Observed and Predicted Changes in Virulence Gene Frequencies at 11 Loci in a Local Barley Powdery Mildew Population

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ABSTRACT

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The aim of the present study was to investigate observed and predicted changes in virulence gene frequencies in a local aerial powdery mildew population subject to selection by different host cultivars in a local barley area. Observed changes were based on genotypic frequencies obtained through a survey comprising 11 virulence loc. Predictions were based on a model where selection forces were estimated through detailed mapping in the local area of host cultivars and their resistance genes, and taking into account the changes in distribution of host cultivars during the year caused by growth of both autumn- and spring-sown host crops. Large differences in gene frequencies were observed between spore samples collected in periods with a different distribution of host cultivars, whereas only minor differences were observed between samples collected at different times within periods with a constant distribution of host cultivars. Significant changes in gene frequencies were observed for virulence genes subject to strong direct selection as well as for genes subject mainly to indirect selection (hitchhiking). These patterns of changes were generally as predicted from the model. The influence of local selection forces on the local aerial population was demonstrated by a sample of aerial spores with a unique genotypic distribution collected in summer 1989. The results emphasize the importance of knowledge concerning sources of samples when analyzing the dynamics in pathogen populations. Further, the results imply that virulence survey data, which are based on random spore samples collected in regions with a uniform distribution of the different host cultivars, form the most favourable basis for predicting the changes in the genetic composition of aerial powdery mildew populations.

Additional keywords: Erysiphe graminis f. sp. *hordei*, host crop distribution, modeling, population dynamics, selection forces.

Barley powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Ém. Marchal) is a haploid, biotrophic pathogen prevalent in many barley growing areas. It reproduces asexually on host cultivars throughout the year in most temperate regions (15), and forms cleistothecia on the ripening host with ascospores being released in autumn (25). Autumn-sown barley is infected by spores produced on volunteer plants of previous barley crops and by ascospores from cleistothecia, whereas spring-sown barley is infected by spores produced on the winter barley crops.

The fungus possesses a number of virulence and avirulence genes, which, in general, are matched by resistance genes in the host cultivars in a "gene-for-gene" relationship (17,21). Barley cultivars possessing different powdery mildew resistance genes give rise to field-specific, subpopulations with different virulence gene frequencies. The aerial powdery mildew population in a specific region at a specific time is made up of a mixture of spores from these subpopulations.

The genetic composition of the aerial population determines to a large extent the efficiency of disease control by means of host resistance (22,29). For this reason, virulence surveys providing information on the current status of the pathogen population are carried out in many countries (16,31). Models have been developed to improve the understanding of the composition of aerial powdery mildew populations (4,20,23,24,30), but detailed studies on how to use survey data to predict changes in gene and genotypic frequencies are lacking. For a comprehensive analysis of genetic changes in the aerial population, detailed information on the distribution of host cultivars and their resistance genes is of major importance (22,29).

The current study was concentrated in a local area in which the acreage of all barley cultivars and their powdery mildew resistance genes could be mapped. Temporal changes in virulence gene frequencies in the corresponding local aerial powdery mildew population were investigated, and observed changes were compared with those predicted based on the model developed by Østergård and Hovmøller (24). In the present paper, the model was extended by including a changing distribution of host cultivars during the year.

THE MODEL

The basic assumptions of the model are 1) the pathogen reproduces only asexually, 2) migration of spores to and from the considered barley area as well as mutations are ignored, 3) the aerial spores are dispersed on the emerging host cultivars in autumn according to the relative area of each cultivar within the area, 4) spores of different genotypes able to infect the same cultivar are assumed to produce the same number of offspring on that cultivar, and 5) avirulent spores are not able to reproduce at all. Let f_i denote the frequency of spores of genotype i in the aerial population being dispersed on the winter barley crops, and let f_{ij} denote the frequency of spores of genotype i establishing colonies on host cultivar j ($i = 1,\ldots,2^n$, $j = 1,\ldots, m$, where n equals the number of virulence loci considered, m equals the number of host cultivars considered, $\Sigma_i f_i = 1$, and $\Sigma_i f_{ij} = 1$). Let u_{ij} denote the probability that spores of genotype i are established on host cultivar j. In the present case, $u_{ij} = 1$ or

0, depending on whether genotype i is capable of reproducing on cultivar j (virulent) or not (avirulent). Then the genotypic frequencies among colonies established on cultivar j equal

$$f_{ij} = f_i \times u_{ij} / w_j, \quad (1)$$

for $i = 1,....,2^n$, $j = 1,...,m$, and where the normalizing factor w_j is defined such that $\Sigma_i f_{ij} = 1$, i.e., w_j equals $\Sigma_i f_i \times u_{ij}$. This factor may be interpreted as the average fitness on cultivar j of aerial spores relative to the fitness of a virulent spore. Equation 1 generalizes the notation previously used by Østergård and Hovmøller (24).

