Potential versus actual contribution of vertical transmission to pathogen fitness

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SUMMARY

Theory predicts that virulent parasites cannot be maintained at high prevalence if they are only vertically transmitted. However, parasites with high rates of vertical transmission that cause severe reduction in host fitness have been reported. *Atkinsonella hypoxylon* is a fungal pathogen capable of both vertical and horizontal transmission that drastically reduces its host's fitness. In contrast with theoretical predictions, field and laboratory observations suggested that the primary mechanism of transmission was vertical. Using randomly amplified polymorphic DNA markers, we investigated the effective contribution of vertical and horizontal transmission to the genetic structure of three natural populations of *A. hypoxylon*. We found high genotypic diversity and low linkage disequilibrium, indicating that most established genotypes are derived from horizontally transmitted, sexual spores. The low contribution of vertical transmission to the parasite's fitness despite its high potential might be due to lower establishment of cleistogamous seeds (through which vertical transmission occurs) or lower vigour of vertically transmitted fungal genotypes. Low establishment of vertically infected hosts might explain the persistence of virulent parasites with high apparent vertical transmission. Our results suggest that caution must be taken when using the potential for vertical transmission to make predictions about the evolution of parasite virulence.

1. INTRODUCTION

The evolution of parasite virulence is thought to depend on the mode of parasite transmission (Fine 1975; Ewald 1987). According to Ewald (1987), vertically transmitted parasites should evolve towards mutualism because the parasite's transmission depends on the host's reproductive success. Although there is empirical evidence that supports Ewald's prediction (Bull *et al*. 1991; Herre 1993), parasites with strong negative effects on host fitness and high vertical transmission have also been reported (Hurst *et al*. 1994; Mangin *et al*. 1995).

Parasites capable of both vertical and horizontal transmission (mixed-strategy parasites) are ideal systems to test theoretical predictions about the relationship between virulence and transmission. Lipsitch *et al*. (1995) suggest that high vertical transmission and high prevalence can occur in virulent parasites if they are also capable of some level of horizontal transmission. According to their model, horizontal transmission will occur at low frequencies when parasite prevalence is high, but it is crucial for the maintenance of the parasite over time (because the prevalence of a virulent parasite will necessarily decrease if only vertically transmitted). Mangin *et al*. (1995) suggested that virulent parasites with vertical transmission could be observed if the host–parasite association is recent and unstable, or if parasite virulence is only expressed after host reproduction. Hurst *et al*. (1994) have shown that cytoplasmic parasites causing sex ratio distortion can also spread in the host population despite their virulence.

Much of the debate about the relationship between transmission mode and parasite virulence is based on theoretical work. The scarcity of empirical evidence is due in part to the difficulty in estimating parasite transmission rates. Vertical transmission is commonly estimated by determining the proportion of offspring that carry the parasite, but this only estimates the potential contribution of vertical transmission to the parasite's fitness. The contribution of vertical transmission to a mixed-strategy parasite's fitness will only be equivalent to the potential transmission when all host offspring successfully become established in the population and remain infected. The importance of vertical transmission to the evolution of mixed-strategy parasites will depend on the number of new infected hosts that become established in the population through vertical transmission, i.e. the parasite's reproductive rate (or R, according to Anderson & May (1992)). Discrepancies between potential for vertical transmission and R should be especially common in plant pathogens that are transmitted vertically through seeds, since many factors can interfere with the actual establishment of infected seeds (e.g. low germination rate, seedling mortality, seedling competition, and adverse environmental conditions). Therefore, a mixed-strategy parasite might be highly virulent despite a high potential for vertical transmission if

horizontal transmission gives rise to most infected hosts in the population.

