POLYMORPHISMS FOR PURELY CYTOPLASMICALLY INHERITED TRAITS IN BISEXUAL PLANTS

HANS-ROLF GREGORIUS

Abteilung für Forstgenetik, Universität Göttingen, Büsgenweg 2, 3400 Göttingen, Federal Republic of Germany

Manuscript received May 28, 1985
Revised copy accepted October 15, 1985

ABSTRACT

It is shown that cytoplasm polymorphisms transmitted only by the ovules can be maintained without gene-cytoplasmic interactions. The necessary prerequisites are asymmetry of the plasmotypes in production of ovules and pollen (sexual asymmetry), incomplete and frequency-dependent fertilization efficiency and differential selfing rates. These factors can generate the negative frequency dependence of cytoplasmic fitnesses required for a stable polymorphism. The model considered allows also for facultative fixation of either of two plasmotypes and, thus, may produce all of the dynamical characteristics known for nuclear selection with two alleles at one locus.

Strong sexual asymmetry, which probably occurs frequently in bisexual plants, may facilitate stable cytoplasmic polymorphisms. However, these polymorphisms may also endanger survival of the whole population in the absence of nuclear interactions. Gene-cytoplasmic interactions avoid this risk and, at the same time, utilize the advantages of sexual asymmetry in maintaining genetic polymorphisms.

A considerable amount of experimental evidence for the existence of cytoplasmic variation has accumulated, particularly during the last decade. This variation may affect all of the principal components of fitness, such as male and female fertility, viability and selfing rate (*e.g.*, see OEHLKERS 1964; EDWARDSON 1970). Population genetic studies concerned with the general causes for the maintenance of cytoplasmic variation are very recent and concentrate on gene-cytoplasmic interaction (CLARK 1984; GREGORIUS and ROSS 1984). This is because almost all cases where cytoplasmic variation affected fitness components also showed nuclear effects. A further reason is that cytoplasmic genetic information is regularly transmitted through the eggs only, so that selection acts similar to a haploid model. In such models constant fitnesses imply fixation of one cytoplasmic type. This also applies to models where the maintenance of cytoplasmic variation is considered on the cellular level. There, it is shown that fixation of a single cytoplasmic type occurs within each cell line due to random effects which act during each cell division on the transmission of mitochondrial or chloroplast DNA. The maintenance of cytoplasmic variation on the population level strongly depends in these models on bipa-

rental transmission of extranuclear genes (BIRKY, MARUYAMA and FUERST 1983; CHAPMAN *et al.* 1982; TAKAHATA and MARUYAMA 1981). Hence, the maintenance of cytoplasmic variation requires frequency-dependent fitnesses which are readily generated by nucleo-cytoplasmic interactions (GREGORIUS and ROSS 1984).

In order to achieve a more comprehensive understanding of why gene-cytoplasmic interactions appear to play such an important role for the maintenance of cytoplasmic polymorphisms, it is essential to study models of purely cytoplasmic selection (*i.e.*, where nuclear effects are absent) which entail frequency-dependent fitnesses. Only if it is possible to demonstrate that frequency-dependent selection is less effective in maintaining polymorphisms for purely cytoplasmic rather than for gene-cytoplasmic inheritance can we explain the observed phenomena. It is of limited biological meaning to merely assume increased fitness of a cytoplasmic type when it becomes rare. This would also be tautological. Instead, it is necessary to identify explicitly the fitness parameters which may give rise to frequency-dependent selection. For monoecious or hermaphrodite plant populations, we know that asymmetry in the production of ovules and pollen (sexual asymmetry), as well as partial self-fertilization, may result in frequency-dependent fitnesses (GREGORIUS and ROSS 1981), and ROSS (1984) has suggested that this is the rule rather than the exception. Therefore, the present paper is concerned mainly with the effect of these two sources of frequency dependence on the maintenance of purely cytoplasmic polymorphisms.

It will be shown that stable, purely cytoplasmic polymorphisms may, indeed, exist and that they depend in an unexpected way on partial selfing and incomplete fertilization of the ovules. Incomplete fertilization is regularly observed in plant populations, and it becomes more pronounced with increasing sexual asymmetry (*e.g.*, ROSS 1969). In its extreme forms, sexual asymmetry may lead to subdioecy, where all plants function either almost as females or almost as males. Thus, the general results derived in this paper will be applied to the question of whether purely cytoplasmic subdioecy can persist. The answer to this question may also provide interesting insights into the significance of cytoplasmic inheritance for the evolution of dioecy.

