PROTOPLASMIC INCOMPATIBILITY IN PODOSPORA ANSERINA:
A POSSIBLE FUNCTION FOR INCOMPATIBILITY GENES

HÉLIAN BOUCHERIE AND JEAN BERNET

Laboratoire de Génétique, Allée des Facultés, 33405 Talence, France

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ABSTRACT

The suppression of protoplasmic incompatibility resulting from nonallelic gene interactions has been obtained by the coupled effect of mutations in the \textit{modA} and \textit{modB} genes (BERNET 1971). Due to their female sterility, \textit{modA} \textit{modB} strains provide an experimental tool to determine whether or not the \textit{mod} and incompatibility loci are involved in a function other than protoplasmic incompatibility. Present results show that \textit{modA modB} female sterility is a nonautonomous trait since heterokaryotic mycelia that include a \textit{modA modB} nucleus and a female fertile nucleus (wild-type, \textit{modA} or \textit{modB}) produce \textit{modA modB} protoperithecia, which are also formed by culture on medium supplemented with specific amino acids. Using \textit{modA modB} strains, which are sterile at 32°C and fertile at 26°C, we have shown that the \textit{mod} genes have no specific sequential timing. Indeed, the \textit{mod} mutations may prevent the achievement of the female sexual cycle at any developmental stage from before early differentiation of protoperithecia until ascospore maturation. Employing different \textit{modA} and \textit{modB} mutations, we have shown that protoperithecia in \textit{modA modB} cultures are generally distributed in female fertile rings; this result indicates that protoperithecia occur only in mycelial areas that have a restricted range of age at the time that \textit{modA modB} thalli complete growth. Furthermore, nonsense mutations of incompatibility genes suppress the \textit{modA modB} female fertile rings or restrict their width, suggesting that incompatibility loci, like the \textit{mod} loci, are involved in protoperithecium formation. Taken together, these results lead to the postulate that \textit{mod} and incompatibility genes do not determine, \textit{sensu stricto}, protoperithecial function, as previously supposed (BOUCHERIE and BERNET 1974), but may be involved in the homeostatic control of stationary cell functions essential for the complete development of the female sexual cycle.

IN \textit{Podospora anserina}, protoplasmic incompatibility resulting from the fusion of cells of unlike genotype is the consequence of the interaction of allelic and nonallelic incompatibility genes (RIZET and ESSER 1953; ESSER 1954; BERNET 1965). Allelic incompatibility depends on five loci \((b,q,s,v,z)\). Nonallelic incompatibility genes were found at nine loci, including the \(v\) locus involved in allelic incompatibility. Five of these \((c,d,e,r\) and \(v)\) were found in the genetic investigation of 16 geographical isolates (BERNET 1967) and four \((f,g,k\) and \(l)\) were produced by mutagenesis (DELETTRE and BERNET 1976). Protoplasmic incompatibility has also been shown in numerous other fungi (CARLILE and DEE 1967; Genetics 96: 399–411 October, 1980.
Three main hypotheses have been proposed to explain the widespread occurrence of protoplasmic incompatibility in fungi. Because it prevents protoplasmic mixing, protoplasmic incompatibility has been postulated to restrict outbreeding (Esser 1971; Esser and Blaich 1973), to protect the organism against invasion by pathological cytoplasmic factors (Caten 1972) or to prevent heterosis by discarding the association of nuclei that would be nonadaptive in homokaryons (Hartl, Dempster and Brown 1975). All three hypotheses have in common the idea that protoplasmic incompatibility is the sole function of incompatibility loci.

A different notion has arisen, however, from investigations of the nonallelic incompatibility systems of Podospora anserina. In this organism, mod mutations suppressing protoplasmic incompatibility have been selected (Belcour and Bernet 1969; Bernet 1971; Labarere and Bernet 1977). In three cases, the mutant mod strains (modA, modB and modC) are defective in protoperithecium formation, thus suggesting that the mod genes, and consequently the incompatibility genes, might have a role other than protoplasmic incompatibility (Boucherie and Bernet 1974; Boucherie, Begueret and Bernet 1976). Confirmation of this general notion has come from the study of two mutations in a fourth mod gene (modD). In addition to female sterility (Labarere and Bernet 1979a), modD strains are defective in the renewal of growth from stationary cells (Durrens et al. 1979). These investigations led to the postulate that proteolytic activity specific to protoplasmic incompatibility (Begueret and Bernet 1973) is normally associated with the destabilization of the stationary physiology preparatory to the renewal of growth or protoperithecium differentiation (Labarere and Bernet 1979b).

