Theor Appl Genet (1989) 77:195–199

Methods for the study of cytoplasmic effects on quantitative traits

J.A. Mosjidis [1] *, J.G. Waines [2], D.M. Yermanos [2] and A.A. Rosielle [3]

[1] Department of Agronomy and Soils, Auburn University, Auburn, AL 36849, USA
[2] Department of Botany and Plant Sciences, University of California at Riverside, CA 92521, USA
[3] Asgrow Seeds, Inc., 634 East Lincoln Way, Ames, IA 50010, USA

Received June 15 1988; Accepted July 25, 1988
Communicated by A.L. Kahler

Summary. The methods used to study cytoplasmic effects in quantitative traits often do not measure quantitative genetic parameters, while those that do are either complicated or do not take into account situations where the expression of cytoplasmic effects does not persist, but decreases in advanced generations. We present two simple models that take cytoplasmic effects and the quantitative genetic parameters into account. One of the models (A) is for cases where cytoplasmic effects remain constant through successive generations, and the second model (B) is for traits where cytoplasm-genotype interactions are present. This model also takes into account the decreasing persistence of cytoplasmic effects with advancing generations, which is often reported in the literature.

Key words: Cytoplasmic effects – Maternal effects – Generation mean analysis – Cytoplasm-genotype interaction – Gene action

Introduction

The differential contributions of parents to their offspring have been studied as far back as Kölreuter (1765, cited in Roberts 1929) and Mendel (1865). This kind of research has traditionally been done by comparing either the F_1 hybrids from reciprocal crosses (Cockerham and Weir 1977) or the F_2 populations derived from the reciprocal F_1's. Reciprocal F_1 populations have confounded maternal and cytoplasmic effects, while a comparison of reciprocal F_2 populations provides unequivocal information on cytoplasmic effects, which also can be achieved by comparing reciprocal backcrosses (Mosjidis and Yermanos 1984). The advantage of making these paired comparisons is its simplicity and straightforward biological interpretation of the results. However, if inferences about the presence of cytoplasmic effects are obtained from isolated sets of reciprocal generations, no general information is obtained about the expression of cytoplasmic effects in later generations. A second limitation is that in nearly all studies of maternal effects, and effects of traits that do not involve the whole plant, or that are measured on a sample taken from the plant, it is assumed that variation within the plant is random. Therefore, averaged over the entire plant, the effects of this variation cancel each other. However, there are reports that indicate the presence of systematic variation within the plant that in some cases need to be taken into account (Jellum and Marion 1966; Zimmerman and Fick 1973; Mosjidis and Yermanos 1985). A third limitation is that with this approach it is not possible to measure genetic effects.

One way to overcome some of these limitations in the study of cytoplasmic effects is to measure the traits on single plants in several reciprocal populations. For example, Bhat and Dhawan (1971) used a split-plot design to determine the presence of cytoplasmic effects in maize (*Zea mays* L.). They tested one set of populations that shared the same cytoplasm, which is assumed to have a constant effect, against another set of genetically equivalent populations but with a different cytoplasm. This approach misses the opportunity to measure the Standard quantitative genetic parameters such as additive and dominant gene action which can not be measured with this approach, and, as well, the results have confounded maternal and cytoplasmic effects.

The first biometrical method used to measure cytoplasmic effects together with genetic parameters was pre-

* To whom correspondence should be addressed

sented by Chandraratna and Sakai (1960). Their model does not account for additive gene action, and the parameter describing cytoplasmic effects is included in the model in an arbitrary way.

Tyson (1973) formulated a model to study cytoplasmic effects that uses generation means analysis as described by Mather and Jinks (1971). His model compares sets of reciprocal backcrosses and F_2's to study maternal and paternal cytoplasmic effects, as well as to measure the standard genetic parameters. This model does not use the F_1's to estimate cytoplasmic effects, nor does it account for the decreasing persistence of differences in reciprocal crosses. Moreover, some of the interactions indicated in the model have an unrecognizable biological meaning (Tyson 1973).

Barnes (1968) described a model, also published later by Mather and Jinks (1971, pp 293–298), that ascribes the differences between reciprocal populations to the genotype of the maternal parent. The model ignores the existence of cytoplasmic effects. The impact of maternal effects would be confounded with cytoplasmic effects if the latter are also present. Muehlbauer et al. (1971) and Jinks et al. (1972) reported differences in reciprocal crosses that persisted only a few generations. Jinks et al. (1972) treated these differences as caused only by a temporary effect of the parental genotypes, that could also interact with the genotypes of the progeny. No cytoplasmic effects were considered. The model described by Jinks et al. (1972) has 17 parameters. Although the model takes into account the lack of persistance of the differences between reciprocal populations, the large number of parameters and the difficulties in asserting a biological meaning to them makes this a complicated and not very useful model.

