Selection with gene-cytoplasm interactions. III. Evolution of dioecy

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Abstract: Gynodioecious populations consist of female (male-sterile) and hermaphrodite individuals, and the male sterility is often inherited through nucleocytoplasmic interactions. In contrast, male sterile mutants in hermaphrodite populations are usually inherited monogenically, without any cytoplasmic effect. Such male sterility may readily evolve to dioecy, and it has been suggested that the cytoplasmic effects in gynodioecious species constitute a barrier to such evolution. This paper confirms this suggestion by showing that all cytoplasm types are expected to occur in both sexes of dioecious populations. In addition, one-locus, two-allele two-cytoplasm models of male/female resource allocation are studied, where the three nuclear genotypes are always unisexual with female (Model I) or male (Model II) heterogamety in one cytoplasm, but may be unisexual or hermaphrodite in the other. There is no selfing in the model. Conditions are found under which one cytoplasm may displace the other, and therefore where one breeding system (e.g., dioecy) may displace the other (e.g, hermaphroditism or subdioecy). For some situations there may be a cytoplasmic polymorphism with males, females and hermaphrodites present. The extreme of this case has dioecy with male heterogamety in one cytoplasm and with female heterogamety in the other. The fixation of hermaphroditism or dioecy may depend upon the starting genotype frequencies. Numerical results often show cyclic or spiral behavior of population trajectories.

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Introduction

Natural populations of gynodioecious species contain female individuals (often in considerable frequencies) in addition to hermaphrodite ones. The females presumably developed from hermaphrodite ancestors by mutation for male sterility. Male-sterile (female) mutants usually show monogenic recessive inheritance, although monogenic dominants, more complex nuclear inheritance, and inheritance involving the cytoplasm are known (Jain 1959). Male sterility in gynodioecious species, however, often involves cytoplasmic effects, and seldom if ever shows simple monogenic inheritance (Lewis 1941; Ross 1978). There have been several attempts to understand why there should be such a difference in mode of inheritance of male sterility between normally hermaphrodite and gynodioecious species. Lewis (1941) held that male sterility inherited solely through the cytoplasm was much easier to maintain in populations than monogenic male sterility. He found that the cytoplasmically inherited females needed to be only

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slightly more seed fertile than the hermaphrodites, whereas the monogenic dominant or recessive females needed to be more than twice as seed fertile. However, other models have shown that it is possible to maintain monogenic recessive females through overdominance (Ho and Ross 1973; Ross and Weir 1975) or inbreeding depression (Valdeyron, Dommée and Valdeyron 1973), so that Lewis' argument may not be generally valid.

In another attempt to explain the differences in mode of inheritance, Ross (1978) distinguished two types of gynodioecy, namely an evolutionarily stable type, which showed no tendency to evolve toward dioecy and was often inherited through nucleocytoplasmic interactions, and an unstable type, which tended to evolve toward dioecy and was inherited through the nucleus only. The cytoplasm probably constituted a barrier to the further evolution of dioecy from gynodioecy. Such further evolution occurred readily under nuclear inheritance (Ross 1978; Charlesworth and Charlesworth 1978; Gregorius, Ross and Gillet 1983), so that gynodioecious species were a selection of genetically atypical male sterility mutants in which further evolution toward dioecy was prevented by the cytoplasm. The present paper confirms this suggestion by showing that dioecy can only evolve if both males and females share the same cytoplasm type, i.e., if inheritance of sex type is nuclear, with no cytoplasmic effect. It thus remains to show whether nuclear dioecy can evolve from an originally nucleocytoplasmically controlled sex polymorphism through loss of one cytoplasm. The models for the evolution of dioecy in the literature attempt to imitate the evolution of dioecy as it apparently occurred in nature. For example, dioecy appears to have frequently evolved from hermaphroditism via nuclear gynodioecy, followed by gradual reduction of seed set in the hermaphrodites (Ross 1982). Models of such evolution have for example a recessive gene for male sterility which is or becomes completely linked to several non-recessive genes for partial female sterility, resulting in dioecy or subdioecy with male heterogamety (Ross 1978; Charlesworth and Charlesworth 1978).

However, in order to understand the way evolution in nature seems to have taken place, it is also necessary to study *alternatives* to what apparently occurs. Thus if an apparently plausible alternative can be shown at least theoretically to be capable of evolving readily, then there are reasons to look for such evolution in nature. If, on the other hand, the apparently plausible alternative turns out after further study to be very implausible, then even this result is valuable, as it provides some support for the accepted mode. Thus in either case it is useful to study alternative models. This paper also presents resource-allocation models of competition between hermaphrodite or gynodioecious populations in one cytoplasm, and dioecious populations in the other. It is shown for example that under some conditions the cytoplasm for dioecy displaces the cytoplasm for hermaphroditism, and vice versa.

