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Gene mapping in fishes: a means to an end

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Abstract

Genetic research in fishes is poised to contribute a vast amount of information on the structural organization and function of vertebrate genomes. Recent advances in molecular biology have made possible the widescale characterization of genomes in all living organisms. This includes defining chromosomes at the cytological level down to their linear composition at individual nucleotide base pairs. Pioneering gene mapping studies into the genomes of fishes will only serve as the starting point for more detailed studies into the function of these genomes. Future research directed at understanding the mechanisms of gene actions and interactions will benefit all areas of biology, including ecology, ethology, evolution, and physiology. Gene mapping data from brown trout and rainbow trout are used to exemplify how basic information on gene transmission in a species may help to localize centromeres onto a genetic map and identify chromosomal regions possessing a high degree of segregation distortion. Genetic maps may also be used to identify differences in recombination levels among individuals and between the sexes when multiple mapping families are utilized in studies. Observations of this type are the antecedents to more complex biological investigations on the genetic architecture underlying these phenomena.

Introduction

Genetics is defined as the study of heredity and embodied in the definition of heredity is the transmission of genetic characters from parents to offspring. Among the several definitions that exist for a map, the most appropriate is the concept that a map is a two-dimensional reconstruction of a part of the earth or heavens. In its totality, a genetic map does portray the ordering and landmarks of the physical entities we know as chromosomes. But in and of itself any map, including a genetic map, can only direct the observer to a specific location or place. It cannot convey to the observer the nature of that location or the activities that occur at that location. To attain an appreciation for the location we recognize that is necessary to actually visit and observe the location. As a biological metaphor genetic maps are only the genesis for understanding the evolutionary complexities of any species.

To use an analogy based upon a world map, we may regard genetic mapping as a multi-layered process spanning the identification of individual landmarks that may be miles apart on several continents, down to the cataloging of individual grains of sand between these landmarks. By equating chromosomes to continents, landmarks to genetic markers, and grains of sand to individual nucleotide base pairs, a measure of the complexity inherent in the construction of genetic maps may be gained. Fortunately, the analogy to 'grains of sand' is somewhat of an exaggeration, as species have far fewer nucleotide base pairs than inferred. Nonetheless, the concept does portray a feeling for the magnitude of organization that is expected at each level. We have yet to witness the completion of a gene map for any vertebrate species, even though this target is nearing completion for our own species, Homo sapiens (IHGSC, 2001). Studies of gene mapping in fishes are largely focused on the second level of organization (i.e., the addition of genetic markers to define linkage groups within the species). However, in the zebrafish (*Danio rerio*) and pufferfishes (*Fugu rubripes*, *Tetraodon nigroviridis*), gene mapping studies are well advanced at the highest level of organization, and a large amount of

raw sequence data exists for either species in public databases.[1] In addition, plans are currently underway to sequence the entire genome of the pufferfish due to its relatively small size (∼400 Mb) (McLysaght et al., 2000) which would make it the first teleost for which complete genome information is available. It is expected that functional gene coding regions will be in a higher density in the pufferfish (given the compact nature of the genome) per number of contigs assembled (Crnogorac-Jurcevic et al., 1997) and thus sequencing efforts are expected to yield more immediate rewards.

Several families of fish (e.g., Callichthyidae, Catostomidae, Cobitidae, Salmonidae) possess members that are derived from ancient polyploid events in their ancestry. Allopolyploid derivative lineages such as the Catostomid fishes (Ferris, 1984) form two sets of bivalents at meiosis I and thus recombination rates among markers in a linkage group approximate normal diploid values. Gene mapping in autotetraploid derivative species (e.g., Salmonid fishes) may be complex due to the unusual meiotic configurations that may still arise between ancient homeologous linkage groups. For example, male salmonids demonstrate a phenomenon known as pseudolinkage, where an excess of recombinant genotypes between a pair of homeologously linked markers is generated when the ancestral homeologs pair as quadrivalents at meiosis I in the males (Wright et al., 1983). These differential pairings appear to be restricted to telomeric regions of the homeologous linkage groups and are therefore of interest as they may signal the sites for preferential chiasmata junctions during meiosis I (Sakamoto et al., 2000). Since marker transmission in polyploid derivative species may be complicated by the nature of chromosome pairings during meiosis, it is important to consider the dynamics of this process and how it may influence the interpretation of genetic maps constructed in such lineages. Most notably, sex-specific maps constructed in such species may be quite divergent (Sakamoto et al., 2000).

Gene mapping is a hierarchical process and utilizes a number of different biological techniques to gain information on the genome. In this paper, some of the techniques that are currently used in the study of genomics will be outlined, and an overview of various types of genetic maps will be presented with consideration given to the source material and markers that are needed to construct these maps. Based upon the knowledge of genetic marker map orders, it is possible to relate physical chromosome morphologies to the linear order of markers on the chromosomes via gene-centromere mapping distances (Johnson et al., 1996). This methodology will be discussed in relation to its utility in defining the location of centromeres on genetic maps. In the latter sections of the paper, an overview of the applications of genetic mapping will be given. Included in those sections will be a discussion of gene transmission dynamics not only within species, but also between the sexes within a species. Highlighted, will be the use of a genetic map and its representative markers in investigating and understanding the process of adaptation, survival, and evolution (i.e., the *raison d'etre* of genetic maps).

Map construction

Genomes may be mapped at three different levels ranging from physical maps that localize large DNA segments onto the cytological karyotype of a species, to genetic maps that depict linear marker orders along a chromosome, and finally, by cataloging and sorting raw DNA sequence along a chromosome. Genetic maps are the most common and are typically composed of linear marker arrangements depicting various linkage groups. Such marker linkage maps are generally produced using inbred/ semi-inbred or outcrossed lines in an intercross or backcross design, where F1 parents are produced by mating two strains that are divergent for some character of interest in a quantitative trait locus (QTL) study. Haploid and diploid gynogenetic or androgenetic family lines have also been used for mapping in fishes. Several different software programs are available for constructing maps (Table 1) and examples of studies utilizing different source mapping families for the construction of genetic maps are summarized in Table 1. Some of the programs also perform QTL analyses on the same data set but require that the phase of the markers be specified in the mapping families. When access to fully inbred lines is not available then the method of producing doubled haploid progeny (Young et al., 1998) provides an efficient way to generate a genetic map using fully homozygous progeny lines. By producing mitotic diploids from two lines and then hybridizing the lines, the F1 may be used to re-establish haploid progeny lines in

[1] Links to genomic databases worldwide can be accessed through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Genomic data for the zebrafish project (zfin.uoregon.edu) and pufferfish project (fugu.hgmp.mrc.ac.uk) may also be accessed via the world wide web.

Table 1. Software available for the construction of genetic linkage maps and the types of reference families that may be used for map construction

Software name	Platform[a]	www URL
CARTHAGENE	P,*	http://www.inra.fr/bia/T/Carthagene/
CRI-MAP	P,M,*	http://bimas.dcrt.nih.gov/sw.html#crimap
LINKMFEX	P	http://www.uoguelph.ca/~rdanzman/software/
JOINMAP	P,M,*[b]	http://www.plant.wageningen-ur.nl/products/
MAPMAKER	P,M,*	http://www-genome.wi.mit.edu/ftp/distribution/software/
MAP MANAGER	P,M	http://mapmgr.roswellpark.org/mapmgr.html
Web site to other mapping software maintained by Brian Yandell		http://www.stat.wisc.edu/biosci/linkage.html#linkage

Mapping source	References and species investigated
Gynogenetic haploids	Postlethwait et al., 1994, *zf*[c]; Kocher et al. 1998, *til*
Inbred strains	Naruse et al., 2000, *med*; Knapik et al., 1996, *zf*
Outbred pedigrees	Sakamoto et al., 2000, *rt*
Mitotic gynogenetic diploids	Kelly et al., 2000, *zf*
Mitotic androgenetic diploids	Young et al., 1998, *rt*
Gynogenetic half-tetrad diploids	Johnson et al., 1996, *zf*; Sakamoto et al., 2000, *rt*; Lindner et al., 2000, *ps*

[a]Available platforms include either a PC-based (P), Mac-based (M) desktop mode, plus multiple mainframe platforms (*) such as UNIX, Linux, etc.
[b]Retailed software.
[c]Species abbreviations: *med* = medaka; *ps* = pink salmon; *rt* = rainbow trout; *til* = tilapia; and *zf* = zebrafish.

the following generation. Linkage assignments will be completely in phase as only homozygous progeny genotypes are produced and the allelic origin can be traced to either donor strain (if the alleles differ between the strains).

