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Towards a Genetic Map for *Anopheles albimanus*: Identification of Microsatellite Markers and a Preliminary Linkage Map for Chromosome 2

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Abstract. Fifty microsatellite loci were identified in the malaria vector *Anopheles albimanus*. Markers segregating in F2 progeny of crosses between laboratory strains of *An. albimanus* were used to construct a preliminary genetic map. More than 300 progeny were genotyped, but the resolution of the map was limited by the lack of polymorphisms in the microsatellite alleles. A robust linkage map for chromosome 2 was established, and additional markers were assigned to the third and X chromosomes by linkage to morphological markers of known physical location. Additional non-informative microsatellite sequences are provided including some showing similarity to those of *An. gambiae*. This study significantly increases the number of genetic markers available for *An. albimanus* and provides useful tools for population genetics and genetic mapping studies in this important malaria vector.

INTRODUCTION

Anopheles albimanus is widely distributed throughout the tropics and subtropics of the Americas, extending from the southern United States to northern Peru and the Caribbean Islands. It is the primary coastal vector of malaria in these regions. Unlike many anophelines, which are members of species complexes, there is no evidence for cryptic species of this vector.[1,2] Nevertheless, *An. albimanus* populations differ in their host preference and vectorial capacity, and barriers to gene flow between Atlantic and Pacific populations have been detected.[3,4] The power of genetic analyses has been limited by a lack of neutral markers for *An. albimanus*. A linkage map consisting of biochemical and morphological markers has been reported,[5] but this contained no microsatellite or SNP markers, and few of the markers are extant for developing the map further.

In this study we report the sequences of 50 new microsatellite markers that will facilitate detailed population genetic studies of this malaria vector. We were able to use a small number of the existing biochemical markers, whose cytological location had been determined,[5] to anchor the microsatellite-based map to chromosome arms of *An. albimanus*. In addition, we have constructed a well-supported low-resolution map for chromosome 2 of *An. albimanus*. The development of this integrated genetic and cytogenetic map will facilitate molecular and population genetic studies in this species.

MATERIALS AND METHODS

Identification of microsatellite markers. Clones were identified by two methods: A genomic plasmid library was created by digesting c-purified *An. albimanus* deoxyribonucleic acid (DNA) with *Sau* 3AI and ligating it into phosphatased *Bam* HI-digested pUC18 plasmids. Colonies were blotted onto nitrocellulose membranes and probed with digoxigenin-labeled microsatellite primers including various di-, tri-, and tetranucleotide repeats (GC10, TA10, ATG8, CTG8, AAC8, GAA8, TCAG6, TGAA6, CTGA6, CAGA6). Hybridization-positive clones were purified and sequenced using M13 forward and/or reverse primers. Additional loci were identified using a procedure to enrich the library for microsatellite-containing plasmids.[6] Primers to amplify regions containing microsatellites were designed using the program Primer3[7] or by eye. DNA sequencing was performed on an Applied Biosystems ABI 377 sequencer (Life Technologies, Carlsbad, CA) using BigDye reagents and standard M13 forward and reverse primers.

Mosquito culture and genetic crosses. Mosquitoes were cultured under conditions described by Benedict.[8] Briefly, crosses were performed in aluminum and gauze cages[9] or pint (approximately 1/2 L) paper cups. Two types of genetic crosses were conducted. In the first, a stock previously selected by insecticide exposure for high levels of oxidase and esterase (HIOX/HIEST)[10] was crossed with PREBST+. The latter resulted from genetic mapping crosses reported elsewhere,[11] and had high frequencies of the dominant alleles of propoxur resistance,[7,12] *ebony,*[11] and *Stripe*.[13] Individual females were separated after blood feeding for oviposition. The F1 families were reared separately and intercrossed to produce the F2 generation. L3 and L4 larvae were scored for the two morphological markers, *ebony* and *Stripe,* or were cultured to the pupal stage and scored for sex and, in some families, *Stripe*, before being frozen for later DNA extraction. In the second set of crosses, a stock originating from Santa Tecla, El Salvador (STECLA) was crossed with PREBST+. STECLA is pure-breeding for the recessive alleles of the PREBST+ markers described above. Individual F1 siblings were crossed to produce F2 families. These were raised to the pupal stage and scored for sex and, in some families, the presence of the *Stripe* phenotype, before being frozen for later DNA extraction.

