EVALUATION OF GENE LINKAGE AND ITS USE IN THE DETERMINATION OF THE HEREDITY OF TRAITS IN SUNFLOWER

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INTRODUCTION

The range of problems studied by the Genetics Section of the Prague Research Institute of Crop Production, includes the problems of sunflower genetics and cytology. The research in this crop, used as a model representing allogamous plants pollinated by insects and suitable for heterosis breeding, is concentrated in the following three fields :

a) study of the hereditarily conditioned quantitative traits of production importance ;

b) the use of cytological methods in the study of pollen sterility ;

c) the determination of the heredity of marker traits, i.e. morphological and anatomic traits easy to define by sight and potentially applicable to the disclosure of other, less marked traits.

The determination of the relationships to the traits which are to be disclosed is a prerequisite for the use of the marker traits. At the level of the genes determining the respective traits, such relationships are realized through gene linkage.

Some methodical and interpretation possibilities of the evaluation of gene linkage are subjected to the study described in this paper.

METHODS

The determination of the dependence of two traits and the expression of the linkage strength between the genes conditioning these traits can be done by several ways. Two basic principles available for this purpose are seen in the procedure based on the segregation ratios transformed into the contingency table and in the procedure in which phenotype frequencies are used for the calculation without further transformation. Both procedures offer some advantages ; the latter one is currently indicated in the basic genetic handbooks Hruby, 1961 ; Nečásek and Cetl, 1980).

The first procedure can be divided into the following steps :

1. determination of the dependence of two traits from the 2×2 or $2\times x^n$ contigency table ;
2. determination of the extent of dependence by means of the association coefficient or contingency coefficient ;
3. determination of the presence of an interaction ;
4. determination of the strength of the linkage by conversion of the association coefficient to the Morgan number.

The first procedure offers the advantage of enabling some continuity in the system of the evaluation of quantitatively and qualitatively conditioned traits, i.e. the traits determined by a small number of genes are evaluated by calculations based, in the essence, on the same principles as the calculation procedures used in quantitative genetics. In addition to this, in the calculation of the strength of the linkage in F_2 generation it is not necessary to use hardly accessible tables or a complicated calculation. If two traits demonstrated only by two phenotypic categories are involved, the tests of dependence are based on the 2×2 contingency table and on the calculation of the testing criterion X^2. If the calculated $X^2_{(1)}$ value exceeds the table value, we believe that the genes underlying both traits cannot be freely combined and that they can be expected to have a gene linkage. The extent of this linkage can be determined by means of the so-called association coefficient whose calculation ensues from the relation :

$$V = \frac{(ad-bc)}{\sqrt{(a+b)(c+d)(a+c)(b+d)}}$$

It is also advantageous to use a simplified method of the conversion of the association coefficient to the Morgan number, determining the strength of the linkage. The overall calculation of the strength of the linkage is quite simple. For instance, when linkage is determined from the F_2 generation in the cis stage (i.e. at increased phenotype frequencies

a, d) and in the absence of interaction, the sequence of calculations looks as follows :

$$X^2_{(1)} = \frac{n(ad-bc)^2}{(a+b)\,(c+d)\,(a+c)\,(b+d)}$$

$$V = \frac{\sqrt{X^2_{(1)}}}{\sqrt{n}} \qquad p = 1 - \sqrt{\frac{3V+1}{2}}$$

The dependence between two traits characterized by several phenotypic categories can be tested by means of the contingency coefficient. In the genetic analysis of the traits conditioned by a small number of genes, the use of the contingency coefficient is of importance in two cases : first, in the case of testing the linkage with a trait conditioned by a single gene but realized by three phenotypes (incomplete dominance) and second, in a trait in which a multi-phenotypic manifestation is conditioned by interaction.

The calculation of the contingency coefficient is based on the value of $X^2_{(1)}$, determined by the following procedure :

$$X^2_{(1)} = \left(\frac{n^2}{b_1 b_2} \sum_{i=1}^{s} \frac{n^2}{a_1}\right) - \frac{nb_1}{b_2}$$

$$C = \frac{\sqrt{x^2_{(1)} \cdot 2}}{\sqrt{x^2_{(1)} + n}}$$

The mentioned methods of the determination of the strength of linkage with contingency tables require previous testing of the segregation ratio aimed at finding whether a change in the distribution of phenotypes is not caused by interaction, since the conversion of the association coefficient to the Morgan number changes with the type of interaction ; this, of course, fully corresponding with different ways of the calculation of the Morgan number for different interactions with the use of the second procedure without the transformation of data into contingency table.