Most mapping studies in fishes are currently focused at the second level of genetic map organization (i.e., the construction of marker linkage maps) (e.g., Kocher et al., 1998; Young et al., 1998; Naruse et al., 2000; Shimoda et al., 1999). A marker linkage map may be defined as a landmark map that is composed of anonymous DNA markers (e.g., most microsatellite markers, AFLP (amplified fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), SNP (single nucleotide polymorphism) markers), or known gene sequence regions marking either introns or exons (e.g., SNP marker derived from an EST (expressed sequence tag) or designed from partially homologous DNA sequence information). In general any marker that identifies a known expressed gene region (e.g., insertion/deletion (INDEL) polymorphism, microsatellite repeat, SNP) may be defined as an expressed sequence marker polymorphism (ESMP). If the marker identifies a known gene using the polymerase chain reaction (PCR), amplifying DNA through a known intron-exon boundary with subsequent detection of an ESMP, the marker may also be classified as an EPIC (exon primed intron capture) marker. Markers may also be developed as STS (sequence tagged sites) from cloned or PCR amplified DNA.

By reference back to our original analogy, we must recognize that a marker linkage map is largely incomplete. It can give us a reference to landmarks along the physical surface of a chromosome, but it is largely characterized by unlinked sequence data spanning 1 cM (a centiMorgan) or more. A centiMorgan is defined as 1% genetic recombination between a pair of markers, and the actual number of base pairs embodied in this distance is dependent upon the species being examined, and even the linkage group being examined. Certain regions of chromosomes may have higher recombination rates than others, and this will make the actual translation of estimated recombination distances into physical map distances variable throughout the genome. A sequenced linkage map of overlapping cloned fragments (contigs) that is characterized as containing large tracts of assembled sequence is generally referred to as a sequenced genome rather than a map. Therefore, the term map will be

used to refer to a collection of genetic markers. The attainment of even a draft status of a sequenced genome requires a large investment of time and money to perform the necessary multiple sequencing runs on a large number of cloned fragments from the genome.

Genetic maps are simply built on the examination of recombination differences among pairs of genetic markers transmitted by a given parent. Implicit in this is the use of doubly heterozygous markers that uniquely mark four chromosome segments in the genome. Each genetic marker position will of course identify one homologous pair of chromosome segments that possess a different allelic marker on each chromosome arm. If the other pair of marker alleles being compared are also derived from the same set of homologs (i.e., from an identical linkage group) then the recombination levels between any pair of linked markers will be reduced inversely to the physical distance they are separated on a chromosome arm. This will be detected as a significant deviation in the expected 1:1:1:1 segregation pattern of genotypic combinations, with parental coupling alleles being in excess to non-parental recombination alleles. In the absence of information on the phase of the markers transmitted by a parent, the phase may be inferred by comparing the genotypic combinations that occur in the highest frequency (i.e., if the genotypes being compared are Aa at locus 1 and Bb at locus 2, then either AB and ab genotypes must occur in the highest frequency, or Ab and aB gametes must occur in the highest frequency). Theoretically, only those markers that are physically close enough to one another to escape recombination via chiasmata cross-overs will map syntenically. Chiasmata cross-overs involve exchange between only one pair of non-sister chromatids that are present at meiosis I.

Markers that are physically close to one another have a reduced probability of having parental phases disrupted during meiosis, and thus, are expected to transmit a high frequency of parental (phase intact) genotypes to their progeny. In contrast, markers that are located far from one another on a linkage group may experience a cross-over event in 100% of the meioses generated. However, if chiasma interference is complete during meiosis (i.e., the formation of one chiasmata junction inhibits the subsequent formation of other junctions) then only two of the four chromatid strands present during meiosis I will experience a cross-over event. Thus, 50% of the potential gametes formed at meiosis II should contain only non-recombinant parental marker orders. Assuming that cross-over gametes are transmitted in equal frequency to non-cross gametes, then total map length is expected to be fairly accurately represented by additive pairwise recombination distance estimates. For sets of markers that are located less than 50 cM from one another (i.e., less than 50% recombination for any pairwise combination) map distances are expected to be additive for any three point recombination estimate (e.g., $A - B + B - C = A - C$, if B is the intercalary marker). Double cross-overs involving the same pair of non-sister chromatids may also occur during meiosis I, which will disrupt a linear map order by shortening the recombination distance estimates among proximally and distally located markers along a linkage group. Non-random or preferential transmission of parental phase chromatids compared to recombinant chromatids will also shorten the apparent map distances between distally located markers and may mimic the results obtained from double cross-over events (see section on Distribution of cross-overs along rainbow trout chromosomes).

Mapping functions such as Haldane's and Kosambi's mapping function (see Ott, 1999 for their formulations) account for the shortened map distances that may occur between any pair of linked markers due to double cross-over events. These mapping functions readjust observed recombination distances upward, thus generating a larger estimate of total genome size than would be obtained by directly summing recombination intervals among linked markers. The Haldane mapping function gives larger interval readjustments than the Kosambi function. Dense marker maps saturated with markers spanning only 1–2 cM intervals are largely equivalent in terms of their genome size estimates regardless of the mapping functions used.

Regional differences in recombination rates are known to bias the direct extrapolation of recombination distances to physical map distances. For example, recombination rates appear to be suppressed around the centromere and increase towards the telomeres (reviewed in Zickler & Kleckner, 1999). Also shorter chromosome arms appear to have proportionately greater map distances (Kaback et al., 1992; IHGSC, 2001) and this appears to be a function of maintaining chiasmata junctions in all paired chromosome arms. By virtue of their smaller physical length, short chromosome arms may experience a greater cross-over rate (per physical DNA length) than longer arms (Zickler & Kleckner, 1999; IHGSC, 2001).

Genetic map construction using information from the segregation of genetically variable markers is

essentially a four stage process. The first stage is composed of scoring progeny genotypes in a source mapping family (see Table 1), and then entering the genotypes into a computer program for analysis of the segregation patterns obtained. The second step is to ascertain the linkage arrangements among the markers tested. Implicit in this analysis is the fact that only one contributing parent is scored for segregation and linkage. The use of androgenetic or gynogenetic haploids or diploids makes this a requisite, and when inbred lines are used, F_1 hybrids between the inbred lines may be backcrossed to one of the homozygous inbred parents resulting in scored segregation of heterozygous loci from the single hybrid parent. Hybrids may also be intercrossed, although the phase assignment of heterozygous progeny genotypes in such crosses is not possible (i.e., the parental origin of the alleles cannot be ascertained). In outcrossed families, however, both parents may be heterozygous for different alleles at marker loci making phase assignments possible. Three of the software programs (CARTHAGENE, JOINMAP, and LINKMFEX) listed in Table 1 currently accommodate linkage analyses using outcrossed pedigrees. When groupings of genetic markers have been statistically identified the markers belonging to any one group may be tentatively regarded as belonging to a linkage group within the species. It is not unusual to initially have more linkage groups identified in the species than may be accommodated by the actual known haploid number of chromosomes in the species. This is due to the fact that clusters of 2 or 3 markers may occur that remain unlinked due to low marker density. These clusters may occur at opposite ends of larger linkage groups, and they will eventually be joined as intervening markers in the linkage group are genotyped.

Once a tentative linkage group has been identified it is necessary to ascertain the map order of the markers along the linkage group. For this analysis, markers are sorted into separate linkage group files, and pairwise recombination distances between the markers in the linkage group may be used to sort the markers into their linear sequence along the linkage group. For outcrossed families, this needs to be done separately for each parent. Once the map order is ascertained, the map distances between the markers on the linkage group can be estimated using either the raw observed recombination levels or adjusted levels based upon a given mapping function (i.e., Haldane or Kosambi). Some of the existing computer programs (e.g., CARTHAGENE, JOINMAP, and LINKMFEX) may also facilitate the construction of genetic maps generated from multiple mapping families or between mapping parents, provided that two or more common markers are used to 'anchor' linkage orientations within either source linkage group.

Radiation hybrid maps may also be used to construct gene maps (e.g., in zebrafish, (Geisler et al., 1999; Hukriede et al., 1999)). Typically small fragments of the genome of any species are established in cell lines. These cell lines are then screened for the presence or absence of specific markers. The absence of co-expression for any given pair of markers is taken as evidence for the localization of these markers in different linkage groups (or more distal regions of a linkage group) compared to markers that are co-expressed in the same hybrid cell line. The map distance used is the cR (or centiRad) which denotes the percentage of cell lines where any two markers are not co-expressed. Radiation hybrid maps have proven to be somewhat more inaccurate in the placement of markers on human linkage groups compared to assignments made using classical genetic maps (IHGSC, 2001).

