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Constructing Linkage Maps with Achiasmatic Gametogenesis

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Abstract: Maximum likelihood (ML) approach is used for estimating recombination frequency based on the achiasmatic model and the corresponding software package is developed for constructing linkage maps for achiasmatic organisms (F_2 populations). The detection of sex-linked markers is done through a chi-square test. Monte Carlo simulations were conducted for comparing estimation of recombination frequency and mapping powers between these two genetic models (chiasmatic and achiasmatic models) when the achiasmata occurs. Simulation results showed that the achiasmatic model could provide unbiased estimations, while the chiasmatic model (without correction) gave under-estimates. The powers of grouping and ordering by the achiasmatic model were greater than those by the chiasmatic model (without correction) for all cases. ML approach based on the achiasmatic model can be used without correcting the data to obtain desirable linkage map powers in achiasmatic organisms.

Key words: linkage mapping; sex linkage; achiasmatic gametogenesis; maximum likelihood

非交叉配子形成体的连锁图谱构建方法

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摘 要: 根据非交叉(achiasmatic)遗传模型,提出采用最大似然法计算遗传交换率的方法,同时开发了构建非交叉生物(F_2 群体)连锁图谱的计算机软件。通过卡方验检可测性连锁分子标记。对于无交叉生物现象,采用蒙特卡洛模拟技术,对交叉(chiasmatic)和非交叉两个遗传模型遗传交换率的估计值和作图效率进行了比较。模拟结果表明,非交叉模型能提供无偏的估计值,而交叉模型则只有实际值的一半。在所有同等的条件下,基于非交叉模型的作图效率均高于基于交叉模型(无校正)的作图效率。对于非交叉配子形成体,采用基于非交叉模型的交换率计算方法能获得理想的作图效率。

关键词: 连锁作图; 性连锁; 非交叉配子发生; 最大似然法

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Achiasmata, a process where the cells nucleus divides, without reaching the stage at which homologous chromatids pair to exchange genetic material, often occurs only in male or female individuals of insects. Such a genetic phenomena may affect the detecting recombination frequency of two loci and constructing accurate linkage maps in such achiasmatic organisms for successful detection of quantitative trait loci (QTL) in organisms. Recombination frequency is usually estimated by the maximum likelihood (ML) approach or the expectation maximization (EM) algorithm[1-6]; however, this calculation is usually based on the assumptions of the possible occurrence of the chiasmata in both male and female gametogenesis (the chiasmatic model) because most organisms are chiasmatic. Currently used software packages [3,7] are based on this genetic model. Morgan[8] first described an extreme case in which recombination is absent in male *Drosophila*. Recently, researchers also found that the achiasmata can occur in either female or male gametogenesis (the achiasmatic model)[9-10]. The occurrence of achiasmata during meiosis will result in the same expected genotype frequencies in a backcross mapping population but different in an F_2 mapping population (*i. e.* F_2 population) as in chiasmatic organisms.

The silkworm, *Bombyx mori* L., domesticated for silk production for about 5 000 years, has been well studied in China, Japan, and India. Like in other organisms, development of molecular markers in silkworm is of importance for constructing linkage maps, mapping QTLs, fingerprinting strains for breeding, and marker-assisted selection[11-14]. Genetic linkage maps in silkworm have been constructed[15-17]. However, a distinguished characteristic in silkworm is the occurrence of achiasmatic oogenesis[17,18]. Heckel *et al.*[9] used biphasic linkage method to map the resistance gene to *Bacillus thuringiensis* toxins in diamondback moth. This method included the identification of linkage groups and mapping resistance within a group. On the other hand, some genes or DNA markers are linked to the sex chromosome Z. The detection of sex-linked markers and construction of sex linkage map is also required[16]. Certain offspring genotypes will not appear in an F_2 population of *B. mori* because of the restriction of crossing-over to males, thus, it is common to use backcross for linkage mapping. Tan *et al.*[18] constructed a linkage map from a backcross population. Yasukochi[17] converted the F_2 data to equivalent of two backcross data sets to construct linkage maps. Shi *et al.*[16] pointed out that the direct use of MAPMAKER for linkage mapping in silkworm was not appropriate. Theoretically, the recombination frequency in an achiasmatic F_2 population calculated with the chiasmatic model is about a half of actual recombination. Therefore, data comparisons between results of these two genetic models are needed to correctly estimate the recombination frequency and for constructing linkage maps for silkworm or other achiasmatic organisms. Software for the achiasmatic model is also needed.

