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Rapid evolutionary changes in a globally invading fungal pathogen (Dutch elm disease)

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Abstract

Two enormously destructive pandemics of Dutch elm disease occurred in the 20th century, resulting in the death of a majority of mature elms across much of the northern hemisphere. The first pandemic, caused by *Ophiostoma ulmi*, occurred as this pathogen spread across Europe, North America and Southwest and Central Asia during the 1920s–1940s. The current pandemic is caused by another *Ophiostoma* species, *O. novo-ulmi*. Since the 1940s, *O. novo-ulmi* has been spreading into the regions previously affected by *O. ulmi*. It has also spread as two distinct subspecies, termed subsp. *americana* and subsp. *novo-ulmi*. This sequence of events has resulted in competitive interactions between these previously geographically isolated pathogens. This article summarizes the biological properties of the Dutch elm disease pathogens and their history of spread. It reviews the remarkable series of genetic events that have occurred during their migrations; including the emergence of genetic clones, the spread of deleterious fungal viruses within the pathogen clones, and the rapid and continuing evolution of *O. novo-ulmi* via horizontal gene flow. The wider role of horizontal gene flow in the evolutionary potential of migratory plant pathogens is discussed.

Introduction

Plant disease epidemics resulting from the introduction of exotic fungal pathogens are a familiar process even to many of the public. Better known examples include epidemics of Potato blight, Chestnut blight and Dutch elm disease. Often they are initiated by human activity. Usually, there is limited resistance in the new host population and excessive aggressiveness in the introduced pathogen, reflecting their lack of prior co-evolution. This results in an explosive outbreak of disease.

Such introduction events present an enormous window of evolutionary opportunity for a plant pathogen. In its original endemic location, a pathogen tends to be subject to routine selection constraints: the multiplicity of selection or mortality factors to which it would normally be subject during the disease cycle, tending to favour ecological balance over time. None of the individual components of selection varies so strongly that this ecological balance is disturbed. Routine selection therefore favours maintenance of a relatively stable, if fluctuating, population structure over time (Brasier 1986). When introduced into a new environment, however, a pathogen will be subject to novel or episodic selection. Episodic selection encompasses a relatively sudden and major quantitative or qualitative change in the selection intensity imposed by a single or a variety of environmental components. Such selection is likely to lead to a significant alteration in a species' population structure (Brasier 1986, 1995). Although routine selection may eventually be restored following a period of episodic selection, the form of ecological balance arrived at may be different from that existing previously. Often, it will result in a chain of additional episodic selection events.

Introduction or sudden migration beyond its normal geographic range represents a major episodic selection event for a plant pathogen, providing sudden exposure to a range of new biotic and abiotic influences such as a

new host population, new vectors, new biological competitors and different climatic and edaphic regimes. These influences may tend to have a destabilising influence on the pathogen population, either through the sudden release of previously imposed selection, or through an increase in selection intensity of some components to the point where they are strongly directional. They may be further compounded both by genetic founder effects, and by novel opportunities for horizontal gene transfer. In such circumstances, the scope for evolutionary development in the pathogen may be large and the rate of evolutionary change at times extremely rapid (Brasier 1995, 2000).

Dutch elm disease, one of the major ecological accidents of the 20th century (Lamb 1979; Heybroek 1993), has resulted in the death of billions of elm trees on two continents. The disease is now providing considerable insights into rapid evolution of a plant pathogen outside its original environment. The consequences for the ecological dynamics of the host, beetle vector and fungal populations have been discussed elsewhere (Brasier 1986, 1996). In this article, we review the spread of the disease and consider the unusual genetic events that have occurred in the pathogen, with special reference to the emergence of pathogen clones and to the role of interspecific gene flow.

The Dutch elm disease pathogens

Elm trees (*Ulmus* spp.) are confined mainly to the temperate regions of the northern hemisphere. China and Japan have about 25 elm species, while Eurasia, North America and the Himalayas each have about 5 or 6 species. Dutch elm disease (so called because the early seminal research was in the Netherlands, see Holmes and Heybroek 1990) the elm's main fungal enemy, is a wilt disease caused by ascomycete fungi of the genus *Ophiostoma*, that spread within the tree's vascular system. The fungus is transmitted from diseased to healthy elms by elm bark beetles of the genus *Scolytus* (Fransen 1935; Webber 1990; Webber and Brasier 1984).