As previously indicated (Bernet 1971), protoplasmic incompatibility is abolished by the coupling of modA and modB mutations. Furthermore, the female sterility of the modA modB double-mutant strains has been shown to depend on the presence, at incompatibility loci, of wild-type genes from different geographic isolates (Boucherie, Begueret and Bernet 1976). Thus, the study of modA modB female sterility might provide a means for identifying a function of both the mod and incompatibility genes apart from the process of protoplasmic incompatibility. The present results show that mod and incompatibility genes are not directly involved in protoperithecium formation. Instead, they seem to play a role in the achievement of stationary functions essential for the complete development of the female sexual cycle.

**MATERIALS AND METHODS**

*Podospora anserina* is basically a heterothallic ascomycete. Asci contain 4 binucleate spores, including the 2 products of a half-tetrad. Due to the high post-reduction rate of the mating-type locus (98%), the spores give rise to heterokaryotic self-fertile mycelia; this phenomenon was termed secondary homothallism (Askeys 1934). Occasional uninucleate ascospores produced the (+ or −) self-sterile strains used in this work. For details see Esser 1974.
**Incompatibility genes and mutations:** At the \(c, d\) and \(e\) incompatibility loci, allelic series were found (Bernet 1967). Consequently, \(c(A), e(F)\) designate the \(c\) and \(e\) genes of the \((A)\) and \((F)\) geographical races. Nonsense mutations from the \(d(A)\) and \(e(C)\) genes (Labarere 1978) were designated by \(d(A)\) and \(e(C)\). Two wild-type alleles (\(R/V\) and \(V/V1\)) have been recognized at the \(r\) and \(v\) loci; the wild-type strains were \(rV\) or \(Rvi\).

The \(modA\) \(modB\) strains: ModA mutations are recessive; \(modA(1)\) is a nonsense mutation, and \(modA(2)\) is a thermosensitive mutation. ModB mutations are dominant (Bernet, Begueret and Labarere 1973); \(modB(1)\) is the most efficient mutation (in the suppression of both protoplasmic incompatibility and protoperithecium formation), and \(modB(2)\) is mutant only at 32°. The \(modA(1)\) \(modB(1)\) strain can produce protoperithecia only below 27°: at 26°, protoperithecia occasionally occur and, at 20°, are distributed in a female fertile ring that surrounds a sterile center (Boucherie, Begueret and Bernet 1976). \(modA(1)\) \(modB(2)\) and \(modA(2)\) \(modB(1)\) strains, which are always female fertile at 26°, are sterile at 32° (Boucherie and Bernet 1974).

The \(modC\) strain: The \(modC(1)\) mutation was selected on the basis of a suppression of protoplasmic incompatibility due to the \(R/V\) nonallelic interaction (Labarere and Bernet 1977). The complete female sterility of the \(modC(1)\) strain is an autonomous trait (Labarere and Bernet 1978).

**Heterokaryotic mycelia:** In order to determine whether or not the \(modA\) \(modB\) female sterility is an autonomous trait, \(modA\) \(modB\) + \(modC\) heterokaryotic mycelia were employed. The heterokaryons were constructed as shown in Figure 1. One inoculum of a homokaryotic strain \((X)\) was deposited in the center of the plate; 36 hr later, 4 inocula of a second strain \((Y)\) of the same mating type were placed 2 to 3 mm outside the margin of the \(X\) thallus (Figure 1A). Then, by means of concomitant growth and anastomosis, a heterokaryotic sector forms (Figure 1B). Apart from a few heterokaryotic cells, it mainly consists, as demonstrated by Bernet (1965), of a mixture of \((X)\) and \((Y)\) hyphae, with those arising from the center colony \((X)\) forming only 5 to 10% of the total. This procedure allows the construction of nonforced heterokaryotic thalli having, in addition, the desired disproportion of \(modC\) and \(modA\) \(modB\) nuclei.

The nuclear ratio was checked by means of a suspension of microconidia, collected from the supposed heterokaryotic areas, that was used to fertilize a homokaryotic mycelium of the opposite mating type. To determine the ratio of \(modC\) or \(modA\) \(modB\) fertilizing microconidia, the genetic content of a sample of 100 perithecia was investigated.

