Assessment of Polymorphism in Zebrafish Mapping Strains

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To assess the level of heterozygosity within two commonly used inbred mapping zebrafish strains, C32 and SJD, we genotyped polymorphic CA-repeat markers randomly dispersed throughout the zebrafish genome. (For clarity purposes we will primarily use the term polymorphic to define polymorphism between strains, and the term heterozygous to address heterogeneity within a strain.) Eight male individuals each from C32 and SJD stocks were typed for 235 and 183 markers, respectively. Over 90% of the markers typed were polymorphic between these two strains. We found a limited number of heterozygous markers persisting in clusters within each inbred line. In the SJD strain, these were mainly limited to a few telomeric regions or regions otherwise distant from centromeres. As expected, centromeric regions were homozygous in the SJD strain, consistent with its derivation from a single half-tetrad individual. In contrast, heterozygous clusters were distributed randomly throughout the genome in the C32 strain, and these clusters could be detected with linked polymorphic markers. Nevertheless, most regions of the C32 strain are homozygous for CA-repeat markers in current stocks. This identification of the heterozygous regions within C32 and SJD lines should permit rapid fixation of these remaining regions in future generations of inbreeding. In addition, we established levels of polymorphism between the inbred, C32 and SJD, strains and three other commonly used strains, the *AB, WIK, and Florida wild type (hereafter referred as EKK), with CA-repeat markers as well as SSCP polymorphisms. These data will maximize the use of these strains in mapping experiments.

Genetically uniform animals have proven to be a valuable tool for research involving both fruit flies, worms, and mice (for review, see Quillet et al. 1991). Clonal laboratory strains that have been used in generation of the zebrafish genetic maps are also ideal reagents for performing mutagenesis and maintaining stocks (Postlethwait et al. 1994; Johnson et al. 1996). The C32 clonal strain was established by Streisinger and colleagues in 1978 (Streisinger et al. 1981). In the years prior to 1991, C32 stocks were maintained by mass mating, with gametes from multiple individuals, to produce successive generations. Beginning in 1991, the C32 isolate used in this study was maintained by six generations of sib matings, prior to this analysis. Another inbred line, designated SJD, was established by sequential early pressure (EP) parthenogenesis (Johnson et al. 1996). Following EP generations, SJD stocks were maintained by six generations of full-sib matings, prior to this analysis.

In 1995, Buth et al. assayed the homogeneity of the C32 strain by allozyme expression. Overall, 38 loci were analyzed and only one locus, malate dehydrogenase (sMdh-A), was found to be heterozygous in C32 fish (Buth et al. 1995). Despite the small size of the sample, they concluded that mutation was the likely source of heterogeneity. This implies that the sMdh-A locus or C32, in general, have high mutation rates. Another explanation for the observed variation in the C32 strain is contamination from other zebrafish stocks. Following multiple generation of inbreeding, most introduced polymorphisms would be fixed in stocks. The remaining regions of persistent heterogeneity can be identified by clustering of polymorphic markers.

Here, we analyzed 235 polymorphic CA-repeat markers in fish of the C32 strain and 183 markers in SJD fish. We conclude that the majority of loci in current stocks of C32 and SJD strains are homozygous. The heterozygous markers we did see were confined to distinct clusters in both strains, rather than isolated loci. However, the size of these regions was small, and such regions could be detected with closely linked markers. We also analyzed the degree of polymorphism between inbred strains, C32 and SJD, and other commonly used zebrafish strains, *AB, WIK, and EKK. We found that the SJD strain exhibited the highest degree of polymorphism, >75%, with all other strains genotyped, whereas the C32 strain was found highly polymorphic with the SJD and WIK strains, but not with the *AB and EKK strains.

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RESULTS

Analysis of Heterogeneity in the C32 and SJD Strains

Eight male C32 and eight male SJD individuals were selected for genotyping. In total, we used 264 CA-

repeat markers for the C32 strain and 203 markers for the SJD strain (Goff et al. 1992; Knapik et al. 1996, 1998; Shimoda et al. 1999), from which we were able to unambiguously score 235 in the C32 strain and 183 in the SJD strain (Fig. 1). If markers were spaced less than a centiMorgan apart according to current genetic maps (Knapik et al. 1998; Shimoda et al. 1999), we considered them as a single locus. Therefore, 235 markers typed in the C32 strain represent 223 loci and 183 markers typed in the SJD strain represent 172 loci. The loci we genotyped were largely dispersed randomly throughout the genome, spaced an average distance of 10 and 13 cM apart for the C32 and SJD strains, respectively. The largest interval between typed markers was 56 cM on linkage group (LG) 21.

After an initial screening of 96 markers, we assayed another 168 for the C32 strain and 107 for the SJD strain, most of which were chosen to fill gaps or to test the extent of polymorphism in the heterogeneous regions. The approximate size of the C32 and SJD alleles are displayed in Table 1. The map positions and regions of heterogeneity are shown in Figure 1.