Several models that can be applied to diallel crosses for obtaining information on maternal effects and reciprocal non-maternal effects have been presented in the literature (Hayman 1954; Griffing 1956; Cockerham 1963). These models suffer from the same limitations common to the use of any diallel analysis (Baker 1978). Furthermore, the inconsistent cytoplasmic effects across matings reported by Robertson and Frey (1984) in oats indicate that diallel crosses would not be suited for this kind of analysis. Generation means analysis would be of more value.

Cytoplasmic effects and maternal effects are two different phenomena. Barnes' model (1968) clearly measures the effect of the maternal genotype on the progeny if cytoplasmic effects do not exist, or if they could be isolated. The purpose of this paper is to present two models that estimate additive and dominance gene effects and their interactions as described by Mather and Jinks (1971, chap. 5), as well as cytoplasmic effects if maternal genotype effects do not exist, or if they could be measured by another model. Thus, the models can be used in conjunction with Barnes' model to obtain estimates of genetic, cytoplasmic, and maternal genotype effects. In this context, cytoplasmic effects are understood to be factors in cytoplasmic DNA. Model (A) deals with cases where cytoplasmic effects remain constant through successive generations, while Model (B) considers the diminished expression of reciprocal differences reported in the literature. The parameters included in these models that measure cytoplasmic effects could also be used in conjunction with Eberhart and Gardner's (1966) model to estimate genetic as well as cytoplasmic effects.

The models

In the terminology used to discuss the models, it is assumed that cytoplasmic effects are due to the transmission of factors present in the cytoplasmic DNA. Mitochondria and plastids have their own genomes (Srivastava 1981), and episomes have been reported to be present in mitochondria (Laughnan and Gabay-Laughnan 1983). It is assumed that only those organelles present in the maternal cytoplasm are transmitted to the offspring, unless otherwise indicated. This seems to be the most common case in angiosperms (Tilney-Basset 1978). It is also assumed that if the trait being studied is expressed in the seeds – these lack, or have negligible, endosperm – or that the trait is not influenced by the endosperm.

Model A. Constant cytoplasmic effects

The terminology used to describe the relationship between genotype and genotypic value follows Fisher (1918). Because there is no advantage to defining the parameters relative to a particular background population (Hayman 1960), we chose to define parameters relative to the midparent. Thus, the midparent is the origin from where allele *B* increases the genotypic value by *a*, and allele *b* decreases it by the same amount. The value *d* of the heterozygote depends upon the degree of dominance. Cytoplasmic effects are assumed to have a constant effect symbolized by *c* that shifts the genotypic value according to the cytoplasm associated with the genotype. In other words, the DNA present in the cytoplasm affects physiological processes, or the expression of a trait controlled by nuclear DNA, in such a way that the genotypic value determined by the nuclear DNA is incremented by an amount *c* due to cytoplasmic DNA. It is assumed that the cytoplasmic DNA in one of the parental lines reduces the genotypic value in the amount symbolized by *c* due to cytoplasmic DNA. It is assumed that the cytoplasmic DNA in one of the parental lines reduces the genotypic value in the amount symbolized by *c*, while the cytoplasmic DNA of the other parental line increases it by the same amount. The genotypic value of

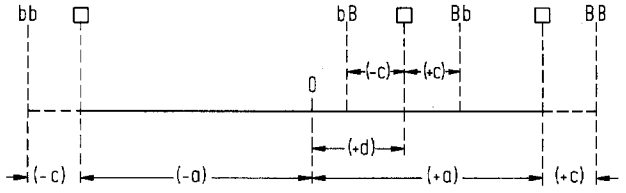

Fig. 1.

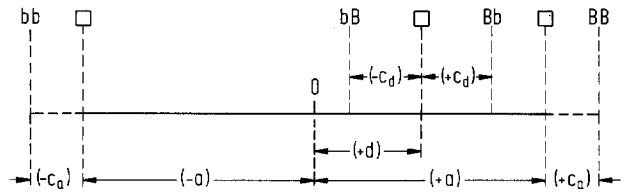

Fig. 2.

Figs. 1 and 2. Relationships between genotypes and genotypic values; the blank spaces represent the value that the genotypes would have had if cytoplasmic effects were not present; **1** model A; **2** model B

the heterozygotes increases or decreases with the same magnitude c, depending on the cytoplasmic DNA received from the maternal parent. These relationships are illustrated in Fig. 1.

The details of how generation means can be calculated are described by Mather and Jinks (1971). Components of some expected generation means are presented in Table 1.