The models presented here assume nucleocytoplasmic control of both male and female sterility. There is ample evidence for such interactions for male sterility, but sufficient evidence is also available that such interactions also govern female sterility, namely in the extensive hybridization experiments of Oehlkers (1964) in *Streptocarpus*. An unconventional and not well understood mode of inheritance of dioecy is known in *Isotoma fluviatilis*, where it is probably of recent origin (McComb 1969). These examples are sufficient to suggest that the evolution of

dioecy has not yet been exhaustively studied.

The logic of the present paper consists in deriving the necessary conditions for the final decisive step in the nucleocytoplasmic evolution of strict dioecy. These conditions suggest that in the final step the mode of inheritance of the sex types is determined by two cytoplasmic types and two nuclear alleles. The models which may explain prior evolution of the above mode of inheritance are not considered here, since they are arbitrary in both their assumptions and degree of complexity.

Cytoplasmic and nucleocytoplasmic dioecy

It is assumed here and throughout that the cytoplasmic genes are inherited only through the ovules, and that the sex expression is independent of the environment. We now show that dioecy cannot develop in a population where males and females always have different cytoplasm types.

Proof: Suppose that dioecy had developed where males and females always had different cytoplasm types. Since the cytoplasm of the males cannot be inherited, there will be no males in the next generation.

Conclusions: Dioecy can only develop in a population where there is at least one cytoplasm type which, together with particular nuclear genes, determines both male and female phenotypes. All cytoplasm types which occur only in males will be lost during the development of dioecy.

Model: Assume that cytoplasm S occurs only in females, and that cytoplasm N occurs in both females and males. Let N-females produce females and males in proportion $x : (1 - x)$, respectively. Let Φ_N, Φ_S be the number of ovules produced by females in N- and S-cytoplasm, respectively, let P_N, P_S be the respective cytoplasm frequencies, and let P'_N be the frequency of N-cytoplasm in the next generation. If all females have the same chance of fertilization, then:

$$P'_N = \frac{\Phi_N \cdot x \cdot P_N}{\Phi_N \cdot x \cdot P_N + \Phi_S \cdot P_S}$$

Thus if $\Phi_N \cdot x = \Phi_S$ there will be no change in frequency in the next generation. This case, however, is unlikely in real populations. If $\Phi_N \cdot x > \Phi_S$ the S-type cytoplasm will disappear, and the population will be dioecious with only one cytoplasm type. If $\Phi_N \cdot x < \Phi_S$ the N-cytoplasm disappears, and the all-female population will die out.

Further conclusion: It is therefore to be expected that all types of cytoplasm will occur in both sexes, and cytoplasmically influenced maleness or femaleness will not be expected to occur.

Evolution of dioecy

The previous section has given the necessary conditions for the evolution of dioecy, namely that all cytoplasm types must be present in both sexes. The situation where dioecy may evolve in only one type of cytoplasm, i.e., nuclear dioecy, has already been studied (e.g. Ross and Weir 1976; Charnov, Maynard Smith and Bull 1976; Charlesworth and Charlesworth 1978; Gregorius,

Ross and Gillet 1983). It remains, therefore, to study the situation where nuclear genes give dioecy in one cytoplasm and hermaphroditism, gynodioecy or some other non-dioecious system in another. We ask whether dioecy can evolve from hermaphroditism or some other system in this fashion?

Models for the evolution of dioecy: We consider a nuclear gene locus with two alleles, where the nuclear genes interact with N-cytoplasm to give hermaphroditism or some other non-dioecious system. We then introduce S-cytoplasm into the population, where the same nuclear genes interact with S-cytoplasm to give dioecy with female heterogamety (Model I) or male heterogamety (Model II). We then find the conditions under which S-cytoplasm replaces N-cytoplasm. We consider a model of male/female resource-allocation with dominance. The population has a gene-locus A with the dominant allele A_1 and the recessive A_2, as in the model of Ross and Gregorius (1983), except that there is no selfing. The present model differs also in that the alleles interact with two cytoplasm types S and N to control the distribution of reproductive resources between female and male sex functions. Each genotype has the same amount of resources, and all resources not devoted to ovules are devoted to pollen. Genotype NA_1A_1 (frequency P_{N11}) devotes R_{N1} of its resources to ovule production, and $1 - R_{N1}$ to pollen production, and so on for all six genotypes (Table 1). It is assumed that all ovules have an equal chance of pollination.

