Viral Pathogens and the Advantage of Sex in the Perennial Grass Anthoxanthum odoratum [and Discussion]

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Viral pathogens and the advantage of sex in the perennial grass *Anthoxanthum odoratum*

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SUMMARY

The ubiquity of sexual reproduction among plants and animals remains one of the major unresolved paradoxes of modern evolutionary biology. In order for sex to be maintained in populations, sex must confer immediate and substantial fitness benefits. Theoreticians have proposed numerous mechanisms to explain how such advantages arise, but experimental data are few. In one well-studied population of the perennial grass *Anthoxanthum odoratum* in a mown North Carolina field, sexual offspring have been found to have significantly higher fitness than asexual offspring. More recent field experiments show that an aphid-transmitted virus, barley yellow dwarf (BYDV)-strain SGV, specifically transmitted by *Schizaphus graminum*, frequently infects *Anthoxanthum* progeny soon after transplantation into the field. BYDV infection is asymptomatic in *Anthoxanthum*, but BYDV-inoculated clones planted directly in the field had significantly lower fitness than healthy controls.

Sexual females have been hypothesized to gain a fitness advantage for their offspring in the presence of pathogens either by providing 'an escape in time' from pathogens preadapted to the parental genotype or through the production of rare genotypes, which escape frequency-dependent infection. When parental clones and seed-derived sexual offspring were planted in identical but separate arrays in sites near where the parent was collected, parental clones were twice as frequently infected as sexual offspring. Factors other than genetic variation may have contributed to differences in levels of infection between sexual and asexual progeny: in this experiment, clonally derived asexual offspring tillers were slightly larger than seed-derived sexual tillers; in field experiments, larger plants were more frequently infected than smaller plants. When different families were planted into a common site, there was evidence that genotypes were less frequently infected when locally rare than when common.

Taken together, the data suggest that BYDV infection generates advantages for rare or sexually produced genotypes in *Anthoxanthum*. The pattern of infection is likely to result from a complex interaction between vector, host, and viral genetics and population structure, vector behaviour, and host and vector dispersal patterns. Sexually produced genotypes appear to benefit because they are both novel and rare, but the observed minority advantage was weak. Other viral, bacterial, and fungal pathogens in this *Anthoxanthum* population may act as frequency-dependent selective forces in different places in the field, collectively generating the substantial and observed overall fitness advantage of rare genotypes. Further study is needed to elucidate their role. Nevertheless, the data do show that viral pathogens, which are often asymptomatic, play a significant evolutionary role in plant populations.

1. INTRODUCTION

Viruses are ubiquitous in wild plant populations. Surveys of wild populations suggest that an average of 10% of individuals in a population are infected (Kelley 1993). But epidemics (in which up to 67% of individuals are infected) are regularly found in small surveys of multiple populations (Kelley 1993). When plants are in their natural environments, it is commonplace for virus replication to occur in individuals that show no detectable abnormalities (Cooper & MacCallum 1984). Viruses are difficult to detect; because the absence of symptoms is often assumed to indicate little fitness cost to infected hosts, it is not surprising that field ecologists often overlook viruses in plant populations.

Since the discovery of an 'intrinsic cost' of sex (Williams 1975; Maynard Smith 1978; Bell 1982), there has been considerable interest in the adaptive significance of sexual reproduction. Theoreticians have proposed that pathogens represent the major selective force favouring the maintenance of genetic variation and sexual reproduction in plant as well as animal populations (Jaenike 1978; Hamilton 1980, 1990; Hutson & Law 1981; Tooby 1982). According to theory, to be maintained in populations, sexual reproduction must confer immediate, substantial (twofold) fitness benefits. The discovery of the cost

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of sex has spawned a number of experimental studies (Antonovics & Ellstrand 1984; Ellstrand & Antonovics 1985; Bierzychudek 1987; Lively 1987; Kelley *et al.* 1988; Kelley 1989*a*,*b*; Lively *et al.* 1990).