Four F2 families were selected for genotyping, each originating from a distinct parental pair. For crosses resulting in multiple isofemale lines, the families with the largest number of progeny were selected. One of these, family AJ-1, originated from the HIOX/HIEST × PREBST+ cross, and the remaining three (families 108N, 121D and 129D) were from crosses between STECLA and PREBST+.

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Analysis of Microsatellites. Genomic DNA was extracted from the parents and progeny of the single pair crosses as described previously.[14] The microsatellite loci were amplified using fluorescently labeled primers with a reaction cycle of 95°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The polymerase chain reaction (PCR) products were resolved by capillary electrophoresis using either an ABI 377 or a CEQ8000 (Beckman Coulter Inc., Fullerton, CA) automatic sequencer. A size standard was included in each lane and allele sizes were determined using the Applied Biosystems GeneScan or CEQ8000 (Beckman Coulter Inc., Fullterton, CA) software. Initially the parental and F1 generations were genotyped at 50 loci to identify informative markers for each family. The F2 progeny were then scored for each of these informative markers and χ^2 goodness of fit tests were performed to identify markers not segregating according to Hardy Weinberg equilibrium. MapMaker/Exp V3.0[15] and JoinMap V3.0[16] were used to identify linkage groups. The nomenclature used for the markers and reported in the GenBank entries is the pattern "Aalbi-CA-####" where C is the chromosome if assigned (X, Y, 2, 3) or U if unassigned, A is the arm if assigned or N if unknown, and #### is an arbitrary identification number. Throughout this manuscript, we will refer to the markers only by their number. Eleven adult *An. albimanus* collected from cattle corrals from six villages from coastal Chiapas province, Mexico between May 1997 and June 1998 as part of a large scale insecticide resistance management trial[17] were also used to further assess the polymorphism of some of the microsatellite markers.

RESULTS

Identification of *An. albimanus* microsatellite markers. Ninety-six sequences were identified by screening a plasmid library for repetitive DNA. The inserts were sequenced and 12 were discarded either because they contained less than four repeat units or because the repeat was at the end of the clone, and hence flanking primers could not be designed. Primers were designed to amplify the microsatellite repeats in the remaining 84 clones. Some plasmids contained more than one repeat, and in these cases, primers were designed to independently amplify each microsatellite repeat. Each primer pair was tested for amplification using genomic DNA extracted from three laboratory strains of *An. albimanus.* Products of the expected size were obtained for 75 loci. Fifty markers were selected for the present study. Table 1 summarizes the procedure for the selection of microsatellite markers used in this study.

Markers were tested for polymorphism using laboratory strains and field collected mosquitoes from six sites in Chiapas, Mexico. More than one allele was detected for 36 of the microsatellites. The primer sequences, expected allele size, and presence or absence of polymorphism for each of the 50 markers are shown in Table 2.

Table 1
Steps for identifying microsatellite markers in *Anopheles albimanus*

Plasmids containing repetitive DNA identified by screening of genomic library	96
Presence of repeats confirmed by sequencing	84
Positive amplification from gDNA	75
Microsatellites tested for polymorphism	50
Number of polymorphic microsatellites	36
Number of markers informative for genetic crosses	25

Linkage analysis. Four F2 families were selected for genotyping. Informative markers were identified by scoring the F0 parents of the crosses and the results confirmed by genotyping of the F1 generation. A total of 303 F2 progeny were analyzed from these four families (94 from family AJ-1, 90 from 108N, 80 from 121D, and 39 from 129D). The number of informative markers ranged from 14 (family 129D) to 23 (family AJ-1).

Sex-linked markers. All markers were analyzed for Hardy Weinberg equilibrium assuming a segregation ratio of 1:2:1 (homozygous parental: heterozygous: homozygous parental) in the F2 progeny. Those markers that significantly deviated from the expected ratios were re-analyzed for sex linkage. Only females are informative for X-linked markers and therefore, for those families where the sex of the progeny was known, the males were excluded from this analysis. Two candidate X linked markers were identified. Marker 0086, which was informative in three families, segregated in a 1:1 ratio of heterozygous: homozygous for the maternal genotype in the female progeny ($P = 0.48$) suggesting that this marker is located on the X chromosome. The *white* locus, which has previously been localized to the X chromosome in *Anopheles albimanus*, also segregated in the ratio expected for a sex-linked marker. This marker was only informative for family AJ-1 whose sex was not determined. Assuming this family has an equal number of males and females in the F2 generation, a ratio of 2:1:1 (homozygous maternal: heterozygous: homozygous paternal) would be expected. The observed ratio for the *white* locus in this family matched this expected distribution ($P = 0.184$). Unfortunately, as none of the families analyzed were informative for both 0086 and *white*, it was not possible to test for linkage between these putative X-linked markers.

