions on the X. F_1 females were generated by mass mating between WE males and Suakoko females and were individually backcrossed with WE males by forcible pair mating (*7*). From such pairs five families (A to E) totaling 248 progeny were obtained; they contained 45, 63, 76, 13, and 51 progeny, respectively. Chromosomal DNA was prepared from each mosquito (*8*), and small portions (typically corresponding to 1/200 of a mosquito) were amplified with a pair of primers (*6*) and scored for the corresponding marker on a sequencing gel. All alleles (electrophoretic bands) behaved as codominant Mendelian factors. For 24 markers, the male progeny received one of the maternal (F_1) alleles and no paternal (WE) allele, indicating linkage to the X chromosome. These markers were designated AGXH (*An. gambiae* X chromosome, Harvard) followed by a clone number; they are identified in Table 1. The AGXH prefix will be omitted hereafter for convenience. Genotypic scoring for one of the markers is shown in Fig. 1 and the results of scoring family A for all loci are summarized in Table 2.

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In general, the alleles derived from the Suakoko female grandparent were designated *a*, and those derived from the WE male grandparent were designated *b* (for exceptions, see Tables 2 and 3). Linkage of the *w* mutation to the *b* allele of each locus was demonstrated in the backcross progeny by the preponderance of b,w and a,w^+ among the hemizygous males. The exceptional males (b,w^+ or a,w) were recombinant, and their frequency indicated the distance between that microsatellite locus and the *white eye* gene. Similarly, two types of rare recombinant female progeny were encountered (b/b, w^+/w or a/b, w/w); the two other predominant types of female progeny (b/b, w/w or a/b, w^+/w) were nonrecombinant (see also Fig. 1).

Effectively, typing all microsatellites and the visible eye marker on the same set of mosquitoes corresponded to a 25-factor cross, permitting us to arrive at a very robust genetic map with only 248 progeny. By considering the total frequency of recombinants between each microsatellite marker and *white eye* in all the families, we could arrange the markers in a linear order representing the order of the loci on the X chromosome (Tables 2 to 4). Three-factor analysis permitted us to assign any two microsatellite markers on the same or opposite sides of *w*, depending on whether the frequency of recombination between these microsatellites was lower or higher than that between either marker and *w*. The genetic distances, as calculated from recombination frequencies between microsatellites and between microsatellites and *w*, were consistent, with due allowance for double recombinants.

Whereas distances between markers can only be determined from recombination frequencies, the order of markers can be determined independently by logic: recombination events delimit nested blocks of contiguous markers, which differ by steps that reveal the order of the markers. Table 3 exemplifies this analysis in 18 highly informative recombinant mosquitoes. Logical analysis of all the recombinants confirmed the frequency-based order of the markers and also revealed rare double recombinants. With the available data, a few markers could not be separated (AGXH-180 from -289; -179 from -1002; -24 from -25 and -80; and -7 from -32 and -81), and thus the map has 19 resolved loci. Figure 2 summarizes all the data, in the form of a genetic map that indicates both the order and the genetic distances between neighboring loci. The total length of the map is ~40 cM, and the average distance between resolved loci is 2.1 cM.

Excellent polytene chromosome preparations can be obtained from *An. gambiae* nurse cells (9). Thus, we were able to anchor the genetic map to the cytological map represented by the polytene banding pattern (Table 1 and Fig. 2). Some microsatellites could be localized to a chromosomal division by virtue of their origin from DNA that was microdissected from that division and subsequently amplified by PCR and cloned (5). Others were localized by in situ hybridization to polytene chromosomes (*10*). Usually, the repetitive microsatellite sequence did not interfere with specific hybridization if the array contained fewer than 11 repeats; however, even then interpretable signals could not be obtained in 2

Table 1. X-linked microsatellite markers.

