THE GENETICS OF WHEAT AND WHEAT RUSTS SINCE FARRER

FARRER ORATION 1976

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University of Sydney, N.S.W. 2006

My selection as Farrer Memorial Medallist for 1976 was indeed an unexpected, but nevertheless, much appreciated, honour. In my letter of acceptance to the chairman of the Trust I stated that I saw the award as not just recognition of my personal efforts but, more importantly, as recognition of the overall efforts of our wheat breeding and wheat rust groups.

When Gregor Mendel, the father of the science of genetics, published his results on inheritance in garden peas in 1866, William Farrer (1845-1906), the man we honour to-day, was a student at Cambridge University. However, the significance of Mendel's work was not appreciated until 1900 and when Farrer became conversant with Mendel's work in 1905, he commented that he had been following the Mendelian principles for many years. Certainly, Farrer realised that when two plants with contrasting phenotypes were intercrossed, segregation did not occur until the F2 or second filial generation after crossing. Furthermore, his entire approach to wheat breeding was based on assumptions that various desirable attributes of different parents could be combined in some of the progenies produced from crosses. Indeed in his address to the Fifth Rust In Wheat Conference in Melbourne in 1989 Farrer gave actual examples of frequencies with which combinations of earliness and rust resistance might be expected in F2 generations from certain hybrids (Russell 1949).

Although Farrer is usually credited with breeding rust resistant wheats there is little evidence that his wheats were resistant to stem rust. Certainly, he may have had rust resistant wheats in his collection, but after the disastrous stem rust epidemic of 1899, the disease almost disappeared for a number of years and he could not select for resistance.

Farrer correctly assumed that leaf rust, which occurred each year, was far less damaging than stem rust and did not warrant much attention at that time. He turned his efforts to the selection of wheats with earliness and improved baking quality and milling yield. For earliness, Farrer used introductions from India, and crossed them with the late maturing high quality Fife wheats from North America. However, his higher quality early maturing selections were lacking in yield and he was forced to make further crosses to older Australian wheats. This approach resulted in the development of Federation which, although not fulfilling Farrer's objectives of baking quality and rust resistance, became a leading cultivar for almost 20 years. Although Farrer's wheats were not rust resistant, they had an increased ability to escape stem rust because they permitted wheat to be grown in drier areas and were early maturing.

In this address I shall attempt to summarise some of the genetic knowledge of wheat and its rust diseases that has accumulated since Farrer's death.

I. THE GENETICS OF WHEAT

About 1915 biologists realised that the genetic determinants, the genes, were situated in the chromosomes - structures occurring in the cell nucleus and staining distinctly with certain chemicals. As far as wheat was concerned the science of cytogenetics was not widely applicable until about 1950 when Dr. E.R. Sears, University of Missouri, isolated the various aneuploids in Chinese Spring (Sears 1954).

Bread wheat, Triticum aestivum, the most important of cultivated wheats, has 42 chromosomes which can be counted in cells taken from roots of freshlygerminated seedlings. At meiosis, the chromosomes form 21 bivalents.

Of the other main forms of cultivated wheat, the durum, or macaroni and emmer forms, T. turgidum, have 28 chromosomes (14 bivalents), and einkorn, T. monococcum, has 14

chromosomes (7 bivalents). These three species are respectively, hexaploid, tetraploid and diploid and form part of a polyploid series which includes wild as well as cultivated forms.

The Evolution of Wheat

Wild forms of diploid and tetraploid wheats occur in eastern Mediterranean countries and Asia Minor, but there are no wild forms of hexaploid wheat. Hexaploid wheat, apparently arose in fields of cultivated emmer, was selected by man, and has co-evolved with him. Earlier in prehistory, both diploid and tetraploid wheats were domesticated by selection of plants with spikes which did not break up on ripening. This permitted increased flexibility in the time available for harvest and enabled sheaf harvesting, but deprived the plants of the means of natural dispersal.

Wild emmer apparently arose from a hybrid of wild einkorn and a related unknown diploid wheat or grass species followed by chromosome doubling to produce the new species with 14 chromosome pairs. The process of polyploidization is not uncommon in plants. Later, cultivated emmer hybridized with a goat grass, Aegilops squarrosa, now commonly a weed of cereals in the Caspian Sea area, to produce a hybrid which, after chromosome doubling, was fertile and was selected by man. It is uncertain whether this process occurred once, or repeatedly, but it is certain that the addition of Aegilops squarrosa to emmer, set the basis for global expansion of the wheat industry and for evolution of the modern bread-making process.

Genomic Relationships

The evidence to deduce the evolution of wheat has been obtained from the study of present-day wild and cultivated forms of wheat, from archaeological records, and from chromosome pairing studies in hybrids between the various species. Diploid wheat shares its basic chromosome set, or genome, with the tetraploid and hexaploid wheats. The chromosome set of tetraploid wheat is present in hexaploid wheat. The relationships of the three forms are designated as follows:

Diploid	T. monococcum	2n = 14	AA
Tetraploid	T. turgidum	2n = 28	AABB
Hexaploid	T. aestivum	2n = 42	AABBDD

Chromosome pairing typical of triploid (AAB) and pentaploid (AABBD) hybrids is shown in Plate 1. Taxonomically, the wheats are closely related to other genera, viz. Secale (rye), Agropyron (wheat-grass), Aegilops (goat grass), Hordeum (barley) and Haynaldia. Chromosome pairing in a hexaploid wheat/rye hybrid confirms the relative lack of homology between the chromosomes of these species. (Plate 1c)