**RESULTS**

**modA modB sterility is a nonautonomous trait**

Heterokaryotic strains \(modA(1)\) \(modB(1)\) + \(modC(1)\) were constructed. The \(modC(1)\) strains employed in the heterokaryotic mycelia were of three genotypes: \(modA^+\) \(modB^+,\) \(modA(1)\) or \(modB(1)\). Due to the complete autonomy of the \(modC\) female sterility (Labarere and Bernet 1978), we expected the protoperithecium that appeared in the \(modA(1)\) \(modB(1)\) + \(modC(1)\) heterokaryotic sectors to be derived from the \(modA\) \(modB\) nucleus. Protoperithecium appeared in all three genetic \(modA\) \(modB\) + \(modC\) heterokaryons, whether the \(modA\) \(modB\) nucleus was the major component or the lesser (see Material and Methods). By contrast, homokaryotic areas of the mycelia \((modC\) or \(modA\) \(modB)\) were completely devoid of protoperithecium. When the cultures were fertilized by appropriate microconidia, perithecia developed only on the heterokaryotic areas. We verified, in a sample of about 100, that, as expected, no perithecia were derived from \(modC\) female nuclei. The perithecia were heterogenous in size. Some developed normally (i.e., having a wild-type number of ascis and mature ascospores), whereas most corresponded to sterile perithecia...
Figure 1.—Construction of heterokaryotic mycelial sectors between the homokaryotic strains X and Y. (■) indicates the position of the X and Y inocula.

arrested at any stage of their growth or containing few asci and ascospores. It could be observed that the development of the perithecia depended on their relative position inside the heterokaryotic area. In heterokaryons that predominantly contained the $modA$ $modB$ nucleus (Figure 2B), perithecia in the center were nearly all aborted, whereas those situated at the limit of the $modC$ homokaryon sector were all wild type. In contrast, when the number of $modC$ nuclei was higher in the heterokaryotic sector (Figure 2A), the perithecia in the center were more developed than those situated in the external areas in close contact with the $modA$ $modB$ homokaryotic sector.

<table>
<thead>
<tr>
<th>homokaryon</th>
<th>heterokaryotic mycelial sector</th>
</tr>
</thead>
<tbody>
<tr>
<td>X $modA(1)$ $modB(1)$ $modC$</td>
<td>$modC$ $modA(1)$ $modB(1)$</td>
</tr>
</tbody>
</table>

Figure 2.—Schematic representation of the perithecia obtained after the fertilization of the $modC + modA$ $modB$ heterokaryotic sectors: the different sizes of the black dots figuring the perithecia give an indication of their relative development. In (A), the most abundant nucleus is $modC$; in (B), $modA$ $modB$ (details in Figure 1).
These results clearly show that the modA modB genetic block that abolishes the formation of protoperithecia has a nonautonomous expression. Thus, it may be suspected that the function impaired by the modA modB mutations is not, sensu stricto, a protoperithecial function. Furthermore, because the relative development of modA modB perithecia is strongly dependent on the frequency of the nucleus (modC) carrying the wild-type mod alleles (modA+ modB+, modA+ or modB+), it can be concluded that the focus of the modA and modB gene action is the protoplasm of the cells surrounding the perithecium, i.e., the stationary cells. In addition, the fact that the presence of the mutations not only prevented the differentiation of protoperithecia, but also inhibited their further development, suggests that the action of two mod genes is not subject to a specific timing.

modA and modB gene action shows no specific timing in the female developmental cycle

The effect of thermosensitive mutations of modA and modB genes on modA modB female fertility was investigated by means of the modA(2) modB(1) and modA(1) modB(2) strains that are fertile at 26°, but sterile at 32°. The strains were grown at 26° and then exposed at the nonpermissive temperature (32°) after the completion of growth, the prerequisite for the differentiation of protoperithecia. No protoperithecia formed. In the reverse situation, when the strains were grown at 32° and exposed at 26° at the onset of the stationary stage, complete female sterility was also observed. This result showed that the action of the modA and modB genes is not restricted to stationary cultures. Furthermore, an area of the mycelium that was exposed to the nonpermissive temperature during growth or immediately after its completion never produced protoperithecia.

Protoperithecia of modA(1) modB(2) and modA(2) modB(1) genotypes were obtained after continuous exposure of the cultures to the permissive temperature. In a first experiment, the cultures were exposed for 36 hr at 32° and then placed at 26° prior to their fertilization. No perithecia developed. In a second experiment, the cultures were fertilized at 26°, shifted 24 hr later for 36 hr to 32° and placed again at 26°. No perithecia developed completely. Indeed, most of them were arrested in the early stages of their development, and those that appeared normal contained a small number of asci and immature ascospores.