Autosomal markers. Of the remaining 23 informative loci, five loci showed segregation disorders in one or more families (Table 3). One of these (0056) never segregated as expected and was therefore removed from the subsequent analysis. In all other cases markers showing segregation disorders were removed from selected families.

Initially each family was analyzed separately, using MapMaker/Exp with a minimum logarithm of the odds (LOD) score of 3.0, to identify putative linkage groups. Three distinct linkage groups (linkage group 1–3) were identified within each of three of the four families. However, in the largest family, AJ-1, a single linkage group, consisting of 15 markers was obtained. The AJ-1 linkage group contained markers from the initial linkage groups 2 and 3. We therefore used Joinmap to integrate the maps from the different families. This confirmed the linkage between groups 2 and 3. This large linkage group, which will hereafter be referred to as linkage group 2, consisted of 14 markers with defined order spanning 124 cM (Figure 1).

Linkage group 1, consisting solely of markers 0143 and 0057, was unaffected by the pooling of the data. This linkage group was not detected in family AJ-1 as marker 0143 was non-informative in this family.

Unassigned markers. Seven markers were unlinked in the pooled analysis. Three of these, 0020, 0022, and 0087, were associated with linkage group 2 in the single family analysis but were only loosely associated with linkage group 2 in the integrated map. The remaining four markers (0078, 0101, 0129, and 0077) consistently could not be assigned.

