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# Evolution of mildew resistance in a hybrid bulk population of barley

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Inbred lines derived from successive generations of a composite cross population of barley, CCV, by single seed descent were screened for variation in mildew resistance with the aim of investigating the effect of natural selection on the population. A significant increase in the frequency of plants resistant to the open-air mildew spores over 10 generations was observed. Tests of reaction to selected mildew isolates of known virulence genotypes showed a similar shift towards increased resistance to single and combined isolates. These results are discussed in relation to the effect of selection on inbreeding species and the usefulness of composite crosses as dynamic reservoirs of genetic diversity.

**Keywords:** barley, composite cross, host–pathogen interaction, powdery mildew, selection.

## Introduction

Hybrid bulk populations or composite crosses (CC) of self-fertilizing crop species have been extensively used as experimental populations in evolutionary studies. These studies include: observing changes in gene frequencies at particular loci in successive generations; comparing the performance of the population with commercial cultivars; comparison of the performance between generations; selecting individual lines periodically and using their performance as an index of change in the population; creating sub-populations from one composite cross and growing them in different localities to study the effect of specific environmental differences on the evolutionary process. Barley is especially suited for such studies because it is diploid and has a relatively small number of chromosomes ($n=7$) all of which are well mapped genetically; it reproduces predominantly by self-fertilization and yet is easily hybridized artificially.

Powdery mildew of barley, caused by *Erysiphe graminis* DCf. sp. *hordei* Marchal, has long been recognized as an important fungal disease of barley in Western Europe. In the United Kingdom, powdery mildew is the principal disease of cultivated barley. The pathogen may attack barley plants at all stages of their growth causing estimated yield losses of up to 25 per cent (Large & Doling, 1962; King, 1972; 1977). The genetic interaction between powdery mildew of barley and its host is recognized as belonging to the gene-for-gene system described by Flor (1956; Moseman, 1957). Many virulence genes in the pathogen population have been observed to occur at frequencies which reflect the frequencies of the corresponding resistance genes in the host population. The number of the virulence genes carried by individual mildew isolates has also been shown to increase with the increased use of varieties that carry multiple resistance genes. Increased fungicide insensitivity in response to the wide-scale application of the triazole fungicides has also been reported (Wolfe & Barrett, 1980; Limpert & Schwarzbach, 1981; Wolfe *et al.*, 1984; Brown, 1989; Brown & Wolfe, 1990).

Several workers have investigated the evolutionary significance of resistance to fungal pathogens in a number of composite cross populations of barley including CCII (Allard, 1990), CCV and CCXXI. The diseases studied include scald caused by *Rhynchosporium secalis* (Oud.) Davis (Jackson *et al.*, 1978, 1982; Muona *et al.*, 1982, 1984; Saghai-Maroof *et al.*, 1983; Webster *et al.*, 1986) net blotch, *Helminthosporium teres* and powdery mildew (Saghai-Maroof *et al.*, 1983; De Smet *et al.*, 1985). These studies consistently showed significant shifts towards increased resistance to the pathogens with time. However, as a result of some inconsistencies in the magnitude of the changes observed in different populations, and the

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dynamic nature of the racial composition of the pathogen population, the general conclusion from these studies has been that the observed changes in resistance are due to the association of the resistance with other traits that confer selective advantage.

Three sub-populations of composite cross five (CCV), which were grown in Cambridge as parallel populations for 15 years, were screened for variation in mildew resistance in order to determine the evolutionary significance of host resistance to the pathogen.

## Materials and methods

Composite cross five (CCV) was synthesized at Davis, California in 1937 by inter-crossing 30 diverse varieties of barley and their $F_1$ hybrids. The hierarchical way in which the varieties and their $F_1$ hybrids were crossed and mixed to create the hybrid population has been described in detail by Harlan *et al.* (1940), Luckett (1982) and Ibrahim (1989). The population has subsequently been grown annually at Davis in large plots under standard agricultural conditions without conscious selection. In 1974, large samples of seeds from $F_{10}$, $F_{20}$ and $F_{30}$ of the Californian CCV population were brought to Cambridge, UK where they have been maintained as parallel populations(Populations 1, 2 and 3) ever since.