These experiments showed that the incubation of a mycelial area at the nonpermissive temperature always inhibits the achievement of the female sexual cycle, thus indicating the constant need for the wild-type mod gene products (modA or modB) throughout the developmental life cycle.

modA modB sterility is conditional

Influence of culture size: The consequences of culture size on the presence and distribution of protoperithecia in modA(1) modB(1) and modA(1) and modB(2) strains are summarized in Figure 3. In modA(1) modB(2) cultures, the female fertile ring reaches the limit of the Petri dish, whereas in modA(1) modB(1) cultures, it is surrounded by a sterile margin 5 mm in width. In this
latter case, when the diameter of the Petri dish was 10 cm, the cultures were completely sterile or (in 20% of the cultures) showed a fertile area where protoperithecia were found in wild-type density. When the diameter increased, the fertile ring was always present and its width (2 cm) and distance to the margin of the thallus were constant; only the diameter of the center sterile area increased. In modA(1) modB(2) cultures, only the diameter of the sterile area covering the center of the plate remained constant (about 2 cm in diameter).

These results show that, in modA modB mutant strains, two factors are important to obtain the formation of protoperithecia. The first of these is a minimal diameter of the cultures; it is the only factor that conditions the female fertility of the modA(1) modB(2) strain. The second condition was found in the case of the modA(1) modB(2) cultures: protoperithecia appear only in areas whose cells have a restricted range of age (about 15 to 72 hr) at the time of the completion of growth. It should be noted that wild-type (or modA or modB) cultures are not subject to such restrictions. Indeed, in our culture conditions, wild-type protoperithecia are distributed at an apparently uniform density over the entire surface of the mycelia, indicating that they develop irrespective of the culture size and the age of the cells.

Effect of amino acids: It is known that the development of the sexual cycle in
TABLE 1

Effect of various amino acids on the relative density of protoperithecia of a wild-type strain and of the modA(1) modB(1) female sterile strain at 26°

<table>
<thead>
<tr>
<th>Amino acid added (10^{-4} M)</th>
<th>Wild-type</th>
<th>modA(1) modB(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>155</td>
<td>140</td>
</tr>
<tr>
<td>asparagine</td>
<td>150</td>
<td>95</td>
</tr>
<tr>
<td>tyrosine</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>leucine</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>isoleucine</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>tryptophan, glycine, proline, methionine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>glutamine, glutamic acid, valine, aspartic acid, alanine</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>serine, histidine</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>arginine</td>
<td>160</td>
<td>0</td>
</tr>
</tbody>
</table>

many fungal species is promoted by the addition of biological molecules, particularly amino acids (Bull and Busheir 1976). We investigated the effect of various amino acids on both the female fertility of the wild-type and the sterility of the modA(1) modB(1) strains. The results are given in Table 1. Five amino acids suppressed modA modB sterility, the most efficient being phenylalanine. Figure 4 summarizes the results obtained with various concentrations of phenylalanine. At 10^{-4} M, protoperithecia were formed at wild-type density, but only in an external fertile ring. At 10^{-3} M, protoperithecia occurred over the entire surface of the mycelia. Phenylalanine apparently can suppress the age conditions that restrict the formation of protoperithecia in modA modB strains.

Effects of incompatibility loci on the modA modB female sterility

As previously reported (Boucherie, Begueret and Bernet 1976), modA

![Figure 4](image-url)
modB female sterility is dependent on the presence of specific wild-type alleles at incompatibility loci. To investigate in detail the relationship between these genes and modA modB sterility, we have chosen for reference the genotype of the (F) geographic isolate for the c, d, e, r and v incompatibility loci: i.e., c(F) d(F) e(F) RV1. modA(1) modB(1) strains carrying these c, d, e, r and v genes, which are nearly sterile in standard conditions (cultured at 27° in 10 cm diameter Petri dishes) always developed protoperithecia distributed in a ring when cultures are grown at 20° (BOUCHERIE, BEGUERET and BERNET 1976). Nonsense mutations in the d and e incompatibility loci, namely d°(A) and e°(C), have been considered. These mutations, which per se had no detectable consequence (LABARERE 1978), were substituted in strains modA(1) modB(1) for the wild-type alleles d(F) and e(F). The results in Figure 5 show that the presence of the nonactive d and e genes results in the disappearance at 20° of the modA modB fertile ring. In the sterile c(F) d°(A) e°(C) basic genotype, the R and V1 genes were replaced by their wild-type alleles, r and V. Since the RV genotype, combining the R and V incompatibility genes, was lethal (LABARERE 1973), these substitutions lead to two additional genetic combinations rV1 and rV. The results in Figure 6 show that the rV1 combination significantly suppressed the female sterility associated (at 20°) with the d°(A) e°(C) modA(1) modB(1) genotype. This example shows that the effect of the r and v loci on the modA modB sterility does not result from the addition of the individual effect of the r and V1 genes, but from their specific interaction.