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Genetic Variation in Hexaploid Wheat

Despite the fact that all hexaploid wheats are cultivated and that the species evolved relatively recently, there is a wide range of variability within the species. Indeed, Farrer and his contemporaries in Europe and North America showed how this variability could be used to produce cultivars more suited to particular environments, or more suited to the requirements of the baking industry. Genetically, the overall characters yield and quality are difficult to understand, but much has been established regarding many other heritable attributes. In general, the genetics of hexaploid wheat are not different from those of diploid species such as barley, but because wheat is a complex of three basic species, the genetic determinants are often duplicated and triplicated. For example, the synthesis of proteins often can be related to three different chromosomes, or red grain colour may be determined by genes situated in one, two or three chromosome pairs. Such duplication of gene action suggests the occurrence of genes of similar function derived from the different progenitors, a process confirmed by Sears (1954, 1966a) after he produced many combinations of wheats deficient in one chromosome pair but having extra doses of other chromosomes. In some instances the extra doses compensated for loss, whereas in other instances they did not. Seven sets of three pairs of compensating

or homoeologous chromosomes were identified. These findings enabled the designation of wheat chromosomes according to genome (A, B or D) and an arbitrary set number (1-7).

The dark red grain colour of some wheats is determined by genes R2, R3 and R1 located in chromosomes 3A, 3B and 3D, respectively. Other wheats such as Chinese Spring with light red grain may carry only one of these factors (e.g. R1R1 r2r2 r3r3) and white grained wheats carry none (r1r1 r2r2 r3r3). Commercial wheats in Australia are white grained, and in Australian breeding programmes where red grained parental genotypes are being used, red grained segregates must be discarded irrespective of other attributes. Depending on the particular red grained parents being used in crosses with white wheats, F2 ratios of red:white seeded segregates can be 63:1, 15:1 or 3:1. Also, some intercrosses of red wheats may produce white seeded F2 offspring.

Use of Aneuploids in Genetic Analysis

Of the various primary aneuploids in wheat viz., nullisomics (2n-2 =40), monosomics (2n-1=41), trisomics (2n+1 =43), and tetrasomics (2n+2=44), the most valuable in genetic analysis are the monosomics which are fertile and give rise to nullisomics in their progenies. Nullisomics are usually weak in vigour and sterile and therefore cannot be maintained. When an "average" Chinese Spring monosomic plant is self pollinated it produces among its progeny about 23% euploid, or normal, plants, 73% monosomics and 4% nullisomics (Figure 1). While the meiotic process in a monosomic plant produces gametes in the frequency 25% n=21 and 75% n-1=20, this frequency is reflected only in functioning female gametes. Since pollen grains with n-1 chromosomes are less able to compete with normal counterparts, most of the pollen effecting pollination is normal. These separate male and female transmission rates can be established by respectively testcrossing a monosomic plant with euploid wheat and determining the frequencies of monosomic and euploid offspring.

If Chinese Spring carries the dominant alleles AA at a particular locus then 20 of the 21 monosomic lines will have genotype AA and the other ("critical") monosomic will be A-, the "-" representing absence of one chromosome carrying allele A. In the latter case, selfing of the monosomic plant will produce some nullisomic offspring which will be -- and will exhibit the recessive phenotype, or an absence of the A phenotype. Since such plants are nullisomic they should also show the morphological features of the particular nullisomic. In the 20 non-critical lines there will be no variation with respect to the A phenotype.

Usually a particular dominant gene, BB, that we wish to locate is present in some cultivar other than those in which monosomics are available. In this case a selected monosomic plant in each of the 21 parent monosomic lines is pollinated with the cultivar of interest. Selected monosomic F1s (75% are monosomic and 25% euploid) will have the genotype Bb in 20 instances and B- in the critical case. The 21 monosomic F1s are phenotypically indistinguishable but upon selfing, F2 populations derived from the first 20 F1s will segregate 1BB + 2Bb : 1bb (3B:1b phenotypes), whereas the F2 population derived from the critical F1 will segregate 24BB:73B-:3-- (97B:3b phenotypes). In this instance the individuals with contrasting phenotypes are nullisomic whereas, in the non-critical lines, the incidence of nullisomy is independent of genetic segregation at the B locus. Hence genes can be located in aneuploids by the detection of statistical departures from hypothetical genetic ratios, or by the precise cytological association of phenotype with chromosome constitution. In some instances statistical departures may not be detected but cytological association should be possible.

Other methods involving the use of monosomics in gene location were described by Unrau (1950) and Sears (1953). One of these involves chromosome substitution, whereby a particular chromosome from one cultivar is maintained in an

unchanged condition as a monosome while it is transferred by backcrossing to a second cultivar. After the desired number of backcrosses, euploid individuals may be derived by self pollination. Intervarietal chromosome substitution provides a means of analysis of quantitative genetic characters in wheat, and although the procedure has been suggested as a means of breeding improved cultivars, I am not aware of any commercial wheats produced in this way.

Once the chromosome location of a particular gene is known its position within the chromosome can be established by telocentric mapping (Sears 1966b) and by conventional genetic mapping. The genes located in wheat and their linkage relationships were summarised by McIntosh (1973) and Sears (1974).

