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Proc. R. Soc. B 2009 **276**, 2195-2208 first published online 18 March 2009
doi: 10.1098/rspb.2008.1930

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Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation

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We report construction of a genetic linkage map of the guppy genome using 790 single nucleotide polymorphism markers, integrated from six mapping crosses. The markers define 23 linkage groups (LGs), corresponding to the known haploid number of guppy chromosomes. The map, which spans a genetic length of 899 cM, includes 276 markers linked to expressed genes (expressed sequence tag), which have been used to derive broad syntenic relationships of guppy LGs with medaka chromosomes. This combined linkage map should facilitate the advancement of genetic studies for a wide variety of complex adaptive phenotypes relevant to natural and sexual selection in this species. We have used the linkage data to predict quantitative trait loci for a set of variable male traits including size and colour pattern. Contributing loci map to the sex LG for many of these traits.

Keywords: linkage map; single nucleotide polymorphism markers; expressed sequence tag linked; bacterial artificial chromosome ends; synteny; quantitative trait loci mapping

1. INTRODUCTION

Fishes of the family Poeciliidae have served as model systems for the study of evolution, ecology, behaviour, tumour genetics and genomics. Natural populations of guppies (*Poecilia reticulata*) have been studied for more than 80 years (Winge 1922*a*), and many of the selective forces driving phenotypic variation between populations are well known (Haskins *et al.* 1961). In addition to a plethora of ecological and evolutionary studies, guppies have been studied historically as a model system for the sex-linked inheritance of a variety of male ornamental traits important for sexual selection and adaptation in the natural populations. The evolution of several interesting behavioural and adaptive strategies of this species in the wild is specifically driven by a delicate balance of natural and sexual selective forces. A variety of studies regarding these aspects of guppy biology have already generated a basis to establish this species as an evolutionary model system (Houde & Endler 1990; Magurran & Nowak 1991; Magurran 2001, 2005; Brooks 2002; Crispo *et al.* 2006; Olendorf *et al.* 2006).

We would like to understand the underlying molecular basis for the extreme male colour polymorphism and other variable traits that have been important for evolution and adaptive success of guppies in their wild habitat (Haskins *et al.* 1961; Reznick & Endler 1982; Endler 1991). Genetic maps are thus needed and will allow the mapping of loci underlying quantitative variation (quantitative trait loci (QTL); Lander & Botstein 1989; Leder *et al.* 2006; Shirak *et al.* 2006), map-based cloning of genes defined by mutant or natural alleles (Karsi & Waldbieser 2004; Kazianis *et al.* 2004*b*) and comprehensive investigation of genome evolution between related strains and species (Gates *et al.* 1999; Naruse *et al.* 2000; Danzmann *et al.* 2005; Stemshorn *et al.* 2005).

Detailed genetic linkage maps have been developed for several commercially and scientifically important fishes, including tilapia, rainbow trout, catfish, Atlantic salmon, medaka, Arctic char, zebrafish, fugu and platyfish (Young *et al.* 1998; Gates *et al.* 1999; Naruse *et al.* 2000; Sakamoto *et al.* 2000; Gilbey *et al.* 2004; Kazianis *et al.* 2004*a*; Woram *et al.* 2004; Kai *et al.* 2005; Lee *et al.* 2005).

The guppy was one of the first vertebrates for which sex-linked inheritance was demonstrated and the first linkage map of the guppy was published in 1927, although it was restricted to some sex-linked loci causing variation in ornamental traits (Winge 1927). There is extensive genetic evidence supporting an XY sex-determination system in the guppy, suggesting that a limited non-recombining region of the guppy Y chromosome harbours the male sex-determining locus in tight genetic linkage to male-advantageous ornamental genes (Winge 1922*a*,*b*, 1927; Winge & Ditlevsen 1947; Haskins *et al.* 1970). A few later studies have also shown genetic mapping of different colour patterns to the sex chromosomes, using ornamental guppy strains (Khoo *et al.* 1999*a–c*). Additional partial linkage maps for the guppy genome have been constructed (Foo *et al.* 1995; Khoo *et al.* 2003) using randomly amplified polymorphic DNA, amplified fragment length polymorphism and microsatellite DNA markers (Watanabe *et al.* 2005; Shen *et al.* 2007), as well as microsatellite markers from the closely related genus *Xiphophorus* (Brummell *et al.* 2006). However, none of

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2008.1930 or via http://rspb.royalsocietypublishing.org.

Received 22 December 2008
Accepted 24 February 2009

Table 1. Description of mapping crosses. (Only informative SNP markers, with each grandparent homozygous for polymorphic alleles, are listed. For details, see table 1 in the electronic supplementary material.)

(*a*) *grandparents and number of offspring in mapping populations*

cross	populations[a]	no. of F_2 individuals	no. of informative markers (both grand-parents homozygous)	additional markers[b] (one grand-parent heterozygous)	subpopulations[c]
Cross76	♀Cumaná×♂QuaII_215-3	230	510	209	—
Cross99	♀QuaII_203-4×♂Cumaná	133	461	151	two F_1 pairs
Cross150	♀QuaII_203-4×♂Cumaná	99	504	123	—
Cross153	♀QuaII_203-4×♂Cumaná	281	495	116	five F_1 pairs
Cross157	♀QuaII_215-3×♂Cumaná	896	577	135	nine F_1 pairs
Cross158	♀QuaII_215-3×♂Cumaná	354	583	167	three F_1 pairs

(*b*) *number of informative markers shared between the six mapping crosses*

cross	158	157	153	150	99	76
158	583					
157	501	577				
153	401	415	495			
150	423	408	379	504		
99	396	389	366	377	461	
76	424	416	399	373	363	510

[a] All Quare originated from Quare 6, the numbers indicate laboratory-reared families from this location.
[b] SNP markers for which one of the grandparents was homozygous, while the other grandparent was heterozygous.
[c] F_2 offspring from each F_1 pair considered as an independent population (to facilitate inclusion of a maximum number of informative markers including those for which either grandparent of the mapping cross showed heterozygous alleles).

these previous maps reproduced the correct haploid chromosome number of the guppy, because of an insufficient number of markers.

Some teleosts show differences in recombination rates between the sexes. An androgenetic haploid linkage map of *Danio* was estimated at 1010 cM, but a gynogenetic map at 2583 cM (Singer *et al.* 2002). By contrast, *Xiphophorus* does not show any sex-specific differences in recombination rates (Kazianis *et al.* 2004*a*; Walter *et al.* 2004). Most previous studies have not revealed consistent differences in recombination rates between guppy sexes (Khoo *et al.* 2003; Watanabe *et al.* 2004; Brummell *et al.* 2006), although one report suggested a greater length in males (Shen *et al.* 2007). We report a detailed and complete genetic linkage map for the guppy, with polymorphic single nucleotide polymorphism (SNP) markers linked to either expressed protein-coding genes (expressed sequence tag, EST) or bacterial artificial chromosome (BAC) genomic clones.

Male guppies exhibit high levels of phenotypic and genetic variability for several traits such as size, shape and colour, which have been implicated to influence female choice, male mating success and their susceptibility to predation (Reznick & Endler 1982; Houde & Endler 1990; Endler 1995; Brooks & Endler 2001*a*; Hughes *et al.* 2005; Lindholm *et al.* 2005). These secondary sexual characters are important for male fitness. We report predictions of QTL peaks for selected traits showing variation in the male progeny in one of our mapping crosses.