We found that most regions in the genome were homozygous in both C32 and SJD strains. The heterozygous regions appeared randomly dispersed in the C32 strain and could be found in the centromeric regions as well as in the regions close to the telomeres. For example, two centromeric markers, Z737 and Z4717, were heterozygous on LG 18 (Fig. 1); at the same time, telomeric markers Z618 and GOF18 were heterozygous on linkage groups 14 and 15, respectively (Fig. 1; Table 1). In contrast, we found that heterogeneous regions in the SJD strain were confined to the ends of the chromosomes and regions distant from the centromeres (e.g., see markers Z3952, Z4830, Z732, and 1243 on linkage groups 11, 12, 15, and 24, respectively, Fig. 1; Table 1). This is consistent with the fact that the SJD strain originated from a single half-tetrad animal. An example of heterogeneity and genotype segregation in the SJD strain is shown in Figure 2A and B. Only one SJD heterozygous region, on LG7, was exceptionally close to its centromere (Fig. 1).

For a number of cases, we assayed additional markers in the regions that were heterozygous within both strains. We hypothesized that if such regions in the C32 strain originated from contamination by another stock, then we would find additional linked markers segregating with heterozygous genotypes. Heterozygous markers detected within the C32 strain are clustered in regions on linkage groups 2, 12, 13, 16, 18, and 23. For example, the C32 heterozygous genotypes segregating for markers Z3424, Z10852, and Z11696 (LG 13) are shown in Figure 2A.

Because most of the markers used in this study were randomly dispersed throughout the zebrafish genome (except for a small number of markers that were used to define the extent of the heterozygous regions), the number of heterozygous regions within each strain reflects the heterogeneity of a strain. As the number of loci typed were 223 and 172 in the C32 and SJD strains, respectively, and the number of heterozygous loci are 20 and 18 in the C32 and SJD strains, respectively, we estimate that ~91% of the genome is homozygous in the C32 strain, whereas the SJD strain is homozygous throughout ~90% of its genome.

Three heterozygous loci were shared by both strains. One region on LG 12 extends through marker Z4830. Another heterozygous cluster shared by the C32 and SJD strains extends through markers Z4670, Z6854, and Z15453 on LG16. Our findings are well within expectations taken that heterozygous clusters distributed randomly in both strains. In this case, we expect ~1%, or two loci to be shared by both strains [(20/223 * 18/172) * 100% = 1% or approximately two loci].

Analysis of Polymorphism Between Zebrafish Strains

To compare polymorphism levels between different strains, we genotyped 4 individuals from the C32, SJD, *AB, WIK, and EKK strains with 73 CA-repeat markers and 40 SSCP markers (Gates et al. 1999; Shimoda et al. 1999). Two telomeric and one centromeric CA-repeat markers per chromosome were selected for genotyping. For single-strand conformation polymorphism (SSCP) analysis, we randomly selected 50 markers (2 per each linkage group), of which 40 were scored unambiguously (Fig. 2C). All SSCP markers derived from the 3′-UTR of either known genes or ESTs that were mapped previously (Gates et al. 1999). To quantify levels of polymorphism between strains, we used polymorphism index (PI) (see Methods section for details). We calculated PI for each individual marker, and then averaged PIs for all CA-repeat or SSCP markers in each strain. Polymorphism indices determined from the CA-repeats genotyping data differ significantly from the SSCP genotyping data in 6 of 10 pairwise comparisons (data sets were compared with one-sided two-sample t-test) (Table 2). In these cases, PIs derived from SSCP data were ~10% lower compared with PIs derived from CA-repeat markers. We suspect that the overall sensitivity of SSCP detection, 50%–90%, could account for these significant differences (Sheffield et al. 1993).

The SJD strain was found to be the most polymorphic with all other four strains analyzed (PI = at least 0.75 for all strains) (Table 2). We found the SJD strain to be mostly polymorphic with the C32 strain (PI = 0.97 if analyzed with CA-repeat markers, and PI = 0.87 if analyzed with SSCP markers). The C32 strain, on the other hand, was moderately polymorphic with the WIK strain (average PI = 0.73 and PI = 0.71 for the CA-repeats and SSCP markers, respectively). It was only ~50% polymorphic with the *AB