Atkinsonella hypoxylon, a common systemic fungal pathogen of the grass *Danthonia spicata*, is an example of a virulent parasite with high potential for vertical transmission. Healthy *D. spicata* produce chasmogamous seeds in aerial inflorescences and cleistogamous seeds at the base of leaf sheaths. Infected plants can produce only cleistogamous seeds because a fungal fruiting body develops in place of the aerial inflorescence. Vertical transmission occurs by vegetative growth of the fungal hyphae into the host's cleistogamous seeds (Clay 1994). Horizontal transmission occurs through outcrossed sexual spores produced on the fungal fruiting body (Diehl 1950; Clay 1994). The fungus severely reduces host fitness when it castrates the aerial inflorescence where 50 to 75 % of the seeds are produced by a healthy plant (Clay 1982). Vertical transmission was assumed to be the most important mode of transmission of *A. hypoxylon* because a high percentage (70–90 %) of the host's cleistogamous seeds carry the infection (Clay 1994) and because conversion of healthy to infected plants is seldom observed in the field. Antonovics *et al.* (1987) observed no conversions of 216 healthy plants to infected over three years. K. C. (unpublished data) observed three conversions of 115 healthy plants to infected in a three-year study.

To better understand the coevolutionary dynamics between *A. hypoxylon* and its host, it is necessary to evaluate the relative contribution of vertical and horizontal transmission to fungal fitness (R). We investigated the genetic structure of three natural populations using 11 randomly amplified polymorphic DNA (RAPD) markers. Since transmission mode and breeding system are coupled in *A. hypoxylon*, the relative contribution of vertical and horizontal transmission to fungal fitness can be estimated by determining whether the fungal genotypes from natural populations are derived primarily from sexual or asexual reproduction. If vertically infected seeds are the primary source of infected plants, the genetic structure of established *A. hypoxylon* populations should be highly clonal with low genotypic diversity and high linkage disequilibria. In contrast, we expect high genotypic diversity and low linkage disequilibrium if horizontal transmission causes the greater proportion of infected plants.

2. METHODS

(*a*) *Sample collection*

Atkinsonella hypoxylon fruiting bodies were collected from three populations within a 10 km radius around Bloomington, IN (USA): TC Steele (TS), Friendship (FR) and Griffy (GR). Populations are located in small clearings within areas of secondary forest. At FR and TS one or two fruiting bodies per infected plant were collected in a 4×4 m^2 grid, with one plant sampled from each 0.5 m^2 subplot. In this study we used 32 isolates from TS and 46 isolates from FR. Isolates from 28 out of the 30 pairs of stromata from the same plant had the same multilocus haplotype, indicating that most plants are infected by a single fungus clone. Therefore, we used only one isolate per plant. Sample sizes varied because not all subplots contained an infected plant and not all isolates were successfully cultured in the laboratory. At GR, fruiting bodies from every infected plant in the population were collected. Isolates from 63 plants that occurred within an area of comparable size to the other populations grids were chosen for the analyses. These sample sizes correspond to approximately 25–50 % of each population.

FR and TS isolates were cultured in the laboratory and DNA was extracted following Van Horn & Clay (1995). DNA from GR isolates were extracted a year later using a smaller-scale procedure (Rizzo & May 1994). DNA was quantified with a fluorimeter and diluted to 10 ng μl^{-1} for polymerase chain reaction (PCR) amplification.

(*b*) *Randomly amplified polymorphic DNA* (*RAPD*) *analyses*

Amplification conditions for RAPD markers were based on the Williams *et al.* (1990) protocol with the following changes in the PCR program. The first two cycles had a longer denaturation period: 94 °C for 5′, 36 °C for 1′, 45 °C for 15′ and 72 °C for 1′ 45″. The other 43 cycles had the denaturation step reduced to 1 min.

A subsample of ten isolates was used to screen 80 RAPD primers from Operon Technologies, Inc. (OP) and University of British Columbia (UBC). Primers which gave consistent polymorphism over DNA concentrations from 5 to 40 ng μl^{-1}, and that yielded the same results in three independent trials were selected for use: OPC11, OPD7, OPG3, OPG19, OPI17, UBC105, UBC290, UBC308, UBC319. Each polymorphic band was considered as one locus with only two possible alleles. Presence or absence of 11 polymorphic bands were scored for each isolate using these markers.