The second procedure of testing of the presence of linkage also includes the cause of the change in the segregation : whether it is a linkage or an interaction. When testing the linkage according to the segregation ratio of phenotypes in the F_2 generation, the sequence of calculations is as follows :

$$X^2_{(1)} = \frac{(a_1+a_2-3\,a_3-3\,a_4)^2}{3\,n};$$

the testing criterion serves for the verification of the non-interaction segregation ratio for the first trait.

$$X^2_{(1)} = \frac{(a_1-3\,a_2+a_3-3\,a_4)^2}{3\,n}$$

verifies the non-interaction segregation ratio for the second trait.

$$X^2_{(1)} \frac{(a_1-3\,a_2-3\,a_3+9\,a_4)^2}{9\,n}$$

covers the presence of a gene linkage.

The sum of all three values of $X^2_{(1)}$ should be in congruence with the value of $X^2_{(3)}$ determined at testing the free combining ability with dominance, i.e. the dihybrid segregation ratio.

If the value of $X^2_{(1)}$ for the first and second gene is insignificant, the cause of the change in segregation ratio lies in linkage. A change in segregation is caused by linkage as well as by interaction in those cases in which the $X^2_{(1)}$ is significant for linkage and at the same time one or both $X^2_{(1)}$ for the verification of the non-interaction segregation ratios.

The strengths of the linkage determined by direct calculation of the Morgan number differ for each linkage stage cis-trans and for cases of separate interactions ; in the stage of cis in the F_2 generation the Morgan number value is determined from the relationship :

$$\frac{a_2 \cdot a_3}{a_1 \cdot a_4} = \frac{2(1-p)^2+(1-p)^4}{1-2(1-p)^2+(1-p)^4}$$

in which the ensuing from the right side of the equation is usually tabulated for the non-interaction segregation ratio (H r u b y, 1961) as well as for the interaction segregation ratios (I m m e r, 1930). The left side of the equation is called the product ratio and when calculating the strength of the linkage only this part of the relationship is calculated. The p corresponding to it can be seen from the respective table. In the cis stage, with a non-interaction segregation ratio, a simplified calculation can be used for the determination of the strength of the linkage: $p = 1-2\sqrt{a_4/n}$.

In the trans stage the determination of the strength of linkage from F_2 is rather difficult and when the linkage is strong, i.e. the frequency of phenotypic category expressed by frequency a_4 is very low, such a determination is factually impossible. The product ratio for the trans stage $\frac{a_1 \cdot a_4}{a_2 \cdot a_3}$ is again converted to the Morgan number, using special tables.

In our opinion, when linkage strength is determined from the segregation ratio in the F_2 generation it is better to use, instead of the product rations, the calculation of the p value from the association coefficient or a simplified calculation based on the phenotype frequency of the twice recessive gene set a_4 as proposed in this study.

RESULTS AND DISCUSSION

Four cases of linkage between the genes underlying the so-called marker traits have been found in sunflower until now. The most important of them is the linkage between the gene for anthocyanic colour and the gene for pollen sterility. Further, a gene linkage was found between the anthocyanic colour of the

plants and the colour of ligulate flowers. The linkage with pollen sterility gained some practical importance in the heterosis breeding of sunflower, based on the production of hybrids from gene-conditioned pollen sterility.

The linkage between the T gene for anthocyanic colour and the pollen sterility gene Ms was discovered by Leclercq (1966). This author presumed the genotype set of a fertile plant with anthocyanic colour to be TTMsMs and that of a sterile green plant ttmsms. The hybridization of both genotypes produced an F_1 generation of fertile red coloured specimens defined as TtMsms. In the F_2 generation the segregation ratio of 157 red fertile to 37 green sterile to 1 green fertile plant was obtained. The segregation ratio in the B_1 generation was 51 : 36 : 1, which also showed a rare occurrence of ttMs-recombinants and suggested that the T-msms genotypes did not occur at all. Leclercq believed the Ms gene, characterized by monofactorial heredity, and the T gene are in a linkage which is very close. The author enumerated the recombinants and got a value close to 1%.