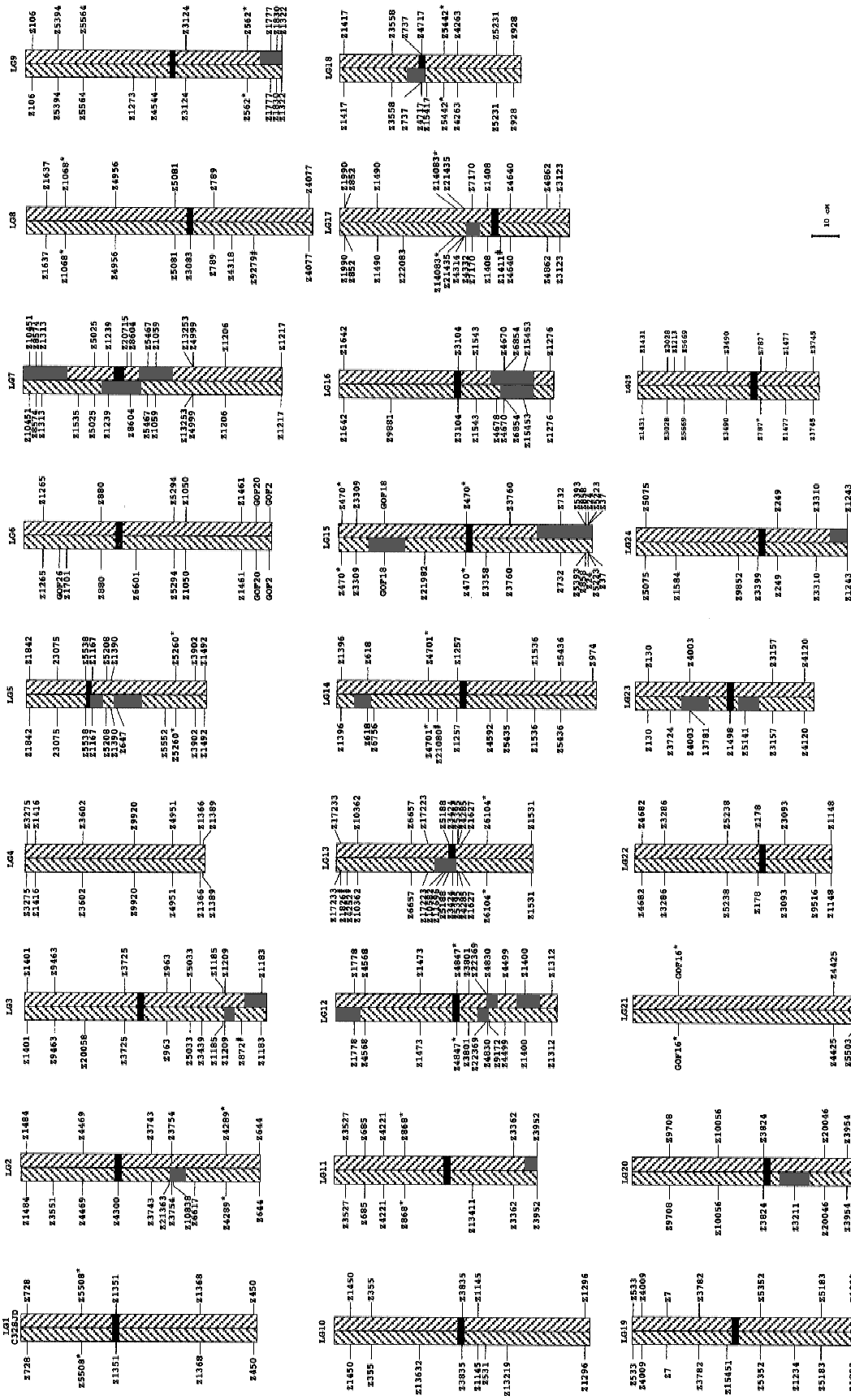

Figure 1 Heterozygosity data for the C32 and SJD strains. Marker positions derived from Knapik et al. (1998) and Shimoda et al. (1999). Centromere positions are from those determined by Johnson et al. (1996), with markers genotyped on both panels. The relationship of markers on LG 4 to its centromere has not been determined. The *left* forward hatched rectangles indicate homozygous regions in the C32 strain and the right backward hatched regions indicate homozygous regions in the SJD strains. Centromeres are depicted as solid black rectangles. The heterozygous regions in both strains are shown in gray. The extent of these regions is given as a midpoint between a heterozygous marker and the next adjacent tested maker that was not polymorphic. Markers that did not amplify an SJD allele, but amplify in the C32 strain are designated by #. If a C32 allele was equal in size to the SJD allele, the marker is denoted by an asterisk (*). Sizes of bands were estimated from acrylamide and agarose gels (see Table 1).

Table 1. Allele Sizes of Markers Used in the Genotyping of the C32 and SJD Strains