Four generations spanning a period of 10 years were selected from each of the three populations (Table 1a). The convention adopted for naming the generations

**Table 1** The generations and year of harvest of the seed samples used in the field experiment (a), the six infection types used to classify the reaction of the plants to powdery mildew (b) and the virulence genotypes of the four mildew isolates used in the laboratory experiment (c). In (b), letters in brackets indicate the reaction classes to which each infection type was assigned when data were pooled to facilitate analysis.

| (a) | Population | Seed generation | | | |
|---|---|---|---|---|---|
| | 1 | $F_{12}$ | $F_{15}$ | $F_{18}$ | $F_{21}$ |
| | 2 | $F_{22}$ | $F_{25}$ | $F_{28}$ | $F_{31}$ |
| | 3 | $F_{32}$ | $F_{35}$ | $F_{38}$ | $F_{41}$ |
| Year of harvest | | 1975 | 1978 | 1981 | 1985 |

| (b) Infection type | Description | Mildew reaction |
|---|---|---|
| 0 | No symptoms | Highly resistant (R) |
| 1 | Necrotic spots, no sporulation | Resistant (R) |
| 2 | Lesions and no sporulation | Moderately resistant (R) |
| 3 | Fewer than 10 sporulating lesions | Moderately susceptible (S) |
| 4 | More than 10 sporulating lesions | Susceptible (S) |
| 5 | Abundant lesions coalesced with profuse sporulation, yellowing | Highly susceptible (S) |

| (c) | CC/1 | CC/99 | CC/124 | CC/128 |
|---|---|---|---|---|
| | — | — | *Va1* | — |
| | — | — | *Va6* | *Va6* |
| | — | *Va7* | — | *Va7* |
| | *Va8* | *Va8* | — | — |
| | — | — | *Va9* | — |
| | *Va12* | *Va12* | — | *Va12* |
| | — | — | — | *Va13* |
| | — | *Vab* | — | — |
| | — | *Vcp* | *Vcp* | *Vcp* |
| | — | — | *Vg* | *Vg* |
| | — | *Vh* | — | — |
| | — | — | *Vk* | *Vr* |
| | — | *Vra* | *Vra* | — |
| | — | *Vv* | *Vv* | — |

consists of the numbers 1, 2 or 3 which refer to the populations followed by the actual generation of the seeds. In the summer of 1986, seed samples from these 12 generations were sown in the field at a spacing of 7 inches (17.5 cm) between plants and 20 inches (50 cm) between rows. An assessment of mildew infection was carried out 6 weeks after sowing. The second lowest leaf of the oldest (longest) tiller was scored using a scale of 0–5 corresponding to the mildew infection types shown in Table 1b. The infection type of the 33 middle plants in each row, plus an extra plant from the third row of each generation, were recorded. This yielded data on a random sample of 100 plants per generation which were later harvested individually to obtain a set of 400 family lines per population.

In a second experiment, seedlings of the family lines and the CCV parental lines were grown in a disease-free, filtered-air glasshouse. A 1-inch (2.5-cm) long segment was cut from the first leaf when approximately 10 days old and artificially inoculated with mildew spores from the four isolates shown in Table 1c. Agar medium (0.5 per cent) containing 100 p.p.m. benzimidazole (senescence inhibitor) was poured into polystyrene boxes measuring $12.3 \times 8.0 \times 2.0$ cm to a depth of 0.6 cm and allowed to cool. The leaves were placed on top of the agar, adaxial surface uppermost. Golden Promise leaf segments, a variety susceptible to the four isolates tested, were included in each box as a control to test the success of the inoculation.

The inoculation method used was essentially as described by Wolfe *et al.* (1984). An aluminium cylindrical tower, 36 inches (90 cm) high and 14 inches (35 cm) in diameter with a small circular hole [diameter = 13/16 inches (2 cm)] on the wall, 5 inches (12.5 cm) from the top, was used. Spores from the isolates were shaken on to a sheet of paper which was then folded into a conical shape. The polystyrene boxes containing the leaf segments were placed inside the sterilized tower and the spores were blown into the tower by placing the narrow end of the cone through the circular hole on the wall and blowing at the other end. The boxes were then incubated in growth cabinets at 15°C and 16 h daylength. Under these conditions, mycelia were clearly visible 7 days after inoculation and spore production became obvious after 10 days. All infected leaf segments, regardless of the size of the pustule or the number of colonies, were scored as susceptible. The segments which were either completely free of infection, or which showed hypersensitive reaction in the form of necrotic spots or patches were classified as resistant. Fifty family lines from each of the $1F_{12}$ and $1F_{21}$ generations and the 30 parental lines were tested.