One of the most well-studied systems used to test hypotheses for the adaptive significance of sexual reproduction is a population of the short-lived perennial bunch grass *Anthoxanthum odoratum* growing in a mown North Carolina field (Antonovics & Ellstrand 1984; Ellstrand & Antonovics 1985; Kelley *et al.* 1988; Kelley 1989*a*,*b*, 1993). In these studies, seeds taken from single inflorescences were germinated, grown to large plants in a glasshouse, and then divided into single tillers to constitute 'sexual progeny'. Vegetative ramets sampled from adults were taken to the glasshouse, grown to large size, and likewise divided into single tillers to constitute 'asexual progeny'. Fitness was assessed as the net reproductive rate, estimated as counts of the number of inflorescences summed over the lifetime of the progeny. The number of inflorescences has been shown to be highly correlated with the number of spikelets, and hence male and female contribution to fitness, there being one seed and two anthers per spikelet. When sexual and asexual progeny were planted directly into the field in arrays that simulated the natural dispersal profile around adults, sexual progeny were more fit than asexual progeny by a factor of 1.55 (Kelley *et al.* 1988).

The pattern of the advantage of sex in numerous experiments suggested that predators or pathogens were responsible for this advantage, but there was little obvious evidence of pathogen or predator attack. However, aphids (*Schizaphus graminum*) were occasionally observed, and in one experiment, the presence of aphids was correlated with advantages for genetic variability in seedlings (Schmitt & Antonovics 1986).

In an initial survey, I tested 45 flowering *Anthoxanthum* plants in this field for a cosmopolitan, aphid-borne RNA virus, barley yellow dwarf luteovirus (Rochow & Duffus 1981), by using direct enzyme-linked immunosorbent assay (ELISA). Twenty percent of adult *Anthoxanthum* tested positive for BYDV-strain SGV (transmitted specifically by *S. graminum* (Kennedy *et al.* 1962)) and 2.2% for BYDV-strain RPV (transmitted specifically by *Rhopolosiphum padi* (Kennedy *et al.* 1962)). BYDV induces yellowing, reddening and brittleness of leaves, and stunting in many crop hosts, but is asymptomatic in *Anthoxanthum* (Kurstak 1981). The virus is phloem-restricted and cannot be spread through contact or through seed. Rather, BYDV consists of multiple different strains, which are specifically transmitted by different aphid species. The virus is transmitted in a persistent manner, and extended feeding on infected plants is necessary in order for aphids to acquire the ability to transmit the virus.

2. PATHOGEN SPREAD IN THE FIELD: THE 'ESCAPE IN TIME' HYPOTHESIS

Pathogen infection can explain short-term advantages of sex if novel genotypes produced by sexual females 'escape in time' from pathogens that are preadapted to or spread preferentially among clones of the parent (Bremermann 1980; Rice 1983). The hypothesis predicts that if sexual and asexual offspring are planted near the parent, asexual offspring will be more frequently infected than sexual offspring.

To test this prediction, both sexual and asexual progeny were generated for each of 18 adult plants sampled for single tillers and several inflorescences in May 1991. Nine parents were sampled at regular 4 m intervals in each of two transects (northern, N, and southern, S) across the central part of the field (figure 1). Tillers sampled from parents were grown in the glasshouse and divided into single tillers to generate 'asexual progeny'. Seeds from the same parents germinated in flats in August 1991 were grown in the glasshouse and divided into single tillers to generate 'sexual progeny'. Because *Anthoxanthum* is self-incompatible and wind-pollinated, individuals deriving from seeds were likely to be highly genetically variable, sometimes full sibs of each other but mostly half-sibs (Kelley *et al.* 1988).

Both sexual and asexual progeny were planted in the field, with minimal disturbance of the vegetation, in 'home sites' (within 1.5 m of the location at which the parent was sampled). All progeny were planted into hexagonal arrays at a fixed density (4.0 cm between adjacent plants) at which advantages for sex were large in previous experiments (Ellstrand & Antonovics 1985; Kelley *et al.* 1988).