Results (not reported) showed that the presence of mutations in the f, g, k, l loci that were produced by mutagenesis (DELETTRE and BERNET 1976) resulted in a complete suppression of the partial female sterility at 20° of the modA(1) modB(1) genotype. Like the nonsense d°(A) and e°(C) mutations, these f, g, k and l mutations have not, outside of the modA(1) modB(1) genotype, any apparent consequence on the female fertility and wild-type growth features.

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**Figure 5.**—Effect of wild-type and mutant alleles of the d and e incompatibility loci on the formation of protoperithecia in double-mutant modA(1) modB(1) strains grown at 20°.
**Genes at r and v loci**

<table>
<thead>
<tr>
<th>RV₁</th>
<th>rV₁</th>
<th>rV</th>
</tr>
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<tbody>
<tr>
<td>![Symbol]</td>
<td>![Symbol]</td>
<td>![Symbol]</td>
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**FIGURE 6.**—Effect of the wild-type alleles of the r and v loci on the formation of protoperithecia in the mutant strain $d^0(A) e^0(C) modA(1) modB(1)$ at 20°C.

**DISCUSSION**

The existence of a specific differentiation of stationary cells that sustains the development of the female sexual cycle: Two features of modA modB sterility are important in trying to understand the effect of the mod mutations on protoperithecum formation. First, whenever the modA modB nucleus is part of a heterokaryotic mycelium containing a nucleus with the modA⁺ or modB⁺ wild-type genes, the formation of modA modB protoperithecia is obtained. This result indicates that the modA modB female sterility is a nonautonomous trait, since a single nucleus is assumed to give rise to each protoperithecium (RIZET and ENGELMAN 1949). In support of the latter assumption is the complete autonomy of the modC mutation responsible for female sterility (LABARERE and BERNET 1978). Second, using thermosensitive mutations of the modA and modB genes, we have shown that the need for the modA or modB wild-type gene product is constant throughout the developmental life cycle practically from the onset of growth up to its completion, the development of protoperithecia or perithecia and ascospore maturation. These two features of the modA modB female sterility show that the function impaired by the modA modB genetic block is not a protoperithecial one, as originally thought (BOUCHERIE and BERNET 1974), but a function of cells that do not undergo sexual differentiation. The results show that this function exists before the initiation of protoperithecum differentiation and lasts during the late stationary stages until the attainment of the next generation by means of ascospores.