## Results

### *Host reaction to natural infection*

The numbers of plants that show the different mildew infection types in the field experiment, re-classified into three groups from the original five, are given in Table 2. Statistical analysis of these data was carried out in four steps in order to examine the following aspects of mildew infection.

**1** Are there any significant differences between the frequencies of the different mildew reaction types in the three rows for each generation?

**2** Are the frequencies of mildew reaction types independent of the generation of the plants?

**3** Are the years of harvest of the parent plants associated with the mildew reaction type of the progeny plants?

**4** Do the three populations differ in their resistance to powdery mildew?

A chi-squared test was carried out in order to test the homogeneity of the distribution of the mildew reaction types in the three rows for each generation. Eleven of the 12 sets of data produced non-significant associations and the significant test on $1F_{21}$ can probably be ascribed to Type I error (Table 2). The row data were pooled to give the infection type frequencies and tested for association between mildew reaction and the generation in each population. The three chi-squared values obtained were significant at the 1 per cent level (Table 2). It appears that within each population, the frequencies of the different mildew reaction types are significantly associated with generations. The generation totals in Table 2 show a marked increase in the frequency of resistant plants in the later generations of all three populations. The shift towards increasingly resistant plants seems to have occurred mainly after 1978.

Three of the four tests for homogeneity in the frequencies of mildew reaction types in concurrent generations in the three populations gave highly significant chi-squared values. The comparison between the three 1978 generations, i.e. $1F_{15}$, $2F_{25}$ and $3F_{35}$ was not significant. This implies that, with the exception of the three generations harvested in 1978, there are significant differences in the frequencies of the different reaction types in concurrent generations from the three populations. A closer look at Fig. 1 reveals that the frequency of resistant plants in the 1975 and 1978 generations is in the range of 18–25 per cent, except for $2F_{22}$ where the frequency is 10 per cent. Within this range, the variation in the frequency of resistant plants appears to be independent of the populations. This

**Table 2** Number of plants showing resistant (R), susceptible (S) and intermediate (I) reaction types to the open-air mildew spores in the field experiment.