Physical mapping involves *in situ* hybridization of cloned segments of genomic DNA to their physical location on a chromosome. Although at the lowest level of genomic discrimination, physical mapping is extremely useful in terms of giving researchers insights into structural arrangements within the genome (i.e., localizations of repetitive DNA (e.g., distribution of specific SINE (short interspersed nuclear elements) families among chromosomes (Perez, Garcia-Vazquez & Moran, 1999)), identification of multi-gene family locations in the genome (e.g., rDNA clusters), localization of linkage group specific repetitive clusters (e.g., chromosomal paint probes))(reviewed in Phillips, 2001). *In situ* hybridization methods can also be of great use in verifying the relative location of specific candidate genes that have been localized to large cloned DNA fragments (e.g., BAC clones). Through the integration of genetic map information with the localization of selected markers from the genetic map, the confirmation of genetic map orders to specific chromosomal landmarks can be confirmed (i.e., the localization of terminal markers in a genetic map linkage group to telomeres and markers with small gene-centromere recombination distances to their relative location on the chromosome). Markers with apparent small gene-centromere map distances may in fact prove to be variable in their placement along chromosome arms dependent upon the frequency and

localization dynamics of chiasmata formation among different linkage groups.

Markers

It is sobering to contemplate that little more than two decades ago, genetic maps in a wide variety of organisms were largely built on information obtained from phenotypic mutant lines and a few biochemical marker loci (reviewed in Lie et al., 1994). Currently, genetic maps are constructed with a wide variety of molecular markers that may broadly be classified as sequence-specific and sequence-independent markers. The latter category is represented by anonymous DNA markers such as AFLP and RAPD markers. These markers have proven to be of great utility in establishing initial framework maps (e.g., in zebrafish, (Postlethwait et al., 1994) and in rainbow trout (Young et al., 1998)), since they are generated with relative ease by the researcher (Ferguson & Danzmann, 1998) and appear to be highly polymorphic in most of the species that have been examined. Since no knowledge of a targeted DNA region is required prior to their utilization it is possible to implement these markers in virtually any species of fish from which genomic DNA may be extracted in a pure form that is amenable to restriction enzyme digestion, ligation, and PCR amplification.

While sequence-independent DNA markers may be beneficial in establishing an initial framework map, it is unlikely that these markers will be of great utility in population-based studies examining gene function and action. This class of marker is characterized by a lack of tractable inheritance across family lines since these markers are predominantly expressed in a dominant/recessive fashion. Thus, the genotypes of individuals expressing a given fragment may not be accurately classified as being either homozygous or heterozygous. This is possible if previous pedigree information confirms that the individual is descended from an individual expressing the fragment and one that is lacking the fragment, but this is tempered by the need to assess the segregation patterns in the progeny of such a mating in the previous generation. If both classes of phenotype are present in the progeny, the individual may accurately be classified as being heterozygous, whereas if all siblings in the family expressed the fragment, then the inferred genotype is that of a homozygote. The labour required to establish inheritance modes in each generation will severely limit the utilization of such markers.

Additionally, AFLP homologies are often difficult to infer between families given the large number of fragments that may be generated with this technique, and reproducibility is a concern with RAPD fragments in certain species of fish (Ferguson & Danzmann, 1998). AFLP fragments may aid in the establishment of sequence-specific markers (Hakki & Akkaya, 2000) if they can be cleanly purified and sequenced. Thus by virtue of their ubiquitous distribution, AFLP markers may be more readily localized to QTL regions in the genome, facilitating the sequence characterization of these regions. Whether the reported localization of AFLP markers to centromeric regions in salmonid fishes (Lindner et al., 2000) is a general finding among all fish species remains to be established.

Sequence-specific markers are the markers of choice in constructing and utilizing genetic maps. Most functional genetic maps use microsatellite (or simple sequence repeat – SSR) markers (e.g., in zebrafish (Shimoda et al., 1999)). SSR markers are numerous and widely spread in the genome. For example, it has been estimated that there are on the order of over 200,000 $(CA)_n$ repeats in the haploid salmonid genome, providing an almost inexhaustible supply of markers (Hamada, Petrino & Kakunaga, 1982). This class of marker is highly polymorphic and is co-dominantly expressed which facilitates the tracking of their transmission dynamics within populations (O'Connell & Wright, 1997). These markers require prior knowledge of the DNA sequence surrounding a repeat core to design primers, which increases their specificity, but also increases their implementation costs (i.e., increased development costs and screening costs as only one polymorphism may be detected per PCR reaction) compared to anonymous sequence markers. Often the microsatellite markers developed in one species will cross amplify in closely related species (e.g., McConnell et al., 2000; Sakamoto et al., 2000) thus lowering the development costs and facilitating the direct comparison of chromosome homologies among these species (e.g., McConnell et al., 2000; Sakamoto et al., 2000).

SNP markers are also being utilized widely in map construction and their frequency will undoubtedly increase in the future as more sequence information is gathered on fish. SNP marker information will be accumulated as a by-product of sequence comparisons among large to medium insert DNA clones (e.g., YACs, BACs, cosmids) once these clones are aligned and re-sequenced from multiple target in-

dividuals. Design-specific SNPs may be generated from EST clones using exon sequences to generate primers that will either amplify across intron/exon boundaries (EPIC marker) or will ramp into UTRs (untranslated regions flanking a gene) to detect an SNP or INDEL. The identification of gene-specific SNPs will increase as more direct sequence and EST data becomes available in fishes. The zebrafish project (http://zfish.uoregon.edu/ with links to: http://www.genetics.wustl.edu/fish_lab/frank/cgi-bin/fish/) and the pufferfish project (http://fugu.hgmp.mrc.ac.uk/) have large public EST databases available on the web and EST sequence data is becoming available on a variety of other fish species (e.g., Atlantic salmon (Davey et al., 2001)) with links to these through GenBank dbEST accession numbers.

STS marker is an 'umbrella' term that may be applied to any marker polymorphism generated via PCR amplification of a small segment of genomic DNA (typically around 500 bp). Initially, the region is often characterized by more complete sequence information (e.g., sequencing terminal regions of BAC clones) accompanied by the discovery of potentially polymorphic DNA regions (i.e., repetitive DNA, SNP discovery in homologous contigs). Primers designed to encompass the putative polymorphisms may then be generated. Even in the absence of detectable internal polymorphisms tail-end primers for clones may still be designed and used to identify potential SNP sites via single strand conformational polymorphism (SSCP) studies (e.g., in zebrafish (Fornzler et al., 1998)). STS tags are most widely used to try and locate large insert clones onto a genetic map.

Undoubtedly, comparative evolutionary studies will be enhanced greatly as details of syntenic marker arrangements among species becomes available (e.g., between human and zebrafish (Barbazuk et al., 2000)). Anonymous markers will provide the greatest amount of information by virtue of the fact that such markers will be most widespread throughout the genome. However, these markers are likely to only be utilized among relatively closely related species where the homology of the marker generation can be confirmed between the pair of species being compared (e.g., microsatellite markers in salmonid fishes). Comparisons on the homologous relationships among functional genes will be possible across more divergent taxa. Information on the organization of multi-gene families and repetitive elements such as LINEs (long interspersed nuclear elements), and SINEs, and any retroposon-like elements in the genome may also be garnered from homologous sequence information among closely related species.

Both the genes themselves and their nature of expression may be studied once the genes have been characterized and sequenced. Thus, if a researcher is interested in studying a given physiological function, and has *a priori* knowledge that a given gene is likely to be of importance in this physiological process, then a genome search for the candidate gene may be initiated. The influence of this candidate gene on the physiological function may be studied in greater detail once the gene has been located. An alternative method, termed a genome scan, utilizes a marker linkage map to localize chromosomal regions (and putative QTL) that may influence the trait. The advantage of the latter scenario is that it may detect multiple regions (in addition to identified candidates) influencing a given trait. New chromosomal regions (devoid of existing candidate loci) may be discovered with this approach.

Chromosome structure, linkage groups, and centromeres

Gene-centromere (or half-tetrad) mapping is a direct method for assessing the relative distances of genetic markers along the length of a chromosome from their centromere. This method allows the researcher to localize the position of a centromere from the actual physical map of the chromosome onto the genetic map. Since genetic map distances are influenced by the structure of a chromosome, the localization of centromeres onto the genetic map helps to orient and interpret recombination patterns along the chromosome. For example, metacentric and acrocentric chromosomes that contain approximately equivalent amounts of DNA may demonstrate different recombination levels dependent upon the localization and size of synaptonemal complexes within each chromosome and their associated recombination nodes (Anderson et al., 1999). Short arms of human chromosomes generally express somewhat elevated recombination rates (and hence map distances) compared to long arms (IHGSC, 2001). Since normal bivalent formation requires at least one synaptonemal complex to form between homologous arms, short arms may simply experience a greater proportion of crossovers within a standard length of DNA (Kaback et al., 1992).