The genotypic frequencies in the next generation of the aerial population, f_i', which is made up of spores produced by the colonies growing on the different cultivars, are weighted averages of the genotypic frequencies in the subpopulations, i.e.,

$$f_i' = \Sigma_j f_{ij} \times w_j \times s_j / w' = f_i \times [\Sigma_j u_{ij} \times s_j] / w' \quad (2)$$

for $i = 1,....,2^n$, $j = 1,...,m$, and where s_j is the relative area of cultivar j within the considered winter barley area, and w' is defined such that $\Sigma_i f_i' = 1$, i.e., $w' = \Sigma_j w_j \times s_j$ is the average relative fitness of aerial spores on host cultivars in that region. Equation 2 also expresses the aerial population at the end of the winter season, if it is assumed that 6) spores for new infections almost entirely come from within the field during an epidemic, and that 7) the relative distribution of green foliage of different cultivars being substrate for the pathogen is constant during the growth season.

When both winter barley and spring barley cultivars are grown in the same region, additional assumptions have to be made for expressing the genotypic distribution, f_i'', in the aerial population at the end of the growth season for both winter barley and spring barley. These assumptions are as follows: 8) The number of spores per unit area infecting the emerging spring barley crop is of the same order of magnitude as the number infecting the emerging winter crop; and 9) the total number of colonies produced by the pathogen during the lifetime of a susceptible host crop is of the same order of magnitude on winter- and spring-sown crops, respectively. Defining s_j as the relative area of cultivar j in summer, the genotypic frequencies in the aerial population of spores dispersed on the emerging winter barley cultivars in the next growth season (equal to f_i''), can be expressed as follows:

$$f_i'' = [\Sigma_{j=\text{winter}} f_i \times u_{ij} \times s_j + \Sigma_{j=\text{spring}} f_i' \times u_{ij} \times s_j] / w'', \quad (3)$$

for $i = 1,....,2^n$, $j = 1,....m$, and where "j = winter" and "j = spring" denotes summation over winter and spring barley cultivars, respectively, and the normalizing factor w'' is defined such that $\Sigma_i f_i'' = 1$.

MATERIALS AND METHODS

Sampling and inoculation techniques. Random samples of aerial powdery mildew spores were collected from winter 1987/88 to winter 1989/90 by exposure of trap plants during a period of 4–8 days at a fixed stand in a local barley area in Denmark (Fig. 1). Wind directions during the exposure periods were measured at 1-h intervals in order to indicate sources of sampled spores. The trap plants consisted of seedlings of barley cultivar Pallas, which possesses resistance gene *Mla*8 (18), but Pallas was considered susceptible, as the matching avirulence gene has not been observed in powdery mildew populations in northwestern Europe (J. H. Jørgensen, *personal communication*). The seedlings were grown in trays in greenhouse under powdery mildew-free conditions, and after exposure they were covered with polyethylene bags and returned to the greenhouse for 7–8 days of incubation. Detached leaves of seedlings containing single colony isolates (clones) were rinsed under tap water and placed on 0.5% water agar containing 35 ppm of benzimidazole. After an incubation period of 4–5 days (16–18 C, relative humidity of 90–95%, and artificial light of 20 $\mu Em^{-2}s^{-1}$ for 18 h per day), each single colony isolate was used as inoculum at a density of 100–200 spores/cm^2 on a differential set of 12 near-isogenic barley lines developed by Kølster et al (19) (resistance genes in