Asexual offspring were planted in a single hexagonal design with six clones surrounding a central clone.

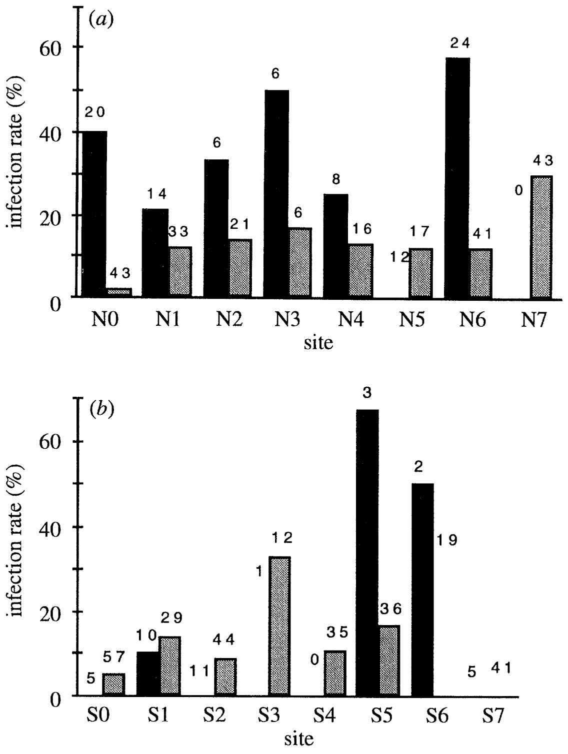

Figure 1. Percentage of asexual (solid) and sexual (stippled) offspring planted into home sites infected with BYDV-strain SGV. Number of plants alive and tested at each site are shown above the bars. Letters beneath the bars designate the different home sites. See text for further details.

Sexual progeny were planted into three designs, which differed in the amount of local genetic variability. Sexual offspring were planted with a single clone of each (seed-derived) genotype planted at the centre surrounded in separate hexagonal plantings with: (*a*) six clones of the same genotype, or (*b*) six maternal half-sib sexual progeny, or (*c*) six unrelated sexual progeny. Each of the three sexual treatments was replicated once each for eight unique sexual genotypes obtained from the female parent sampled at that site. Thus, the three sexual treatments (*a–c*) were each replicated eight times for each parent (if tillers were available) and the asexual treatments were replicated six times. A total of 3255 ramets was planted on 22–30 November 1991.

All material was serologically tested for BYDV-strain SGV, immediately before planting the experiment, by using direct ELISA with sera supplied by AGDIA (Elkhart, Indiana, U.S.A.). Four of 18 parental clones had been naturally infected in the field before sampling and tested positive for BYDV-strain SGV (N2, N3, S4, S6). Tillers were weighed before planting.

A frost immediately after transplantation killed many plants. With few individuals alive in each treatment, there was little difference among the three sexual treatments, since plants had few neighbours. The three sexual treatments were therefore considered as a single treatment in statistical analyses. All plants were surveyed on 3–7 June 1992, the number of vegetative and reproductive tillers were counted, and a small sample of leaf was taken from each living plant for serological testing.

Sexual and asexual progeny planted into home sites were tested for BYDV-strain SGV. The results showed that sexual and asexual progeny were rapidly and frequently infected after transplantation into home field sites (figure 1). Asexual progeny were more than twice as frequently infected as sexual progeny. Overall, 28.3% of asexual progeny and 11.4% of sexual progeny were infected with BYDV-strain SGV (difference: $G = 20.2$, d.f. $= 1$, $p < 0.001$). Excluding parental clones that were infected before the experiment, 26.5% of asexual and 11.7% of sexual progeny tested positive for BYDV-strain SGV (difference: $G = 13.2$, d.f. $= 1$, $p < 0.001$). Local 'hot spots' of infection occurred within short distances, with infection rates as high as 67%. Excluding the sites in which parental clones were infected before the experiment, there was significant among-site variation in the frequency of infection ($G = 71.3$, d.f. $= 22$, $p < 0.001$) and among-site variation in the relative frequency of infection of asexual and sexual progeny ($G = 25.2$, d.f. $= 11$, $p < 0.01$). Although these among-site differences in the infection rates were significant, in almost all cases, asexual progeny were more frequently infected than sexual progeny.