Cross-over localization appears to be related to the actual size of the synaptonemal complex that is formed during meiosis I, and to the relative position of the complex along the chromosome arm. Cross-over or recombination nodes are characterized by DNA excision and mismatch repair enzymes that may use homologous DNA from non-sister chromatid strands to repair double-stranded nicks, thus generating cross-overs (Goyon & Lichten, 1993). By localizing the presence of the mismatch repair protein MLH1 to foci within the synaptonemal complex (SC) of mice, Anderson et al. (1999) have demonstrated that cross-overs appear to localize towards the telomeric ends of short SCs, and were located in the middle of longer SCs, when only single foci were observed within each SC. Multiple foci were localized non-randomly within the SC. Invariably, multiple foci were only located on longer SCs and at opposite ends of the SC indicating that positive chiasma interference was involved in their formation (see also the review by Zickler & Kleckner, 1999). Positive chiasma interference (or the presence of one cross-over event inhibiting the occurrence of a subsequent cross-over event in the same region) has also been reported in fishes (Thorgaard, Allendorf & Knudsen, 1983; Streisinger et al., 1986; Morishima, Nakayama & Arai, 2001).

Single locus analysis

Gene-centromere map distances are obtained by scoring the genotypes of gynogenic diploid full-sib progeny created following the disruption of the normal process of meiosis in females. Since female teleosts do not extrude their second polar body until fertilization of the egg is initiated, it is possible to prevent the loss of this second polar body using either heat shock or pressure shock methodologies (Chourrout, 1980; Streisinger et al., 1981; Pelegri & Schulte-Merker, 1999). This procedure 'captures' the results of any cross-over events in two of the four chromatids involved in homologous chromosome pairings during meiosis I. Since sister chromatids carry identical gene or DNA copies, any genetic marker regions that have not experienced a cross-over between one of the two sister chromatids and the centromere, will retain identical gene copies for that region following gynogenesis. This is due to the fact that sister chromatid strands (attached at the centromere) do not separate until meiosis II is completed. By suppressing the meiosis II division with either heat or pressure shock, the genetic state of sister (and non-sister chromatid recombinations due to cross-over) can be characterized along the length of chromosome. Since recombination events are expected to be primarily random along the length of a chromosome, the most common gametic vectors observed in the progeny should be parental ditypes. However, if a species experiences a large number of recombination events during meiosis, then the phase of the parental genotypes needs to be known, in order to accurately assess recombination nodes within the species.

By using heterozygous genetic markers to screen gynogenetic diploid families, it is possible to ascertain their relative position along the chromosome. Those markers further away from the centromere will experience the greatest number of cross-over or chiasmata formation events between the marker and the centromere, whereas those markers that are relatively close to the centromere may not experience any cross-over events. Heterozygous markers close to the centromere may appear only as alternate homozygous genotypes in the progeny of a gynogenetic family since only sister chromatid strands are present in any given individual following meiosis II suppression. If a cross-over event involving non-sister chromatids has occurred between the marker and centromere the resulting diploid gynogenetic progeny will appear to be heterozygous (Figure 1). By counting the number of progeny that are either heterozygous or homozygous for a given marker region an estimate of the relative gene-centromere distance of that marker may be obtained under various models of chiasma interference. Assuming complete chiasma interference (i.e., with only a single cross-over event between non-sister chromatids), gene-centromere distance may be obtained as $y/2$, where, y is the percentage of heterozygous progeny observed in the total sample. Thus, if all the progeny were heterozygous for a given genetic marker, the relative gene-centromere distance estimate for this marker would be 50 cM suggesting that it is located telomerically on a linkage group (Figure 2).

Chiasma interference will determine the accuracy of gene-centromere estimates. If interference is complete then gene-centromere map distances will be an accurate representation of the marker orientation on the linkage group. However, the final map distance estimate must be regarded as only an approximation of the true map distance. This is due to the fact that the additive map distances obtained using recombination intervals in mapping families may often exceed, or even be less than 50 cM (i.e., the maximum estimate obtainable via gene-centromere methods)

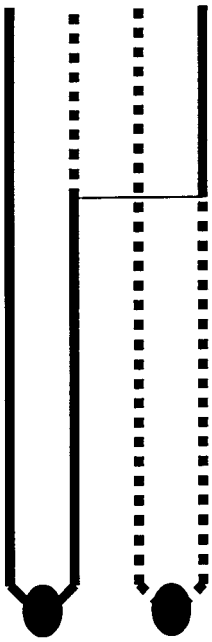

Figure 1. Possible state of chromatid configurations following a single cross-over exchange during meiosis I. Markers distal to the cross-over junction will be in a heterozygous state (shown as solid and hatched adjacent chromatids) following meiosis II suppression in gynogenetic diploids. Markers proximal to the cross-over junction will be homozygous (shown as either identical solid chromatids or hatched chromatids).

depending upon the size of the linkage group investigated. Regional suppression of cross-over events (e.g., around the centromere) (Johnson et al., 1996; Choo, 1998) will also cause an underestimation of marker-centromere distances, for markers proximal to the centromere.

In the absence of complete chiasma interference, the number of heterozygous progeny observed in a gynogenetic family will be reduced. For example, if two cross-over events were always observed during meiosis along a given linkage group (Figure 3) then the maximum number of heterozygous progeny that would be detected at telomeric marker positions would be 50% (assuming random chiasma pairings). Thus, the occurrence of multiple cross-over junctions along the length of a chromosome will tend to uncouple genetic and gene-centromere map distances, and will appear to shorten the relative distance estimates obtained from gynogenetic progeny.

Gene-centromere mapping has the immediate benefit of orienting mapping data into a framework that may be related to a physical map of the genome (Figure 2). Thus, it is possible to relate the additive recombination distances obtained with the genetic map into an image of the physical location of the markers along the chromosome. Assuming that a large amount of information has been obtained on the placement of markers in the genome, it may be possible to define the 'ends' of linkage groups without gene-centromere information. However, it is impossible to ascertain whether a given linkage group appears to represent a metacentric, sub-metacentric, or an acrocentric chromosome without information on the relative location of the centromere. Information on the centromeric location may also help to interpret differences in the regionalization of recombination events. Centromeric regions may possess lower levels of recombination (Choo, 1998) while telomeric regions of the chromosome may experience increased cross-over events (IHGSC, 2001).

Two locus analysis

Map distances may be obtained using gene-centromere distances among linked markers following an assessment of the number of double or multiple cross-over events between a pair of markers being considered. In the absence of information from a genetic map, all pairwise combinations of genotypes that may be produced between a pair of linked markers needs to considered. For example, for two doubly heterozygous markers of genotype Aa at a locus proximal to the centromere, and Bb for a more distal locus on the same side of the centromere, nine genotypic combinations are possible (Table 2). Significant deviations from the expectations shown in Table 2 would signify linkage for the pair of markers examined. The phase of the markers could be inferred from the orthogonal pairs that are in excess. For example if the phase is AB and ab, then AABB and aabb genotypes should be detected in excess of expectations, with the absence of AAbb and bbAA genotypes. If the phase were Ab and aB then the reciprocal combination should exist. Estimates of the genetic distance between the markers can be obtained by summing the apparent single cross-over (SCO) cells between the proximal and distal markers along with apparent double cross-over cells that reverse-ordered. These would be characterized as the homozygous genotypes for the more distal marker in combination with a heterozygous genotype for the proximal marker. Such a combination is unexpected as heterozygous genotypes are expected to be more distally located along the chromosome. In the example shown in Table 2, if the markers were linked and the