If nonallelic interaction is suspected, the genetic parameters that estimate it (Mather and Jinks, 1971) can be included along with c. The same is valid for paternal cytoplasmic effects and for maternal genotype effect (Barnes, 1968). The values for paternal cytoplasmic effects are listed under c_p (Table 1). If maternal environmental effects are being studied, the backcrosses P_1/F_1^1 or P_1/F_1^2 and P_2/F_1^1 or P_2/F_1^2 should be included.

Model B. Interaction of cytoplasm with genotype

As in model A, the midparent is the origin used in measuring the effects of the cytoplasm and the genotypes for a single locus. The diagrammatic representation of the relationship between the genotypes and the genotypic and cytoplasmic values for model B is presented in Fig. 2.

In model B, it is assumed that there is an interaction between the nuclear and cytoplasmic genomes. Cytoplasmic DNA in one parental line would affect physiological processes or the expression of a trait controlled by nuclear DNA in such a way that the genotypic value in the homozygous individuals would increase in the magnitude symbolized by c_a, while the cytoplasm of the other parent reduces the genotypic value of homozygous individuals

Table 1. Components of some expected generation means on an additive-dominance model in the presence of maternal and/or paternal cytoplasmic effects

Generation[a]	Mating	Parameters				
		m	a	d	c	c_p
P_1	P_1/P_1	1	1	0	1	1
P_2	P_2/P_2	1	−1	0	−1	−1
F_1^1	P_1/P_2	1	0	1	1	−1
F_1^2	P_2/P_1	1	0	1	−1	1
F_2^1	F_1^1/F_1^1	1	0	0.5	1	1
F_2^2	F_1^2/F_1^2	1	0	0.5	−1	−1
BC_1^1	F_1^1/P_1	1	0.5	0.5	1	1
BC_1^2	F_1^2/P_1	1	0.5	0.5	−1	1
BC_2^1	F_1^1/P_2	1	−0.5	0.5	1	−1
BC_2^2	F_1^2/P_2	1	−0.5	0.5	−1	−1
F_3^1	F_2^1/F_2^1	1	0	0.25	1	1
F_3^2	F_2^2/F_2^2	1	0	0.25	−1	−1
[b]BC_1^1	P_1/F_1^1	1	0.5	0.5	1	1
[b]BC_1^1	P_1/F_1^2	1	0.5	0.5	1	−1
[b]BC_2^2	P_2/F_1^1	1	−0.5	0.5	−1	1
[b]BC_2^2	P_2/F_1^2	1	−0.5	0.5	−1	−1

[a] The superscript indicates that the cytoplasm transmitted by the maternal parent is from P_1 or P_2

[b] Backcrosses needed for the study of maternal environment effects

by the same amount. The heterozygous individuals would have their genotypic value increased or decreased by the amount symbolized by c_d, depending on the cytoplasm received; i.e., there is a nonadditive relationship of cytoplasms and degree of heterozygosity. The model can be readily extended to include the parameters for nonallelic interactions (Hayman 1958; Mather and Jinks 1971), paternal cytoplasmic effects, and/or maternal environmental effects (Barnes 1968). If biparental transmission of organelles has been reported for the species, the parameters c_{ap} and c_{dp} that estimate paternal cytoplasmic effects should be included. The parameters c_{ap} and c_{dp} are equivalent to c_a and c_d respectively: c_{ap} measures the increment or reduction of the genotypic value of the homozygotes, and c_{ad} measures the increment or reduction of the genotypic value of the heterozygotes; both are due to the paternal cytoplasmic DNA.

From the relationships between genotypes and the genotypic and cytoplasmic values depicted in the model, values for each parameter in each population can be obtained (Mather and Jinks 1971). The values for the parameters m, a, and d are the same as those indicated in model A (Table 1). The values for the other parameters are presented in Table 2.

Table 2. Components of some expected generation means on an additive-dominance model in the presence of interactions between the genotype and the maternal and/or paternal cytoplasm