In addition to estimating recombination frequency, to construct the linkage maps among a number of markers one also needs to order markers within each linkage group. Marker ordering is normally completed by the global algorithm, which is equivalent to the traveling salesman problem (TSP) algorithm[3,19,20]. However, the above methods require computation of a statistic on each of the $n!/2$ possible marker orders for n markers. Other approaches[21-24] do not need to consider all possible orders and therefore can be used for ordering a large number of markers within a linkage group with much less computational intensity.

The objectives of the present study are to use ML approach to estimate recombination frequency and to develop the corresponding software for constructing linkage maps based on the achiasmatic model for organisms having achiasmata during gametogenesis. The efficiency of detection of sex-linked markers and construction of sex linkage map will be examined via simulation. The esti-

mation of recombination frequency and mapping powers will be compared between the two genetic models using or not using the 2 × correction factor for the chiasmatic model.

1 Methodology

1.1 Detection of sex linked marker

Before linkage mapping, detection of sex-linked markers is needed. If we set sex-linked genotype of female parent (P_1, ZW) to be *AO* and of male parent (P_2, ZZ) to be *aa*, where *O* means no gene or marker on chromosome W, then the expected sex-linked genotype frequencies in an F_2 population can be derived as in Table 1. Thus, the goodness-of-fit test (or χ^2 test) can be used to determine the sex-linked markers. The degrees of freedom are 2 for a co-dominant marker and 1 for a dominant marker.

Table 1 Expected genotypic frequencies of a single marker, sex linked and the non-sex linked in an F_2 population

Marker	Female		
Sex-linked	$P(AO)=0.50$		$P(aO)=0.50$
Autosomal	$P(AA)=0.25$	$P(Aa)=0.50$	$P(aa)=0.25$
	Male		
Sex-linked	$P(AA)=0.00$	$P(Aa)=0.50$	$P(aa)=0.50$
Autosomal	$P(AA)=0.25$	$P(Aa)=0.50$	$P(aa)=0.25$

1.2 Recombinant individuals for non-sex linked markers

Assume the genotype of female parent (P_1) as *AB/AB* and of male parent (P_2) as *ab/ab*, and the occurrence of achiasmatic oogenesis for two linked markers on an autosome[9,16,17], then the expected genotype frequencies in an F_2 population can be derived as in Table 2. The F_1 female individuals only produce two possible non-recombinant gametes *AB* and *ab*, while the F_1 male individuals produce two possible non-recombinant gametes *AB* and *ab*, and two recombinant gametes *Ab* and *aB*. Nine genotypes could appear in a regular F_2 population with two co-dominant markers, while seven genotypes could appear in an F_2 population with achiasmata in female individuals at the same conditions.

Table 2 Expected gamete frequencies for non-sex linked markers in an F_2 population with chiasmatic and achiasmatic gametes in female individuals

Female	Male			
Achiasmatic	*AB* (0.5(1-r))	*Ab* (0.5r)	*aB* (0.5r)	*ab* (0.5(1-r))
AB(0.5)	*AB/AB*	*AB/Ab*	*AB/aB*	*AA/ab*
ab(0.5)	*ab/AB*	*ab/Ab*	*ab/aB*	*ab/ab*
	Male			
Chiasmatic	*AB* (0.5(1-r))	*Ab* (0.5r)	*aB* (0.5r)	*ab* (0.5(1-r))
AB (0.5(1-r))	*AB/AB*	*AB/Ab*	*AB/aB*	*AB/ab*
Ab (0.5*r*)	Ab/AB	Ab/Ab	Ab/aB	Ab/ab
aB (0.5*r*)	aB/AB	aB/Ab	aB/aB	aB/ab
ab (0.5(1-r))	*ab/AB*	*ab/Ab*	*ab/aB*	*ab/ab*

r = recombination frequency between marker loci *A* and *B*.