(*c*) *Data analysis*

Since *A. hypoxylon* is haploid, allele frequencies were obtained by a direct count of isolates with presence or absence of the band at each locus. Genetic diversity between populations was estimated using Nei's G_{st} index (Nei 1973).

Each isolate was assigned a 'clone type' based on its multilocus haplotype. Genotypic diversity was calculated for each population using Stoddart & Taylor's (1988) $\hat{G}$ estimate. For this index, genotypic diversity is maximized when every isolate in the sample is a different clone, that is, $G_{max} = N$ (sample size). $\hat{G}/G_{max}$ gives a standardized estimate of genotypic diversity that allows comparisons between populations. The expected genotypic diversity under the assumption of panmixia (G) was compared with $\hat{G}$ using the t-test described in Stoddart & Taylor (1988).

An exact test of linkage disequilibrium for each pair of loci was calculated with the program GENEPOP (Raymond & Rousset 1995). GENEPOP calculates the probability (p) of finding the observed contingency table if allele frequencies at the two loci are independent using the Markov chain method. The variance associated with p is estimated by GENEPOP through multiple simulations. We assumed that the two loci were in linkage disequilibrium if $p \pm$ s.e. < 0.05.

Linkage disequilibrium can result from lack of sexual recombination, physical linkage or sampling of subpopulations together (Maynard Smith *et al.* 1993). To distinguish between these we tested for linkage among pairs of loci two ways: (i) including all isolates from each population, and (ii) using a 'clone-corrected' sample, where each clone observed in the population is represented only once (Chen *et al.* 1994). Since isolates were sampled within a very small area, it is unlikely that we sampled isolated subpopulations together. Markers that are physically linked should have $p < 0.05$ in

both calculations. In contrast, when the linkage disequilibria is caused by asexual reproduction, we should see evidence of linkage among loci only when all the isolates are used because the clone-corrected sample would have removed the effect of multiple sampling the same haplotype.

When a population is reproducing asexually all alleles should be inherited together, causing an association across all loci in the haplotypes. We estimated the degree of multilocus structure using the method described in Brown *et al.* (1980). K is the average number of loci at which two random haplotypes in a population have different alleles at a locus. The variance in K depends on the number of loci considered and the degree of association between them. Therefore, an expected variance of K, (V_e), under the assumption of random segregation can be estimated. Observed variance of K, (V_o), should not be significantly different from V_e if the population is in panmixia. Populations were assumed to be reproducing asexually when V_o fell outside the 95 % confidence limit of V_e, (L).

3. RESULTS

Fifty-five clones were identified among the three populations. At FR we identified 25 clones among 46 isolates, at GR 27 clones among 63 isolates, and at TS 20 clones among 32 isolates. Within populations, most clones occurred only once (figure 1). G_{st} varied between 0.04 and 0.02, indicating a lack of genetic isolation between populations. Clones that occurred more than once were the ones expected under random mating given the observed allele frequencies and the assumption of no linkage disequilibrium (data not shown).

Table 1 shows the observed and expected genotypic diversity indices for each population. $\hat{G}/G_{max}$ are comparable to values reported for other pathogenic fungi (Chen *et al.* 1994; McDonald *et al.* 1994). $\hat{G}$ was not significantly different from the expected G in any population (table 1), indicating that we cannot reject the null hypothesis that these populations are panmictic.

