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Estimating relative fitness in asexually reproducing plant pathogen populations

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Summary. A mathematical model is presented and analysed to find the conditions under which changes in gene frequencies can be used in asexually reproducing populations for estimating fitness of single genes, for example, for estimating the fitnesses of unnecessary virulence genes relative to their corresponding avirulence genes. It is concluded that the underlying distribution of relative fitness of clones (genotypes) has to be unimodal and that many populations consisting of a mixture of distinguishable clones then provide the best experimental data for estimating relative fitness of single genes. An improved statistical test procedure, i.e. generalized logistic regression, is suggested for analysing changes in gene frequencies in population experiments with a mixture of distinguishable clones. A population study of *Erysiphe graminis* f.sp. *hordei* (Klug-Andersen 1980) provides data to illustrate the procedure in the case where the population consists of a large number of genotypes. A bimodal distribution of "genotypes" possessing the virulence gene is indicated here.

Key words: Logistic regression – *Erysiphe graminis* f. sp. *hordei* – Gene-for-gene system – Unnecessary virulence gene

Introduction

In cereal growing areas where fungal diseases are prevalent, it is customary to use cultivars possessing major resistance genes for disease control.

Hence, a knowledge of the genetics of the fungal virulence genes is important for breeding and management programmes. In host-pathogen systems with gene-for-gene interactions, e.g. barley-barley powdery mildew (*Erysiphe graminis* f. sp. *hordei*) (Wolfe and Schwarzbach 1978), wheat-wheat stem rust (*Puccinia graminis tritici*) (Watson and Luig 1968), and flax-flax rust (*Melampsora lini*) (Flor 1956), the pathogen populations seem to change their genetic composition rapidly in favour of virulence genes corresponding to newly introduced resistance genes in the crop. When the cultivars possessing these resistance genes are withdrawn, the corresponding virulence gene frequencies may decrease. This has led to the hypothesis that "unnecessary" virulence genes (virulence genes not needed for survival on a host cultivar (Flor 1956; Watson 1958)) decrease the fitness of individuals possessing them (Vanderplank 1968). If pathogen genotypes possessing unnecessary virulence genes usually have relative fitnesses less than 1 and, therefore, are likely to decrease in frequency, then it is much easier to control plant diseases by means of resistance genes than if unnecessary virulence genes persist in populations in high frequencies.

The hypothesis of selection against unnecessary virulence genes in fungi whose primary mode of reproduction is asexual (e.g. *Erysiphe graminis* f. sp. *hordei*) can be interpreted as follows. Assume that the fitness values of a large number of different genotypes (clones) from a field population were estimated from field observations or laboratory experiments in similar environments. Then according to the hypothesis, genotypes possessing an unnecessary virulence gene, i.e. a gene not needed for survival in the field, would on average have fitness values below the values for genotypes possessing the corresponding (allelic) avirulence gene (Fig. 1 A). If, however, some genotypes possessing the virulence gene have values above the avirulent group (Fig. 1 B), then the hypothesis breaks down. Consequently, it is important to measure general fitness as well as its different components and to evaluate the possibilities for estimating fitness of single genes when, in fact, genotypes are the unit of selection. Attention