2. MATERIAL AND METHODS

(a) *BAC library screening*

All of the clones were obtained from a guppy BAC library, prepared from males of the Cumaná population, with an average insert size of 160 kbp (constructed by Bio S&T, Montreal, Canada).

(b) *SNP discovery from BACs*

Plasmid DNA was isolated from overnight cultures of random BAC clones with a REAL Prep96 plasmid kit (Qiagen, Hilden, Germany) and sequenced at both ends with Big Dye Terminator chemistry using the standard pIndigoBAC-5 vector-specific sequencing primers (forward 5′GGATGTGCTGCAAGGCGATTAAGTTGG3′, reverse 5′CTCGTATGTTGTGTGGAATTGTGAGC3′) on a 3730xl DNA analyser (Applied Biosystems, Darmstadt, Germany).

The resulting sequence trace files in ABI format were processed with pregap4 (Staden package), using the phred base-calling algorithm (Ewing *et al.* 1998). Vector sequence and low-quality insert sequence were trimmed (Staden 2000). BAC end sequences were blasted against each other, to identify high copy number, repeat-rich sequences, which were excluded from further marker development. PCR primers were designed from all unique sequences using Primer3 (Rozen & Skaletsky 2000). PCR products were generated with genomic DNA pooled from six individuals representing each strain and sequenced. These sequences were compared and SNP markers were selected that distinguish the Cumaná and Quare strains (see table 1*a*,*b* and the electronic supplementary material, table 1, for details on the number of informative markers in each mapping cross).

(c) *Mapping panel and genotyping*

Several reciprocal mapping crosses were set up between the Cumaná and Quare-6 wild populations, which were maintained in the laboratory for few generations. Cumaná is derived from a high-predation stream in Cumaná, Venezuela (Alexander & Breden 2004) and Quare-6 is derived from a low-predation population in the lower Quare River, Trinidad (Kelly *et al.* 1999). F_1 individuals from each intercross were mated in single pairs. The crosses with Quare ♀ × Cumaná ♂ were found to be more successful in producing fertile F_1

pairs, compared with reciprocal crosses. A final set of six populations with the highest number of F_2 offspring were selected for genotyping. Only one of these, Cross76, was from a Cumaná ♀ × Quare ♂ pair of grandparents. Quare-6 females used were from two separate families, Quare-6 II-203-4 and Quare-6 II-215-3 maintained in our laboratory (table 1*a*).

A total of 2060 individuals from the mapping populations including the F_1 and F_2 offspring from the six intercrosses were genotyped for 224 EST-linked (Dreyer *et al.* 2007) and 819 BAC-linked markers developed in this work. The information about the primer sequences and accession numbers, of all the SNP markers generated and used in this study, is provided in tables 2 and 3 in the electronic supplementary material. A fraction of the markers were linked with coding genes (electronic supplementary material, table 4; additional information is available at http://guppy.weigelworld.org/weigeldatabases/). Genomic DNA was isolated from the tail muscle of each fish using Qiagen DNeasy96 kit (catalogue no. 69582) according to the manufacturer's instructions. Genotyping was done using the MassArray MALDI-TOF mass spectrometry assay (Sequenom, San Diego, CA, USA).

Mapping population was analysed for the marker genotypes and the markers were classified as informative if both grandparents in a cross were homozygous for alternative alleles (table 1; see table 1 in the electronic supplementary material). Between 461 and 583 markers were in this category, depending on the cross. Among the remaining markers, those for which either of the grandparents was heterozygous formed the second category. This comprised 116–209 additional markers per cross (table 1*a*,*b*).

(d) *Generation of the linkage map*

Calculation of the linkage groups (LGs), order and distance between the markers was carried out with the software JoinMap4 (Van Ooijen 2006). Based on the first set of informative markers, LGs were calculated using the independence logarithm of the odds (LOD) at a minimum of 4.0 and recombination frequencies at a maximum of 0.40 as the threshold for each cross. The correct haploid number of 23 LGs was obtained for each of the six mapping populations. The map order of each LG was estimated with the regression mapping algorithm using Kosambi's (1944) mapping function. An initial framework map was generated for each cross. For comparison of the basic framework map, maximum-likelihood mapping, which uses Haldane's mapping function, was also used. The marker order was the same with both mapping functions, but occasionally maximum-likelihood mapping resulted in larger inter-marker distances. For the final combined map, all map distances were computed with regression algorithm using Kosambi's mapping function.

To integrate the maximum number of additional markers from the second category into the framework map, the F_1 pairs with a large number of F_2 offspring from each intercross were analysed as a separate mapping population. For this, the genotypes of all markers were used to calculate the linkages (table 1*a*), applying the fixed marker order obtained from the framework map when required (JoinMap; Van Ooijen 2006).

This resulted in individual F_1 pair-specific maps with additional markers incorporated into the framework map for each cross. The multiple maps obtained from each pair of grandparents were combined to obtain a single map using the map integration function of JoinMap4 (Van Ooijen 2006). This calculated the virtual numbers of recombinant and non-recombinant gametes estimated in each population for each pair of loci, which is then averaged across populations to obtain the mean recombination frequencies and corresponding LOD score values. The integrated maps for each of the six mapping crosses were studied individually and the best order of the markers was confirmed for each LG. Consensus maps from the six grandparent crosses were then compared and markers whose positions were ambiguous or that added significantly (more than 20 cM) to the map length were removed. Each of the 23 LGs from different populations was then combined, to obtain the final integrated map. For calculating the map orders, the consensus framework maps for each LG were given as fixed orders (JoinMap; Van Ooijen 2006).

(e) *Phenotype analyses*

All mature males were submerged in tricaine as terminal anaesthetic, and photographed with a digital single lens reflex camera under incident light, at an average age of 120 days. Lateral views, each with a coloured scale bar, were used for the measurements of the colour patterns on male body and fins, as well as for the size estimates of each male. The measurements of the phenotypes of all males were performed using Cell Imaging software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). This allows semi-automatic identification of each area of similar pixel density on the fish, and calculates associated parameters for it. The parameters measured for each colour pattern were area size, perimeter length, mean colour intensity, mean hue (red, blue and green) and position of the centre of mass with respect to a reference point that was arbitrarily set to the middle of the eye. The final trait values used for QTL mapping calculations were direct measurements as well as derived numerical values from each property of the trait. For example, for the anterior orange spot, we used its area, mean colour intensity and mean hue individually as well as the products of these measurements. Here we present the results of the QTL mapping of area (mm^2) of each colour trait. Besides recording all colour patterns on males, specific length measurements (mm) between fixed points on each fish were taken. Variation in trunk length was measured as the gap between fixed points on the snout and the caudal or dorsal fin; the width was measured as the height of the anterior and posterior caudal peduncle and the gap between dorsal fin and gonopodium. The caudal peduncle was surrounded by fine margins at its dorsal–ventral, anterior–posterior boundaries and the total area of the enclosed surface was estimated. These length measurements and their ratios were used for QTL mapping of size and shape variation among males.

(f) *QTL predictions*

All estimations of genotype–phenotype correlations for QTL mapping were performed with the MapQTL5 software (Van Ooijen 2004). Under the null hypothesis, i.e. when the segregating QTL has no effect, the Kruskal–Wallis (KW) statistic is distributed approximately as a chi-square distribution with the number of genotype classes minus 1 as the degrees of freedom. The power of the test depends on the degrees of freedom and the number of individuals in the test. We used a KW score (K^*) of 10.0 and a significance level of 0.005 as the threshold for all our tests. The traits with significant K^* values on any LG were further analysed using interval mapping (IM) and multiple-QTL model

Table 2. Marker distributions across linkage groups among six mapping crosses. (no., the number of markers; *L*, the genetic length of each linkage group, for every cross.)

