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A microsatellite linkage map of *Drosophila mojavensis*

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Abstract

Background: *Drosophila mojavensis* has been a model system for genetic studies of ecological adaptation and speciation. However, despite its use for over half a century, no linkage map has been produced for this species or its close relatives.

Results: We have developed and mapped 90 microsatellites in *D. mojavensis*, and we present a detailed recombinational linkage map of 34 of these microsatellites. A slight excess of repetitive sequence was observed on the X-chromosome relative to the autosomes, and the linkage groups have a greater recombinational length than the homologous *D. melanogaster* chromosome arms. We also confirmed the conservation of Muller's elements in 23 sequences between *D. melanogaster* and *D. mojavensis*.

Conclusions: The microsatellite primer sequences and localizations are presented here and made available to the public. This map will facilitate future quantitative trait locus mapping studies of phenotypes involved in adaptation or reproductive isolation using this species.

Background

Evolutionary biologists have struggled to determine the number and types of genetic changes that lead to speciation. Recent advances in molecular techniques facilitate a more thorough investigation into these issues. For example, by mapping quantitative trait loci (QTLs) affecting interesting traits, we can explore the genetic basis of phenotypic variation between two populations that may lead to reproductive isolation.

One hallmark species used in studies of speciation and ecological adaptation is the desert cactophilic *Drosophila mojavensis*. *D. mojavensis* belongs to the mulleri complex of the *repleta* species group within the subgenus Drosophila. Unlike many well-studied Drosophila, its ecological niche has been well documented, and extensive cytogenetic work has been done on it and its close relative, *D. arizonae* [see e.g., [1]]. With regard to speciation, *D. mojavensis* has been the subject of many genetic and phenotypic studies of mate choice [e.g., [2-5]], hybrid sterility and inviability [6,7], and variation in sperm and female sperm-storage organ length [8,9]. However, all of these studies have been forced to use a handful of either allozyme or morphological mutant markers. Microsatellites have been isolated from this species before [10], but they are unmapped and their sequences are not available.

Here, we present a microsatellite-based linkage map of the five major chromosomes of *D. mojavensis* using a new set of markers. We mapped 25 microsatellites to the X chromosome and 65 microsatellites spanning the four major autosomes. We also use our results to confirm the conservation of Muller's chromosome elements [11] across approximately 65 million years of evolutionary

divergence between *D. melanogaster* and *D. mojavensis* [see [12]]. Muller [11] had suggested that chromosomal elements conserve their identities (ie, complement of genes) across all Drosophila species, and several subsequent studies have supported this idea [e.g., [13-15]], though only one study involving the *repleta* group [16].

Results and Discussion

Primers were successfully developed for a total of 116 markers. Of these, 26 did not distinguish between the two isofemale lines that were used for mapping and were therefore not pursued. We mapped 25 microsatellites onto the *X*-chromosome, 10 onto chromosome *2*, 7 onto chromosome *3*, 13 onto chromosome *4*, and 10 onto chromosome *5*. Twenty-five more microsatellites were confirmed to be autosomal but could not be mapped because of segregating polymorphism within our lines. Microsatellites were named based on their localizations, where the fifth character of the name was an X if *X*-linked, A if unmapped autosomal, or a number indicating a specific autosome. The distribution of microsatellites across the chromosomes suggests a possible excess of repetitive sequences on the *X*-chromosome (27.8% observed vs. 20% expected assuming all chromosomes are similar in size, chi-square test, p = 0.07; 27.8% observed vs. 18.7% expected assuming chromosomes are the same size as *D. melanogaster* homologous chromosome arms, chi-square test, p = 0.03).

Using two female-parent backcrosses, we constructed a recombinational map of the *Drosophila mojavensis* genome using 34 of our microsatellites: 13 on the *X*-chromosome, 7 on chromosome *2*, 4 on chromosome *3*, 7 on chromosome *4*, and 3 on chromosome *5*. Recombinational distances are presented in Figure 1. *DMOJX040* was not placed in the figure because it was only 0.7 cM from *DMOJX030*. The recombinational lengths of the chromosomes generally exceed the homologous chromosome arms in *D. melanogaster* and some other Drosophila species. For example, the *X*-chromosome in *D. mojavensis* spanned 130.8 cM, while the *X*-chromosome in *D. melanogaster* spans only 73 cM. Even within the *repleta* species group, *D. buzzatii* has an *X*-chromosome that spans 109 cM [17] and *D. hydei*'s *X* spans 116 cM [12]. Similarly, *D. mojavensis* chromosome *2* could only be assembled into three pieces that recombine freely from each other. This difference between species in recombinational length most likely indicates an overall greater recombination rate per megabase in *D. mojavensis*, but we cannot exclude dramatic differences in sequence lengths of the chromosome arms.

