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Estimation of linkage in trisomic inheritance

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Abstract Based on F_2 families derived from selfed F_1 trisomic plants we have developed a genetic model to estimate linkage relationships between pairs of loci located on the extra chromosome. Genotypic frequencies of each class expected in a trisomic F_2 family have been calculated and the maximum-likelihood equations for recombination-fraction estimation have been derived for a variety of genetic situations. Morton's test of homogeneity was used to compare recombination fractions estimated between loci exhibiting trisomic segregation to those obtained in families where the same loci showed Mendelian segregation. This method has been applied to an analysis of morphological, isozyme and RAPD data from faba bean (*Vicia faba* L.).

Key words Trisomic · Linkage · Maximum-likelihood estimates · *Vicia faba* L.

Introduction

A fully informative genetic map of a species useful for scientific and breeding purposes has to combine both linkage and cytogenetic data. The most common method to relate linkage maps to specific chromosomes is the use of aneuploids (Helentjaris et al. 1986 used monosomics in maize and Young et al. 1987 used trisomics in tomato) and substitution lines (Sharp et al. 1989). The availability of above mentioned cytogenetic tools depends largely on the karyotypic characteristics of a given species. In a broad sense, substitution lines are used in species of allopolyploid origin and for monosomics in eupolyploids, whereas strictly diploid material requires the use of trisomics.

Trisomics are found in a range of species and their use in gene localization has been widely proven (McClintock and Hill 1931 in maize; Lesley 1932 in tomato; Blakeslee and Avery 1934 in *Datura*). Although trisomics can be obtained from different sources (polyploids, translocation stocks), asynaptic mutants have proven to be very convenient since, like those known in *Vicia faba* L., they are easily maintained, sufficiently fertile and widely crossable (Cabrera et al. 1989). By crossing them to normal lines, trisomics with a given genetic background can be obtained in a constant and reliable way. Thus, successful identification and characterization of five out of the six possible primary trisomics in *V. faba* (Martìn and Barceló 1984), obtained by crosses between the asynaptic line Vf 6 and a normal diploid parent, enabled the localization of some morphological, isozyme and RAPD markers on their respective chromosomes (Torres et al. 1995; Satovic et al. 1996).

Different types of genetic populations are widely used for gene mapping (F_2 and backcross populations, haploid populations, doubled-haploid populations, recombinant inbred lines, etc.), and suitable statistical procedures and software have been developed for processing the data. The segregation ratios of the loci is expected to be Mendelian in all these cases. However, linkage can also be detected in families derived from plants trisomic for a chromosome on which these loci are located and thus exhibit aberrant, so-called trisomic, segregation ratios. Although a genetic map can be established by joint segregation analysis in families derived from trisomic plants, a part of the

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information seems to be lost due to the lack of a suitable statistical method to estimate the linkage between loci exhibiting trisomic inheritance. We have developed maximum-likelihood equations to estimate the recombination fraction in trisomic inheritance. This method has been tested with results obtained in faba bean F_2 families derived from trisomic F_1 plants.

Materials and Methods

Genetic material

V. faba primary trisomics for chromosomes 3, 4, 5, and 6 were obtained by crossing Vf 6 (an asynaptic line always used as the female parent) with different mutant types as pollen parents, all of them from the collection of genetic variants at the E.T.S.I.A.M. (Escuela Técnica Superior de Ingenieros Agrónomos y Montes) in Córdoba. The F_1 plants with $2n + 1$ chromosomes were identified by studying the meiotic metaphase and were characterized as outlined by Martín and Barceló (1984). Seven F_2 families derived from these trisomic plants were scored for six morphological, 13 isozyme and 282 RAPD marker phenotypes.

The details of isozyme and RAPD analysis, as well as the results of linkage analysis and genetic mapping, were previously described by Torres et al. (1995) and Satovic et al. (1996).