Genetic Control of Chromosome Pairing

A major landmark in the development of wheat cytogenetics was the discovery that strict bivalent pairing is largely controlled by a single gene, Ph, situated in the long arm of chromosome 5B (Riley and Chapman 1958, Sears and Okomoto 1958). In the absence of chromosome 5B homoeologous chromosomes undergo meiotic pairing and this is particularly well illustrated in intergeneric hybrids such as bread wheat/rye when normal 28-chromosome hybrids and nulli-5B 27 chromosome hybrids are compared (Plate 1 c and d). Moreover, Ph control is suppressed in hybrids of wheat and certain genotypes of Aegilops speltoides and related species (Riley et al. 1961, Kimber and Athwal 1972, Kimber and Sallee 1973). More recently, lines of Chinese Spring with apparent point mutations at the Ph locus were obtained by Wall et al. (1971) and Sears (personal communication).

Transfer of Genes from Related Species to Wheat

Wheat breeders are often confronted with a lack of genes controlling some character of economic interest and genes for disease resistance may be taken as excellent examples of this. Breeders may search for the desired variability firstly within collections of hexaploid wheats, but they are also dependent on related species as sources of such genes. In

order to carry out such transferences hybrids between wheat and such species must be obtained. Bridging crosses to a third species may be necessary in some instances.

Gene transference by homologous chromosome recombination

If genes determining a desired character are situated in species whose chromosomes are homologous with those of wheat, gene transference simply involves backcrossing to wheat with concurrent selection for the desired phenotype. Many breeders, including Farrer, have made crosses between tetraploid wheats and hexaploid wheats, and rust resistances in well known cultivars such as Gabo, Madden, Mengavi, Timgalen, and the North American wheats, Hope and Thatcher, were derived in this way.

More recently, genes were transferred to wheat from the diploid progenitors, T. monococcum (The and Baker 1975) and Aeg. squarrosa (Kerber and Dyck 1969) and the derivatives are now being used as sources of rust resistance in our breeding programme.

Transference of genes located in non-homologous chromosomes

In recent years the transference of genes to wheat from species with chromosomes not homologous with those of wheat has attracted a great deal of attention and developments in this field, commonly known as chromosome engineering, have paralleled developments in knowledge of wheat cytogenetics in general. When the possibility of transferring genes for disease resistance from related (alien) grasses was first entertained in the 1950s it was thought that such resistances would present more permanent protection against wheat pathogens. However, we now realise that pathogens have the ability to overcome alien resistances in the same way as they adapted to resistances already present in the wheats. Hence genes from alien sources simply add to the number available for use in breeding.

When species such as cereal rye are crossed with hexaploid wheat the F1 plants are highly sterile but after chromosome doubling either, spontaneously or by treatment with the chemical, colchicine, fertile polyploids result (Figure 2).

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If rust resistance, or some other attribute, is present in the polyploid, or triticale in this example, a programme of backcrossing to wheat with concurrent selection for the character usually results in the production of normal wheat-like plants which carry an extra chromosome (Figure 3). Such lines can be made
jure 3 homozygous, and disomic, for the added character but they are usually genetically unstable and the additional chromosomes have detrimental effects on agronomic performance. Occasionally such alien chromosomes may spontaneously replace a pair of wheat homoeologues, or such chromosome substitution can be performed by cytogenetic manipulation.

Disomic alien chromosome substitution lines are more stable than disomic alien addition lines and there is at least one group of commercial wheat cultivars with a pair of rye chromosomes replacing a pair of wheat homoeologues (Zeller 1973). Generally, however, alien substitution lines lack somewhere in agronomic performance.

Sears (1956) reported the transference of leaf rust resistance from a chromosome of the grass, Aegilops umbellulata, to a wheat chromosome following X-ray treatment of pollen from a plant carrying an alien chromosome. In the following 10 years a number of disease resistance genes were transferred to wheats following radiation of spikes or seeds (Knott 1961, Driscoll and Jensen 1963, Sharma and Knott 1966). One of these was destined to be the parent of the Australian wheat cultivars, Eagle, Kite and Jabiru which carry gene Sr26 derived from Agropyron elongatum.

By the mid- to late 1960's wheat cytogeneticists realised that various alien chromosomes behaved as wheat homoeologues, and that removal of 5B pairing control by mutation, deletion or suppression would permit alien chromosomes to pair and recombine with those of wheat. An Aegilops comosa derivative with resistance to wheat stripe rust was produced by Riley et al. (1968) by suppressing Ph with Aegilops speltoides while Sears (1972, 1973) produced multiple transfers of leaf rust

resistance from two different chromosomes of Agropyron elongatum following the removal of chromosome 5B.

Additionally, certain instances of spontaneous translocation were reported. Two of these, a group of cereal rye derivatives (Zeller 1973) and Agent, an Agropyron elongatum derivative (Smith et al. 1968) have been used in commercial wheats in Europe and North America, respectively. Sears (1972) indicated that some spontaneous translocations involve a process of centric fusion following simultaneous misdivision of wheat and alien chromosomes.

Spikes of some of the alien species from which disease resistance has been obtained are shown in Plate 2.

