SOMATIC RECOMBINATION IN *USTILAGO HORDEI* DURING THE PARASITIC PHASE ON BARLEY

by

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ABSTRACT

The question investigated in this study was whether or not somatic recombination can occur in Ustilago hordei whilst it is in the parasitic phase on barley. The investigation was carried out in two parts.

In the first and major part, barley seeds were inoculated artificially with mixtures of nutritionally deficient mutants of U. hordei. This was done in such a way that infection of the mature plants could only occur if at least one recombination event had taken place asexually, between at least two infective dikaryons, prior to teliospore formation. One smutted plant was found. Detailed analysis of the teliospores from this infection was carried out.

The second part of the study was designed to demonstrate that more than one infective dikaryon can simultaneously occupy the host. Again, mixtures of nutritionally deficient mutants were used to inoculate barley seeds but this time so that the origin of teliospores from any infection could be traced back to the parental types in the infective dikaryon.

It was concluded that somatic recombination can in fact occur whilst the fungus occupies the host tissue.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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LITERATURE REVIEW

The extreme variability amongst plant pathogenic fungi has been known for a long time. Even where normal sexual reproduction is impossible because the alternate host is absent or mating types are incompatible, the pathogenic organisms often continue to show extensive variability. This is especially true of the smuts, which may be more variable than any other group of plant pathogenic fungi (Holton,1968; Cherewick,1958). Here variation abounds in both morphology and physiology.

In many cases where sexual reproduction is absent, the diversity of progeny types cannot be explained on the basis of gene mutation alone. For this reason, parasexuality (Pontecorvo, 1956), otherwise known as somatic recombination, has been suggested as the mechanism by which these pathogenic fungi maintain their variability. Parasexuality may be thought of as genetic recombination where no fine coordination between recombination, segregation and reduction exists. In fact, the operation of parasexuality has been implicated in a number of plant pathogens, including several rusts (Bartos et al.,1969; Bridgmon,1959; Ellingboe,1961; Sharma and Prasada,1969; Vakili and Caldwell,1957; Waterhouse,1952; Watson,1957; Watson and Luig,1958, 1959, 1962;), smuts (Rowell,1955; Kozar,1969; Day and Anagnostakis,1971) and Verticillium (Ingram,1968; Hastie,1962), Cephalosporium (Tuveson and

Coy,1961), Phytophthora (Wilde,1961; Leach and Rich,1969) and Fusarium (Buxton,1956) species.

Implicit and perhaps more important than variability in a discussion of plant pathogenic fungi is the means by which new races are originated. There are many cases in the rusts, for example, where new races have been observed to arise asexually (Bartos et al.,1969; Bridgmon, 1959; Bridgmon and Wilcoxson,1959; Bugbee et al.,1968; Ellingboe,1961; Flor,1957, 1960; Little and Manners,1967; Nelson et al.,1955; Nelson,1966; Sharma and Prasada, 1969; Vakili and Caldwell, 1957; Waterhouse, 1952; Watson, 1957; Watson and Luig, 1958, 1959, 1962). It has been known for many years that new races and biotypes of parasitic fungi arise through hybridisation, mutation, and heterokaryosis but it seems not unreasonable to suspect that somatic recombination is also responsible in large part for the origin of new races (Person, 1958).

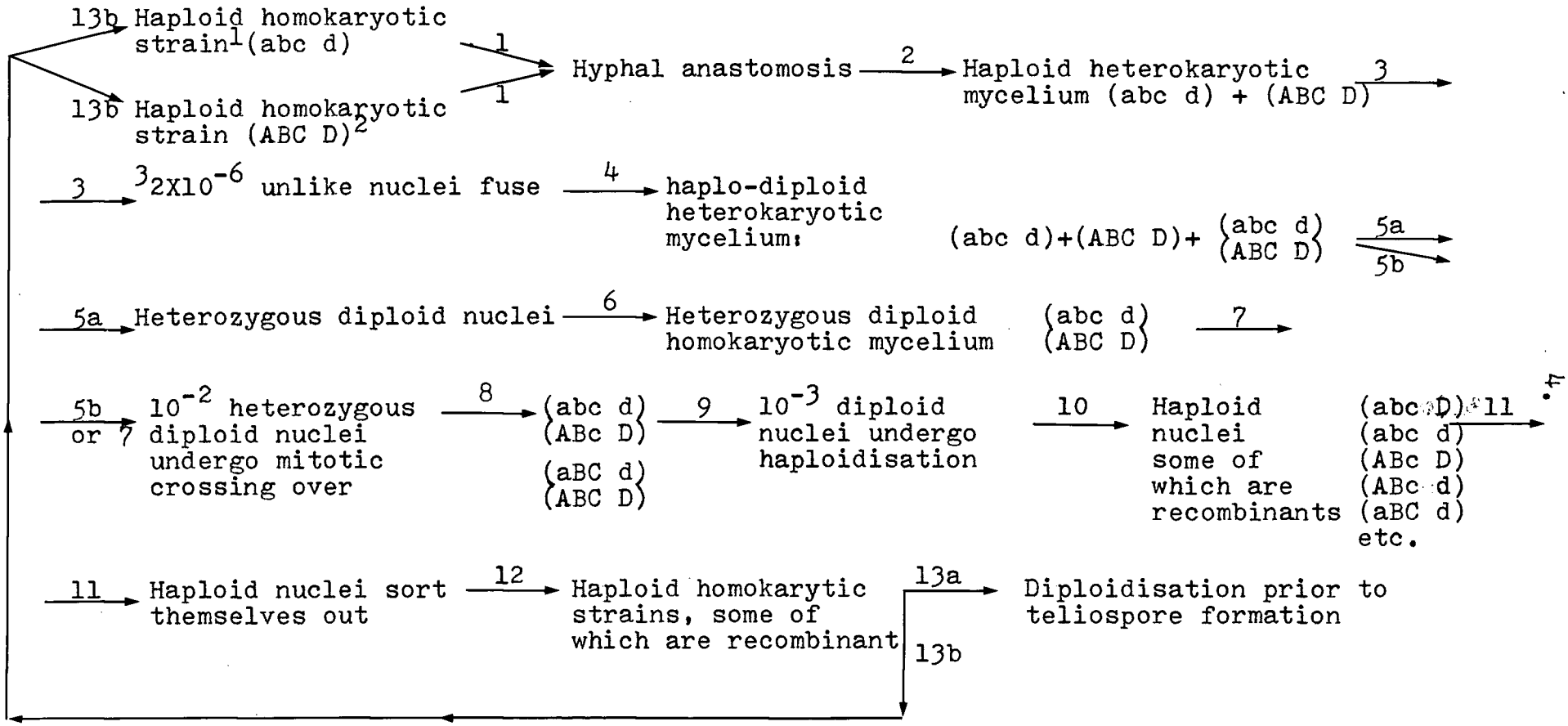
The parasexual cycle has been shown to operate in a variety of fungi including Aspergillus nidulans, (Pontecorvo and Raper, 1952), A. niger, and Penicillium chrysogenum, (Buxton, 1956), Coprinus species and Schizophyllum commune (Casselton, 1965), as well as in the phytopathogenic fungi mentioned above. It appears therefore, legitimate to conclude that the parasexual cycle is not a rare oddity but that its occurrence is fairly widespread

in nature and that it may well play a part in the variation of host-parasite relationships. The precise extent of the operation of the parasexual cycle in nature cannot be gauged since many species in which it might occur have so far not been suitable for experimentation of this kind. A major difficulty in assessing the occurrence of parasexuality in nature is due to the lack of criteria for its detection. Nevertheless, sufficient evidence is available to make a study of the process of somatic recombination in a pathogen such as Ustilago hordei, a smut, worthwhile.