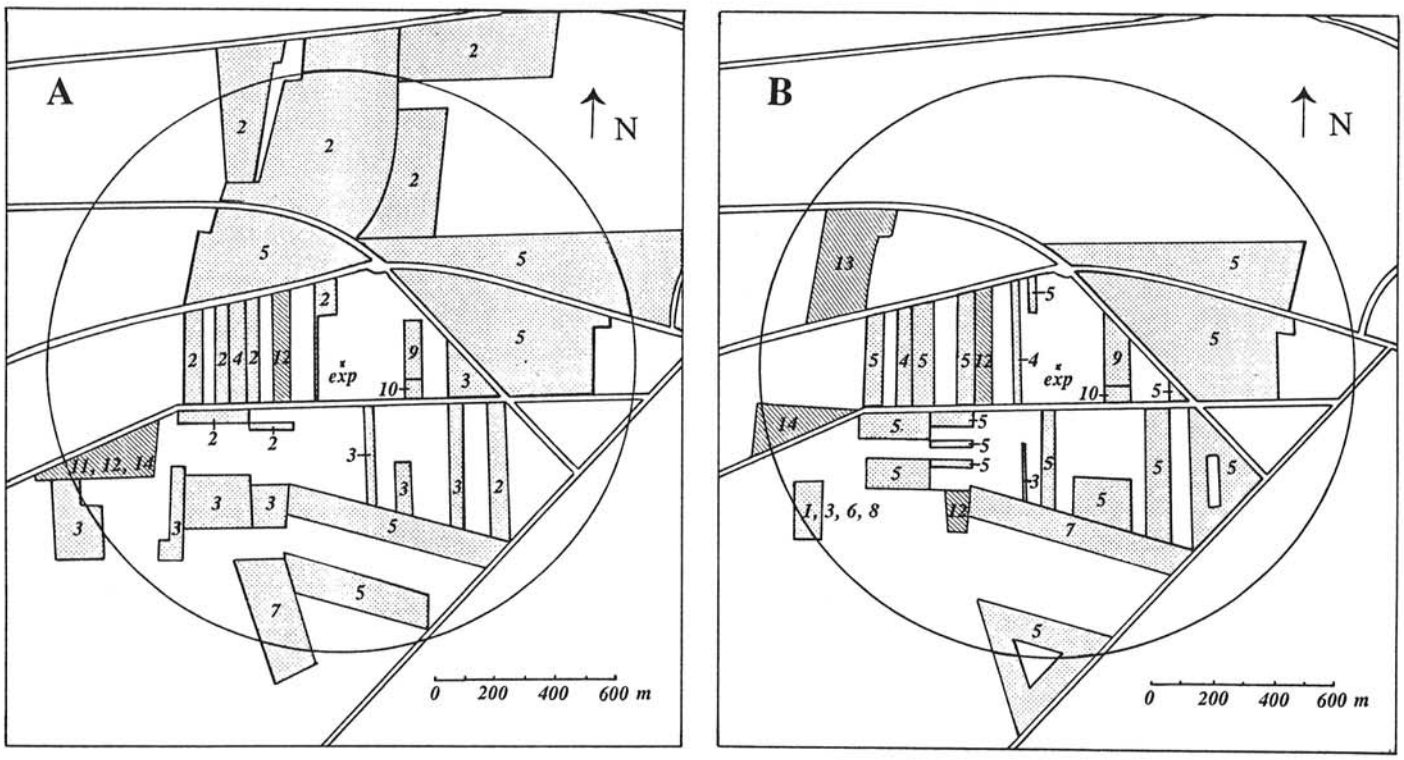

Fig. 1. Map of barley fields in summers of **A,** 1988 and **B,** 1989 within a radius of 1,000 m from the fixed stand of exposure. Hatched fields were grown with barley cultivars sown in autumn of 1987 or 1988 (winter barley), and dotted fields were grown with barley cultivars sown in spring of 1988 or 1989 (spring barley). The number(s) on fields refers to cultivars shown in Table 1, and 'exp' indicates the fixed stand of exposure.

parentheses): P01 (*Mla*1), P02 (*Mla*3), P03 (*Mla*6, *Mla*14), P04B (*Mla*7, *MlNo*3), P08B (*Mla*9), P10 (*Mla*12, *Ml*(*Em*2), P11 (*Mla*13, *MlRu*3), P14 (*Mlra*), P16 (*Mlk*) (unpublished), P21 (*Mlg, MlCP*), P23 (*MlLa*), and P24 (*Mlh*). The lines were represented by detached leaf segments placed on 0.5% water agar containing 35 ppm of benzimidazole. After inoculation, the leaf segments were incubated for 10–12 days under the conditions described above.

Identification of virulence genotypes. Based on the gene-for-gene hypothesis (7), virulence genotypes were recorded by the presence of virulence and avirulence alleles at 12 virulence loci for each single-colony isolate. The two alleles at each virulence locus were designated V_i (virulence) and A_i (avirulence), respectively, corresponding to resistance gene *Mli*, i = *a*1, *a*3, *a*6, *a*7, *a*9, *a*12, *a*13, *ra*, *k*, *g*, *La*, and *h*, and they were identified from disease reactions scored on the differential lines using infection types 0–4 according to Torp et al (27). Isolates giving rise to infection type 4 on the lines P01, P02, P08B, P14, P16, P23, and P24, possessing one single resistance gene each, implied the presence of the virulence genes V_{a1}, V_{a3}, V_{a9}, V_{ra}, V_k, V_{La}, and V_h, respectively, while all scores below infection type 4 were considered to imply the presence of an avirulence gene. The differential lines P03, P04B, P10, and P21 each had two genes for powdery mildew resistance, implying that two different infection types below 4 could be observed on each line. Infection type 4 implied the presence of virulence genes corresponding to both resistance genes in each of the lines, whereas type 2-3n implied the presence of the virulence genes corresponding to the "main" resistance genes, i.e., V_{a6}, V_{a7}, V_{a12}, and V_g, respectively, and avirulence to the second gene in the lines (not considered in the present analysis). On P11, also possessing two powdery mildew resistance genes, infection types higher than or equal to 1n implied the presence of V_{a13}. In the following, V_{ra} was not considered, as it was observed at a fixed frequency of 100% in all samples.

Distribution of host resistance genes. The distribution of host cultivars within a radius of 1,000 m from the fixed stand of exposure was described by mapping barley fields in the summers of 1988 and 1989 (Fig. 1). The cultivars were described by their powdery mildew resistance genes and sowing time. Cultivars sown in autumn were referred to as "winter barley," and those sown in spring were referred to as "spring barley". For each cultivar, the areas sown from autumn 1987 to autumn 1989 are given in Table 1.

Estimation of selection forces. The selection forces operating on the pathogen population from spring 1988 to autumn 1989 were estimated from the relative area of groups of barley cultivars with different powdery mildew resistance genes in four growth periods (Table 2). This table is derived from Table 1 with the cultivars being pooled according to their resistance genes and sowing time (autumn or spring). Resistance gene *Ml*(*ra*) and unknown resistance genes were considered as genes not exerting selection on the pathogen population. The genes *Mla*14, *Ml*(*CP*), *Ml*(*He*), and *Ml*(*Ab*) were omitted as it was not possible unambiguously to estimate allele frequencies at the corresponding virulence loci.