AGXH Marker	Allel.*	Cytol.†	5′ primer (5′→3′)	Repeats	3′ primer (5′→3′)
145	3 (0)		TGGTGGAATGTGAGACACAG	(GT) 11	ATGATGGTCGATCCTTGTCC
77	3 (1)	4B	TGGGACTGTAAGTGTCTCCC	(GT) 10	TATCAGTGAGGCCGAGTTGC
503	2 (3)	(4)	AGGTTAGAGTGAGCAACCAC	(GT) 30	GCACTGCATCTCTCCAATAC
71	2 (2)		GCGGAGTTATTTCCTGAACC	(GT) 8	ACAGGCCAAGCAAATGCAGG
180	2 (1)	3B	GTATGTTGTGATCTCCTGCC	(GT) 10	AAAACGAGCCACCACCAGAG
106	2 (1)		CTCTTGGCTTACGCTCCTTG	(GT) 4+12+8	GGGAATGAAGATGAGAAGCC
289	2 (1)		CTGCGAACTTTGCTGATTCG	(GA) 9	TTCGCCAAACTGACAACTGC
19	2 (2)	(3)	CTTTTTCTCCCCATTATCTC	(GT) 9	CTGCAGTGTCCATTACGTAC
38	2 (1)		AGTGACTACGCTTCTCGGAG	(GT) 7+4	AAGTCATACTCTTGCGCCCG
1002	2 (1)	3C	GATCGGTATATGCTTCCCGC	(GA) 23	AATAAGCCACGGCGTATCCC
179	2 (1)		CCATCCCCTCGACAGACC	(GT) 10+6	AACGACGTAAGCTGACACGG
131	2 (2)		TTCCCACACTTTCTCCCAGG	(GT) 48	ATAATGCGCTGCTCCCAAGG
24	2 (2)	(3)	GGAGGCTAAAATCACGGTTG	(GT) 36	GATCGGCAAGACTATCGGCC
25	2 (4)	(3) 3D	GCCGAAAACATTCCAACAGG	(GT) 9	CAGTTATGTCGGCATGCTAC
80	2 (3)	2B	TGCTCTCTCCTACATCGAGG	(GT) 9	GCCAGTGCTCTAGATTAACG
99	2 (1)	2C	CGGGAATTTGTTGCTTCCTG	(GT) 8	TCGCCCTCTTTCTCCATCTC
49	3 (2)	1D	CAGCGCCTCCATATAGAACG	(GT) 5+4	GATCATTCAGCTGAACCTGC
7	2 (0)	(1) 1C	CACGATGGTTTTCGGTGTGG	(GT) 8	ATTTGAGCTCTCCCGGGTG
32	3 (4)		CGGTGCGTGTTCCTCGTGC	(GT) 29	TATGGTGTGGTTTCCCGTCC
81	2 (0)		CACTGTAAATCGGAAGCGCG	(GA) 7	CGGGCGGTTAAAGAAAACGG
8	2 (1)	(1)	GGATGTGCTCCCAATACAAG	(GT) 4+6	CTTATCGCACTGCAAGTGTC
37	2 (1)	1C	ATGTCTTGCTCACCTCGAGC	(GA) 9	TAAGTTGGGCGTCTTGCTGG
100	2 (0)		AGAAAGGAAATGTAACGCGG	(GA) 7	CTTTCATCTTGGCTGGCTGC
412	2 (0)	(6)	GCATGCACCTGTTGGGACAG	(GT) 16+4	AAACCTTACCCAAAACACAG

*Number of alleles detected by PCR amplification in five families (see Table 4), each originating from one Suakoko female and one WE male. Additional alleles (numbers in parentheses) were found in five females, each randomly chosen from colonies AA, G3, Mopti, Suakoko, and WE. †Cytological origin according to the division and subdivision where the marker hybridizes on polytene chromosomes or (in parentheses) according to the numbered chromosomal division from which the marker was cloned (*5*).