| | | Mildew reaction | | | | Tests of association† | |
|---|---|---|---|---|---|---|---|
| Generation | Row | R | S | I | N | $\chi^2(1)$ | $\chi^2(2)$ |
| Population 1 | | | | | | | |
| $F_{12}$ | 1 | 7 | 15 | 11 | 33 | | |
| | 2 | 11 | 15 | 7 | 33 | | |
| | 3 | 4 | 23 | 7 | 34 | | |
| Total | | 22 | 53 | 25 | 100 | 7.011 | |
| $F_{15}$ | 1 | 4 | 26 | 3 | 33 | | |
| | 2 | 7 | 21 | 5 | 33 | | |
| | 3 | 7 | 22 | 5 | 34 | | |
| Total | | 18 | 69 | 13 | 100 | 2.217 | |
| $F_{18}$ | 1 | 23 | 3 | 7 | 33 | | |
| | 2 | 20 | 6 | 7 | 33 | | |
| | 3 | 19 | 7 | 8 | 34 | | |
| Total | | 62 | 16 | 22 | 100 | 2.116 | |
| $F_{21}$ | 1 | 29 | 1 | 3 | 33 | | |
| | 2 | 21 | 7 | 5 | 33 | | |
| | 3 | 26 | 1 | 7 | 34 | | |
| Total | | 76 | 9 | 15 | 100 | 10.916* | 129.65*** |
| Population 2 | | | | | | | |
| $F_{22}$ | 1 | 4 | 25 | 4 | 33 | | |
| | 2 | 2 | 27 | 4 | 33 | | |
| | 3 | 4 | 27 | 3 | 34 | | |
| Total | | 10 | 79 | 11 | 100 | 1.066 | |
| $F_{25}$ | 1 | 5 | 22 | 6 | 33 | | |
| | 2 | 10 | 18 | 5 | 33 | | |
| | 3 | 6 | 23 | 5 | 34 | | |
| Total | | 21 | 63 | 16 | 100 | 2.790 | |
| $F_{28}$ | 1 | 15 | 5 | 13 | 33 | | |
| | 2 | 17 | 6 | 10 | 33 | | |
| | 3 | 15 | 10 | 9 | 34 | | |
| Total | | 47 | 21 | 32 | 100 | 2.946 | |
| $F_{31}$ | 1 | 12 | 15 | 6 | 33 | | |
| | 2 | 13 | 11 | 9 | 33 | | |
| | 3 | 15 | 6 | 13 | 34 | | |
| Total | | 40 | 32 | 28 | 100 | 6.745 | 87.41*** |
| Population 3 | | | | | | | |
| $F_{32}$ | 1 | 10 | 14 | 9 | 33 | | |
| | 2 | 10 | 8 | 15 | 33 | | |
| | 3 | 5 | 19 | 10 | 34 | | |
| Total | | 25 | 41 | 34 | 100 | 8.220 | |
| $F_{35}$ | 1 | 4 | 21 | 8 | 33 | | |
| | 2 | 8 | 17 | 8 | 33 | | |
| | 3 | 8 | 18 | 8 | 34 | | |
| Total | | 20 | 56 | 24 | 100 | 2.057 | |
| $F_{38}$ | 1 | 18 | 10 | 5 | 33 | | |
| | 2 | 12 | 16 | 5 | 33 | | |
| | 3 | 10 | 13 | 11 | 34 | | |
| Total | | 40 | 39 | 21 | 100 | 7.376 | |
| $F_{41}$ | 1 | 18 | 10 | 5 | 33 | | |
| | 2 | 13 | 11 | 9 | 33 | | |
| | 3 | 13 | 9 | 12 | 34 | | |
| Total | | 44 | 30 | 26 | 100 | 4.158 | 24.39*** |

†Tests of association: (1) between mildew reaction type and rows in each generation (4 d.f.); (2) between mildew reaction type and generation in each population (6 d.f.).
The chi-squared values for testing the association between the year of harvest and the reaction type were 31.23***, 5.25, 19.60** and 32.56*** for 1975, 1978, 1981 and 1985 respectively (d.f. = 4). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

would suggest that until 1978 all three populations had a similar level of resistance to powdery mildew and that the significant chi-squared value for the comparison between the 1975 generations could be the result of the exceptionally low frequency of resistant plants in $2F_{22}$. In the 1981 and 1985 generations, however, the frequency of resistant plants ranged between 40 and 62 per cent, with the generations from Population 1 showing the highest frequency in both years. Furthermore, within each of these years, the population versus mildew reaction comparisons gave highly significant chi-squared values, which suggests that the rate of increase in the frequency of resistant plants was not the same in the three populations.

### *Host reaction to selected mildew isolates*

The frequencies of the $1F_{12}$, $1F_{21}$ and parental lines resistant to the four isolates tested are given in Table 3. Of the 30 parental lines, at least two were resistant to any one of the four mildew isolates tested. In the period between $1F_{12}$ and $1F_{21}$, the frequency of lines resistant to isolates CC/1, CC/99 and CC/128 has more than tripled. The frequency of plants resistant to CC/124 appears to have remained unchanged at 10–12 per cent in both generations. Comparing these two generations with the parental lines, the $1F_{12}$ family lines show closer similarity to the parental lines in their reaction to the four mildew isolates than the $1F_{21}$ lines. However, only 22 per cent of the $1F_{12}$ lines were resistant to CC/1 compared with 37 per cent in the parental lines.