We recombinationally mapped some markers in a second cross because of segregating variation within the lines. Figure 1 presents the most conservative map, where all markers were mapped against each other for any particular chromosome. However, we have some additional information about the linkage of other microsatellites. Specifically, we observed that *DMOJ4200* is freely recombining from all the *4*-chromosome markers between and including *DMOJ4010* and *DMOJ4060*. Also, the following markers are freely recombining from each other on chromosome *5*: *DMOJ5100*, *DMOJ5200*, *DMOJ5300*, and *DMOJ5400*.

To evaluate the conservation of Muller's elements across 65 million years, we used BLAST [18] to identify segments homologous to the sequences flanking the 65 microsatellites in *D. mojavensis* that were mapped to chromosome. We identified segments mapped to *D. melanogaster* chromosomes similar to 23 of the sequences isolated from *D. mojavensis* (see Table 1). The inferred homology of the arms are as follows (melanogaster:mojavensis): *X:X*, *2L:3*, *2R:5*, *3L:4*, *3R:2* [19,20]. Based on the BLAST results, all 23 *D. mojavensis* sequences matched *D. melanogaster* sequences on the homologous chromosome arms. This observation strongly supports the conservation of Muller's elements between the subgenera Drosophila (*D. mojavensis*) and Sophophora (*D. melanogaster*).

Conclusions

We have developed and mapped a panel of 90 variable microsatellites for genetic studies in a model system for ecological genetics and speciation: *Drosophila mojavensis*. Thirty-four of these microsatellites have been placed onto a detailed linkage map of this species. We also confirmed that Muller's chromosome elements were conserved between *D. melanogaster* and *D. mojavensis*, species separated by 65 million years of independent evolution, in 23 of 65 sequences tested. Given the long-term interest in this species for studies of adaptation and speciation, the construction of a linkage map and presentation of variable microsatellite sequences will facilitate future work in this area.

Methods

Isolation of microsatellite sequences

We used a modification of Hamilton et al's [21] enrichment technique to increase the proportion of microsatellites in the genomic DNA insert library prior to cloning [see also [22]]. This procedure uses a subtractive hybridization, in which streptavidin-coated magnetic beads and biotinylated oligonucleotide repeats retain single-stranded genomic DNA fragments containing repeat sequences.

Genomic DNA was isolated from approximately 30 *D. mojavensis* individuals from a mixture of strains using the Puregene™ DNA Isolation Kit (Gentra Systems). Except where indicated, we used reagent concentrations and