At the start of the experiment, there were slight size differences between asexual and sexual tillers, although these were not statistically significant (asexual tiller mean mass 0.60 g, sexual tiller mean mass 0.47 g, ANOVA square-root transformed data $f = 3.34$, d.f. $= 1, 208$, $p < 0.07$).

3. PATHOGEN SPREAD IN THE FIELD: THE 'MINORITY ADVANTAGE HYPOTHESIS'

According to the 'minority advantage' or Red Queen hypothesis, sex will be favoured if rare genotypes produced by sexual females escape pathogens that are spread in a frequency-dependent manner. It was not possible to test this hypothesis in the earlier experiment, because the frost eliminated differences between 'minority' and 'majority' treatments. Consequently, a second experiment was planted near the first, using the seeds of nine parents (described above) germinated in the glasshouse, grown, and then divided into single tillers. Because BYDV is not seed-transmitted, this procedure ensured that all starting material was free of virus infection. In each of five sites, located at 2 m intervals, genotypes from each of the nine families were represented in three treatments with a genotype planted in hexagonal arrays (4.0 cm spacing) surrounded by (*a*) identical clones, (*b*) maternal half-sibs, and (*c*) unrelated individuals. The minority advantage hypothesis predicts that virus infection will be more frequent in treatments with less genetic variability. All planting material was tested for BYDV-strain SGV before planting the experiment (to ensure that plants were virus-free). A total of 945 tillers were planted on 21–23 December 1991 and surveyed during the period 20 May–2 June 1992, at which time a small leaf sample was taken and serologically tested for BYDV-strain SGV by using direct ELISA with sera supplied by AGDIA (Elkhart, Indiana, U.S.A.).

After approximately six months in the field in one site (site 1), 29% of the plants became infected with BYDV-strain SGV (figure 2) and genotypes were 1.63 times less likely to be infected when rare (unrelated treatment) than when common (identical treatment). At the other four sites, the incidence was small, with

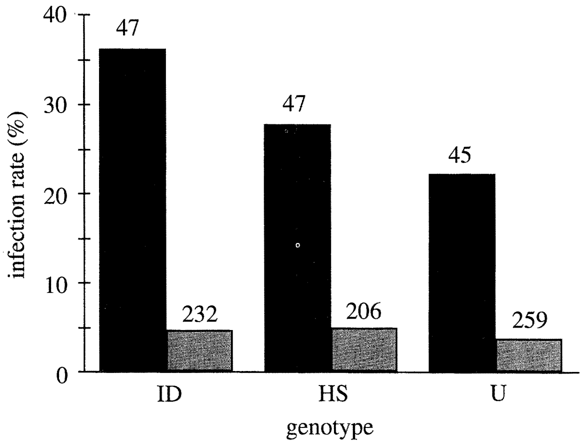

Figure 2. Frequency of BYDV-strain SGV infection in the three local variability treatments shown for site 1 (solid bars) and sites 2–5 (stippled bars) separately. ID, a genotype planted in the centre of a hexagon surrounded by six clonal copies; HS, the same genotype planted in the centre surrounded by six maternal half-sibs; U, the same genotype planted in the centre surrounded with six unrelated individuals. Numbers of plants alive and serologically tested are shown above the bars.

only slightly more plants infected in the less compared with the more genetically variable plantings; no minority advantage was seen. Although minority advantage was seen in one site, these differences in infection frequency were not statistically significant, perhaps because of the small sample size (site × treatment × infection frequency interaction $G = 4.8$, d.f. $= 8$, n.s.; site × infection frequency interaction $G = 56.8$, d.f. $= 12$, $p < 0.01$; treatment × infection frequency interaction $G = 6.0$, d.f. $= 10$, n.s.).