The expected genotype frequency and conditional probability given each genotype for four linked marker types on an autosome, Co-dominant/Co-dominant (C/C), Co-dominant/Dominant (C/D), Dominant/Dominant in coupling phase (D/D(C)), and Dominant/Dominant in repulsion phase (D/D(R)) can be derived (Table 3). However, the recombination frequency for the D/D(R) marker type is unestimable because of the uninformative value for expected frequency and the conditional probability.

1.3 Recombinant individuals for sex linked markers

Given the genotype of female parent (P_1) as *ab/OO* and male parent (P_2) as *AB/AB* for two sex linked markers A and B, the female F_1 individuals only produce two gametes *AB* and *OO*, while the male F_1 individuals produce two possible non-recombinant gametes *AB* and *ab* and two recombinant gametes *Ab* and *aB*. The expected genotype frequency and conditional probability given each genotype for four marker types on sex chromosome can be derived. Both female and male individuals are informative in the estimation of recombination frequency for the C/C marker type.

The female individuals are informative and can be considered as haploids for other marker types, while male individuals are complicated and may not be informative in both chiasmatic and achiasmatic F_2 populations.

Table 3 Expected genotype frequencies and their corresponding conditional probabilities of recombination given genotypes in an achiasmatic F_2 progeny for different non-sex linked marker types

Genotype	Observed count	Expected frequency	$P_i(R/G)$ †
C/C			
AABB	n_1	0.25(1-r)	0.0
AABb	n_2	0.25r	1.0
AaBB	n_3	0.25r	1.0
AaBb	n_4	0.5(1-r)	0.0
Aabb	n_5	0.25r	1.0
aaBb	n_6	0.25r	1.0
aabb	n_7	0.25(1-r)	0.0
C/D			
*AAB_*	n_1	0.25	r
*AaB_*	n_2	0.25(2-r)	$r/(2-r)$
Aabb	n_3	0.25r	1.0
*aaB_*	n_4	0.25r	1.0
aabb	n_5	0.25(1-r)	0.0
D/D(C)			
*A_B_*	n_1	0.75-0.25r	2r/(3-r)
*aaB_*	n_2	0.25r	1.0
A_bb	n_3	0.25r	1.0
aabb	n_4	0.25(1-r)	0.0
D/D(R)			
*A_B_*	n_1	0.50	r
*aaB_*	n_2	0.25	r
A_bb	n_3	0.25	r
aabb	n_4	0.0	0.0

†: Conditional probability of being recombined given certain genotype(s).

1.4 Estimation of recombinant frequency and marker grouping and ordering

The recombination frequency between two sex-linked or non-sex-linked marker loci can be directly estimated by the maximum likelihood (ML) approach. Once the matrix of recombination frequency is obtained among all marker loci, the distance matrix can be converted based on Haldane's mapping function[25]. A specific criterion ranging from 50-70 cM are suggested for grouping markers based on Monte Carlo simulations[26]. The global and approximate ordering approaches can be used for marker ordering within each group[3,19-23].

2 Simulation Procedures

To enable the comparison of the mapping efficiencies between the two genetic models, several factors, flanking marker distances, population sizes, and linked marker types, were chosen for this simulation study. For generality, ten loci were located evenly on each of eight autosomal linkage groups and one sex linkage group with known flanking marker distances (5 cM and 10 cM). Under the assumptions of no interference and normal segregations, F_2 marker data with three marker types (C/C, C/D, and D/D(C)) were separately generated for each combination of different population sizes (100, 150, 200, 250, and 300) and two spacing distances (5 cM and 10 cM).