	Cross76		Cross99		Cross150[a]		Cross153[a]		Cross157[a]		Cross158[a]	
LG	no.	*L* (cM)	no.	*L* (cM)	no.	*L* (cM)	no.	*L* (cM)	no.	*L* (cM)	no.	*L* (cM)
01	16	27.50	18	32.70	25	32.70	20	32.50	28	30.46	30	38.10
02	23	28.80	27	29.90	29	43.30	31	29.90	35	40.29	41	41.40
03	14	19.90	17	21.90	17	19.30	14	22.20	15	22.69	26	35.80
04	15	31.60	15	49.10	19	39.80	11	43.50	22	54.23	31	51.70
05	8	20.70	14	23.80	10	17.50	15	29.50	18	30.02	21	24.50
06	29	36.00	25	45.70	30	38.70	31	45.40	35	49.60	32	41.90
07	37	37.00	29	41.70	30	38.90	37	34.30	26	33.04	34	38.70
08	28	29.30	19	28.80	28	37.60	26	32.00	30	33.15	34	31.00
09	28	33.80	26	53.50	23	50.80	33	52.00	33	52.75	34	50.80
10	19	34.10	17	31.30	21	52.00	23	40.30	24	41.24	28	50.20
11	20	25.40	20	38.80	15	33.80	25	32.90	28	40.90	24	38.80
12	14	25.80	15	30.20	15	35.40	19	29.80	25	27.55	22	34.10
13	19	35.80	19	44.90	18	43.20	23	37.50	30	42.54	28	42.40
14	15	27.20	14	24.90	20	36.80	19	33.90	31	31.21	30	32.00
15	21	36.00	16	47.70	15	48.20	19	32.80	21	52.85	28	45.40
16	13	24.00	11	30.60	17	24.40	19	29.40	22	30.43	17	31.70
17	26	32.50	24	39.40	24	34.50	30	27.80	32	34.63	30	30.40
18	20	29.00	20	35.80	19	45.00	25	34.80	23	38.16	22	33.90
19	22	23.50	17	32.60	19	31.40	29	30.80	24	28.81	27	33.30
20	10	30.30	11	37.30	14	31.90	18	30.00	21	32.60	18	31.80
21	17	23.00	12	26.70	14	28.30	16	25.40	23	37.69	22	32.50
22	15	10.70	11	15.50	13	28.20	25	20.80	23	26.29	24	30.30
23	23	28.10	18	28.70	16	35.60	20	27.90	30	28.90	23	33.00

[a]Combined map from individual F_1 pair subpopulations for the cross.

(MQM) functions of the MapQTL5 software. A genome-wide permutation test was performed for each trait with 1000 iterations, to establish LOD thresholds at a *p*-value of 0.05 for each LG. Following the initial round of IM, markers with the highest LOD score for a trait were selected as cofactors, and MQM analysis was performed. The analysis was repeated, until no further significant peaks were obtained. The automatic cofactor selection function of MapQTL5 was then used to select the significant set of cofactors and MQM was repeated, until no more peaks with higher significance were obtained. All peaks with a LOD score above the calculated threshold for their specific LG were considered to represent the QTL linked with the trait. The markers closest to the maximum significant interval in the QTL likelihood map are listed with their positions, LOD values. The estimates of the additive and dominant effects and the estimated mean distributions of the quantitative trait associated with each genotype, for all significant loci, are also shown. The estimated mean trait value is received as an output from the final mapping of the trait through MQM analysis, and it takes into account the additive and dominance effects of all the cofactors influencing a trait. For an F_2 population, in addition to the three QTL means, the associated additive and dominance effects for every cofactor marker are also modelled (Van Ooijen 2004). Because there are roughly as many heterozygous as homozygous (Cumaná and Quare) genotypes, the positive and negative additive effects do not always average to 0, affecting the overall values around which the three QTL means are estimated and sometimes giving a negative estimate mean trait value. A correlation matrix was established to identify the size and shape traits that co-segregated in a significant manner. To test whether the size and shape traits covary, Pearson correlation coefficients were calculated for all trait–trait comparisons, for the 16 size- and shape-related traits. This analysis used the trait scores for each individual in the mapping panel. The correlation matrix retained the magnitude and direction (positive and negative) of each correlation coefficient (see table 5 in the electronic supplementary material).

3. RESULTS

(a) *A complete genetic map of the guppy*

The linkage map obtained from each of the mapping crosses represents the correct number of 23 haploid chromosomes for the guppy (Winge 1922*b*). The individual maps were found to be consistent regarding the marker orders and overall map lengths (table 2), and were combined to obtain a final consensus linkage map for the species (figure 1). The sum of intervals between all loci on the final combined map was 850 cM. Two approaches were used to estimate map length: first, the genome length was estimated by adding $2s$ (s is the average space between adjacent markers on the linkage map) to the length of each group to allow for chromosome ends (Fishman *et al.* 2001). This yielded a corrected genome length of 896 cM. By the second approach, the genome length was calculated by multiplying the length of each LG by $(m+1)/(m-1)$, where m is the number of markers in each group. The estimated map length is the sum of the revised length of all LGs (Chakravarti *et al.* 1991) and was calculated to be 902 cM by this method. The average of the two corrected genetic length estimates was 899 cM and includes 790 markers on 23 LGs (table 3).

In addition, the positions of a few markers were uncertain (from 2 to 7 per LG; see table 6 in the electronic supplementary material), either because they were