Several additional or more general symbols are used, as follows:

$G_{i|K}$ = frequency of A_i among ovules with K cytoplasm ($K = N$ or $K = S$)

g_i = frequency of A_i among all pollen

P_{Kij} = frequency of the genotype KA_iA_j in zygotes

$P_{ij|K}$ = frequency of A_iA_j among all zygotes having K cytoplasm

$p_{i|K}$ = frequency of A_i among all zygotes having K cytoplasm

P_K = frequency of K cytoplasm

R_{K1} = resources used for ovule production by KA_1A_1 and KA_1A_2

R_{K2} = resources used for ovule production by KA_2A_2

For the present model, with two cytoplasms and no selfing, we have:

$$
\begin{aligned}
G_{1|K} &= p_{1|K} \cdot R_{K1}/\bar{R}_K \\
G_{2|K} &= (R_{K2}P_{22|K} + \tfrac{1}{2}R_{K1}P_{12|K})/\bar{R}_K = 1 - G_{1|K} \\
g_1 &= [(1 - R_{N1})(P_{N11} + \tfrac{1}{2}P_{N12}) + (1 - R_{S1})(P_{S11} + \tfrac{1}{2}P_{S12})]/(1 - \bar{R}) \qquad (1) \\
g_2 &= 1 - g_1
\end{aligned}
$$

	Genotype					
	NA_1A_1	NA_1A_2	NA_2A_2	SA_1A_1	SA_1A_2	SA_2A_2
Resources for ovule production	R_{N1}	R_{N1}	R_{N2}	R_{S1}	R_{S1}	R_{S2}
Frequency	P_{N11}	P_{N12}	P_{N22}	P_{S11}	P_{S12}	P_{S22}

TABLE 1: *General features of the models for the evolution of dioecy*

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where

$$\bar{R}_K = (1 - P_{22|K})R_{K1} + P_{22|K}R_{K2}$$
$$\bar{R} = \bar{R}_N P_N + \bar{R}_S P_S.$$

The frequencies in the next generation are given by

$$P'_{ii|K} = G_{i|K}g_i, \quad P'_{12|K} = G_{1|K}g_2 + G_{2|K}g_1, \quad P'_K = P_K\bar{R}_K/\bar{R} \tag{2}$$

For populations which are at polymorphic equilibrium and have one cytoplasm only (Ross and Gregorius 1983), $R_i < 1/2 < R_j$, which yields

$$\bar{R} = \bar{R}^* = \tfrac{1}{2},$$

$$g_1 = g_1^* = 2(1 - R_1)p_1^* = \frac{1 - \sqrt{(R_1 + R_2 - 2R_1R_2)(1 - 2R_1)/(R_2 - R_1)}}{2R_1}$$

for $R_1 > 0$, and $g_1^* = (R_2 - \frac{1}{2})/R_2$ for $R_1 = 0$. It can easily be shown that this equilibrium is globally stable.

Results and Discussion

APPENDIX A obtains the conditions for the maintenance of each cytoplasm, and APPENDICES B and C are concerned with Models I and II, respectively. The features of these models are presented in Table 2. Table 3 and Fig. 1 summarize the results, and show for Model I (female heterogamety) that quite simple conditions determine which type of population is maintained. If genotype NA_1A_1 puts more than half and NA_2A_2 less than half its resources into ovules, then N-cytoplasm is lost and dioecy evolves where all plants have S-cytoplasm only (i.e., normally nuclear dioecy). Thus this case and others to be considered represent competition between breeding systems and cytoplasms. That such competition occurs in nature can be inferred from the extensive results of Michaelis with *Epilobium*. Nucleus and cytoplasm were apparently adjusted to each other in such a way that the various taxa were hermaphrodite, and the transfer of

	Genotype			
	NA_1A_1, NA_1A_2	NA_2A_2	SA_1A_1, SA_1A_2	SA_2A_2
Model I	$0 < R_{N1} < 1\ (H)$	$0 < R_{N2} < 1\ (H)$	$R_{S1} = 1\ (F)$	$R_{S2} = 0\ (M)$
Model II	$0 < R_{N1} < 1\ (H)$	$0 < R_{N2} < 1\ (H)$	$R_{S1} = 0\ (M)$	$R_{S2} = 1\ (F)$

Abbreviations: H = hermaphrodite, F = female and M = male.