My involvement in chromosome engineering has not been in producing translocation lines but in studying the best means of exploiting them in commercial wheats. As far as Australia is concerned, the Eagle/Kite resistance is the only one of these alien chromosome transfers which has reached commercial production. The others appear to have various agronomic defects which cause them to be rejected by breeders during the selection process. It seems they must be studied more carefully in their new wheat backgrounds. One example from our experience is the spontaneously translocated Agropyron derivative, Agent. This red seeded line not only carries leaf rust resistance but in our first year of observation, we found it was very resistant to stem rust. The two resistances, attributable to genes designated Lr24 and Sr24, are inherited together as part of the alien chromosome sector. After four years of breeding work it has become apparent that the rust resistances were also associated with red grain colour which apparently was also inherited from Agropyron. Since red seeded wheats are not grown in Australia an alternative approach had to be sought if the Agent resistances were to be exploited. We were fortunate that one group of Sears' multiple alien transfers involved the 3Ag source chromosome of Agent, and two of these, 3D/Ag#3 and 3D/Ag#14 (Sears 1973), have given white seeded rust resistant

progenies. From the genetic data provided by Sears (1973) it appears that the translocation points in these two lines are distal to gene Rl in Chinese Spring, whereas in Agent and other lines produced by Sears, the translocation points are proximal to Rl. Consequently in these latter lines R^{Ag}, Lr24 and Sr24 are inherited as a non-recombinable unit. Since wheat chromatin extends beyond the Rl allele in the former lines, recombination within wheat chromatin allows the rust resistance allele to recombine with rl hence permitting white seededness. Whether the white seeded derivatives have other relative advantages due to the smaller segments of alien chromosome remains to be tested.

Since chromosome engineering in wheat can now be conducted by cytogenetic manipulation, rather than the "sledge-hammer" effect of radiation, its potential for wheat breeding is much more attractive. Nevertheless, considerable study and possibly further cytogenetic modifications may be necessary before genetic attributes produced by such techniques can be exploited in commercial wheats.

The 1974 Farrer Medallist, Dr. Helen Newton Turner described genetic diversity in plants and animals as "Hidden Treasure" and stressed the need for preservation of that diversity expecially in regions such as Turkey where the modern agricultural revolution is rapidly expanding. Frankel and Hawkes (1975) suggest that much of the natural variability in crop plants will be lost by 1985. If chromosome engineering is to play an important part in wheat breeding in the future, comprehensive collections of the related species will be essential.

II. THE GENETICS OF WHEAT RUSTS

"The greatest single undertaking in the history of applied plant pathology was to be the attack on the rust diseases of cereals" (Large 1962).

There are three rust diseases of wheat, viz., stem or black rust, leaf or brown rust, and stripe or yellow rust for which the fungal species Puccinia graminis f. sp. tritici,

P. recondita and P. striiformis, respectively, are responsible. Stripe rust is a disease of cooler environments and does not occur in Australia. As Farrer correctly concluded, stem rust is the more destructive of the two Australian rusts (illustrated late 3 in Plate 3). Therefore, the major part of our research and breeding efforts have been directed toward its biological control by breeding for resistance.

Biotrophic pathogens such as P. graminis and P. recondita infect and reproduce on restricted ranges of host plants. During the 1890's it was realised that different forms of P. graminis were specialised to the various cereals and grasses. The form affecting oats was restricted mainly to oats, whereas the wheat form attacked mainly wheat. About 1914, E.C. Stakman and co-workers in Minnesota found that cultures of the wheat form could be further distinguished by their ability to reproduce on different wheat genotypes. Using 12 wheat genotypes as testers these workers identified many distinguishable entities which they designated as "races" (Stakman et al. 1962). Similar variation was found in relation to the wheat-attacking forms of P. recondita and races of this pathogen were distinguished using eight differential genotypes (Browder 1971).

Life Cycles in Australia

P. graminis tritici. P. graminis is an heteroecious, or two-host pathogen, but on mainland Australia only the asexually reproducing, dikaryotic, red urediospore stage, produced on the graminaceous hosts, is important. The sexual stage derived from the black teleutospores is insignificant because the alternate hosts, common barberry (Berberis vulgaris) and Mahonia spp. are rare. Urediospores are dispersed over short and long distances by air movement.

Because the urediospore is not a truly resting stage, survival of the pathogen in Australia from one crop season to the next, that is, from about December until July or later, is dependent on its ability to remain within green tissues of self-sown wheat or barley and various grass species. The

relative importance of cereals and grasses on overseasoning is not known, but we attach greater significance to the former. Obviously, practices which reduce the amount of overseasoning rusts are important in disease control. Plate 4 illustrates overseasoning (infected) cereals on a roadside and in a potato crop in southern N.S.W. in a drought year (month of July).

late 4

P. recondita. P. recondita is also heteroecious but its alternate hosts Thallictrum spp. and Isopyrum spp. are not common in this country. The survival of this organism is more dependent on self sown wheat than is P. graminis tritici.

Nature of the Host Pathogen Interaction

A rust pustule resulting from infection of a wheat leaf by a single urediospore is truly an association of two organisms. Both host and pathogen contribute to its existence. As agriculturalists, we are firstly conscious of large pustules - the compatible interactions involving host and pathogen. At this arbitrary reference point the host is susceptible to the pathogen and the pathogen is virulent to the host. Because wheat is inbreeding all plants in an arbitrary susceptible "pure-line" host are genetically identical, and if urediospores of the single pustule are collected and increased, large numbers of genetically identical spores can be generated and can be stored in a refrigerator.

Suppose the host genotype is grown in a large field area and a rust epidemic is initiated using the above culture of the pathogen. The rate with which, and degree to which, the epidemic develops will depend upon:-

- the amount of inoculum used to initiate the epidemic
- the environment which includes the availability of free moisture for sufficient time to ensure maximum spore germination and infection, and high temperatures favouring development of the disease after infection
- the time available before the crop matures.

Since different combinations of these factors are responsible for variation in disease incidence from year to

year, "rusty" and "non-rusty" years are experienced. Before resistant wheats were introduced, rusty years occurred about once in four seasons in northern N.S.W. and Queensland, and as infrequently as once in 15 years in the Victorian Wimmera. Farrer did much to reduce the effects of stem rust by producing earlier maturing wheats which had increased abilities to "escape" damage, and which could be grown in drier environments that were less conducive to rust development.