Table 2 shows the probability that each pair of loci is segregating randomly as determined by GENEPOP. Probabilities above the diagonal are from the analyses including all isolates in the populations, and the values below the diagonal are from the 'clone-corrected' analyses. Since three loci were fixed in the GR population, only 28 pairwise comparisons were possible and none of them were significantly different from independent assortment. In the FR population, six out of 45 possible pairwise comparisons (one locus fixed) were significantly different from independent assort-

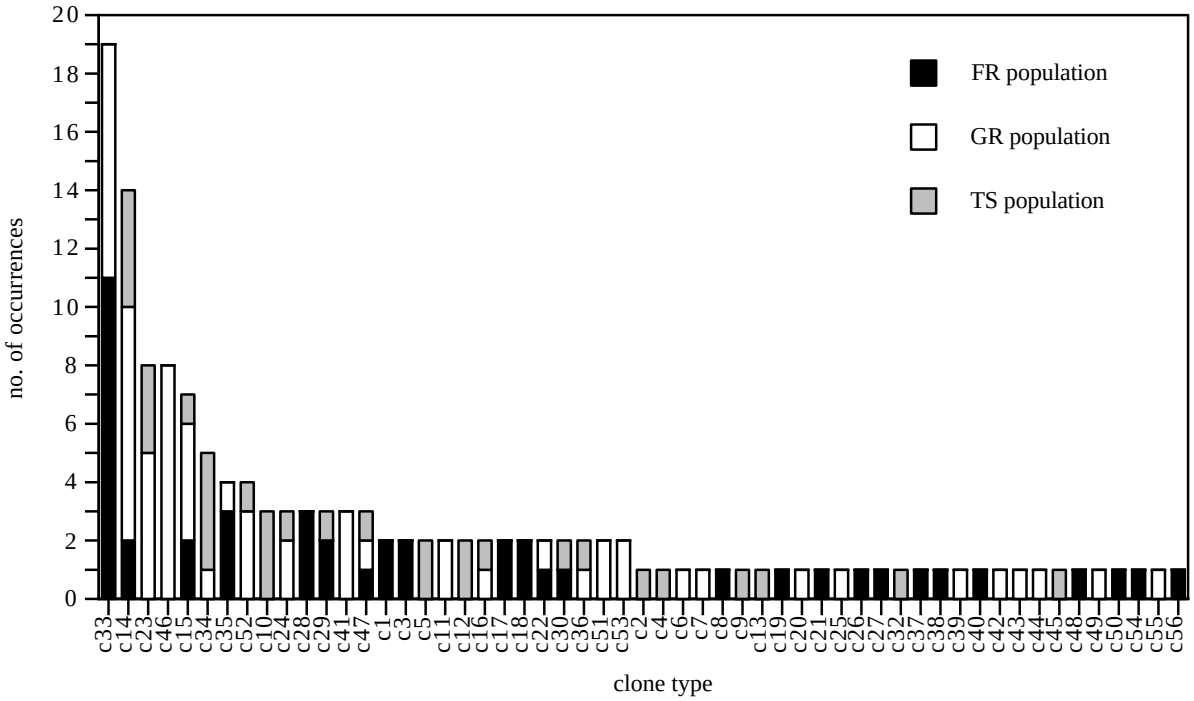

Figure 1. Distribution of clone types in the TS, FR and GR populations (clones are ordered from the most frequent to the least frequent).

Table 1. *Stoddart and Taylor's index of genotypic diversity for the FR, TS and GR* Atkinsonella hypoxylon *populations*

($\hat{G}$ is the observed genotypic diversity ± s.e.; G_{max} is the maximum genotypic diversity (equals sample size); $\hat{G}/G_{max}$ is the standardized observed genotypic diversity ± s.e. G is the expected genotypic diversity under the hypothesis of panmixia and p is the probability associated with the t-test that compared observed and expected genotypic diversity. For explanation, see text.)

population	$\hat{G}$	$\hat{G}/G_{max}$	G	p
FR	11.63 ± 3.47	0.25 ± 0.08	18.13	0.067
TS	14.22 ± 2.79	0.44 ± 0.08	18.89	0.105
GR	14.03 ± 2.34	0.22 ± 0.04	12.18	0.432

Table 2. *Probability of obtaining the observed contingency tables under the null hypothesis that loci are segregating independently*