Before describing the present study, a brief outline of the steps in the parasexual cycle as visualised by Pontecorvo (1956) will be given and the evidence for them considered. Three steps appear to be essential: (a) Heterokaryosis, (b) formation of heterozygous diploid nuclei and their multiplication and (c) occassional segregation and recombination at mitosis in conjunction with haploidisation of diploid nuclei. A more detailed explanation of the parasexual cycle is given in Figure 1.

FIGURE 1

STEPS IN THE PARASEXUAL CYCLE AS THEY MAY OCCUR IN U. HORDEI. (AFTER PONTECORVO).



1. abc/ABC represent 3 linked genes and d/D another one unlinked
2. The genotypes of the various kinds of nucleus are enclosed in parentheses.
3. The occurrence figures are those given for Aspergillus nidulans.

There is adequate evidence documented for each of the major steps involved. Heterokaryosis entails association, separation and subsequent regrouping of genetically unlike nuclei in multinucleate cells as a result of hyphal fusions. In the Basidiomycetes, dikaryosis, which is simply heterokaryosis involving two different nuclei, is a step in the sexual process and as such is a prerequisite for infection of barley by U. hordei.

The formation of heterozygous diploids in vegetative cells, or diploidisation, is not so clearly demonstrable in smuts although there is conclusive evidence for it occurring in other fungi. In Verticillium (Ingram, 1968; Tolmsoff, 1972) vegetative diploids occur in nature whilst artificial, genetic methods have been devised for the selection of spontaneously occurring heterozygous diploids in Aspergillus nidulans (Roper, 1952), Ustilago maydis, (Holliday, 1961b), and U. violaceae (Day and Jones, 1968). Cytological evidence for diploidisation is contradictory. Ehrlich (1958), working with U. maydis, observed that the mycelium resulting from a cross of two haploid lines in the host was initially uninucleate. There was a progressive increase, however, in the percentage of dikaryotic cells until eleven days after inoculation at which time the trend reversed and uninucleate cells again predominated. These changes were interpreted as being due to dikaryotisation

followed by nuclear unions. The latter were inferred from measurement of nuclear size, which corresponded well with that of chlamydospore nuclei. Thus it was concluded that karyogamy occurred prior to spore formation. Indirect evidence for diploidisation was also obtained by Leach and Rich (1960) in Phytophthora infestans where hyphal fusions were observed cytologically and production of parental and recombinant types of zoospores was obtained. The actual presence of diploid nuclei, however, was never observed.

Bakerspigel (1965), on the other hand, obtained inconclusive results upon cytological investigation of 19 different species and 14 million nuclei for evidence of nuclear fusions in heterokaryotic mycelium. He concluded that such fusions possibly occur at a rate lower than 1 in 10^7. Thus the evidence for the occurrence of heterozygous diploids in vegetative cells of filamentous fungi has been almost exclusively genetic. It seems certain that diploidisation is a rare event.

Finally, the evidence for segregation and recombination at mitosis and for haploidisation is extensive. These phenomena have been followed in diploid strains during both the sexual and parasexual cycles (Pontecorvo, 1956). Mitotic haploidisation has been induced in U. violaceae by p-fluorophenylalanine (Day and Jones, 1968)

and the resulting haploid lines show a wide range of genotypes, both recombinant and parental for markers in the original diploid. It also occurs spontaneously but at a much lower frequency, indicating that diploid nuclei are relatively stable through many generations of somatic cells. This obviously would enhance the opportunity for mitotic crossing-over and segregation to occur. The first demonstration of mitotic recombination in fungi followed the observation of homozygous diploid segregants from heterozygous diploids in A. nidulans. This was followed by a similar demonstration in U. maydis, where auxotrophic segregants, still diploid, were obtained from prototrophic heterozygous diploids (Holliday, 1961) and another in U. violaceae (Day and Jones, 1968).

It seems certain then that somatic recombination, an integral part of the parasexual cycle, is an important feature of the life cycle of certain fungi. Indeed, Raper (1959) has implicated its significance in all Basidiomycetes, which of course includes the smuts.

INTRODUCTION

The knowledge that somatic recombination occurs in culture both spontaneously and after induction in Ustilago maydis (Rowell, 1955; Holliday, 1961b, 1965) and U. violaceae (Day and Jones, 1968, 1969) has stimulated similar investigations of the process in another smut, namely Ustilago hordei. Dinoor and Person (1969) obtained growth of the dikaryotic phase of U. hordei in culture. The authors indicate that haploid recombinant sporidia have been obtained from "forced dikaryons" and in view of the known parasexuality in the two species mentioned above, conclude that somatic recombination can occur in vitro in U. hordei also.

Further evidence that the parasexual cycle, as well as the normal sexual cycle, occurs in U. hordei was obtained by Kòzar (1967, 1969a). Compatible combinations of mutant sporidia were used to infect the barley host. After some time the parasite was recovered as sporidia or as teliospores. Evidence indicates that the nuclei within the infective dikaryon undergo fusion and produce diploid sporidia characterised by an indefinite Bauch test (see Materials and Methods) and biochemical "wild type" appearance. If these diploid infection sporidia were to undergo haploidisation, thereby providing somatic recombination of parental

biochemical markers, all the requirements of the parasexual cycle would have been fulfilled (see Literature review). In fact recombinant types were observed amongst sporidia extracted from the host prior to teliospore formation. The difficulty in inferring somatic recombination occurring in vivo, however, lies in the fact that recombination could possibly have occurred in the inoculum mixture prior to penetration of the host. Alternatively, it may have occurred in the in vitro grown cultures after they had been extracted from the host.

In an effort to clarify the process, Dinoor (unpublished) utilised a "double inoculation technique". In this technique some cultures were used on the seed (e.g. $ad^{-}arg^{-}leuc^{+}ad^{-}$) and others were injected into the seedlings grown from these inoculated seed (e.g. $ad^{-}arg^{-}leuc^{-}+leuc^{-}$). Recovery of wild type mutants, which could only have arisen by at least two consecutive recombinational events, one of which would necessarily be asexual, taking place in the host prior to teliospore formation, would demonstrate the operation of somatic recombination inside the plant. Preliminary results were encouraging but the work was discontinued owing to the lack of repeatability in the experiment. In addition, the numbers of plants used were unrealistically small for the detection of such a rare phenomenon (see Literature review).