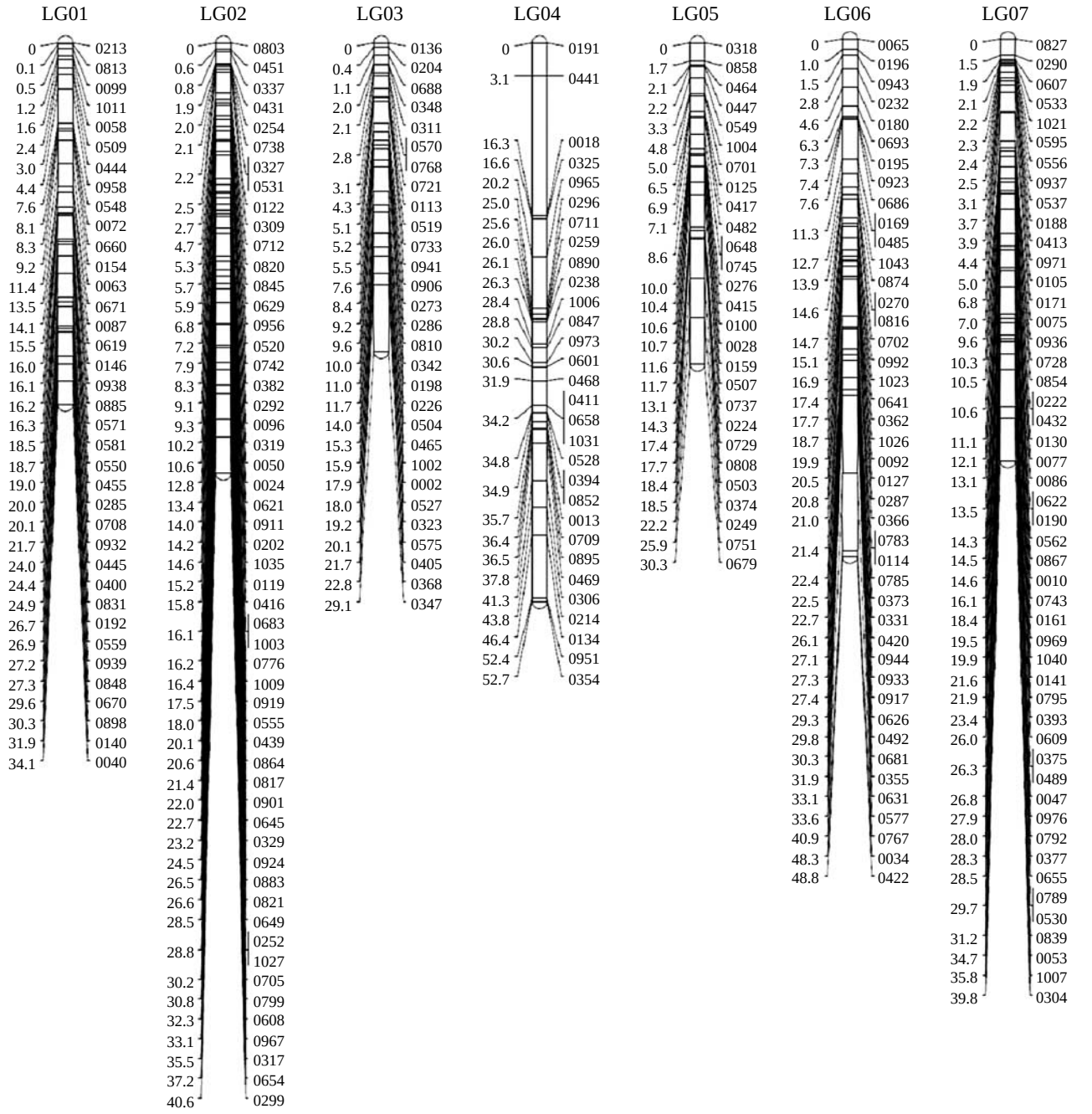

Figure 1. The genetic linkage map of the guppy genome. The numbers to the right on each bar are the markers and the numbers on the left indicate the genetic distance in centimorgans (cM). The numbering of LGs is based on homology with medaka chromosomes. The markers showing no recombination in between them on an LG are shown at the same locus. The putative location of the sex-determining locus (*Sex*) is shown at the distal end of LG12 (sex chromosome).

successfully typed in only a small number of individuals, or because they were informative in only a few individuals.

A few markers were excluded from the final map because they would have added significantly to the map distances. These markers mapped terminally on the LG, with abnormally large distances (more than 20 cM) to the nearest markers, rendering their positions doubtful. Examples include marker 0484 on LG01, marker 0003 on LG16, marker 0145 on LG03 and marker 0046 on LG20 (see table 6 in the electronic supplementary material).

The largest gap on the framework map is 13.2 cM, on LG04. The number of mapped loci ranges from 23 to 54 per LG, and the average length of an LG is 39 cM, ranging from 29 to 58 cM, with an average of one marker per 1.1 cM. Based on the predicted genome size of 700 Mbp (Khoo *et al.* 2003), we expect the average inter-marker distance to be approximately 1 Mbp (table 3).

(b) *Distribution of crossovers*

Cytogenetic studies have established the acrocentric nature and similar size of all 23 pairs of guppy chromosomes (Nanda *et al.* 1993; Traut & Winking 2001). With 23 haploid chromosome arms, the predicted genome size should be in the range of 1150 cM (50×23) under the complete interference model (Villena & Sapienza 2001; Guyomard *et al.* 2006; Moen *et al.* 2008). Our estimates of the total genome length are slightly lower than this. We estimated the average proportions of F_2 offspring inheriting recombinant gametes, from meiosis in both F_1 parents. The mean

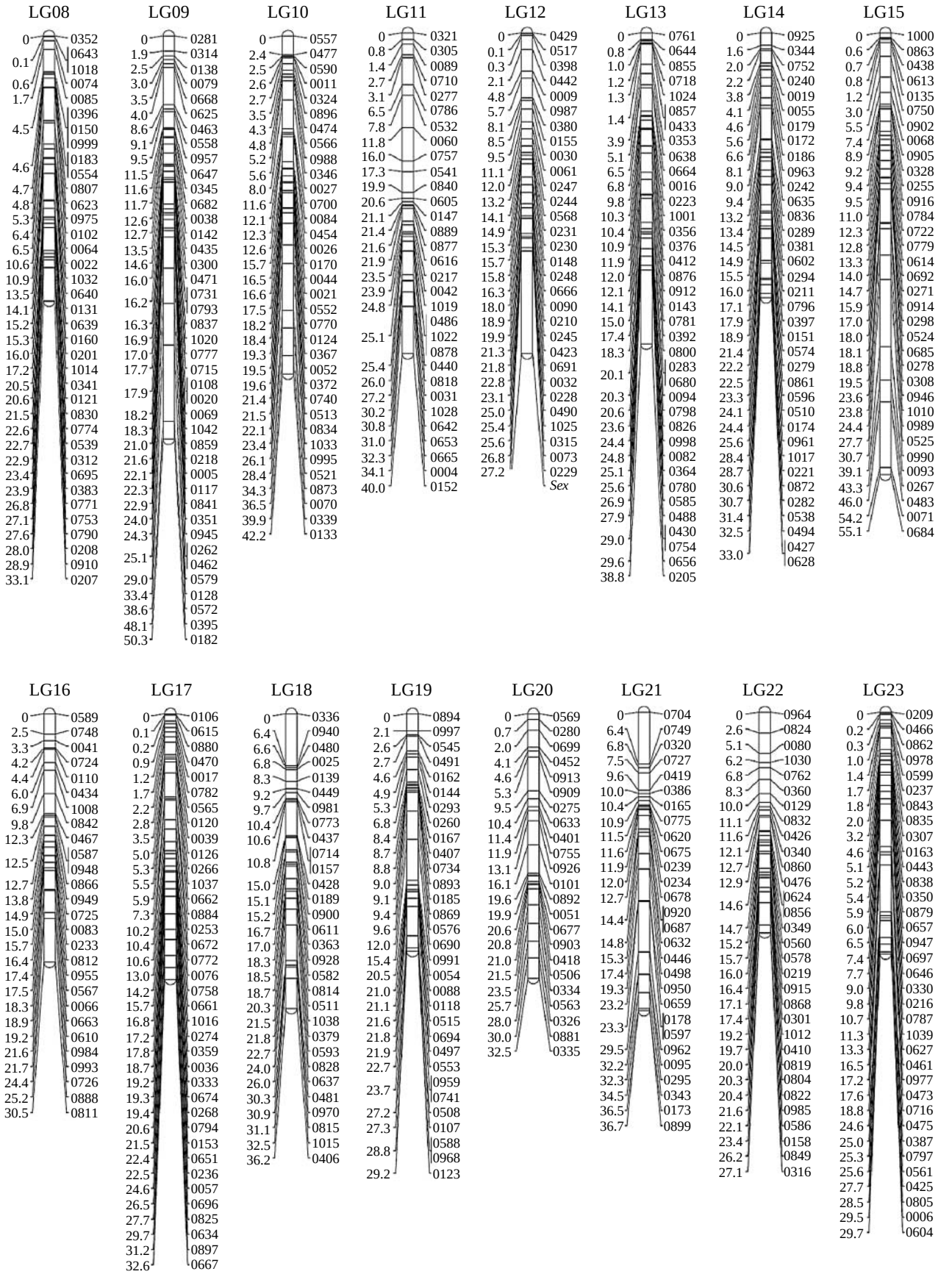

Figure 1. (*Continued*.)

frequency of non-recombinant and single recombinant gametes, across all LGs, was 44 per cent. The frequency of F_2 offspring inheriting two recombined gametes (one from each F_1 parent) for a chromosome was 10 per cent. Moreover, the distribution of the estimated proportion of individuals with two recombined gametes was roughly correlated with the genetic size of the LG. A very low proportion of F_2 individuals (0.43%) inherited recombined chromosomes with more than two crossover events (table 4). These results demonstrate

Table 3. Guppy genetic map overview.