Marker name	Linkage group	Size of C32 allele	Size of SJD allele	Marker name	Linkage group	Size of C32 allele	Size of SJD allele	Marker name	Linkage group	Size of C32 allele	Size of SJD allele
Z1351	1	200	180	Z1273	9	130		Z1276	16	300	270, 302
Z1368	1	110	112	Z1322	9	320	240, 300	Z15453	16	215, 225	200, 210
Z450	1	150	158	Z1777	9	200	218, 222	Z6854	16	165	180
Z5508	1	200	200	Z1830	9	160	160, 168	Z1408	17	100	250
Z728	1	210	208	Z3124	9	140	150	Z14083	17	210	210
Z10838	2	205, 225		Z4544	9	160		Z1411[a]	17	150	
Z1484	2	210, 209	196	Z5394	9	100	140	Z1490	17	130	120
Z21363	2	2100		Z5564	9	250	260	Z1990	17	190	180
Z3551	2	240		Z562	9	200	200	Z21435	17	300	310
Z3743	2	220	216	Z1145	10	210	199, 204	Z22083	17	210	
Z3754	2	130, 134	150	Z1296	10	280	310	Z3123	17	140	160
Z4289	2	250	250	Z13219	10	190		Z4314	17	250	
Z4300	2	175		Z13632	10	275		Z4332	17	190, 210	
Z4469	2	140	165	Z1450	10	260	240	Z4640	17	170	156
Z644	2	160	154	Z355	10	255	250	Z4862	17	200	206
Z6617	2	115		Z3835	10	150	200	Z7170	17	150, 200	220
Z1183	3		130, 140	Z531	10	110		Z852	17	250	244
Z1185	3	140	136	Z13411	11	240		Z1417	18	160	140
Z1209	3	152, 160	150	Z3362	11	160	180	Z15417	18	110	
Z1401	3	190	150	Z3527	11	170	200	Z3558	18	250	230
Z20058	3	110		Z3952	11	145	157, 165	Z4263	18	320	318
Z3439	3	100		Z4221	11	200		Z4717	18	218, 200	218
Z3725	3	280	300	Z685	11	200		Z5231	18	180	170
Z5033	3	260	240	Z868	11	120	120	Z5442	18	150	150
Z872[a]	3	220		Z1312	12	100	115	Z737	18	160, 168	150
Z9463	3	170	180	Z1400	12	110	106, 118	Z928	18	120	130
Z963	3	200	202	Z1473	12	150	120	Z1234	19	130	
Z1366	4	130	150	Z1778	12	170, 200	180	Z15451	19	120	170
Z1389	4	180	150	Z22369	12	205	200	Z1803	19	190	200
Z1416	4	150	142	Z3801	12	195	180	Z3782	19	120	170
Z3275	4	290	300	Z4499	12	250	252	Z4009	19	300	260
Z3602	4	90	110	Z4568	12	170	180	Z5183	19	150	154
Z4951	4	240	250	Z4830	12	190, 198	190, 198	Z533	19	200	194
Z9920	4	100		Z4847	12	130	130	Z5352	19	150	1154
Z1167	5	186, 190	184	Z9172	12	120		Z7	19	144	140
Z1390	5	150	165	Z10362	13	180	174	Z10056	20	195	180
Z1492	5	200	192	Z10582	13	210, 230		Z20046	20	170	180
Z1842	5	200	150	Z11696	13	194, 200		Z3211	20	120, 135	
Z23075	5	188	190	Z1531	13	200	140	Z3824	20	230	270
Z3902	5	230	215	Z1627	13	270	240	Z3954	20	255	175
Z2499	5	290, 310	290, 310	Z17223	13	180	200	Z4329	20	150	160
Z5208	5	350	360	Z17223	13	150	190	Z9708	20	230	220
Z5260	5	230	230	Z1826[a]	13	150		GOF16	21	200	200
Z5538	5	250	320	Z4252[a]	13	140		Z1162	21	130	130
Z5552	5	180		Z4285	13	130	140	Z15491	21	190	
Z647	5	190, 200		Z5188	13	120, 130, 210	120	Z4425	21	150	130
GOF2	6	185	165	Z5395	13	200	170	Z4910	21	180	186
GOF20	6	250	270	Z6104	13	150	150	Z5503	21	220	
GOF26	6	190		Z6657	13	180	184	Z722	21	100	
Z1050	6	130	150	ZZ3424	13	190, 210	250	Z1148	22	210	230
Z1265	6	110	100	Z1257	14	150	140	Z178	22	110	
Z1461	6	122		Z1396	14	150	135, 150	Z3093	22	150	180
Z1701	6	1701		Z1536	14	220	200	Z3286	22	104	100
Z5294	6	350	348	Z21080[a]	14	250		Z4682	22	270	268
Z6601	6	140		Z4592	14	135		Z5238	22	220	222
Z880	6	150	152	Z4701	14	250	250	Z9516	22	200	
Z10451	7	210	218, 232	Z5435	14	130		Z130	23	100	110
Z1059	7	180	180, 184	Z5436	14	100	85	Z13781	23	270, 290	
Z1206	7	140	160	Z618	14	118, 120	150	Z1498	23	180	
Z1217	7	208	200	Z6756	14	200		Z3157	23	130	110
Z1239	7	200, 260	240	Z974	14	190	200	Z3724	23	130	
Z1313	7	160	130, 166	Z21982	15	200		Z4003	23	242, 250, 270	242
Z13253	7	180	176	Z24	15	150	120, 144	Z4120	23	160	150
Z1535	7	110		Z3309	15	100	150	Z5141	23	100, 112	
Z20715	7		130	Z3358	15	200		Z1243	24	210	202, 26
Z4999	7	180	200	Z37	15	110	104, 106	Z1584	24	190	