TABLE 2: *Resource allocation and phenotypes for each genotype in Model I (female heterogamety) and Model II (male heterogamety)*

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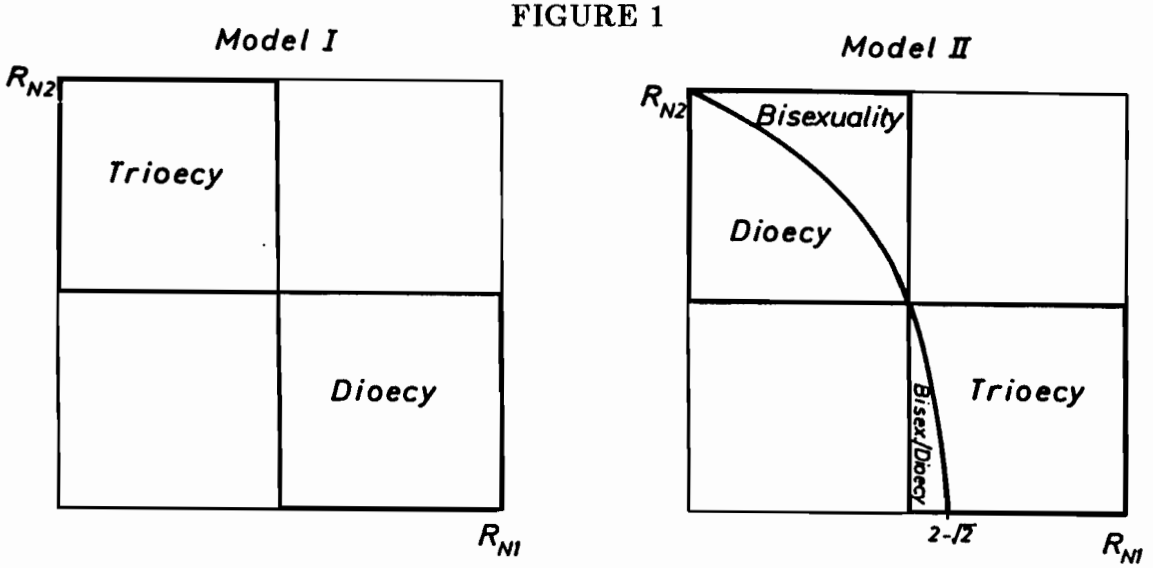
FIGURE 1



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a foreign nucleus into a particular cytoplasm by repeated backcrossing resulted in male sterility, inviability or other disturbances (Michaelis 1954). In Model I fixation of hermaphroditism (or monoecy, etc.) cannot occur, and therefore facultative fixation (fixation of hermaphroditism or dioecy, according to the starting frequencies) also cannot occur. If the resource allocations in N-cytoplasm are reversed, then trioecy (presence of females, males and hermaphrodites) occurs, and all six genotypes and both cytoplasms are present.

For male heterogamety (Model II) the results are more complicated. If genotype NA_1A_1 puts less than half and NA_2A_2 more than half its resources into ovules, then either dioecy or hermaphroditism and therefore one cytoplasm type will become fixed, according to whether NA_2A_2 puts less than or more than a certain quantity N^* of its resources into ovules. N^*

$$\text{Let } N^* = \frac{1 - 2R_{N1} + \frac{1}{2}R_{N1}^2}{1 - \frac{3}{2}R_{N1}}$$

	Fixation of dioecy ($R^*_{S\|N} > R_N$, $R^*_{N\|S} < R^*_S$)	Fixation of bisexuality ($R^*_{S\|N} < R^*_N$, $R^*_{N\|S} > R^*_S$)	Stable trioecy ($R^*_{S\|N} > R^*_N$, $R^*_{N\|S} > R^*_S$)	Facultative fixation ($R^*_{S\|N} < R^*_N$, $R^*_{N\|S} < R^*_S$)
Model I: $R_{S1} = 1$, $R_{S2} = 0$	$R_{N2} < \frac{1}{2} < R_{N1} < 1$	cannot occur	$R_{N1} < \frac{1}{2} < R_{N2}$	cannot occur
Model II: $R_{S1} = 0$, $R_{S2} = 1$	$R_{N1} < \frac{1}{2} < R_{N2}$ and $R_{N2} < N^*$	$R_{N1} < \frac{1}{2} < R_{N2}$ and $R_{N2} > N^*$	$R_{N2} < \frac{1}{2} < R_{N1}$ and either $R_{N1} > 2 - \sqrt{2}$ or $R_{N2} > N^*$	$R_{N2} < \frac{1}{2} < R_{N1} < 2 - \sqrt{2}$ and $R_{N2} < N^*$