TABLE 2

Anopheles albimanus microsatellite markers

Microsatellite	Sequence Acc. No.	Repeat	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Size range	Informative	Alleles (field samples)*
0143	FJ170378	(GGT) 6	CGAACACGTTTATCGGTGTG	TGCGAAACGATGACAATCAG	77–80	Y	77, 80
0019	FJ170339	(CT) 12	TGAAGAAAAGGGTGAATGTTG	CGTAATGCCACTCACTCAGC	98–114	N	98, 110, 104, 114, (114, 118, 120, 128)
0086	FJ170340	(TG) 12	CCGGAACACGATACCTGTC	GGGTAGAACCGAAACCGAAC	88–100	Y	88, 96, 98, 100
0022	FJ170341	(TC) 30	GATCCGATTCTCCCTTTTCG	CAAGGAAGGAGACGAACGAG	97–107	N	97, 99 (91, 93, 95, 97, 99, 105, 107)
0114	FJ170342	(AC) 27	CCAGCTGGGACCAATTAAAC	CAGCGAGACTCACGGTCAC	128–166	Y	132, 164
0025†	FJ170343	(TC) 19	GGTTTCCAGCCTCCATTCTC	CCTTACTGTGCTGGAACACG	104–120	Y	104, 120
0032	FJ170375	(CA) 11	CGGTAAAGGGGAAATGAGTG	ATAAACCCCTGCCATCATCA	161–163	Y	161, 163
0078	FJ170344	(CT) 15	GTCATTTTACGGCGGAACTG	GCGCACCTAATGGACGTAG	71–83	Y	71, 73, 75, 77, 81, 83
0057	FJ170345	(CT) 19	TAGTGGGCACCACACCATTC	CTGCAAATTCACGCTAAACG	149–155	Y	149, 155
0034	FJ170346	(GAC) 6	TGTCGGTGCTCCGAGTAATAG	GGCGTTGTTGTTGTTGGTC	144–147	Y	144, 147
0056	FJ170347	(GT) 8 + 1	TTCAATTCGTTCCTTGATTGG	GGCCCAACTCTGGACATTC	138–140	Y	138, 140 (138, 140)
0087	FJ170348	(AG)13	TGATGAACCACTTCGACGAC	GTCTCGGGATTCTGTTTTCG	114–124	Y	114, 120
0135	FJ170349	(GT) 11	GCAGCGTTAGTTCAGTGTGC	AGCGAGCGAGCGTATTATGT	215–219	Y	215, 219
0100	FJ170350	(CCT) 3 + 9	CACCCTTCTTACCGTTCGAC	ACCTAACCCCAACCCGTTAC	100, 109	Y	100, 109
0101	FJ170351	(AG) 16	GTGGGCTCATGAAGTGGTTC	GAGTCGATCAGCGAAAGCAC	133–147	Y	133, 145, 147
0107	FJ170368	(GA) 16 + 5 + 11	TCCGTACTGCTCACATACGC	GCGGAACTGACTGAATGATG	121–146	Y	121, 137, 146 (133, 140, 143, 146)
0108	FJ170368	(TC) 9	ACCCTTTCTCGCCGTCTC	GCTTCCTGCTCTGGTAATCG	66–72	N	66, 68, 70 (68, 72)
0115	FJ170352	(GA) 17 + 10 + 38	GCGAAATACACAGAGCGAAATA	CCTTTCCCTCGTTTTTCG	158–190	Y	158, 190
0109	FJ170352	(TCG) 5	ATGATGGGCACAGTATGACG	CACACATGACCAACGAATCC	97–102	Y	97, 102
0113	FJ170353	(TG) 5 + 9	TTCGGGAGCATCGTCTACAT	TATACCAACCGAACGCATCA	111–125	Y	111, 123, 125
0117	FJ170354	(CT) 20	AGGCCGTAAGGAAGGAAGTC	AAAATTTTCCATCCCAACAGG	114–123	Y	114,123
0125	FJ170353	(GA) 5 + 3 + 4	ACATTCCTGGGAAGGACCAC	CCGGCTTCATTCACTCACTC	74–84	Y	74, 84
0124	FJ170355	(GT) 16	ACTTGCAACTCCCGAAAGC	GTTTCGCTCGCTGAAAGATG	155–157	Y	155, 157
0128	FJ170356	(CT) 15	GTCATCGTGCTCTCGTGATG	AATTTGGACGATTTCTCAAGG	136–140	Y	136, 140
0129	FJ170357	(GA) 5 + 3 + 8 + 14	AATGATGCCGCTTGATC	TAGTTCTCACGGCACGATG	237–289	Y	237, 240, 275, 277, 289
0004	FJ170358	(AC) 6	TCGCTTTCAACCCATCTACC	CTTTGGTTATCCCCCACAGA	96–110	N	96, 109, 110, (96, 102, 104)
0037	FJ170358	(GA) 18	CAGCTGCTGTCACCATCG	TTTAGTGCATGCCTTTGAGC	110	N	110
0038	FJ170359	(GA)7 + 9	GCTGGACATGCTTGACATTG	GCGGAGAGGGCTCTGTTTAC	103–116	Y	103, 116
0008	FJ170360	(TG) 17	TCCGTAGCGTTCAGAGGAGT	AAAATGGGGGAGGTTTATGG	102–110	Y	102, 104, 106, 108, 110,
0045	FJ170361	(TC) 8	AACGCGCTACGCACTCTAAT	AGTGGAGAAAAGGAGCCACA	116–120	N	116, 118, 120
0046	FJ170362	(TC) 24	CCAGAACGAGCGAAATGAAG	ACGGAGAGAGCGAAAGAAAC	84–87	N	84, 87
0012	FJ170373	(CA) 6	GGTGCATTCCAAATCCAAA	AAGCGGTAATTGGGTTATGC	67–69	N	67, 69
0077	FJ170363	(TG) 11 + 13	CGAGAACTTAGAGAACTAGAAGCTG	CGTGCGTTAGTATTTGTGTGTG	86–110	Y	86, 110
0020	FJ170364	(GA) 12	GTGAAGAGCCTTCCATCTCG	CGACCGAAAGGTTTGGTAAC	83–97	Y	83, 95, 97,
0022	FJ170341	(CA) 2 + 4	TCATCTCCTTTAGCGTTCGTG	TGCGTATGTGGCTGAGAAAG	143–153	Y	143, 153
white exon 2	L76302	(TC) 26	TCGGTTCGCCCTATCAATC	AATCAGCTGGGTTGTAGTTGG	205–225	Y	205, 225
0064	FJ170366	(CAG) 6	GAACAAGAAGCGCAAAAAGC	GGCTGAAACCAGAATTGCAG	97–102	N	97, 102 (97, 102)
0065	FJ170366	(CA) 9	TGGTCGCCAATGTGTTTCTA	TCGTCAGCGAAGATGATGAG	192	N	192
0066	FJ170366	(CT) 16	ATAGTCGCTTGCAGCACTCC	TGGCGACCATTTGTTTTACA	186	N	186
0083	FJ170377	(TC) 11	TGGTGGTGCATAACTCATCG	GCTGCTGTTGTTGTGGTGAG	93	N	93
0144	FJ170367	(AC) 11	CCACGCGGTACACATACAAC	GCAACGCAAAATTGAAACAA	143	N	143
0106	FJ170368	(GAT) 8	TTAAAACCGCTCTCGACTCC	GCGTGGAGACTAACCGAGAG	143	N	143
0111	FJ170368	(GTC) 5	GACCGCTCGGTAGTTCTGC	TAATATACGCGGCCAGCAAC	121	N	121
0112	FJ170352	(GCA) 2 + 3	TCCCTGTCATCTGCTGAACC	GAGGACTGGATGGAAACCAC	109	N	109
0120	FJ170379	(GA) 16 + 4 + 11	GCTCACATACGCACGCTAAA	CAGCGGAACTGACTGAATGA	134	N	134
0036	FJ170369	(GTC) 2 + 1 + 6	CGGTCGGAAGGTTGTTTTAG	AGCAGCCGCACAATCTTTAT	147	N	147
0005	FJ170370	(ATC) 5	AACTGCTGCTGCTACTGCTG	CCTCATGTTTGCTTGGTTCA	110	N	110
0048	FJ170376	(CTG) 8	TCGTCGCATAACCAATTAGCG	TAATTCCCGTTTGCTCATCC	88	N	88
0016	FJ170371	(AC) 32	GGCTTACCGCCAGTGATAAT	GAATCGGCCTTTTTCACAGA	133	N	133
0017	FJ170374	(CGT) 8	ATAGTTCGGTTCGGTTGACG	CTGACGGGCGCATAATGA	103	N	103
0079	FJ170372	(CA) 4	CGAATTTTTGGACTTTTTCC	ATGTTCGACGAAACAACGAA	97	N	97