Table 1. (*Continued*)

Marker name	Linkage group	Size of C32 allele	Size of SJD allele	Marker name	Linkage group	Size of C32 allele	Size of SJD allele	Marker name	Linkage group	Size of C32 allele	Size of SJD allele
Z5025	7	214, 220	214	Z3760	15	100	160	Z249	24	160	150
Z5467	7	290	220, 300	Z470	15	130	130	Z3310	24	150	170
Z8574	7	210	200, 210	Z5223	15	180	220, 260	Z3399	24	290	
Z8604	7		140	Z5393	15	225	211, 215	Z5075	24	220	200
Z1068	8	120	120	Z732	15	140	110, 134	Z9852	24	280	
Z1637	8	160	140	Z858	15	290	310, 330	Z1213	25	130	
Z3083	8	110		ZGOF18	15	180, 190	170	Z1431	25	150	200
Z4077	8	270	270	Z1543	16	150		Z1477	25	190	
Z4318	8	150		Z1642	16	220	240	Z3028	25	250	240
Z4956	8	260	262	Z3104	16	230	250	Z3490	25	120	150
Z5081	8	230	210	Z4670	16	140, 150	140, 150	Z3745	25	130, 150, 154	130, 154
Z789	8		205	Z4678	16	200		Z5669	25	260	250
Z9279[a]	8	150		Z9891	16	150		Z787	25	120	120
Z106	9	200	202								

[a]Markers that did not amplify an SJD allele but amplified in the C32 strain.

and EKK strains. Among the three noninbred strains, *AB, WIK, and EKK, the most polymorphism was found between WIK and *AB (average PI = 0.77 and PI = 0.68 for the CA-repeats and SSCP markers, respectively), and WIK and EKK (average PI = 0.81 and PI = 0.71 for the CA-repeats and SSCP markers, respectively).

DISCUSSION

After analysis of 223 loci in the C32 strain and 172 loci in the SJD strain randomly dispersed throughout the zebrafish genome, we concluded that C32 and SJD strains each have limited heterogeneity. A total of 91% of the genome is homozygous in the C32 strain, whereas 90% of the genome is homozygous in the SJD strain.

Although the C32 strain was originally constructed by a heat-shock method, which yields fish from single haploid gametes, we identified a number of heterozygous genomic regions. These regions are small and can be detected by tightly linked polymorphic markers. For example, the marker cluster Z10582, Z11696, Z3424, and Z5188 on LG 13 is heterozygous within the C32 strain. This heterozygous region is bounded above by Z17223 and below by Z5395. Thus, the genetic distance of this heterozygous region is ~7 cM. Because heterozygous C32 markers were grouped in regions rather than randomly distributed throughout the genome, we infer that most heterogeneity in the C32 strain is due to an old strain contamination, rather than random mutations. Subsequent generations of inbreeding presumably fixed the alleles at most loci.

Heterogeneous regions in the SJD strain were confined to the ends of the chromosomes and regions distant to the centromeres, rather then dispersed randomly throughout the genome. This is consistent with the fact that the SJD line was derived by two sequential rounds of parthenogenesis. Because this strategy resulted in a line derived from a single meiotic half-tetrad, alleles near the centromere tend to be fixed more often than at telomeric loci. Subsequent generations of full-sib matings presumably helped fix alleles in more distal regions. By utilizing markers known to be heterozygous in existing strains, we can select for the most homozygous animals in future matings to increase the efficiency of inbreeding. This should maximize the effectiveness of these animals in mapping experiments and stock maintenance.

Another valuable asset of the SJD strain is its high degree of polymorphism with all strains used in this study, most prominently as compared with the C32 strain (PI = 0.97 and 0.87 for the CA-repeat markers and SSCP markers, respectively). In addition, we found that the C32 strain is ~70% polymorphic with the WIK strain. Among noninbred strains, a high degree of polymorphism was found between the *AB and WIK strains, as well as between the WIK and EKK strains (Table 2). The degree of polymorphism between strains can be inferred from the origin and maintenance of the strains. The original University of Oregon stocks that were used to make the AB line, and its derivatives, *AB and C32, were purchased from United States pet stores, fish were typically acquired from farms in Florida. Presumably, this explains the low degree of polymorphism we observe between *AB, C32, and EKK, a more recent acquisition from the Florida zebrafish trade. In contrast, SJD and WIK are derived from recent acquisitions from the wild (Johnson and Zon 1999). Because the zebrafish is native to numerous watersheds throughout much of India and southeast Asia, it is not surprising that SJD and WIK show a high degree of