Linkage estimation

The estimation of linkage in trisomic inheritance, and the comparison of recombination fractions between a pair of loci in an F_2 family derived from an F_1 plant trisomic for the chromosome where these loci are located and another F_2 family derived from the same cross but trisomic for another chromosome, would involve the following steps.

Estimation of the recombination fraction

The expected frequencies of each genotypic class observed in a F_2 family derived from a trisomic F_1 plant can be calculated by knowing the gametic frequencies of two linked loci situated on the extra chromosome.

Sánchez-Monge (1972) developed formulae for the gametic segregations of the eight different trisomic types heterozygous for two linked loci considering random chromosome association (RCA) and random complete chromatid association (RCCA). The RCA model assumes that the meiosis has a normal first-anaphase whereas the RCCA model takes into account the random distribution of the six chromatids within each mother cell after the occurrence of crossing-over. Since in our studies the rate of chromatid association seemed to be minimal we have based our statistics on the RCA model. Nevertheless, it is possible to develop similar maximum-likelihood equations to estimate recombination fractions considering the RCCA model.

The gametic frequencies for gametes of constitution n (gametes carrying a normal haploid set of chromosomes) and $n + 1$ (gametes carrying an additional copy of a particular chromosome of the haploid set) of the three possible trisomic types expected considering the RCA model are shown in Table 1.

Taking into account the frequencies of the different gametic types produced by trisomic plants, the frequencies of each genotypic class expected in a F_2 family derived by selfing a F_1 trisomic plant can also be calculated. It is possible to develop the maximum-likelihood equations to calculate the recombination fraction between a pair of genes exhibiting trisomic segregation in a similar way to that used by Allard (1956) who derived maximum-likelihood equations for the estimation of recombination fractions when the segregation of each gene was normal diploid.

Table 1 Gametic frequencies of different trisomic types considering the random chromosome association model (RCA)

Gametic types produced by the following trisomics			Gametic frequency (RCA)[a]
AB/AB/ab	*Ab/Ab/aB*	*AB/ab/ab*	
Gametes n:			
AB	*Ab*	*ab*	$1/3 \cdot (2 - r)$
Ab	*ab*	*aB*	$1/3 \cdot r$
aB	*AB*	*Ab*	$1/3 \cdot r$
ab	*aB*	*AB*	$1/3 \cdot (1 - r)$
Gametes n + 1			
AB/AB	*Ab/Ab*	*ab/ab*	$1/3 \cdot (1 - r)$
AB/Ab	*Ab/AB*	*aB/ab*	$1/3 \cdot r$
AB/aB	*Ab/ab*	*Ab/ab*	$1/3 \cdot r$
AB/ab	*Ab/aB*	*AB/ab*	$1/3 \cdot (2 - r)$

[a] r = recombination fraction between the two loci considered

Critical ratios expected in a F_2 family derived from a trisomic plant depend on four critieria.

(1) The type of marker segregation (co-dominant vs dominant markers).

(2) The transmission rate of the extra chromosome. The transmission rate of the extra chromosome (i.e. the percentage of trisomics in a F_2 family derived from a trisomic plant) depends on both female and male transmission rates. The transmission of the extra chromosome in *V. faba* is always through the female parent and, thus, the male transmission rate is considered to be equal to zero (González 1985; Cabrera et al. 1989).

(3) The parent carrying the dominant allele of each locus. The genotype of a trisomic F_1 plant will be either *AAa* or *Aaa*. When two loci are considered, the phase between them (coupling or repulsion) has to be taken into account giving three possible F_1 genotypes (*AB/AB/ab*, *Ab/Ab/aB*, *AB/ab/ab*) as shown in Table 1.

(4) The trisomic segregation model. As mentioned above we have considered only the RCA model. Moreover, only diploid F_2 plants were taken into account due to the very low number of trisomic F_2 plants (see Discussion).

Table 2 presents the frequencies of each genotypic class in F_2 families derived from trisomic plants of different types. Considering the segregation type of each of the loci (co-dominant or dominant) and the phase between them (coupling or repulsion), ten different maximum-likelihood equations can be developed (Table 3).