On the basis of the linkage relationship, a scheme of the maintenance of sterile genotypes was determined for hybrid production. In this scheme the heterozygotic fertile red phenotype with the TtMsms genotype set, crossed with the twice recessive genotype of a green sterile plant ttmsms, gives two phenotypic categories in the progeny. About one half of the plants should be green and sterile, i.e. suitable for the production of heterozygotic hybrids, and the other half of useless plants would remain red and fertile. Hence from the genetic point of view, the principle of the maintenance of sterile genotype corresponds with repeated back crossing. Some complication in the use of anthocyanic disclosure is seen in the recombinants of fertile plants without anthocyanic colour. The originally determined 1% proportion of such phenotypes would not do much harm but the actual number of the plants with undesired phenotypic manifestation was often as high as five per cent or higher.

The discrepancy between the assumption and the fact was attributed to a reduced penetration of the ms gene under field conditions. Change in the strength of linkage as the cause of this phenomenon was not taken into consideration because such a situation would necessarily include an increased occurrence of the fourth phenotypic category of red sterile plants whose existence was not demonstrated.

Our opinion concerning the explanation of the increasing proportion of green fertile plants in the progeny of crosses used for the maintenance of sterile genotypes includes the assumption of a strong linkage between the T and Ms genes, requiring the involvement of a higher number of genes in the realization of the phenotypic manifestations of a trait. The starting points are the determination of the existence of a higher number of Ms genes and an apparent difference in the gene basis of the phenotypes with the anthocyanic colour of whole plants and phenotypes with red hypocotyl.

The explanation of the increased proportion of green fertile phenotypes can be demonstrated as follows : the crossing of the genotypes $AsTtMs_1ms_1Ms_2ms_2 \times Asttms_1ms_1ms_2ms_2$, i.e. fertile, red × sterile, green with red hypocotyl, gives, in the B_1 generation, rise to the phenotype categories red, fertile : green, fertile : green, sterile in a 3 : 3 : 2 ratio. All green fertile plants have a red hypocotyl ; of the green sterile plants $\frac{1}{4}$ have a non-coloured hypocotyl. The presence of a single dominant gene Ms suffices for the manifestation of fertility and only one sterility gene is in linkage with gene T. The general existence of these prerequisites was demonstrated by some authors. The proposed explanation reckons with a fairly high proportion of green fertile phenotypes. However, in fact, when back crossing was frequently repeated, the proportion of fertile green plants was observed in some cases to increase rapidly to the detriment of sterile plants. The consideration of the involvement of a higher number of genes enables the explanation of the increase in the proportion of one recombinant category without the presence of the other recombinant category even in cases when the linkage remains very close. This fact is hard to accept by the hypothesis in which only two genes are considered. Among other things, concurrent presence of red plants with green and red hypocotyl was observed during exact study of phenotype manifestations after back crossing ; the recombinants characterized by fertility mostly belonged to the coloured hypocotyl phenotype category. This finding is also in agreement with our opinion described above.

The linkage between the genes underlying the colouring of ligulate flowers with the gene underlying the anthocyanic colour of tubular flowers, or whole plants, was determined in our trials.

We studied the results of the crossing of green plants with yellow ligulate and red plants with whitish yellow ligulate flowers. Another cross consisted of the combination of green plants with orange yellow flowers and red plants with whitish yellow flowers. For the first case, only red plants with yellow ligulate flowers occurred in the F_1 generation. The progeny of the second combination consisted of red plants with orange yellow flowers. Segregation in the F_2 generation is shown in Table 1.

Table 1

Parent phenotypes	Red yellow (orange)	Green yellow (orange)	Red whitish yellow	Green whitish yellow	$\chi^2_{(3)}$ 9:3:3:1
Green, orange yellow × red,	28	14	9	0	5.30
whitish yellow	46	24	22	0	10.34*
Sum	74	38	31	0	14.78*
Green, yellow ×	19	13	11	1	6.10
red, whitish	15	5	4	0	1.78
yellow	27	17	16	0	10.04*
Sum	61	35	31	1	14.89*

The table shows that in both cross types the basic segregation ratio of the dihybrid was distorted by linkage. The strength of the linkage could not be determined, since in the trans stage the number of plants was too low for calculating the percentual proportion of recombinants in the F_2 generation. Besides the undeniable fact of the existence of linkage between genes L (or *lo, la*) and T (or t), the occurrence of phenotypes in the segregating generation also clearly indicates that the genes responsible for orange yellow and yellow colour have about the same distance from gene T ; this supports the hypothesis of the involvement of the multiple allelism relationship among alleles L, *lo, la*.