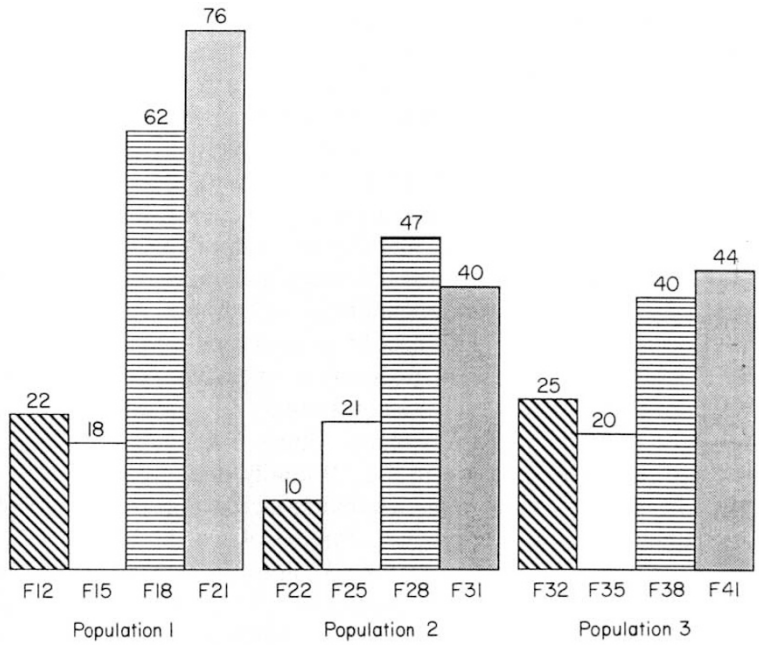


**Fig. 1** Percentage frequency distribution of plants resistant to natural mildew infection in field experiment. Bars with the same shading represent generations harvested in the same year.

Resistance to CC/128 also appears to be slightly higher in the parental lines (16 per cent) than in the $1F_{12}$ lines (12 per cent). In contrast, the frequency of plants resistant to three of the four isolates was consistently higher in the $1F_{21}$ lines than both the parental and $1F_{12}$ lines, while the resistance to isolate CC/124 was lower by 6 per cent in the $1F_{21}$ lines than the parental lines. A more striking feature of the data in Table 3 is that although only two of the 30 parent lines were resistant to isolate CC/128, the frequency of plants resistant to this isolate has increased to 74 per cent in $1F_{21}$.

Combinations of resistance to more than one isolate in single families are of particular interest because they shed some light on the inheritance of the resistance to the four isolates. In the extreme case where the resistance to the four isolates is controlled by independent factors, and the host reaction to the isolates can be classified as either resistant or susceptible, there would be 16 ($2^4$) possible phenotypes (family types). However, the virulence genotypes of the isolates used indicate that all lines resistant to isolates CC/99 and CC/128 are also likely to be resistant to CC/1, whose identified virulence genes ($V_{a8}$ and $V_{a12}$) form a subset of the virulence genes in the other two isolates (Table 1c).

The total number of phenotypes observed was 12 of which nine were present in the parental lines (Table 3). There were 10 family types in the $1F_{12}$ lines, two of which were new combinations not present in the parental lines. In the $1F_{21}$ lines, of the seven family types observed, five were similar to the parental types, one was unique and one was similar to one of the two new combinations found in $1F_{12}$. Hence, there were only three phenotype combinations not observed in the parental lines which appeared in the later generations. This represents only a modest increase in the diversity of phenotypes as a result of recombination and segregation in the years between the initiation of the

**Table 3** Numbers of the 12 combinations of reaction to the four mildew isolates, CC/1, CC/99, CC/124 and CC/128 in the CCV parent lines (P) and the family lines extracted from $F_{12}$ and $F_{21}$.

| | Type | | | | | | |
|---|---|---|---|---|---|---|---|
| Reaction | CC/1 | CC/99 | CC/124 | CC/128 | P | $F_{12}$ | $F_{21}$ |
| 1 | R | R | R | R | 1 | 1 | 2 |
| 2 | R | R | R | S | 2 | 1 | 0 |
| 3 | R | R | S | R | 0 | 2 | 31 |
| 4 | R | S | R | R | 0 | 0 | 1 |
| 5 | R | S | R | S | 3 | 1 | 2 |
| 6 | R | S | S | R | 1 | 0 | 0 |
| 7 | R | S | S | S | 4 | 6 | 1 |
| 8 | S | R | R | S | 1 | 1 | 0 |
| 9 | S | R | S | S | 1 | 3 | 0 |
| 10 | S | S | R | S | 2 | 2 | 0 |
| 11 | S | S | S | R | 0 | 1 | 3 |
| 12 | S | S | S | S | 15 | 31 | 10 |
| Total | | | | | 30 | 49 | 50 |

composite cross and the harvest of the twelfth generation. On the other hand, there has been a substantial decrease in the frequency of the phenotype for joint susceptibility to the four isolates from 50 and 63 per cent in the parental and $1F_{12}$ lines respectively, to 20 per cent in the $1F_{21}$ lines. In contrast, the frequency of multiple resistant types increased over generations. The distribution of the phenotypic combinations (Table 3) clearly indicates that the reactions to the four mildew isolates are not independent of each other.