Sex-linked markers were detected by chi-square value at probability level of 0.001. Recombination frequency between each pair of sex-linked markers was estimated by the ML approach. Recombination frequency between each pair of non-sex-linked markers was estimated using the ML approach for the achiasmatic model and the EM algorithm for the chiasmatic model[6]. Cutoff criteria ranging from 60-70 cM (significant at least 0.005) was used for grouping autosomal marker loci for these two genetic models. The seriation approach[23] was used for constructing both non-sex and sex-linked maps because of its fast computation.

The grouping power (GP) and ordering power (OP) are defined as following, $GP = \frac{g}{n} \times 100\%$ and $OP = \frac{o}{n} \times 100\%$, where n is the total simulation number, g is the number of correct groups for a specific linkage group, and o is the number of correct marker orders for the same link-

age group[26]. One thousand simulations were conducted for each of fifteen combinations. The standard errors for grouping and ordering powers were calculated based on the properties of binomial distributions[27]. All simulations were conducted using programs written in C + +.

3 Results

3.1 Separation of sex-linked markers

Co-dominant markers on the sex chromosome could be effectively detected from markers on autosomes by a Chi-square test for different population sizes of F_2. Furthermore, there was a low rate (≤0.05%) of markers on autosomes being grouped into the sex-linked markers (Table 4). Dominant markers on sex chromosome could also be effectively detected in an F_2 population when population size is 200 or larger. The rate of autosomal dominant markers being grouped into the sex-linked markers was much higher than that of autosomal co-dominant markers; however, all the false-grouping rates were still very low (0.62%-1.02%).

Table 4 Detection powers and false-grouping rate (FGR) for sex-linked markers

Marker type	Parameter (%)	Sample size				
		100	150	200	250	300
Co-dominant	Power	100	100	100	100	100
	FGR†	0.03	0.01	0.00	0.01	0.01
Dominant	Power	75.2	92.7	98.7	99.6	100
	FGR	0.84	1.02	0.62	0.77	0.82

†: The rate of a marker actually on autosome but grouped as a sex-linked marker.

3.2 Estimation of linked-marker distances

Estimates of distances between two sex-linked markers were unbiased for various population sizes and marker types (data not presented). The C/C marker type provided more precise estimations than the other three marker types, which gave the same precisions for different population sizes. The major reason is that both male and female individuals are informative in an F_2 population for the C/C marker type, while only the female individuals are informative in an F_2 population for the other marker types.

The chiasmatic model (without conversion) gave under-estimated distance (approximately one-half of actual value), indicating that it is not appropriate based on the chiasmatic model when occurrence of achiasmata oogenesis or spermatogenesis does exist unless one corrects the results by a factor of two.

3.3 Comparisons of mapping powers between the two genetic models

Mean powers of grouping and ordering and their standard errors over all nine linkage groups and 1000 simulations for the two genetic models are summarized in Table 5. Grouping power and ordering power obtained by the achiasmatic model are greater than those by the chiasmatic model without correction for all cases ($P = 0.05$). The achiasmatic model provides desirable grouping powers ranging from 97.9%-100% if all mar-kers are co-dominant, and 90.3%-99.5% if each pair of markers belongs to the C/D or D/D(C) marker type. An F_2 population size of 100 was good for achieving a desirable grouping power for the C/C marker type and 150 for the other two marker types for various marker distances. A desirable ordering power could be reached for various flanking marker distances for the C/C marker type when population size is 150 or above. Lower ordering powers (< 90%) could be obtained for the C/D and D/D(C) marker types when population size is 200 or less. The results indicated that the C/D and D/D(C) marker types would result in higher chances of inverted marker orders than the C/C marker types. It seems that marker type has a great impact on the ordering powers than population size and flanking marker distance, suggesting that the use of co-dominant markers (SSR and RFLP DNA markers) should achieve more desirable mapping powers than dominant markers

(RAPD and AFLP DNA markers). The chiasmatic model without correction also provided desirable grouping and order powers for the C/C marker type when population sizes reach 200; however, since the chiasmatic model without correction gave under-estimates of recombination, the linkage maps and marker information would still provide poor results for QTL mapping or gene mapping. The grouping and ordering powers were very similar for the two models when the correction factors were applied to data in the chiasmatic model (data not shown).