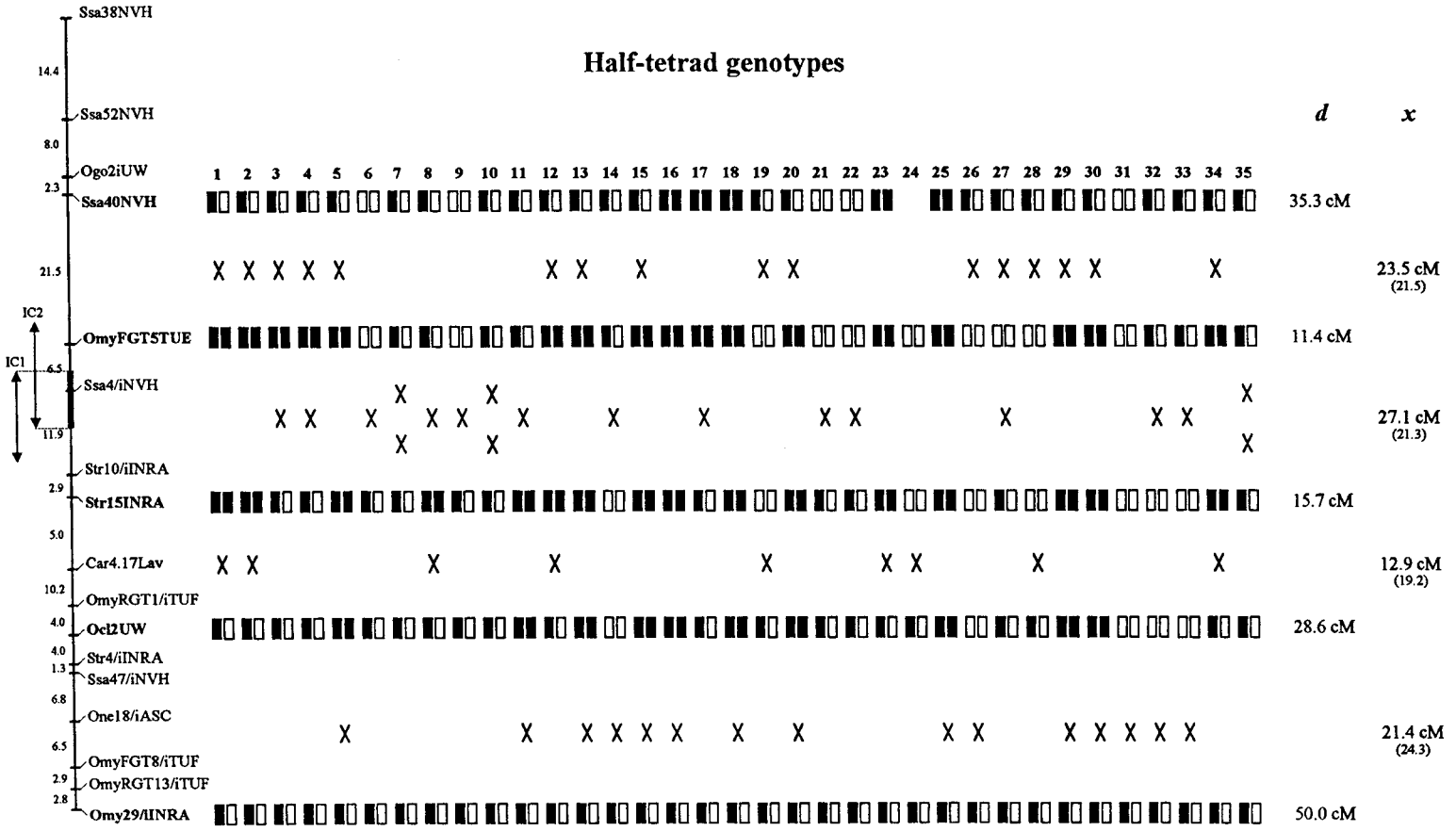
LG12
Half-tetrad genotypes
d
x
Ssa38NVH
14.4
Ssa52NVH
8.0
Ogo2iUW
2.3
Ssa40NVH
21.5
IC2
OmyFGT5TUE
IC1
6.5
Ssa4/iNVH
11.9
Str10/iINRA
2.9
Str15INRA
5.0
Car4.17Lav
10.2
OmyRGT1/iTUF
4.0
Ocl2UW
4.0
Str4/iINRA
1.3
Ssa47/iNVH
6.8
One18/iASC
6.5
OmyFGT8/iTUF
2.9
OmyRGT13/iTUF
2.8
Omy29/iINRA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
35.3 cM
23.5 cM
(21.5)
11.4 cM
27.1 cM
(21.3)
15.7 cM
12.9 cM
(19.2)
28.6 cM
21.4 cM
(24.3)
50.0 cM

Figure 2.

phase was Ab and aB, then AaBB and Aabb would be reverse-ordered since the heterozygous genotypes should occur at the more distal B locus. An estimate of the map distance between A and B may then be obtained as: (AABb + aaBb + ((AaBB + Aabb) * 2))/(total number of progeny * 2). Three strand double cross-overs are indistinguishable from single cross-overs that give rise to AABb and aaBb genotypes, and are expected to be as frequent as the 2 strand and 4 strand double cross-overs, that generate the AaBB and Aabb reverse ordered genotypes. Hence, an estimate for their frequency may obtained by doubling the frequency of the rarer genotypes (Thorgaard, Allendorf & Knudsen, 1983) (see also, Da et al., 1995; Zhao & Speed, 1998).

It is possible to use the genetic map order obtained from source mapping families to cross-reference the gene centromere distances obtained (Figure 2). Genetic map distances are expected to relate directly to gene-centromere distances under a model of complete or almost complete chiasma interference. However, genetic map distance estimates may exceed gene-centromere estimates for longer chromosome arms. Longer chromosome arms will have a greater likelihood of generating larger synaptonemal complexes with multiple recombination nodes (Anderson et al., 1999). For example, if double cross-overs are common during meiosis I, then telomerically located markers may in fact appear to be closer to the centromere than intercalary markers since 50% of all non-sister chromatid exchanges will place sister chromatids adjacent to one another in the telomeric segment of the chromosome, while non-sister chromatids will co-segregate in intercalary regions (Figure 3). The model assumes that local chiasma interference is complete (i.e., two exchanges cannot take place in the same interval).

Interference

Gene centromere data obtained from a variety of teleosts suggests fairly strongly that chiasma interference levels are high (Thorgaard, Allendorf & Knudsen, 1983; Allendorf et al., 1986; Arai et al., 1991; Johnson et al., 1996; Lindner et al., 2000; Morishima, Nakayama & Arai, 2001) and linear map orders generated from recombination intervals agree well with observed gene-centromere estimates (Johnson et al., 1996). However, chiasma interference may not be complete yet appear to be so, if any additional chiasmata that is formed involves one of the two chromatids that initially exchanged segments (i.e., partial negative chromatid interference). If, for example, strands 2 and 3 were involved in a non-sister chromatid exchange proximal to the centromere (type 2–3″ – Figure 3) then a more distal cross-over may be constrained to only involve strands 2 and 4 or strands 1 and 3. Either configuration would result in only a single cross-over point being detected in the resulting gametic vectors (i.e., 2–3″ + 1–3′ or 2–3″ + 2–4′ configuration in Figure 3), and would result in the segregation of heterozygous chromatids at the telomeres. Thus, multiple chiasma formations may not necessarily lead to double cross-over events with respect to genetic marker inheritance if the pairings are regulated such that negative chromatid interference operates on one of the pairing strands. One additional scenario may result in the preservation of apparent gene-centromere and linear map order orientation. Telomeric regions in the linkage group will still be heterozygous in gynogenetic diploids if an odd number of cross-over events are always produced between any pair of non-sister chromatids.

←

Figure 2. Centromere linkage analysis in brown trout. Linkage group number 12 (LG12) from the microsatellite brown trout linkage map (unpublished data) is shown on the left. The construction of LG12 was generated from four female mapping parents contributing independant outcrossed families. Five microsatellite markers (bold-faced) were analyzed using 35 half-tetrad progeny derived from a single female. Half-tetrad genotypes are shown at the right of each marker position. Alternate alleles inherited from the donor female are depicted as clear and dark rectangles so that alleles with the same shading indicate the parental phase (Ssa40NVH has missing data for progeny 24). Distances to centromere (d) were estimated assuming complete chiasma interference (i.e., $d = y/2$ where y is the proportion of heterozygous genotypes). These results indicate that the centromere lies within the map interval defined by OmyFGT5TUF and Str15INRA. The centromere position (thick line) was estimated as the region where the 95% confidence intervals inferred from OmyFGT5TUF (IC1: (3.4–16.5)) and Str15INRA (IC2: (9.3–25)) overlap (see Johnson et al., 1996 for confidence interval construction). Map distances between adjacent markers may be estimated by assessing the number of crossovers (X) required to account for the observed genotypes. For any two markers lying on the same side of the centromere, a single crossover event may be inferred when the more proximal marker is homozygous while the more distal marker is heterozygous (e.g., Ssa40NVH and OmyFGT5TUF, progeny 1). If the markers lie on opposite sides of the centromere (e.g., OmyFGT5TUF and Str15INRA), simple (e.g., progeny 3) and doubly (e.g., progeny 7) heterozygous half-tetrads may be generated by single and double cross-overs, respectively. Map distances between adjacent markers (x) were then calculated as the number of inferred cross-overs within each marker interval over twice the number of progeny. Resulting distances were fairly consistent with the estimates obtained from recombination estimates using the family pedigrees (shown in parentheses for the interval indicated on the linkage map).

