A GENETIC MAP FOR THE ONLY SELF-FERTILIZING VERTEBRATE

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Figure S1. Phylogenetic relationship of hermaphroditic *Kryptolebias marmoratus* and *K. hermaphroditus* strains and a sister gonochoristic species, *K. caudomargimatus* with an outgroup, *K. brasiliensis*. The tree was constructed by neighbor-joining (NJ) method based on mitochondrial DNA sequences (a part of 12S rRNA, corresponding to nucleotide 17 to 332 in KP998194). Tree topology did not change when analyzed with maximum likelihood (ML) or maximum parsimony (MP) methods (data not shown). Numbers at the nodes (NJ/ML/MP) represent percentage recovery of those nodes per 10,000 bootstrap replicates. Only values above 60% are shown. The data from the present study are indicated in bold red. The hermaphroditic mangrove killifish is clearly separated into two groups, *K. marmoratus* and *K. hermaphroditus* (formerly *K. ocellatus*). The accession numbers are: hKb (AY946281) and hKm (AY946280) (Vermeulen and Hrbek, 2005); hKc (AF092294, Hrbek and Larson, 1999); mKc (AF002428), mKm (AF002429), and mKo (AF002430) (Murphy *et al.* 1999); Lee, Km (AF283503, Lee *et al.* 2001). Scale bars indicate genetic distance.









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			44898LG5 32280LG5 21244LG5 44157LG5 43102LG5
13.6		-	15666LG5 34193LG5 25/50LG5 33496LG5 2319LG5 7656LG5 26345LG5 6368LG5 3203LG5 14643LG5
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21.0		-	4674LG5 24241LG5 50161LG5Xma13(6.67 32411LG5 28966LG5
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27.8		\sim	27865LG5
29.3			42701LG5 20330LG5 32417LG5 8169LG5 14172LG5
30.3			39494LG5 27840LG5 51871LG5 4829LG5 59177LG5 33657LG5 53469LG5 29754LG5 3439LG5
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			14894LG5 10457LG5 59858LG5 37344LG5 14753LG5
39.8			5002LG5 7677LG5 53311LG5 28674LG5
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47.1	\vdash	-	51431LG5 57797LG5 20956LG5 17796LG5 10907LG5 106LG5 27376LG5 28640LG5 26004LG5 21701LG5
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51.2	\vdash	-	7380LG5 493LG5Xma13 19.15 20053LG5 53081LG5 22074LG5 16934LG5Xma13 18.35 9393LG5 10962LG5 31820LG5Xma13 17.34 31532LG5Xma13 17.31
			48970LG5 29028LG5 51858LG5
52.2			126033LGD 3196LGD 26728LGD 10712LGD
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			26254LG5 19553LG5 58732LG5 8804LG5 34873LG5
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61.5~		1	58879LG5 25240LG5 6273LG5 31868LG5 26179LG5 17009LG5 8257LG5 19375LG5 33824LG5 43194LG5
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LG7