modA and modB and incompatibility loci are involved in a homeostatic control of the stationary physiology: The modA modB female sterility is extremely sensitive to various extrinsic and intrinsic factors. This feature has not generally been found in cases of female sterility described in Podospora (LABARERE and BERNET 1978; LABARERE and BERNET 1979a; DURRENS et al. 1979) or in other fungi (TURIAN 1975; SCOTT 1976; JOHNSON 1978).
Among the mutations of the \textit{modA} and \textit{modB} genes, \textit{modA(1)} and \textit{modB(1)} show the most severe effects. \textit{modA(1)} is a nonsense mutation (Begueret 1973), whereas \textit{modB(1)} was recognized, in a sample of more than 60 dominant mutations of the \textit{modB} gene, by having the most marked consequences (Boucherie and Bernet 1974). Thus, the coupling of the \textit{modA(1)} and \textit{modB(1)} mutations showed the strongest suppression of both protoplasmic incompatibility and protoperithecium formation. Despite the drastic conditions for the selection of \textit{modA(1)} and \textit{modB(1)}, the \textit{modA(1) modB(1)} strain cannot be considered as a female sterile strain since nearly all wild-type female fertility can be restored under several conditions: at low temperature, by culture on a starvation medium or in the presence of dihydrostreptomycin (Boucherie and Bernet 1974; Boucherie, Begueret and Bernet 1976) or by the addition of specific amino acids to the culture medium (present results. Together, these results clearly indicate that the extreme conditionality of the \textit{modA modB} female fertility is an important and significant feature of the action of the \textit{mod} genes. The potential for forming normal protoperithecia and perithecia is not dependent on the \textit{mod} genes. Furthermore, the trigger mechanism for protoperithecium formation, which is normally associated with the completion of growth, is not defective in \textit{modA modB} strains. Thus, the results support the idea that the \textit{mod} gene products are not direct intermediates in the protoperithecium forming process, but instead have a regulatory role. In effect, it appears that the \textit{mod} genes are involved in a homeostatic control of stationary functions that, as a possible specific role, ensure the complete development of the female sexual cycle in a large range of conditions. Two examples for this control may be given. First, most of the \textit{modA modB} strains never produced protoperithecia at 32° on a rich medium (standard laboratory conditions) or in the absence of some specific amino acids. Thus, it can be deduced that the wild-type \textit{mod} genes (\textit{modA} and \textit{modB}) may have the role of sustaining the development of the female sexual cycle under conditions that would be, in their absence, completely unfavorable for protoperithecium formation. Second, the formation of protoperithecia in \textit{modA modB} cultures is subject to two conditions: a minimal size of the thallus and restrictive age conditions at the time of growth completion. Since wild-type protoperithecia are always distributed over the entire surface of the mycelia, it may be deduced that the \textit{mod} genes play the role of insuring the formation of protoperithecia (and their further development) as independently as possible of the age of the cells and of the relative mycelial extension.

The extent of the female-fertile rings of the \textit{modA modB} cultures is also dependent on the presence of specific genes at the incompatibility loci. For example, nonsense mutations at the \textit{d} and \textit{e} incompatibility loci, which have no consequence \textit{per se} on female fertility, reduce the extent of the fertile areas in the \textit{modA(1) modB(1)} strains. For the \textit{r} and \textit{v} incompatibility loci, a nearly complete restoration of the female fertility of the \textit{modA(1) modB(1)} genotype was obtained only by means of the nonallelic \textit{rV1} combination. This observation suggests that these loci (which carry nonallelic \textit{R} and \textit{V} incompatibility genes) have a role through a specific interaction between the \textit{r} and \textit{v} gene products (for
instance, rV1) rather than by the addition of the individual effect of the genes (r and V1). The same conclusion was previously drawn (Boucherie, Begueret and Bernet 1976) by investigating different wild-type genes from the c, d or e loci (involved in the C/D and C/E incompatibility systems); the female fertility of the modA(1) modB(1) strain depended on the specificity of the c/d and c/e combinations. Since mutations in the f, g, h and l loci, which are incompatibility loci created by mutation (Deletrre and Bernet 1977) increased the female sterility of the modA modB strains, it is now clear that all incompatibility loci involved in nonallelic interactions have an effect on modA modB sterility. Thus, it can be postulated that the incompatibility loci may represent the active components of the homeostatic system that controls the development of the female sexual cycle; consequently, the modA and modB gene products may be considered as intermediates of the incompatibility genes’ action.

*Protoplasmic incompatibility may be a pathological deviation in a process sustaining the differentiation of stationary physiology: From the results discussed above, it can be concluded that nonallelic protoplasmic incompatibility and the female sterility associated with the modA modB mutations are under the control of identical genes: the modA and modB genes and the incompatibility loci. Thus, it may be deduced that protoplasmic incompatibility is the deviant expression of functions depending on mod and incompatibility genes, which, as postulated above, are involved in the achievement of the stationary physiology. The idea of a possible relationship between incompatibility and stationary physiology is an enlargement of a previous suggestion (Labaree and Bernet 1979b) that inferred that the proteolytic activity responsible for the cell lysis specific to protoplasmic incompatibility (Begueret and Bernet 1973) is normally associated with stationary functions, such as protoperithecium formation and growth renewal. It appears that protoplasmic incompatibility occurs only as a trivial consequence of mutations affecting the polygenic system that we suspect of being involved in the control of the stationary physiology. The existence of racial divergences in correlation with the occurrence of strong incompatibility systems between Podospora wild-type strains isolated in a restricted geographic area has been previously outlined (Bernet et al. 1960; Bernet 1965); these divergences have been explained by the severe inbreeding that results from the secondary homothallism.*

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**LITERATURE CITED**


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