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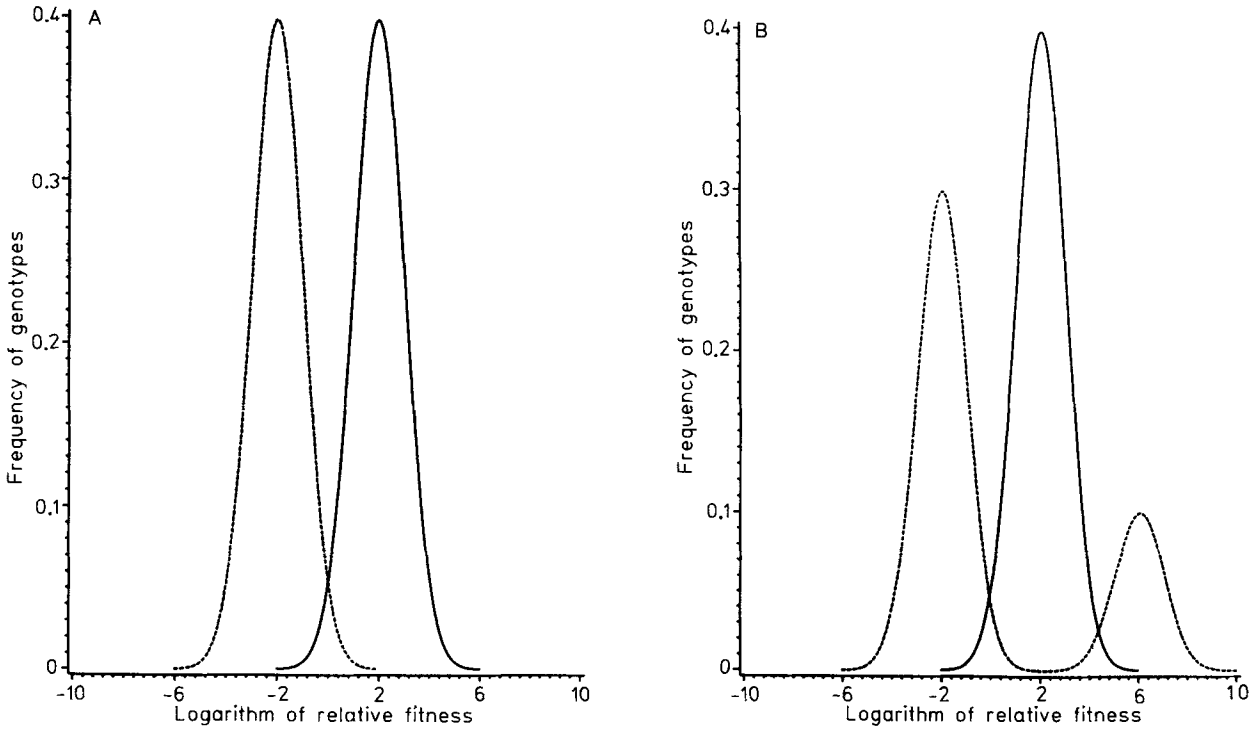

Fig. 1 A, B. Hypothetical distribution of logarithmic relative fitness values among genotypes (clones) possessing a virulence gene (dotted line) or an avirulence gene (full line) corresponding to a certain resistance gene. **A** Unimodal distribution within pathogenicity group. **B** Bimodal distribution for the group possessing the virulence gene

has recently been given to this point by Wolfe and Knott (1982) and Alexander et al. (1985).

In this paper, estimation of relative fitness from changes in relative frequencies of genotypes in laboratory populations reproducing asexually is considered. A statistical test procedure is suggested on the basis of a simple mathematical model and it is applied to experimental results from a population study of *Erysiphe graminis* f.sp. *hordei* (Klug-Andersen 1980). This experiment will be described first in order to provide the experimental background for the mathematical model.

Data example (Klug-Andersen 1980)

A laboratory population of *Erysiphe graminis* f.sp. *hordei* was initiated using about 10^4 colonies sampled from a field experiment on cultivar mixtures. Sexual reproduction of the fungus had not occurred during the field experiment for about 30 generations. The laboratory population was grown for 16 conidial (asexual) generations on the barley cultivar 'Pallas' which contains no known major resistance genes. Each generation, spores from infected 'Pallas' seedlings were transmitted to new seedlings when the infections began to sporulate (every nine days). One hundred and five, 109 and 110 single colonies (designated infections) were isolated from generations 2, 9 and 16, respectively, and tested for their reactions on 11 differential cultivars, some of which were included in the cultivar mixture. This corresponds to determining the gene combination of each infection at 11 pathogenicity loci. Among the 324 infections tested, 63 different combinations were observed (Table 1).

