tention of heterogeneity is supported by the work of Sokoloff *et al.* (*5*), who have reported heterogeneous rates of glucose utilization within specific brain structures, often related to the pattern of cytoarchitecture. This heterogeneity of venous oxygen saturation, tissue oxygen tension, and glucose utilization does not appear to lead to large regional (for example, pons versus medulla) differences in oxygen consumption. Our results indicate that regional oxygen consumption in anesthetized cat brain is relatively uniform despite this heterogeneity.

Our blood flow values are similar to those reported by others for the anesthetized cat (*8*) although slightly lower than that reported for conscious animals (*10*). Chloralose as well as barbiturate anesthesia tend to reduce both cerebral blood flow and regional differences in that flow (*11, 12*). These results may be due to the tendency of anesthesia to reduce perfusion of most brain regions to a basal value, one similar to that found in the pons and medulla. The relative homogeneity of regional blood flow in the brain may be due to a reduction in the rate of cerebral metabolism (and possibly in neuronal activity) to a relatively uniform level throughout the brain. Small differences in microflow revealing a heterogeneity of capillary blood flow in the brain—which may be related to differences in capillary length, flow, or both (*13*)—may also be rendered more uniform by anesthesia.

Our regional oxygen consumption values for the brain are similar to those global values reported by others (*5, 12*). Our study reveals a lack of regional differences in brain oxygen consumption. Studies of significantly smaller brain structures have shown heterogeneity of glucose utilization (*5*). This heterogeneity appears to be averaged out in structures as large as the thalamus or lenticulate nuclei. Anesthesia may have depressed regional brain oxygen consumption and metabolism so that metabolic rate throughout the gray matter became more uniform at a lower level. The moment-by-moment differences in microflow (*9*), which may be dampened by anesthesia, when combined with a depression of differences in activity among the various types of neuronal and glial components of a region, may result in a relatively uniform oxygen consumption throughout the anesthetized brain. Although there were no regional differences in cerebral oxygen extraction, flow, and consumption, there were significant differences between animals that might be due to differences in age, sex, weight, strain, and depth of anesthesia.

Our study demonstrated that the supply of oxygen to the various brain regions studied was at least 2.5 times the metabolic needs throughout the brain. Oxygen extraction was relatively low in any given region. This is consistent with hypotheses that the brain is able to maintain adequate blood flow under most circumstances to meet its relatively constant metabolic needs (*14*). The relation of oxygen supply to demand in the brain is higher and more uniform than in the heart. In the heart, the subendocardial region of the left ventricle has a lower oxygen supply-to-demand ratio than the rest of the organ, and the ratio is much lower throughout the heart than the brain (*7*). Whereas under α-chloralose anesthesia all brain regions have an adequate and uniform oxygen supply-to-demand relation, it is not possible to predict what would happen under stressful conditions, such as hypoxia. Different brain regions may have very different blood flow and oxygen extraction reserves.

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Multiple Mating-Type Specificities in the Flax Rust *Melampsora lini*

Abstract. *The results of selfing and intercrossing two strains of the flax rust fungus* Melampsora lini *indicate that this species does not possess a simple (+) and (−) mating system. Instead, the data are consistent with the assumption that the genetic control of mating type in* Melampsora lini *is similar to that in the mushroom* Schizophyllum commune, *in which mating type is determined by two factors, each of which is controlled by two linked loci.*

In heterothallic rust fungi (Uredinales), the haploid, monokaryotic infections (pycnia) develop dikaryotic aeciospores only after they are fertilized with "nectar" (a liquid exudate containing haploid pycniospores) from another pycnium of a different mating type. In 1927, Craigie (*1*) proposed that the pycnia of sunflower rust, *Puccinia helianthi*, and wheat stem rust, *P. graminis*, were of two mating types, which he designated (+) and (−). Since then, a (+) and (−) mating system, assumed to be controlled by two alleles at a single locus, has been accepted as common to all heterothallic rusts (*2*). Under this system, 50 percent of crosses between two pycnia should result in aecia formation, irrespective of whether the pycnia being crossed are from the same or different strains.