The determination of the strength of the linkage was enabled by experiments with hybridization analysis in cis stage and with an increased number of individuals in the F_2 generation, in the trans stage. Crossing in the cis stage was only performed in the combination of red, yellow × green, whitish yellow, since only one plant with a twice recessive ttlala genotypes was available. For the combination of green, orange yellow × red, whitish yellow in the trans stage, the amount of plants used for initial crossing was increased. Both these analyses demonstrated the existence of linkage and gave a prerequisite for approximate determination of the strength of the linkage between the genes for the colouring of ligulate flowers and anthocyanic colouring of the plant. The crossing of a red plant with yellow ligulate flowers and a green plant with whitish yellow flowers was followed, in the F_2 generation, by segregation in the ratio of 144 : 18 : 13 : 53 which corresponds to linkage expressed by the 13% value of recombinants. Segregation of progenies in the F_2 generation produced by the crossing of a green plant with orange yellow flowers and a red plant with whitish yellow flowers is shown in Table 2.

The summarized segregation ratio of 403 : 177 : 230 : 1 corresponds to linkage which can be expressed as 7% of recombinants.

Table 2

F_2 generation after crossing		Red orange yellow	Red whitish yellow	Green orange yellow	Green whitish yellow
160×220	243	45	16	25	0
	244	54	26	25	0
	245	43	20	20	0
	246	7	4	8	0
	247	29	16	10	0
220×160	248	46	16	33	0
	249	42	20	22	0
	250	44	17	27	0
	251	50	22	29	1
	252	43	20	31	0
160×220 sum		178	82	88	0
220×160 sum		229	95	142	1

Although the results suggest some differences between the position of the L and *lo* genes from the point of view of linkage strength, it can be said that the comparison of the Morgan number, determined in two contrasting stages, may always indicate certain difference in the obtained value. In addition to this, the possible occurrence of another (at least one) phenotype of green, whitish yellow would increase, in the trans stage, the proportion of recombinants to as much as 10%. The range of the linkage determined for genes T and L is from 7 to 19% ; in the case of genes T and *lo* the exactness of the determination of linkage strength is within the range from 4 to 10%. Hence these data coincide, and it can be generally concluded that the value expressing the strength of the linkage between the genes for the anthocyanic colouring of the plant and genes for the colouring of ligulate flowers ranges about 10%.

The experiments also included the study of the linkage relationship of the trait of ligulate flowers shape in two alternatives of manifestation, i.e. normal flowers and tubular ligulate flowers. No relationship was found between the traits of ligulate flower shape and colour. The phenotype ratio in the F_2 generation corresponds to a linkage strength expressed as 44.2% of recombinants, and this is, in the essence, a value characterizing free combining ability. Similarly, between the genotypes underlying flower shape and anthocyanic colouring, the alleles are freely combinable, because 45.2% of the recombinants ensuing from the segregation ratio of 92 : 37 : 23 : 13 fully proves this conclusion. This finding well corresponds with the data asserted by Stoenescu and Vrânceanu (1976) who found a linkage between the Ms_2 genes for pollen sterility and Fl for ligulate flower shape, and this linkage had a strength of 20%. It can be generally stated that the Ms_1, T and L genes

are located on one chromosome and genes Ms_2 and Fl occur jointly on another chromosome.

In relation with the linkage relationships an example can be given, in which the determination of linkage becomes the decisive criterion for the determination of the correctness of one of two or more different opinions concerning the heredity of a trait. In sunflower a discrepancy was found between our opinion which is in agreement with the conclusions published by Leclercq (1968) and Stoenescu, (1974), and the conception of trait as proposed by Fick (1976). The point of contradiction is the colour of the ligulate flowers, manifesting itself in the variant of yellow, orange yellow and whitish yellow. An allelic relationship of genes was derived from our results, whereas Fick introduced a two-gene hypothesis. The results of both works are the same in the case of the crossing of yellow × orange yellow and orange yellow × whitish yellow, in which the monogenic basis of the phenotypic manifestation is involved. In our trials, like in those performed by Stoenescu, a monogenic manner of heredity was determined in the cases of the crossing of yellow × whitish yellow. Fick believed that there was an interaction of two genes of recessive epistasis type. Fick's opinion is based on the analysis of the F_2 generation in which he allegedly obtained a segregation ratio of 216 : 79 : 86 and the orange yellow phenotype occurred after the autogamic pollination of the yellow F_1 generation produced from the crossing of yellow × whitish yellow. The segregation ratio shows good correspondence with the theoretical ratio of 9 : 3 : 4 (X_2^2 =1.71). With the existence of such a relationship, each cross between the alternative genotypes could be expressed by the following genotype sets : yellow × orange yellow AABB × aaBB, orange yellow × whitish yellow aaBB × aabb, yellow × whitish yellow AABB × aabb. Then genotypes A—B are realized in yellow ligulate flowers, aaB by orange yellow, and A—bb are whitish yellow.

