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Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: How to explain a cytotype polymorphism?*

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Abstract

Although cytoplasmic incompatibilities have been used as a means of eradicating the mosquito *Culex pipiens*, the population dynamics of these sterilities in relation to the coexistence of multiple incompatible cytotypes in a single area has not been investigated, except in the case of two unidirectionally incompatible cytotypes. An analytical model of the evolution of n cytotypes in an infinite panmictic population has been developed in order to investigate polymorphic equilibrium. A necessary criterion for the stability of such an equilibrium is established; it is shown that a stable polymorphism cannot exist between incompatible cytotypes. This result is discussed in the light of population dynamics and genetics of *Culex pipiens*, and of our present knowledge on incompatibilities. The consequences of a geographic structuring and of homogamy are considered. A careful reconsideration of previous experimental results disclosed probable nuclear effects and a serious experimental weakness: with the common procedure of backcrossing hybrid females to males of constant genotype it is not possible to rule out probable nuclear effects with paternal expression. It is concluded that incompatibilities in *Culex pipiens* may have a nuclear-cytoplasmic determinism.

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Introduction

Cytoplasmic incompatibility is a sterility phenomenon that has been observed between strains of the mosquito *Culex pipiens* (Ghelelovitch, 1952; Laven, 1967a) and other insect species (e.g. Brower, 1976; Wade and Stevens, 1985; Hoffmann *et al.*, 1986; Hoffmann and Turelli, 1988). These sterilities are characterized by a drastic decrease of female fertility. In *Culex pipiens*, a female mated with an "incompatible" male produces a normal number of eggs, often fertilized, but none or very few of the larvae hatch. Ghelelovitch (1952) and Laven (1953, 1957, 1967a) have shown that these sterilities are maternally inherited and independent of nuclear genes, hence they were named "cytoplasmic incompatibilities". A rickettsia-like symbiont, *Wolbachia pipientis*, may be responsible for this phenomenon (Yen and Barr, 1971, 1973; see also Louis and Nigro, 1989; O'Neill, 1989), but no conclusive results have yet been obtained (Subbarao, 1982).

Incompatibilities are the manifestation of an asymmetrical interaction between parental cytoplasms. In the mosquito *Culex pipiens*, a cross between two strains (S_1 and S_2) is said to be: (a) bidirectionally incompatible when no or very few offspring are produced in the two reciprocal crosses ($♀S_1 \times ♂S_2$ and $♀S_2 \times ♂S_1$), (b) unidirectionally incompatible when one of the crosses produces a normal number of offspring, the other being sterile, and (c) compatible when the two reciprocal crosses are fertile (Table 1).

A cytoplasm, characterized by its crossing type (compatible, unidirectionally or bidirectionally incompatible) with different cytoplasms, will be referred to as a cytotype (=cytoplasmic crossing type), and named c_i throughout the text. All known cytotypes are self-compatible.

After Ghelelovitch (1952) and Laven's (1953, 1967a) pioneering studies, cytoplasmic incompatibilities have been investigated as a means of eradicating *Culex pipiens* (Barr, 1966). The original idea was to release males that were sterile with the local females. It was based on two assumptions: all females in the area of release were sterile with released males (Barr, 1966; Laven, 1967b; Thomas, 1971), and it was thought that cytoplasmic incompatibilities were restricted to crosses between mosquitoes collected from distant geographic areas (Laven, 1967a,b). However, studies on small geographic scales, such as those of Barr (1980) in California, Raymond *et al.* (1986) and Magnin *et al.* (1987) in southern France, revealed that incompatible cytotypes do coexist. Barr (1980) found three cytotypes in natural

Table 1. The different possible results of a cross between two strains S_1 and S_2.

Type of cross	Cross	
	♀S1 × ♂S2	♀S2 × ♂S1
Compatible	Fertile	Fertile
Unidirectionally incompatible	Fertile Sterile	Sterile Fertile
Incompatible	Sterile	Sterile

populations from Los Angeles (California) and Magnin *et al.* (1987) found eight in southern France. We have investigated whether such a polymorphism could be stable.

The problem of the coexistence of two unidirectionally incompatible cytotypes in a panmictic population was considered by Caspari and Watson (1959) and later by Fine (1978) with some modifications. They found that when cross $♀c_1 \times ♂c_2$ is sterile and cross $♀c_2 \times ♂c_1$ is fertile, c_1 cytotype is eliminated, independent of its original frequency, because $♀c_1$ are sterilized by $♂c_2$, while $♀c_2$ are fertile with all males in the population. However, if c_1 cytotype has an advantage over c_2, independent of the incompatibility phenomenon, an unstable polymorphic equilibrium exists, and one of the factors that will determine which cytotype remains is its initial frequency in the population. In addition, Fine (1978), in the context of unidirectional incompatibility, considered the rate of appearance of one cytotype (aposymbiotic) in the offspring of females infected by *Wolbachia pipientis*.