During a recent study of the inheritance of pathogenicity in the flax rust organism *Melampsora lini*, I found that crosses between pycnia of one strain (CH_5) and those of an unrelated strain (I) resulted in aecia formation 47 out of 48 times. Since this result is clearly inconsistent with a (+) and (−) mating system, I undertook a study to investigate more fully the genetic control of mating type in *M. lini*.

Strains CH_5 and I were used in this study. Strain CH_5 is a hybrid strain derived from intercrossing strains C and H. Strain C was obtained by self-fertilizing New Zealand race 5 (*3*). Strain H is phenotypically the same as race 228 of North American origin (*4*), but was shown to be genotypically different (*5*) from the race 228 used by Flor (*6*). Strain

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I is race 271 (North American series).

In the crossing scheme, 150 pycnia of each strain were used as recipients of nectar; of these, 100 received nectar from pycnia of the same strain (selfings) and 50 received nectar from pycnia of the other strain (intercrossings). Flax plants (*Linum usitatissimum*, variety Hoshangabad) lacking genes known to confer resistance to *M. lini* were inoculated with haploid basidiospores by suspending germinating teliospores of a parent rust strain over the plants for several hours in a humidity chamber. Seven or eight days later, when the pycnial infections derived from the basidiospores were visible (but before they had produced nectar), all leaves with more than one infection were removed from the plants.

At this point, the plants were transferred from the greenhouse to a growth cabinet (23°C during "day," 18°C at "night"), where they were placed under frames enclosed with clear cellophane to exclude insects that might be attracted to the nectar and make uncontrolled crosses. Crosses were made about 5 days after the first appearance of nectar by transferring pycniospores from one pycnium to another. This was accomplished by adding a drop of tap water to the tip of a plastic toothpick, and touching this drop to the nectar of the donor pycnium and then to the nectar of the recipient pycnium. A different toothpick was used for each transfer. A pycnium used as donor in one test was not used in any other test.

In compatible crosses, recipient pycnia first developed a ring, or partial ring, of orange tissue that subsequently broke through the epidermis to release aeciospores, usually on the fourth or fifth day after transfer of the nectar. Recipient pycnia that did not produce aeciospores either underwent no apparent change and continued to produce nectar, or formed an orange crust or scab and lost nectar. Pycnia were scored for aeciospore production 8 days after receiving nectar.

In studies of mating type in Uredinales, special care must be taken to prevent experimental error, which can occur if rust stocks become contaminated with other strains, if insects make uncontrolled crosses, if the crossing technique is poor, or if the environmental conditions are unsuited for aeciospore development in compatible crosses. In the present study, the possibility of contamination was reduced by producing teliospores of the parent strains from uredospores obtained by asexual multiplication of a single pustule of uredospores on plants kept isolated in a greenhouse several hundred meters from any other rust-infected plants. A further check on insect activity (in addition to the cellophane covers described above) was possible because usually fewer than one-third of the pycnia on the plants in any one pot were used in the crossing study. Since with few exceptions the uncrossed pycnia did not develop aeciospores, insect activity was either nonexistent or very low. Finally, the observation that only 2 out of 100 crosses between strains CH_5 and I failed to produce aeciospores suggests that the crossing technique and the environmental conditions were quite adequate.

Table 1. Results of selfing and intercrossing *M. lini* strains CH_5 and I.

Cross*	Response of recipient pycnia	
	Aeciospores produced	Aeciospores not produced
$CH_5 \times CH_5$	21	79
$I \times I$	35	65
$CH_5 \times I$	48	2
$I \times CH_5$	50	0

*Recipient strain is given first.

Table 1 gives the results of the crossing study. Of particular interest is the finding that crosses between pycnia of strain CH_5 with those of strain I once again resulted in aecia formation in nearly every instance. This observation suggests that strain CH_5 produces pycnia with mating-type specificities unlike those possessed by pycnia of strain I, indicating that *M. lini* does not possess a simple (+) and (−) mating system. This conclusion is supported by the results of the two selfings, where, in each case, the proportion that produced aecia was significantly less than the 50 percent expected for a (+) and (−) mating system ($P < .001$ for CH_5 selfings, $P < .01$ for I selfings).