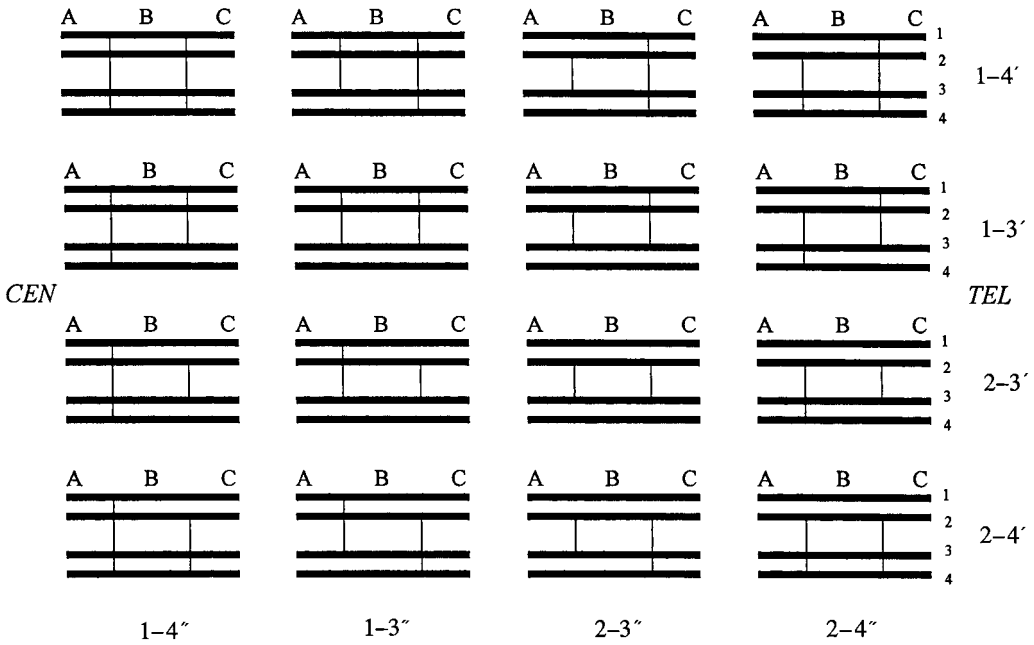

Figure 3. Distribution of possible double cross-over configurations assuming positive chiasma interference in either region I (telomeric = *TEL*) or II (centromeric = *CEN*). Any pair of chromatids involved in a cross-over event in region I (e.g., 1–4′) may also experience a cross-over involving identical strands in region II (i.e., 1–4″) or cross-overs may involve three other combinations (i.e., 1–3″, 2–4″ or 2–3″). All 16 combinations are depicted.

Table 2. Expected frequencies of cross-over and non cross-over half tetrads for two linked markers along a chromosome

	BB NCO or 4 st DCO	Bb SCO or 3st DCO	bb NCO or 4 st DCO
AA NCO or 4 st DCO	AABB $(1-m)/2 \cdot (1-n)/2$	AABb $(1-m)/2 \cdot n$	AAbb $(1-m)/2 \cdot (1-n)/2$
Aa SCO or 3 st DCO	AaBB $m \cdot (1-n)/2$	AaBb $m \cdot n$	Aabb $m \cdot (1-n)/2$
aa NCO or 4st DCO	aaBB $(1-m)/2 \cdot (1-n)/2$	aaBB $(1-m)/2 \cdot n$	aabb $(1-m)/2 \cdot (1-n)/2$

The frequency of non-sister exchanges (single cross-over (SCO) or three strand double cross-overs (3 st DCO)) at the first locus (A) is given as m. The frequency of similar half tetrads at locus B is given as n. Non cross-over half tetrads (NCO) or four strand double cross-over (4 st DCO) half tetrads are calculated as: (1−SCO)/2, assuming equal frequencies of either NCO half tetrad.

Table 3. Maternal non cross-over and cross-over strands detected in the progeny of two rainbow trout mapping families

Linkage group	Family	No. of markers	Length (cM)[a]	NCO[b]	SCO	DCO	TCO	QCO
2	Lot 25	6	28.3	37	7	3	–	–
5	Lot 25	7	39.5	27	19	2	–	–
8	Lot 25	4	26.2[c]	33	14	–	–	–
15	Lot 25	9	70.0	18	21	8	1	–
	Lot 44	7	70.0	30	39	13	2	1
A	Lot 25	4	45.1	37	11	–	–	–
B	Lot 44	5	48.6	62	25	2	1	0
C	Lot 25	5	50.0	30	16	1	–	–
Fi	Lot 25	5	65.5	39	4	4	1	–
G	Lot 25	6	26.2	37	11	–	–	–
	Lot 44	3	26.2	68	18	4	–	–
H	Lot 25	6	28.7	21	17	6	3	1
	Lot 44	5	28.7	60	23	1	–	–
N	Lot 25	6	35.0	33	11	4	–	–
	Lot 44	5	26.8	65	23	2	–	–
Oi	Lot 25	7	54.3	34	8	3	–	–
	Lot 44	10	65.3	24	36	7	1	–
Oii	Lot 25	4 (1)[d]	27.5	35	11	1	–	–
		5 (2)	43.1	38	7	1	–	–
P	Lot 25	5	41.6	23	23	2	–	–
R	Lot 44	5 (1)	42.0	41	40	4	2	–
		3 (2)	45.7	58	29	–	–	–

[a]Distances include initial gene-centromere mapping estimates for the most proximal marker.
[b]Non cross-over (NCO) chromatids, single cross-over chromatids (SCO), double cross-over chromatids (DCO), triple cross-over chromatids (TCO), and quadruple cross-over chromatids (QCO) detected in the progeny. Cross-over haplotypes were determined with GENOVECT using LINKMFEX (see Table 1). A maximum number of 48 and 90 progeny were screened in Lot 25 and 44, respectively. Chromatid vectors were only counted if they were not missing a genotype from more than one marker.
[c]Gene-centromere data is insufficient to orient this linkage group with respect to the centromere.
[d]Indicates the presence of a metacentric chromosome possessing markers on both arms of the chromosome spanning greater than 20.0 cM distance. Each separate arm is indicated by either a number 1 or 2 given in parentheses.

Distribution of cross-overs along rainbow trout chromosomes

Insights into the distribution of potential cross-over sites along the linkage group may be gained by comparing the genotypic vector of genetic markers along a linkage group. These may then be compared across linkage groups to ascertain if differences exist among linkage groups within a family, and even across families if multiple mapping families have been used in a study. An examination of cross-over distributions among linkage groups in rainbow trout reveals a surprising asymmetry in the transmission of parental genotypic vectors among linkage groups. Although the female linkage map for this species is still relatively incomplete (Sakamoto et al., 2000, unpublished data) certain linkage groups possess a sufficient number of markers to examine recombination points along the length of the chromosome. Recombination levels appear to be low across most linkage groups, as the greatest number of gametic vectors observed are parental. Only linkage groups 5, 15, H, Oi, P, and R (one arm), show recombinant vectors in approximately the same proportion as parental vectors (Table 3). For linkage groups H and Oi only one of the two female mapping parents express equal gametic vectors. Within linkage group 15 (both Lot 25 and Lot 44), H (lot 25), Oi (Lot 44), and R there is also a higher frequency of multiple recombination sites.

These findings are unexpected since cross-over gametes are expected in equal proportion to non cross-over gametes assuming complete chiasma interference. While gametic vectors in most linkage groups support a model of complete interference, the

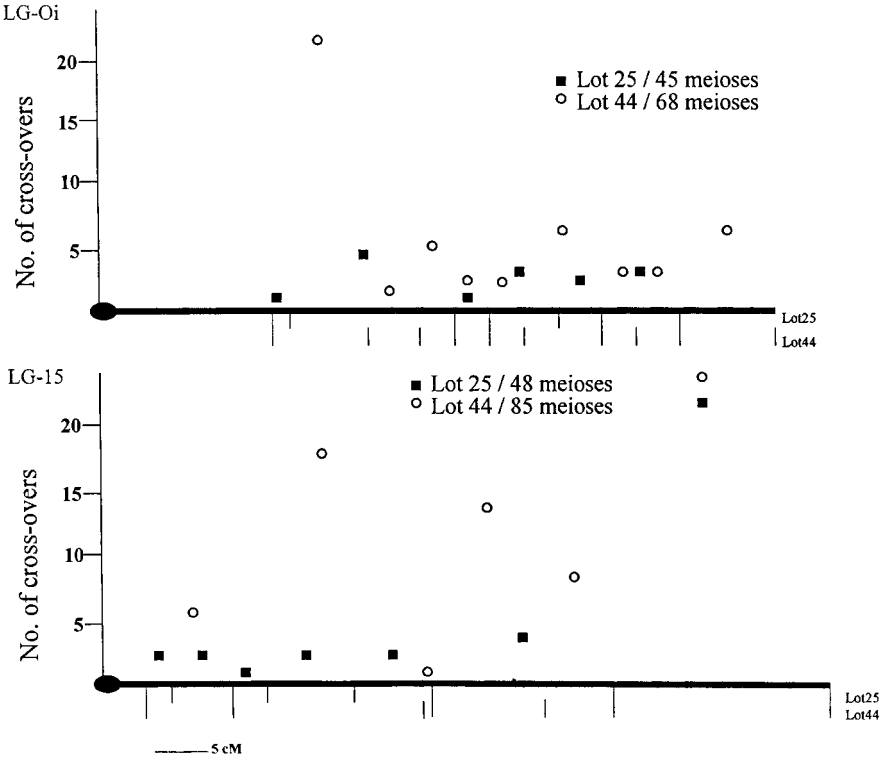

Figure 4. The number of cross-over detected in two maternal parents, along linkage groups Oi and 15 in rainbow trout (data taken from Sakamoto et al., 2000; and unpublished). The total number of meioses scored in each parent are indicated. Map reference markers for each female are shown along the abscissa for each linkage group and markers are positioned using averaged map distances between the two females (calculated with MAPDIS from LINKMFEX) according to the scale shown. For the marker most proximal to the centromere, the gene-centromere mapping distance was used.

data reveals a bias towards the survivorship of progeny inheriting a complete non-recombinant parental homologue. Whether this arises due to differential progeny survivorship (i.e., selection), or is determined by differential meiotic disjunction is unclear.