Here, a more general situation is examined, and an undetermined number of cytotypes is considered. It is obvious that in a population where males bearing a particular cytotype are sterile with females bearing any other cytotype, while the reciprocal crosses are fertile, that particular cytotype will eliminate the others. Thus no polymorphic equilibrium can exist in this simplistic case. In more complex situations, when many cytotypes with a large variety of relationships of sterility are present, it is not clear whether a polymorphic equilibrium is possible, and whether this equilibrium is stable or not. In order to investigate this problem in a general way, an analytical model has been developed.

The model

We have considered an infinite panmictic population with non-overlapping generations, in which n ($n \geq 2$) cytotypes are present, at frequencies $p_1, \ldots, p_n$ ($\Sigma_{i=1}^{n} p_i = 1$; for all i, $p_i > 0$) at generation g and frequencies $p'_1, \ldots, p'_n$, at generation $g + 1$. The relative numbers of offspring obtained in crosses $♀c_i \times ♂c_j$ and $♀c_j \times ♂c_i$ are described by the parameters ϕ_{ij} and ϕ_{ji}, respectively (these parameters can reflect fertility and viability differences).

Consider the matrix A of the relative numbers of offspring in crosses $♀c_i \times ♂c_j$:

$$A = A(\phi_{ij}) = \begin{array}{c} \\ i \end{array} \overset{\begin{array}{c} j \end{array}}{\begin{vmatrix} \phi_{11} & \cdot & \phi_{1n} \\ & \cdot & \\ \cdots & \phi_{ij} & \\ \phi_{n1} & & \phi_{nn} \end{vmatrix}}$$

and the vector P of frequencies:

$$P = \begin{vmatrix} p_1 \\ \vdots \\ p_i \\ \vdots \\ p_n \end{vmatrix}$$

Assuming an unbiased sex ratio among the offspring of all possible crosses, the frequency of cytotype c_i at generation $g + 1$ will be:

$$p_i' = p_i \frac{\sum_{j=1}^{n} p_j \phi_{ij}}{\sum_{j=1}^{n} \sum_{k=1}^{n} p_k p_j \phi_{kj}} \tag{1}$$

Equation (1) also describes the change of frequency of a nuclear allele in an autosomal one-locus multiple-alleles model, with ϕ_{ij} being the fitnesses of the different genotypes. The problem of equilibrium stability in such a model has been thoroughly investigated by Kimura (1956), Mandel (1959), Kingman (1961), Tallis (1966), Lewontin *et al.* (1978) and others, assuming an increase in mean fitness from generation to generation, and the symmetry of the matrix A (for all i, j, $\phi_{ij} = \phi_{ji}$), since the fitness of a genotype AB is supposed to be the same whether the mother (or the father) gives the A or B allele. These assumptions are not valid for cytoplasmic incompatibility, because unidirectionally incompatible crosses are common, and therefore the matrix A is not symmetric, and mean fitness can decrease from generation to generation. Thus, another criterion, valid for an asymmetric matrix, has been established.

Equation (1) is equivalent to

$$p_i' = p_i \frac{(A \cdot P)_i}{(A \cdot P \mid P)} \tag{2}$$

where $(A \cdot P \mid P)$ is the inner product between $A \cdot P$ and P vectors, and $(A \cdot P)_i$ the i^{th} element of $(A \cdot P)$ vector. A is said to be the matrix of cytotypes.

An equilibrium point is characterized by:

$$\forall i, p_{i_e}' = p_{i_e} \tag{3}$$

$$\overset{p_{i_e} \neq 0}{\Leftrightarrow} \forall i, (A \cdot P_e)_i = (A \cdot P_e \mid P_e) \tag{4}$$

where P_e is the vector of equilibrium frequencies (p_{i_e}).

There is an equilibrium between the n cytotypes when condition (4) is respected and when for all i, $p_{i_e} > 0$.

For example, a system with three cytotypes described by the following matrix:

$$A = \begin{vmatrix} 1 & 1 & 0 \\ 0 & 1 & 1 \\ 1 & 0 & 1 \end{vmatrix}$$

has a unique polymorphic equilibrium when $p_1 = p_2 = p_3 = 1/3$.