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	LGIO
	6357 3881 4412 35612 50358 47885 11641 3927 56198 49698
A	5036 51386 22604 49298 39018 30186 16592 11360 56399 15052 45168 667 24761 40008 20172
/H\	12998 10884 38844 10268 35509 3920 60176 53835 49893 16949
0.0 / []	30651 53019 43645 28 7966 28654 4869 31560 52195 44973
	42458 42908 42256 46299 55 24625 18685 7889 534 47884
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	33035 23316 37057 41483 26082 35358 3584 5 5202 32750 27002 7405 4402
- 1.1 J	22236 32334 44758 23142 5409 14225 30265 56669 41799 33988
	2928 48004 46135 16769 19416 58913
3.2	42647 47283 47191 39874 22193 5430 2226 40736 53775 9662
4.2	11808LG10Xma19 22.82 54375 54686 46533 43426 56928
5.3-	35345 37742 32119 16825 4025 35830
6.3	1026 46652 8375 13874 21806 135221 35466 54064
0.9-11 1-1	44411 19355 59329 4825 46245 47569 33016 436 33703 54075
7.4	38545 45935LG10Xma19 22.83 32291 1143 48564 4927 53117 5249 58743 29960
	34834 14098 24370 17333 8167 3517 53994
10.6	4045 58943 24862 28199 44123 6832 29414
13.8 14.8	17944 12245 36815 19665 1274
15.9	16905 45788 29243 16868 5929 54598 53758 39923 16439 8707
	145383 6525 6783 42618 45337 49558 50957
17.5 J	142161 39949 30463 39172 32412
18.0	4 1446 16473 42568 134469 51217 48225 52134 60217
21.2	120902 H 49849 14879
23.3	439366 38869 60103 19227 46167 34716 46388 55776
24.4	10610 37220 48700 22108 8768LG10Xma19 12.73 37127 22013 36452 35739LG10Xma19 13.25 20788
	4 51516 34068 27800 38307 6919 46331 36038 31715
28.6	11607 12720
29.7	10243 46164 5719 2561 40002 18309 14357 59138 26673 18505
30.0	48974 5060LG 10Xma19 9.58
30.7	63339 29783LG10Xma19 9.12 49411 49246 38535
31.3 //	131966 46176 23021 23263 34671 12097
33.9 J/H	30095 32907 19287LG10Xma19 6.04 57884 51545 36931 33566 25099 32922 15963
36.0	54067 53414 28893 16765 14305
37.1	4 15630 39057 - 57329
^{39.2}	440138 20220 00927 42902 03721 13175 40392 153140 14605 26497 1384 31030
40.2 /H	21439 2984 22574 45330 53224 9326
41.3 /H	43122 15310 17356 39808 14216 58473 41961 18172 42353 58087
42.3-	U 41867 46594 11270LG10Xma19 16.10 25373 29472 31199 39295 12034 55149 46929 46925 2490 44654
43.4	22548 47843 39555 22015LG10JH557918.1 47025 1022 4898LG10LH557918.1 16100 55949 50698
44.5	20904 20046 9 20211 39245
45.5	H 13509 50812 J 43115 4429 54904 46104LG10Xma19 22.61 16828
48.7	127590 23851 36227 567 153640 48938 24959
49.7	- 60040 H 9493 31590 30669 34690 60671
51.1	418649 14243 448167 18073 46251
51.8	17979 52415 15437 22369 36089 45681 35394 60658 2739 6035
52.9	16721 57460 45389 56529 37242 10961
53.9	H 42851 8350 35143 717 10718 H 47241 12811 52182 1896 14840
58.2	14440/ 003 37837 45847 20816 11783 28566 38534
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59.2	60142 9680 35553 21180 2644 46418 12101LG10Xma14/7.65 13504 29445 46500
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LGI3















LG19



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LG20

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2.1-11		N١٩	11119 31554 11631 44552 43904
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14.6		Vr	19536 44890 24426 46492 34325
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. N		1//	38180 4218LG20Xma6 22.18 40697 47981 194
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20.0		V/.	49257 5360 26596
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- //		[//	46375 52892 48475 40447 15342
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384			52778 34977 54098 2858 40594
18			54730 25927 51691LG20Xma6 15.85
39.5		Шг	46025 5715 12894 48530 36995
40.5		WIIA	13933 58468LG20Xma6 14.44 12591
41.5		WA	58144 41165 52782 38238 16095
42.6		WWA	00165LG20Xma6I13.50 50173 18991
43.6	-	UMH:	23510 40488
			32369 14624 35023 58613 34227
811			58620 32600 41363 29185LG20Xma6 12.43 360
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52.9		$[\]$	58045 42373 54867 59047
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- 14333LG23

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49559LG23 20979LG23

39821LG23 2011CG23 44319LG23 27781LG23 10613LG23 28891LG23

39971 Loca 2007 29991 Loca 2007 46271 Loca 2007 46471 Loca 2007 44431 Loca 2007 471141 Loca 24434 Loca 244398 Loca 2452 471141 Loca 24431 Loca 24439 Loca 2452 Loca 2452 471141 Loca 24431 Loca 24431 Loca 24432 Loca 2452 Loca 2452 47141 Loca 2452 Loca 24432 Loca 2452 Loca 2452 Loca 2452 47141 Loca 2452 Loca 2452 Loca 2452 Loca 2452 Loca 2452 47141 Loca 2452 Loca 24522 Loca 2452 Loca 24





13.5 13.5 13.5 14.5 15.5 42733LG24 37521LG24 57436LG24 60361LG24 32937LG24 40634LG24 7681LG24 45406LG24 24107LG24 37409LG24 48942LG24 53097LG24 33962LG24 59297LG24 51559LG24 24107L624 31409L624 3974L624 49794L624 49794L624 Xms9j9.23 9059L624 2746L624 31391L624 33259L624 49794L624 Xms9j9.23 9059L624 50223L624 41647L624 1648L624 50223L624 41647L624 1648L624
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Figure S2. A Genetic linkage map of *Kryptplebias marmoratus/K. hermaphroditus* constructed with JoinMap 4.1. We obtained identical marker grouping into LGs as the PHP program and marker ordering consistent with the PHP program as shown in Figure 2.