TABLE 3: *Summary of the results*

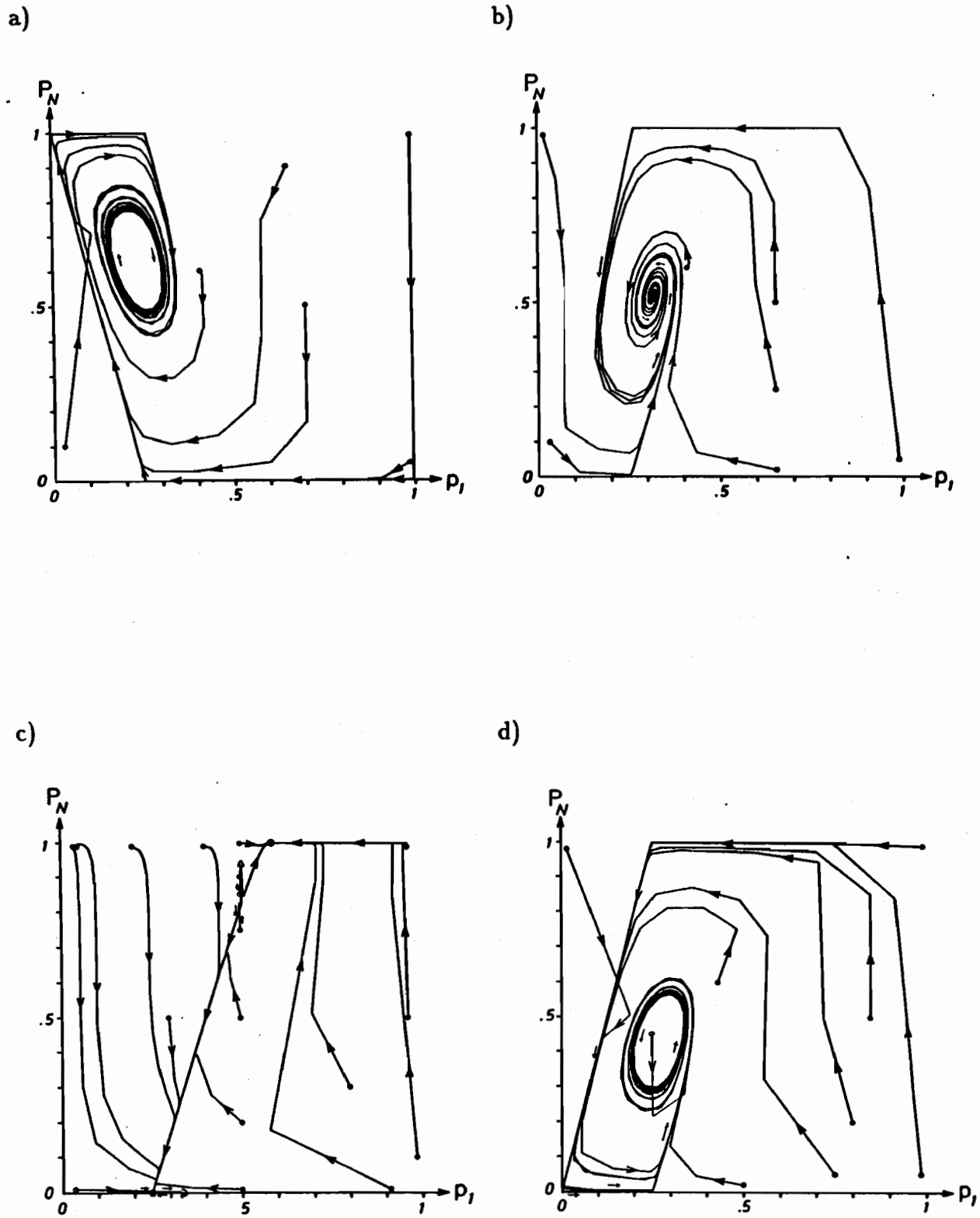
depends upon the resource allocation of genotype NA_1A_1. For example, if NA_1A_1 puts 0.4 of its reproductive resources into ovule production, then dioecy will be fixed if NA_2A_2 puts less than 0.7 of its resources into ovules, and hermaphroditism will be fixed if this amount is more than 0.7. Thus only the more asymmetric hermaphrodite populations can assert themselves against a dioecious population. Similar restraints occur for the other two cases in Model II, and dioecy can evolve more readily for female than for male heterogamety.