However, there is not always a single equilibrium position. A system of cytotypes described by the matrix:

$$A = \begin{vmatrix} 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 1 & 1 \\ 1 & 0 & 0 & 1 \end{vmatrix} \tag{5}$$

will be in equilibrium when

$$\begin{cases} p_1 + p_2 = 1/2 \\ p_2 + p_3 = 1/2 \\ p_3 + p_4 = 1/2 \end{cases}$$

These conditions correspond to an infinity of equilibria (mathematically a line in a four-dimensional space).

In order to determine the stability of an equilibrium point P_e, we can consider a small deviation from P_e,

$$\delta P = \begin{vmatrix} \delta p_1 \\ \vdots \\ \delta p_i \\ \vdots \\ \delta p_n \end{vmatrix}$$

and determine whether P has a tendency to deviate more from the equilibrium point P_e (unstable equilibrium), or to return to it (stable equilibrium). The stability of an equilibrium is equivalent to the following condition:

for all i,

$$\frac{dp_i}{dt}\,\delta p_i \leq 0 \tag{6}$$

We show in Appendix A that a necessary condition for a stable equilibrium is:

for all i, j,

$$\phi_{ij} + \phi_{ji} \geq \phi_{ii} + \phi_{jj} \tag{7}$$

This is an extension of the necessary condition for stable polymorphism of mendelian alleles: for all i, j, $\phi_{ij} > (\phi_{ii} + \phi_{jj})/2$ (Mandel, 1959; Lewontin *et al.*, 1978). As in that situation, the necessary condition for stable equilibrium $\overline{\phi_{ii}} > \overline{\phi_{ij}}$ follows, where $\overline{\phi_{ii}}$ is the mean of ϕ_{ii} on all i and $\overline{\phi_{ij}}$ the mean of ϕ_{ij} on all i, j.

Discussion

The model indicates that, in an infinite panmictic population in which there is an incompatible cross, and therefore $\phi_{ij} + \phi_{ji} < \phi_{ii} + \phi_{jj}$ for some i, j, a polymorphism of incompatible cytotypes cannot be maintained because no stable equilibrium exists. Inevitably, one cytotype will sooner or later disappear, and the process of cytotype elimination will continue with n-1 cytotypes, as long as condition (7) is not respected, i.e. until all coexisting cytotypes are compatible.

The model also assumed that the frequencies of cytotypes in females and in males are equal, i.e. that the nature of cytoplasms does not affect the sex-ratio in offspring. In strongly incompatible crosses, the few offspring produced are often parthenogenetic females (Jost, 1970; Yen and Barr, 1973; Curtis and Suya, 1981;

Raymond *et al.*, 1986). However, these females are too scarce (about 0.1% of the eggs) to bias the frequencies of cytotypes in females in relation to the frequencies in males.

Populations are not infinite and panmictic, and we will now consider how deviations from these assumptions affect the predictions of the model. Obviously, in a finite population, the process of loss of cytotypes will accelerate as the population size decreases. The panmixia hypothesis deserves further comments, as homogamy and geographical structuring of cytotypes are possible.

Strict homogamy among cytotypes will prevent loss of polymorphism. If a female accepts mating only with a "compatible male", incompatible cytotypes can coexist, because they will never meet. However, laboratory experiments seem to indicate that females do not discriminate between compatible and incompatible males (Curtis and Adak, 1974; Curtis *et al.*, 1982), and field releases of incompatible males gave rise to high percentages of incompatible egg rafts (Laven 1967b, Curtis *et al.*, 1982), indicating that strict homogamy is not the rule. It seems that low homogamy would not have any stabilizing effect (calculations not shown), at least when for most $i, j, \phi_{ii} \geq \phi_{ij}$, as is observed in experimental crosses.

Absence of panmixia, due to population structuring, may lead to a more or less stable coexistence of cytotypes. It can be assumed that *Culex pipiens* populations are structured as a neighbourhood, because distant mosquitoes have a lower probability of mating than close ones. The size of a *Culex pipiens* neighbourhood is not known, but non-occasional migrations over distances of several kilometers are well-documented (Subra, 1972; Curtis *et al.*, 1982) and repetitive long distance migrations have also been recorded (Highton and Van Someren, 1970). The structuring of natural populations of *Culex pipiens* may have important consequences. If two bidirectionally incompatible cytotypes are present in a panmictic population, the rarest is eliminated. If one cytotype is predominant in an area and the other in an adjacent area, immigrant cytotypes will be eliminated from each other cytotype's area, and the cytotype differences between sub-populations will be maintained.