This experiment was repeated in the following year at an adjacent site in the field. Preliminary results showed that minority advantage was observed in four of six sites in the field, with statistically significant heterogeneity found in one of those sites (data unpublished).

4. FITNESS CONSEQUENCES OF BYDV INFECTION

For BYDV infection to act as an important selective force favouring sex, viral infection must reduce individual fitness. The fitness consequences of BYDV-strain SGV infection can be estimated by comparing the performance of cloned parental (asexual progeny) and (sexual) progeny tillers used in the 'escape in time' experiment separately for (i) parents that had been naturally infected in the field with BYDV-strain SGV (before clonal propagation), and (*b*) parents that were not infected (tables 1 and 2).

When planted into sites close to where parents were sampled (home sites), asexual offspring that were initially free of BYDV infection had 26% higher fecundity than sexual progeny of those same parents. However, asexual progeny that were initially infected (because they had been vegetatively propagated from BYDV-infected parents) had almost fourfold lower fecundity than seed-derived sexual progeny, when both were planted into sites close to where their parents were sampled. These differences were highly statistically significant.

Table 1. *The fitness consequences of virus infection*

(The relative fitnesses of asexual and sexual offspring are shown separately for sites in which all asexual offspring were BYDV-infected because they were clonally propagated from virus-infected parents, and for sites in which asexual offspring were initially free of BYDV-infection. Differences were tested for statistical significance by using a *t*-test, which assumed different variances.)

Parents uninfected with BYDV-SGV	Asexual offspring ($n = 118$)	Sexual offspring ($n = 429$)
vegetative size	2.25	2.16
fecundity	1.08	0.86**
Parents initially infected with BYDV	Asexual offspring ($n = 14$)	Sexual offspring ($n = 90$)
vegetative size	1.71	1.77
fecundity	0.14	0.51*

Symbols: **, $p < 0.025$; *, $p < 0.05$.

Table 2. *Patterns of infection*

(Data are vegetative and reproductive tiller number for plants infected during the first six months of the experiment. Differences between the means were tested for statistical significance by using a *t*-test, which assumed different variances.)

	BYDV serological test result	
	positive ($n = 38$)	negative ($n = 95$)
vegetative size	2.37	1.99^{+}
fecundity	1.47	1.15*

Symbols: $^{+}$, $p < 0.06$; *, $p < 0.05$.

Because these parental clones had been infected in the field, it was possible that these were less fit genotypes (using 'fitness' in its everyday sense), which had been more susceptible to infection. However, plants that became infected during these field experiments were consistently larger and manifestly more fit than those that did not become infected (table 2). Genotypes with initially higher fitness appeared to be more prone to infection than less fit genotypes. These data seem to indicate that, in the absence of pathogen attack, asexual offspring were slightly more fit than sexual offspring in local home sites.

To measure the fitness consequences of BYDV infection directly, nine seeds obtained from the females sampled in this field were germinated in August 1993, grown to large size, and then divided into single tillers. Three tillers of each genotype were individually inoculated with BYDV-strain SGV by placing seven aphids (*Schizaphus graminum* obtained from field collections and cultured in the glasshouse on a BYDV-SGV infected *Anthoxanthum* plant) on each tiller and allowing them to feed for ten days. The BYDV-infected *Anthoxanthum* used as a source for virus infection was originally sampled from the North Carolina field, and then vegetatively propagated in the glasshouse. To serve as negative controls, single tillers of *Anthoxanthum* were mock-inoculated by placing seven virus-free individuals of *S. graminum* (cultured in the glasshouse on virus-free *Anthoxanthum* plants) onto plants and allowing them to feed for ten days. To serve as an additional negative control, single tillers were left uninoculated. After feeding, aphids were removed from individual plants in the inoculation and mock-inoculation treatments, and two weeks later all plants were tested for BYDV-SGV infection by using direct ELISA. Plants that tested positive, along with negative controls, were propagated for three months in the glasshouse, then divided into single tillers, individually weighed, and planted directly into the field in a randomized block design on 15–18 March 1994. All individuals are surveyed on 15 May 1994, at which time the number of vegetative and reproductive tillers were counted to provide estimates of vegetative size and fecundity, respectively.