Although only two strains of *M. lini* were used in this study, the results allow rejection of some postulated genetic controls of mating type in this species. First, the data are not consistent with mating type being determined by multiple alleles at a single locus, since under such a model 50 percent of selfings are always expected to produce aecia, and since the results of selfing strains CH_5 and I are not in agreement with this expectation. Second, the data are not consistent with a simple two-locus model. If genes at two loci control mating type, with an allelic difference at both loci being necessary for compatibility (aeciospore formation), then 25 percent of selfings should produce aecia—assuming that the genes at the two loci are inherited independently of each other. Although the results of the CH_5 selfings are in agreement with this expectation ($P = .30$ to $.50$), the results of the I selfings are not ($P < .05$).

The results of selfing strain CH_5 differ significantly from the results of selfing strain I ($P < .05$). A similar finding was reported by Flor (*6*), who made extensive studies of the genetics of pathogenicity in *M. lini*. He reported that with one strain, only 15 to 20 percent of selfings produced aecia, whereas in two previous studies with different strains, approximately 50 percent of selfings produced aecia. If it is assumed that the experimental error in these results is small, then any model that is proposed for the genetic control of mating type in *M. lini* must permit different proportions of selfings to be compatible.

A model that meets this requirement is based on the incompatibility system in *Schizophyllum commune*, which, like *M. lini*, is a basidiomycete. In *S. commune*, mating type of monokaryons is determined by two factors, both of which must differ for a mating to be compatible. Each factor is controlled by two linked loci, with an allelic difference between two monokaryons at either or both of the loci controlling each factor giving a different factor (*7*). Under such a system, 25 percent of selfings are expected to be compatible if the parent strain is heterozygous at just one of the loci controlling each factor. However, if the parent strain is heterozygous at both loci controlling a factor, then the proportion of selfings expected to be compatible will be greater than 25 percent. The maximum proportion of compatible selfings will occur when the parent strain is heterozygous at all four loci controlling mating type, with the value depending on the amount of recombination between the two loci controlling each factor. Such a model can account for the present data on mating type in *M. lini*, but must still be considered tentative.

Since *M. lini* does not possess a simple (+) and (−) mating system, it is pertinent to reexamine the evidence for a (+) and (−) system in other rust species. Whitehouse (*8*) noted that "no tests appear to have been made to determine whether the heterothallism of the Uredinales is of the two-allelomorphic or the multiple-allelomorphic type"; that is, no study involving crosses between pycnia of different strains has been reported. A search of subsequent literature indicates that Whitehouse's statement is still valid for rust species other than *M. lini*. The initial proposal by Craigie (*1*) that *P. helianthi* possesses a (+) and (−) mating system was based on the observation

that about half of 175 paired pycnia produced aecia. In a later report by Craigie (*9*), more detailed data were given. In two studies with *P. helianthi*, the ratios of pycnia producing aecia to those that did not produce aecia were 108:138 and 15:33. Similarly, in two studies with *P. graminis*, ratios of 24:35 and 30:44 were obtained. Each of these do not differ significantly from a 1:1 ratio except the 15:33 ratio, which differs significantly at $P < .01$. In a further study with *P. helianthi*, Brown (*10*) observed 288 pairs of coalescing pycnia and found that 110 produced aecia and 178 did not, which differs from 1:1 at $P < .001$. In all five sets of data, the number of crosses that produced aecia was less than the number that did not. Brown does not give the source of the teliospores he used in his tests, but Craigie's *P. helianthi* teliospores were collected from a field of sunflowers (*Helianthus annuus*), and those of *P. graminis* came from wild barley (*Hordeum jubatum*). Therefore it is possible that these teliospores may have included more than one strain, in which case some of the fertilization tests carried out in Craigie's study could have been between pycnia of the same strain and others between pycnia of different strains. This would give misleading results if the mating system were not of a (+) and (−) kind.

Although the earlier studies clearly established the heterothallic nature of several rust species, they did not determine with any certainty that only two types of thalli occur. Thus the suggestion that heterothallic rust species possess a (+) and (−) mating system, which has become widely accepted, is not well supported by the present data.