Among the 30 parental lines, 15 were susceptible to all the isolates, seven were resistant to one, five were resistant to two, two were resistant to two and one was resistant to all four of the isolates. These frequencies showed a minor deviation in the 50 family lines from $1F_{12}$. In contrast, there was a dramatic increase in the frequency of plants resistant to three isolates in the $1F_{21}$ family lines. Thirty-one of the 32 lines resistant to three isolates in $1F_{21}$ were resistant to CC/1, CC/99, and CC/128. In fact, this phenotype, which is one of the three new combinations that were not present in the parental lines, has become the most abundant phenotype by $1F_{21}$.

### *Correlations between mildew reaction phenotypes*

The number of phenotypic combinations between the types of natural infection by mildews and reaction to the four isolates is 48 ($2^4 \times 3^1$). In order to carry out tests of association, this was reduced to 16 by excluding the reaction to isolate CC/124 for which no significant shift across generations was observed, and, by pooling the intermediate (*I*) and susceptible (S) reaction types to natural infection into one group designated S′. The pooling had the added advantage of making the scale used to score plants in the field experiment more compatible with that of isolate tests where all lines with any amount of mildew growth were classified as susceptible. The frequencies of the 16 phenotype combinations and the chi-squared values for the tests of association, none of which show significance, are given in Table 4a. It appears that the reaction of the family lines to the individual mildew isolates is independent of the reaction of their parent plants to natural infection.

The four isolates tested, taken together, are known to possess a substantial proportion of the virulence genes present in the air spora. It is therefore likely that family lines with triple or quadruple resistance to these isolates would show resistance to natural infection from the air spora. To test this, the data were pooled and the reaction to isolate CC/124 was excluded as above. In addition, the $1F_{12}$ and $1F_{21}$ data were pooled to avoid expected frequencies of less than five in the contingency table. The family lines were then grouped into two classes, those showing resistance to more than

**Table 4** Tests of association (d.f. = 1) between the reaction of the family lines to natural infection (RNI) and to (a) individual mildew isolates, (b) the four isolates together (see text)

| (a) Generation | | Reaction to isolates | Reaction to natural infection R | S′ | $\chi^2$ |
|---|---|---|---|---|---|
| $F_{12}$ | CC/1 | R | 3 | 8 | |
| | | S | 7 | 31 | 0.41 |
| | CC/99 | R | 4 | 4 | |
| | | S | 6 | 35 | 5.15* |
| | CC/128 | R | 2 | 2 | |
| | | S | 8 | 37 | 1.87 |
| $F_{21}$ | CC/1 | R | 29 | 8 | |
| | | S | 11 | 2 | 0.23 |
| | CC/99 | R | 27 | 6 | |
| | | S | 13 | 4 | 0.20 |
| | CC/128 | R | 31 | 6 | |
| | | S | 9 | 4 | 1.27 |
| (b) RNI | Multiple resistance | | Single or no resistance | | |
| R | 31 | | 19 | | 23.82*** |
| S′ | 7 | | 42 | | |

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

one isolate and those which are susceptible to all or resistant to just one isolate. A test of association between these two classes and the reaction to natural infection yielded a significant chi-squared value ($n=99$, data for one family line from $1F_{12}$ were missing). More than 80 per cent of the lines resistant to two or more isolates were derived from parent lines which were resistant to the mildew in the field. Sixty-nine per cent of the plants which showed mildew resistance in the field had progeny lines with multiple resistance to the mildew isolates (Table 4b).

## Discussion

The major findings of the survey of variation in powdery mildew resistance in Cambridge CCV can be summarized as follows.

**1** There have been large directional shifts towards increased resistance both to natural infection and to selected mildew isolates.