THE MODEL AND ITS PREDICTIONS

Consider a hypothetically infinite bisexual (monoecious, hermaphrodite, etc.) plant population that reproduces in separated generations and in which the basic selection parameters are the number f of ovules, the number m of pollen grains available for cross-fertilization, and the proportion z of all ovules that are self-fertilized. For the moment let us assume that self-fertilization occurs prior to cross-fertilization, which allows us to consider z as a constant. Other modes of self-fertilization implying frequency dependence of the selfing rates can also be included, provided they do not affect the ranking of these rates for different genotypes (H.-R. GREGORIUS, M. ZIEHE and M. D. ROSS, unpublished results). For the questions to be answered here it is irrelevant whether or not cross-fertilization occurs at random; it is only required that all ovules

available for cross-pollination (these are the $(1 - z)\cdot f$'s) have the same probability q of being fertilized. This assumption is meaningful in all situations, where the pollination vectors are not directed, such as is the case for anemogamy or hydrogamy; but it applies to insect-pollinated species, provided each flower is equally likely to be visited. Basically, the probability q increases with the average number of pollen grains $\bar{m}$ produced per plant which are available for cross-pollination. Hence, $q = q(\bar{m})$ is assumed to be an increasing function of $\bar{m}$.

In order to study purely cytoplasmic selection, consider two cytoplasmic genotypes (plasmotypes) C_1 and C_2, which are transmitted via the ovules only and which are characterized by constant ovule fertilities f_i, pollen fertilities m_i and selfing rates $z_i (i = 1, 2)$. Furthermore, let P_i denote the relative frequency of zygotes carrying the C_i plasmotype, so that

$$\bar{m} = m_1 \cdot P_1 + m_2 \cdot P_2 .$$

Consequently, the relative frequency P_i' of C_i in the next generation obeys the transition equation

$$P_i' \cdot \bar{w} = P_i \cdot (z_i \cdot f_i + (1 - z_i) \cdot f_i \cdot q(\bar{m})), \tag{1}$$

where the population fitness $\bar{w}$ results from summation over i of the right side of (1).

The dynamic characteristics implied by this transition equation can be more easily analyzed if (1) is rewritten in the form

$$P_1'/P_2' = (P_1/P_2) \cdot g(\bar{m}),$$

where (2)

$$g(\bar{m}) = (f_1/f_2) \cdot (z_1 + (1 - z_1) \cdot q(\bar{m}))/(z_2 + (1 - z_2) \cdot q(\bar{m}))$$

is an increasing or decreasing function of $\bar{m}$ depending on whether $z_2 - z_1$ is positive or negative.

There are two important conclusions that we can draw immediately from (1) if we consider that C_1 increases or decreases in frequency according to whether g is greater or less than 1. If $z_1 = z_2$ or if $q = 1$ (*i.e.*, complete fertilization) then $g = f_1/f_2$ so that the plasmotype with the lower ovule fertility is lost, and thus, the cytoplasmic polymorphism cannot persist if either the pollen production is sufficiently large to guarantee fertilization of all ovules or if both plasmotypes self-fertilize to the same degree. Moreover, if $f_1 = f_2$, $q < 1$ and $z_2 < z_1$, then $g > 1$ for all frequencies, and we find that for identical ovule fertilities and incomplete fertilization the plasmotype with the lower selfing rate is lost. Finally, if the fertilization probability q is constant over the whole range of frequencies, then g is also constant, and thus, with the exception of the biologically irrelevant case $g = 1$, fixation of one of the plasmotypes will occur even for incomplete fertilization.

Hence, to protect a cytoplasmic polymorphism it is necessary that fertilization is incomplete and frequency dependent and that the plasmotypes differ in ovule fertility, pollen fertility and selfing rate. In general, "protectedness" re-