*Eleven field samples from Chiapas state in Mexico were genotyped for a small number of markers. The allele sizes obtained are given in brackets.
†This marker is the same as marker 2–25 described in Molina-Cruz et al.[4]

TABLE 3

Markers showing segregation disorders* in *Anopheles albimanus* progeny

Locus	Family	Segregation ratio (all progeny)†	*P* value of χ^2 test – autosomal loci‡	Segregation ratio (females only)†	*P* value of χ^2 test – sex linked loci
0086	AJ-1	36:30:20	0.001	n.k.	0.1§
	108N	42:26:21	0	20:24:0	0.546¶
	121D	42:16:21	0	22:16:0	0.947¶
	129D	19:5:14	0	9:4:0	0.165¶
0056	AJ-1	19:55:17	0.132	–	–
	121D	24:46:6	0.003	16:21:2‖	–
	129D	3:23:13	0.041	3:7:3‖	–
0109	AJ-1	22:45:21	0.966	–	–
	108N	23:36:28	0.251	–	–
	121D	19:38:17	0.922	–	–
	129D	16:16:6	0.045	7:5:1‖	–
0101	108N	16:51:17	0.144	–	–
	121D	18:52:7	0.002	8:25:4‖	–
	129D	12:23:4	0.103	–	–
0008	108N	18:48:24	0.549	–	–
	121D	16:43:15	0.373	–	
	129D	6:16:16	0.045	3:4:7‖	–
0077	AJ-1	11:55:2	0	n.k.	0‡
	121D	25:38:12	0.108	–	
	129D	14:21:3	0	6:10:12‖	–
white	AJ-1	46:20:13	0	n.k.	0.184‡

*χ^2 tests were performed on each marker for each individual family, according to the expected distributions indicated below. Only loci not segregating in Hardy Weinberg distribution in one or more families are listed. Markers not segregating in the expected 1:2:1 ratio for autosomal loci were tested for sex linkage. In families 108N, 121D, and 129D tests for sex linkage were performed using only the female progeny. Markers for which homozygous paternal genotypes are observed in the female F2 progeny cannot be located on the X chromosome. These are marked with‖. n.k. = not known.