The history of the field population indicated that each 11-locus combination represented a single genotype (clone) with a large relative fitness or growth rate on the variety mixture. It is likely that more than 63 genotypes were present in the field as well as in the laboratory population (the maximum number being 2^{11}), but their frequencies were so small that they did not influence the population development, or were lost by chance. The latter is supported by the fact that the number of different genotypes detected in generations 2, 9 and 16 were 43, 37 and 28, respectively. Finally, it is unlikely that new combinations had arisen during the 16 generations by mutation (Torp and Jensen 1985) or by somatic recombination (Hermansen 1980).

The pathogenicity locus corresponding to one of the resistance genes, *Ml-a9*, in the variety mixture was arbitrarily chosen to illustrate the procedure for estimating relative fitness. The genotypes were grouped according to which of the alleles at this pathogenicity locus they possessed: the virulence gene, that was unnecessary in the laboratory population, or the avirulence gene. Twenty-eight genotypes possessed the virulence gene and 35 genotypes possessed the avirulence gene (Table 1). The virulence gene frequency was 43%, 18% and 30% in generations 2, 9 and 16, respectively. This indicates that nearly half of the infections found in the field population possessed the virulence gene partly necessary in the cultivar mixture. Further, the changes in environment from the field to

Table 1. Sixty-three different genotypes on 11 pathogenicity loci and their number observed in generations 2, 9 and 16 in a population of *Erysiphe graminis* f.sp. *hordei* grown on the barley cultivar 'Pallas'. The genotypes are divided into those possessing the virulence gene corresponding to resistance gene *Ml-a9* and those not possessing it (data from Klug-Andersen (1980)). Sample sizes are 105, 109 and 110, respectively

28 genotypes with virulence gene			35 genotypes with avirulence gene			Identification on remaining 10 loci
Generation			Generation			
2	9	16	2	9	16	
			2	0	0	1
			0	0	1	2
1	3	1				3
2	0	0				4
			0	1	1	5
8	5	2[a]	1	1	1	6
2	2	1	8	14	4[a]	7
			1	1	0	8
3	0	0	1	0	0	9
			1	0	0	10
1	0	0				11
2	1	0				12
2	0	0				13
3	0	0				14
			0	1	0	15
2	2	0	2	13	4[a]	16
			0	1	0	17
			8	2	4[a]	18
4	1	1[a]				19
0	1	1				20
1	1	0				21
			1	0	0	22
			3	3	4[a]	23
			0	1	0	24
			1	1	0	25
			0	0	1	26
1	0	0	1	0	0	27
			2	0	0	28
			2	3	1[a]	29
			0	1	0	30
			0	1	0	31
			0	2	0	32
1	0	0	3	3	4[a]	33
1	0	2	3	3	10[a]	34
			5	8	10[a]	35
1	0	0				36
1	1	12[a]				37
0	0	3				38
3	0	1				39
0	0	1				40
1	0	0				41
			0	2	0	42
0	0	2	1	1	0	43
0	1	3	2	1	4[a]	44
			0	1	0	45
0	0	2	0	1	0	46
4	1	1[a]	10	22	28[a]	47
0	1	0				48
1	0	0	0	1	0	49
			1	0	0	50
			1	0	0	51

[a] This genotype is considered singly in the analysis
The remaining genotypes are pooled within pathogenicity group

the laboratory (e.g. a change in host cultivar) had a pronounced effect on the genetical composition of the population leading to a halving in the frequency of infections possessing the virulence gene at generation 9. By generation 16, however, the frequency of infections possessing the virulence gene had increased again, indicating that a particular genotype had a high relative fitness under these laboratory conditions.

Before the further analysis of these data are made, a mathematical and a statistical model dealing with the analysis of such data are considered.