Dutch elm disease was unknown in Europe and North America before 1900, yet there have been two enormously destructive pandemics of disease in the northern hemisphere in the 20th century. These have been caused by the spread of two different species of fungal pathogen, *Ophiostoma ulmi* and *O. novo-ulmi*, respectively. Their geographic origins remain unknown, despite a number of expeditionary searches, but both species are believed to have come from Asia (Brasier 1990; Brasier and Mehrotra 1995).

O. ulmi and *O. novo-ulmi* differ markedly in many important behavioural and genetical properties, such as their optimum temperatures for growth, colony morphologies, molecular fingerprints, toxin production and pathogenicity to elms (see Brasier 1991). From their behavioural differences, it appears that they have different ecological strategies in their original locations. For example, the optimum temperature for growth of *O. novo-ulmi* is ca 22 °C and that of *O. ulmi* 28 °C. This suggests that *O. novo-ulmi* may be adapted to a temperate and *O. ulmi* to sub-tropical environment (Brasier and Mehrotra 1995). Another major difference is that *O. ulmi* is a moderate and *O. novo-ulmi* a highly aggressive pathogen of European elms. American elms, however, are generally more susceptible to Dutch elm disease than European elms (Gibbs et al. 1975). In consequence; *O. ulmi* caused significantly greater damage to American elms than it did to European elms. *O. ulmi* and *O. novo-ulmi* are genetically divergent based on molecular polymorphism studies; while studies on DNA sequences of their cerato-ulmin genes (cerato-ulmin is a glycoprotein implicated as a wilt toxin) and rDNA genes indicate that they are anciently divergent taxa (Pipe et al. 1997, 2000; Bates et al. 1983).

O. novo-ulmi is not a single entity but exists as two subspecies: subsp. *novo-ulmi* and subsp. *americana* (Brasier and Kirk 2001) (previously known as the Eurasian and North American races; EAN and NAN, Brasier 1979). These have different geographical distributions (see below). On average, subsp. '*novo-ulmi*' is slightly less pathogenic than subsp. *americana*, but both are very aggressive pathogens of European and North American elms (Brasier 1991). The two subspecies also differ in a number of other properties, such as their colony morphologies (Figure 2), their sexual fruitbody (perithecial) morphology (Brasier and Kirk 2001) and their molecular fingerprints (e.g. Bates et al. 1993; Pipe et al. 1997).

Intercontinental migration of the Dutch elm disease pathogens

The two Dutch elm disease pandemics of the 20th century represent major migratory events for the

pathogens involved. The history of these migrations will now be summarized.

First pandemic: O. ulmi. The first pandemic of Dutch elm disease, caused by *O. ulmi*, began in Northwest Europe around 1910 (Figure 1a). Thereafter the disease spread rapidly eastward on a series of epidemic fronts across Europe and into Southwest Asia. It was also introduced to the UK and North America around 1927 and into Central Asia in the late 1930s as a result of a series of importations of infested elm timber (Peace 1960; Brasier 1990). Initially, the spread of *O. ulmi* resulted in an intense epidemic in Europe. During the 1940s this first epidemic unexpectedly declined after losses of 10–40% of the elms in most European countries (Peace 1960). This decline is now thought to have involved the spread of deleterious fungal viruses in the *O. ulmi* population (Mitchell and Brasier 1994). In North America, however, no such decline occurred.