Table 2. Number of progeny of family A with the indicated genotypes at 25 sex-linked loci. The first column identifies each mosquito as male or female, and the second (*n*) reports numbers of progeny for each genotype. The genotypes are indicated in the remaining columns, one column for each marker except for markers not resolved by present data (the prefix AGXH is omitted from the headings for convenience). For each marker, the maternal genotype was *a/b* and the paternal *b*; in the case of AGXH-145 (asterisk), the paternal genotype was *c*, and therefore the female progeny were actually either *a/c* or *b/c*. At the *white eye* locus, the maternal genotype was +/− (w^+/w) and the paternal was −(*w*). Recombinant markers are shaded. The last row (T) shows the total number of recombinants between each locus and *w*.

					19					7,8		
					38			24		32		
					106			25		37		
		77			180	179		80		49		
Sex	*n*	145*	503	71	289	1002	131	99	*w*	81	100	412
m	3	a	a	a	a	a	a	a	+	a	a	a
m	1	b	b	b	b	b	a	a	+	a	a	a
m	1	b	b	b	b	a	a	a	+	a	a	a
m	2	b	b	b	a	a	a	a	+	a	a	a
m	1	b	b	a	a	a	a	a	+	a	a	a
m	1	a	a	a	a	a	a	a	+	a	a	b
m	5	b	b	b	b	b	b	b	−	b	b	b
m	2	a	a	b	b	b	b	b	−	b	b	b
f	9	a/b	a/b	a/b	a/b	a/b	a/b	a/b	+/−	a/b	a/b	a/b
f	1	b/b	b/b	b/b	b/b	b/b	b/b	b/b	+/−	a/b	a/b	a/b
f	2	b/b	b/b	b/b	b/b	b/b	b/b	a/b	+/−	a/b	a/b	a/b
f	1	b/b	a/b	a/b	a/b	a/b	a/b	a/b	+/−	a/b	a/b	a/b
f	11	b/b	b/b	b/b	b/b	b/b	b/b	b/b	−/−	b/b	b/b	b/b
f	1	a/b	a/b	a/b	a/b	b/b	b/b	b/b	−/−	b/b	b/b	b/b
f	1	a/b	a/b	b/b	b/b	b/b	b/b	b/b	−/−	b/b	b/b	b/b
f	1	b/b	b/b	b/b	b/b	b/b	b/b	b/b	−/−	a/b	a/b	a/b
f	1	b/b	b/b	b/b	b/b	b/b	b/b	b/b	−/−	b/b	a/b	a/b
T	45	13	12	8	6	4	3	1	0	1	2	3

out of 10 cases, possibly because of the existence of additional repetitive sequences. Table 1 and Fig. 2 report our current cytological assignments of 14 microsatellite markers. In most cases the assignments are consistent with the genetic map, although two slight discrepancies were observed (inversions of blocks of markers: 180, 1002, and 25, and also 80 and 99; Fig. 2). We have no explanation for these discrepancies, but we cannot exclude the possibility of small undetected chromosomal rearrangements in the stocks used for genetic mapping, especially in the WE strain, which has multiple inversion polymorphisms. The distal divisions, 4 and 3, account for 80% of the genetic map of the X chromosome.

The integrated genetic and cytological map presented in Fig. 2 permitted us to localize the *w* mutation to within 1 cM from markers on either side, in region 1D to 2C of the polytene X chromosome. Recently, the *An. gambiae* homolog of the