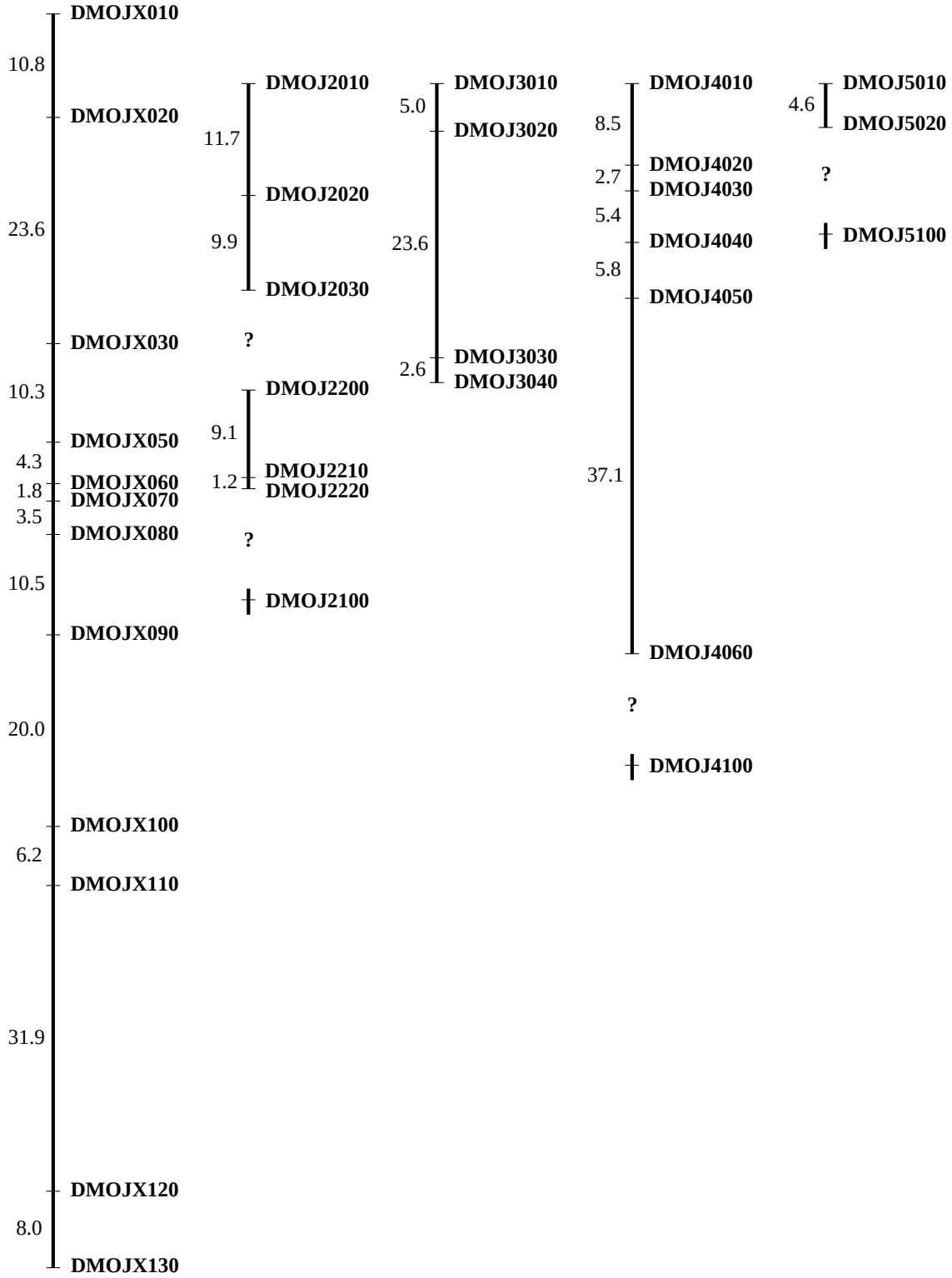

Figure 1
Linkage map of the five major chromosomes of *Drosophila mojavensis*. From left to right, are the *X*-chromosome, chromosome *2*, chromosome *3*, chromosome *4*, and chromosome *5*. Kosambi recombinational distances between markers are on the left of each chromosome, and the microsatellite names are on the right. A question mark appears between markers or groups when markers were assigned to the same chromosomes but freely recombined from each other.

Table 1: Ninety microsatellites mapped in *Drosophila mojavensis*. Microsatellites assigned to chromosome "A" were autosomal but could not be mapped to a particular autosome because of variation segregating within the lines used for mapping. We present the BLAST expect (E) value in the column after the microsatellite name only for the 23 microsatellites used in the Muller's chromosome element comparison.