Prediction of virulence genotype frequencies. When using winter samples to predict the frequencies of genotypes in the next winter, assumptions in addition to those being basis for Equation 3 have to be made, i.e., that host cultivars grown in winter possess either unknown resistance genes or resistance genes exerting only weak selection on the pathogen population. Then Equation 3 can be simplified to:

$$f_i'' = f_i' \times [\Sigma_{j=\text{winter}}\, s_j + \Sigma_{j=\text{spring}}\, u_{ij} \times s_j]/w'', \qquad (4)$$

TABLE 1. Barley cultivars within a radius of 1,000 m from the fixed stand of exposure according to Fig. 1, their powdery mildew resistance genes, and the area on which they were sown from autumn of 1987 to autumn of 1989

Cultivar and number[a]	Powdery mildew resistance gene(s)	Area sown (hectares)				
		Autumn 1987	Spring 1988	Autumn 1988	Spring 1989	Autumn 1989
1: Sewa	*Mla*3, *Mlg* (12)[b]	...	0.0	...	0.6	...
2: Triumph	*Mla*7, *Ml*(*Ab*) (27)	...	50.6	...	0.0	...
3: Catrin	*Mla*7, u^c (12)	...	17.6	...	0.8	...
4: Ida	*Mla*9, *Mlk*, *Mlg* (12)	...	2.0	...	3.0	...
5: Grit	*Mla*12 (12)	...	59.3	...	57.6	...
6: Camir	*Mla*12, *Ml*(*He*) (12)	...	0.0	...	0.6	...
7: Natasha	*Mla*12 (12)	...	4.3	...	8.0	...
8: Alis	*Mla*12, *MlLa*, u^c (12)	...	0.0	...	0.6	...
9: Cerise	*MlLa*, *Mlg*, *Ml*(*CP*) (12)	...	1.6	...	1.5	...
10: Golden Promise	*None* (30)	...	0.5	...	0.3	...
11: Mammut	*Mla*6, *Mlra*, *Mla*14 (13)	1.4	...	0.0	...	0.0
12: Igri	*Mlra*) (13)	2.9	...	4.0	...	2.5
13: Ermo	*Mlra*, u^c (1)	0.0	...	5.5	...	0.0
14: Andrea	u^c (1)	1.4	...	5.2	...	7.0

[a] Number refers to fields shown in Figure 1.
[b] The number in parentheses represents the reference source of information.
[c] Unknown resistance genes.

TABLE 2. Frequency of groups of barley cultivars within the local barley area in four periods from spring of 1988 to spring of 1990

Cultivar[a] group	Resistance gene(s)	Relative acreage of cultivar groups A–H			
		May 1988–Oct 1988	Nov 1988–Apr 1989	May 1989–Oct 1989	Nov 1989–Apr 1990
A	*Mla*3, *Mlg*	0.00	0.00	0.01	0.00
B	*Mla*7	0.48	0.00	0.01	0.00
C	*Mla*9, *Mlk*, *Mlg*	0.01	0.00	0.03	0.00
D	*Mla*12	0.45	0.00	0.75	0.00
E	*Mla*12, *Ml*(*La*)	0.00	0.00	0.01	0.00
F	*Mlg*, *Ml*(*La*)	0.01	0.00	0.02	0.00
G	—[b]	<0.01	0.00	<0.01	0.00
H	—[b]	0.05	1.00	0.17	1.00

[a] Groups A–G refer to spring barley cultivars, and group H refers to winter barley cultivars.
[b] Unknown resistance genes or resistance genes not exerting selection on the aerial powdery mildew population from spring of 1988 to spring of 1990.

where f_i' and f_i'' denote the genotypic distribution in the aerial population in winter and the following summer, respectively. Equation 4 was used to calculate expected changes in genotype frequencies in the aerial population from winter to next winter for i=1,...., 2^{11}, and for the parameters s_j, j = A,..,G being equal to the relative area on which groups of spring barley cultivars were grown in summer and s_H being equal to the relative area of winter barley cultivars in summer (Table 2). The normalizing factor w'' equals to $\Sigma_j w_j \times s_j + s_H$. Predicted virulence genotype frequencies in a subpopulation of spores produced by colonies established on cultivar Ida in summer (f_{iIda}') were calculated according to Equation 1 for i = 1,..., 2^{11} and the normalizing factor w_{Ida}' equal to $\Sigma_i f_i' \times u_{iIda}$. Expected changes in gene frequencies were in all cases derived from the calculated genotypic frequencies.

Statistical analysis. Tests of homogeneity in gene frequencies between samples were carried out by likelihood ratio tests (G-tests) by considering each single locus, and overall tests considering all 11 loci were performed by summing up test values for the individual tests (26).