(*a*) *summary of the integrated genetic map of the guppy*

no. of individuals	2060
no. of linkage groups	23
no. of markers on the map	790
marker distribution	34 markers/LG
total map length	899 cM[a]
average length/LG	39 cM
average marker density	1 marker/1.1 cM
estimated physical spacing	1.19 cM Mbp^{-1}
range of marker distribution	23–54
range of length/LG	29–58 (cM)

(*b*) *length and marker distribution on linkage groups*

linkage group	no. of markers	length (cM)
LG01	37	36.04
LG02	54	42.34
LG03	29	31.12
LG04	30	55.57
LG05	27	32.41
LG06	43	50.97
LG07	49	41.64
LG08	37	34.98
LG09	41	52.57
LG10	34	44.47
LG11	31	42.29
LG12	29	29.14
LG13	37	40.93
LG14	36	34.95
LG15	34	57.82
LG16	27	32.72
LG17	37	34.47
LG18	30	38.47
LG19	31	31.21
LG20	23	35.00
LG21	28	39.01
LG22	31	29.01
LG23	35	31.60
total	790	899[a]

[a] The total map length is the derived estimate, incorporating the correction for chromosome ends.

crossover interference and comply with the predictions based on the known acrocentric nature of guppy chromosomes.

(c) *Identification of the sex LG*

LG12 was identified as the sex LG in the guppy, since the markers showed clear sex-linked segregation and recombination between X- and Y-linked alleles was suppressed (Tripathi *et al.* in press). The sex-determining locus (*Sex*) mapped genetically to the distal end of LG12 in all crosses (figure 1), marker 0229 being mapped closest to this locus; although the gap between marker 0229 and *Sex* could not be predicted with certainty. This gap is expected to include the differentiated region of the Y chromosome and has not been taken into account in genome length calculations, due to its uncertain length. None of the known molecular markers on LG12 are present exclusively on the Y chromosome, although X- and Y-linked alleles for all markers on this LG show distinct segregation, due to reduced recombination. The putative Y-specific region at the distal end of this chromosome is also predicted to contain a few colour pattern loci in tight linkage with the master sex-determining locus (Winge & Ditlevsen 1947; Lindholm & Breden 2002).

Table 4. Distribution of the predicted number of recombination events per linkage group in the combined mapping population. (The values represent the percentage of F_2 individuals showing null, 1, 2 or more recombination (rec.) events for the respective linkage groups. The results obtained from the six mapping crosses have been averaged.)

LG	no rec.	1 rec.	2 rec.	>2 rec.
LG01	47.33	43.74	7.96	0.24
LG02	40.80	45.60	10.44	0.79
LG03	62.23	34.66	2.72	0.10
LG04	31.39	43.62	21.20	0.95
LG05	44.01	43.72	9.70	0.64
LG06	34.21	45.72	18.22	0.46
LG07	42.61	46.26	9.57	0.39
LG08	43.35	46.89	7.91	0.46
LG09	32.13	45.99	19.64	0.56
LG10	38.59	47.23	12.52	0.41
LG11	44.90	45.10	9.03	0.24
LG12	49.76	47.23	2.43	0.15
LG13	35.83	46.17	14.81	0.80
LG14	49.73	42.70	6.89	0.17
LG15	37.07	42.94	16.50	0.87
LG16	47.79	42.31	8.20	0.42
LG17	43.92	46.74	8.22	0.28
LG18	42.18	44.71	12.14	0.24
LG19	48.74	45.97	3.69	0.40
LG20	41.58	46.00	10.19	0.56
LG21	47.72	41.84	9.47	0.24
LG22	56.65	38.98	3.59	0.19
LG23	47.77	44.81	5.87	0.39
average	43.93	44.30	10.04	0.43

(d) *Synteny with medaka*

Our markers included 332 markers linked to coding genes with significant similarity to sequences in the medaka genome (as determined by BLASTN, *e*-value $\leq 10^{-5}$, identity $\geq 80\%$). Of these, 276 (83%) could be assigned to specific LGs on the guppy map. For each guppy LG, we found between 2 and 16 markers that belong to a single medaka chromosome (table 5; see table 4 in the electronic supplementary material). We can thus infer orthologous chromosomes in these two species, which have been separated for approximately 100 million years (Volff 2005; Volff *et al.* 2007). We used this information to name guppy LGs corresponding to the medaka chromosome with the maximum number of shared markers (table 5; see table 4 in the electronic supplementary material). Since medaka (24 chromosomes) has one chromosome more than the guppy, LG02 of the guppy corresponds to chromosomes 2 and 21 of the medaka, while LG21 of the guppy corresponds to chromosome 24 of the medaka. Despite ample evidence for collinear blocks, the orthology between medaka chromosomes and guppy LGs is not absolute, since, on average, approximately a quarter of gene-linked markers from a given LG in the guppy mapped to a different medaka chromosome (table 5; see table 4 in the electronic supplementary material).

(e) *QTL predictions*

We mapped QTL influencing a selected set of size, shape and colour traits, segregating in one of the mapping

Table 5. Synteny between medaka chromosomes and guppy linkage groups (LGs). (Distribution of the markers linked with coding genes found common between guppy linkage groups and medaka chromosomes. The numbers in italics along the diagonal indicate the number of markers showing major syntenic relationships between guppy LGs and corresponding medaka chromosomes, and comprise 190 EST-linked markers.)

guppy LG	medaka chromosomes																							
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
01	*14*				1			2										1	2	1				
02		*3*											1		2						*5*			
03			*5*													1	1							
04				*8*		1											1		1	1				
05			1		*4*		1									1						1		
06	1					*7*	2	1					1											
07	2				1		*16*			1							1							
08	1		1					*13*		1							1		1			1		
09	1								*12*					1		1	1							
10									1	*12*				2						1				
11							1			1	*11*		1											1
12												*10*		1						1				
13									1				*6*				1			1		1		
14			1		1		1		1					*9*		3	1			1	1	1		
15															*2*									
16		1								1	1	1				*9*	1							
17																1	*6*				1			
18					1		1								2			*3*				1		
19		1				1													*11*					
20		1								1										*4*				
21							1				1			1						1				5
22							1									1		1				*10*		
23					2			2			2												*5*	

crosses (Cross157, Quare ♀ × Cumaná ♂). The non-parametric KW test showed a clear correlation between quantitative trait and markers on specific LGs. Traits with a high KW significance were mapped independently using MQM mapping. MQM mapping resulted in the detection of loci influencing the traits on some additional LGs and further strengthened the confidence of the QTL prediction (table 7 in the electronic supplementary material). This resulted in selection as cofactors, of a subset of markers from each contributing LG, showing the highest correlations with the trait (see table 8 in the electronic supplementary material).