For linkage groups with multiple cross-overs, insights into the localization of recombination sites within the linkage group can be obtained by comparing the distribution of cross-over points along the chromosome. Regional differences in recombination rates are known to exist among linkage groups in vertebrates (Nachman & Churchill, 1996) and also within linkage groups. For example, human males have somewhat elevated recombination rates closer to the telomeres than females, and both sexes have elevated rates in intercalary regions of their chromosomes compared to the centromeres (IHGSC, 2001). This distribution may be related to the propensity to form recombination nodes distally along a chromosome with subsequent chiasmata junction formations progressing medially (Zickler et al., 1992; Scherthan et al., 1996). Since larger chromosomes are expected to possess larger synaptonemal complexes and perhaps multiple synaptonemal complexes (Anderson et al., 1999), recombination levels are expected to be elevated across larger chromosomes in the genome.

The distribution of recombination nodes was compared across two of the larger linkage groups in rainbow trout (linkage groups Oi and 15, Figure 4). Since the rainbow trout map is still far from complete it cannot be unequivocally stated that these linkage groups are in fact the largest within the genome. A comparison across both mapping families is however, possible, with the markers currently genotyped. While recombination levels appear fairly uniform across all marker positions within the Lot 25 female for linkage group Oi, levels are clearly elevated in the lot 44 female for the most proximal markers to the centromere. Conversely, a survey across linkage group 15 reveals higher recombination levels for the most distal map interval in both mapping females (Figure 4). As a percentage of total meioses scored this difference is most

pronounced in the Lot 25 female. Thus, both mapping females provide evidence for an enhanced level of chiasma formation closer to the telomere in linkage group 15 with the distribution of additional sites dispersed throughout the remainder of the chromosome. As more mapping data is collected, insights into the localization of recombination nodes across linkage groups will be obtained. This will facilitate studies on the degree to which such nodes may be inherited, and their possible role in chromosomal evolution among fishes.

Sex-specific differences in recombination rates

Currently, there is a paucity of information on sex-specific recombination differences collected in teleost species. According to Haldane's rule (1922), gonochoristic species are expected to demonstrate sex-specific recombination differences with the heterogametic or sex-determining sex exhibiting lower recombination levels. Current mapping studies in fishes support this rule (Knapik et al., 1998; Sakamoto et al., 2000; Waldbieser et al., 2001), although the observation of slightly lower female recombination rates in the tilapia species used for mapping by Agresti et al. (2000) is confounded by the fact that one Z-W and two X-Y sex-determining species were used in the creation of the mapping family. In species with an X-Y pattern of sex determination, recombination ratios ranging from 1.6:1 (F:M) in zebrafish (based upon a comparison with the female haploid map of Postlethwait et al. (1994) to the sex-averaged microsatellite linkage map of Knapik et al. (1998)) to 3.25:1 (F:M) in rainbow trout (Sakamoto et al., 2000) have been reported. The recombination differences observed in rainbow trout are the largest sex-specific differences known for any vertebrate species. A combination of site-specific control of chiasmata formation in males coupled with the dynamics of meiotic segregation in this ancient tetraploid lineage may account for such large differences in this species (Sakamoto et al., 2000).

Interestingly, a female Japanese flounder (*Paralichthys olivaceus*) used in a mapping study by Coimbra et al. (in review) showed depressed levels of recombination compared to the male mapping parent. Since the female was produced by gynogenetic means it is unclear whether the recombination levels observed were artificially influenced by the genetic origin of the female, or whether this difference is general to the species. Japanese flounder was reported to be a male-determined gonochoristic species, since gynogenesis induces all-female production in the hatchery (Yamamoto, 1995, 1999). It was observed, however, that certain gynogenetic crosses did occasionally produce fertile males suggesting that some type of polygenic sex determination or environmental sex determination may occur in the species.

While comparative data on recombination differences between the sexes is lacking for the vast majority of fish species, insights garnered from our own species highlight the importance of obtaining such data. Recombination levels reported in humans when standardized against a genome sequence of 1 Mb are 1.83X greater in females than males (mean $= 0.9\,\mathrm{cM\,Mb^{-1}}$ in males, and $1.68\,\mathrm{cM\,Mb^{-1}}$ in females) (Yu et al., 2001). Recombination levels vary dramatically across different regions of the human genome, spanning a range of $0-7.9\,\mathrm{cM\,Mb^{-1}}$ in males, and $0-8.8\,\mathrm{cM\,Mb^{-1}}$ in females (Yu et al., 2001). This is not unusual and has been reported in other species (Nachman & Churchill, 1996). In fish, large differences in recombination rates have been observed between the sexes within specific linkage groups and between linkage groups. For example, recombination rates are greatly reduced in male medaka (*Oryzias latipes*) for markers along the sex chromosome, compared to those of the female, and this difference appears to be under physiological control (Matsuda et al., 1999). In rainbow trout, males appear to express larger recombination levels in their telomeres compared to females but these differences appear variable across different linkage groups (Sakamoto et al., 2000). While data on sex specific differences in recombination rates is largely lacking in fish species, the construction of sex-specific genetic maps will be of great evolutionary interest, as they will help to shed light on conserved recombination nodes, or hotspots, and these regions may help to define nodes where chromosomal rearrangements (i.e., translocation fusions/fissions) may have occurred between related species.

Many fish species are also susceptible to environmental sex determination (Strussmann & Patino, 1995) and some degree of environmental sex determination may also exist for species considered to be purely gonochoristic (e.g., in sockeye salmon (Craig, Foote & Wood, 1996)). Thus, the relative influence of environmental factors in altering sex-specific recombination rates is largely unknown. In addition, several teleost species (e.g., members of the family Cichlidae

and Poecilidae) exhibit some form of multigenic sex determination (Kallman, 1984) that may potentially be modified via environmental factors. The examination of sex-specific recombination differences among such sex modulated species will be of interest.

Transmission genetics

In addition to the information obtained on the evolutionary relationships among homologous genes, and their nature of expression, linkage maps also provide a window on the transmission dynamics of different chromosomal regions. Once a marker linkage map arrangement is known, there are a number of processes that may be indirectly examined by comparing observed progeny genotypic counts to those expected according to various models of allelic transmission (i.e., in both diploid and tetraploid species). Most important in this regard, is the simple examination of random Mendelian segregation of the alleles along any given linkage group. Among all linkage groups there should be a demonstrated conformation of a 1:1 segregation ratio for any pair of heterozygous alleles transmitted by either parent. Significant deviation from uniform segregation ratios may localize the presence of lethal or semi-lethal alleles on one of the two homologs of a linkage group. This would be supported if several adjacent markers along the linkage group demonstrated similar segregation distortion for the same chromosome arm. Once linkage relationships are firmly established, it is also possible to measure the degree of segregation disequilibrium that may exist among markers on different linkage groups. Using linkage disequilibrium methodologies (Weir, 1990) pairwise combinations of alleles at unlinked loci may be compared to assess whether there is any propensity for given pairs of alleles to occur more frequently than expected according to random segregation in the progeny.

Transmission genetics are fundamental in establishing genetic maps, but an investigation of the dynamics of this process also provides us with additional insights into allelic properties within the genome. The most powerful application of transmission genetics relates to the ability of genetic markers to provide us with information on the 'nature and disposition' of other genetic elements that are syntenically housed with the marker along a given length of chromosome. Naturally, the further away a given marker is from an element on a chromosome, the greater the probability that the marker will be uninformative in this regard, due to recombination between the two elements. With increased marker saturation, gene maps will become extremely useful in the study of a wide variety of processes in fishes ranging from ecology (life-history variation and fitness related studies for example) to functional proteomics. Knowledge obtained from gene maps will allow us to 'localize' the positions of genes influencing a given trait more precisely (i.e., QTL mapping) (Cheverud & Routman, 1993) and will facilitate the examination of various QTL variants more accurately, since it will be possible to accurately establish pedigrees among a strain or group of fish under study. Pedigree mapping is an essential element to any multi-generational study of gene effects that might be conducted at the organismal level.