Generation[a]	Mating	Parameters			
		Maternal cytoplasm		Paternal cytoplasm	
		c_a	c_d	c_{ap}	c_{dp}
P_1	P_1/P_1	1	0	1	0
P_2	P_2/P_2	−1	0	−1	0
F_1^1	P_1/P_2	0	1	0	−1
F_1^2	P_2/P_1	0	−1	0	1
F_2^1	F_1^1/F_1^1	0.5	0.5	0.5	0.5
F_2^2	F_1^2/F_1^2	−0.5	−0.5	−0.5	−0.5
BC_1^1	F_1^1/P_1	0.5	0.5	0.5	0.5
BC_1^2	F_1^2/P_1	−0.5	−0.5	0.5	0.5
BC_2^1	F_1^1/P_2	0.5	0.5	−0.5	−0.5
BC_2^2	F_1^2/P_2	−0.5	−0.5	−0.5	−0.5
F_3^1	F_2^1/F_2^1	0.75	0.25	0.75	0.25
F_3^2	F_2^2/F_2^2	−0.75	−0.25	−0.75	−0.25
[b]BC_1^1	P_1/F_1^1	0.5	0.5	0.5	0.5
[b]BC_1^1	P_1/F_1^2	0.5	0.5	−0.5	−0.5
[b]BC_2^2	P_2/F_1^1	−0.5	−0.5	0.5	0.5
[b]BC_2^2	P_2/F_1^2	−0.5	−0.5	−0.5	−0.5

[a] The superscript indicates that the cytoplasm transmitted by the maternal parent is from P_1 or P_2

[b] Backcrosses needed for the study of maternal environment effects

Estimation of the parameters

The parameters *m* (mean), *a* (additive gene effects), *d* (dominance gene effects), *c* (maternal cytoplasmic effects), c_a (maternal cytoplasmic effects on the homozygotes), c_d (maternal cytoplasmic effects on the heterozygotes), are estimated by weighted least squares. The weights are the reciprocal of the error variance of each generation mean.

A general procedure that can be used to estimate the parameters and the conformity of the data to either model is presented here. The procedure is expressed in the matrix form. The parameters to be estimated (*m, a, d, c, ca, cd, dm, hm,* etc.) are found using:

$$\hat{P} = (V' W^{-1} V)^{-1} V' W^{-1} M$$

P is the vector of the parameters to be estimated. The matrix V consists of the values of the parameters to be estimated according to the model being fitted. The diagonal matrix W contains the error variance of each population which will be used to calculate the weights (reciprocal value of each error variance) in the weighted linear regression analysis. M is the vector of the observed generation means. The expected values of the generation means $\hat{M}$ is

$$\hat{M} = V \hat{P}$$

The adequacy of the model can be tested by calculating χ^2, which is

$$\chi^2_{k-n} = (M - V\hat{P})' W^{-1} (M - V\hat{P})$$

where k is the number of generation means and n is the number of parameters estimated.

The comparison of the observed means with their expected values is done assuming that the sum of squares, minimized in the fitting process, is distributed as a χ^2, with the degrees of freedom equal to the number of estimated generations minus the number of estimated parameters.

The standard errors of the estimates P are the square root of the diagonal elements of $(V' W^{-1} V)^{-1}$ (Beaver and Mosjidis 1988). A t-test can be computed to determine the significance of each parameter.

Discussion

Model A could be used to study cases where a trait is controlled exclusively by factors in the DNA present in the cytoplasm which exhibit uniparental inheritance.

Model B probably describes a situation more likely to be found in nature. This model assumes that there is interaction between nuclear and cytoplasmic genomes. It describes this interaction in very general terms; namely, the genotype of homozygous individuals interacts with cytoplasmic genomes in such a way that the magnitude of this interaction is different from that which heterozygous individuals have with the same cytoplasmic genomes. The basis for such an interaction came from our knowledge of the integrated action of both organelles and nuclear genomes and their structural and catalytic functions. Thus, there is evidence indicating that nuclear DNA operates in consonance with chloroplast DNA in the development of the chloroplast, since several proteins found exclusively in the chloroplast are coded by nuclear DNA or contain polypeptides coded by both nuclear and organelle genes (Srivastava 1981; Lax et al. 1984). Similarly, the adenosine triphosphatase and cytochrome oxidase complex in mitochondria contain polypeptides coded by both nuclear and mitochondrial DNA. Also, there is now evidence indicating that the nucleus contains integrated sequences which are homologous with chloroplast DNA sequences, and that these sequences are incorporated at specific sites within the nuclear genome (Timmis and Scott 1983).

According to the assumptions underlying model B, it can be predicted that some of the differences observed in reciprocal crosses will diminish by further advancing the filial generations. This model also predicts that advanc-

ing the backcross populations, either by selfing or backcrossing, will bring about an increment in the cytoplasmic effects associated with the homozygotes and a reduction in the fraction associated with the heterozygotes. The reason for these predictions is that if cytoplasmic effects are found to be associated with the heterozygotes, their value, when advanced populations are compared, will be smaller because the degree of heterozygosity diminishes in those populations.

Acknowledgements. The senior author expresses gratitude for the financial support of the Fulbright Commission for Educational Interchange between the USA and Chile, the California Agricultural Experiment Station, and US-AID Title XII Bean/Cowpea CRSP.

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