LOD scoring

Calculation of the maximum-likelihood equations was carried out using the MATHEMATICA computer program (MATHEMATICA 1.2.1 f33 enhanced, Wolfram Research, Inc.).

For each recombination fraction the corresponding *LOD* score can be calculated as shown by Lander and Botstein (1986)

$$LOD(r) = \log \frac{P(r)}{P(r = 0.5)} = \sum_{i=1}^{c} a_i \cdot \log \frac{f_i(r)}{f_i(r = 0.5)} \quad (1)$$

where,

$P(r)$ = probability that the observed data would arise assuming a recombination fraction r,

Table 2 Expected frequencies of various diploid genotypes occurring among the selfed progeny of trisomic individuals (*AB*/*AB*/*ab*, *Ab*/*Ab*/*aB* and *ab*/*ab*/*AB*)

Genotype	Observed frequency[a]	Expected frequency in F_2 assuming an F_1 of the following genotypes		
		AB/*AB*/*ab*	*Ab*/*Ab*/*aB*	*ab*/*ab*/*AB*
AB/*AB*	e	$(1/9)\cdot(2-r)^2$	$(1/9)\cdot r^2$	$(1/9)\cdot(1-r)^2$
AB/*aB*	f	$(2/9)\cdot r\cdot(2-r)$	$(2/9)\cdot r\cdot(1-r)$	$(2/9)\cdot r\cdot(1-r)$
AB/*Ab*	g	$(2/9)\cdot r\cdot(2-r)$	$(2/9)\cdot r\cdot(2-r)$	$(2/9)\cdot r\cdot(1-r)$
AB/*ab*	h	$(2/9)\cdot(1-r)\cdot(2-r)$	$(2/9)\cdot r^2$	$(2/9)\cdot(1-r)\cdot(2-r)$
Ab/*aB*	i	$(2/9)\cdot r^2$	$(2/9)\cdot(1-r)\cdot(2-r)$	$(2/9)\cdot r^2$
Ab/*Ab*	j	$(1/9)\cdot r^2$	$(1/9)\cdot(2-r)^2$	$(1/9)\cdot r^2$
Ab/*ab*	k	$(2/9)\cdot r\cdot(1-r)$	$(2/9)\cdot r\cdot(2-r)$	$(2/9)\cdot r\cdot(2-r)$
aB/*aB*	l	$(1/9)\cdot r^2$	$(1/9)\cdot(1-r)^2$	$(1/9)\cdot r^2$
aB/*ab*	m	$(2/9)\cdot r\cdot(1-r)$	$(2/9)\cdot r\cdot(1-r)$	$(2/9)\cdot r\cdot(2-r)$
ab/*ab*	n	$(1/9)\cdot(1-r)^2$	$(1/9)\cdot r^2$	$(1/9)\cdot(2-r)^2$

[a] Symbols used correspond to those used by Allard (1956)

$P(r = 0.5)$ = probability that the observed data would arise assuming non-linkage,
i = genotypic class,
c = number of classes,
a_i = observed number of individuals in class i,
$f_i(r)$ = expected frequency of class i assuming a recombination fraction r,
$f_i(r = 0.5)$ = expected frequency of class i assuming non-linkage.

Estimation of recombination fraction from pooled data

In the present study, in order to justify the linkage with a $LOD > 3$, different families segregating for the same loci were pooled. Due to the origin of our material (F_2s derived from plants trisomic for different chromosomes) a pair of loci could show trisomic inheritance in a given family whereas in another family their segregations would be normal diploid. Since maximum-likelihood equations to estimate recombination fractions are different for diploid and trisomic segregations, it is not legitimate to add observed frequencies from both families when two or more normal diploid families are analyzed. Consequently, new maximum-likelihood equations are needed where the recombination fraction would depend on both sets of data. These equations can be derived by combining those shown in Table 3 for trisomic segregations and those developed by Allard (1956) for diploid segregations. A joint likelihood is established by setting the sum of the derivatives of the two likelihoods to zero. *LOD* scores can be calculated once the recombination fractions are estimated.