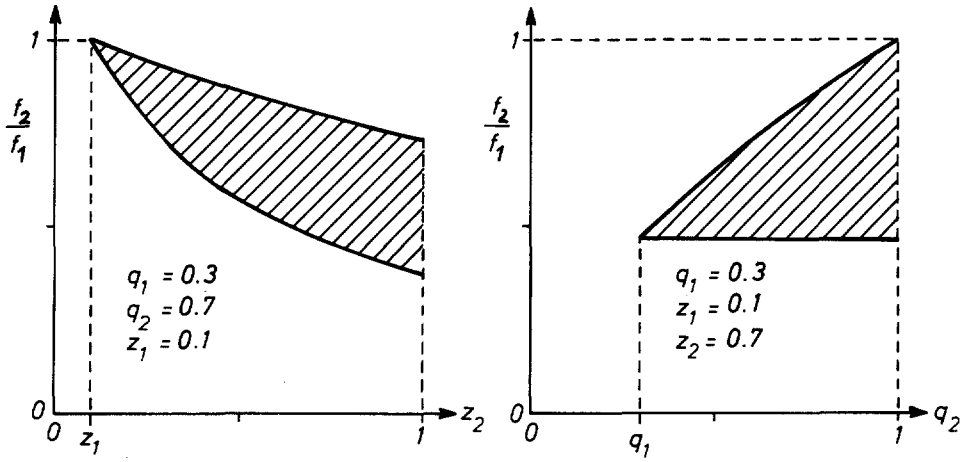

FIGURE 1.—Regions for the ovule fertility ratio f_2/f_1 where the cytoplasmic polymorphism is protected, given particular values for the selfing rates z and the fertilization probabilities q.

quires that each plasmotype increases in frequency when it is rare. This is tantamount to $g > 1$ for small P_1 and $g < 1$ for small P_2. If $P_i = 1$, then $\bar{m} = m_i$ and $q = q(m_i)$, $g = g(m_i)$. For convenience, put

$$q(m_i) = q_i, \qquad g(m_i) = g_i, \qquad i = 1, 2.$$

Consequently, the cytoplasmic polymorphism is protected if, and only if, $g_1 < 1 < g_2$, *i.e.*, if

$$(z_1 + (1 - z_1)q_1)/(z_2 + (1 - z_2)q_1) < f_2/f_1 < (z_1 + (1 - z_1)q_2)/(z_2 + (1 - z_2)q_2).$$

Assuming without loss of generality that $z_1 < z_2$, the above inequality requires that $f_2 < f_1$ and $q_1 < q_2$, the latter of which implies that $m_1 < m_2$. In other words, for the protection of the cytoplasmic polymorphism, it is necessary that the plasmotype with the larger ovule fertility has the lower selfing rate and pollen fertility. Given appropriate values for z_i and q_i, such that $(z_1 - z_2)(q_1 - q_2)$ is positive, the left and right side of the above inequality can be conceived of as the extremes of an open interval in which the ovule fertility ratio f_2/f_1 has to lie in order to yield a protected polymorphism. This interval shrinks as the difference between z_1 and z_2 or between q_1 and q_2 becomes smaller, so that protectedness will also be limited to a very narrow range of ovule fertility ratios (see Figure 1).

The interval is largest if $q_2 = 1$, $z_1 = 0$, and $q_1 = 0$, in which case protectedness requires simply that $f_2 < f_1$ and $z_2 > 0$. In this case C_1 plants produce practically no pollen and, therefore, may be classified as females, whereas C_2 plants produce enough pollen to fertilize all of their ovules in the absence of the females; C_2 plants are thus ideal bisexuals. Hence, the case $q_2 = 1$, $z_1 = q_1 = 0$ represents ideal gynodioecy, and this cytoplasmically controlled ideal gynodioecy is stable if the bisexual plants are self compatible and produce fewer ovules than the females. LEWIS (1941) already obtained a similar result;

however, this author has failed to recognize that his result holds only if the bisexual plants are self-compatible and produce abundant pollen.

If the polymorphism is protected, a unique polymorphic equilibrium exists in the present model. However, this equilibrium need not be locally stable since the internal dynamics are strongly dependent on the type of fertilization function q used. It can be shown that, even for simple linear dependence of q on $\bar{m}$, regular and chaotic fluctuations around the equilibrium frequencies may occur. This cannot happen for a polymorphic equilibrium if the polymorphism is not protected. Nonprotectedness in the presence of an internal equilibrium occurs if, and only if, $g_2 < 1 < g_1$, *i.e.*, if

$$(z_1 + (1 - z_1)q_2)/(z_2 + (1 - z_2)q_2) < f_2/f_1 < (z_1 + (1 - z_1)q_1)/(z_2 + (1 - z_2)q_1).$$

This condition implies facultative fixation of the plasmotypes, *i.e.*, if P_1^* denotes the equilibrium frequency of C_1, then C_1 becomes fixed or is lost according to whether it starts with frequencies $P_1 > P_1^*$ or with $P_1 < P_1^*$. It is necessary for facultative fixation that if $z_1 < z_2$, then $q_2 < q_1$ and $f_2 < f_1$, *i.e.*, the plasmotype with the lower selfing rate has the larger ovule and pollen fertility. In summary, the above results demonstrate that purely cytoplasmic selection with two plasmotypes can lead to the same dynamical characteristics as found in nuclear selection theory with one locus and two alleles.