RESULTS

Eight samples representing the local aerial population were collected in different exposure periods from February 1988 to November 1989 (Table 3). The number of trapped aerial spores (represented by mean number of colonies on trap plants) varied depending on season and weather conditions during exposure, but, nevertheless, a relatively high number of colonies were collected in all sampling periods. The sample size varied between 74 and 212, and a relatively high number of different genotypes was observed in each sample, implying much genetic variation in the pathogen population. Seven of the samples were collected in periods in which only winter barley cultivars were expected to be the source of spores. These samples were collected in three different periods facilitating an analysis of changes in gene frequencies within and between winter periods. According to the model, however, selection takes place only when a new crop emerges. In the present case, this occurred in the autumn of 1987, 1988, and 1989 and in the spring of 1988 and 1989 (Table 4). This implies that the model predict constant gene and genotypic frequencies within the three winter periods and changing frequencies between the periods. Using the notation of the model in the actual situation, the initial aerial population in summer of 1987 was changed through two steps to the population in summer of 1988, which again was changed through two steps to the population in summer of 1989. In this study, however, the changes in gene and genotypic frequencies from winter of 1987/88 to winter of 1988/89 and from winter of 1988/89 to winter of 1989/90 were predicted. The predictions were based on Equation 4 and the fact that the genotypic frequencies in the aerial population were expected not to change from summer of 1988 to winter of 1988/89, i.e., $f_i(S88) = f_i'(S88)$, and from summer of 1989 to winter of 1989/90, i.e., $f_i(S89) = f_i'(S89)$

TABLE 3. Details of eight samples of single colony isolates of barley powdery mildew collected by exposure of trap plants of cultivar Pallas to the aerial population in the local barley area described in Fig. 1

Sample designation[a]	Date of exposure	Colonies on primary leaf per seedling (mean no.)	Sample size (no. isolates)	11-locus genotypes (no.)
W87/88a	4–11 Feb 1988	0.2	74	32
W87/88b	16–20 May 1988	4.0	212	63
W88/89a	11–18 Nov 1988	0.1	77	34
W88/89b	10–15 Mar 1989	2.0	117	53
W88/89c	25 Apr–2 May 1989	0.8	106	51
Vol89(Ida)	22–29 Aug 1989	2.3	97	41
W89/90a	2–9 Nov 1989	1.6	101	55
W89/90b	9–15 Nov 1989	3.6	107	47

[a] Sample designation refers to the expected source of sampled spores and the growth season in which the host plants were present: "W" refers to crops of winter barley cultivars and "Vol" to volunteer plants, respectively.

TABLE 4. Expected selection events (→) and notation for genotype frequencies in aerial population from summer of 1987 to winter of 1989/90

	Summer 87		Winter 87/88		Summer 88		Winter 88/89		Summer 89		Winter 89/90
Genotype frequencies	$f_i(S87)$	→	$f_i'(S87)$	→	$f_i''(S87)$ $=f_i(S88)$	→	$f_i'(S88)$	→	$f_i'(S88)$ $=f_i(S89)$	→	$f_i(S89)$

TABLE 5. *P* values for test of homogeneity of gene frequencies between samples originating from the same sources, and between groups of samples originating from different sources

Samples/ groups to be compared	Virulence gene											
	V_{a1}	V_{a3}	V_{a6}	V_{a7}	V_{a9}	V_{a12}	V_{a13}	V_k	V_g	V_{La}	V_h	Σ[a]
W87/88a W87/88b	0.926	0.178	0.821	0.685	0.609	0.275	0.465	0.988	0.022	0.016	0.308	0.137
W88/89a W88/89b W88/89c	0.263	0.895	0.249	0.370	0.995	0.437	0.037	0.648	0.097	0.258	0.575	0.430
W89/90a W89/90b	0.144	0.936	0.344	0.035	0.224	0.097	0.505	0.546	0.220	0.239	0.688	0.158
W87/88 W88/89	0.397	0.064	0.000	0.000	0.011	0.000	0.057	0.000	0.095	0.947	0.268	0.000
W88/89 W89/90	0.281	0.002	0.571	0.001	0.000	0.000	0.028	0.072	0.969	0.471	0.652	0.000

[a] Overall test based on summed chi square values for all 11 loci.

(Table 4). The expected stability in the genetic composition of the aerial population from summer to winter could be ascribed to simplified selection forces due to the presence of winter barley cultivars possessing either unknown resistance genes, or only resistance genes for which the corresponding virulence genes were fixed in the aerial population.

Analysis of changes in the composition of the observed aerial population were based on gene frequencies derived from the observed genotypic frequencies (data not shown). Only minor differences in gene frequencies were observed between spore samples originating from the same sources, i.e., samples collected in periods between two selection events (cf. Table 4), and this