As the rate of appearance of new cytotypes seems high (mutations have been found among the descendants of individual females by French (1978), Subbarao *et al.* (1977), and Barr (1980) (but see below for another possible interpretation of some of these results), the polymorphism observed in natural populations of *Culex pipiens* is perhaps a transient polymorphism maintained by an equilibrium between mutation and selective replacement.

Thus, we may think that geographical structuring and/or instability of cytotypes are sufficient to explain the cytotype polymorphism of natural populations. Estimations of the frequency of incompatible egg rafts in natural populations could be used to test these hypotheses. For instance, if population structuring is the driving factor, then incompatibilities should be geographically restricted to areas of contact between cytotypes. If cytotypes are unstable owing to high mutation rates, incompatibilities should be widespread in natural populations.

Unfortunately, although cytoplasmic incompatibility is well-documented in laboratory experiments and eradication attempts, studies of egg rafts collected from the

wild in order to assess incompatibility in natural populations have been done only a few times, in particular in California (Barr, 1980, 1982) and southern France (Perrot, Rousset and Raymond, unpublished); see also Ishii and Sohn (1987) in Sweden. There is not enough data to conclude in favour of any interpretation.

An alternative explanation for multiple incompatible cytotypes is, however, possible. The generally accepted hypothesis of purely cytoplasmic determination of the incompatibilities in *Culex pipiens* (Ghelelovitch, 1952; Barr, 1966; Laven, 1967a; Irving-Bell, 1983; see also the experiments of Krishnamurthy and Laven (1976), Subbarao *et al.* (1977), Doyle and Ellis (1979), Raymond *et al.* (1986), and others) suffers a theoretical weakness: in such a system, a nuclear gene restoring, even partially, the compatibility of a cross otherwise sterile, would be selected for. Therefore, nuclear genes (restorers) limiting the expression of incompatibilities should be expected.

The fact that no restorer has been described may have at least two causes.

First, most strains used to analyze cytoplasmic inheritance had a long laboratory history (more than 20 years for the strain Hamburg used by Ghelelovitch (1952), Laven (1953, 1957, 1967a) and Barr (1966)), and restorers may have been lost, so that only cytoplasmic factors remained. This will explain why incompatibility is less common between strains recently established from natural populations (i.e. less than a few months old), than between older strains, as noted by Irving-Bell (1983). This phenomenon has been observed in strains from California (Barr, 1966; and personal communication), Africa (Eyraud and Mouchet, 1970), Southeast-Asia (Thomas, 1971) and Brazil (Espinola and Consoli, 1972); most of these strains had different cytotypes that were distinguished only by crosses with older laboratory strains (e.g. Barr, 1966).

Second, paternal nuclear effects could not be revealed by the backcrosses performed by Ghelelovitch (1952), Laven (1953, 1957, 1967a), Barr (1966) and Irving-Bell (1983), because the male genome does not change from generation to generation. If a cross between two strains ♀A × ♂B is compatible because ♂B bears a nuclear restorer efficient *in males* with a B cytotype, the replacement of the A nuclear genome by a B one in males and females with an A cytotype will not affect their crossing properties with males and females with a B cytotype. As male cytotype affects the nature of sperm (Yen and Barr, 1974; Barr, 1980), it would not be surprising that nuclear restorers also affect the nature of sperm, and therefore have a paternal, cytotype-specific expression. Consequently, all published experiments supposedly made to prove the exclusively cytoplasmic nature of incompatibilities, failed to disprove a possible nuclear effect with paternal expression.

Evidence of nuclear influence on cytoplasmic incompatibilities can be found several times in the literature:

– Dobrotworsky (1955) obtained the following results between two strains, Me and Lo: ♀Me × ♂Lo was fertile (97.8% egg hatch), ♀Lo × ♂Me was sterile (0% egg hatch), and ♀Lo × ♂(♀Me × ♂Lo) was partially fertile (38.3% egg hatch). Thus, as parental cytoplasms were the same in the last two crosses, the strain Lo seems to bear a nuclear "restorer" inducing partial compatibility between females carrying

Lo cytotype and males carrying Me cytotype. Considering a third strain, Se, Dobrotworsky found that ♀Lo × ♂Se is fertile (99.4% egg hatch) and ♀Lo × ♂(♀Me × ♂Se) is fertile (91% egg hatch). Therefore, it seems that strain Se possesses genes restoring the compatibility between cytoplasms Lo and Me.