The objective of the present study was to provide an unequivocal demonstration of somatic recombination, if indeed it does occur, in operation during the development of U. hordei inside the host plant. As in the two previously described studies, barley seeds were inoculated artificially with mixtures of nutritionally deficient mutants. In this study, however, the gametic matings were arranged in such a way as to preclude the occurrence of a recombinational event prior to penetration of the host. Furthermore, the possibility of a recombinational event occurring after extraction of sporidia from the host was eliminated by the selection process employed in this study (see Methods).

MATERIALS AND METHODS.

A. STRAINS

1. Ustilago hordei (Pers.) Lagerh.

The mutants used in this study were induced by Hood (1966) using UV irradiation and were derived from the wild type strains I_4^+ and E_3^-. It was assumed that the majority of these mutations were point mutations rather than chromosomal aberrations, since the former are known commonly to result after UV irradiation.

The particular auxotrophic strains used were chosen (1) for ease of detection i.e. non-leakiness, (2) for the absence of any evidence of close linkage with other markers such as the mating type locus or other nutritional mutations and (3) for their non-pathogenicity on Hannchen and Vantage barley cultivars when present in the homozygous state. Table 1 shows the strains chosen. It should be noted that the double mutant strains used in each case were derived from the corresponding single mutants.

2. Hordeum vulgare L.

For this study, two varieties of barley, Hannchen and Vantage, were chosen. These varieties are known to be highly susceptible to infection from crosses between the wild type strains of the fungus from which the auxotrophic mutants (see Table 1) were derived (Thomas, 1965).

In addition to the laboratory stock of Vantage seed, some Vantage was obtained from Buckerfields Ltd., New Westminster. Care was taken to distinguish between these two types of Vantage since their genetic ancestry was likely to be different.

B. CULTURE OF THE FUNGUS.

1. Media.

A modified Vogel's (1956) medium was used. Complete medium contained: 5g. Difco yeast extract, 5g. salt-free casein hydrolysate (N.B. Co.), 10g. dextrose, 50mg. tryptophane, 20ml. Vogel's salt solution (ibid), 10ml. vitamin solution (Holliday, 1961), 1 litre distilled water. For solid medium 2% Difco bacto agar was added to make agar plates and 2.5% to make slants.

Minimal medium contained 20ml. Vogel's salt solution and 10g. dextrose per litre of distilled water.

Supplemented minimal medium was prepared according to Holliday (1961). The individual growth factors were added to the minimal medium as required: amino acids 100mg., purines and pyrimidines 10mg., and vitamins 1 mg. per litre.

All media were sterilised by autoclaving for 15 minutes at 15lb. pressure and 121 degrees Centigrade before use.

TABLE 1

Mutants of U. hordei used in this study.

Experiment	Strains	Genotype	Mating type	Nutritional requirement
Somatic Recombination	AdU40	$Ad^{-}Nia^{+}$	a	Adenine
	NiaX95	$Ad^{+}Nia^{-}$	a	Niacin
	2-15	$Ad^{-}Nia^{-}$	A	Adenine + Niacin
	MetV364	$Met^{-}Ser^{+}$	a	Methionine
	SerV138	$Met^{+}ser^{-}$	a	Serine
	2-34	$Met^{-}Ser^{-}$	A	Methionine + Serine
	LeuV417	$Leu^{-}Arg^{+}$	a	Leucine
	ArgV240	$Leu^{+}Arg^{-}$	a	Arginine
	2-53	$Leu^{-}Arg^{-}$	A	Leucine + Arginine
	ArgV242	$Arg^{-}Leu^{+}$	a	Arginine
	LeuV417	$Arg^{+}Leu^{-}$	a	Leucine
	2-55	$Arg^{-}Leu^{-}$	A	Arginine + Leucine
Multiple Infection	$I4^{+}$	* +	A	-
	$E3^{-}$	* +	a	-
	ArgV35	Arg^{-}	A & a	Arginine
	ProV324	Pro^{-}	A & a	Proline
	PanV271	Pan^{-}	A & a	Pantothenic Acid
	PdxV26	Pdx^{-}	A & a	Pyridoxine
	MetV375	Met^{-}	A & a	Methionine
	LeuV4	Leu^{-}	A & a	Leucine
	IlvU26	Ilv^{-}	A & a	Isoleucine and Valine

* Denotes wild type.

Each culture was routinely tested for its nutritional requirements and its mating type prior to use.

2. Growth of the fungus.

Shake cultures were maintained in Delong culture flasks fitted with Morton stainless steel closures placed in a New Brunswick Psychrotherm incubator-shaker. These flasks contained liquid medium to approximately 40% of their total capacity. All incubation of cultures, liquid or solid, was made at 22 degrees Centigrade (±2degrees).

3. Techniques.

a. Mating type tests.

This fungus exhibits bipolar incompatibility and therefore it is important to establish the mating types of all cultures involved. Compatibility of the sporidial lines was determined by the Bauch test referred to by Fischer and Holton (1957). Here, however, the tests had to be carried out on complete medium owing to the reluctance of strains carrying more than one auxotrophic marker to produce suchfäden (infection hyphae) on minimal medium.

b. Random spore analysis and replica plating.

Sporidia recovered from smutted heads of barley were taken from the shake cultures when the concentration of sporidia approached 10^6-10^7 per ml. One or two drops of achromycin (10mg/ml.) were added to each shake culture to prevent bacterial proliferation. Counts of the sporidia were made using a Spencer haemocytometer with improved Neubauer ruling

and appropriate dilutions were made to give approximately 100 colonies per plate. Sterile conditions were ensured throughout.

When these colonies became visible (about 3-4 days), colonies were picked off with sterile toothpicks and planted to "master" plates of complete medium on grids of 25 sections. For each analysis made, approximately 200 colonies were tested. After 3-4 days incubation, the colonies were ready for replication.

Replica plating was performed according to Lederberg and Lederberg's technique (1952), otherwise known as the "Velvet Pad Method". Colonies were replicated to minimal medium, to each of the required supplemented media and to lawns of I_4^+ and E_3^- on complete medium in order to determine the mating type (see B3a. above). After 4-5 days the plates were examined to determine the biochemical characteristics of the colonies.

c. Tetrad analysis.

Isolation of the four products of meiosis from the budding promycelium and their subsequent transfer to new media to set up separate cultures was done by standard procedure (Dickinson, 1926) using a De Fonbrune micromanipulator and microforge. A slight modification of the method was employed to eliminate the difficult and hazardous process of single teliospore isolation. Instead, a small drop of

the teliospore suspension was placed on the agar block, having first greatly diluted the suspension. In this way 5-10 teliospores were planted to each agar block and after germination, the most suitable one could be chosen for micromanipulation, leaving the rest untouched in the centre of the block. The tetrads could then be analysed for their requirements on supplemented minimal media.

C. HOST INOCULATION AND PLANTING.

1. Techniques.

a. Basic method of seed inoculation.