Figure S3. A comparison of LG1 linkage maps of *Kryptplebias marmoratus/K. hermaphroditus* constructed with either JoinMap 4.1 or the PHP programs. The marker grouping and locus ordering are consistent in both programs. However, the distances between markers were calculated differently in some cases because the algorithms used in two programs were distinct.



Figure S4. A comparison of genetic maps of *Kryptplebias marmoratus/K. hermaphroditus* constructed with a whole set of markers (whole) and with a subset of markers whose sequences showed conservations with platyfish (conserved). Marker grouping and marker ordering are consistent in both maps. For 18 LGs, the markers were grouped together and were ordered identically (e.g. LG1). The results corroborate the ordering obtained in the whole map. The exceptions were LG4 (together with LGs 7 and 13) where the markers were in two groups or LG20 (together with LGs 9 and 21) where most markers were in one group with a single marker not linked to those.



Figure S5. Metaphase chromosomes of *Kryptolebias hermaphroditus* obtained from primary cultured fibroblasts of a HY strain adult. Two sets of representative Giemsa stained samples shown were composed of 48 chromosomes. Total of 23 spreads were counted and 48 was a mode (data not shown). Except for one pair of subtelocentric (ST) and two pairs of submetacentric (SM) chromosomes, all were acrocentric chromosomes gradually changing in size. One pair of SM chromosomes had less stained areas in their short arms, probably representing a nucleolus organizing region (NOR).



100 markers

Figure S6. A Genetic linkage map of *Kryptplebias marmoratus/K. hermaphroditus* based on RAD sequencing. The map was coalesced into 24 linkage groups (LGs). LGs were named in decreasing order of total number of markers, relative to which each vertical bar (grey) was drawn. Each bin (location of co-segregating markers) occupies length relative to total number of co-segregating markers, middle of which blue horizontal lines were plotted. Green horizontal bars, drawn on the right of the grey bars, represent numbers of recombination events and were plotted between adjacent bins. Assuming relatively even distribution of markers, these grey bars represent physical maps with density of green bars as relative frequency of recombination events along the chromosomes.



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Figure S7. Conserved synteny of *Kryptolebias marmoratus/K. hermaphroditus* linkage groups (Kma LGs) to either platyfish (*Xiphophorus maculatus*, Xma) or medaka (*Oryzias latipes*, Ola) chromosomes, suggesting basically one to one relationship (24 orthologous chromosome pairs). Dotted lines indicate homology of mapped Kma markers to either Xma or Ola mapped genome sequences identified by the blastn program with default parameters. For platyfish, blast hits with a cut-off e-value of 1.0E-9 are shown. Many Kma LGs show intrachromosomal rearrangements probably due to recurred inversions after divergence from common ancestors between either Xma or Ola. Some Kma markers show homology to sequences on non-orthologous chromosomes (indicated by red letters in parenthesis). For example, a marker at 8.2 cM on Kma LG1 (Kma1) has a homology to a sequence on Xma chromosome 20 at 7.8 Mb. Two such markers, 5064 (represented by 1948 on Kma LG1) and 34956 (represented by 1052 on Kma LG2), had homologous sequences on another orthologous chromosomes (Ola23-Kma18-Xma17 and Ola19-Kma17-Xma10, respectively), indicating translocations happened after the *K. marmoratus/K. hermaphroditus* lineage diverged from platyfish. Similarly, markers 18609 (represented by 5464 on Kma LG2) and 38898 (represented by 2302 on Kma LG8), had homologous sequences on orthologous platy chromosomes (Xma18 and Xma1, respectively) but on non-orthologous medaka chromosomes (Ola22 and Ola19, respectively), indicating translocations happened after the medaka lineage diverged from cyprinodontiformes.

File S1 A genotyped panel of the map based on 9,904 markers and 49 F_2 DNA (a Microsoft excel file). Twenty-four linkage groups (LGs) are separated and bins of co-segregating markers have been ordered. Genotype symbols represent following: a, DAN homozygous; b, PAN-RS homozygous; h, heterozygous; -, missing genotype.

File S2 Nucleotide sequences of the markers used for mapping (a Microsoft excel file).