**2** There are differences between the three populations in the rate of increase of the frequency of resistant plants.

**3** There is an indication of the time when the major part of the differential survival and reproduction in the population which led to the shift could have taken place.

**4** Some insight into the diversity of mildew-resistance genes in the parental line and the subsequent generations of the population.

That severe mildew attack can cause differential viability and fecundity in individual plants, depending on their resistance, is well established. Last (1962) carried out detailed studies of the physiological disruption caused by mildew infection in barley and reported a reduction in root growth of up to 50 per cent. Barley seedlings are rarely killed by mildew attack in the field but produce fewer tillers that are likely to survive to maturity and produce ears (Burdon, 1987). These two effects combined, lead to a commonly observed effect of the disease in the field, i.e. fewer fertile ears per plant. Other components of grain yield, including the number of grains per ear and grain size, are also affected by mildew infection, but to a lesser extent (Brooks, 1972; Griffiths *et al.*, 1975).

In the relatively dry climate of Southern California where CCV was initially produced, mildew epidemics are not very common. In contrast, in the wet, temperate climate of Cambridge where yield losses of up to 20 per cent due to mildew attack have been reported (Last, 1955), epidemics are common and mildew resistance could confer selective advantage in the CCV populations which essentially represent mixtures of highly inbred plant genotypes. This could have led to the observed shift towards increased resistance to powdery mildew in the three populations.

In the field experiment, however, it was observed that the proportion of resistant plants in the latest generation of Population 1 was higher than those in the latest generations of Populations 2 and 3. Since the three populations have been maintained under similar conditions with regard to sowing dates, harvest dates and all other agricultural practices, the discrepancies in the rate of increase of resistant plants in the three populations could be an effect derived from differences in the genetic composition of the initial populations. Population 1 was initiated from seed samples from $F_{10}$ of the original CCV population at Davis, California, while Populations 2 and 3 originated from $F_{20}$ and $F_{30}$ seeds respectively. The initial seeds of Population 1, therefore, had undergone only 10 generations of selfing when they were first introduced to Cambridge, compared with 20 and 30 generations for those of Populations 2 and 3 respectively. Three possible ways in which this could influence the subsequent evolutionary changes in the three populations are discussed below.

**1** A number of studies involving the original CCV population at Davis have shown that the population was evolving towards increased fitness, measured as grain yield (Allard & Jain, 1962; Allard *et al.*, 1971, 1972). In this process, the genetic diversity of the population decreases as the fitter genotypes contribute more to subsequent generations (Allard & Jain, 1962; Brown, 1978) and diversity in mildew resistance genes could also decline with time. It is possible, therefore, that in the case of Population 1 the initial seeds were more diverse in their genetic make-up and that the subsequent selection for plants resistant to powdery mildew under Cambridge conditions had more genetic variation to operate on, leading to a faster rate of increase in the frequency of resistant plants compared with Populations 2 and 3.

The absence of significant differences in the frequency of resistant plants in $1F_{12}$, $2F_{22}$ and $3F_{32}$, the closest available generations to the three initial generations, does not support this argument. However, it is possible that this could be the result of the extensive changes in race composition that occurred in the mildew population in the period between the introduction of CCV to Cambridge and the summer when the tests were carried out. The major trend in these changes has been that virulence genes in the pathogen population tend to increase or decrease depending on the resitance genes deployed in the current commercial varieties. Wolfe (1984), Wolfe *et al.* (1981), Wolfe & Barrett (1980), Wolfe & Schwarzbach (1978) have given examples of several virulence genes that have

become widespread in Western Europe as a result of matching resistance genes being widely used in commercial varieties of barley. In addition, the use of varieties with multiple resistance genes has led to an increase in the frequency of isolates carrying many virulence genes.