The barley seeds were inoculated with the fungus according to the method outlined by Tapke (1942). All seeds were treated with 0.12% formalin (i.e. 40% formaldehyde diluted 1:320 with water) for one hour in order to sterilise the seed surface and loosen hulls, which is thought to aid infection. The seeds were then washed in running tap water for 30 minutes. The wet seeds were spread thinly on paper towels to air dry for 24-48 hours.

Sporidial cultures were inoculated to fresh agar slants and allowed to grow for 3-4 days. Fresh inoculum from slants was placed in the appropriate quantity of liquid complete medium and multiplied in shake culture for 48-60 hours until the cultures appeared thick.

The barley seeds to be inoculated were counted into batches of about 100 and placed in flat-bottomed #7 snap-cap crystalite vials. Sporidial inoculum was poured over the seeds using equal quantities of the two mating types in each vial. The vials were placed uncovered in a dessicator jar (without dessicant), and evacuated for 20 minutes at 20 lb. vacuum, in order to draw the inoculum under the seed hulls. Excess inoculum was poured off and the seeds dumped into labelled #2 Manilla coin envelopes. These envelopes were left unsealed so that the seeds could air dry at room temperature. After a minimum of three days, the seeds were sown.

b. Experimental plan.

The first and main experiment was designed to detect somatic recombination occurring in the fungus during its development inside the host. To this end, seeds were inoculated in the manner shown in Table 2. The seeds were then sown in the field in May, 1972. Each row was 10 feet long and contained about 100 seeds. The rows were spaced one foot apart. After about three months the barley plants were examined carefully for the presence of smut and any smutted heads collected.

The second experiment was carried out as a supportive study to the first experiment. It was designed to demonstrate that multiple infection, a prerequisite for

TABLE 2

Plan for seed inoculation in somatic recombination experiment.

Test number	Combination	Cross[a] A	Cross[a] a	Variety	Number of rows
1[e]	1	2-15	AdU40 + NiaX95	H	10
				V_b	6
				V^b	4
	2[c]	2-15	AdU40	H	10
				V_b	6
				V^b	4
	3[c]	2-15	NiaX95	H	10
				V_b	6
				V^b	4
	4[c]	AdU40[d]	NiaX95	H_b	10
				V^b	10
2	1	2-34	MetV364 + SerV138	H	20
				V_b	10
				V^b	10
	2	2-34	MetV364	H	20
				V_b	10
				V^b	10
	3	2-34	SerV138	H	20
				V_b	10
				V^b	10
	4	MetV364[d]	SerV138	H	20
				V_b	10
				V^b	10
3	1	2-53	LeuV417 + ArgV240	H	20
				V_b	10
				V^b	10
	2	2-53	LeuV417	H	20
				V_b	10
				V^b	10
	3	2-53	ArgV240	H	20
				V_b	10
				V^b	10
	4	LeuV417[d]	ArgV240	H	20
				V_b	8
				V^b	12

TABLE 2 (CONT.)

Test	Combination	Cross[a] A	Cross[a] a	Variety	Number of rows
4	1	2-56	ArgV242 + LeuV417	H	20
				V_b	10
				V^b	10
	2	2-56	ArgV242	H	20
				V_b	10
				V^b	10
	3	2-56	LeuV417	H	20
				V_b	10
				V^b	10
	4	ArgV242[d]	LeuV417	H	20
				V_b	8
				V^b	12

H = Hannchen
V = Vantage

a. As stated above, equal quantities of the two opposite mating types were used in all combinations i.e. when two cultures of the same mating type, a_1 and a_2, are used in one cross, Volume of A = Volume a_1 + a_2 and Volume a_1 = Volume a_2

b. The Vantage seed designated V^b was obtained from Buckerfield's Ltd. (see A2 above).

c. Combinations 2, 3 and 4 in each test constitute the necessary controls for this experiment i.e. the pair-wise combinations of each of the cultures used in one test.

d. Although the cultures used in Combination 4 of each test were of the one mating type and therefore incombatible, it was still necessary to make this control (see C2 below).

e. Each test was divided into two identical parts in order to provide replicates. Replicates were treated separately in different batches and sown in different parts of the field.

TABLE 3

Plan for seed inoculations in multiple infection experiment

Test Combination		A I_4	a E_3	a ArgV35	a ProV324	a PanV271	a Pdxv26	a MetV375	a LeuV4	a IlvU26
A	1	+	+							
	2	+		+						
	3	+		+	+					
	4	+		+	+	+				
	5	+		+	+	+	+			
	6	+		+	+	+	+	+		
	7	+		+	+	+	+	+	+	
	8	+		+	+	+	+	+	+	+

TABLE 3 (CONT.)

Test Combination

		A ArgV35	A ProV324	A PanV271	A PdxV26	A MetV375	A LueV4	A IlvU26	a E_3
B	1	+							+
	2	+	+						+
	3	+	+	+					+
	4	+	+	+	+				+
	5	+	+	+	+	+			+
	6	+	+	+	+	+	+		+
	7	+	+	+	+	+	+	+	+

Test B formed a reciprocal (with respect to mating types) replication of Test A.

Once again, equal quantities of the two mating types were used in each combination as explained in Table 2.

the success of the first experiment, can actually occur. Crosses were made according to the schedule shown in Table 3. All the inoculations were made to the Hannchen cultivar and in each case one row of 50 seeds was planted. Each row was $4\frac{1}{2}$ feet long and the rows were spaced 6 inches apart. This experiment was performed in the greenhouse in October, 1972. Seeds were grown at 75 degrees Fahrenheit ($\pm$5 degrees) using a 15 hour day as suggested by Schafer et al. (1962). During its growth, the crop had to be treated with insecticide for aphids and spider mites and with sulphur dust for powdery mildew. These problems and the crowding of the seeds in the bench caused a number of plants to die but sufficient data was still obtained owing to the provision of a replicate being made. Smutted heads were collected after about 4 months.

2. Rationale.

The somatic recombination experiment was designed to detect the occurrence of this phenomenon in the fungus within the infected host plant prior to teliospore formation. The method depends on the occurrence of double infection. The mutant strains chosen for each test were known not to permit sporulation when brought to homozygosity. For example, in one test, the markers were arranged in the following way:

Strain 1 : A Leu^-Arg^-
Strain 2 : a Leu^-Arg^+
Strain 3 : a Leu^+Arg^-

where A and a are the two mating types.

When these strains are mixed together and poured over the susceptible barley seeds for inoculation, two matings are expected, both equally likely (providing that there is no differential in tendency to mate and that the two 'a' strains were present in equal quantities and the quantity of 'A' mating type equalled that of 'a' mating type):

#1 X #2 to give dikaryon Leu^-Arg^- + Leu^-Arg^+
and #1 X #3 to give dikaryon Leu^-Arg^- + Leu^+Arg^-.

Thus, those plants which have been infected singly, or carry a single infecting genotype, should show no smut at maturity, since neither dikaryon is capable of causing infection alone; both dikaryons are homozygous for one or the other of the two nutritional markers. Consequently, any plant which does have smut would be of interest and investigation of the teliospores would be informative.