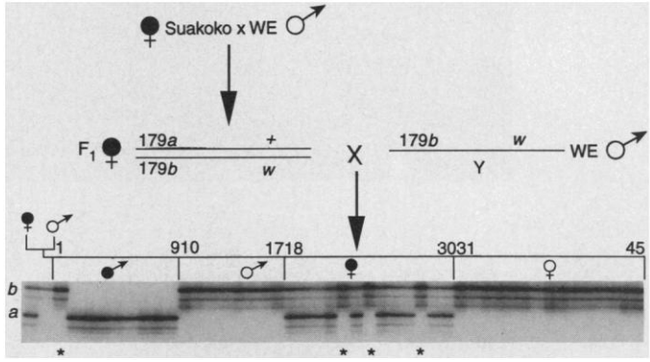

Fig. 1. Genetic crosses and microsatellite typing for marker AGXH179 in family A. The microsatellite allele originally provided by Suakoko is shown as 179*a* or *a*; this is linked to the wild-type dark purple eye allele (*+*). Conversely, the 179*b* or *b* allele from the WE strain is linked to the white eye allele (*w*). Each lane of the autoradiogram corresponds to a parent (♀, ♂) or one of the 45 progeny, which are grouped by sex and eye phenotype. Because of PCR artifacts and impurities in the primers, each microsatellite allele yields a cluster of closely spaced bands, but the *a* and *b* alleles can be distinguished unambiguously. Note that, in general, purple-eyed mosquitoes (filled symbols) show the *a* allele if male, and *a* plus *b* if female; white-eyed mosquitoes (open symbols) show exclusively the *b* allele, as expected from the parental linkage relations. Exceptional individuals (asterisks) are recombinant.

Table 3. Selected individual progeny that are informative for ordering microsatellite markers. Conventions are as described in Table 2. The third column indicates expected recombinant genotype at each locus. Mosquitoes are identified by family (A through E) and numerical code. For simplicity, maternal genotypes are designated *a/b* and paternal *b*, even if the alleles of different families are not identical. Exceptions are paternal genotypes of family A at marker 145 (*c*) and family C at marker 145 (*a*). In some cases (asterisks) the mother was homozygous, and thus recombination could not be detected with this marker. ND, not determined. Note that mosquito B:26 can be identified unambiguously as a double recombinant.

Code	Sex	Rec.	145	77	503	71	106	180 289	19	38	179 1002	131	24 25 80	99	*w*	49	7	32 81	8	37	100	412
B:21	m	a	a	b	b	b	b	b	b	b	b	b	b	b	-	b	b	b	b	*	*	b
B:19	m	a	a	a	b	b	b	b	b	b	b	b	b	b	-	b	b	b	b	*	*	b
A:12	m	a	a	a	a	b	b	b	b	b	b	b	b	b	-	b	b	b	b	b	b	b
B:30	m	a	a	a	a	a	b	b	b	b	b	b	b	b	-	b	b	b	b	*	*	b
B:28	m	a	a	a	a	a	a	b	b	b	b	b	b	b	-	b	b	b	b	*	*	b
B:27	m	a	a	a	a	a	a	a	b	b	b	b	b	b	-	b	b	b	b	*	*	b
B:20	m	a	a	a	a	a	a	a	a	b	b	b	b	b	-	b	b	b	b	*	*	b
E:24	m	a	a	a	a	a	a	a	a	a	b	b	b	b	-	b	*	b	b	b	b	ND
B:26	m	a	b	b	b	a	a	a	a	a	a	b	b	b	-	b	b	b	b	*	*	b
A:24	f	b/b	b/c	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	a/b	a/b	+/-	a/b	a/b	a/b	a/b	a/b	a/b	a/b
D:01	m	b	b	b	b	b	b	b	b	b	b	b	b	a	+	a	a	a	a	a	a	ND
E:04	m	b	b	b	b	b	b	b	b	b	b	b	b	b	+	a	*	a	a	a	a	ND
A:45	f	a/b	b/c	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	-/-	a/b	a/b	a/b	a/b	a/b	a/b	a/b
C:64	f	a/b	b/a	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	*	-/-	b/b	a/b	a/b	a/b	a/b	a/b	a/b
D:04	m	a	b	b	b	b	b	b	b	b	b	b	b	b	-	b	b	b	a	a	a	ND
C:47	f	b/b	a/a	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	*	+/-	a/b	a/b	a/b	a/b	b/b	b/b	b/b
A:31	f	a/b	b/c	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	b/b	-/-	b/b	b/b	b/b	b/b	b/b	a/b	a/b
C.38	f	b/b	a/a	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	a/b	*	+/-	a/b	a/b	a/b	a/b	a/b	a/b	b/b