Homogeneity of recombination test

Finally, a test of homogeneity of recombination fractions estimated from the data of different F_2 families is carried out as proposed by Morton (1956).

$$(2\ln 10)\left[\sum_{i=1}^{N} LOD_i(r) - LOD_p(r)\right] \quad (2)$$

$LOD_i(r) = LOD$ given the maximum-likelihood estimate of the recombination fraction r for population i, and,
$LOD_p(r) = LOD$ given the maximum-likelihood estimate of the recombination fraction r for data pooled from all N populations.

The test is asymptotically distributed as χ^2 with $(n - 1)$ degrees of freedom.

Results

To illustrate our method we will describe the following example. A RAPD marker OPC-19_{1157} (*A*) and an isoenzymatic locus *Prx*-1 (*B*) segregated jointly in two F_2 families, 6×159 T4 and 6×159 T5, derived from plants trisomic for chromosomes 4 and 5, respectively (Tables 4 and 5). The female parent showed the presence of the RAPD band and the slow-migrating allele for *Prx-1*. Thus, we considered that the loci were in repulsion phase and that the F_1 trisomic genotype is expected to be *Ab*/*Ab*/*aB*. In 6×159 T4 both loci exhibit normal diploid segregation whereas in 6×159 T5 both loci fit to critical trisomic ratios indicating their location on chromosome 5. The recombination fraction between these loci estimated on data from family 6×159 T4 was 0.11 and its corresponding *LOD* score was 3.46.

Joint segregation data for OPC-19_{1157} and *Prx-1* 6×159 T5 are shown in Table 4. Using the adequate maximum-likelihood equation for this situation (Table 3, equation no. 6), the recombination fraction was estimated as $r = 0.149$ and the corresponding *LOD* score was 1.9986, as shown in Table 5.

Equation (3) is a joint maximum-likelihood equation established by setting the sum of the derivatives of the two likelihoods (one for trisomic segregation, mentioned above, and the corresponding one for normal diploid segregation as shown by Allard 1956) to zero. The estimate of the recombination fraction considering both sets of data (6×159 T4 and 6×159 T5) is then $r = 0.13$ with a corresponding *LOD* score of 5.43.

$$2\cdot\left(\frac{2-2\cdot r}{2\cdot r-r^2}\right)+13\cdot\left(\frac{2\cdot r-1}{1-r+r^2}\right)+12\cdot\left(\frac{-2\cdot r}{1-r^2}\right)$$

$$+5\cdot\left(\frac{2}{r-1}\right)+1\cdot\left(\frac{1-2\cdot r}{r-r^2}\right)+0\cdot\left(\frac{2}{r}\right)$$

Table 3 Summary of the maximum-likelihood equations for determining recombination fractions between two loci located on the extra chromosome from F_2 families derived from trisomic F_1 plants (considering only normal diploid progeny)

1. Co-dominant–Co-dominant (*AB*/*AB*/*ab*)

$$(e)\cdot\left(\frac{-2}{2-r}\right)+(f+g)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(h+i)\cdot\left(\frac{4\cdot r-3}{2\cdot r^2-3r+2}\right)+(j+l)\cdot\left(\frac{2}{r}\right)+(k+m)\cdot\left[\frac{2\cdot r-1}{r\cdot(r-1)}\right]+(n)\cdot\left(\frac{-2}{1-r}\right)=0$$

2. Co-dominant–Co-dominant (*Ab*/*Ab*/*aB*)

$$(e+n)\cdot\left(\frac{2}{r}\right)+(f+m)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-1)}\right]+(g+k)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]\cdot(h+i)\cdot\left(\frac{4\cdot r-3}{2\cdot r^2-3r+2}\right)+(j)\cdot\left(\frac{-2}{2-r}\right)+(l)\cdot\left(\frac{-2}{1-r}\right)=0$$