Controls were necessary in this experiment to ensure that the individual pair-wise matings (i.e. #1X#2, #1X#3, #2X#3) on the susceptible host gave no result. These controls would also provide a check for mutation of the mutant cultures back to wild type. Otherwise the experiment consisted simply of inoculating with the mixtures of #1, #2 and #3, looking for smutted heads and checking these

for markers present in single teliospores.

The method dictated by this rationale cannot entirely exclude the possibility of an asexual recombination event occurring during inoculation, whilst the sporidia are resting on the surface of the seed prior to penetration. The time span involved, however, is so brief that, for the purposes of this study, such a likelihood may be subordinated to the greater possibility of the event occurring whilst the fungus is actually inside the plant.

Since the success of the somatic recombination experiment wholly depended upon the occurrence of double infection, it was considered necessary to show by experiment that more than one infection could in fact occur. The experiment was set up in a similar manner to that described by Person and Cherewick (1964). The mutants were known to produce infection on Hannchen when crossed with either I_4^+ or E_3^- (depending on their mating type). The sporidial matings were arranged in such a way that up to seven different genotypes, each capable of being distinguished from the others by virtue of their nutritional requirements, were present together in the inoculum mixture. In this way, it can be deduced, by random spore analysis, whether any smut on a plant has been produced by either one, two, three, four, five, six or seven infections.

RESULTS

A. SOMATIC RECOMBINATION EXPERIMENT.

1. Harvesting.

TEST 1. (see Table 2)

It was noticed that many of the 8000 plants in this test were smutted, which was not expected. Therefore, the original cultures used were retested and it was found that the Ad U40 culture had reverted to wild type. This test was consequently disregarded.

TEST 2.

Out of 16000 plants, none were smutted.

TEST 3.

One plant out of 16000 was smutted. It occurred in combination 1 of the test and was therefore of great interest. It had two smutted tillers and was designated 501 since this was the number of the row in which it was found.

TEST 4.

Out of 16000 plants, none were smutted.

N.B. None of the control combinations in tests 2, 3 or 4 showed smut.

2. Random Spore Analysis.

Table 4 shows the results of the first random spore analysis that was made.

TABLE 4

Preliminary random spore analysis of smut from head 501.

	Leu^+Arg^+	Leu^+Arg^-	Leu^-Arg^+	Leu^-Arg^-	Unknown	Total
Number of colonies:	30	24	25	26	21	126
% Total:	23.8	19.1	19.9	20.6	16.6	100

It can be seen from the table that 17% of the colonies recovered did not have any of the expected requirements i.e. they did not grow on minimal medium or on medium supplemented with arginine, leucine or leucine + arginine.

These unknown colonies were investigated by a process of elimination after the manner described by Holliday (1961). They were successively tested on media lacking first amino acids, then bases, then vitamins. Once it was established that the unknown requirement was for a vitamin, it was possible to narrow the investigation down still further, by a similar process, to nicotinic acid (niacin) as being the missing nutrient. It was assumed that this was the result of a spontaneous mutation since the original cultures, when retested, showed no trace of a niacin requirement.

With this knowledge, a second random spore

analysis was carried out, the results of which are shown in Table 5.

TABLE 5

Second random spore analysis of smut from head 501.

Genotype as deduced from auxonography	Mating type A	Mating type a	Total number	% of total colonies
$Leu^{+}Arg^{+}Nia^{+}$	23	18	41	20.2
$Leu^{-}Arg^{-}Nia^{-}$	7	5	12	5.9
$Leu^{-}Arg^{+}Nia^{-}$	9	6	15	7.4
$Leu^{+}Arg^{-}Nia^{+}$	18	19	37	18.2
$Leu^{-}Arg^{+}Nia^{+}$	22	8	30	14.8
$Leu^{+}Arg^{-}Nia^{-}$	6	11	17	8.4
$Leu^{+}Arg^{+}Nia^{-}$	7	7	14	6.9
$Leu^{-}Arg^{-}Nia^{+}$	17	20	37	18.2
Totals	109	94	203	100.0

In this case, the requirements of all the colonies were discovered and fitted with the expectations if there had been a mutation to Niacin deficiency in one of the parental cultures. It was observed, however, that the different genotypes did not occur in equal frequencies, as would be expected on the basis of random gene assortment at meiosis.

A possible explanation for the discrepancy (i.e. the severe deficiency of all Nia^- containing colonies) could have been differential rates of multiplication of the various genotypes after spore germination. In order to find out if this was the case, a competition experiment was performed.

In this experiment, a $Leu^+Arg^+Nia^+$ culture and a $Leu^-Arg^-Nia^-$ culture were each inoculated to 5ml. of liquid complete medium. These were allowed to multiply in shake culture for 24 hours. The cells were then counted so that the concentrations could be adjusted and equal numbers of cells of each type added to 50 ml. of liquid complete medium in a DeLong culture flask. The mixture was shaken at constant temperature for three hours after which time the cells were plated out onto complete medium to a concentration of about 100 colonies per plate. Master plates were made after three days and these were later replicated to minimal medium.

It was found that twice as many of the colonies selected were of the $Leu^+Arg^+Nia^+$ type as of the $Leu^-Arg^-Nia^-$ type, confirming the suspicion of differential rates of multiplication for the two genotypes. Evidently then, the discrepancy was caused not by a deficiency of Nia^- carrying genotypes after spore germination but merely by their slower rate of division.

3. Tetrad Analysis.

Great difficulty was experienced in obtaining complete tetrads after micromanipulation of the 501 teliospores. Out of at least 100 attempts, only 10 complete tetrads were obtained, with the help of J.V. Groth.

Table 6 shows the results of the tetrad analysis.

TABLE 6

Tetrad analysis of 501 teliospores after micromanipulation.

Tetrad type	Leu	Arg	Nia	Number obtained	Tetrad type	Leu	Arg	Nia	Number obtained
1	-	+	+	1	4	+	-	+	1
	-	-	-			+	+	-	
	+	+	+			-	-	+	
	+	-	-			-	+	-	
2	-	+	+	1	5	+	-	+	1
	-	+	-			+	-	-	
	+	-	+			-	+	+	
	+	-	-			-	+	-	
3	-	+	+	2	6	+	-	+	4
	-	-	+			+	+	+	
	+	+	-			-	-	-	
	+	-	-			-	+	-	

Arg = Arginine; Leu = Leucine; Nia = Niacin.
- indicates a requirement for the nutrient.
+ indicates no requirement for the nutrient.

B. MULTIPLE INFECTION EXPERIMENT.

Smutted plants were not obtained from every combination in both tests (see Table 3) but at least one smutted head was obtained for each type of inoculation. Where more than one smutted head was obtained for a particular combination, each head was tested individually. The results of the analysis appear in Table 7. It can be seen that in only one head out of the 20 examined was there any evidence of multiple infection. Combination 4 in Test B showed definitely that two different infective dikaryons had participated in producing the smut infection.