(Each column heading indicates one locus. Locus nomenclature follows commercial names of primers used. If more than one polymorphic band was scored from the same primer they are distinguished as a, b, etc. For each pair of loci there are three rows of probabilities. The first row corresponds to probabilities from GR population, second row from FR population and third row from TS population. Probabilities above the diagonal were obtained when all isolates found in each population were included. Probabilities below the diagonal were obtained when the 'clone corrected' sample was used (see text). Probabilities below 0.05 are in bold face. Comparisons that were not possible because the locus was fixed are indicated with an 'X'. Standard errors associated with each of the probabilities were all smaller than 0.004 and are not shown. All probabilities and standard errors were calculated with GENEPOP)

	OPG19	OP117	OPD7	OPC11	OPG3	UBC105	UBC308	UBC290	UBC319a	UBC319b	UBC319c
OPG19		X	0.778	0.566	1.000	0.251	1.000	0.548	X	0.546	X
		0.159	0.493	**0.031**	0.088	**0.020**	0.110	1.000	1.000	0.499	X
		1.000	0.411	0.139	1.000	1.000	0.285	1.000	1.000	1.000	1.000
OPI17	X		X	X	X	X	X	X	X	X	X
	1.000		1.000	0.152	0.206	1.000	**0.007**	0.320	1.000	1.000	X
	1.000		1.000	0.103	1.000	1.000	0.556	0.630	**0.004**	1.000	1.000
OPD7	0.696	X		0.082	0.650	1.000	0.654	1.000	X	1.000	X
	1.000	1.000		0.302	1.000	0.167	0.636	**0.036**	0.105	1.000	X
	0.424	1.000		0.534	1.000	0.343	1.000	0.552	1.000	1.000	1.000
OPC11	0.691	X	0.665		0.306	1.000	0.360	0.515	X	0.548	X
	0.184	1.000	0.604		0.714	0.183	0.142	**0.005**	0.212	0.120	X
	0.517	0.224	0.515	X	1.000	0.274	0.680	0.069	0.594	0.589	1.000
OPG3	1.000	X	1.000	0.136		1.000	1.000	1.000	X	1.000	X
	0.563	0.505	0.550	1.000		1.000	**0.006**	0.660	0.317	1.000	X
	1.000	1.000	1.000	1.000		1.000	1.000	0.403	0.121	1.000	1.000
UBC105	0.550	X	1.000	1.000	1.000		0.265	0.559	X	0.139	X
	0.050	1.000	0.308	0.483	1.000		1.000	1.000	0.288	1.000	X
	1.000	1.000	0.304	1.000	1.000	X	1.000	0.129	1.000	1.000	1.000
UBC308	1.000	X	0.626	0.366	0.556	0.544		1.000	X	1.000	X
	0.383	0.094	0.593	0.428	0.161	1.000		1.000	0.213	0.633	X
	0.264	1.000	1.000	0.616	1.000	1.000		0.255	0.560	0.055	0.488
UBC290	0.692	X	1.000	1.000	0.629	1.000	0.629		X	0.563	X
	1.000	0.279	0.120	0.204	0.621	0.521	1.000		0.339	0.559	X
	0.582	1.000	0.587	1.000	0.478	0.472	0.070		0.627	0.129	0.162
UBC319a	X	X	X	X	X	X	X	X		X	X
	1.000	0.553	0.229	0.661	0.287	0.432	0.163	0.625		1.000	X
	1.000	0.053	1.000	1.000	0.161	1.000	1.000	1.000		1.000	1.000
UBC319b	0.499	X	1.000	0.538	1.000	0.214	1.000	1.000	X		X
	1.000	1.000	1.000	0.071	1.000	1.000	1.000	0.279	0.552		X
	1.000	1.000	1.000	0.223	1.000	1.000	0.524	0.210	1.000		1.000
UBC319c	X	X	X	X	X	X	X	X	X	X	
	X	X	X	X	X	X	X	X	X	X	
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.480	1.000	1.000	

Table 3. *Estimation of multilocus structure using the method of Brown* et al. (*1980*)