Preliminary results from this experiment showed that, although there was no significant size difference between BYDV-infected and healthy tillers at the time of planting, BYDV-infected plants were 12% smaller (mean size, infected : healthy plants 1.7 : 1.9 tillers, $t = 2.12$, d.f. 341, $p < 0.025$) and had 80% fewer inflorescences than healthy plants (infected : healthy 0.006 : 0.03 mean inflorescence number, $t = 1.62$, d.f. = 254, $p < 0.06$). Thus although growth of the host may be reduced only a little by the pathogen, the effect of the latter on its host's genetic fitness is quite severe.

5. DISCUSSION

Other studies of the consequences of sex, in a dioecious freshwater snail with sexual and parthenogenetic populations (Lively 1987) and coexisting sexual and clonal fish (Lively *et al.* 1990) have implicated parasites in favouring genetic diversity. More recently, studies of a perennial herb, *Eupatorium chinense* (Yahara & Oyama 1993) found considerably higher rates of infection by the geminivirus tobacco leaf curl in agamospermous polyploid populations (30% plants) than in related sexual diploid populations (3%). The results here directly implicate a pathogenic RNA virus as a factor favouring sex in a plant population. The idea that parasites (pathogens) are important to the maintenance of sexual reproduction appears to be quite general.

Despite the intensity with which this *Anthoxanthum* population has been studied and the apparent ubiquity of BYDV infection, BYDV managed to escape detection because it caused no overt foliar symptoms. The absence of symptoms was not equivalent to the absence of fitness consequences. BYDV significantly reduced both fecundity and growth. Studies of other natural virus–host combinations have shown an association between viral infection and reduced growth and fecundity. MacKenzie (1985) found that *Primula vulgaris* clones infected with arabis mosaic virus had significantly lower growth and survivorship than uninfected clones when both were transplanted back into the field. Yahara & Oyama (1993) found that *Eupatorium chinense* plants naturally infected with tobacco leaf curl geminivirus had significantly lower growth rates and higher mortalities and produced significantly few seeds than uninfected plants. In the first study to directly compare the fitness of healthy and virally infected plants, Kelley (1993) found that clones of *Anthoxanthum odoratum* inoculated with brome mosaic bromovirus had significantly lower growth and fecundity, and higher mortality rates, than healthy controls. Plants inoculated with *Anthoxanthum* latent blanching hordeivirus had lower growth and higher mortality, but had slightly higher fecundity than uninfected controls. The fitness consequences of virus infection will depend on the specific host–viral strain combination.

The results of earlier studies that showed an association between viral infection and lower fitness might have been caused by the increased susceptibility of genotypes of low fitness to infection. In particular, one of the major factors proposed to explain advantages to sexual reproduction is mutational damage, whereby sex functions as a DNA repair mechanism (Michod & Levin 1988) or as the provider of an efficient elimination of deleterious mutations that have to be judged by their effects (not being detectable for repair) (Kondrashov 1984, 1988). According to this hypothesis, plants with mutational damage (clonally propagated asexual genotypes) will be less robust and will be more susceptible to viral infection. However, in this study, *Anthoxanthum* plants that became infected were significantly more robust (larger) than plants not infected close to the time of infection. Further, minority advantage (escape from pathogens) was found here in experiments in which all individuals were (sexually) seed-derived. Interestingly, Oyama and Yahara (1993) also found that most fit individuals were preferred by the whitefly vector of tobacco leaf curl geminivirus and that these plants were preferentially infected. Viral infection may thus primarily affect the fitness of Sisyphean genotypes (Williams 1975).