TABLE 7

RANDOM SPORE ANALYSIS MADE FROM THE MULTIPLE INFECTION EXPERIMENT

Test	Combination	Head	Colony types 1 Mating type Number	A	a	2 Mating type Number	A	a	3 Mating type Number	A	a
	1	1	WT 96	40	56	Arg$^-$ 90	57	33	-		
	2	1	WT 104	44	60	Arg$^-$ 95	50	45	-		
		2	WT 104	54	50	Arg$^-$ 93	45	48	-		
	3	1	WT 93	57	36	Arg$^-$ 105	51	54	-		
A	6	1	WT 109	57	52	Leu$^-$ 91	40	51	-		
		2	WT 127	69	58	Leu$^-$ 72	25	47	-		
	7	1	WT 175	86	89	Ilv$^-$ 18	8	10	-		
		2	WT 175	98	77	Ilv$^-$ 15	2	14	-		
	8	1	WT 180	96	84	-					
	1	1	WT 107	56	51	Arg$^-$ 92	47	45	-		
	2	1	WT 90	38	52	Arg$^-$ 110	53	57	-		
		2	WT 110	53	57	Arg$^-$ 84	34	50	-		
	4	1	WT 105	51	54	Arg$^-$ 22	10	12	Pan$^-$ 68	26	42
	5	1	WT 100	51	49	Pan$^-$ 99	48	51			
B	6	1	WT 136	63	73	-			-		
		2	WT 190	97	93	-			-		
	7	1	WT 198	103	95	-			-		
		2	WT 192	107	85	-			-		
		3	WT 195	94	101	-			-		
		4	WT 183	98	85	-			-		

WT = wild type

Refer to Table 3 for the sporidial crosses made in each combination.

DISCUSSION

The detection of a rare recombinational event in a micro-organism requires conditions that facilitate recognition of a novel phenotype in a vast population having the parental phenotype. The selection system employed in this study met this requirement: out of 12000 plants in which somatic recombination could have occurred, it was easy to pick out the one plant in which it had taken place (subject to confirmation by the isolation of the original markers).

Nutritionally deficient mutants were used in this study as markers for investigating the process of somatic recombination. Analysis for their presence, subsequent to the putative somatic recombination event, provided confirmation that this one smutted plant was indeed produced through somatic recombination rather than by mutation. An additional safeguard against the latter possibility was provided in the controls of the experiment (see Results, C2). Even though two spontaneous mutations were observed during the course of the experiment, namely the adenine reversion to wild type and the mutation to niacin requirement in one of the parental cultures of 501, none were observed in the pair-wise control combinations of Test 3, in which 501 was found. In these there was no chance of smut being produced unless wild type recombinants had been formed as a result of

some back mutation occurring in one of the parental cultures. From the results, then, it is possible to say that somatic recombination must have occurred in the infective dikaryons of 501 whilst they occupied host tissue.

In dealing with an obligate parasite such as U. hordei, it is not easy to give the exact sequence of events as Pontecorvo (1956) has done for the Ascomycetes. An attempt, however, will be made to explain the possible mechanism by which these results may have been obtained.

Certain deductions can immediately be made from the tetrad analysis (see Table 6). Since non-parental ditypes are as numerous as parental ditypes, no matter which two markers we consider and no matter what we assume the genotype of 501 teliospores to be, it can be concluded that there is no linkage between the markers concerned; i.e. the loci determining arginine, leucine and niacin requirements are either all situated on different chromosomes or sufficiently far apart on a single chromosome that recombination would appear to be random. This is supported by the fact that in no tetrad are all three pairs of markers tetratype. This means that there can have been no three-strand double cross-overs at meiosis, which is highly unlikely if the three genes were linked.

As was intimated in the literature review (p. 5), segregation of markers for which a diploid is heterozygous

occurs during vegetative multiplication, not only as a consequence of mitotic crossing-over between linked genes but also as a consequence of a process of haploidisation, in which whole chromosomes, not chromosome parts, reassort at random. Thus somatic "recombination" of unlinked genes, on different chromosomes, should still occur. This, then, is the form of somatic recombination thought to be active in this study.

Details of the process of haploidisation are almost completely unknown. Haploidisation could be a consequence of an accidental breakdown at mitosis in the separation of chromatids to the two poles: in a proportion of cases one daughter nucleus arises with a single set of chromosomes (Pontecorvo, 1956). Breakdowns of this kind are well-known in higher organisms and result in aneuploids.

A further deduction which can be made from the tetrad data is that since all three markers are segregating in all the tetrads, the 501.teliospores must have been heterozygous for all three markers. The wild type sporidia obtained in both the random spore analyses and the tetrad analysis bear testimony to this since wild types can only be generated if there is at least one wild type allele at each locus in the diploid teliospore. Furthermore, the fact that wild type sporidia were obtained at all indicates that there must have been two successive recombination events;

one taking place asexually in the plant (somatic recombination) and the other in the teliospores (meiotic recombination). This can be inferred because one member of each possible dikaryon (the 'A' mating type) is mutant at both the original marker loci. Since each of the 'a' mating type members is mutant at one or other of these loci, it is impossible for a wild type offspring to be generated by a single meiotic event. The following diagrammatic representation will help to make this clearer:

DIKARYON	TELIOSPORES
#1: $Leu^-Arg^-A + Leu^-Arg^+a$	.....Segregation for Arg^- only, not for Leu^-.
#2: $Leu^-Arg^-A + Leu^+Arg^-a$	.....Segregation for Leu^- only, not for Arg^-

Thus, if any teliospore segregates for both Leu^- and Arg^- (thereby generating some wild type offspring) all three nuclei must have participated in at least two recombinational events.

With the above information and allowing for the fact that a mutation to niacin requirement occurred (see Results A2) in one of the parental cultures of 501, an hypothesis may be put forward which can explain how the present results were obtained. Originally, three sporidial types were present in the inoculum mixture of 501:

#1 : $Leu^-Arg^-Nia^+$ A
#2 : $Leu^-Arg^+Nia^+$ a
#3 : $Leu^+Arg^-Nia^+$ a

The possible ways in which somatic recombination could have occurred between these types are shown in Table 8.

Now, assuming free association of all nuclear types when it comes to teliospore formation, subject only to mating type restrictions, it is possible to determine the various ways in which karyogamy could occur in order to produce teliospores heterozygous at all three loci. This is shown in Table 9.

TABLE 8

Possible ways in which somatic recombination could have occurred to give rise to teliospores 501.

Mutation to Nia⁻ in:	Event	Dikaryons									
		1					2				
		Nuclear Type	Leu	Arg	Nia	Mating Type	Nuclear Type	Leu	Arg	Nia	Mating Type
#1	Diploidisation*	1	-	-	-	A	1	-	-	-	A
		2	-	+	+	a	3	+	-	+	a
	Haploidisation	4	-	-	-	a	4	-	-	-	a
		5	-	+	+	A	8	+	-	+	A
		6	-	-	+	a	6	-	-	+	a
		7	-	+	-	A	9	+	-	-	A
		2	-	+	+	a	6	-	-	+	a
		1	-	-	-	A	9	+	-	-	A
#2	Diploidisation*	1	-	-	+	A	1	-	-	+	A
		2	-	+	-	a	3	+	-	+	a
	Haploidisation	6	-	-	+	a	6	-	-	+	a
		7	-	+	-	A	8	+	-	+	A
		4	-	-	-	a	6	-	-	+	a
		5	-	+	+	A	8	+	-	+	A
		2	-	+	-	a	6	-	-	+	a
		1	-	-	+	A	8	+	-	+	A

TABLE 8 (CONT.)