Discrete genetic polymorphism for male size is characteristic of some species in the family Poeciliidae (Kallman 1989). Guppies are sexually dimorphic for adult size and males are highly variable in size at maturity (Reznick & Endler 1982). Size variation in male guppies is a quantitative trait, approximately normally distributed within populations and characterized by high heritability (Reznick *et al.* 1997; Nakajima & Taniguchi 2002; Hughes *et al.* 2005). While the females continue to grow throughout their adult lives, males reach their maximum body size at approximately three months of age, when the growth rate sharply decreases. We used the genetic map to predict QTL for various parameters related to the size and shape of males. We directly measured the distances between fixed landmarks on each adult male and used these as quantitative traits (figure 2*a*). We used the derived ratios of specific length measurements and caudal peduncle area, to map QTL, which reflect the relative proportions and geometry of the body and are indicative of shape variations.

For all of the length measurements and the caudal peduncle area, there was a major effect QTL in the proximal region of LG12. This peak accounted for a total of 20–30% variation in the length traits, with additional contributions from QTL on three to five other LGs. In combination, all the significant peaks could explain 35–45% of the total variation for the length traits, while peaks on five different LGs, including LG12, could explain 43 per cent variance in caudal peduncle area. QTL on LG12, 09, 02, 07, 18 and 04 seemed to contribute predominantly to most of the size-related traits (see table 8*a* in the electronic supplementary material).

The derived quantitative traits reflecting the relative proportions and aspect ratios of the fish were used to map QTL affecting body shape. Ratios of various length measurements and the area of the caudal peduncle were calculated, to represent components of the overall geometry of the fish. QTL for these ratios, indicative of shape variation, also mapped to the proximal region of LG12 for many traits, confirming the presence of at least one major locus influencing size and shape in the pseudo-autosomal region of the sex chromosome (see table 8*b* in the electronic supplementary material). This QTL explained 5–21% of the total variance for different ratios. QTL on LG01, 02, 04, 07, 08, 09, 11, 12, 18 and 22 seemed to contribute predominantly to most of the shape-related traits. In combination, all the significant peaks could explain 17–49% of the total variation in different shape-related traits (see table 8*b* in the electronic supplementary material).

A correlation matrix generated to analyse the correlation coefficients between all the size and shape-related

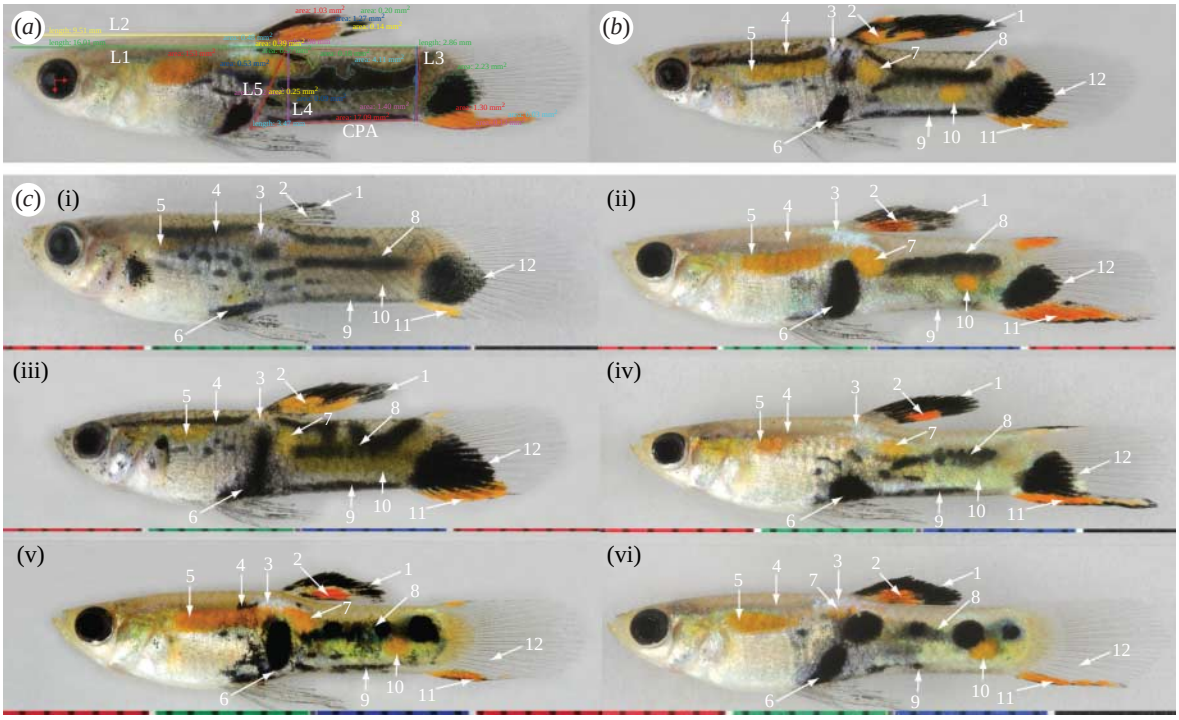

Figure 2. Variable quantitative traits segregating in Cross157 F_2 males. (*a*) The length measurements between fixed points on each fish, used for QTL predictions for the size and shape variation (table 7*a*,*b* in the electronic supplementary material). L1, snout to hind fin; L2, snout to dorsal fin; L3, posterior caudal peduncle height; L4, anterior caudal peduncle height; L5, gonopodium to dorsal fin; CParea, caudal peduncle area. (*b*) The polymorphic colour traits used for QTL predictions (table 7*c* in the electronic supplementary material). The numbers mark following traits: (1) dorsal fin black; (2) dorsal fin orange; (3) central blue white spot; (4) anterior main black stripe; (5) anterior orange spot; (6) black spot by gonopodium; (7) central orange spot; (8) posterior main black stripe; (9) posterior ventral black stripe; (10) posterior orange spot; (11) hind fin lower orange; (12) hind fin caudal black spot. (*c*) The segregation of colour pattern variation among males of Cross157. (i)–(vi) Arrows show the extreme variation for different traits among six representative F_2 males.

traits indicated that all the size traits and the area of the caudal peduncle showed very high correlations (80–97%) among themselves, and with selected shape traits (see table 5 in the electronic supplementary material). Since most of these traits also show a major effect QTL on LG 12, this locus could be responsible for the coregulation of the size and shape variation.

Highly polymorphic colour patterns of guppy males have been long known to play important direct and indirect roles in various aspects of their biology, influencing sexual selection through female preference (Hughes *et al.* 1999; Brooks & Endler 2001*a*,*b*; Eakley & Houde 2004; Miller & Brooks 2005) and natural selection due to a variable susceptibility to predation (Endler 1991; Karim *et al.* 2007; Reznick *et al.* 2008). Guppy males exhibit diverse phenotypic patterns, including variation in colour, number, shape, size and position of spots, and this polymorphism is known to have a substantial genetic component (Winge 1922*a*; Winge & Ditlevsen 1947; Haskins *et al.* 1961; Brooks & Endler 2001*a*; Lindholm *et al.* 2004). We have mapped some of the colour traits prevalent in two geographically distant populations (Quare and Cumaná), segregating in Cross157. The selected traits that have been used for QTL mapping include all prominent orange or black spots and stripes as well as an iridescent patch on the body of the fish. These patterns exhibit visible polymorphism, segregating among F_2 males of this cross (figure 2*b*), and show distinct patterns of expression having dominant, suppressive, mutually exclusive or co-dominant effects over other traits (Tripathi *et al.* 2008). These colour patterns are potentially important for the fitness and adaptive success of the parental populations in the wild. We detect significant QTL peaks on various LGs underlying some of these colour traits. The results of the MQM mapping for the area of the colour traits are shown (see table 8*c* in the electronic supplementary material).