† Segregation ratio: homozygous maternal:heterozygous:homozygous paternal.

‡ *P* value for 1:2:1 distribution (homozygous maternal:heterozygous:homozygous paternal).

§ *P* value for 2:1:1 distribution (homozygous maternal:heterozygous:homozygous paternal).

¶ *P* value for 1:1 distribution (homozygous maternal:heterozygous).

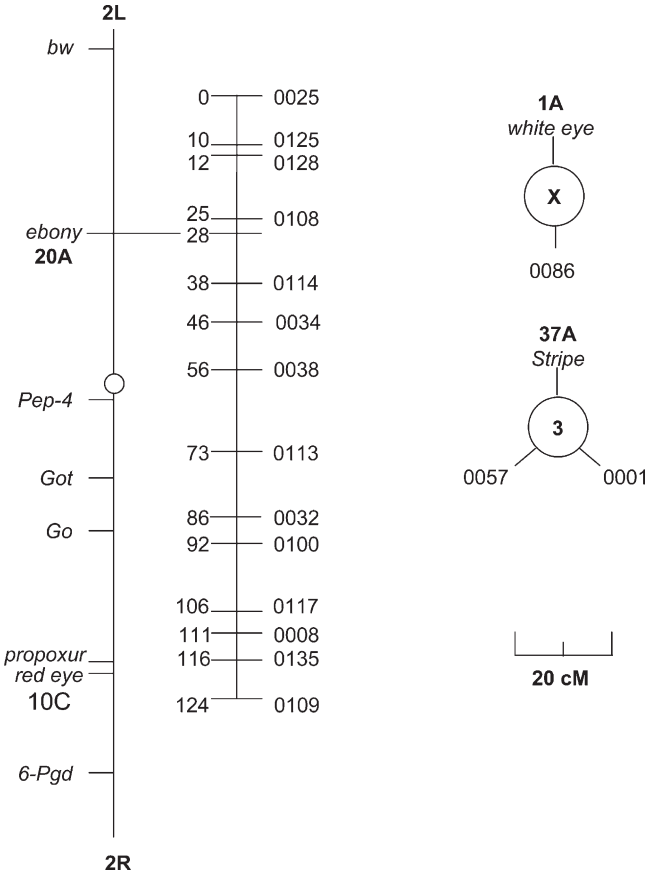

FIGURE 1. Linkage groups for *Anopheles albimanus*. The original linkage map for chromosome 2 based on morphological and isoenzyme markers is shown on the left.[5] The cytological position of the morphological markers used in the present study is given. For linkage groups 1 (chromosome 3) and X, no order or genetic distance is intended. Distances are in cM.

Anchoring of linkage groups to *An. albimanus* chromosomes. One family, AJ-1, was informative for the semidominant morphological marker, *ebony,* that had been mapped using deficiencies to division 20A on the left arm of chromosome 2.[18] The F1 parents were both heterozygous at this locus, and the F2 progeny segregated in the expected 1:2:1 ratio. The *ebony* locus was genetically mapped between markers 0108 and 0014, thereby anchoring linkage group 2 to the second chromosome.

Stripe is a polymorphic gene whose various alleles are common in wild mosquitoes. It has been located by deletion analysis to chromosome 3R in division 33B.[18] Two families, AJ-1 and 108N, were informative for this second morphological marker. The female parent of both families was homozygous for the dominant allele whereas the male carried the recessive allele. As heterozygotes could not be distinguished from homozygotes, the expected ratio of *Stripe*:wild type is 3:1. This ratio was observed in family AJ-1 but not in family 108N ($P = 0.024$). When the *Stripe* locus was included as a genetic marker, weak linkage was found to linkage group 1 in family 108N (LOD 2.0) possibly indicating that this linkage group is found on chromosome 3. No linkage was observed in family AJ-1, but this is perhaps expected as this family was non-informative for the markers putatively located on chromosome 3.