Mutation to Nia⁻ in:	Event	Dikaryons 1 Nuclear Type	Leu	Arg	Nia	Mating Type	2 Nuclear Type	Leu	Arg	Nia	Mating Type
	Diploidisation*	1	-	-	+	A	1	-	-	+	A
		2	-	+	+	a	3	+	-	-	a
	Haploidisation	6	-	-	+	a	6	-	-	+	a
#3		5	-	+	+	A	9	+	-	-	A
		6	-	-	+	a	4	-	-	-	a
		5	-	+	+	A	8	+	-	+	A
		2	-	+	+	a	4	-	-	-	a
		1	-	-	+	A	8	+	-	+	A

* This event was inferred

TABLE 9

Possibilities for 501 teliospore formation

Mutation to Nia⁻ in:	Karyogamy between nuclear types:	Teliospore genotype Leu	Arg	Nia	Mating Type	Teliospore Type
#1	#2 + #9	-	+	+	a	A
		+	-	-	A	
	#3 + #7	+	-	+	a	B
		-	+	-	A	
#2	#3 + #7	+	-	+	a	B
		-	+	-	A	
	#2 + #8	+	-	+	A	C (=B)
		-	+	-	a	
#3	#2 + #9	-	+	+	a	A
		+	-	-	A	
	#3 + #5	+	-	-	a	D (=A)
		-	+	+	A	

With respect to the nutritional markers, it can be seen that there are only two basic types of teliospore possible for 501, A or B, regardless of where we postulate the mutation to Nia⁻ to have occurred. Therefore, whilst we can say that the mutation to Nia⁻ did occur, it is impossible to say yet exactly where. The meiotic segregation patterns expected from teliospore types A and B offer further evidence on this point, as shown in Table 10.

When the tetrad types given in this table are compared with those actually obtained in this study (see Table 6), it

will be seen that all the possible types of tetrad were present if both types of teliospore, A and B, had germinated from the 501 head. By inspection of Table 9, only one of the three possibilities for teliospore formation fulfills this requirement, namely that where the mutation to Nia^- has occurred in strain 1, the double mutant. It is concluded, therefore, that a mutation to niacin requirement occurred in parental strain #1 and that this was followed by the somatic and meiotic recombination events described above in the series of tables 8, 9, and 10.

It must be realised that this whole hypothesis is based on the conventional idea of parasexuality involving diploids (see Figure 1). This need not necessarily be the case, as Hartley and Williams (1971) have pointed out. They envisage a mechanism for generating genetic variation by errors in migration of chromosomes at dikaryotic mitosis. Thus an exchange of whole chromosomes between haploid cells could occur without altering the normal chromosome complement of the two nuclei. Somatic recombination could therefore be explained without invoking the hypothetical process of diploidisation. To prove that somatic recombination is a mitotic process involving diploids requires the isolation of stable diploids in which segregation can be studied. This has been done successfully in U. maydis (Holliday, 1961b), as mentioned below, but the event of diploidisation

can only be inferred in U. hordei so far. For the purposes of this hypothesis, however, it is felt that this inference is justified.

TABLE 10

Table showing the meiotic segregations expected from germination of teliospore types.

Tetrad Type	Teliospore Type A Leu⁻Arg⁺Nia⁺ Leu⁺Arg⁻Nia⁻			Tetrad Type	Teliospore Type B Leu⁺Arg⁻Nia⁺ Leu⁻Arg⁺Nia⁻		
1	-	+	+	4	+	-	+
	-	-	-		+	+	-
	+	+	+		-	-	+
	+	-	-		-	+	-
2	-	+	+	5	+	-	+
	-	+	-		+	-	-
	+	-	+		-	+	+
	+	-	-		-	+	-
3	-	+	+	6	+	-	+
	-	-	+		+	+	+
	+	+	-		-	-	-
	+	-	-		-	+	-

At this point, consideration should be given to the difficulties experienced in micromanipulation of the 501 teliospores. It has already been mentioned that the various sporidial types are subject to differential rates of multiplication (see Results A2) depending on the nutritional requirements which they carry. This explains why the

allele ratios (the proportions of recovery of the two homologous strands) depart from 1:1, bearing in mind that the segregants are recovered many nuclear generations after the event which produced them. It is suggested that this slower rate of growth and consequent lowered viability of some of the sporidial types, notably of those carrying the niacin requirement, may account for the widespread failure to recover all four meiotic products after teliospore germination in this experiment. Germination type is known to be affected by the nutrient content of the medium (Holton et al., 1968) and variations in spore germination amongst the smut fungi are extensive (ibid.). Wild type teliospores, the offspring from which have no nutritional requirements, are sensitive enough to manipulation, so it is not unreasonable to conclude that teliospores carrying one or more nutritional markers would be extremely difficult to handle. The only 10 complete tetrads obtained (Table 6), however, do present unequivocal evidence for somatic recombination occurring in the fungus whilst it resided within the infected host plant.

Additional evidence that this was indeed the case comes from the multiple infection experiment. The results shown in Table 7 demonstrate clearly that at least two infective dikaryons can occupy an infected host plant at the same time. Furthermore, Person and Cherewick (1964)

showed that in U. kolleri and U. avenae, multiple infections do occur and that more than one genotype of the pathogen may be found within a single diseased plant. In fact, the majority of diseased plants had been infected at least twice. The dangers of extrapolation of results between organisms must be realised but it seems fairly certain from these two pieces of evidence that multiple infection can occur generally in the smuts and therefore that somatic recombination may also be a general phenomenon in this group of fungi. This will be discussed at greater length below.

The actual event of somatic recombination in U. hordei seems to be different from that described for U. maydis. So far as is known, none of the cultures of U. hordei that have been tested is unstable or reacts like a bisexual culture, although Kozar (1969b) did obtain indications that some diploids may have been recovered along with haploid sporidia. In other words, workers failed to find any definitely diploid sporidial cultures like those described for U. maydis (Holliday, 1961b). This could simply indicate that any diploids that may be formed in the dikaryons are very transitory by virtue of extreme instability. Although in most recorded instances the diploids persist as a clone, a rare diploidisation event followed immediately by haploidisation could produce much of the genetic diversity implicit in parasexuality, with no direct evidence of the presence of

a fusion nucleus. So this is certainly not evidence against the occurrence of somatic recombination in U. hordei.