Asexual population development

An asexually reproducing fungus growing on a genetically uniform host cultivar is considered at the life stage when infections can be observed. It is assumed that generations are discrete, i.e., that the vegetative generations are separated. The fungus population consists of a number (m) of genotypes, i.e., groups of infections of common origin (clones). The change in frequencies of the genotypes will be studied mathematically to give a framework for estimating the relative fitness (equivalent to relative growth rate) of these genotypes and of groups of genotypes pooled according to a set of marker genes. This includes estimating the fitness of unnecessary virulence genes relative to their corresponding avirulence gene.

The most simple model describing the change in frequency of different genotypes assumes that the changes are only due to fitness differences between the genotypes, i.e. mutation is neglected and the genotypes do not interact. If the frequency of infections of genotype i in the n'th generation is designated p_{ni}, then the number of infections in generation $n+1$ can be expressed as

$$p_{n+1i} = w_{ni}\, p_{ni}/K_n \qquad (1)$$

where w_{ni} is the relative fitness of genotype i in the n'th generation with $w_{n1} = 1$. In fact, all fitnesses mentioned throughout this paper will be relative fitnesses. K_n is the relative increase in population size from generation n to generation $n+1$. K_n is a function of the fitnesses and the frequencies in generation n (see Crow and Kimura 1970). Equation (1) can be modified in some special cases.

Constant fitness

If the fitness of genotype i is constant from generation to generation, i.e., $w_{ni} = w_i$, then p_{ni} can be determined from the initial frequencies as

$$p_{ni} = w_i^n\, p_{0i}/K_n \qquad (2)$$

with K_n being a function of the fitnesses and the frequencies in the initial generation only. In a popula-

tion grown over many generations, the genotype with the largest fitness eventually reaches a frequency of 1, whereas all other genotypes reach a frequency of 0.

Two genotypes in the population (m = 2)

Equation (1) can be simplified, using the short notation $p_n = p_{n1}$ and $b_k = w_{k1}/w_{k2}$, to

$$\log(p_n/1 - p_n) = \log(p_0/1 - p_0) + \sum_{k=1}^{n} \log b_k. \quad (3)$$

If the fitness of genotype 1 relative to genotype 2 is constant from generation to generation ($b_k = b$), then and only then does the most fit genotype increase monotonically in frequency and Equation (3) can be simplified to the equation given by Haldane (1924) and later by Leonard (1969):

$$\log(p_n/1 - p_n) = \text{logit } p_n = \text{logit } p_0 + n \log b. \quad (3a)$$

The graphical representation of the relationship between generation number and the logarithm of the relative frequency of genotype 1 to genotype 2 is a straight line through logit p_0 with slope log b.

Two pathogenicity groups in the population

Genotypes may be pooled into two groups according to pathogenicity with respect to a resistance gene not present in the host cultivar. If the genotypes numbered 1 to x all possess the unnecessary virulence gene and the genotypes numbered x + 1 to m all possess the corresponding avirulence gene, then the frequency of infections possessing the virulence gene in the n + 1'th generation, designated q_{n+1}, is the sum of the frequency of each genotype possessing the virulence gene, i.e.

$$q_{n+1} = \sum_{i=1}^{x} p_{n+1\,i} = \left[\sum_{i=1}^{x} w_{ni}\, p_{ni}/q_n\right] q_n/K_n. \quad (4)$$

The expression in the brackets in Equation (4) (denoted f_n) is a weighted average fitness of genotypes possessing the virulence gene in generation n. This parameter describes the change in the genotypic composition of the considered population specified by the particular initial composition of genotypes. Thus, if two populations consist of identical genotypes but their initial composition differs, the population development in identical environments will also differ during the first generations until the genotype with the largest relative fitness predominates. Since f_n is a function of p_{ni}/q_n, it will change from generation to generation even if w_{ni} is constant. Therefore, the change in frequency of the virulence gene will be in accordance with Equation (3) with b_k equal to

$$\left[\sum_{i=1}^{x} w_{ki}\, p_{ki}/q_k\right] \Big/ \left[\sum_{i=x+1}^{m} w_{ki}\, p_{ki}/(1 - q_k)\right] \quad (5)$$

and the frequency will neither increase nor decrease monotonically. If, however, the values p_{ni}/q_n in Equation (4) are small and for i = 1 to x and i = x + 1 to m, respectively, nearly identical and if w_{ni} are constant over generations, then f_n is approximately constant and the change in frequency of the virulence gene will be in accordance with Equation (3a) with b approximately equal to

$$F = \left[\sum_{i=1}^{x} w_i/x\right] \Big/ \left[\sum_{i=x+1}^{m} w_i/m - x\right]. \quad (5a)$$