Name	BLAST E-value	Chromosome	Size	Primers	Repeat motif	GenBank Accession
DMOJX010		X	132	attgtgttgcgccttagggc gtgataatttgttgatttgggtgcatgtc	$(ca)_{11}$	AY578823
DMOJX020		X	142	ctctgccactcaacggacc ctcttcagtgttgcctagggtatac	$(ca)_7$	AY578824
DMOJX030		X	124	aagctatgcctagttgtcacttcc caaacggcatttcatataagaatctatctcac	$(ag)_9$	AY578825
DMOJX040		X	147	aggcatgccttagtttgtgtcac cacacatattaagcattgtattacaatcgtccc	$(ac)_{15}$	AY578826
DMOJX050		X	143	accaagcaaaagccaattgcac caaagctttgccggcattagc	$(ac)_{12}$	AY578827
DMOJX060		X	128	caattgtggagttgcgtttgcac cacgcatttctgattgaccatacac	$(ac)_{12}$	AY578828
DMOJX070	1e-19	X	163	gccactcgttgttgtcccta atagttctttgctctatatgcgtgtg	$(ca)_4$	AY578829
DMOJX080		X	202	ccactccacgttcgcttgac cacaatgtagagctccctttaatctcc	$(ac)_{14}$	AY578830
DMOJX090		X	149	ccttcaactttgtcgctccagac caatcaaagcgaacgtagctcaatg	$(ca)_9$	AY578831
DMOJX100		X	160	tttgtatataaggcggaaaggcgg gtctgtatataatttaataagttgttacgtaaaagaactcac	$(gt)_{20}$	AY578832
DMOJX110	2e-09	X	98	cagtggcgctcaaaaggtctg gggatgtatggggtctatgggtg	$(ca)_9$	AY578833
DMOJX120		X	110	gcctgcatttgtgcatctgc caagtgtttgccaaagctgcag	$(tg)_{13}$	AY578834
DMOJX130		X	130	tgggctacacttcagcaaacattc tcatgtgcaaaggtagccaagc	$(ca)_{11}$	AY578835
DMOJX500		X	100	caattattgcatagccacgccc cgagcacttttccaatttttggc	$(gt)_{11}$	AY578836
DMOJX501		X	136	ctcgagcgaattttcctattggatttc ccaacgagccatttcctcacg	$(ca)_6$	AY578837
DMOJX502		X	107	attaaagtgcattaaatgacacagccac ctctgccttcacgtgtgc	$(ca)_{10}$	AY578838
DMOJX503		X	136	gcgaagaattgcaaataccccttgtag aagcaaatatacacaacatacacatgtgc	$(gt)_{10}$	AY578839
DMOJX504		X	126	catcaatctctagaatgcctcacgc gagtgactcactttaaagcgagctc	$(gt)_8$	AY578840
DMOJX505	0.004	X	128	tcgcttgtttccgtttagtaaccg tacgcgtatgcgtatgcatgc	$(ac)_{10}$	AY578841
DMOJX506	2e-15	X	149	actgcctacactgctctgtctc acaggctttacacatggccaaataac	$(tc)_{16}$	AY578842
DMOJX507		X	166	ttttctttgcatcggcttagttgc cacatatggaaatgcagcacgaac	$(tc)_{15}$	AY578843
DMOJX508	4e-43	X	248	aagcagcctagctgaacagttg cattgggaagagctgatgtatagacg	$(ac)_{12}$	AY578844
DMOJX509		X	157	agctgattaacgaagcagatttcgtg ttccattcatgaaccactcacacatc	$(ga)_{18}$	AY578845
DMOJX510		X	169	atttggctgctgcgagacg gatttgtgccactgtgcaatgg	$(ag)_{18}$	AY578846
DMOJX511		X	177	gcttcagtgagcctcaaatgaaac tgcagctggcatgggtataag	$(ct)_{16}$	AY578847
DMOJ2010		2	157	acgagtttgccatgaactggattg aaagccgaaacttgtattcatttggc	$(ac)_{14}$	AY578848
DMOJ2020		2	153	attgacttagcgtgtgagcgtg cgctgtctcatttacataggtcgg	$(gt)_7$	AY578849
DMOJ2030		2	199	tgacgcgccaatcagttgac gattcaaggtgtcatatctatatgtgtgtagg	$(gt)_{16}$	AY578850
DMOJ2100	4e-05	2	187	ggcgctcccttaatcacagatac agcatgtgtctgcttgctgt	$(ag)_{17}$	AY578851

Table 1: Ninety microsatellites mapped in *Drosophila mojavensis*. Microsatellites assigned to chromosome "A" were autosomal but could not be mapped to a particular autosome because of variation segregating within the lines used for mapping. We present the BLAST expect (E) value in the column after the microsatellite name only for the 23 microsatellites used in the Muller's chromosome element comparison. *(Continued)*