Suppose epidemics can be produced at will and suppose further, that representative P. graminis clones are collected from various regions around the country. If then, separate epidemics are initiated by inoculating each of these clones into stands of the standard susceptible genotype it is expected that some will develop more rapidly than others. The clones may show differential pathogenicity. On the other hand, if pustule sizes differ markedly we say the clones show differential virulence or avirulence. This implies host resistance.

Suppose, thirdly, that a single clone is used to produce a series of epidemics in different cultivars. Again, a range of responses can be anticipated. In some cultivars the epidemic may develop very rapidly whereas in others it may develop less rapidly. Relative to the former, the latter genotypes may be considered "slow rusters". Other genotypes might develop little rust and would be considered resistant. This implies pathogen avirulence.

In the field situation, environment, host, and pathogen will vary simultaneously and may interact. For example, some variants of the pathogen may be favoured by high temperatures, or some resistance mechanisms may be more effective at cooler temperatures. Experimentally, we attempt to simplify the environment-host-pathogen system and to evaluate one variable at a time. This is achieved through the induction of field epidemics in relatively isolated, irrigated conditions at Castle Hill, using a limited number of pathogen strains, or by controlled inoculations, usually of seedlings, in the glasshouse.

Seedling rust reactions are usually described in terms of infection type (i.t.), the most common system being that described by Stakman et al. (1962) for stem rust. These vary from i.t. "0" where no macroscopic symptoms are evident or i.t. ";" (fleck) for small necrotic spots without sporulation, through i.t. "1" for small pustules with underlying necrosis to
Plate 5 i.t. "4" for the largest compatible pustules (Plate 5). One further variant is i.t. "X" for the mesothetic interaction involving a range of pustule sizes. Experience has shown that hosts producing i.t.s "0", ";", "1" and usually "2" will sustain no, or very little, disease. Others producing i.t.s "2", "3" and "4" will develop some disease ranging over degrees of resistance and susceptibility. Frequently, but not invariably, seedling i.t.s "2" and "3" can be related to degrees of resistance in mature plants in the field. Furthermore, genotypes producing i.t. "4" in the seedling stage may exhibit mature plant resistance.

Plant pathologists and writers (e.g. Day 1973) commonly describe i.t.s "0" - "2" as representing resistant host responses and i.t.s "3" and "4" as representing susceptible responses. Because different workers tend to rate infection types differently and because seedling infection types occur on a continuous scale, there is no clear-cut distinction between incompatibility and compatibility. Infection type "3" is relatively compatible compared with i.t. "1", but relatively incompatible compared with i.t. "4". Hence infection type data may be interpreted differently by different workers but obviously, there will be fewer disagreements as larger differences are considered.

In discussing infection types the response of a range of host genotypes to a single clone of the pathogen was considered. A range of pathogen clones can similarly produce a range of infection types on a single host genotype. Furthermore, infection types produced by a single pathogen clone and a single host genotype can be modified by the environment. Infection type is the means by which both the pathogen and the host are

assessed in a stipulated environment.

Genetics of host reaction and of pathogen avirulence and virulence

Inheritance studies in wheat have demonstrated the presence of at least 30 loci (*Sr* genes) involved in reaction to *P. graminis* and at least 20 loci (*Lr* genes) for reaction to *P. recondita*. McIntosh (1973) lists many of these genes. With both diseases, several instances of multiple allelism, or close linkage, of resistance genes have been reported. Resistance in any one host genotype is usually determined by one or relatively few host genes.

When rust resistant cultivars were first produced, agriculturalists found that only relatively short periods of time elapsed before they became susceptible. This was caused by the increase of new or previously rare variants of the pathogen on the previously resistant cultivars.

Working with flax rust (*Melampsora lini* : *Linum usitatissimum*), H.H. Flor (1956) showed that avirulence and virulence in *M. lini* with respect to a particular flax genotype was dependent on the presence and absence of a dominant allele for avirulence. Furthermore, avirulence and virulence for different flax genotypes was dependent on allelic variation at different *M. lini* loci. This "gene-for-gene" relationship, later demonstrated for wheat stem rust (Loegering and Powers 1962) and wheat leaf rust (Samborski and Dyck 1968), serves as the genetic basis for modern approaches to plant disease genetics and disease resistance breeding. As indicated in Figure 4 incompatibility is dependent on the presence of an allele determining resistance in the host and the corresponding allele for avirulence in the pathogen. Other combinations of host and pathogen genotypes result in compatibility and, generally, cannot be distinguished. Therefore an incompatible interaction, relative to a "compatible" standard, immediately indicates that host and pathogen carry at least one set of corresponding alleles for resistance and avirulence respectively. On the other hand,

Figure 4

a compatible interaction indicates no genetic information about host or pathogen since it results from virulence in the pathogen, lack of resistance in the host,or both.

The following generalities can be deduced from the gene-for-gene relationship:-

1. The incompatible infection type is characteristic of particular interacting genes. For example, the interaction of host gene Sr5 in wheat with its corresponding fungal avirulence gene usually produces i.t. "0", Sr11 produces i.t. ";1", Sr9b produces i.t. "2" or "23", and Sr15 produces i.t. "X".

2. When more than one set of corresponding genes producing incompatibility are involved, the infection type produced is similar to, or lower than, the single set with the lowest infection type. Plate 6 illustrates the complementary effect resulting from the combination of Sr22 and Sr15 when seedling leaves are infected with a P. graminis tritici clone with avirulence alleles corresponding to both host resistance alleles.