3. Co-dominant–Co-dominant (*AB*/*ab*/*ab*)

$$(e)\cdot\left(\frac{-2}{1-r}\right)+(f+g)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-1)}\right]+(h+i)\cdot\left(\frac{4\cdot r-3}{2\cdot r^2-3r+2}\right)+(j+l)\cdot\left(\frac{2}{r}\right)+(k+m)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(n)\cdot\left(\frac{-2}{2-r}\right)=0$$

4. Co-dominant–Dominant (*AB*/*AB*/*ab*)

$$(e+g)\cdot\left[\frac{2\cdot r}{(r-2)\cdot(r+2)}\right]+(f+h+i)\cdot\left(\frac{2\cdot r-1}{r^2\cdot r-2}\right)+(j)\cdot\left(\frac{2}{r}\right)+(k)\cdot\left[\frac{2\cdot r-1}{r\cdot(r-1)}\right]+(l+m)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(n)\cdot\left(\frac{-2}{1-r}\right)=0$$

5. Co-dominant–Dominant (*Ab*/*Ab*/*aB*)

$$(e+g)\cdot\left[\frac{2\cdot(r-2)}{r\cdot(r-4)}\right]+(f+h+i)\cdot\left[\frac{2\cdot(r-1)}{r^2-2\cdot r+2}\right]+(j)\cdot\left(\frac{-2}{2-r}\right)+(k)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(l+m)\cdot\left[\frac{2\cdot r}{(r-1)\cdot(r+1)}\right]+(n)\cdot\left(\frac{2}{r}\right)=0$$

6. Dominant–Co-dominant (*Ab*/*Ab*/*aB*)

$$(e+f)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(g+h+i)\cdot\left(\frac{2\cdot r-1}{r^2-r+2}\right)+(j+k)\cdot\left[\frac{2\cdot r}{(r-2)\cdot(r+2)}\right]+(l)\cdot\left(\frac{-2}{1-r}\right)+(m)\cdot\left[\frac{2\cdot r-1}{r\cdot(r-1)}\right]+(n)\cdot\left(\frac{2}{r}\right)=0$$

7. Co-dominant–Dominant (*AB*/*ab*/*ab*)

$$(e+g)\cdot\left[\frac{2\cdot r}{(r-1)\cdot(r+1)}\right]+(f+h+i)\cdot\left[\frac{2\cdot(r-1)}{r^2-2r+2}\right]+(j)\cdot\left(\frac{2}{r}\right)+(k)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(l+m)\cdot\left[\frac{2\cdot(r-2)}{r\cdot(r-4)}\right]+(n)\cdot\left(\frac{-2}{2-r}\right)=0$$

8. Dominant–Dominant (*AB*/*AB*/*ab*)

$$(e+f+g+h+i)\cdot\left[\frac{2\cdot(r-1)}{r^2-2\cdot r+8}\right]+(j+k+l+m)\cdot\left[\frac{2\cdot(r-1)}{r\cdot(r-2)}\right]+(n)\cdot\left(\frac{-2}{1-r}\right)=0$$

9. Dominant–Dominant (*Ab*/*Ab*/*aB*)

$$(e+f+g+h+i)\cdot\left(\frac{2\cdot r}{r^2+4}\right)+(j+k)\cdot\left[\frac{2\cdot r}{(r-2)\cdot(r+2)}\right]+(l+m)\cdot\left[\frac{2\cdot r}{(r-1)\cdot(r+1)}\right]+(n)\cdot\left(\frac{2}{r}\right)=0$$