Genetic markers have been used to examine the influence of genetic variation on adaptation and fitness related traits in a wide variety of organisms in the past 40 years. Many initial studies, fueled largely by the discovery of allozyme variation at biochemical loci, attempted to elucidate whether genetic variation *per se* was of direct adaptive value to an organism. These studies were limited in their nature given the paucity of genetic variants that could be used in any species, and while supportive evidence was obtained suggesting an enhanced influence of heterozygosity on the traits studied, the evidence was weak (Britten, 1996). Debate focused on whether a dominance model (Davenport, 1908) (masking of lethal or low performance alleles) or overdominance model (East, 1908) (superior performance of heterozygotes) of gene action could most accurately account for observed phenotypic effects. While the latter model is difficult to envision physiologically, overdominance can be approximated in a hybrid genome by the combined expression of either maternally or paternally derived dominant acting alleles (associative overdominance model), or in their altered performance due to epistatic interactions (Wade, 2000). The dominance model is supported by studies that show enhanced performance effects in F_1 generation hybrids (heterosis) with consequent rapid breakdown of the heterosis following outcrossing (Armbruster et al., 1999).

Lack of marker availability hampered the ability of researchers to accurately track an assess associations with heterozygosity in these initial studies. Differential recombination rates between experimental families will have generated inconsistent results with respect to marker-QTL associations. Furthermore,

population based studies would have been unlikely to accurately assess associations unless significant marker-QTL linkage disequilibrium existed within the population (Allendorf & Leary, 1986). Given the molecular methodologies that are currently available, more detailed gene maps will become available in fish. Information on the location of markers in relation to their proximity to functional genes will help to interpret any observed associations between allelic and phenotypic variation in the fish being studied. As a consequence, it is anticipated that research examining the influence of genetic variation on life-history variation and adaptation in fishes will experience a resurgence.

Linkage disequilibrium and genomic co-adaptation

One of the central ideas embodied in the notion of gene action and interaction is the concept that certain genetic elements may become co-adapted (Templeton, 1986; Armbruster et al., 1999). Implicit in this, is the notion that certain blocks of genetic elements may become functionally integrated to work together more efficiently, and that recombination within this block will greatly diminish the overall fitness of an individual. An example of this are the tandem clusters of *Hox* genes that occur in living organisms. They are not only physically integrated into paralogous arrays on their respective linkage groups, but they are also integrated into a temporal unit with respect to the sequential expression of the array during ontogeny (Amores et al., 1998; Meyer & Malaga-Trillo, 1999). Significant rearrangements have occurred in the *Hox* gene clusters of certain teleost lineages that appear to have involved whole genome duplications (Amores et al., 1998; Aparicio, 2000). While knowledge of functional allelic variants and the study of mutations at individual *Hox* genes in fish is still sparse, it is anticipated that such studies will reveal a rich catalogue of associated allele-specific developmental patterns. Allelic integration sites may be scattered throughout the genome, and evidence for their existence may be obtained by examining linkage disequilibrium among segregated markers in a population (e.g., HLA alleles in humans (Huttley et al., 1999)). The detection of significant linkage disequilibrium is likely to be extremely rare, however, as even a low level of recombination is likely to disrupt allelic associations. Most of the evidence for disequilibirum in the human genome was localized to linked markers with a low level of recombination (<4%)(Huttley et al., 1999). Thus, in the absence of multi-generational non-random associations among alleles within individuals, linkage disequilibirum will be undetectable.

Even in the absence of significant linkage disequilibrium, certain genetic regions may experience selection on combinations of resulting allelic vectors due to differential epistatic interactions among alleles, or because of the segregation of sub-viable or semi-lethal alleles. Semi-lethal alleles are unlikely to contribute towards the establishment of linkage disequilibrium as they are expected to be removed from the population via selection. If an allelic variant is modified due to epistatic interactions with other alleles in either a favourable or negative fashion (dependent upon the suite of interacting) alleles, then the given allele may persist in a large population for a long time. These alleles may not, however, generate significant disequilibrium due to the fact that reciprocal performance effects may be expressed dependent upon family background. Thus, allelic segregation differences within families need to be estimated each generation, to detect such differences.

Segregation distortion

By examining the degree of segregation distortion across mapping families, it may be possible to ascertain whether certain chromosomal regions consistently express non-random segregation patterns. While the causes of distortion cannot be determined by the demonstration of the phenomenon, the repeated expression of this pattern will suggest that genomic incompatibilities exist. The presence of a segregating lethal, or semi-lethal allele may be implicated, or reduced viability resulting from new allelic combinations may be implicated (i.e., co-adaptation).

Comparisons of segregation distortion in rainbow trout using the Sakamoto et al. (2000) mapping families (plus unpublished data), revealed that certain linkage groups exhibit higher levels of distortion than others (Table 4). When significance levels are corrected for the number of linkage groups examined, markers on linkage groups 5, 15, Oi, Q, and R showed significant segregation distortion in two or more of the four mapping parents, while linkage groups 8 and Fi possessed a single marker region with significant distortion. Interestingly, linkage groups 5 and 15 are homeologous to one another, as well as 8 and R (in at least one of their chromosome arms). It is unknown whether these differences arise as a result of gamete inviability or from differential mortality during onto-

Table 4. Number of markers showing significant segregation distortion across rainbow trout linkage groups (taken from Sakamoto et al., 2000 and unpublished data)

Linkage groups	2	5	15	8	A	C	Fi	Fii	H	I	J	K	L	M	N	Oi	Oii	Q	R
Map25F																			
marker no.	9	–	11	7	–	–	8	10	6	6	2	–	6	–	–	8	8	–	7
Map 25F Significant	2		2	1			2	1	4	3	1		1			2	2		1
$p < 0.05, p < 0.002$	0		1	1			0	0	0	0	0		0			2	0		1
Map44F																			
marker no.	2	6	8	–	–	6	–	3	–	2	4	–	–	–	–	11	–	6	12
Map44F Significant	1	2	1			2		1		1	1					3		2	4
$p < 0.05, p < 0.002$	0	2	0			0		0		0	0					1		1	0
Map 25M																			
marker no	–	–	9	–	3	–	5	6	–	6	4	–	–	2	–	4	–	–	10
Map 25M Significant			1		3		1	2		1	1			1		1			1
$p < 0.05,\ p < 0.002$			1		0		1	0		0	0			0		0			1
Map44M																			
marker no.	–	8	10	–	–	–	–	–	–	–	4	4	–	–	4	–	–	5	7
Map44M Significant		2	1								4	1			4			4	7
$p < 0.05,\ p < 0.002$		2	1								0	0			0			1	6

Two mapping families (Map25 and Map44) were used to construct the map and the segregation distortion detected in either the female (F) or male (M) parent from each cross is shown. The number of markers detected in each linkage group with greater than 2 cM recombination in the female map and greater than 1 cM recombination in the male map are shown. The number of markers with significant segregation distortion ($p < 0.05$) are shown in the top row, while markers with a significant deviation at a Bonferroni corrected ($p < 0.002$) level are shown in the bottom row.

geny, since genotypic vectors were not compared in developing embryos prior to hatching. Some of the distortion detection in males may also be attributed to pseudolinkage arising from multivalent formations during meiosis. Aberrant segregation may be detected at telomerically duplicated loci involved in multivalent formations. However, this phenomenon cannot account for all the observed cases of distortion across linkage groups. For example, in linkage group A with the Map25 male, and linkage group R with the Map44 male, all the markers surveyed show segregation distortion (Table 4), including those that map proximal to the centromere. Also, it is uncertain that the increased recombination rates observed on linkage groups 15 and R have contributed directly to the distortion, although this must remain a possibility .

Conclusion

Fish represent the most diverse group of vertebrates on the planet, with a myriad of morphological, behavioural, and physiological differences between them. The degree to which major orthologous genes are conserved and rearranged among linkage groups (Morizot, 1994; Barbazuk et al., 2000; McLysaght et al., 2000) will provide us with the means to understand this complexity. Studies on the genomics of fishes will supply us with the means to recognize truly novel changes that have occurred among species, and it will also highlight those regions of the genome that are recalcitrant or plastic among species, thus facilitating more detailed analyses of chromosomal rearrangements (Schoen, 2000). With this increased understanding we will be able to relate the two-dimensional map to the three-dimensional phenotype.

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