Note that the constraints on p_{ni}/q_n will often be found for only a few generations, most likely the first generations after a cross between two isolates.

In conclusion, Equation (1) describes the general relationship between the genotypic frequencies in two successive generations. If the fitnesses are constant from generation to generation, the frequencies in the n'th generation can be expressed by the initial frequencies. If genotypes are pooled with respect to alleles at a pathogenicity locus, then the weighted average fitness determines the change from generation to generation in the frequency of the virulence gene (Equation (4)). In general this parameter varies over generations depending on the most prevalent genotypes. Similar results will be obtained if pooling is done on basis of combinations of pathogenicity loci. The short term composition of the population will depend on the initial frequencies of the genotypes and on their fitnesses, whereas the long term composition of the population will be determined by one single genotype, the one with largest fitness.

Estimation of relative fitnesses

The observations from population experiments on changes in relative frequencies consist of an array of numbers for each generation assayed. The relative frequency of infections belonging to a genotype or group of genotypes will on average change from generation to generation according to Equation (1) (or Equation (4)). Two types of variation will influence the observed development: the sampling error, described by a multinomial distribution with parameters given by the mathematical model, and an experimental error due to small changes in the environment during the experiment. The latter might be described by a random element in the parameters of the multinomial distribution (McCullagh and Nelder 1983).

When infections can be classified into clones (genotypes), a relevant hypothesis to test is whether Equation (2) (or Equation (3a)) describes the observed changes satisfactorily, i.e., whether the fitnesses are constant. Omitting the non-multinomial variation, the statistical model is equivalent to a logistic regression

model in the case of two categories, and otherwise equivalent to a generalized logistic regression model. Estimates of the relative fitness can, therefore, be obtained easily using most statistical packages. Unfortunately, non-multinomial variation might lead to a significant test value of the test for constant fitnesses and then the estimated fitnesses can be interpreted only as averages over generations. This implies that when additional hypotheses on the fitnesses are tested (e.g., $w_i = 1$ for all i), a modification of the test value by, for example, the heterogeneity factor suggested by Finney (1971) should be used. In the following it is assumed that the fitnesses are constant over generations.

If the population consists of m genotypes m – 1 relative fitnesses can be estimated, the remaining genotype having fitness set to 1. If the genotypes are classified into those possessing an unnecessary virulence gene and those possessing the corresponding avirulence gene, the logarithm of the estimates can be plotted as in Fig. 1. (Note that the frequency of infections of each genotype in the actual population is not taken into account.) The mean logarithmic fitnesses of genotypes possessing the virulence gene and the avirulence gene are

$$\left[\sum_{i=1}^{x} \log w_i\right]/x \quad \text{and} \quad \left[\sum_{i=x+1}^{m} \log w_i\right]/m - x,$$

respectively, where x is the number of genotypes possessing the virulence gene. If the fitnesses are distributed as in Fig. 1 A (unimodal) then the "superiority" of the virulence gene is expressed by the difference between these means:

$$\log\left[\prod_{i=1}^{x} w_i^{\frac{1}{x}} \Big/ \prod_{i=x+1}^{m} w_i^{\frac{1}{m-x}}\right] = \log W. \qquad (6)$$

Equation (6) defines an estimate (W) of the fitness of a virulence gene relative to its corresponding avirulence gene. If the fitnesses of genotypes are distributed as in Fig. 1 B (bimodal for one of the pathogenicity groups) then the mean values are meaningless and it is not possible to describe the difference between genotypes possessing the virulence and the avirulence gene by just one parameter.