10. Dominant–Dominant (*AB*/*ab*/*ab*)

$$(e+f+g+h+i)\cdot\left[\frac{2\cdot(r-2)}{r^2-4\cdot r+4}\right]+(j+k+l+m)\cdot\left[\frac{2\cdot(r-2)}{r\cdot(r-4)}\right]+(n)\cdot\left(\frac{-2}{2-r}\right)=0$$

$$+1\cdot\left(\frac{2\cdot(r-1)}{r\cdot(r-2)}\right)+12\cdot\left(\frac{2\cdot r-1}{r^2-r+2}\right)+14\cdot\left(\frac{2\cdot r}{(r-2)\cdot(r+2)}\right)+5\cdot\left(\frac{-2}{(1-r)}\right)+2\cdot\left(\frac{2\cdot r-1}{r\cdot(r-1)}\right)+0\cdot\left(\frac{2}{r}\right)=0 \quad (3)$$

The test of homogeneity of the recombination proved non-significant (χ^2 0.13 with 1 *df*). This led us to the conclusion that the linkage was maintained in the two tested populations (Table 6).

Discussion

Selfing a trisomic F_1 plant may yield disomic (i.e. normal diploid), trisomic, and tetrasomic plants. Their respective frequencies will depend on female and male transmission rate. Nevertheless, if the male transmission rate is zero (as in the case of faba bean) the F_2 family derived from a trisomic plant is expected to consist only of disomic and trisomic individuals. Although, in theory, the same number of diploid and trisomic plants could be expected, the observed number of trisomics is always significantly lower. González (1985) summarized the possible causes as: (1) loss of the

Table 4 Joint segregation data for OPC-19_{1157} (*A*/*a*) and *Prx-1* (*B*/*b*) in the family 6×159 T5 (derived from the plant trisomic for chromosome 5)

Marker		Diploid plants					Trisomic plants			
		Prx-1					*Prx-1*			
		BB	*Bb*	*bb*	Total		*BBB*	*B_b*	*bbb*	Total
OPC-19_{1157}	*A_*	1	12	14	27	*A_*	0	3	1	4
	aa	5	2	0	7	*aaa*	0	0	0	0
	Total	6	14	14		Total	0	3	1	

Table 5 Calculation of the *LOD* score that corresponds to a recombination fraction $r = 0.15$ between OPC-19_{1157} (*A*/*a*) and *Prx-1* (*B*/*b*)

Genotypic class	Observed	Expected frequency		Ratio of odds	*LOD*
		If linked $r = 0.15$	If unlinked $r = 0.50$		
A_BB	1 (e + f)	0.0305	0.0833	0.3655	− 0.4372
A_Bb	12 (g + h + i)	0.4164	0.3889	1.0708	0.3565
A_bb	14 (j + k)	0.4420	0.4167	1.0608	0.3590
aaBB	5 (l)	0.0807	0.0278	2.9036	2.3147
aaBb	2 (m)	0.0280	0.0556	0.5044	− 0.5945
aabb	0 (n)	0.0024	0.0278	0.0876	0.0000
Total	34	1.0000	1.0000		1.9986

Table 6 Test of homogeneity of the recombination-fraction estimates between OPC-19_{1157} and *Prx-1*

F_2 family	*r*	*LOD*
6×159 T4 (diploid segregation of both loci)	0.11	3.46
6×159 T5 (trisomic segregation of both loci)	0.15	2.00
6×159 (pooled data)	0.13	5.43
χ^2 Test of homogeneity (1 df)	0.13 ns	

extra chromosome during meiosis, (2) reduced viability of n + 1 gametes, (3) abnormal development of 2n + 1 embryos, (4) reduced germination capacity of 2n + 1 seeds, and (5) reduced vigor of 2n + 1 plants.

The percentage of trisomic plants in the families studied varied from 7 to 35%, with an average of 19%. Thus, in most cases the number of trisomic plants was insufficient to obtain reliable estimates of recombination fractions between loci located on the extra chromosome. This is the reason why only the diploid F_2 plants are chosen for the detection of linkage between loci exhibiting trisomic segregation.