Table 5 Grouping power (GP,%) and order power (OP,%) and their standard error (SE) under two genetic models for different linked marker types, marker distances, and population sizes

Distance	Sample size	C/C		C/D		D/D(C)	
		GP (SE)	OP (SE)	GP(SE)	OP(SE)	GP(SE)	OP (SE)
Chiasmatic model without conversion							
5 cM	100	82.5(0.4)	73.7(0.5)	38.9(0.5)	19.1(0.4)	36.5(0.5)	19.1(0.4)
	150	89.7(0.3)	87.8(0.3)	49.9(0.5)	36.6(0.5)	43.6(0.5)	33.7(0.5)
	200	97.4(0.2)	97.1(0.2)	73.9(0.5)	64.0(0.5)	68.1(0.5)	60.8(0.5)
	250	99.2(0.1)	99.1(0.1)	84.5(0.4)	78.7(0.4)	79.2(0.4)	75.7(0.4)
	300	99.8(0.0)	99.8(0.1)	92.8(0.3)	89.2(0.3)	89.6(0.3)	87.4(0.3)
10 cM	100	72.7(0.5)	70.9(0.5)	25.3(0.4)	18.9(0.4)	19.5(0.4)	15.0(0.4)
	150	84.2(0.4)	83.9(0.4)	37.6(0.5)	34.3(0.5)	28.4(0.5)	26.5(0.4)
	200	95.6(0.2)	95.6(0.2)	63.1(0.5)	61.0(0.5)	53.3(0.5)	52.1(0.5)
	250	98.9(0.1)	98.9(0.1)	77.9(0.4)	76.8(0.4)	68.0(0.5)	67.5(0.5)
	300	99.8(0.0)	99.8(0.0)	89.6(0.3)	89.1(0.3)	83.4(0.4)	83.2(0.4)
Achiasmatic model							
5 cM	100	99.2(0.1)	89.3(0.3)	93.2(0.2)	45.5(0.5)	94.1(0.2)	49.2(0.5)
	150	99.8(0.0)	97.6(0.2)	97.5(0.2)	71.5(0.5)	96.9(0.2)	74.6(0.5)
	200	99.9(0.0)	99.5(0.1)	99.2(0.1)	85.9(0.4)	98.8(0.1)	88.2(0.3)
	250	99.9(0.0)	99.8(0.0)	99.4(0.1)	92.3(0.3)	99.1(0.1)	94.6(0.2)
	300	100(0.0)	99.9(0.0)	99.5(0.1)	95.6(0.2)	99.3(0.1)	96.9(0.2)
10 cM	100	98.6(0.1)	96.1(0.2)	91.2(0.3)	68.6(0.5)	92.2(0.2)	71.0(0.5)
	150	99.9(0.0)	99.6(0.1)	97.2(0.2)	88.6(0.3)	96.0(0.2)	89.1(0.3)
	200	100(0.0)	100(0.0)	98.8(0.1)	95.4(0.2)	98.1(0.1)	96.0(0.2)
	250	100(0.0)	100(0.0)	99.3(0.1)	97.9(0.2)	98.8(0.1)	98.0(0.1)
	300	100(0.0)	100(0.0)	99.4(0.1)	98.8(0.1)	99.0(0.1)	98.7(0.1)

3.4 Mapping powers for sex-linkage maps

The simulations (results not presented) showed that grouping powers of sex linkage group ranged from 99.5% to 100% and ordering power from 90.7% to 100% across different population sizes and marker distances when co-dominant markers were used. Grouping powers of sex linkage group ranged from 35.7% to 97.2% and ordering power from 28.1% to 96.6% across different population sizes and marker distances when co-dominant and dominant markers in coupling phase were used. Mapping powers for sex linkage group were similar to those for non-sex linkage groups for co-dominant markers, while mapping powers for sex linkage group were lower than those for non-sex linkage groups for the other two types of DNA markers because only the female individuals were informative using either model.