– The hypothesis of "instability of cytotypes" has been proposed (Subbarao *et al.*, 1977; French, 1970, 1978) to explain non-conventional results. French used three strains here referred to as Th, As and Aw. As and Aw derived from the same population. As males were selected for incompatibility with Th females, and Aw males were selected for compatibility with Th females. He found that ♀Th × ♂As is sterile (0% egg hatch), ♀Th × ♂Aw is partially fertile (57% egg hatch), and ♀Th × ♂(♀Aw × ♂As) gives only 6.7% egg hatch. These results were interpreted as the consequence of relaxing selection of Aw males for compatibility with Th females, associated with a segregation of cytoplasmic determinants in the Aw strain. Such an interpretation implies that Aw males should have become nearly incompatible with Th females, but this was unfortunately not verified. Another interpretation is that some Aw males contained a nuclear "restorer" responsible for the partial compatibility of the ♀Th × ♂Aw cross, and which was absent in As males. French's other results are consistent with this second interpretation. To test this hypothesis, the following cross: ♀Th × ♂(♀(((♀As × ♂Aw) × ♂Aw) · · ·) × ♂Aw) should have been studied. In the presence of nuclear restorers, this cross would have been partially fertile.

– Subbarao *et al.* (1977) have also interpreted some results by the hypothesis of "segregation of cytotypes" existing in individual mosquitoes. Subbarao *et al.* (1977) and later Subbarao (1982) commented on these assumptions, and found them insufficient, because the segregations were not in agreement with the high number of supposed determinants, *Wolbachia pipientis*, in an egg. They assumed the existence of some "controlling particles" segregating in small numbers and controlling the segregation of *Wolbachia*. As previously, the data of Subbarao *et al.* (1977) may be explained by a polymorphism of nuclear restorers efficient in males, and probably recessive; however, in this case as before, specific well-planned experiments (repetitive backcrosses taking into account the possibility of a nuclear restorer with paternal expression) can be carried out to discriminate cytoplasmic *vs* nuclear inheritance (details not shown).

In any case, cytoplasmic segregation is not a likely explanation of Dobrotworsky's results, since these do not correspond to a rare polymorphism (as in Subbarao *et al.*, 1977), and there is no selection involved (as in French, 1978).

It is obvious that nuclear effects on incompatibility have not been sufficiently investigated, and that there is some evidence of their existence. Thus, cytoplasmic incompatibility may be a new case of nuclear-cytoplasmic conflict (involving nuclear and cytoplasmic polymorphisms), as cytoplasmic male-sterility in Angiosperms (Couvet *et al.*, 1986), feminising bacteria in Isopods (Legrand *et al.*, 1985), sex-ratio factors in *Drosophila* (Poulson and Sakaguchi, 1961), and many other "sex-ratio distorters" (Cosmides and Tooby, 1981).

In the experiments analyzed above, nuclear effects are expressed through males. The presence of such nuclear "restorers" would explain why polymorphism in a strain is generally recognized by differences between males, while females do not differ (Sasa *et al.*, 1966; Subbarao, 1982). Moreover, cytotypes are certainly less unstable than previously concluded from experiments (reviewed in Subbarao, 1982) which generally cannot separate paternally expressed nuclear effects and cytoplasmic effects, even if they are not as stable as thought by Laven (1967a). In many cases, the "cytotype polymorphism" could be a nuclear polymorphism.

The model developed has shown that cytotype polymorphism cannot be explained by stabilizing selection. Thus, in order to better understand the dynamics of cytotype polymorphism, we must take into account the rate of appearance of new cytotypes, the structure of natural populations of *Culex pipiens*, and a possible nuclear-cytoplasmic determinism of incompatibility which may lead to an overestimation of cytotype polymorphism. The more stable cytotypes are, the more localized, geographically, incompatibilities will be, and the easier nuclear restorers could be selected. As restorers could reduce selective pressures between cytotypes, and decrease the impact of incompatibilities in natural populations, their influence on cytotype polymorphism needs to be investigated.

Appendix A

Consider the variation of frequency in one generation

$$\Delta p_i = p_i\left(\frac{(A \cdot P)_i}{(A \cdot P \mid P)} - 1\right) \tag{A.1}$$

On a continuous scale, (A.1) gives:

$$f_i(P) = \frac{\mathrm{d}p_i}{\mathrm{d}t} = p_i\left(\frac{(A \cdot P)_i}{(A \cdot P \mid P)} - 1\right) \tag{A.2}$$

On an equilibrium point,

$$\forall i, f_i(P) = 0 \overset{p_{i_e} \neq 0}{\Leftrightarrow} \forall i, (A \cdot P_e)_i = (A \cdot P_e \mid P_e) \tag{A.3}$$

where P_e is the equilibrium frequencies vector $P_e = (p_{i_e})$.