2 The frequency of heterozygous individuals in highly selfing hybrid populations like CCV decreases with time. The expected frequency of heterozygotes at a single locus with two alleles after nine generations (at $F_{10}$) of complete selfing is $(\frac{1}{2})^9 = 0.20$ per cent. This proportion, which increases with an increase in the number of loci, the number of alleles per locus and the rate of out-crossing, become $(\frac{1}{2})^{19}$ and $(\frac{1}{2})^{29}$ in $F_{20}$ and $F_{30}$, respectively. Jain & Allard (1960) conducted a study to determine changes in the frequency of heterozygotes in successive generations from the original CCV population. They showed that heterozygotes at a number of loci did not decline as fast as expected given the amount of inbreeding common in CCV under Californian conditions. Hence, all the original introductions of CCV seeds could have had some individuals heterozygous at the mildew-resistance loci, but the frequency of heterozygotes in the $F_{10}$ seed sample from which Population 1 originated would have been higher than in the $F_{20}$ and $F_{30}$ seed samples. In the subsequent generations the heterozygote individuals would segregate and release new allelic combinations.

3 In a population of finite size, changes in genetic composition can occur from generation to generation due to sampling leading to erosion of variation. In very large populations, the rate of loss of variation may not be very large on a single locus basis, but when combinations at different loci are considered, especially in an inbreeding species, there may be a substantial loss of combinations of genes. Therefore, if reasonably large, same-sized populations of an inbreeding species are maintained under identical conditions, drift in the combination of alleles is likely to happen although individual alleles may not show significant frequency shifts.

There are a number of explanations for the apparent absence of correlations between the resistance of the family lines to the single isolates and the resistance of their parent plants to the open-air mildew spores. A particular family line may be susceptible to the mildew spores in the air but resistant to one or more of the single colony isolates because it possesses resistance genes that have been overcome by virulence genes present in the air spora. On the other hand, a line could be resistant to the spores in the air but susceptible to one or more of the isolates for one of two reasons; (a) the virulence genes in the isolate to which the particular line is susceptible may be absent or in very low frequency in the open-air mildew population; (b) the barrier and dilution effects of neighbouring plants could lead to that particular plant not being exposed to compatible mildew genotypes. Furthermore, the confounding effect of the different scoring scales in the two experiments may have contributed to the absence of correlation.

The virulence genes in the four isolates tested, taken together, may represent a substantial proportion of the total virulence spectrum of the air spora. It is therefore likely that family lines showing triple or quadruple resistance to the isolates would also show resistance to the open-air mildew spores. The tests carried out confirmed this (Table 3); in both generations, 90 per cent of the lines that showed combined resistance to three or more isolates were also resistant when exposed to the open-air mildew spores.

A major problem in the interpretation of the shift towards increased resistance observed in the three populations of CCV is to determine whether it is due to selection for resistance *per se*. Populations of strongly inbreeding species like barley are generally divided into a number of families, members of which have largely the same multi-locus genotype (Allard *et al.*, 1972; Brown *et al.*, 1980). When the genetic composition of such populations is observed to change systematically and consistently over time with respect to a single character, it is tempting to ascribe such changes to the action of selection on that character. However, there is always the danger that selection acts on another character and that the observed character is 'hitch-hiking'. In the Cambridge populations of CCV several studies have shown consistent and systematic changes in a range of characters (Edwards, 1975; Luckett, 1982; Ibrahim, 1989) and apart from the changes in the date of flowering, the agent that produces these changes has remained elusive. In the case of increased resistance to powdery mildew, however, we do have a source which is always present, can produce substantial differences in reproductive output in different genotypes (Burdon, 1987; Brooks, 1972) and is known to have changed during the period in which the genetic changes occurred. Taken together, these factors provide strong circumstantial evidence that natural infection by powdery mildew has led to an increase in the frequency of barley genotypes resistant to one or more common pathogen genotypes present in the air spora and that other characters may be hitch-hiking with the resistant phenotypes.

Other surveys of genetic variation at loci controlling morphological and quantitative characters in the Cambridge CCV populations have indicated that, in spite of the detectable effect of natural selection favouring particular phenotypes, a substantial amount

of genetic variation still exists in the populations (Allard, 1988; K. M. Ibrahim & J. A. Barrett, in preparation). This raises the possibility of using composite crosses for conservation of crop genetic resources (Allard, 1990). The extent of the shift in mildew resistance reported in this study, and estimated losses of diversity of 50–75 per cent reported in other composite crosses (Marshall & Brown, 1975), precludes the use of composite crosses for long-term conservation of total genetic diversity. However these populations may be useful as dynamic mass reservoirs of exploitable genetic diversity that keep pace with the evolution of pathogen populations.

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