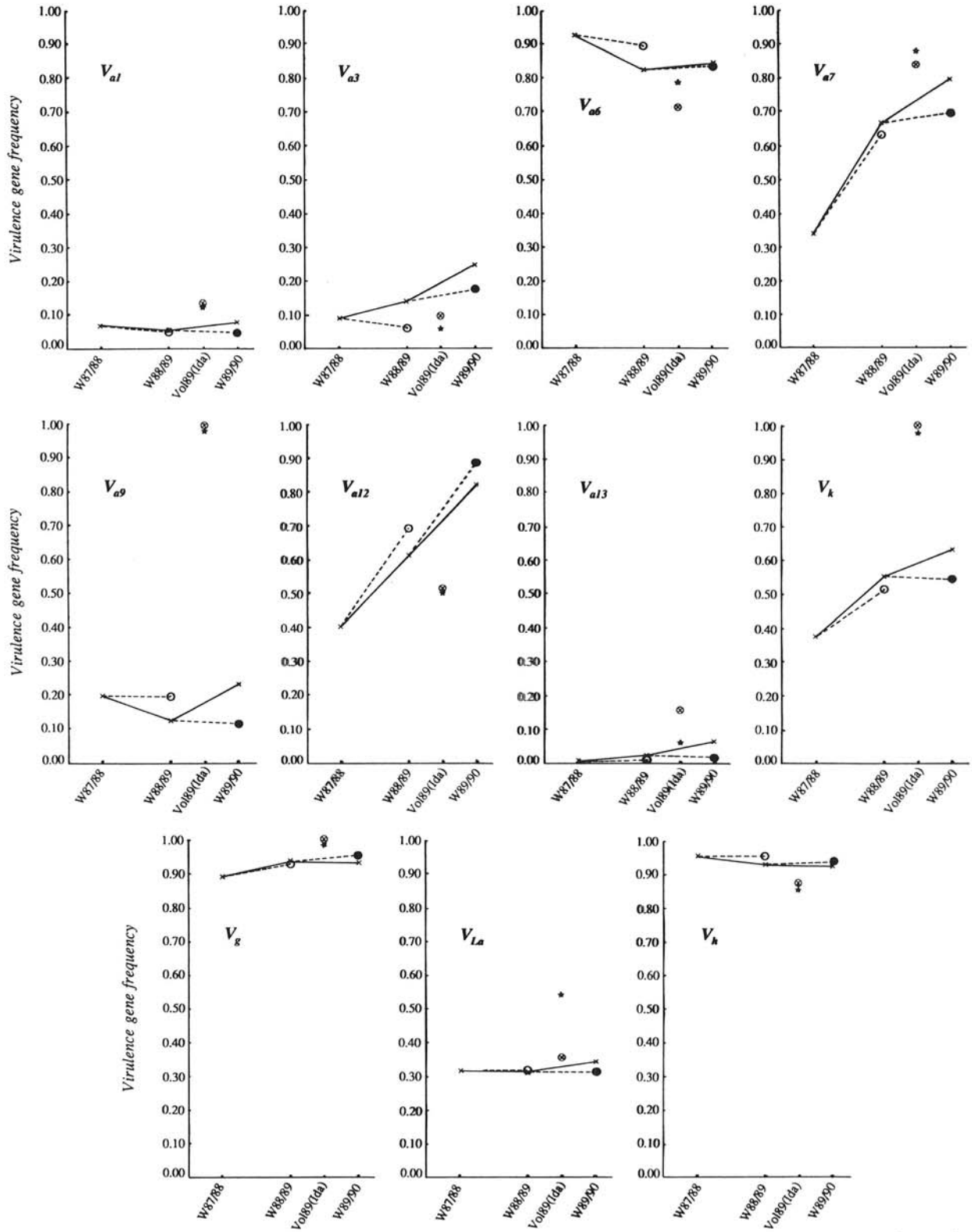

Fig. 2. Observed and predicted virulence gene frequencies in random samples of aerial powdery mildew spores. Observed changes in gene frequencies between winter samples (×——×) and observed gene frequencies in a sample of spores primarily originating from cultivar Ida in summer of 1989 (★). Predicted changes in gene frequencies in the aerial population from winter of 1987/88 to winter of 1988/89 based on the genotypic frequencies in sample W87/88 (× - - - ○), from winter of 1988/89 to winter of 1989/90 based on the genotypic frequencies in sample W88/89 (× - - - ●). Predicted gene frequencies in a subpopulation of spores produced on cultivar Ida based on genotypic frequencies in sample W88/89 and selection forces given by resistance genes present in Ida (⊗).

was the case even when the samples were collected at different times over several months. The large degree of homogeneity between samples was supported by an overall test comprising all 11 loci (Table 5). For simplicity, the samples originating from the same sources were pooled for the further analysis: the two samples collected in winter of 1987/88, denoted W87/88a and b, respectively, were pooled to W87/88; the three samples collected in winter of 1988/89, denoted W88/89a, b and c, respectively, were pooled to W88/89; and the two samples collected in winter of 1989/90, denoted W89/90a and b, respectively, were pooled to W89/90.

In contrast, large differences in gene frequencies were observed between samples originating from different sources (Fig. 2, Table 5). From winter of 1987/88 to winter of 1988/89, a significant increase in virulence gene frequency was observed for V_{a7}, V_{a12}, and V_k, and to a lesser extent, V_{a3}, V_{a13}, and V_g, a significant decrease was observed for V_{a6} and V_{a9}, whereas no significant changes were observed with respect to allele frequencies at the three remaining loci. These changes were confirmed by the overall test comprising all 11 loci (significant at $P = 0.000$). The increased frequency for V_{a7} and V_{a12} coincided with a large relative area grown with cultivars possessing resistance genes *Mla*7 and *Mla*12, respectively (direct selection), whereas the changes in gene frequencies for the remaining loci where changes occurred could not be explained by direct selection. The observed changes in virulence gene frequencies were generally concordant with predicted gene frequencies, which were calculated according to Equation 4 using the genotypic frequencies in W87/88 and the local selection forces described by the relative areas of host cultivars shown in Table 2. Differences occurred for V_{a6} and V_{a9}, for which the observed decreases in gene frequency were larger than expected, and for V_{a3}, for which a nonsignificant increase in frequency was observed but a small decrease was expected.