As previously reported (Torres et al. 1995; Satovic et al. 1996) joint segregation analysis of morphological, isozyme and RAPD markers can be carried out on data pooled from different families of the same cross (in the case of RAPD markers when the pollen parent was the dominant homozygote) or from all the segregating families (in the case of morphological and isozyme loci, and RAPD markers when Vf 6 was the dominant homozygote). By this approach the problem of the reduced fertility of trisomic F_1 plants, and consequently the relatively limited F_2 population size, was solved, revealing a number of linkages justified considering a *LOD* score of 3 as threshold.

However, for the analysis of linkage using the program MAPMAKER V2.0 (Lander et al. 1987), only markers which exhibit a typical Mendelian ratio (1 : 2 : 1 or 3 : 1) can be included. The reason is to avoid the bias of the estimates of the recombination fraction caused by segregation distortion of a specific locus. This makes it impossible to take full advantage of the data obtained from different families. When a set of markers is localized on a specific chromosome, the joint segregation data just from the family where the markers exhibited normal segregation ratios are sometimes not sufficient to consider the linkage significant (i.e. $LOD < 3$). Nevertheless, by applying our model, we could pool the data of families with both normal and trisomic segregating markers, so obtaining a higher *LOD* and proving the existence of linkage.

Asynaptic mutants are known in a range of species and as in our case, they can be, a reliable source for the production of trisomics. In order to develop a saturated

map of a given species, F_2 families derived from trisomic plants could be obtained and analyzed. This approach would imply the use of the same asynaptic line as the female parent in a number of crosses in order to justify the pooling of data from all the families studied. Secondly, joint segregation analysis in trisomic F_2 families allows both the estimation of linkage relationships and the localization of linkage groups on specific chromosomes simultaneously. Finally, by applying our statistical procedure the estimation of linkage in trisomic inheritance is also possible taking full advantage of all the available data.

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References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24 : 235–278

Blakeslee AF, Avery AG (1934) Three genes located in the 21-22 chromosome of the Jimson weed. J Hered 25 : 393–404

Cabrera A, Cubero JI, Martín A (1989) Genetic mapping using trisomics in *Vicia faba* L. FABIS Newslett 23 : 5–7

González JA (1985) Tetraploidia, aneuploidia y asinapsis en *Vicia faba* L. PhD thesis, Dept. of Genetics, Faculty of Sciences, University of Córdoba

Helentjaris T, Weber DF, Wright S (1986) Use of monosomics to map cloned DNA fragments in maize. Proc Natl Acad Sci USA 83 : 6035–6039

Lander ES, Botstein D (1986) Mapping complex genetic traits in humans: new methods using a complete RFLP linkage map. Cold Spring Harbor Symp Quant Biol 51 : 49–62

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1 : 174–181

Lesley JW (1932) Trisomic types of the tomato and their relations to the genes. Genetics 12 : 449–460

Martín A, Barceló P (1984) The cytology and morphology of *Vicia faba* trisomics. In: Chapman GP, Tarawai SA (eds.) Systems for cytogenetic analysis in *Vicia faba* L. Martinus Nijhoff, The Hague, pp 63–76

McClintock B, Hill HE (1931) The cytological identification of the chromosome associated with the R-G linkage group in *Zea mays*. Genetics 16 : 175–190

Morton NE (1956) Sequential tests for the detection of linkage. Am J Hum Genet 7 : 277–318

Sánchez-Monge E (1972) Linkage in trisomic inheritance. INIA/Ser Prod Veg 2 : 23–39

Satovic Z, Torres AM, Cubero JI (1996) Gene mapping of new morphological, isozyme and RAPD markers in *Vicia faba* L. using trisomics. Theor Appl Genet 93 : 1130–1138

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78 : 342–348

Torres AM, Satovic Z, Canovas J, Cobos S, Cubero JI (1995) Genetics and mapping of new isozyme loci in *Vicia faba* L. using trisomics. Theor Appl Genet 91 : 783–789

Young ND, Miller J, Tanksley SD (1987) Rapid chromosomal assignment of multiple genomic clones in tomato using primary trisomics. Nucleic Acids Res 15: 9339–9348