The condition for stability (Equation 6) is:

$$\forall i, \frac{\mathrm{d}p_i}{\mathrm{d}t}\,\delta p_i \leq 0 \tag{A.4}$$

As

$$\forall i, \frac{\mathrm{d}p_i}{\mathrm{d}t} = f_i(P_e + \delta P) = f_i(P_e) + (\delta f_i)_{\delta P} = (\delta f_i)_{\delta P} \tag{A.5}$$

equation (A.4) can be written

$$\text{for all } i,\ (\delta f_i)_{\delta p} \cdot \delta p_i \leq 0 \tag{A.6}$$

The calculation of $(\delta f_i)_{\delta P}$ gives:

$$(\delta f_i)_{\delta P} = \delta p_i \times \left(\frac{(A \cdot P_e)_i}{(A \cdot P_e \mid P_e)} - 1\right) + p_{i_e}\left(\frac{(A \cdot \delta P)_i}{(A \cdot P_e \mid P_e)} - \frac{(A \cdot P_e)_i}{(A \cdot P_e \mid P_e)^2}((A \cdot \delta P \mid P_e) + (A \cdot P_e \mid \delta P))\right) \tag{A.7}$$

and from the equilibrium condition (A.3)

$$(\delta f_i)_{\delta P} = \frac{p_{e_i}}{(A \cdot P_e \mid P_e)}((A \cdot \delta P)_i - (A \cdot \delta P \mid P_e) - (A \cdot P_e \mid \delta P)) \tag{A.8}$$

This can be written

$$(\delta f_i)_{\delta P} = \frac{p_{i_e}}{(A \cdot P_e \mid P_e)}\left(\sum_{j=1}^{n} \phi_{ij}\,\delta p_j - \sum_{j=1}^{n}\sum_{k=1}^{n}(\phi_{kj}\,\delta p_j p_{k_e}) - \sum_{j=1}^{n}\sum_{k=1}^{n}(\phi_{jk} p_{k_e}\,\delta p_j)\right) \tag{A.9}$$

Consider the following perturbation, on two cytotypes c_{i0}, c_{j0}:

$$\delta p_{i_0} = \varepsilon > 0,\ \delta p_{j_0} = -\varepsilon,\ \forall k \neq i_0, j_0,\ \delta p_k = 0 \tag{A.10}$$

then, the equilibrium condition (A.6) can be written for $i = i_0$:

$$\frac{p_{i_0 e}}{(A \cdot P_e \mid P_e)}\left(\phi_{i_0 i_0} - \phi_{i_0 j_0} - \sum_{k=1}^{n}(\phi_{k i_0} - \phi_{k j_0}) p_{k_e} - \sum_{k=1}^{n}(\phi_{i_0 k} - \phi_{j_0 k}) p_{k_e}\right) \leq 0 \tag{A.11}$$

therefore

$$\left(\phi_{i_0 i_0} - \phi_{i_0 j_0} - \sum_{k=1}^{n}(\phi_{k i_0} - \phi_{k j_0}) p_{k_e} - (A \cdot P_e)_{i_0} + (A \cdot P_e)_{j_0}\right) \leq 0 \tag{A.12}$$

and from (A.3)

$$\left(\phi_{i_0 i_0} - \phi_{i_0 j_0} - \sum_{k=1}^{n}(\phi_{k i_0} - \phi_{k j_0}) p_{k_e}\right) \leq 0 \tag{A.13}$$

Similarly, with $i = j_0$:

$$\left(\phi_{j_0 j_0} - \phi_{j_0 i_0} - \sum_{k=1}^{n}(\phi_{k j_0} - \phi_{k i_0}) p_{k_e}\right) \leq 0 \tag{A.14}$$

Summing (A.13) and (A.14) we get

$$\phi_{i_0 i_0} + \phi_{j_0 j_0} - \phi_{i_0 j_0} - \phi_{j_0 i_0} \leq 0 \tag{A.15}$$

If the equilibrium is stable, this is true for any perturbation (A.10) i.e. for any i_0, j_0. So for all i, j

$$\phi_{ii} + \phi_{jj} \leq \phi_{ij} + \phi_{ji} \tag{A.16}$$

This is a necessary condition for stability. Thus, a sufficient condition for instability is $\phi_{ij} + \phi_{ji} < \phi_{ii} + \phi_{jj}$ for some i, j.

The criterion (A.16) holds for possible paternal transmission of cytotype: a paternal transmission in $♀c_i \times ♂c_j$ cross will be equivalent, with regard to cytotype transmission, to a decrease in the relative number of offspring ϕ_{ij}, and an equivalent increase in the relative number of offspring of the symmetrical cross ϕ_{ji}, because symmetrical crosses are equally probable. Then, if $\phi_{ij} + \phi_{ji}$ remains constant, equation (A.16) is unaffected.