The sample Vol89(Ida) was collected in August of 1989 at a time when primarily volunteer plants of spring barley cultivar Ida (*Mla*9, *Mlk*, and *Mlg*) were present. Predicted gene frequencies in the subpopulation of spores produced on Ida were calculated according to Equation 1 using the genotypic frequencies in W88/89 and selection forces given by resistance genes present in Ida (Fig. 2). There was a high agreement between observed gene frequencies in the sample Vol89(Ida) and the predicted gene frequencies among powdery mildew spores produced on Ida, i.e., for the three virulence genes matching the resistance genes in Ida, V_{a9}, V_k, and V_g, and for six nonmatching genes V_{a1}, V_{a3}, V_{a6}, V_{a7}, V_{a12}, and V_h. Differences were observed only for V_{a13}, for which the observed frequency was smaller than predicted, and for V_{La}, for which the observed frequency was larger than predicted.

From winter of 1988/89 to winter of 1989/90, a significant increase in virulence gene frequency was observed for V_{a3}, V_{a7}, V_{a9}, V_{a12}, and V_{a13}, and to a lesser extent V_k, whereas no significant changes were observed with respect to allele frequencies at five loci (Fig. 2, Table 5). The differences in gene frequencies were significant at $P = 0.000$ in the overall test comprising all 11 loci. In six cases the observed patterns of changes were concordant with the predicted changes calculated according to Equation 4, i.e., on basis of the genotypic frequencies in W88/89, and average selection forces in the local area (Table 2). Some differences occurred with respect to V_{a3}, V_{a7}, V_{a9}, V_{a13}, and V_k, for which all observed frequencies were larger than the expected frequencies. However, this coincided with relatively high predicted frequencies in the subpopulation of spores produced on Ida, except for V_{a3}. Differences between observed and predicted gene frequencies in the aerial population in winter of 1989/90 may, therefore, be ascribed to a relatively large influence on the pathogen population of the genotypic distribution in the subpopulation of spores produced on volunteer plants of Ida in autumn of 1989.

DISCUSSION

Selection caused by host cultivars possessing resistance genes has been shown to have great impact on the composition of the aerial powdery mildew population (9,11,22,29,32). This was confirmed by the present results, where large changes in virulence genotype and gene frequencies in a local aerial population were observed during a 2-yr period. Generally, the changes in gene frequencies at each locus showed the same trend in both years even though selection forces were different in the 2 yr. Changes were observed for virulence genes subject to direct selection by matching host resistance genes, as well as for genes generally not subject to direct selection, e.g., for V_k, V_g, and V_{a6}. The increases in frequencies of V_k and V_g and the decrease in frequency of V_{a6} from winter of 1987/88 to winter of 1988/89 coincided with positive and strong gametic disequilibria in the sample W87/88 between V_{a7} and V_k, and between V_{a12} and V_g, as well as negative gametic disequilibria between V_{a7} and V_{a6}, and between V_{a12} and V_{a6} (data not shown). This demonstrates that the dynamics of virulence gene frequencies may be influenced to a large extent by indirect selection (hitchhiking). Other investigations have shown that gametic disequilibrium between virulence genes is common in aerial populations of barley powdery mildew in Europe (5,9,28).

The observed changes in gene frequencies were generally as predicted from the model developed by Østergård and Hovmøller (24), which is extended in the present paper by taking into account a changing distribution of host cultivars during the year. A high degree of concordance between observed and predicted gene frequencies existed for virulence genes subject to strong direct selection as well as for unselected loci and for loci mainly under indirect selection, illustrating the predictive power of the model.

The contribution of spores from different subpopulations established on different host cultivars may change during the growth season. For instance, the subpopulation of spores produced on cultivar Ida (*Mla*9, *Mlk*, and *Mlg*) in summer of 1989 entered the aerial population in larger proportions than conditioned by the relative area on which this cultivar was sown. This was demonstrated by the sample Vol89(Ida) obtained in August, for which most of the virulence gene frequencies were concordant with those predicted from spores produced on Ida, i.e., the frequencies of the three genes matching the resistance genes in Ida, and the six nonmatching genes V_{a1}, V_{a3}, V_{a6}, V_{a7}, V_{a12}, and V_h. The frequencies of the latter six were strongly deviating from the observed gene frequencies in the aerial population distributed on Ida in spring 1989; this illustrates the potential influence of hitchhiking in aerial powdery mildew populations. The sample Vol89(Ida) was much influenced by cultivar Ida, an early ripening cultivar (6), and this gave rise to volunteer plants with a relatively high incidence of powdery mildew disease compared to disease severity on volunteer plants of other spring barley cultivars in the area. Furthermore, the wind was blowing from the west for 60–70% of the time within the exposure period for this sample, which indicates a relatively large influence of sources west of the fixed stand of exposure (Fig. 1). The results emphasize the importance of knowing the sources of samples when changes in gene and genotypic frequencies in an aerial powdery mildew population are analyzed.

In winter of 1989/90, differences between observed and predicted gene frequencies in the aerial population were larger than in the previous winter. However, in most cases the diffeences could be explained by a potential influence of the subpopulation of spores produced by Ida on the aerial population in winter of 1989/90.