The parameter W can be interpreted as the expected fitness of a randomly chosen genotype possessing the virulence gene relative to a randomly chosen genotype possessing the avirulence gene. Further, W will measure the real relative influence of the genes at the considered pathogenicity locus if the genotypes 1 to x are the same as those x + 1 to m except for the considered pathogenicity locus (randomised gene background), and if the fitness function over loci is approximately multiplicative.

If infections cannot be classified into genotypes (clones) and only the relative frequencies of two groups of genotypes can be observed, then it is not possible to construct Fig. 1 and to test whether the distribution of fitness is unimodal within the pathogenicity group. Further, it is impossible to estimate W, and the only estimate of relative fitness which can be obtained is that of b_k in Equation (3). This parameter is not generally expected to be constant over generations (cf. Equation (5)). Further, it depends strongly on the initial composition of the population. In the case where the first offspring generation from a cross (or offspring from sexual reproduction on the population level) is studied, the parameter F (Equation (5 a)) can be estimated approximately. If the relative fitness values of all genotypes within the same pathogenicity group (not identifiable) are nearly equal, i.e. in accordance with Fig. 1 A with a small variance, then F is close to W. If the fitness function is multiplicative and the gene background randomised, then F equals W.

Data analysis (continued)

In the laboratory population described (Table 1), 63 genotypes were assumed to be present. It was impossible to estimate the relative fitness of each of the 63 genotypes because the number of observations was small compared to the number of classes. Therefore, all genotypes observed less than six times in total were pooled within each of the two pathogenicity groups defined by the virulence/avirulence gene corresponding to the resistance gene *Ml-a9*. This resulted in 16 groups of genotypes, of which 14 were single-genotype groups (Table 1). The logarithm of the relative fitnesses of the 14 single genotypes and the 2 groups of genotypes were estimated by generalized logistic regression. The distribution of the non-logarithmic values is shown in Fig. 2. To estimate the fitnesses, it was necessary to assume that they were constant from generation to generation, i.e., the logarithm of the expected number of infections of genotype i in generation n could be expressed as

$$c_n + a_i + b_i\, n$$

where c_n corresponds to the sample size in generation n, a_i corresponds to the initial proportion of genotype i and b_i corresponds to the relative fitness. The expected numbers generated under this assumption deviated (measured with the multinomial variance) considerably from the observed numbers (test value $\chi^2 = 31.01$, df = 15). This does not, necessarily, imply that the relative fitnesses varied over generations. First, at least two of the fitness estimates were based on groups of genotypes within which the average fitness was expected to vary. Second, the multinomial variance accounts for the sampling error only and not for small random changes in population development. As a consequence, the estimates shown in Fig. 2 were just used for ranking the genotypes.

One genotype (No. 37, Table 1), when possessing the virulence gene, had a superior fitness of $w_i = 1.41$ (cf. Fig. 2) and thus it is expected to predominate the population in the long run. The same combination of genes (V + 37) has also increased in frequency in other laboratory populations grown