This consideration is apparently acceptable and can resist, to some degree, to the arguments available to those who believe that a trait is conditioned by multiple allelism. The crossing of orange yellow × whitish yellow, evaluated as allelism test, would produce a progeny with yellow flowers in the case of the aaBB × AAbb combination, but when the parental genotypes are aaBB × aabb it is impossible to judge whether the alleles belong or do not belong to the same gene. However, our results concerning the linkage between the anthocyanic colouring of the plant and the colour of ligulate flowers clearly indicate that if the studied genes are not the alleles of the same locus, then they must be located close to each other on the chromosome. This firm fact inevitably suggests that even if the two-gene hypothesis is assumed to hold good, the crossing of the yellow and whitish yellow phenotype cannot yield orange yellow colour. This is due to the fact that the heterozygotic genotype in the F_1 generation would produce, in the AB gene linkage, only two types of gametes, AB, ab, whose combination gives a yellow to whitish yellow ratio of 3 : 1 in the F_2 generation. It is entirely impossible in the case of allelism or linkage of two genes for the colouring of ligulate flowers, to imagine the occurrence of the orange yellow phenotype after the hybridization of plants with the yellow and whitish yellow manifestation of the traits. Hence, the only thing to believe is that Fick confused the orange yellow colour with a shade of yellow which only slightly reminds of rich orange colour. Some variability in the shades of the yellow colour of ligulate flowers is generally known. However, if the proclaimed orange yellow phenotype was, in fact, yellow, then the results of Fick's experiment corresponds to the monogenic basis of the trait even in the case of the yellow × whitish yellow combination. The two-gene theory appears not to be realistic. Therefore the relationship of multiple allelism of genes L, *lo*, la is more probable than their close linkage. Hence the opinion asserted by us, concerning the hereditary basis of the colouring of ligulate flowers, is valid.

The considerations on the heredity of the yellow, whitish yellow and orange yellow colour can be further evolved. It has been said, so far, that the genes for the three mentioned variants of the trait are alleles of the same locus : hence Fick's opinion in which a genotype denoted as LLLaLa underlines the yellow colour, LLlala or lllala is related with whitish yellow colour, and llLaLa with orange yellow colour, is not justified. However, it was revelead during the study of the colour of ligulate flowers that the yellow, whitish yellow and orange yellow colours of the petals can be determined by two genes. Such a finding could not be obtained in direct crossing of the studied phenotypes, but only indirectly in crossing with the red coloured flowers. However, the two considered genes underlying the heredity of the non red colour do not bear any relation to the two genes presented in Fick's hypothesis. This can be explained as follows : in the crossing of whitish yellow x yellow, genotypes aab_2b_2CC x aaBBcc give an F_1 generation with a uniform yellow colour, $aaBb_2Cc$, and in the F_2 generation the yellow to whitish yellow ratio is 12 : 4.

This situation suggests two facts. First, the results from which Fick derived the opinion concerning two genes remain explained in the one-gene way in the form of the Bb_2 set, and second, it is evident that the interaction of genes B and C cannot be demonstrated