The present findings on protectedness of a cytoplasmic polymorphism contrast with those of GREGORIUS and ROSS (1984), where it was found that such a polymorphism cannot be maintained without gene-cytoplasmic interactions. However, in this work it was assumed that all ovules are fertilized, and, as we now see, it is exactly this assumption that makes it necessary to invoke gene-cytoplasmic interactions. Incomplete and frequency-dependent fertilization success appear to be indispensible prerequisites for cytoplasmic polymorphisms without nuclear interactions.

Subdioecy: Subdioecy is a form of extreme sexual asymmetry that comes close to genuine dioecy. Herewith, females (subfemales) may produce some pollen and males (submales) may produce some ovules (WESTERGAARD 1958). This definition is very imprecise since it does not specify how strongly sexually asymmetric a population should be in order to be classified as subdioecious. One objective criterion may be deduced from the fact that in genuinely dioecious species a population cannot survive if the sex ratio is too strongly distorted, so that either there are not enough females to produce a sufficient number of offspring or there are not enough males to guarantee adequate fertilization. If this principle is transferred to subdioecy it would imply that neither the submales nor the subfemales alone can form a viable population. Only if they occur together can the populations survive.

Recalling that the ovule and pollen fertilities f_i and m_i already contain the effects of survival from the zygotic to the reproductive stage (GREGORIUS and ROSS 1981), the above criterion would require that

$$z_i \cdot f_i + (1 - z_i) \cdot f_i \cdot q_i < 1 \quad \text{for} \quad i = 1, 2.$$

This is because the population fitness $\bar{w}$ equals $z_i f_i + (1 - z_i) f_i q_i$ if only C_i plants

are present [see (1)], and because the population cannot persist if $\bar{w} < 1$. Hence, for stable subdioecy the cytoplasmic polymorphism must be protected, and at equilibrium, where $g = 1$, one obtains from (2)

$$f_1(z_1 + (1 - z_1)q^*) = f_2(z_2 + (1 - z_2)q^*),$$

where q^* is the fertilization probability at equilibrium. Consequently, at equilibrium the population fitness is equal to

$$\bar{w}^* = f_i(z_i + (1 - z_i)q^*).$$

For $q_1 < q_2$ it follows that $q_2 > q^*$ and, therefore,

$$\bar{w}^* = f_2(z_2 + (1 - z_2)q^*) \leq f_2(z_2 + (1 - z_2)q_2) < 1,$$

so that the population dies out. Thus, we may conclude that, for purely cytoplasmically inherited subdioecy, a population cannot be maintained if the subfemales or submales occur alone nor if they occur together. Cytoplasmically inherited subdioecy can only persist if at least one of the sex types is capable of maintaining a population alone; this, however, poses the question as to whether this sex type should still be classified as a submale or as a subfemale. According to the above-mentioned principle, such sex types should be classified as bisexual, and this principle offers a quantitative and biologically meaningful way of defining subdioecy.

DISCUSSION

Apparently all previous population genetic studies of polymorphisms for purely cytoplasmically inherited traits referred to gynodioecy (LEWIS 1941; LLOYD 1974). One reason for this trend is that early reports of cytoplasmic inheritance were concerned with gynodioecy (*e.g.*, CORRENS 1928). Another possible reason may be that the vast majority of selection models treated in population genetics are built on sexually symmetric selection—usually in the form of autosomal viability selection—in which case, cytoplasmic inheritance via only one gametic sex is similar to asexual, haploid reproduction. With asexual reproduction, however, it is very difficult to maintain genetic polymorphisms, as is well known. On the other hand, gynodioecy is, by definition, an extremely sexually asymmetric system of reproduction, and therefore, it is not too surprising that the results differ essentially from those obtained for sexually symmetric selection.

Yet, as is clearly demonstrated in the present paper, sexual asymmetry alone is not sufficient for explaining polymorphisms in purely cytoplasmically inherited traits. Although it introduces the indispensible frequency dependence of the cytoplasmic fitnesses, it has to be supplemented by frequency-dependent fertilization efficiency of the pollen and by differential selfing rates for the plasmotypes. These are probably the most interesting aspects of the present model since they imply that complete or constant fertilization efficiency, as well as self-incompatibility, may prevent the persistence of cytoplasmic polymorphisms.