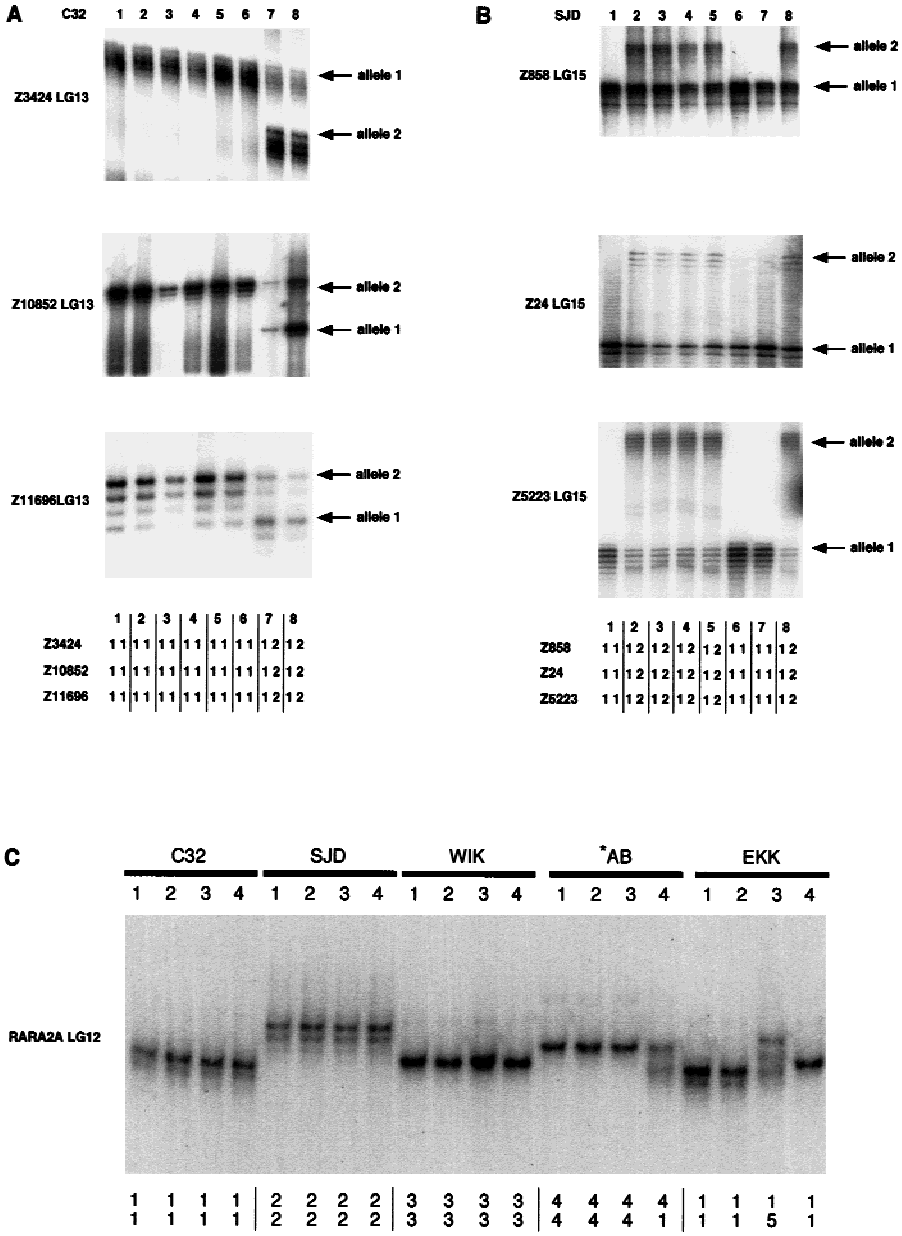

Figure 2 Examples of polymorphisms scored in the C32, SJD, *AB, WIK, and EKK individuals. (*A*) acrylamide gel data for markers Z3424, Z10852, and Z11696 on LG 13 heterozygous in the C32 strains and reconstructed genotypes for these heterozygous markers; (*B*) acrylamide gel data for markers Z858, Z24, and Z5223 on LG 15 and reconstructed genotypes for these heterozygous markers; (*C*) acrylamide gel data for SSCP marker rara2a in the C32, SJD, WIK, *AB, and EKK strains with alleles scored underneath.

polymorphism with respect to each other and with respect to *AB, C32, and EKK. Our interstrain polymorphism results are in good agreement with those reported by Knapik et al. (1998). Using CA-repeat markers, they determined 48%, 82%, and 78% heterozygosity rates for AB×EKK, AB×IN, and EKK×IN pairs, respectively (compare with 58%, 77%, and 81% polymorphism rates for *AB×EKK, *AB×WIK, and EKK×WIK, respectively, derived in this study). The differences in the genetic makeup of IN and WIK, and AB and *AB may account for the subtle variations in polymorphism rates determined in this study and by Knapik et al. (1998).