Estimated mean values of the quantitative traits (Van Ooijen 2004) associated with homozygous and heterozygous genotypes, at all significantly contributing loci, indicated obvious genetic polymorphisms linked with the segregating traits, in the two parental populations. For many of the size, shape (figure 3*a*) and colour (figure 3*b*) traits, a clear correlation with either Cumaná or Quare alleles could be detected. For several traits, the alleles showed obvious additive or dominant effects. For instance, the lengths L2, L3 and L4 appear to be positively correlated with the presence of Quare alleles on LG12 and intermediate values in heterozygotes suggest additive effects of both alleles. The ratio of L1 over L2 maps to a QTL on LG04, but for this trait the Cumaná dominates the Quare allele (figure 3*a*). Also, with respect to the orange area on the dorsal fin, an allele on LG16 of Cumaná is dominant over the corresponding Quare allele. The total percentage of variance explained (PVE) by all significantly contributing QTL for a trait, ranged between 9 and 32 per cent for the colour traits.

Furthermore, fine mapping of the loci underlying the quantitative traits, broadly mapped in this study, will

(*a*)

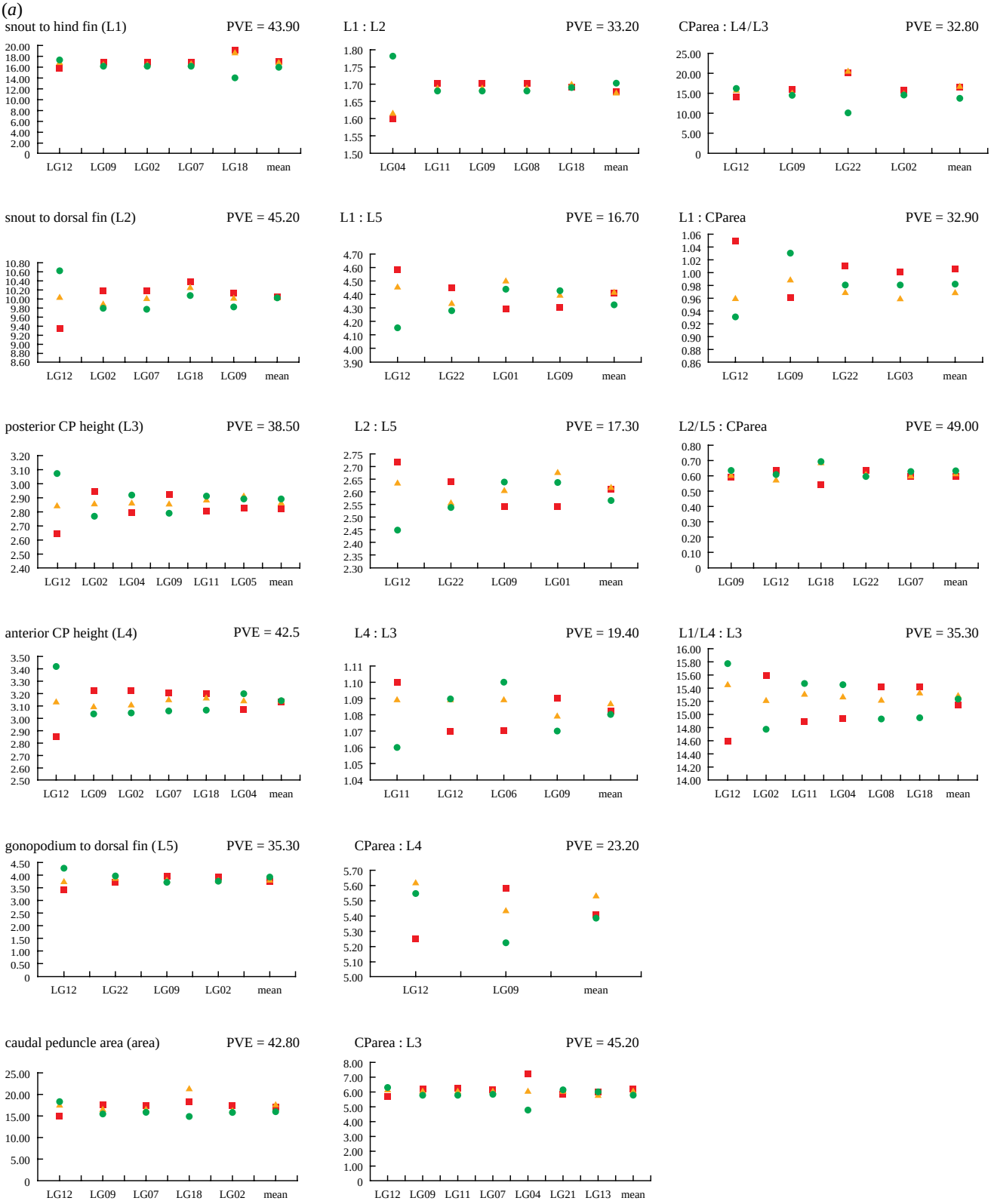

Figure 3. Segregation of colour pattern, size and shape QTL in adult male guppies. The estimated mean values of the distribution of quantitative trait associated with the paternal (Cumaná), maternal (Quare) and heterozygous genotypes among F_2 males of Cross157 are plotted for each contributing linkage group that has a significant QTL (see §2 for explanation of estimated mean trait values). 'Mean' refers to the average value of the trait when all loci significantly linked with the trait have homozygous Cumaná, or, heterozygous or homozygous Quare alleles. The total percentage of variance explained (PVE) by all significantly contributing QTL for each trait is shown. The linkage groups for each trait are shown in descending order of the PVE. Only the loci closest to the significant maxima in the QTL likelihood map, with a LOD higher than the estimated threshold based on the permutation test for respective linkage groups and explaining at least 2.5% of the total variance, are shown here. (*a*) Segregation of size (mm) and shape (ratio) QTL in Cross157 males. (*b*) Segregation of colour pattern QTL in Cross157 males, using the area (mm^2) of each component as trait keys. Red, Cumaná alleles; yellow, heterozygous; green, Quare alleles; *x*-axis, linkage group; *y*-axis, estimated mean trait value.