Table 4. Total number of recombinants in five mosquito families. In each family (A through E) the number of recombinants from each marker relative to *w* is listed, out of the number of progeny indicated in the second column. Mothers were heterozygous except as indicated by asterisks (where recombination could not be detected because of maternal homozygosity). The 412 marker could not be scored in families D and E for technical reasons. Thus, a total of 248 progeny were scored for most markers, except as indicated (†, 172 progeny; ‡, 197; §, 185; ‖, 184; and ¶, 121). ND, not determined. The last two rows show the total number of recombinants between each microsatellite and *w* (All) or between adjacent microsatellites (Adj.). Note that the order of markers inferred by recombination frequencies is the same as that inferred from the nested blocks of recombinant markers (Table 3).

Fam.	Prog.	145	77	503	71	106	180 289	19	38	179 1002	131	24 25 80	99	*w*	49	7	32 81	8	37	100	412
A	45	13	13	12	8	6	6	6	6	4	3	1	1	0	1	1	1	1	1	2	3
B	63	22	20	18	13	11	10	7	4	4	3	3	0	0	0	0	0	0	*	*	3
C	76	27	20	17	11	7	7	5	5	3	2	0	*	0	0	1	1	1	2	2	5
D	13	4	3	3	2	2	2	1	1	1	1	1	0	0	0	0	0	1	1	1	ND
E	51	21	18	16	11	8	8	6	5	1	1	1	1	0	0	*	2	2	2	2	ND
All	248	87	74	66	45	34	33	25	21	13	10	6	2†	0	1	2‡	4	5	6§	7§	11‖
Adj.		13	8	20	11	1	8	4	8	3	4	4	2†	1	1‡	0	1	1§	1§	4¶	