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References

Barr, A. R. 1966. Cytoplasmic incompatibility as a means of eradication of *Culex pipiens* L. Proc. Calif. Mosq. Cont. Assoc. 34: 32–35.

Barr, A. R. 1980. Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens pipiens*. Nature 283: 71–72.

Barr, A. R. 1982. Symbiot control of reproduction in *Culex pipiens*, pp. 153–158. *In* Recent developments in the genetics of insect disease vectors. Stipes Publishing Company, Champaign, IL.

Brower, J. H. 1976. Cytoplasmic incompatibility: occurrence in a stored-product pest *Ephestia cautella*. Ann. Ent. Soc. Am. 69: 1011–1015.

Caspari, E. and G. S. Watson. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution 13: 568–570.

Cosmides, L. M. and J. Tooby. 1981. Cytoplasmic inheritance and intragenomic conflict. J. theor. Biol. 89: 83–129.

Couvet, D., F. Bonnemaison and P.-H. Gouyon. 1986. The maintenance of females among hermaphrodites: the importance of nuclear-cytoplasmic interactions. Heredity 57: 325–330.

Curtis, C. F. and T. Adak. 1974. Population replacement in *Culex fatigans* by means of cytoplasmic incompatibility. I. Laboratory experiments with non-overlapping generations. Bull. W.H.O. 51: 249–255.

Curtis, C. F. and T. B. Suya. 1981. Variations in cytoplasmic incompatibility properties in Tanzanian populations of *Culex quinquefasciatus*. Ann. Trop. Med. Parasitol. 75: 101–106.

Curtis, C. F., G. D. Brooks, M. A. Ansari., K. K. Grover, B. S. Krishnamurthy, P. K. Rajagopalan, L. S. Sharma, V. P. Sharma, D. Singh, K. R. P. Singh and M. Yasuno. 1982. A field trial on control of *Culex quinquefasciatus* by release of males of a strain integrating cytoplasmic incompatibility and a translocation. Ent. Exp. Appl. 31: 181–190.

Dobrotworsky, N. V. 1955. The *Culex pipiens* group in South-Eastern Australia. IV. Crossbreeding experiments within the *Culex pipiens* group. Proc. Linn. Soc. New South Wales 80: 33–43.

Espinola, H. N. and R. A. B. Consoli. 1972. Cruzamentos entre colonias de *Culex pipiens fatigans* Wiedmann procedentes de diferentes partes do Brasil. Rev. Bras. Malariol. Doenças Trop. 24: 165–172.

Eyraud, M. et J. Mouchet. 1970. Incompatibilités cytoplasmiques entre des souches de *Culex pipiens fatigans* Wied. d'Afrique, d'Asie et d'Amérique. Cah. O.R.S.T.O.M., sér. Ent. Méd. Parasitol. 7: 69–82.

Fine, P. E. M. 1978. On the dynamics of symbiote-dependent cytoplasmic incompatibility in culicine mosquitoes. J. Invert. Pathol. 30: 10–18.

French, W. L. 1970. Evidence for the segregation of cytoplasmic genes in *Culex pipiens*, an advanced form of animal life. Genetics 64: s22.

French, W. L. 1978. Genetic and phenogenetic studies on the dynamic nature of the cytoplasmic inheritance system in *Culex pipiens*. Genetics 88: 447–455.

Ghelelovitch, S. 1952. Sur le déterminisme génétique de la stérilité dans le croisement entre différentes souches de *Culex autogenicus* Roubaud. C. R. Acad. Sci. Paris. 234: 2386–2388.

Highton, R. B. and E. C. C. Van Someren. 1970. The transportation of mosquitoes between international airports. Bull. W.H.O. 42: 334–335.

Hoffmann, A. A. and M. Turelli. 1988. Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects. Genetics 119: 435–444.

Hoffmann, A. A., M. Turelli, and G. M. Simmons. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution 40: 692–701.

Irving-Bell, R. J. 1983. Cytoplasmic incompatibility within and between *Culex molestus* and *Cx. quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 20: 44–48.

Ishii, T. and S. R. Sohn. 1987. Highly polluted larval habitats of the *Culex pipiens* complex in central Sweden. J. Am. Mosq. Cont. Assoc. 3: 276–281.

Jost, E. 1970. Genetische Untersuchungen zur Inkompatibilität im *Culex pipiens* Komplex. Theor. Appl. Genetics 40: 251–256.