Notice that Table 3 allows some extreme values for resource allocation in N-cytoplasm, such that unisexual genotypes may occur. For example, in Model I fixation of dioecy could occur if NA_2A_2 is male ($R_{N2} = 0$) and NA_1A_1 hermaphrodite ($\frac{1}{2} < R_{N1} < 1$). Thus a population of males and hermaphrodites (androdioecy) could be displaced by a dioecious one in this fashion. The hermaphrodite could be almost female, and such a type of subdioecy with well differentiated males but incompletely differentiated fermales is known in nature, e.g., in *Mercurialis annua* (Kuhn 1939; see also the review by Westergaard 1958). Notice also that subdioecy in N-cytoplasm cannot compete with true dioecy in S-cytoplasm. The mean fitness of plants in S-cytoplasm is higher (although higher mean fitness does not always accompany selection in similar models (Ross and Gregorius 1983)). Another example of interest in Model I is the case of stable trioecy. The relationship $R_{N1} < \frac{1}{2} < R_{N2}$ allows not only hermaphroditism in N-cytoplasm, but also gynodioecy, androdioecy, subdioecy and true dioecy. When R_{S1} is high R_{N1} must be low, and when R_{S2} is low R_{N2} must be high, so that there is now an additional example where a type of sexual asymmetry is required for maintaining a polymorphism for a dominant gene (see Gregorius 1984 for another example). For true dioecy the genotypes A_1A_1 and A_1A_2 are male in N- and female in S-cytoplasm, so that there is male heterogamety in N and female heterogamety in S-cytoplasm. This report is probably the first to show that both male and female heterogamety may occur within a population, and there are reports of both male and female heterogamety in some species, e.g., *Silene otites* (Westergaard 1958).

Several special cases have been studied numerically. Stable trioecy in Model I is perhaps of particular interest, since it encompasses several special situations. For example, the situation where $R_{N1} = 0.4$, $R_{N2} = 0.6$ yields hermaphroditism in N-cytoplasm and dioecy with female heterogamety in S-cytoplasm. For both the relatively extreme starting frequency $P_{N11} = 0.99$ (with all other frequencies 0.002), and also for moderate starting frequencies for all six types, the population develops extreme frequencies over the generations. These frequencies suggest that although trioecy is theoretically protected for large populations, fixation would be expected in populations of realistic size (cf. similar results in Gregorius and Ross 1984). Another example of this special case shows greater asymmetry ($R_{N1} = 0.1$, $R_{N2} = 0.9$), such that the population is subdioecious in N- and strictly dioecious in S-cytoplasm. Fig. 2a shows that extreme frequencies again develop for relatively extreme starting values, but for starting frequencies toward or in the interior the graph shows a spiral pattern, and ultimately develops a cycle.

We now consider some special cases of Model II. For the analogous case to that last considered we have subdioecy with female heterogamety in N-cytoplasm ($R_{N1} = 0.9$, $R_{N2} = 0.1$), and dioecy with male heterogamety in S cytoplasm. The conditions for stable trioecy are fulfilled,

FIGURE 2



and the graph in Fig. 2b shows that an equilibrium is rapidly approached via a spiral. Genotype SA_1A_1 is missing in all generations after the first, and the equilibrium population contains two cytoplasm types and one female, one male, two nearly female and one nearly male genotypes. Another interesting case is that for facultative fixation ($R_{N1} = 0.54$, $R_{N2} = 0.3$). Fig. 2c confirms that there are two different equilibria, depending on the initial frequencies. Starting frequencies which differ only slightly may lead to different equilibria, and approach to equilibria may take hundreds or even thousands of generations for populations of a realistic size. Finally, the case of stable trioecy also includes strict dioecy in both cytoplasms ($R_{N1} = 1$, $R_{N2} = 0$) as a special case. Fig. 2d shows the development of a cycle in this case.

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Appendix A: The maintenance of a cytoplasm.

The maintenance of the S-cytoplasm, say, is guaranteed if $\bar{R}_S > \bar{R}_N$. In essence it is sufficient if this inequality is realized for small P_S. Hence, if $\bar{R}_S > \bar{R}_N$ as P_S approaches zero, S is protected; otherwise, if $\bar{R}_S < \bar{R}_N$ at this limit then S is not protected. However, even if P_S is very small and thus P_N is close to 1, the $P_{ij|S}$'s as well as the $P_{ij|N}$'s and thus $\bar{R}_S$ and $\bar{R}_N$ still may change. Assuming that in the absence of the S-cytoplasm the $P_{ij|N}$'s always converge to a limit with $\bar{R}_N = R^*_N$, the objective is to investigate for very small P_S the dynamic properties of the $P_{ij|S}$'s and the accompanying $\bar{R}_S$ at this limit. If it holds that $\bar{R}_S$ converges to a limit $R^*_{S|N}$ then $R^*_{S|N} > R^*_N$ or $R^*_{S|N} < R^*_N$ would imply protectedness or non-protectedness of S, respectively.