DMOJ2200	3e-21	2	148	gtcgctccatagagttctacaagtttg cgcctccaagtaattcacgaagc	$(gt)_9$	AY578852
DMOJ2210		2	139	cccagcaagtgtactctactcaag tgctgcatcaataaagaaggcaaac	$(ca)_8$	AY578853
DMOJ2220	0.002	2	136	gttggctttggctattggactgag tgtgcaatgtgactggcaactg	$(gt)_6$	AY578854
DMOJ2300	1e-05	2	114	aattgacagcactccgtggc gttcagcgccggccttac	$(ca)_{12}$	AY578855
DMOJ2301		2	158	ctcttagcggcaggtgtcaag aatcttatcgaaaatatgcaacacgatgg	$(caa)_{11}$	AY578856
DMOJ2302	8e-17	2	193	ctctcgctgtttctcttgtctcttatac aactgatttaccgctcgctatacag	$(ac)_9$	AY578857
DMOJ3010	4e-05	3	218	gcccgccggagttcaatag atgtgtatggccagtgctacattt	$(ct)_8$	AY578858
DMOJ3020	4e-23	3	94	acgtggattacgaacacgagc tttggccaatttgagcaactgc	$(ca)_{14}$	AY578859
DMOJ3030		3	91	cctagtttctttggccaccctac cgcagtgaaacgcatggaaac	$(ca)_{11}$	AY578860
DMOJ3040		3	94	gtcaggtgtcagcagcagc gcctcaacagcacctactgag	$(ac)_{12}$	AY578861
DMOJ3100		3	87	ctgatttgtcaccacagggactc gctaatcgaagcacacacatgtattcag	$(ca)_{10}$	AY578862
DMOJ3101	7e-25	3	150	aacggcggcatccgttg actgtcatcgcacaaatgatttgta	$(gt)_{12}$	AY578863
DMOJ3102		3	210	ctctctgtagcaaaaggcttttgtaacc tgctgtgtgcagcacgaac	$(ca)_{11}$	AY578864
DMOJ4010		4	90	agccagtgcaatgccagc gcctggaccttgtgggc	$(ca)_{16}$	AY578865
DMOJ4020		4	121	cagcagctgccttatgtcagc aataaatcgcagcagccaggac	$(ac)_{10}$	AY578866
DMOJ4030	7e-05	4	137	gtagttgttgtaggcacgcataca aatgagaatgagaactggaacggg	$(ca)_{10}$	AY578867
DMOJ4040	1e-07	4	161	gcaacatgtgctccactcgttc ttttcccacacttcttgcagcag	$(ca)_{11}$	AY578868
DMOJ4050		4	196	atcgcatagaaagacactcatacgc ctggaggcaagggaagtttcg	$(ca)_9$	AY578869
DMOJ4060		4	211	cgagactcgctgataagtaaagcc gattgtaattttggccgtgcgc	$(ac)_{41}$	AY578870
DMOJ4100		4	126	cgcagacatatttgtctcccagc ttcgtagccaagacaaactcacaac	$(ga)_{11}$	AY578871
DMOJ4200	3e-10	4	120	gcttcaagccttgtgatttgttg caagaagaacaagcgcattatgcaaa	$(ct)_{24}$	AY578872
DMOJ4300		4	165	ggaaagaataccaacgcctatggc gtccgcagacagccagc	$(ca)_{12}$	AY578873
DMOJ4301		4	133	acatttggctgttacctggcac ccaatgccagtgagtttctctctc	$(ca)_{12}$	AY578874
DMOJ4302	2e-42	4	218	gtgtgtgcgtggatgtgttttac gacagcactgaacagattatagataagcc	$(ag)_{18}$	AY578875
DMOJ4303		4	174	cacggcaacacttgcagttacc ccattgctcatagcccgtttacc	$(ag)_{23}$	AY578876
DMOJ4304		4	171	ggcacattgccacaagtgtac tctgtgccggaaatcgtcaac	$(ga)_{20}$	AY578877
DMOJ5010	6e-04	5	117	ggcatagggaccgcagc gtaaatattcgccaaacacctacatgc	$(ac)_8$	AY578878
DMOJ5020	4e-06	5	144	ctacaggtatgaagaacctgaaccc acaacagcctacacgcactc	$(gt)_{11}$	AY578879
DMOJ5100	8e-18	5	156	agacaacttgactgttgctcgc tgacactgattggtcgctgtg	$(gt)_8$	AY578880
DMOJ5200	2e-26	5	154	tcgcacaactggcgcatg atttttacagcacgcttaacaagaattttcac	$(ca)_{13}$	AY578881
DMOJ5300		5	97	gtggtggacatcaaccagcc tgagccaactttgagcataaattagcc	$(ca)_9$	AY578882

Table 1: Ninety microsatellites mapped in *Drosophila mojavensis*. Microsatellites assigned to chromosome "A" were autosomal but could not be mapped to a particular autosome because of variation segregating within the lines used for mapping. We present the BLAST expect (E) value in the column after the microsatellite name only for the 23 microsatellites used in the Muller's chromosome element comparison. *(Continued)*