Plate 6

3. The assumption of a gene-for-gene relationship permits the allocation of hypothetical genotypes to hosts and pathogens without genetic knowledge of either. Where host lines carrying known resistance alleles are available (e.g. Sr5, Sr6, Sr7b, Sr8 in wheat) these can be used as testers in the identification of pathogen collections. This constitutes the genetic basis of strain identification. Collections which produce the same array of interactions with an arbitrary set of host testers are classified as the same race or strain. Each year at Castle Hill, up to 3,000 isolates of P. graminis or P. recondita from throughout Australasia are typed with respect to about 25 host testers. This strain survey provides information as to the particular strains, where they occur and their approximate proportions in the various states. Survey results can be considered in relation to the cultivars being grown, and in annual cultivaral recommendations.

Similarly, pathogen clones with known combinations

of virulence genes (strains) can be chosen to screen large collections of wheats in order to detect new sources of resistance. These wheats are then assessed under epidemic conditions in the field before use as parents in the wheat breeding programme and, may be genetically analysed to determine the number and linkage relationships of the genes involved.

Basis of Variability in Rust Pathogens

Although sexual reproduction on alternate hosts is not an important factor in the production of phenotypic variability in P. graminis tritici and P. recondita in Australia, the pathogens are nevertheless quite variable. Even before resistant varieties were used, Australian populations of these organisms, like populations elsewhere, were variable as demonstrated when clones were allowed to infect the respective sets of differential host genotypes. However, the combinations of virulence and avirulence (races or strains) that Waterhouse first observed were different from those occurring elsewhere and, furthermore, were quite distinctive from those occurring within Australia at the present time. A number of mechanisms have affected variability in P. graminis tritici populations.

Migration. When Waterhouse began studies of P. graminis tritici in 1919 he identified a small number of variants on the basis of their behaviour on the Stakman set of host differentials. In 1926 a new variant was detected in Western Australia. It rapidly moved to eastern Australia and by 1935 predominated throughout the country. Earlier types became extinct. In 1954 a second major change occurred. The new type predominated and the 1926 form disappeared. In 1968, a further distinctive group was found and these, or related types, currently co-exist with those derived from the 1954 group. Differences between these four, historical groups of variants were not greatly affected by the genotypes of host cultivars being grown. In each case new types appear to have originated from outside the country and once established here, became predominant because they were, in turn, more competitive than older types under

Australian conditions. There is considerable evidence to suggest that the variants appearing in Australia in 1968 originated from the Angola, Mosambique, Madagascar region of Africa.

Mutation: One characteristic of the genetic code is mutability which involves rearrangements of the order of bases constituting the code. Genes affecting virulence or avirulence in Puccinia are undoubtedly no different in this respect. Following the release of resistant cultivars in Australia, and elsewhere, virulent variants of the pathogens have regularly followed. The new types are usually similar to earlier types except for the added virulences necessary to overcome the particular host resistance genes.

From annual survey results it is apparent that mutation frequencies depend on the particular genes involved. Additionally, spontaneous mutants are occasionally encountered in the laboratory. Presumably mutation frequencies will also vary with the genotype of parental clones since if avirulence is dominant, two changes in homozygous, but one change in heterozygous, dikaryons are necessary for the expression and identification of virulence. In general, induced mutation studies in the laboratory using chemical mutagens reflect the relative natural mutation frequencies. However, in certain instances where natural variants are known it has been impossible to obtain virulent mutants even after recurrent mutagen treatments. It is therefore not possible to use the failure of recovery of induced mutants as a reliable indication that mutations to virulence are unlikely to occur in the field. Because of the lack of appropriate means of selection it has been virtually impossible to study mutation from virulence to avirulence in the rust pathogens.

Somatic Hybridization: When two readily distinguishable P. graminis clones are mixed and cultured on a host which is susceptible to both, new types may arise from the mixture (Watson 1970). Although this mechanism of variability appears

to have been important in the occurrence of some putative hybrid rusts adapted to certain grass species (Luig and Watson 1972) there appears to be only one group of important Australian P. graminis tritici types where such a mechanism for variability can be implicated.

Progressive Increases in Virulence: In a dikaryotic (or diploid) organism such as P. graminis, if avirulence and virulence for a particular host resistance gene are determined by alleles at a single locus, there are two disease phenotypes, incompatibility and compatibility, which can be expressed if either avirulence or virulence is dominant, or three disease phenotypes if either is not dominant and an intermediate level of interaction occurs. Watson and Luig (1968) described two instances with wheat stem rust where more than three levels of interaction were recognised. More recently, we (Luig and McIntosh unpublished) have identified additional instances of multiple levels of interaction involving wheat and P. graminis tritici. The genetic basis and evolutionary significance of these progressive changes are not known. Systems of strain classification based on two categories of pathogen phenotype, avirulence and virulence, cannot account for this type of variability.

Selection: The survival and predominance of successive groups of P. graminis tritici strains occurring in 1926, 1950 and 1968 were not greatly influenced by the genotypes of the wheats being grown. These appear to have been successful because they were more competitive than earlier types. However, as resistant cultivars were introduced after 1938, changes within groups were largely influenced by host genotypes. When wheats such as Yalta, Gabo and Charter with resistance gene Sr11 were first released all rusts in the country possessed the corresponding gene for avirulence and mutant variants with virulence had not been observed. When mutant types were exposed to these cultivars they were able to increase without competition and, because of the widespread use of such cultivars, the majority of surviving types had to possess

virulence for Srll in order to survive. Most subsequent changes therefore involved strains which were virulent for Srll initially because Srll was present in the "susceptible" wheats and later because virulence for Srll had reached a very high frequency.