Barley yellow dwarf virus is restricted to plant phloem, where it is thought to clog phloem and restrict the flow of metabolites to plant roots (Goodman *et al.* 1986). The physiological basis for the fitness reduction in *Anthoxanthum* is not yet understood and is currently under investigation in our laboratory. However, our studies have revealed that aphids (*S. graminum*) can detect the presence of virus in infected plants and, when given a choice, prefer to feed on BYDV-SGV-infected compared with mock-inoculated plants (Kirkley 1993). Aphid colonies initiated on BYDV-infected, mock-inoculated, or healthy tillers of *Anthoxanthum* grew the most rapidly on infected plants (S. E. Kelley, unpublished data). The fitness reduction observed in the field may thus result both from direct physiological debilitation and from that caused by aphid feeding.

Interactive effects of viral infection and aphid behaviour on plant host fitness may be quite general. Blua *et al.* (1994), using an unrelated aphid–host–virus system (*Aphis gossypii* feeding on *Cucurbita pepo* infected with zucchini yellow mosaic potyvirus) also found enhancement of aphid colony development, which they attributed to increased amino acid concentrations in the phloem of virus-infected plants.

When novel sexual genotypes and old asexual genotypes were planted in sites close to where the parent had been sampled, asexual genotypes were more frequently infected. This home-site disadvantage for asexual offspring may have been caused by the fact that clonally propagated 'asexual' tillers were slightly larger than seed-derived 'sexual' tillers. In our laboratory studies, when given a choice between large and small plants, aphids preferred to feed on larger plants; the degree of preference was related to the size discrepancy between plants (Kirkley 1993). Further, in field experiments, larger plants were more frequently infected than smaller plants. Alternatively, feeding preference of local aphid clones for cloned parental genotypes may have resulted in asexual progeny being more frequently infected than sexual progeny. However, our laboratory studies of aphid movements showed no preferential movement toward particular host-plant genotypes (Kirkley 1993). These studies did not exclude the possibility that aphid feeding preferences might be expressed during the period of probing or through differential feeding durations, which would be of critical importance in determining whether plants became infected. Feeding preference of different aphid clones needs further study. A difficulty of the hypothesis that aphid preference is responsible for the 'escape in time' is that the preference for cloned parental over sexual tillers was seen for parents that were initially uninfected. These parents may have initially escaped infection in their home sites because they were resistant to feeding by the local aphid clones or resistant to infection by the local BYDV variants. In such a case, novel sexually produced genotypes might be more vulnerable to aphid feeding and viral infection.

In some sites, genotypes were less frequently infected when locally rare than when locally common. Frequency-dependent infection may also partly explain the disadvantage for asexual genotypes in home sites. Power (1991) noted that rates of aphid movement (*Rhopolosiphum padi* and *Sitobion avenae*) increased in more genotypically diverse plantings of barley varieties compared with less diverse ones, and that symptoms of BYDV infection rates were consequently reduced. A similar mechanism may be operating for *S. graminum* on *Anthoxanthum*, but it needs to be explicitly demonstrated in this system (and its adaptive significance explored).

In experiments reported here, minority advantage was evident only in a few sites, yet the observed advantage of sex in this population is widely observed (Kelley *et al.* 1988). Other viral, bacterial, and fungal pathogens are certainly acting as selective forces and, if the findings for BYDV are typical, are likely to be favouring rare or novel genotypes. J. Bever (personal communication) has recently found that live soils (from this North Carolina field) cultured by different plant species are often pathogenic for those same plant species in the subsequent generations. Kristi Westover (in my laboratory) has found that plant species pairs generally perform less well when planted in soils from beneath the same than different species combinations, and that this effect is at least in part attributable to the living soil components. Other viruses may be spread in *Anthoxanthum* which heretofore have escaped detection. Experiments are planned to assay for other possible sources of enhanced fitness advantage for sexual compared with asexual progeny.