Eight of the most important resistance genes in the host cultivars grown in the local area were taken into account for the estimation of selection forces, whereas five resistance genes of minor importance were omitted, as it was not possible to estimate the allele frequencies at the corresponding virulence loci unambiguously. Four out of the five resistance genes, i.e., *Mla*6, *Mla*14, *Ml*(*CP*), and *Ml*(*He*) were expected not to influence the predicted changes in gene frequencies, as they were present on a small area only. The omission of *Ml*(*Ab*), which was combined with *Mla*7 on a large area in spring and summer of 1988, may have exaggerated the predicted selection in favor of genotypes with V_{a7}, as the frequency of V_{Ab} was approximated to 35% in spring of

1988. However, there was a good concordance between observed and predicted frequencies of V_{a7}, suggesting that other selection forces formed a contrast to this simplification.

Knowledge about the genotypic frequencies in the aerial population at the beginning of the barley growth season, when the entire pathogen population is forced to change host crop, is of great importance in the present model, which include a changing distribution of host cultivars during the year. Under Danish conditions, the barley growth season begins in autumn, when the winter barley crops emerge and volunteer plants from previous barley crops are eradicated. In this study, samples were collected mainly in winter, but because of minimal selection on winter barley cultivars, the model is applicable. The selection for virulence genes is assumed to take place each time the aerial population is dispersed on barley cultivars within the considered area, but the selection has major impact only on the pathogen population during the first pathogen generation after emergence of the host crop. The reason is that only a small proportion of the powdery mildew spores produced on the hosts within each pathogen generation enter the aerial population, while the major part remains within the host crop (3,32).

The selection induced by host cultivars possessing powdery mildew resistance genes are defined in the present study such that only spores of virulent genotypes are able to make successful infections on the cultivars, whereas spores of avirulent genotypes are not. This assumption was not fulfilled in all cases, as powdery mildew resistance gene *MlLa* allows avirulent genotypes to reproduce slightly, i.e., the relative fitness is greater than zero (14). However, as there was no indication of discrepancies in results due to the assumption that fitness values were either zero for avirulent genotypes or one for virulent genotypes, no additional parameter describing the fitness of different genotypes was included in the model.

Other types of selection, e.g., selection due to differences in the rate of reproduction of spores successfully infecting different host cultivars, may be of only minor importance for changes in the composition of the aerial population on a short-term basis. Such a selection, which was not taken into account in the present study, can take place throughout the life of the host and is influenced by the level of aggressiveness of specific clones of the pathogen and by the level of nonspecific resistance in the host (17).

It may be argued that some of the assumptions in the present model are too simplistic, e.g., that the number of spores per unit area infecting the emerging spring barley crop is of the same order of magnitude as the number infecting the emerging winter crop, and that the total number of colonies produced by the pathogen during the life of a susceptible host crop is of the same order of magnitude on winter- and spring-sown crops, respectively. The latter assumption may be complicated by the fact that early in the season, before the spring barley epidemic is established, winter barley cultivars may contribute relatively much to the aerial population. Late in the season, the opposite may be true because of earlier senescence of the winter crop. In general, rather simplified assumptions were chosen to be able to illustrate the features of the most important aspects of selection and to evaluate to which extent a rather simple model can explain the patterns of changes in gene frequencies in a parasitic pathogen population.

The spore samples used for analysis of changes in the genotypic distribution in the aerial population from one winter season to the next were expected to represent all the subpopulations of spores produced by the winter barley hosts in the local area. This assumption was not strictly fulfilled, as winter barley cultivars did not constitute an even distribution at different distances from the stand of exposure in combination with wind directions not being equally frequent within the exposure periods. However, this may only be of minor importance, as the winter barley cultivars in the local area were expected not to exert selection on the pathogen population, i.e., the different subpopulations on host cultivars were expected to have a rather similar genotypic distribution. Further, in some exposure periods, wind came partly from directions in which no source of spores was present within the local area, e.g., in late April of 1989 (sample W88/89c) and in November of 1989 (sample W89/90a), where wind came from the north and east for 20–30% of the time. However, if some of the trapped spores came from outside the local area, they did not influence the samples to such an extent that samples differed significantly from other samples collected within each winter period.

Migration of spores may be considered on a local scale between adjacent local areas, on a regional scale between sources present in different regions within a country, and on an interregional scale between sources present in different countries (2,8). If the distribution of host cultivars is different in adjacent local areas, different local aerial populations are expected, but in the present study, it was not possible to estimate the extent of migration between such local populations.

The extent of migration on the local scale has implications for the predictive value of virulence survey data obtained by sampling random spores in larger regions. For example, large differences between local populations, e.g., due to different selection forces in different local areas within the region and a low rate of migration, will reduce the predictive value of regional survey data. It is suggested that sampling of random spores in virulence surveys is carried out by mobile spore traps or by stationary trap plants at isolated stands of exposure, which are two sampling methods not influenced very much by each local pathogen population (10). Then the genotypic distribution in samples of the aerial population in summer or early autumn and data on the distribution of host cultivars and their resistance genes in winter could serve as basis for predictions of the genetic composition of the aerial population infecting the emerging spring barley cultivars in the next spring. This may improve the impact of virulence surveys with respect to evaluating the degree of disease control by different host resistance genes in the growth seasons to come.

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