Chance: Because the rusts go through boom and bust periods, not only of rusty and non-rusty years, but also from one crop year to the next, chance is a most important factor affecting strain survival. Although common strains are more likely to survive from one season to the next, the particular areas where oyerseasoning occurs and the particular self-sown host genotypes involved, will have significant effects. In some years the main strains surviving after extended periods of drought are quite different from those present beforehand.

Breeding for Resistance

The combined effects of the above factors on the pathogen need consideration when deciding the strategies of rust resistance breeding. Because we believe mutation is the most important predictable variable with respect to the pathogen, our breeding approach has been, and continues to be, one of assembling combinations of resistance genes in wheats such that when single mutational changes occur in the rusts there will be further host resistance genes to match other avirulence factors in such mutants. In order to achieve this we must anticipate the virulence gene combinations of future strains. The gene-for-gene relationship demands that important future strains must have a minimum of those virulence genes necessary to overcome presently resistant host cultivars, but they can carry other less predictable virulence genes which will be characteristic of the parent clones in which the critical mutations arise. Presumably, these parental strains will be similar to the contemporary predominant strains. In the laboratory, such anticipated strains are produced with chemical mutagens and are used to add additional genes to currently field-resistant cultivars.

However, the use of induced mutant strains has to be confined to the glasshouse. Additionally from annual strain surveys, clones with important combinations of virulence genes are occasionally identified and these can be used in the glasshouse and field at Castle Hill to supplement more common strains in the selection of host lines with new combinations of resistance genes. For example, rare strains virulent on the semi-dwarf wheats, Oxley and Condor, have been collected on several occasions, and even though such strains have not increased to significant proportions in the wheat belt, they are playing a major role in our selection programme.

Justification for a multigenic resistance approach is based on genetic analysis of the most resistant wheats from overseas breeding programmes. Almost without exception, these carry combinations mainly of known resistance factors. However, even if the biological approach of multiple gene resistance is valid there are various human, agronomic and economic reasons preventing its optimum application. Firstly, genes being used in multigenic resistances are also being used as single genes in other cultivars. In effect, we may be presenting considerable hurdles to the pathogen in some instances, but ladders are also being provided in other instances for the pathogen to overcome those hurdles by cumulative single step changes. Secondly, if a multigenic strategy is to be effective it may be necessary on occasions to withdraw cultivars from cultivation even before they become susceptible, especially if improved disease resistance is based largely on backcrossing. As far as the pathogen will be concerned, to-day's highest hurdle may be to-morrow's longest ladder. Those involved in cultivaral recommendations are fully aware of the problems in withdrawing susceptible cultivars - let alone resistant cultivars.

Although experience has clearly shown that single gene resistances are vulnerable, breeders are still content to release cultivars with resistances based on single genes. The increasing use of cultivars with resistance based on gene Sr26 derived from Agropyron elongatum is one example of a

particularly concerning situation. The life of this gene, for which virulence in P. graminis tritici is not known, might be greatly extended if it was used in multiple gene combinations rather than alone. On the other hand, if Sr26 continues to confer resistance, breeders will increase its use as a single gene resistance and disease losses as a result of virulence in the pathogen could be huge as a consequence.

A major consequence of the breeding of stem rust resistant wheats for the northern Australian wheat belt has been that an area which was vulnerable to frequent rust epidemics before 1940 can now cultivate prime quality wheats with considerable reliability of freedom from serious stem rust damage. The sacrifice for almost thirty-five years of rust resistance has been a regular turnover of cultivars and the loss of effectiveness of a number of resistance genes. Despite the pessimism that wheat breeders may be rapidly depleting the available resistance gene resources, I believe new resistance sources are still becoming available in adequate numbers to enable a continuation of present procedures. Additionally, greater attention to post-seedling resistances will increase the benefits to be derived.

What are the alternative procedures? The production of cultivars with multigenic resistances is only one strategy; others have been suggested but their theoretical and practical bases for Australian conditions at least, are not well substantiated. The deployment of resistance genes to designated geographical areas as a means of obtaining host diversity, and the use of genetic mixtures instead of "pure-line" cultivars have been suggested. Geographical gene deployment cannot work in practice without very strict controls over cultivars and since the genotypes of many of the resistant parents being used in breeding programmes are unknown and cannot be readily determined the allocation of such genes to designated areas is impossible. Furthermore, the prevented use of a cultivar in a particular area on the basis of non-permissive

resistance genes could be a serious economic liability to the industry. If heterogeneity is desired, the best we might expect to achieve could be an agreed allocation of potential parental genotypes to designated breeders in the hope that some genetic variability will result.

The use of multiline cultivars in disease control is based on contrasting philosophies. One group of multiline proponents suggest that breeders produce mixtures of genotypes which are resistant to all current strains of the pathogen. If one component of the mixture becomes susceptible in a particular crop year it is suggested that since plants of that genotype are interspersed with other genotypes which are resistant, the rate of spread of the new strain will be markedly reduced and, in any case, most of the mixture will be resistant. In the following year the susceptible genotype is replaced with an additional resistant line. This strategy of resistance breeding is being pursued by the CIMMYT organisation (Breth 1976) in an attempt to reduce the rust vulnerability of cultivars of the "8156" series which are grown on very large areas throughout the world.