If the sexual asymmetry is very strong, purely cytoplasmic inheritance may

endanger the survival of the whole population. This may happen in subdioecious populations, where the females may produce some pollen or the males some ovules. In general, the pollen of the females is not sufficient to fertilize enough of their ovules to allow a population consisting only of such females to survive. Similarly, the number of ovules produced by the males will not suffice to maintain a population. It is shown that, even if females produce large numbers of ovules and males large numbers of pollen grains and even if the sex polymorphism is protected, the population fitness at equilibrium is too low to guarantee survival. Hence, a subdioecious population that was previously viable because the subdioecy was controlled by gene-cytoplasmic interactions may become extinct if, for any reason, it loses its nuclear polymorphism at the relevant loci (for an example of where a nuclear mutant replaces the wild type and reduces population fitness, see ROSS and GREGORIUS 1983).

The role played by sexual asymmetry and differential selfing in the maintenance of cytoplasmic polymorphisms seems also to remain significant for gene-cytoplasmic interactions. This is supported by the results of GREGORIUS and ROSS (1984), where it is shown that sexual symmetry and identical selfing rates [CLARK'S (1984) models all belong to this category] prevent protection of a cytoplasmic polymorphism. The type of model considered there is the same as the present one, with the difference that the pollen production is always sufficient to fertilize all ovules. The latter assumption, which *per se* inhibits stable polymorphisms for purely cytoplasmically inherited traits, does not necessarily do so for gene-cytoplasmic inheritance, provided sexual asymmetry holds. Moreover, identical selfing rates up to complete self incompatibility are not insurmountable obstacles to a stable cytoplasmic polymorphism with gene-cytoplasmic interactions. If certain relationships exist between the ovule fertilities of the nucleo-plasmic genotypes, protectedness is even independent of the selfing rates (GREGORIUS and ROSS 1984).

In summary, it appears that sexual asymmetry is important for the persistence of cytoplasmic polymorphisms, whether the control is purely cytoplasmic or gene-cytoplasmic. The danger for the survival of cytoplasmically polymorphic populations which may result from strong sexual asymmetry and associated frequency-dependent fertilization efficiency can be avoided by gene-cytoplasmic interactions. Since marked sexual asymmetry in flowering of bisexual plants is apparently widespread (ROSS 1984), this may help to explain why purely cytoplasmically inherited traits, the variants of which cannot be attributed to selection-mutation or selection-migration balance, appear to be so rare.

The author greatly appreciates the comments of two anonymous reviewers.

LITERATURE CITED

BIRKY, C. W., T. MARUYAMA and P. FUERST, 1983 An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics **103:** 513–527.

CHAPMAN, R. W., J. C. STEPHENS, R. A. HANSMAN and J. C. AVISE, 1982 Models of mitochondrial DNA transmission genetics and evolution in higher eucaryotes. Genet. Res. **40:** 41–58.

CLARK, A. G., 1984 Natural selection with nuclear and cytoplasmic transmission. I. A deterministic model. Genetics **107:** 679–701.

CORRENS, C., 1928 Bestimmung, Vererbung und Verteilung des Geschlechts bei den höheren Pflanzen. Handb. Vererbungsw. *2:* 1–138.

EDWARDSON, J. R., 1970 Cytoplasmic male sterility. Bot. Rev. **36:** 341–420.

GREGORIUS, H.-R. and M. D. ROSS, 1981 Selection in plant populations of effectively infinite size. I. Realized genotypic fitnesses. Math. Biosci. **54:** 291–307.

GREGORIUS, H.-R. and M. D. ROSS, 1984 Selection with gene-cytoplasm interactions. I. Maintenance of cytoplasm polymorphisms. Genetics **107:** 165–178.

LEWIS, D., 1941 Male sterility in natural populations of hermaphrodite plants. New Phytol. **40:** 56–63.

LLOYD, D. G., 1974 Theoretical sex ratios of dioecious and gynodioecious angiosperms. Heredity **32:** 11–34.

OEHLKERS, F., 1964 Cytoplasmic inheritance in the genus *Streptocarpus*. Adv. Genet. **12:** 329–370.

ROSS, M. D., 1969 Heteromorphism and the evolution of dioecy in *Epigaea repens*. Heredity **24:** 162–165.

ROSS, M. D., 1984 Frequency-dependent selection in hermaphrodites: The rule rather than the exception. Biol. J. Linn. Soc. **23:** 145–155.

ROSS, M. D. and H.-R. GREGORIUS, 1983 Outcrossing and sex function in hermaphrodites: a resource-allocation model. Am. Nat. **121:** 204–222.

TAKAHATA, N. and T. MARUYAMA, 1981 A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res. **37:** 291–302.

WESTERGAARD, M., 1958 The mechanism of sex determination in dioecious flowering plants. Adv. Genet. **9:** 217–281.

Communicating editor: M. T. CLEGG