(avg$K$ is the average number of loci at which haplotypes differ in the allele present; E(V_e) is the estimated variance of K under the assumption of no linkage disequilibrium; V_o is the observed variance in K; var (V_o) is the estimated variance of V_o; and L is the 95% confidence limit for V_o.)

population	avg$K$	E(V_e)	V_o	var (V_o)	L
FR	2.738	1.818	2.557	0.136	2.557
TS	2.824	1.917	2.073	0.220	2.856
GR	2.162	1.400	1.393	0.057	1.879

ment when all isolates were used in the analysis. One comparison remained significant when analysed with the clone-corrected data. In the TS population, only one out of 55 possible pairwise comparisons was significantly different from independent assortment when all isolates were used in the analysis. The same comparison was significant when the clone-corrected data were used. FR was the only population to show some evidence of asexual spread of clones. However, two or three significant comparisons at $p < 0.05$ are expected by chance when 45 comparisons are performed. Since genetic differentiation between populations was small, linkage disequilibrium was also calculated for the three populations pooled (data not shown). Five out of 55 pairwise comparisons were significantly different from independent assortment when all isolates were used but none of them remained significant when the clone-corrected data were tested. The lack of associations among loci in the clone-corrected sample when all three populations were pooled suggests that the markers are not physically linked. The significant associations observed in the clone-corrected analysis from TS and FR might be due to founder effect, drift or local selection.

If mutations occur at high frequency, we could see high genotypic diversity even in asexual populations. However, without sexual recombination, new mutations should be associated with a particular haplotype, and haplotypes should differ at a single locus. In all three populations we found that the average number of loci at which haplotypes differed (K) was higher than two (table 3), indicating that these populations are undergoing recombination. There was no evidence of multilocus association in any of the populations. The observed variance in K was not significantly greater than the expected variance, since all V_o were equal to or less than L (table 3).

4. DISCUSSION

(*a*) *Established fungal clones are derived primarily from horizontal transmission*

Field observation of new infections and frequency of vertically infected seeds suggested that the predominant mode of transmission of *A. hypoxylon* is vertical. Since vertical transmission is accomplished through asexual reproduction, fungal populations should be highly clonal with low genotypic diversity and high linkage disequilibria. However, our results indicate that the three populations do not differ significantly from panmixia and have high genotypic diversity. Thus, established genotypes are derived primarily from sexually produced, horizontally transmitted spores. Furthermore, we observed little genetic differentiation between all three populations, indicating the occurrence of gene flow and outcrossing, which are only possible in this system through horizontal transmission.

At FR there was evidence of a higher relative contribution of asexual reproduction (vertical transmission) to parasite fitness. FR had the lowest genotypic diversity and the t-test between the expected and observed genotypic diversity approached significance ($p = 0.067$). Also, FR was the only population where significant associations between loci were found when all clones were used, but not when the clone-corrected sample was analysed. Moreover, the estimated V_o is at the limit of the 95% confidence interval of V_e (L). In this population, one clone (c33) occurred 11 times, representing 24% of the sample (figure 1). Based on the observed allele frequencies and the assumption of panmixia, clone 33 was expected to be the most common clone, but it was expected to occur only 4.9 times. The observed excess could be explained by: (i) higher survival of seeds infected with this fungal clone than seeds infected with other clones, (ii) greater success of this clone at infecting the plant genotypes present in the population, or (iii) non-random sampling. Nevertheless, we can conclude that most genotypes in established populations of *A. hypoxylon* are derived from horizontally transmitted sexual spores.