The contrasting multiline procedure, pursued by oat breeders at Iowa State University, involves the use of mixtures of genotypes with resistance only to some strains, but which are susceptible to others. These workers have claimed that no significant losses to oat crown rust have occurred in such multiline cultivars since their introduction in 1968.

In recent years we have been advised by our pathologist colleagues, to breed for more permanent types of resistance - perhaps not complete resistance, but genetically more complex kinds of resistance that the fungi are not so inclined to overcome. Some people have nominated examples of parent lines which might be useful in deriving this kind of resistance. Recent work in our laboratory has suggested that resistance in two groups of such lines - Hope and Thatcher

derivatives - is controlled mainly by single genes. However, if we are to accept that Hope and Thatcher carry this more desirable permanent type of protection then we must be prepared for some losses in rusty years. Hope derivatives and Thatcher were protecting the North American spring wheat areas in the 1950's when race 15B caused losses estimated at 25%. The main consolation there was that durum wheats without these resistances, but grown in the same areas, suffered losses of 75%.

Robinson (1973) defined "horizontal" resistance as resistance beyond the pathogen's capacity for change. Although I have more respect for our enemy than to assume that permanent resistance can be a reality, I do believe that further research in this direction is warranted.

Acknowledgements

I express my gratitude to those who have advised, encouraged, helped and, particularly, financed me over the years. Firstly, I am deeply indebted to my academic advisers and colleagues, particularly Professor Irvine Watson, Dr. Eldred Baker and Dr. Harold Luig, much of whose work I have described. Secondly, my thanks to my technical assistant, Mrs. Partridge, to Mr. Oates, Officer-in-Charge at Castle Hill, and to colleagues and staff both at the Castle Hill Research Station and at the University for assistance in many ways. Photographs were produced by Mr. D.J.S. Gow. Finally, I thank the Commonwealth Wheat Industry Research Council, representing the taxpayers of Australia, who have supported my professional career.

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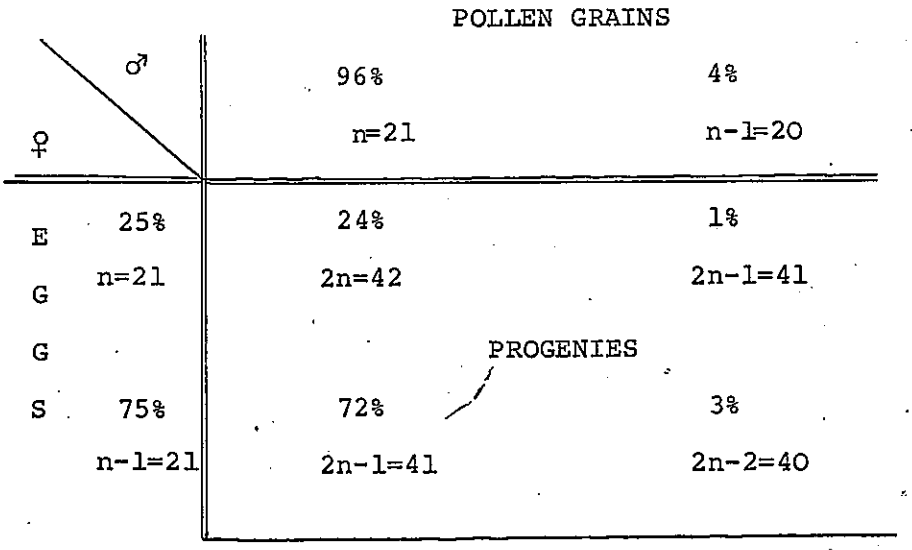

Figure 1. The breeding behaviour of an "average" Chinese Spring monosomic wheat plant when self-pollinated.

Legends for Figures 2 - 4.

Figure 2. Synthesis of octoploid triticale.

Figure 3. Synthesis of a wheat monosomic alien addition line.

Figure 4. The interactions produced when pathogen clones with avirulence (AA or Aa) and virulence (aa) are used to infect host seedlings with the corresponding genotypes for resistance (RR or Rr) and susceptibility (rr)

Legends for Plates 1 - 6.

Plate 1. Metaphase I chromosome pairing in hybrids : a) T. turgidum/T. monococcum (AAB) with 7 bivalents and 7 univalents (2n=21); b) T. aestivum/T. turgidum (AABBD) with 14 bivalents and 7 univalents (2n=35); c) T. aestivum cv. Chinese Spring/Secale cereale (ABDR), with 2 bivalents and 24 univalents (2n=28); d) T. aestivum cv. Chinese Spring/Secale cereale deficient for chromosome 5B, with 1 trivalent, 4 bivalents and 16 univalents (2n=27).

Plate 2. Spikes of some alien species which have contributed genes for disease resistance to wheat.

Plate 3. Wheat leaf rust (left) and wheat stem rust (right).

Plate 4. Typical situations in which overseasoning rust was found in southern N.S.W. Left, - a common roadside scene; right, - a mature potato crop with self-sown cereals as weeds.

Plate 5. Range of infection types produced when a series of wheat seedling genotypes is infected with P. graminis tritici. Left to right, infection types "0", ";", "1", "2", "3", "4", "X".

Plate 6. Infection types produced when host genotypes (l. to r.) Sr15 Sr15 Sr22 Sr22, sr15 sr15 Sr22 Sr22, Sr15 Sr15 sr22 sr22, sr15 sr15 sr22 sr22, were infected with a culture of P. graminis tritici with both corresponding genes for avirulence. Note the complementary effect of the combined resistance genes.