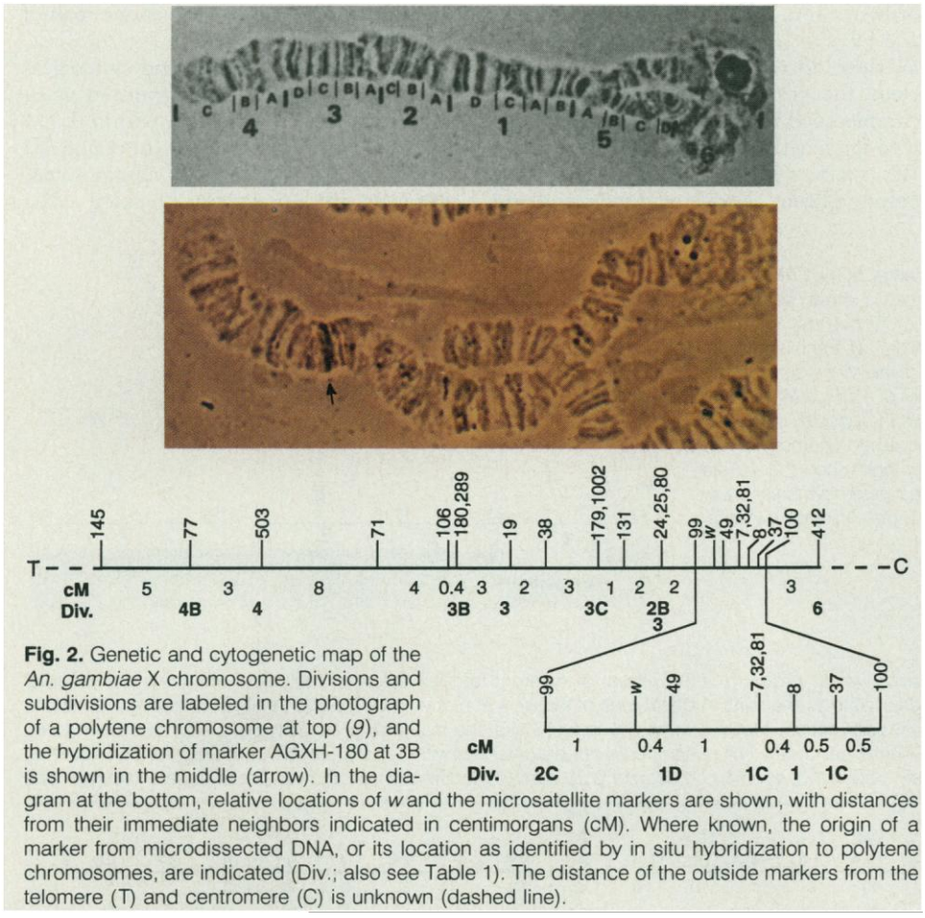

Fig. 2. Genetic and cytogenetic map of the *An. gambiae* X chromosome. Divisions and subdivisions are labeled in the photograph of a polytene chromosome at top (*9*), and the hybridization of marker AGXH-180 at 3B is shown in the middle (arrow). In the diagram at the bottom, relative locations of *w* and the microsatellite markers are shown, with distances from their immediate neighbors indicated in centimorgans (cM). Where known, the origin of a marker from microdissected DNA, or its location as identified by in situ hybridization to polytene chromosomes, are indicated (Div.; also see Table 1). The distance of the outside markers from the telomere (T) and centromere (C) is unknown (dashed line).

Drosophila white gene has been cloned independently and mapped by in situ hybridization to region 2A (*11*), thus further validating our map and suggesting that the white eye phenotype indeed is caused by a mutation in this locus.

Current methods make feasible the detailed molecular and genetic analysis of organisms that have received scant attention to date. The procedures that we have adopted can be used to analyze the autosomes and produce a similarly detailed genetic map. This will permit localization and characterization of important genes, such as those that control the susceptibility or refractoriness of mosquitoes to the malaria parasites (*12*).

Note added in proof: We now have a total of 31 microsatellite markers resolvable at 23 loci, with a total map distance of about 44 cM. A genetic map consisting of 53 restriction fragment length polymorphic DNA markers has been developed for *Aedes aegypti*, the yellow fever vector (*13*).