The outcome of this study has important implications. Primarily, it has shown that this event is not simply a laboratory artifact. Secondly, it is the first report of somatic recombination occurring in vivo. Since the organism used here is a parasite, this suggests that a new and previously unsuspected source of variability is potentially available for all economically damaging fungi. The parasexual cycle in fungi provides a complete ersatz for sexual recombination in that it provides for both recombination and sheltering of gene variation (Pontecorvo, 1958). In the latter respect, it is more versatile than sexual reproduction because it includes two ways of storing gene variation (heterokaryosis and heterozygosis) instead of only one. For example, the formation of diploids could aid in the retention of favourable mutations or in the elimination of unfavourable ones. Too much emphasis, however, should not be placed on the storage of variation in the Basidiomycetes, since all diploids reduce to haploids in each generation by meiosis.

More important here perhaps, is the realisation that the parasexual cycle operating in plant pathogenic fungi could provide an additional vehicle for the reassortment of virulence genes. The use of nutritionally deficient mutants here is a tool in the investigation of processes of importance in the reassortment of genes for pathogenicity.

Since multiple infection can occur in barley, there is also the possibility of a synthesis in the host, by parasexual recombination, of biotypes possessing genes for virulence from more than two parental biotypes.

As mentioned above (see Literature Review), there are many reports of new races of rusts arising asexually but in the majority of cases the authors are reluctant to attribute their occurrence to parasexual recombination. For example, Flor (1964) states categorically that "there was no indication that parasexual processes were involved in the pathogenic variation of Melampsora lini" even though he did obtain several asexual variants upon mixing urediospores of two rust races. Instead he explained these variants on the basis of nuclear exchange followed by single gene mutations or deletions. The importance of mutations in the origin of new races has been mentioned by a number of other authors (Christensen, 1959; Day, 1960, 1966; Fischer and Holton, 1957; Parmeter et al, 1963; Toxopeus, 1956; Zimmer et al., 1963). Whilst it is realised that mutations are undoubtedly a factor in the development of new races, it is felt that one cannot rule out somatic recombination as another possibility, as Flor points out in his 1971 paper.

Other workers at various times have suggested somatic hybridisation, heterokaryosis, nuclear pairing regardless of sex and cytoplasmic influences as being singly or severally responsible for the production of new asexual

variants in the rusts. More recently, Bartos et al. (1969), Hartley and Williams (1971), and Sharma and Prasada (1969) have explained similar results on the basis of parasexual recombination, as it has been defined here. It seems that neither nuclear exchange nor any of the other mechanisms suggested above can account for both the origin and diversity of new strains among the progeny of mixtures of spores. Somatic recombination, on the other hand, can accomodate both of these factors and as such may be an important mode of origin of new strains in both rusts and smuts.

It is interesting to note at this point that in biochemical studies with species of Ustilaginales, mitotic analysis could have considerable advantage over standard meiotic analysis (Halisky, 1965). Mitotic recombination could become important in the mapping of chromosomes of organisms like U. hordei, which have so far frustrated all attempts at meiotic analysis, in the same manner as it has been employed in chromosome studies of A. nidulans (Pontecorvo and Roper, 1952) and U. violaceae (Day and Jones, 1969). Furthermore, in vitro production of somatic recombinants could be used as a means of circumventing passage through the host, barley in this case, thus obviating the necessity of a three month generation interval. Standard parasexuality, despite the many unknowns, may also prove to be a useful tool for the elucidation of genetic controls of and the biosynthetic processes involved in virulence. In any case, well-marked

arms in all the chromosomes are necessary before the true nature of "virulence" can be determined. There is little doubt that this prerequisite could be achieved through somatic recombination.

The results of this study suggest certain lines for further research into this subject. With this system we could study the extent of penetration and development of avirulent biotypes in resistant plants, using the method of sporidial extraction prior to teliospore formation described by Kozar (1967). Further, by inoculating with various combinations of virulence genes, we could investigate (a) the possibility that inexpressible genes for virulence from an avirulent isolate will be recovered by a biotype with other expressible genes and (b) the possibility that two phenotypically avirulent biotypes will recombine asexually in the host to produce a virulent biotype, which will thus be able to reproduce.

This work also has implications for the study of fungi whose sexual phase is not common but in which there is a considerable diversity of races. Since various mechanisms of variation may be operational and since variation is complex and dynamic, the identification of any one mechanism, when all are inter-related, is problematic. Mutation, heterokaryosis and somatic recombination are all nuclear mechanisms thought to produce versatility in Fungi Imperfecti. Versatility

is extremely important to the survival of all plant pathogens, since without it, extension of host range to include new resistant varieties is impossible. The fact that parasexual recombination can determine variation in the host range, at least in the Fusaria, has been shown adequately by Buxton (1956). Therefore a wide field of research has been opened, both fundamental and applied, by work on somatic recombination.

Nevertheless, it is only possible to speculate on the importance of parasexuality in the survival and evolution of plant pathogens in nature, since evidence of parasexuality in natural populations is wanting. It seems certain, however, that the phenomenon is not confined to a particular taxonomic unit or to any specific group of pathogens because it occurs in fungi producing disease on a wide range of hosts (see Literature Review). The group to which it may have the greatest significance is the Fungi Imperfecti, where it may be a major evolutionary mechanism. More complete realisation of its potential requires research not only into the genetics and cytology of recombination events per se but also into problems of anastomosis, incompatibility, heterokaryosis and extranuclear inheritance. There is no doubt for example, that the number of haploid strains of fungi greatly outweighs the number of diploid strains in nature. In fact, there is only one report of a naturally occurring stable diploid (Ingram, 1968). If the

parasexual cycle provides such a good source of variability, why are diploids not more common in nature? What genetic controls are imposed on parasexuality in nature?

On the whole, the parasexual cycle seems less perfect but more flexible than the sexual cycle. It would be surprising if a system with potentialities as great as those of sexual reproduction were merely a laboratory curiousity. Indeed, the results of this study would indicate that it is not. That parasexuality probably does play a relevant part in natural populations is demonstrated by the work of Luig and Watson (1971) in Australia. Here sexual reproduction in wheat rust is virtually non-existent owing to the absence of the barberry host. Yet the rust has been able to attack successively all the resistant wheat varieties that have been introduced, causing great concern. There is little doubt that asexual recombinants and mutants of the fungus provide the necessary variability.

In conclusion, it may be said that variation in smut fungi, in all its ramifications, has its most fundamental impact on pathogenicity. This study has shown that somatic recombination, albeit a very rare event, may contribute to the production of new and more virulent races of smut. Furthermore, it has shown that somatic recombination can occur in pathogenic fungi whilst they are occupying the host plant. The importance of this finding is easy to perceive

when one considers that most of the fungi pathogenic to cultivated plants and, incidentally, most of those employed in industrial fermentation processes, are asexual. It is realised, however, that although currently recognised processes of parasexuality largely conform to those described for A. nidulans (see Literature Review), other viable processes of recombination are not precluded. One thing is certain: many more explorations, using parasexual recombination in plant pathogenic fungi, into the modification of host range, inter-genic effects on virulence and blocks in pathogenicity appear warranted.

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