(*b*) *Transmission mode and coevolution between plant and pathogen*

Among close relatives of *A. hypoxylon* there are species capable of only vertical transmission, only horizontal transmission, or a combination of both (Clay 1988). Transmission mode and virulence seem to be correlated within their tribe (Balansieae), with vertically transmitted fungi being more mutualistic (Schardl & Clay 1997). Alkaloids produced by these fungi can protect their hosts against herbivory, supporting the idea of an evolutionary trend towards mutualism in this group (Clay 1988; Maynard Smith & Szahmary 1995). Since in *A. hypoxylon* a particular fungus genotype is vertically transmitted through selfed seeds, the opportunity for selection towards mutualism is enhanced. Therefore, the mixed strategy of *A. hypoxylon* could be interpreted as a transient polymorphism evolving from horizontal to vertical transmission. Our results do not support this hypothesis. In addition to the reduction in fecundity of infected plants, we found that horizontal transmission, rather than vertical, contributes more to *A. hypoxylon* fitness. Thus, it remains to be understood why the vertical transmission capability is maintained and whether the mixed strategy is evolutionarily stable.

Coevolutionary models have focused primarily on the importance of sexual reproduction for hosts. Sexual recombination would allow hosts to escape pathogens that can quickly adapt to common host genotypes (Bell 1982; Hamilton *et al.* 1990). The importance of sexual

reproduction for pathogens has received less attention. It is assumed that genetic variation in pathogen populations can be maintained through mutation if pathogen populations are very large and if they have shorter generation times than their hosts. However, *A. hypoxylon* population sizes and generation times are similar to their host's. Sexual reproduction in such pathogens may significantly increase the selection pressure on the host to maintain sexual reproduction (similar to sexually reproducing crop pathogens that make selection for resistant host varieties more difficult (Groth & Roelfs 1982)). Selection pressure by *A. hypoxylon* might help to explain the mixed-mating system of *D. spicata* through a two-fold effect: (i) selection for maintenance of outcrossing (chasmogamous) flowers to avoid infection, and (ii) selection for maintenance of cleistogamous flowers as the only alternative for producing seeds once an individual is infected.

(*c*) *Potential versus actual contribution of vertical transmission to pathogen fitness*

Although field observations suggested that vertical transmission was the predominant mechanism of transmission, established *A. hypoxylon* populations are derived primarily from horizontally transmitted sexual spores. The higher contribution of horizontal transmission to fungal fitness, despite the rare observation of new infected plants in the field, may be explained by: (i) lower establishment success of cleistogamous than chasmogamous progeny in natural populations (cleistogamous seed germination was 67% versus 95% for chasmogamous seeds from the same maternal parents (P.X.K., unpublished data)), (ii) senescence of the vegetatively propagated fungal genotype (Esser & Tudzynski 1980) (14% of the seedlings derived from CL seeds that carried the fungus were not infected (P.X.K., unpublished data)), and (iii) cumulative effects of many years with low annual rates of horizontal transmission, since the fungus–grass association is perennial.

To understand the epidemiology of mixed-strategist parasites it is important to estimate the relative contribution of vertical and horizontal transmission to parasite fitness. Our results caution against using observed rates of transmission to make conclusions about the evolutionary importance of each transmission mode. The actual contribution of the two mechanisms to parasite fitness is what is important to the evolution of virulence. Investigation of the parasite's population genetic structure to estimate long-term contribution of vertical and horizontal transmission to parasite fitness might be helpful in other host–parasite interactions where correlation between mating system and transmission mode occurs and direct observations of transmission rates are difficult. This will allow more meaningful tests of the theory regarding the importance of transmission mode to the evolution of parasite virulence.

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REFERENCES

Anderson, R. & May, R. 1992 *Infectious diseases of humans. Dynamics and control*. Oxford University Press.

Antonovics, J., Clay, K. & Schmitt, J. 1987 The measurement of small-scale environmental heterogeneity using clonal transplants of *Anthoxanthum odoratum* and *Danthonia spicata*. *Oecologia* **71**, 601–607.

Bell, G. 1982 *Masterpiece of nature*. Berkeley: University of California Press.

Brown, A., Feldman, M. & Nevo, E. 1980 Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics* **96**, 523–536.

Bull, J., Molineux, I. & Rice, W. 1991 Selection of benevolence in a host–parasite system. *Evolution* **45**, 875–882.