DMOJ5400	5e-08	5	109	cttggatttcagctcagtcgctc cgccacaatcagtcataggtcc	$(gt)_{10}$	AY578883
DMOJ5500		5	121	ggaagcgtcgactgcatacc gtgttgaaacgtatgtgtttgtgcc	$(ca)_{10}$	AY578884
DMOJ5501	1e-04	5	99	cgtgccacgtaaactcttgcc gaaggcaattcaattagttttgagagttatccc	$(ac)_9$	AY578885
DMOJ5502		5	115	gcatattgacaaggacgagctgtc tctgagtgcgtccattactttgtatc	$(gt)_{12}$	AY578886
DMOJ5503		5	150	gtatacgacatgttggcactgcc ttgcaagctgggcgtaagc	$(tg)_{10}$	AY578887
DMOJA500		A	185	gagactgtttgacgcccgc tcgatagacatgagtttggtctagaaacc	$(tg)_8$	AY578888
DMOJA501		A	140	tcagtagcctctgctacggc cgaacggaaattatgaactagtcagcc	$(tc)_{30}$	AY578889
DMOJA502		A	138	ctgaaagttctggcagcaagagt gtgtaatttagttgttagacgcgattgagag	$(ct)_{14}$	AY578890
DMOJA503		A	153	taaggctctgtttcgtaactttgcc ctgtcaatgtgctaaacattgcaacc	$(ca)_9$	AY578891
DMOJA504		A	222	aatcatctgccccctttccac ggaaaatgatgctcaggcaggt	$(ac)_{13}$	AY578892
DMOJA505		A	181	ccatagtgcgatgcacgcttc gccatagcccatagtagccaag	$(tg)_{10}$	AY578893
DMOJA506		A	147	attaatgcaggccggaaagtcg gctcgctctgcgtcgttatg	$(gt)_{11}$	AY578894
DMOJA507		A	134	tcagccgggatgttaactaacttg atgcttaccagagcgaatggc	$(ac)_{12}$	AY578895
DMOJA508		A	196	ctctgcgacatgtagactacgc gataaagttgaacttttactaccgatgcattc	$(tg)_{10}$	AY578896
DMOJA509		A	186	gctgagaaacaaatttcgcatgcc tgttgttgtcctttaacgaacgttcc	$(tg)_{18}$	AY578897
DMOJA510		A	105	cacacagccagacttgacgttag gcttttgattttgtcatagccattgctaaac	$(tg)_{12}$	AY578898
DMOJA511		A	162	cttttctggctattacgagagcagc aaaacataatgtaattgagctgacaaagcaac	$(tg)_{30}$	AY578899
DMOJA512		A	120	gatgagaaataggcgttgctgtcc gcatatgatgaaggctgagagctc	$(ca)_{11}$	AY578900
DMOJA513		A	120	gctcagctaacagaaacaccca gccgtagctgcagcatct	$(tg)_{13}$	AY578901
DMOJA514		A	125	atggcgcaactcggtcg gcagcacatttggctgctg	$(gt)_{12}$	AY578902
DMOJA515		A	203	gaccgaacagcgcagcc cacaaacctacataaacaccgcagtc	$(ac)_{11}$	AY578903
DMOJA516		A	163	ggctgtaccaagcacacactc cgctcgtgtcgtcgtcttc	$(ca)_{13}$	AY578904
DMOJA517		A	87	gaaaacagctgcaaacccgtaaag gctctcttaagcgctcaactatatagac	$(ca)_{15}$	AY578905
DMOJA518		A	118	gtatgtatgggcatacagcggg cttggttctatgatatgatgacgtgtct	$(ca)_7$	AY578906
DMOJA519		A	182	atgaataggaatccagccagcg agcgctttgcgtgcctac	$(ca)_{14}$	AY578907
DMOJA520		A	164	tttcggcgcaaggtcgtc ttagcttctttaccggcatcatgc	$(gt)_8$	AY578908
DMOJA521		A	96	ttttgtttaggttttgcgcctaacc ttttccataatttgtgcgtgtgcc	$(ac)_9$	AY578909
DMOJA522		A	122	cctttcgagtgcctccacaac gtcccactacatattgctacagctg	$(ca)_{11}$	AY578910
DMOJA523		A	147	gcgtaagcacagttggactctc tgtctgcggagttttatgctgtaa	$(ct)_{23}$	AY578911
DMOJA524		A	135	tcgagagagattcgatcgagagc cctgtttgcattatgtgggtgtc	$(ac)_{12}$	AY578912