REFERENCES AND NOTES

1. J. L. Weber and P. E. May, *Am. J. Hum. Genet.* **44**, 388 (1989); M. Litt and J. A. Luty, *ibid.*, p. 397.
2. We recommend the use of the symbols *w* and w^p for the extant *white eye* and *white eye*^{pink} alleles, respectively. It should be noted that the w^p but not the *w* mutation is the same as that described by Mason [G. F. Mason, *Genet. Res.* **10**, 205 (1967)]. Unlike the extant *white eye* [C. F. Curtis, *R. Soc. Trop. Med. Hyg.* **70**, 281 (1976)], Mason's *white eye* mutation (no longer in existence) was epistatic to c^+ (*collared*) and not allelic with w^p [J. B. Kitzmiller and G. F. Mason, in *Genetics of Insect Vectors of Disease*, J. W. Wright and R. Pal, Eds. (Elsevier, Amsterdam, 1967), pp. 3–15]. The symbols Mason used for his *white eye* and *pink eye* genes, respectively, were *w* and *p*. The system we are suggesting contradicts the original assignment of the *w* symbol, but is consistent with current usage and the conventions of genetic terminology in *Drosophila* [see also (*11*)].
3. We have examined mosquitoes from five strains. One is the widely available G3 strain, which was initially established from material collected in Gambia and maintained since the mid-1970s at the London School of Hygiene and Tropical Medicine. It is thought to have received several admixtures since then. Strain AA was selected as an isofemale line from G3; it was lost earlier this year. Strain WE originated from the 2X colony maintained in the London School of Hygiene and Tropical Medicine. The Suakoko (2La) and Mopti strains were selected by the laboratory of M. Coluzzi (Rome) from material collected in Liberia and Mali, respectively. The karyotype of Suakoko is Xag, 2R+, 2La, 3R+, 3L+; for Mopti it is Xag, 2Rbc, 2La, 3R+, 3L+; the rest of the strains show extensive inversion polymorphisms.
4. Genomic DNA was prepared from 50 adult Suakoko mosquitoes and digested with Sau 3AI or Hpa II. Size-selected fragments [200 to 500 base pairs (bp)] were then cloned into the Bam HI or Acc I sites of M13mp18, respectively. Recombinant plaques were screened with an oligonucleotide labeled at the 5′ end, usually $(GT)_{15}$. Single-stranded DNA was prepared from positive plaques and sequenced by the dideoxynucleotide termination method [F. Sanger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)]. Recovery of microsatellite-containing clones indicated the existence of at least 5000 GT-repeat arrays per genome; the density on the X appears to be double that on the autosomes.
5. Microdissected and PCR-amplified DNA from specific chromosomal regions [L. Zheng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11187 (1991)] was treated with Sau 3AI and cloned into the Bam HI site of M13mp18. Recombinant plaques were screened as above.
6. A pair of oligonucleotides were designed for each clone on the basis of sequence analysis by computer (Probepicker, Harvard Genome Facility) and were synthesized in a MilliGen Cyclone Plus DNA synthesizer (Millipore). The oligonucleotides were dissolved in water at a working concentration of 20 μM. Genomic DNAs from single mosquitoes (~10 ng, 1/200 of the total) were used as templates for PCR reactions (total volume, 10 μl), in a Falcon microtiter plate (Becton Dickinson) as described [T. J. Hudson *et al.*, *Genomics* **13**, 622 (1992) and Instructions provided by M. J. Research, Inc., Watertown, MA], except that the enzyme was Taq polymerase (Promega). An equal volume of 98% formamide loading solution [J. Sambrook *et al.*, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989)] was added, the samples were denatured at 65°C for 10 min and chilled on ice, and 2.5-μl portions were loaded into a 6% polyacrylamide gel containing 8 M urea. After electrophoresis the gel was exposed to Kodak XAR-5 film.
7. R. H. Baker and J. B. Kitzmiller, *Am. Zool.* **1**, 435 (1961).
8. Genomic DNA from single mosquitoes was prepared by a modification [L. H. Miller *et al.*, *Science* **237**, 779 (1987); P. Romans *et al.*, *J. Med. Entomol.* **28**, 147 (1991)] of the procedure for preparation of fruit fly DNA [W. Bender *et al.*, *J. Mol. Biol.* **168**, 17 (1983)]. The DNA was resuspended in 200 μl of water and incubated at 65°C for 10 min before storage at −80°C.
9. M. Coluzzi *et al.*, *R. Soc. Trop. Med. Hyg.* **73**, 483 (1979).
10. The polytene chromosomes were prepared from semigravid ovaries of adult mosquitoes according to the procedure of Green and Hunt [C. A. Green and R. H. Hunt, *Genetica* **51**, 187 (1980)] with the following modifications. The squashes were left in a moist chamber overnight at 4°C to flatten the chromosomes. After being dipped in liquid nitrogen, the cover slips were removed, and the slides were dehydrated through a graded series of ethanol, dried, and processed for in situ hybridization. The slides were baked at 60° to 65°C for 20 min (to reduce nonspecific hybridization of the repeats), treated with 4% paraformaldehyde for 2 min, and again dehydrated. The probe was prepared with the BRL Bionick labeling kit according to instructions. After dilution with an equal volume of 20% dextran sulfate, the probe was hybridized to the chromosomes at 95°C for 10 min and then in a humid chamber overnight at 42°C. The signal was detected with the BRL in situ hybridization and detection kit according to instructions, except that all steps were carried out at 42°C.
11. N. J. Besansky, personal communication.
12. F. H. Collins *et al.*, *Science* **234**, 607 (1986); K. D. Vernick and F. H. Collins, *Am. J. Trop. Med. Hyg.* **40**, 593 (1989).
13. D. W. Severson *et al.*, *J. Hered.*, in press.

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