Chen, R., Boeger, J. & McDonald, B. 1994 Genetic stability in a population of a plant pathogenic fungus over time. *Molec. Ecol.* **3**, 209–218.

Clay, K. 1982 Environmental and genetic determinants of cleistogamy in a natural population of the grass *Danthonia spicata*. *Evolution* **36**, 734–741.

Clay, K. 1988 Clavicipitaceous fungal endophytes of grasses: coevolution and the change from parasitism to mutualism. In *Coevolution of fungi with plants and animals* (ed. K. Pirozynski & D. Hawksworth), pp. 79–105. New York: Academic Press.

Clay, K. 1994 Hereditary symbiosis in the grass genus *Danthonia*. *New Phytol.* **126**, 223–231.

Diehl, W. 1950 *Balansia* and the Balansiae of America. *US Dep. Agric. Monogr.* **4**. 1–82.

Esser, K. & Tudzynski, P. 1980 Senescence in fungi. In *Senescence in plants*. (ed. K. Thimann), pp. 67–83. Boca Raton: CRC Press.

Ewald, P. 1987 Transmission modes and evolution of parasitism–mutualism continuum. *Ann. NY Acad. Sci.* **503**, 295–306.

Fine, P. 1975 Vectors and vertical transmission: an epidemiologic perspective. *Ann. NY Acad. Sci.* **266**, 173–194.

Groth, J. & Roelfs, A. 1982 Effects of sexual and asexual reproduction on race abundance in cereal rust fungus populations. *Phytopathology* **72**, 1503–1507.

Hamilton, W., Axelrod, R. & Tanese, R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natn. Acad. Sci. USA* **87**, 3566–3573.

Herre, E. A. 1993 Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**, 1442–1444.

Hurst, G., Purvis, E., Sloggett, J. & Majerus, M. 1994 The effect of infection with male-killing *Rickettsia* on the demography of female *Adalia punctata* L. (two-spot ladybird). *Heredity* **73**, 309–316.

Lipsitch, M., Nowak, M., Ebert, D & May, R. 1995 The population dynamics of vertically and horizontally transmitted parasites. *Proc. R. Soc. Lond.* B **260**, 321–327.

McDonald, B., Miles, J., Nelson, L. & Pettway, R. 1994 Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. *Phytopathology* **84**, 250–255.

Mangin, K., Lipsitch, M. & Ebert, D. 1995 Two virulent microsporidians in *Daphnia magna* and persistence of vertically transmitted diseases. *Parasitology* **111**, 133–142.

Maynard Smith, J., Smith, N., O'Rourke, M. & Spratt, B. 1993 How clonal are bacteria? *Proc. Natn. Acad. Sci. USA* **90**, 4384–4388.

Maynard Smith, J. & Szahmary, E. 1995 *The major transitions in evolution*. San Francisco: W. H. Freeman.

Nei, M. 1973 Analysis of gene diversity in subdivided populations. *Proc. Natn. Acad. Sci. USA* **70**, 3321–3323.

Raymond, M. & Rousset, F. 1995 An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Rizzo, D. & May, G. 1994 Nuclear replacement during mating in *Armillaria ostoyae* (Basidiomycotina). *Microbiology* **140**, 2115–2124.

Schardl, C. & Clay, K. 1997 Evolution of mutualistic endophytes from plant pathogens. In *The Mycota. Vol. IV: plant relationships* (ed. G. Carroll & P. Tudzynski) pp. 221–223. Berlin: Springer (In the press.)

Stoddart, J. & Taylor, J. F. 1988 Genotypic diversity: estimations and prediction in samples. *Genetics* **118**, 705–711.

Van Horn, R. & Clay, K. 1995 MtDNA variation in the fungus *Atkinsonella hypoxylon* infecting sympatric *Danthonia* grasses. *Evolution* **49**, 360–371.

Williams, J., Kubelik, A., Livak, K., Rafalski, L. & Tingey, S. 1990 DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* **18**, 6531–6535.

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