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Limitations of Metabolic Activation Systems Used with in vitro Tests for Carcinogens

Abstract. *Important differences between the metabolic activation of 7,12-dimethylbenz[a]anthracene in intact cellular systems and in liver homogenates suggest that the use of homogenates in conjunction with short-term assays for carcinogens could yield misleading results.*

Percival Pott first recognized chemical carcinogens more than 200 years ago (*1*) when he examined the environmental history of cancer victims. Since recent exposures to chemical carcinogens have been discovered (*2*) in the same manner, the development of reliable and rapid means of recognizing carcinogenic chemicals before human exposure occurs is a prime objective of research in carcinogenesis. The *Salmonella* mutagenicity assay designed by Ames *et al.* (*3*) represents a major step toward this goal (*4*). Nevertheless, if the full potential of such tests in assessing risk to man is to be realized, further development of the metabolic activation system used in the assay may be necessary. At present, we feel there is a need for caution concerning the interpretation of results from this and other short-term tests that incorporate rat liver homogenates for metabolic activation of test compounds. This follows from the studies of activation of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA), which we now report, indicate that the activation in such systems differs from that of intact cellular systems and from that of a target tissue for this carcinogen.

Since the interaction of chemical carcinogens with DNA is considered a critical event in the carcinogenic process, we investigated the metabolic activation occurring in various systems by comparing the products of the binding of DMBA to DNA in these systems. The "bay region" (that is, the region between positions 1 and 12 in DMBA) diol epoxide of DMBA (DMBA-3,4-diol-1,2-epoxide) is apparently responsible for the binding of this carcinogen to DNA in mouse skin (*5–8*), and the carcinogenic potency of its precursor, the 3,4-diol (*9*), indicates that this diol epoxide is also the ultimate carcinogenic form of DMBA. Thus, the ability of an in vitro system to generate DNA adducts through this metabolite can be used to assess how accurately the system simulates target tissue activation.

DNA was isolated from mouse skin and various cells in culture exposed to [^{3}H]DMBA or recovered from incubation mixtures consisting of calf thymus DNA, [^{3}H]DMBA, cofactors, and rat liver microsomes or S9 fraction. After purification, the DNA was enzymatically hydrolyzed to hydrocarbon-deoxyribonucleoside adducts, and these were subjected to chromatography on Sephadex LH-20 columns eluted with a methanol-water gradient (*10*). This yielded chromatographic profiles of the type illustrated in Fig. 1 where hydrocarbon-deoxyribonucleoside adducts elute after 250 ml of eluant has passed through the column but the early eluting radioactivity is as yet uncharacterized (*5–7*). The DMBA-DNA adducts from mouse embryo cells exposed to [^{14}C]DMBA were included in each chromatogram in the present study since these adducts are chromatographically identical to the adducts formed in a target tissue, mouse skin (*5, 11*).

At a high ratio of DMBA to microsomal protein (for example, 320 nmole/mg) binding of DMBA to DNA (catalyzed by Aroclor-induced rat liver microsomes) occurs through DMBA-5,6-oxide rather than through the diol epoxide (*5*). However, at lower DMBA concentrations (for example, 24 nmole/mg), some adducts are generated which elute in coincidence with the ^{14}C-labeled diol epoxide adducts (Fig. 1a), although other adducts are also present (*11*). An even wider variety of adducts is generated when microsomes are replaced by the cruder S9 fraction (Fig. 1b), but the same general principle seems to apply. At high DMBA concentrations, DMBA-5,6-oxide is responsible for much of the binding; at lower DMBA concentrations (Fig. 1b), ^{3}H-labeled adducts eluting in coincidence with the ^{14}C-labeled diol epoxide adducts are present although they represent only a small fraction of the total binding (*12*). In contrast to these findings with the subcellular fractions from liver, no dose-dependent qualitative changes in adducts have been observed in mouse embryo cells in culture exposed to doses of DMBA yielding the same levels of binding obtained (up to 70 μmole of DMBA bound per mole of DNA phos-

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