4 Discussion

Appropriately detecting the recombination frequency and constructing linkage maps are of fundamental importance for mapping genes or QTLs. For most organisms, crossing-over occurs in both female and male gametes during meiosis. The widely used software packages are suitable for linkage mapping under the chiasmatic model. In the present study, we used simulation technique to

compare the estimated recombination frequency based on two genetic models for achiasmatic F_2 populations. The chiasmatic model could give an under-estimation for the recombination frequency in an achiasmatic F_2 population unless correction factors are applied. We found that the estimated recombination frequency by the chiasmatic genetic assumption was close to half of that by the true genetic assumption (0.51 in this study). Shi et al. [16] also found the similar results. Therefore, when a conversion factor of twice the recombination frequency obtained by the chiasmatic model was used, currently used software packages should provide similar mapping results to ours if they have this option available. Additionally, even if the correction factor is applied, the EM or other iteration algorithms are needed for calculation of recombinant frequency using chiasmatic model even for C/C marker type while only ML approach is needed under the achiasmatic model for an achiasmatic F_2 population (Table 2). Thus, the calculation of recombination frequency using the achiasmatic model should be faster than using the chiasmatic model for an achiasmatic F_2 population. Theoretically, the F_2 genotypes are the combination of two BC populations; however, the use of the converted BC-typed data from an F_2 population for estimating recombination frequency could have two major drawbacks. (1) If D/D(C) type markers are used, the genotype *ab/AB* (second line, Table 2) is confound with all genotypes of the first line in Table 2. Thus, only genotypes *ab/aB* and *ab/ab* are informative, so that about 75% of F_2 individuals will be considered as not informative. All individuals in an F_2 population can be used under achiasmatic model, thus, higher statistical power will be resulted. (2) Such a conversion may also require converted BC-typed data for QTL mapping, which would loss the efficiency in detecting dominant QTL effects.

Some DNA markers are located on sex chromosome in silkworm or other insects. Thus, the detection of sex-linked markers and non-sex-linked markers becomes important before the construction of linkage maps. Sex-linked markers can be effectively detected by a Chi-square test from expected Mendelian segregation ratios. The detection power is based on population size, marker type, segregation normality, and the probability level. The efficiency of classifying sex-linked markers will also be influenced by distorted segregation. The detection of recombination frequency of sex-linked markers for ZW type should also be applicable for XY type. If a marker is located on sex chromosome X or Z, heterogametic F_2 individuals are informative and can be considered as haploids, while homogametic F_2 individuals may not always be informative for dominant markers. A Chi-square test can also be used to detect sex-linked markers in a backcross population. Our software package implemented with seriation approach [23] (LinkMap in QGAStation version 1.0) has been developed for constructing linkage maps for organisms with achiasmata during gametogenesis and can be downloaded at website http://ibi.zju.edu.cn/software/qga/index.htm. The software we developed offers several advantages over the others: (1) It can automatically detect sex-linked markers; (2) It is suitable for linkage mapping for both chiasmatic and achiasmatic models; (3) It provides fast computation for a large number of markers.

Mapping QTLs for traits of importance is another important issue in genomics projects. Due to achiasmatic gametogenesis, currently used methods such as interval mapping (IM) [28], composite interval mapping (CIM) [29], multiple interval mapping (MIM) [30], mixed-model-based composite interval mapping (MCIM) [31,32], and other methods should be modified accordingly. The methods for mapping sex-linked QTL also need to be considered. This issue remains a challenge.

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