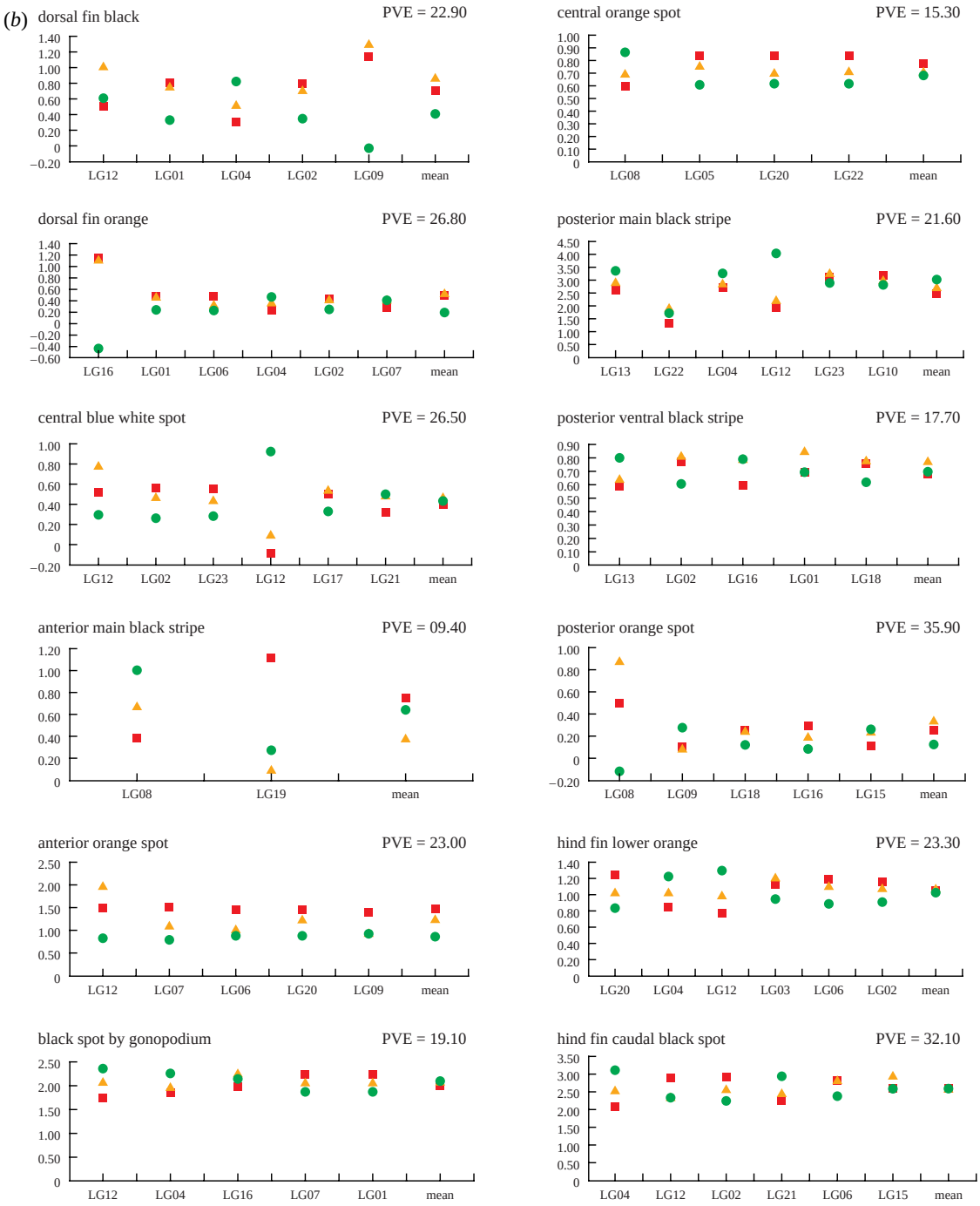

Figure 3. (*Continued.*)

require more individuals segregating for the phenotypes and a higher marker density.

4. DISCUSSION

We have reported here a dense genetic linkage map of the guppy, which provides a basis for further genetic and molecular studies of sex linkage of male-advantageous genes, as well as natural variation of quantitative adaptive traits. The synteny information facilitates selection of candidate genes during fine mapping of QTL, since the guppy genome has not yet been sequenced.

Based on crosses involving ornamental guppy strains, quite variable lengths of the genetic map had been estimated (Khoo *et al.* 2003; Watanabe *et al.* 2005; Shen *et al.* 2007). Our genetic map has a length of 899 cM, which is shorter compared with that of the medaka at 1354 cM (Naruse *et al.* 2000), or even the closely related *Xiphophorus* at 2486 cM (Kazianis *et al.* 2004*a*). Both species have 24 chromosomes compared with a haploid set of 23 in guppies and marginally higher estimated genomic DNA content (Cimino 1974; Lamatsch *et al.* 2000). Owing to the absence of an EST-linked map for the platyfish genome, we could not compare the synteny between guppy and platyfish genomes. In a comparative study with common microsatellite markers, the guppy was also found to have lower rates of recombination than *Xiphophorus*, which is in the same family as the guppy (Brummell *et al.* 2006). An explanation for the relatively short genetic length of the

guppy map could be sequence inversions between the Cumaná and Quare populations, due to significant divergence (Alexander & Breden 2004), which might result in reduced recombination frequency.

We have tested microsatellite markers from the sex chromosomes of platyfish, in addition to a set of selected candidate genes from the sex chromosome, but did not find any evidence of its sex linkage in the guppy (data not shown). Although from the present data, it seems likely that the sex-determining loci in platyfish and guppy are not homologous, we cannot prove it until we have additional markers from the diverged gonosomal region of guppy sex chromosomes, and more gene-linked markers from the sex chromosome of platyfish.

The differentiated region of the sex chromosome is likely to consist of expanded heterochromatin-rich sequences. Hence, due to the uncertain gap between the last mapped markers and the *Sex* locus, we have not included it in genome length estimates. Several additional markers that were placed with confidence on an LG, but could not be assigned to their accurate position (see table 6 in the electronic supplementary material), may add to the total genetic length when correctly positioned on the map. Therefore, the present calculated length could be a slight underestimate.

The multiple QTL mapped in this work affect traits that contribute to phenotypic variation of males in the two mapping populations. Several QTL were located on LG12, indicating that loci responsible for visible polymorphism in size, shape and colour patterns are enriched on the sex chromosome. This is in agreement with the previous knowledge of physical linkage of major colour pattern loci to sex chromosomes in the guppy (Winge 1927; Winge & Ditlevsen 1947; Haskins *et al.* 1970; Brooks & Endler 2001*a*; Lindholm & Breden 2002). We do not believe that the enrichment for QTL reflects exceptional gene density on the sex chromosome, based on the extensive conserved synteny of the major portion of the guppy sex chromosome with an autosome of medaka.

While we do not yet have any markers from the Y-specific segment, due to the suppressed recombination between X and Y chromosomes, the reduced recombination facilitates the identification of QTL mapping to the sex LG. Unfortunately, for the same reason, it is difficult to resolve the precise locations of the sex-linked QTL. Based on the visual analysis of their segregation, we can predict certain Cumaná-derived alleles affecting quantitative colour traits (1, 2, 3 and 9 in figure 2*b*) to be in tight linkage with the *Sex* locus. Not all of these mapped to the expected region in the present QTL analysis, due to the absence of Y-linked markers.

The multiple genes detected for most of the traits mapped in this study probably reflect the complex nature of the traits, and indicate that their final expression may require a range of biological pathways and involve products of many genes. Furthermore, there will undoubtedly be additional loci, as a single mapping cross does not generally segregate all of the quantitative loci affecting any given trait (Mackay 1996).

The MQM mapping results for selected shape traits and for areas of multiple colour traits suggest that multiple QTL of minor effect (lower LOD scores) contribute to each colour trait, while fewer large effect QTL with higher LOD scores may explain the selected shape traits.

In summary, the guppy genetic map will allow efficient use of the extensive genomic information available for several reference teleost fishes. In the long term, it will help to further our understanding of the genetic basis of adaptive evolution in this species and to explore the processes governing the evolution of genomes.

All experiments were performed in agreement with German regulations for keeping lower vertebrates in the laboratory (Regierungspräsidium Tübingen).

We wish to thank Felix Breden and David Reznick for providing guppies, Jakob Hodyl, Bartosz Pieczara and Gertrud Scheer for their help with photography and image analysis, and Norman Warthmann for help with bioinformatics tools. Supported by a Gottfried Wilhelm Leibniz Award (DFG) to D.W. and the Max Planck Society. Sequence data from this paper have been deposited with GenBank Data Libraries under accession numbers FH888484–FH893712.

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