DISCUSSION

The present manuscript catalogues a novel set of microsatellite markers for *An. albimanus* and presents a preliminary genetic map for chromosome 2. Two markers were also assigned to each of the additional chromosomes (X and 3) by linkage to physically mapped morphological markers: 32 microsatellite markers could not be assigned to any chromosome largely due to lack of polymorphism in the parents of the crosses. The bias towards informative markers on chromosome 2 was unexpected, but a similar dichotomy in the distribution of randomly identified microsatellite markers between the autosomal chromosomes was also observed in *An. gambiae*.[19] We are unable to offer an explanation for why this is so. Similarly perhaps, during the period in which fairly intense screens for morphological mutations were performed in *An. albimanus*, morphological markers were found on all arms except 3L though numerous enzyme polymorphisms were found on that arm.[5] We feel certain that further surveys for both would detect markers on chromosome 3 and that this is probably simply a sample size effect which is exaggerated by the need for several markers to be clearly linked.

We compared the size and orientation of the chromosome 2 linkage group as determined here with that estimated previously.[5] Because no chromosome rearrangements exist in any of the stocks used in the mapping crosses (nor have any been observed in spite of extensive examination of natural populations), and recombination rates between males and females are the same, we determined that the minimum length of chromosome 2 is 124 cM. The previously published map estimated a length of 168 cM for linkage group 2.[5] Although the calcula-

tions of lengths may not be directly comparable, the map that we present appears to contain most of the linkage group.

The use of the marker *ebony* that is common to both our microsatellite and previous linkage maps allows us to give a probable orientation for the chromosome 2 map as shown in Figure 1 and which we reason as follows: The total distance from *ebony* to the end of 2L was estimated to be approximately 40 cM by its distance from the sub-telomeric marker *brown larva* (*bw*).[20] The most distant markers from *ebony* in our study are 0025 (28 cM distant) and 0109 (96 cM distant). Unless the estimate of the location of *bw* was grossly underestimated relative to the telomere, there is insufficient recombination distance for a marker to be located on 2L at a distance as great as that of 0109. In contrast, approximately 109 cM exists between *ebony* and the end of 2R. Therefore, the marker-deficient area of our chromosome 2 map likely lies primarily at the telomeric end of 2R.

In situ hybridizations have previously demonstrated that within arms, the gene compositions of *An. gambiae* and *An. albimanus* are largely conserved.[20] However, the arms are arranged in different chromosomes: 2R of *An. gambiae* and *An. albimanus* are syntenic, however 3R of *A. gambiae* is syntenic with 2L of *An. albimanus*. This conclusion is supported by the occurrence of a BLAST hit of the *An. albimanus* microsatellite 0113, located on chromosome 2R in *An. albimanus*, on *An gambiae* division 12E, chromosome 2R. Furthermore, 0128, located on chromosome 2L in *An. albimanus* has a BLAST hit on division 29C, chromosome 3R of *An. gambiae*. Further confirmation of synteny can be found by comparing the location of isozymes mapped previously[21] with their map location in *An. gambiae. Propoxur resistance* (insensitive AChE), 6-*phosphogluconate-dehydrogenase*-2, *phosphoglucomutase*, *aconitase*, and *hexokinase* all map to the chromosomes previously identified as syntenic.

Because the genome of *An. gambiae* has been sequenced, it provides a useful source of information with which to expand our conclusions. Recently, further sequencing has identified over 100 additional sequences containing repeat elements (Table S1, Genbank numbers are within the file). The sequence and synteny of chromosome arms has allowed us to identify 68 untested microsatellites from this new sequence data set that have similarity to *An. gambiae* sequences (by BLAST similarity), and their probable location in the genome of *An. albimanus* will provide a useful starting point for those wishing to add loci to the existing map. In addition, single nucleotide polymorphisms segregating in F2 progeny from the same crosses are being genotyped in the Centro Regional de Investigación en Salud Pública en Chiapas leading to improved resolution of the existing map.

Genetic maps are limited by the number of progeny and the number of markers. In this case, the limited number of informative markers clearly restricts the resolution of the map. In future studies, genetic crosses of populations at the extremes of the geographical distribution of *An. albimanus* should increase the number of polymorphic markers and lead to a more densely populated map. Indeed, a further six markers that were non-informative in the genetic crosses were polymorphic in a small number of field-collected mosquitoes analyzed from Chiapas, Mexico.

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