File S3 A program used for linkage grouping and bin ordering. The original genotyping panel with 49 F₂ individuals and 9,937 markers (map49F2_42.txt), PHP scripts, and an outline of the program (MappingOutline.pdf) were provided. Please consult "Readme.txt" for running the scripts.

Files S1, S2, and S3 are available for download at www.g3journal.org/lookup/suppl/doi:10.1534/g3.115.022699/-/DC1

Table S1. Number of tags classified by presence (1) or absence (0) in each sample.

	K. marn	noratus	K.herma	phroditus			
sample	DAN	VOL	PAN-RS	HY	Кс	number of tags	
	1	1	1	1	1	35415 —	identical 9240
	0	1	1	1	1	98	polymorphic 26175
	1	0	1	1	1	116	(total SNPs 53544)
	1	1	0	1	1	170	
	1	1	1	0	1	212	
	1	1	1	1	0	16426	
	0	0	1	1	1	672	
	0	1	0	1	1	8	
	0	1	1	0	1	5	
	0	1	1	1	0	132	
	1	0	0	1	1	4	
	1	0	1	0	1	7	
	1	0	1	1	0	162	
	1	1	0	0	1	489	
	1	1	0	1	0	235	
	1	1	1	0	0	279	
	0	0	0	1	1	72	
	0	0	1	0	1	49	
	0	0	1	1	0	1752	
	0	1	0	0	1	38	
	0	1	0	1	0	33	
	0	1	1	0	0	22	
	1	0	0	0	1	22	
	1	0	0	1	0	29	
	1	0	1	0	0	45	
	1	1	0	0	0	1975	
	1	0	0	0	0	347	
	0	1	0	0	0	391	
	0	0	1	0	0	646	
	0	0	0	1	0	717	
	0	0	0	0	1	18679	
total	57214	57217	57489	57361	56859	79247	

		(a																										
ts per LG	us per La	P (chi square	0.775	* 0.018	0.607	0.559	0.364	0.645	0.594	0.237	0.084	0.912	0.107	0.077	0.759	0.571	0.364	0.93	0.372	0.093	0.057	0.894	0.252	0.775	0.686	0.594	1	
ombination event		0	12	15	4	6	9	12	15	12	0	3	3	9	3	15	3	3	4	17	S	Ξ	0	0	0	15	293	
			52	15	21	27	24	22	24	8	20	25	30	26	26	21	28	23	27	17	29	26	21	26	23	24	565	
		7	4	6	4	<u>8</u>	6	5	0	9	6	Ξ	9	17	0	12	ω	12	œ	15	15	12	2	12	4	0	308	
0	De L	m	-	0	0	0	0	0	0	7	0	0	0	0	0	_	0	_	0	0	0	0	—	—	7	0	6	
		4	0	0	0	0	0	0	0	_	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	
average map	distance between	adjacent bins (cM)	81.1	1.16	1.17	1.24	II.I	1.16	1.22	I.26	1.19	1.12	1.20	1.37	1.13	I.20	1.26	I.20	1.16	81.1	1.27	1.23	I.36	I.33	I.52	1.16	1	(1.22)
	average markers	per bin	11.70	10.74	11.16	I 0.48	11.67	9.48	11.57	8.61	8.38	9.67	11.56	9.22	9.88	9.95	11.11	9.28	10.50	9.63	8.17	9.10	8.57	9.02	8.03	6.74	1	(9.76)
		gaps≥3	m	m	3(2)	4,3	m	3(2)	3(2)	3(2)	m		4,3	3(3)			3(2)	3(2)		4,3	ъ	4,3	5,4	6, 3(2)	8, 5, 3	m	1	
tota	bins	per LG	46	47	43	44	39	46	37	49	50	43	36	45	42	4	36	43	38	4	48	42	44	4	39	39	1019	(42.5)
tota	markers	per LG	538	505	480	46	455	436	428	422	419	416	416	415	415	408	400	399	399	395	392	382	377	370	313	263	9904	(412.7)
	ς	(Kosambi)	54.5	54.4	50.4	54.5	43.2	53.4	45.2	61.7	59.6	48.3	43.3	61.8	47.3	49.4	45.3	51.4	44.2	48.4	60.8	51.5	59.8	54.7	59.3	45.2	1247.6	(52.0)
tota	•ecombination	events	53	53	49	53	42	52	44	60	58	47	42	60	46	48	44	50	43	47	59	50	58	53	57	44	1212	(50.5)
	-	۲C	_	2	m	4	Ŋ	9	7	8	6	0	=	12	13	4	15	16	17	8	61	20	21	22	23	24	sum	(average)