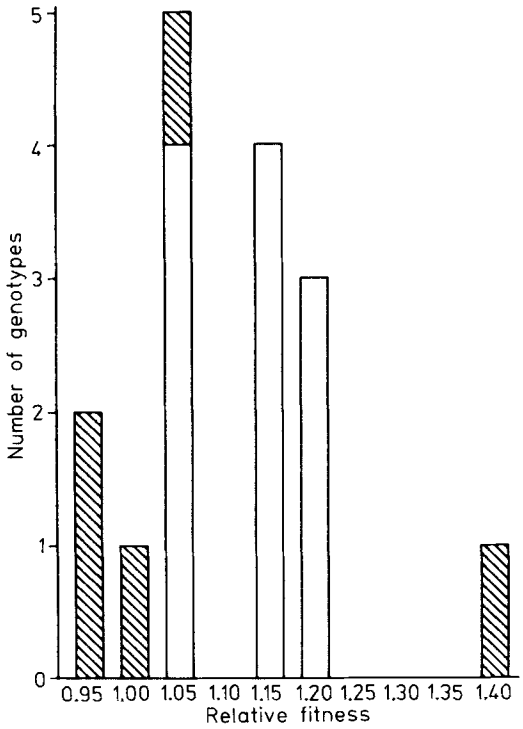

Fig. 2. Histogram showing relative fitness values of 16 genotypes and genotype classes classified according to pathogenicity corresponding to resistance gene *Ml-a9* (see Table 1), open bars for genotypes possessing the avirulence gene, and hatched bars for genotypes possessing the virulence gene. Only interval midpoints are shown

on 'Pallas' (Klug-Andersen pers. comm.). This may suggest that this particular genotype is well adapted to 'Pallas', e.g., by possessing a virulence gene against a hitherto unknown resistance gene in 'Pallas' with a small effect. Except for this genotype (V + 37), the genotypes possessing the unnecessary virulence gene tended to be slightly less fit than those possessing the avirulence gene (Fig. 2). The relative fitness W of the virulence gene was equal to 0.96 but the indication of a bimodal distribution makes this average parameter irrelevant. Further, its value depended to a large extent on which genotypes were pooled.

In gene background No. 47 (defined by 10 loci), the effect of the virulence and the avirulence gene could be compared, the fitness estimates w_i being 0.97 and 1.18, respectively. This may suggest that in this gene background, the avirulence gene was the most fit on the cultivar 'Pallas'. In the remaining cases with a common gene background, the number of observations in one or the other pathogenicity group were too small to make any conclusive comparison.

Discussion

For disease control purposes it is important to investigate the fitness of different pathogen genotypes, possibly under field conditions. It has been suggested that country-wide surveys are the best way to evaluate the hypothesis of selection against "unnecessary" virulence genes (Parlevliet 1981; Grant and Archer 1983). However, in most pathosystems, too little is known about the complex processes determining the evolution of the pathogen population. One problem is that the degree of sexual reproduction as well as its contribution to genetic variability of the pathogen population is often unknown. If asexual reproduction is predominant, the validity of estimating the fitness of certain genes, when the unit of selection is the genotype, is questionable as shown from the analysis here. If selection on a field population has been constant for some generations and has then altered (e.g. if a cultivar is withdrawn and replaced by another possessing a new set of resistance genes), then the genotypes which were most fit under the earlier selection regime continue to predominate the population for some time. After many generations the genotypes most fit in the new environment come to predominate the population and these "new" genotypes may by chance possess either of the genes at a pathogenicity locus, where virulence was necessary under the earlier selection regime but is not unnecessary. Therefore, the hypothesis of selection against unnecessary virulence genes should be studied under laboratory conditions, possibly using isolates recently collected from field populations.

Construction of laboratory population

Several components of fitness can be measured (Østergård 1983), the product of all being the average number of offspring infections from each infection. This general fitness can, in relative terms, be measured from changes in the relative frequencies of genotypes. The construction of the laboratory populations for measuring these relative fitnesses is important for the results obtained. The most important difference is between a mixture of known and distinguishable isolates (genotypes) and a population of an unknown number of genotypes. The former is by far the most informative.

Ideally, relative fitness should be estimated from binary mixtures of mother and mutant isolates differing in pathogenicity, since the variation due to gene background can be taken into account. A bias may, however, be introduced in that new mutants in general are genotypically unbalanced and here all mutants will possess the unnecessary virulence gene. Unfortunately, only a few comparisons of mother-mutant isolates have been published; Grant (1983), for example, has studied one mother-mutant pair of *Erysiphe graminis* f.sp. *hordei*.