For P_S close to zero and the $P_{ij|N}$'s at equilibrium it holds by equation (1) that

$$g_1 = (1 - R_{N1})p^*_{1|N}/(1 - R^*_N) \quad \text{and} \quad g_2 = 1 - g_1$$

and by equation (2)

$$P'_{ii|S} = (P_{ii|S} \cdot a_i + P_{12|S} \cdot b_i)/\bar{R}_S,$$

$$P'_{12|S} = (P_{11|S} \cdot d_1 + P_{22|S} \cdot d_2 + P_{12|S} \cdot c)/\bar{R}_S,$$

where

$$a_i = R_{Si} \cdot g_i,\ b_i = R_{S1} \cdot g_i/2,\ c = R_{S1}/2, \quad \text{and} \quad d_i = R_{Si} \cdot (1 - g_i).$$

Moreover, since $2 \cdot c = R_{S1}$ and $d_i + a_i = R_{Si}$,

$$\bar{R}_S = P_{11|S} \cdot (a_1 + d_1) + P_{22|S} \cdot (a_2 + d_2) + P_{12|S} \cdot 2 \cdot c.$$

The above transition equations can be conceived of as a three dimensional, normalized linear transition equation with state vector z, given by $z_1 = P_{11|S}$, $z_2 = P_{22|S}$, $z_3 = P_{12|S}$, and the transition matrix M with first row $(a_1, 0, b_1)$, second row $(0, a_2, b_2)$ and third row (d_1, d_2, c). Clearly, $\bar{R}_S = e^\top Mz$, where $e^\top = (1,1,1)$, so that the previous transitions can now be written in the matrix form

$$z' = Mz/e^\top Mz.$$

If M is irreducible, then, by the Perron-Frobenius theorem, there exists a positive fixed point z^* of the above transition which is globally attractive, and $e^\top Mz^* = R^*_{S|N}$ is the maximum characteristic value of M. Hence, $R^*_{S|N}$ is the maximum solution x of the characteristic equation

$$(x - a_1)(x - a_2)(x - c) - (x - a_1)b_2d_2 - (x - a_2)b_1d_1 = 0$$

or equivalently

$$x^3 - Ux^2 + Vx + W = 0,$$

where

$$U = a_1 + a_2 + c, \qquad V = a_1a_2 + c(a_1 + a_2) - b_1d_1 - b_2d_2$$

$$\text{and} \quad W = a_1b_2d_2 + a_2b_1d_1 - ca_1a_2.$$

Since $a_ic = b_i(a_i + d_i)$ and $c = b_1 + b_2$ we have $V = a_1a_2 + a_1b_1 + a_2b_2$ and $W = 0$, so that we obtain

$$R^*_{S|N} = \tfrac{1}{2} \cdot U + \sqrt{\tfrac{1}{4} \cdot U^2 - V},$$

which yields

$$\begin{aligned} R^*_{S|N} = &\tfrac{1}{2} \cdot (R_{S1}g_1 + R_{S2}g_2 + \tfrac{1}{2} \cdot R_{S1}) \\ &+ \tfrac{1}{2} \cdot \sqrt{\tfrac{1}{4} \cdot R^2_{S1} + g_2(R_{S1}g_1 - R_{S2}g_2)(R_{S1} - R_{S2})}. \end{aligned} \tag{A1}$$

Replacing S by N and N by S in all of the above statements yields the analysis for the maintenance of the N-cytoplasm.

Appendix B: Model I ($R_{S1} = 1$, $R_{S2} = 0$)

(1) Establishment of the S-cytoplasm:

In this case P_S is assumed to be very small. Then $R^*_N = \frac{1}{2}$, and by (A1)

$$R^*_{S|N} = \tfrac{1}{2}(\tfrac{1}{2} + g_1) + \tfrac{1}{2}\sqrt{\tfrac{1}{4} + g_1(1 - g_1)}$$

where

$$g_1 = (1 - \sqrt{(R_{N1} + R_{N2} - 2R_{N1}R_{N2})(1 - 2R_{N1})/(R_{N2} - R_{N1})})/(2R_{N1})$$

for $R_{N1} > 0$, and $g_1 = (R_{N2} - \frac{1}{2})/R_{N2}$ for $R_{N1} = 0$.

Hence, the S-cytoplasm and thus trioecy becomes established if $R^*_{S|N} > R^*_N$, i.e. if

$$\tfrac{1}{2} + g_1 + \sqrt{\tfrac{1}{4} + g_1(1 - g_1)} > 1$$

which is always true. Consequently, Model I implies *per se* the establishment of trioecy.