We found significant statistical deviations between the PIs derived from the analysis of CA-repeat markers and those derived from the SSCP markers (Table 2). Overall, we made 10 pairwise comparisons and, in 6 cases, we found significant differences between average PIs (p values range from 0.06 to 0.001). In the remaining four cases, the PIs derived from SSCP analysis were always lower, but the differences were not significant. The above variations may be attributed to the overall sensitivity of the SSCP technique. In 1993, Sheffield et al. reported that the SSCP sensitivity could vary widely from as high as 97% to as low as 3% depending on the size of PCR product. The greatest sensitivity was seen when using ~150-bp PCR fragments. The sensitivity substantially dropped with either increased or decreased size of PCR products. The average size of SSCP markers used in this study is ~300 bp. The expected reported sensitivity for this size is ~50%–75% (Sheffield et al. 1993).

In summary, we found that most regions in both inbred strains, C32 and SJD, are homozygous. Our identification of rare heterozygous regions in these strains will help to increase the efficiency of inbreeding. The C32 and SJD strains were found highly polymorphic compared with other widely used strains, which should maximize usefulness of the C32 and SJD stocks in standard mapping experiments.

METHODS

Fish Stocks

C32 strain is derived from Streisenger's clone (Streisinger et al.

Table 2. Degree of Polymorphism Between Different Zebrafish Strains

	C32	SJD	*AB	WIK
	0.97			
SJD	$t = 1.881$ ($P < 0.03$)	—	—	—
	0.87			
	0.53	0.92		
*AB	$t = 0.354$ ($P < 0.36$)	$t = 2.205$ ($P < 0.02$)	—	—
	0.51	0.80		
	0.73	0.86	0.77	
WIK	$t = 0.297$ ($P < 0.38$)	$t = 1.565$ ($P < 0.06$)	$t = 1.872$ ($P < 0.03$)	—
	0.71	0.77	0.68	
	0.55	0.86	0.58	0.81
EKK	$t = 0.219$ ($P < 0.41$)	$t = 1.843$ ($P < 0.04$)	$t = 0.169$ ($P < 0.43$)	$t = 3.146$ ($P < 0.001$)
	0.54	0.77	0.57	0.71

Average polymorphism indices derived from the analysis of CA-repeat markers indicated at the top of each cell. The bottom number in each cell indicates an average polymorphism index derived from the analysis of SSCP markers. Significance of the differences were compared using one-sided two-sample *t*-test. See methods section for calculations of polymorphism indices.

1981). Prior to 1991, C32 strain was passaged generation to generation with gametes from multiple individuals. Presumably, such mass matings may have allowed for a single contaminating individual to contribute heterogeneity to the stock. Since 1991, an isolate has been maintained by inbreeding strategies, typically a single full-sib pair was used to propagate the strain from generation to generation. This mating scheme would tend to restore homozygosity at most loci. SJD strain was derived from the India or Darjeeling line, a recent isolate from wild. The scheme followed to inbreed SJD was two generations of full sib matings, followed by two sequential generations of Early Pressure half-tetrad parthenogenesis. Using two sequential generations of half-tetrads ensures that all subsequent animals in the strain are derived from a single meiotic half-tetrad, homozygous at each centromere. Following the EP generations, stocks were maintained by a further five generations of full-sib mating to further inbreed stocks, before assays of heterozygosity shown here.

*AB and WIK strains were kindly provided by David Grunwald (University of Utah, Salt Lake City) and then further maintained by a random mating within each strain. Origination of these strains were described elsewhere (Johnson and Zon 1999). The Florida wild-type strain or EKK was purchased from EKK Will Waterlife (Gibsonton, FL).

PCR Amplification of CA-Repeat and SSCP Markers

Forward primer of a pair was end-labeled at the 5′ end with [γ-^{32}P]ATP and utilized in PCR reactions according to the published conditions (Knapik et al. 1998). PCR products were separated by electrophoresis through 6% polyacrylamide gels. Gels were dried and exposed to film for 2–24 hr at room temperature.

SSCP PCR reactions were performed as described elsewhere (Gates et al. 1999). PCR products were loaded on 5% (39:1 acrylamide/bisacrylamide) nondenaturing gels and run at 40W at 4°C for 2–3 hr, transferred to Whatman filter paper, dried, and exposed to film overnight. In addition, samples that were not possible to score from the SSCP acrylamide gels were rerun on 10% MDE (FMC Bioproducts) gels overnight at 500–800 volts, depending on the size of the products, transferred to Whatman filter paper, dried, and exposed to film overnight.