by the crossing of whitish yellow and yellow, since the 12 : 4 ratio cannot be formally differentiated from the monohybrid ratio of 3 : 1. Only crossing with a genotype including all the three genes, ABC (red-coloured phenotype), it is possible to determine the presence of gene C and, consequently, the two-gene interaction of B and C. Analogically, the crossing of orange yellow x yellow aab_1b_1CC x aaBBcc produces in the F_1 generation the yellow phenotype $aaBb_1Cc$ and in the F_2 generation the 12 : 4 segregation ratio apparently indicates monogenic heredity. The combination of whitish yellow x orange yellow aab_2b_2CC x aab_1b_1CC gives orange yellow aab_1b_2CC in the F_1 generation and in the F_2 generation the segregation is really monohybrid, 3 : 1. The previous finding concerning the non red colour of ligulate flowers justifies the conclusion that the alleles L, *lo, la* are congruent with B, b_1, b_2 and are located in the same place on the chromosome. Gene C which may also be involved in the manifestation of the genotype, is in duplicate relation with gene L in the determination of the yellow phenotype and cannot be found in the direct analysis of the variants of the non red colour. In addition to this, gene C is in no linkage with gene L, so that there is also no linkage with gene T. Both cases — the linkage of genes L and T, as well as the indirect determination of gene C, can be used as exemples of complicated heredity of an apparently very simply hereditarily conditioned trait whose background cannot be identified by current analytic method based on the crossing of two alternative genotypes.

REFERENCES

Fick G. N., 1976, *Genetics of floral colour and morphology in sunflowers,* Journ. of Heredity, **67**, *4.*

Hrubý K., 1961, *Genetika,* Academia, Praha 1961.

Immer F. R., 1930, *Formulae and tables for calculating linkage intensities,* Genetics, **15**.

Leclercq P., 1966, *Une stérilité mâle utilisable pour la production d'hybrides simples de tournesol,* Ann. Amélior. Plantes, **16**, *2.*

Leclercq P., 1968, *Hérédité de quelques caractères qualitatifs chez le tournesol,* Ann. Amélior. Plantes, **18**, *3.*

Nečásek J., Cetl I., 1980, *Obecná genetika,* SPN. Praha, 1980.

Stoenescu F., 1974, *Genetica* in : *Floarea-soarelui.* Edit. Acad. R.S.R., Bucureşti.

Stoenescu F., Vrânceanu A. V., 1976, *Linkage studies between ms genes and four marker genes in sunflower,* Proc. VII Intern. Sunflower Confer., Krasnodar.

EVALUATION DU LINKAGE GÉNIQUE ET SON UTILISATION POUR DÉTERMINER L'HÉRÉDITÉ DES CARACTÈRES CHEZ LE TOURNESOL

Résumé

Le présent ouvrage analyse les différentes méthodes d'évaluer le linkage entre les gènes et relève l'importance de la méthode basée sur le calcul de l'intensité du linkage en F_2 (la phase „cis") à l'aide du coefficient d'association. Les auteurs passent en revue les groupes de linkage connus chez le tournesol entre Ms_1 T et L, et entre Ms_2 et Fl, avec des considérations sur l'association des gènes T et L. Le phénomène de linkage est utilisé pour analyser certaines opinions concernant l'hérédité de la couleur des fleurs ligulées, conditionée par le gène L, ainsi que pour expliquer les écarts des rapports prévus pour certains phénotypes, en tenant compte de la relation entre les gènes Ms_1 et T. Cette étude fait partie des thèmes de recherche en collaboration avec le sous-réseau FAO pour la génétique du tournesol.

EVALUACIÓN DEL LINKAGE DE LOS GENES Y SU EMPLEO EN DETERMINAR LA HEREDIDAD DE LOS CARACTERES AL GIRASOL

Resúmen

In este papel hay consideraciones sobre lás diferentes métodos de determinación del linkage (enlazamiento) de los genes. Está evidenciado el método por el cual se calcula la intensidad del linkage en la generación F_2 (fas „cis") con la ayuda del coeficiente de asociación. A continuación. se pasan de revista los grupos de linkage conocidos del girasol, entre los genes : Ms_1 T y L, Ms_2 y Fl, y se hacen apreciaciones sobre la asociación entre la gene T y L. El fenómeno de linkage está empleado para confróntar algunas opiniones concernientes a la heredidad del color de las flores liguladas condicionada por la gene L y a la vez para explicar las desviaciones de las relaciones esperadas de unos fenotipos, tomando en consideración la relación entre la gene Ms_1 y T. Este estudio es parte de la temática de investigación en cooperación de la subred FAO para la genética del girasol.

are located on one chromosome and genes Ms_2 and Fl occur jointly on another chromosome.