In addition to vector behavior, host passaging effects may explain the observed patterns of infection in both experiments. Barley yellow dwarf luteoviruses (and the majority of plant viruses) have single-stranded RNA genomes. Single-stranded RNA genomes are known to have extremely high mutation rates (on the order of 1000-fold higher than eukaryotic genomes) because of the absence of a proofreading mechanism (Steinhauer & Holland 1987). Selection on such variation occurring during replication over a short or extended period within one host, or through passaging among hosts, has been repeatedly shown to lead to changes in the host range, vector properties, symptom expression (generally the measure of virulence), within-host virion concentration, or infectivity of virus strains (Dawson 1967; Chiko 1984; Shepherd *et al.* 1987; Hajimorad *et al.* 1991; reviewed in Yarwood 1979). In the course of infecting individual hosts, or through passaging through particular host genotypes, BYDV may evolve to infect particular genotypes more frequently, or cause more severe fitness reductions in host genotypes. The consequences of host passaging is currently under investigation in our laboratory.

Because BYDV is not seed-transmitted, it is clear that reproduction via seed will generate immediate and substantial fitness advantages for seed-derived offspring of BYDV-infected parents, but presumably these advantages would accrue to both sexual and apomictic seed offspring. However, because in the *Anthoxanthum* experimental system asexual offspring are derived through clonal propagation, these offspring would carry the virus if the parent had been naturally infected in the field. In past experiments, this may have contributed to the magnitude of the advantage for sexual offspring that was unrelated to the issue of sexual reproduction and the maintenance of genetic variability. Nevertheless, advantages of genetic variability occur in *Anthoxanthum*. Several experiments have shown advantages for rarity in *Anthoxanthum* using only seed-derived planting material (Antonovics & Ellstrand 1984; Schmitt & Antonovics 1986; see above). Further, advantages for sexual offspring in experiments (Kelley *et al.* 1988) occurred in 21 of 25 sites. Infection rates of flowering adults in this field are typically 20% or less and so carry-over of virus infection is not likely to fully explain the advantage of seed-derived sexual offspring in this system. Nevertheless, an improved estimate of the relative fitness of sex could be obtained in an experiment with a two-generation design: the first generation used to produce virus-free parents, and the second generation of seed-derived sexual and clonally derived asexual offspring planted directly into the field in designs stimulating a natural dispersal profile.

Ecologists have tended to overlook the consequences of RNA viruses for plant populations. Yet, in this study, numerous epidemics of virus infection occurred quickly, over short distances, with significant fitness consequences for the host population, and with no obvious signs of pathogen or predator attack. Viruses appear to be pervasive in plant populations (MacClement & Richards 1956; Barnett & Gibson 1975; Hammond 1981), have significant fitness consequences (Gibbs 1980; MacKenzie 1985), and have the potential for rapid mutation and evolution which may enable them to readily adapt to new hosts (Holland *et al.* 1982; Zimmern 1988). Clearly, RNA viruses in plants represent a good model system to investigate the evolutionary and ecological role of pathogens in natural communities.

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Discussion

J. Shykoff (*Swiss Federal Institute of Technology, Zurich, Switzerland*). What does Dr Kelley know about the variability of viruses in his field system? If there are different foci of foundress aphids invading the grass population bringing different viruses, then virus genotypes in the different field plots could be completely different. Genotypes specific infectivity or virulence could produce the inconsistent results he observed for the three neighbour treatments?

S. E. Kelley. Dr Shykoff brings up an excellent point. Different viral strains and different aphid clones located in throughout the field could explain the inconsistent results. In a recent experiment, we found evidence that particular genotypes appeared to be resistant to infection by local aphid vectors/BYDV-variants, but not to infection by aphid vectors/BYDV-variants found at greater distances from where the genotypes had been sampled. This might indicate the presence of different BYDV strains locally distributed throughout the field and the subsequent buildup of local resistances. Alternatively, it might be caused by resistance to feeding by local aphid clones. Additional experiments are planned to sort this out. BYDV strains might not only be differentially infective, but might have different fitness consequences for infected hosts and this deserves further investigation as well.