(2) Establishment of the N-cytoplasm:

Now P_N is assumed to be very small. Then $R^*_S = \frac{1}{2}$, $g_1 = 0$ and by (A1), interchanging N and S,

$$R^*_{N|S} = \tfrac{1}{2}(\tfrac{1}{2}R_{N1} + R_{N2}) + \tfrac{1}{2} \cdot |\tfrac{1}{2}R_{N1} - R_{N2}| = \max\{\tfrac{1}{2}R_{N1}, R_{N2}\}.$$

Hence, the N-cytoplasm cannot become established so that dioecy is locally stable if $R^*_{N|S} < R^*_S$, i.e. if $\max\{\frac{1}{2}R_{N1}, R_{N2}\} < \frac{1}{2}$, which is equivalent to

$$R_{N2} < \tfrac{1}{2} \quad \text{and} \quad R_{N1} < 1.$$

In summary, we thus obtain that dioecy may evolve after the introduction of the S-cytoplasm if $R^*_{S|N} > R^*_N$ and $R^*_{N|S} < R^*_S$, i.e. if $R_{N2} < \frac{1}{2}$ and $R_{N1} < 1$.

Appendix C: Model II ($R_{S1} = 0$, $R_{S2} = 1$)

(1) Establishment of the S-cytoplasm.

Now, $R_N^* = \frac{1}{2}$, and g_1 is the same as in Model I. Although, in this case, M is reducible, it is easy to show that $\bar{R}_S$ still converges to $R^*_{S|N}$ given by equation (A1), so that

$$R^*_{S|N} = 1 - g_1.$$

Hence, the S-cytoplasm becomes established if $1 - g_1 > \frac{1}{2}$, i.e. if $g_1 < \frac{1}{2}$. This can be written as $1 - R_{N1} < \sqrt{(R_{N1} + R_{N2} - 2R_{N1}R_{N2})(1 - 2R_{N1})/(R_{N2} - R_{N1})}$. Squaring both sides and rearranging yields

$$R_{N1}(R_{N2} - R_{N1})(2(1 - 2R_{N1}) + R_{N1}^2 - R_{N2}(2 - 3R_{N1})) > 0.$$

This inequality is always realized if $R_{N1} > 2 - \sqrt{2}$ and $R_{N2} < \frac{1}{2}$. Otherwise, the inequality is realized if either $R_{N1} < \frac{1}{2} < R_{N2} < (1 - 2R_{N1} + \frac{1}{2}R_{N1}^2)/(1 - \frac{3}{2}R_{N1})$ or $(1 - 2R_{N1} + \frac{1}{2}R_{N1}^2)/(1 - \frac{3}{2}R_{N1}) < R_{N2} < \frac{1}{2} < R_{N1} \leq 2 - \sqrt{2}$.

(2) Establishment of the N-cytoplasm:

Now $R_S^* = \frac{1}{2}$ and $g_1 = \frac{1}{2}$. Therefore, by (A1)

$$R^*_{N|S} = \tfrac{1}{2}(R_{N1} + \tfrac{1}{2}R_{N2}) + \tfrac{1}{4}\sqrt{R_{N1}^2 + (R_{N1} - R_{N2})^2}.$$

The N-cytoplasm cannot become established if $R^*_{N|S} < R_S^*$. This is equivalent to

$$R_{N1} + \tfrac{1}{2}R_{N2} < 1 \quad \text{and} \quad 1 - R_{N1}(2 - \tfrac{1}{2}R_{N1}) - R_{N2}(1 - \tfrac{3}{2}R_{N1}) > 0,$$

so that $R_{N2} < (1 - R_{N1}(2 - \frac{1}{2}R_{N1}))/(1 - \frac{3}{2}R_{N1})$ for $R_{N1} < \frac{2}{3}$, and $R_{N2} > (1 - R_{N1}(2 - \frac{1}{2}R_{N1}))/(1 - \frac{3}{2}R_{N1})$ for $R_{N1} > \frac{2}{3}$. The last inequality, however, cannot be realized since $1 - \frac{3}{2}R_{N1} \geq 1 - R_{N1}(2 - \frac{1}{2}R_{N1})$. Moreover, $1 - R_{N1}(2 - \frac{1}{2}R_{N1}) \leq 0$ for $R_{N1} \geq 2 - \sqrt{2}$, and $R_{N1} + \frac{1}{2}R_{N2} < 1$ for $R_{N1} < 2 - \sqrt{2}$, so that the N-cytoplasm cannot become established if

$$R_{N1} < 2 - \sqrt{2} \quad \text{and} \quad R_{N2} < (1 - R_{N1}(2 - \tfrac{1}{2}R_{N1}))/(1 - \tfrac{3}{2}R_{N1}).$$

In summary, dioecy may evolve after the introduction of the S-cytoplasm if

$$R_{N1} < \tfrac{1}{2} < R_{N2} < (1 - R_{N1}(2 - \tfrac{1}{2}R_{N1}))/(1 - \tfrac{3}{2}R_{N1}).$$