In relation with the linkage relationships an example can be given, in which the determination of linkage becomes the decisive criterion for the determination of the correctness of one of two or more different opinions concerning the heredity of a trait. In sunflower a discrepancy was found between our opinion which is in agreement with the conclusions published by Leclercq (1968) and Stoenescu, (1974), and the conception of trait as proposed by Fick (1976). The point of contradiction is the colour of the ligulate flowers, manifesting itself in the variant of yellow, orange yellow and whitish yellow. An allelic relationship of genes was derived from our results, whereas Fick introduced a two-gene hypothesis. The results of both works are the same in the case of the crossing of yellow × orange yellow and orange yellow × whitish yellow, in which the monogenic basis of the phenotypic manifestation is involved. In our trials, like in those performed by Stoenescu, a monogenic manner of heredity was determined in the cases of the crossing of yellow × whitish yellow. Fick believed that there was an interaction of two genes of recessive epistasis type. Fick's opinion is based on the analysis of the F_2 generation in which he allegedly obtained a segregation ratio of 216 : 79 : 86 and the orange yellow phenotype occurred after the autogamic pollination of the yellow F_1 generation produced from the crossing of yellow × whitish yellow. The segregation ratio shows good correspondence with the theoretical ratio of 9 : 3 : 4 (X_2^2 =1.71). With the existence of such a relationship, each cross between the alternative genotypes could be expressed by the following genotype sets : yellow × orange yellow AABB × aaBB, orange yellow × whitish yellow aaBB × aabb, yellow × whitish yellow AABB × aabb. Then genotypes A—B are realized in yellow ligulate flowers, aaB by orange yellow, and A—bb are whitish yellow.

This consideration is apparently acceptable and can resist, to some degree, to the arguments available to those who believe that a trait is conditioned by multiple allelism. The crossing of orange yellow × whitish yellow, evaluated as allelism test, would produce a progeny with yellow flowers in the case of the aaBB × AAbb combination, but when the parental genotypes are aaBB × aabb it is impossible to judge whether the alleles belong or do not belong to the same gene. However, our results concerning the linkage between the anthocyanic colouring of the plant and the colour of ligulate flowers clearly indicate that if the studied genes are not the alleles of the same locus, then they must be located close to each other on the chromosome. This firm fact inevitably suggests that even if the two-gene hypothesis is assumed to hold good, the crossing of the yellow and whitish yellow phenotype cannot yield orange yellow colour. This is due to the fact that the heterozygotic genotype in the F_1 generation would produce, in the AB gene linkage, only two types of gametes, AB, ab, whose combination gives a yellow to whitish yellow ratio of 3 : 1 in the F_2 generation. It is entirely impossible in the case of allelism or linkage of two genes for the colouring of ligulate flowers, to imagine the occurrence of the orange yellow phenotype after the hybridization of plants with the yellow and whitish yellow manifestation of the traits. Hence, the only thing to believe is that Fick confused the orange yellow colour with a shade of yellow which only slightly reminds of rich orange colour. Some variability in the shades of the yellow colour of ligulate flowers is generally known. However, if the proclaimed orange yellow phenotype was, in fact, yellow, then the results of Fick's experiment corresponds to the monogenic basis of the trait even in the case of the yellow × whitish yellow combination. The two-gene theory appears not to be realistic. Therefore the relationship of multiple allelism of genes L, *lo*, la is more probable than their close linkage. Hence the opinion asserted by us, concerning the hereditary basis of the colouring of ligulate flowers, is valid.

The considerations on the heredity of the yellow, whitish yellow and orange yellow colour can be further evolved. It has been said, so far, that the genes for the three mentioned variants of the trait are alleles of the same locus : hence Fick's opinion in which a genotype denoted as LLLaLa underlines the yellow colour, LLlala or lllala is related with whitish yellow colour, and llLaLa with orange yellow colour, is not justified. However, it was revelead during the study of the colour of ligulate flowers that the yellow, whitish yellow and orange yellow colours of the petals can be determined by two genes. Such a finding could not be obtained in direct crossing of the studied phenotypes, but only indirectly in crossing with the red coloured flowers. However, the two considered genes underlying the heredity of the non red colour do not bear any relation to the two genes presented in Fick's hypothesis. This can be explained as follows : in the crossing of whitish yellow x yellow, genotypes aab_2b_2CC x aaBBcc give an F_1 generation with a uniform yellow colour, $aaBb_2Cc$, and in the F_2 generation the yellow to whitish yellow ratio is 12 : 4.

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