Mixtures of distinguishable isolates (genotypes) with differences in gene background as well as in pathogenicity loci could also be studied. In this case one can choose between binary mixtures and mixtures of more than two isolates. If binary combinations of isolates are chosen for study, it is necessary to study many of them in order to rank all the relative fitnesses, as in Fig. 1. This extra effort may be well rewarded by the additional precision of the fitness estimates gained by the study of binary mixtures. Binary mixtures have been studied extensively for different purposes (Osoro and Green 1976; Meah 1982). If the relative fitness of a virulence

gene (W) is to be estimated, a unimodal distribution of fitnesses within each pathogenicity group is necessary.

When the single genotypes cannot be identified, the information necessary to construct Fig. 1 cannot be obtained and the shape of the fitness distribution cannot be determined. The laboratory population in this case should be initiated in one of two ways. First, the population can consist of the offspring from a cross. Then from the change in virulence gene frequency in the first few generations, the parameter F can be estimated (Equation (5a)). This is a reasonable estimate for the relative fitness of the virulence gene assuming that the fitness values of the unknown genotypes are distributed as in Fig. 1A with a small variance within each pathogenicity group. Experiments on this basis have been performed (Leonard 1969; Munk 1986). In the long run, the genotype with the largest fitness will predominate and by comparing different crosses one can gather information on whether some gene combinations are, in general, best in a given environment.

Second, the laboratory population can be a sample from a field population. If the field population has already been through many vegetative generations and if many pathogenicity loci or other marker genes are assayed, then it is reasonable to work with the marker gene combinations as genotypes and the laboratory population can be considered as a mixture of "known" genotypes. In this paper, the analysis of the experimental results from Klug-Andersen (1980) was performed in this way. The sample size should, however, be very large to obtain reasonable fitness estimates since the number of "genotypes" may be large. When nothing is known about the composition of a field population sampled, conclusions based on gene frequency changes over only a few generations are spurious and such experiments should be avoided.

Sampling and testing

When the laboratory population has been constructed it is important to consider the sampling method carefully. Different methods of determining the composition of the experimental population have been applied. When colour mutants are available, the composition can be estimated in the actual population (Brown and Sharp 1970; Falahati-Rastegar et al. 1981). Otherwise, the transfer of spores ideally from single infections to test plants with known resistance genes has to be carried out (e.g. Osoro and Green 1976; Meah 1982). It is here important to control the spore density since it has been demonstrated that relative fitness changes with spore density (Katsuya and Green 1967; Stähle 1986). A mathematical model for this situation has been analysed (Østergård 1982). Finally, the experimental design should include replicates to check the repeatability of the frequency estimates in each generation.

The statistical method previously recommended for analysing relative fitness in binary mixtures is the linear regression of the logistic transformation of virulence gene frequencies against generation number (Leonard 1969). This method assumes constancy of fitness over generations. Since it is important, when estimating the relative fitness, to evaluate the constancy of fitness over generations, this method is inappropriate. Further, tests for equal fitness of virulence and corresponding avirulence genes using the linear regression method could be misleading if the number of infections assayed differs from generation to generation because then the weightings of the different frequency estimates should also differ. Other more elaborate statistical methods such as logistic regression (and generalized versions of this) are now available taking account of different numbers of infections sampled and making it possible to analyse the hypothesis of time constancy (for an example, see Meah 1982). However, the binomial or multinomial variance applied in these models is often too small to account for the actual variation. Ad hoc methods for overdispersion can be applied in such cases (McCullagh and Nelder 1983).

Conclusion

When using population studies for estimating relative fitness in asexually reproducing populations, the study of a population for which all genotypes included can be identified is recommended. When genotypes cannot be uniquely identified and are pooled on basis of a number of assayed genes, these groups of genotypes will in the long run evolve according to the most fit genotype but initially the other genotypes "disturb" the expected pattern and this influence cannot be measured. In the case of sexual reproduction, it is appropriate to estimate the fitness of virulence genes from an experimental design with unidentified genotypes, as long as it is realised that the fitness values of genes at loci in linkage disequilibrium are confounded.

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