Statistical Analysis

To make a quantitative assessment of polymorphism, we introduced polymorphism index (PI). For a particular marker, PI between two strains, A and B, is calculated as follows:

$$\mathrm{PI}_{\mathrm{AB}} = 1 - \left[\sum_{i=1}^{s} (q_{Ai} l_{Ai} + q_{Bi} l_{Bi})/n \right]$$

in which $i = 1, 2, \ldots, s$ alleles shared in both strains; q_{Ai} is the frequency of the ith allele in strain A; q_{Bi} is the frequency of the *i*th allele in strain B; l_{Ai} is the number of alleles *i* in strain A; l_{Bi} is the number of alleles *i* in strain B; and, finally, *n* is the total number of individuals analyzed. Because the true allele frequencies for markers used in this study are unknown, we gave equal weight to every allele. Then, the above formula simply reduced to the following:

$$\mathrm{PI}_{\mathrm{A,B}} = 1 - \left[\sum_{i=1}^{s} \left(\frac{1}{k_{Ai}} l_{Ai} + \frac{1}{k_{Bi}} l_{Bi} \right)/n \right]$$

in which k_{Ai} is number of different alleles observed in strain A and k_{Bi} is the number of different alleles observed in strain B. For example, the PI for the SSCP marker rara2a (Fig. 2C) between the *AB and EKK strains was calculated as follows:

$$\mathrm{PI}_{*\mathrm{AB,EKK}} = 1 - \left[\sum_{i=1}^{1} \left(\frac{1}{k_{*ABi}} l_{*ABi} + \frac{1}{k_{EKKi}} l_{EKKi} \right)/n \right]$$
$$= 1 - \left\{ \frac{1}{2} * 1 + \frac{1}{2} * 7 \right\}/16 = 0.75.$$

Average PIs calculated for the same strain from either the CA repeats or SSCP analysis were compared by a one-sided two-sample t-test. The hypothesis tested was as follows: H_0: $u_A = u_B$ and H_1: $u_A \neq u_B$, H_0 rejected if $\alpha > 0.05$. All calculations were performed in Microsoft Excel 98.

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REFERENCES

Buth, D.G., M.S. Gordon, I. Plaut, S.L. Drill, and L.G. Adams. 1995. Genetic heterogeneity in isogenic homozygous clonal zebrafish. *Proc. Natl. Acad. Sci.* **92:** 12367–12369.

Gates, M.A., L. Kim, E.S. Egan, T. Cardozo, H.I. Sirotkin, S.T. Dougan, D. Lashkari, R. Abagyan, A.F. Schier, and W.S. Talbot. 1999. A genetic linkage map for zebrafish: Comparative analysis and localization of genes and expressed sequences. *Genome Res.* **9:** 348–359.

Goff, D.J., K. Galvin, H. Katz, M. Westerfield, E.S. Lander, and C.J. Tabin. 1992. Identification of polymorphic simple sequence repeats in the genome of the zebrafish. *Genomics* **14:** 200–202.

Johnson, S.L. and L.I. Zon. 1999. Genetic backgrounds and some standard stocks and strains used in zebrafish developmental biology and genetics. *Methods Cell Biol.* **60** 357–359.

Johnson, S.L., M.A. Gates, M. Johnson, W.S. Talbot, S. Horne, K. Baik, S. Rude, J.R. Wong, and J.H. Postlethwait. 1996. Centromere-linkage analysis and consolidation of the zebrafish linkage maps. *Genetics* **142:** 1277–1288.

Knapik, E.W., A. Goodman, O.S. Atkinson, C.T. Roberts, M. Shiozawa, C.U. Sim, S. Weksler-Zangen, M.R. Trolliet, C. Futrell, B.A. Innes et al. 1996. A reference cross DNA panel for zebrafish (*Danio rerio*) anchored with simple sequence length polymorphisms. *Development* **123:** 451–460.

Knapik, E.W., A. Goodman, M. Ekker, M. Chevrette, J. Delgado, S. Neuhauss, N. Shimoda, W. Driever, M.C. Fishman, and H.J. Jacob. 1998. A microsattelite linkage map for zebrafish (*Danio rerio*). *Nat. Genet.* **18:** 338–343.

Postlethwait, J.H., S.L. Johnson, C.N. Midson, W.S. Talbot, M. Gates, E.W. Ballinger, D. Africa, R. Andrews, T. Carl, J.S. Eisen et al. 1994. A genetic linkage map for the zebrafish. *Science* **264:** 699–703.

Quillet, E., P. Garcia, and R. Guyomard. 1991. Analysis of the production of all homozygous lines of rainbow trout by gynogenesis. *J. Exp. Zool.* **257:** 367–374.

Sheffield, V.C., J.S. Beck, A.E. Kwitek, D.W. Sandstrom, and E.M. Stone. 1993. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* **16:** 325–332.

Shimoda, N., E.W. Knapik, J. Ziniti, C. Sim, E. Yamada, S. Kaplan, D. Jackson, F. de Sauvage, H. Jacob, and M.C. Fishman. 1999. Zebrafish genetic map with 2000 microsatellite markers. *Genomics* **58:** 219–232.

Streisinger, G., C. Walker, N. Dower, D. Knauber, and F. Singer. 1981. Production of clones of homozygous diploid zebra fish (Brachydaniorerio). *Nature* **291:** 293–296.

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