reaction conditions suggested by Hamilton et al [21]. The enrichment procedure was repeated seven times. For each enrichment, one of the following enzymes was used with *Nhe*I to digest *Drosophila mojavensis* genomic DNA: *Sau*3AI, *Bfuc*I, *Rsa*I, *Alu*I, or *HpγCH4*III. Linker sequences were ligated to the digested DNA to provide a PCR priming site. We then hybridized the digested, linker-ligated DNA to a biotinylated oligonucleotide repeat motif, either $(CA)_{15}$ or $(AG)_{15}$, and recovered the microsatellite-enriched DNA. The DNA was amplified via PCR, and fragments between 300 and 800 bp were recovered from an agarose gel for cloning. We then used the Invitrogen TOPO-TA cloning kit to clone the DNA into plasmids and transform into *E. coli*. We omitted the chemiluminescent screen and used pUC19 primers to amplify *D. mojavensis* DNA inserts directly from colonies. Each 50 μl reaction volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 μM pUC forward and reverse primers, and 1.0 unit *Taq* polymerase (Ampli*Taq*, Perkin Elmer). DNA was added by touching a sterile toothpick to a colony and swirling the toothpick into the reaction mix. We used the following thermal profile: 95°C for 5 min; 30 cycles of 94°C for 60 s; 55°C for 30 s, 55°C for 30 s, 72°C for 30 s; rapid thermal ramp to 40°C. PCR products were sequenced with an ABIPrism® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit, and products were visualized on ABI sequencers in the LSU Museum of Natural Science, Pennington Biomedical Research Center, or the Department of Biological Sciences' genomics facility.

Fly stocks

Several inbred lines of *D. mojavensis* were tested to determine the most suitable lines for constructing a microsatellite map based on microsatellite allelic differences between strains, bearing the same chromosomal arrangements, and lack of segregating microsatellite alleles. In the end, we selected the lines A993 (Rancho El Diamante, Sonora) and A924 (St. Rosa Mtns., AZ), obtained from Dr. William J. Etges. These lines were further brother-sister mated for 9–12 generations to ensure thorough inbreeding and a reduction of segregating alleles.

Microsatellite assay conditions

We designed two primers for each microsatellite-bearing sequence, one bearing an M13(-29) tail. A 10 μL PCR reaction was then performed using 0.5 μM of each primer, 1.0 μL of dNTPs, 1.0 μL of 10X PCR buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 0.4 μL of IRDye (LiCor), 1U *Taq* DNA polymerase, and 0.5 μL from a single fly DNA preparation (Puregene). We sometimes added 1.0 μL of 10 mM $MgCl_2$ to the reaction or more polymerase to optimize the results of the PCR. A touchdown PCR cycle was performed [23], and amplifications were visualized on acrylamide gels on our LiCor DNA analyzer.

Assignment to linkage groups

Virgin females and males of the A993 and A924 lines were crossed and offspring reared. DNA was isolated from the parents and progeny using the Puregene™ DNA Isolation Kit (Gentra Systems). We determined if markers differentiating the lines were *X*-linked or autosomal by comparing the F_1 males to the F_1 females and parent strains. For *X*-linked markers, males consistently bore one allele while females consistently bore two. Autosomal markers were further tested using 20 progeny of a male-parent backcross. Because there is no recombination in Drosophila males, the offspring all inherited a nonrecombinant chromosome from one of the original lines. By comparing genotypes across the male-parent backcross progeny, markers were assigned to linkage groups. We also used the NCBI Basic Local Alignment Search Tool [BLAST: [18]] to identify putatively homologous sequences in *D. melanogaster*. Sequences bearing an expect (E) value below 0.01 were scored, as E-values are nearly identical to probability (p) values in that range.

Recombinational mapping within linkage groups

Virgin F_1 females (progeny of the cross described in "Assigning to linkage groups") were backcrossed to males of one of the pure lines (A924). To ascertain the recombinational distances between the markers on each chromosome, we genotyped the parents and 200 progeny with each marker previously assigned to a linkage group. Recombinational distances were estimated in Kosambi centiMorgans using Mapmaker [24].

Authors' contributions

RS maintained all fly cultures and performed all reactions and analyses involved in the recombinational mapping of microsatellites. SDS and MAFN produced the microsatellite genomic libraries, sequenced the clones, and designed the primers. All authors contributed to the preparation of this manuscript.

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