Kimura, M. 1956. Rules for testing stability of a selective polymorphism. Proc. Natl. Acad. Sci. U.S.A. 42: 336–340.

Kingman, J. F. C. 1961. A mathematical problem in population genetics. Proc. Camb. Phil. Soc. 57: 574–582.

Krishnamurthy, B. S. and H. Laven. 1976. Development of cytoplasmically incompatible and integrated (translocated incompatible) strains of *Culex pipiens fatigans* for use of genetic control. J. Genet. 62: 117–129.

Laven, H. 1953. Reziprok unterschiedliche kreuzbarkeit von stechmücken (Culicidae) and ihre deutung als plasmatische vererbung. Z. indukt. Abstamm.-u VererbLehre. 85: 118–136.

Laven, H. 1957. Vererbung durch kerngene und das problem der Ausserkaryotischen vererbung bei *Culex pipiens*. II Ausserkaryotische Vererbung. Z. indukt. Abstamm.-u VererbLehre. 88: 478–516.

Laven, H. 1967a. Speciation and evolution in *Culex pipiens*, pp. 251–275. *In* J. W. Wright and R. Pal (eds.), Genetics of insect Vectors of Disease. Elsevier, North Holland, Amsterdam.

Laven, H. 1967b. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. Nature 216: 383–384.

Legrand, J. J., E. Legrand-Hamelin and P. Juchault. 1985. Le déterminisme du sexe chez les crustacés, en particulier les isopodes. Bull. Soc. Zool. Fr. 110: 31–48.

Lewontin, R. C., L. R. Ginzburg and S. D. Tuljapurkar. 1978. Heterosis as an explanation for large amounts of genic polymorphism. Genetics 88: 149–170.

Louis, C. and L. Nigro. 1989. Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationships with unidirectional cross-incompatibility. J. Invert. Pathol. 54: 39–44.

Magnin, M., N. Pasteur and M. Raymond. 1987. Multiple incompatibilities within populations of *Culex pipiens* L. in southern France. Genetica 74: 125–130.

Mandel, S. P. H. 1959. The stability of a multiple allelic system. Heredity 13: 289–302.

O'Neill, S. L. 1989. Cytoplasmic symbionts in *Tribolium confusum*. J. Invert. Pathol. 53: 132–134.

Poulson, D. F. and B. Sakaguchi. 1961. Hereditary infections in *Drosophila*. Genetics 46: 890–891.

Raymond, M., N. Pasteur, M. Magnin, G. Pasteur and G. Sinègre. 1986. Cytoplasmic incompatibility in the mosquito *Culex pipiens* L. from southern France: implication for the selection and dispersal of insecticide resistance genes in natural populations. Genetica 70: 113–118.

Sasa, M., A. Shirasaka and J. Kurihara. 1966. Crossing experiments between *fatigans*, *pallens* and *molestus* colonies of the mosquito *Culex pipiens s.l.* from Japan and Southern Asia, with special reference to hatchability of hybrid eggs. Jap. J. Exp. Med. 36: 187–210.

Subbarao, S. K. 1982. Cytoplasmic incompatibility in mosquitoes, pp. 313–341. *In* Recent developments in the genetics of insect disease vectors. Stipes publishing Co., Champaign, IL.

Subbarao, S. K., B. S. Krishnamurthy, C. F. Curtis, T. Adak and R. K. Chandrahas. 1977. Segregation of cytoplasmic incompatibility properties in *Culex pipiens fatigans*. Genetics 87: 381–390.

Subra, R. 1972. Etudes écologiques sur *Culex pipiens fatigans* Wiedemann, 1828 (Diptera, Culicidae) dans une zone urbaine de savane soudanienne ouest-africaine. Longévité et déplacements d'adultes marqués avec des poudres fluorescentes. Cah. O.R.S.T.O.M., sér. Ent. méd. et Parasitol. *10*: 3–36.

Tallis, G. M. 1966. Equilibria under selection for k alleles. Biometrics 22: 121–127.

Thomas, V. 1971. Studies on cytoplasmic incompatibility in Southeast Asian *Culex pipiens fatigans*. Southeast Asian J. Trop. Med. Pub. Hlth. 2: 469–473.

Wade, M. J. and L. Stevens. 1985. Microorganism-mediated reproductive isolation in flour beetles (Genus *Tribolium*). Science 227: 527–528.

Yen, J. H., and A. R. Barr. 1971. New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature 232: 657–658.

Yen, J. H. and A. R. Barr. 1973. The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. J. Invert. Pathol. 22: 242–250.

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