Second pandemic: O. novo-ulmi. In the early 1970s, there was a severe new Dutch elm disease outbreak in Britain and neighbouring parts of Europe, caused by the previously unknown *O. novo-ulmi*. Later, sample surveys across much of the northern hemisphere indicated that a second pandemic of Dutch elm disease, caused by *O. novo-ulmi*, had actually begun in the 1940s at two very different locations: the Moldova–Ukraine region in Eastern Europe (subsp. *novo-ulmi*) and the southern Great Lakes area in North America (subsp. *americana*) (Figure 1b) (Brasier 1990, 1996). Thereafter, subsp. *novo-ulmi* migrated steadily westward across Europe, reaching the Netherlands by the mid-1970s; and eastward to Southwest Asia. In the 1970s, it arrived some distance away in Central Asia, probably as a result of an importation jump. Likewise, subsp. *americana* spread steadily outward across the North American continent, reaching both the east and west coasts by the 1970s and 1980s (Figure 1b). In another importation jump, subsp. *americana* was introduced from Canada into Britain on diseased elm logs during the 1960s (Brasier and Gibbs 1973). It quickly spread to the Netherlands, France, Spain and many other countries of Western Europe. The geographical ranges of the two *O. novo-ulmi* subspecies now overlap in several parts of Europe (Figure 1a; cf. also Figure 8).

The spread of *O. novo-ulmi* has resulted in a catastrophic epidemic in which most mature European elms have died. Some 28 million elms have been killed in the UK alone. In North America, where the destructive impact of *O. novo-ulmi* has been even greater, the losses have amounted to hundreds of millions of elms.

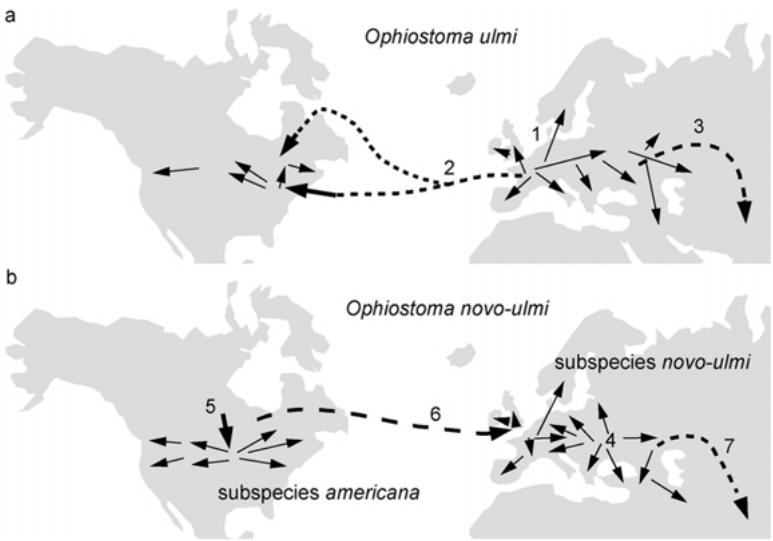

Figure 1. Intercontinental spread of *O. ulmi* and *O. novo-ulmi* in the first and second pandemics of Dutch elm disease. Solid arrows, natural migrations from probable sites of initial introduction. Dashed arrows, subsequent spread via additional importation events. (a) Spread of *O. ulmi*. 1: Its appearance in Northwest Europe around 1910. 2: Introduction to North America in the 1920s. 3: Introduction from Krasnodar to Tashkent, late 1930s. (b) Spread of EAN and NAN races of *O. novo-ulmi*. 4, 5: Original centres of appearance of the subsp. *novo-ulmi* and *americana* in the Romania–Moldova and southern Great Lakes regions, respectively. 6: Introduction of subsp. *americana* from Toronto area to Britain, ca. 1960. 7: Introduction of subsp. *novo-ulmi* to Tashkent area, 1970s. Adapted from Brasier (1990).

Across North America, Europe and Southwest Asia, recurrent cycles of elm recovery via emerging young seedlings and root suckers, followed by further attacks by *O. novo-ulmi*, are predicted to occur well into the future (Brasier 1986, 1996).

Discovery of *O. himal-ulmi*

A series of international surveys have been carried out to try and locate the geographic origins of *O. ulmi* and *O. novo-ulmi* (Figure 1). China was long considered a likely origin for Dutch elm disease, but a survey across Central China and Xinjiang Province on the Central Asian border, in 1986, revealed no evidence of the pathogens (Brasier 1990). They are also absent from Japan. However, a survey in the Western Himalayas led to the discovery of an entirely new, endemic species of Dutch elm disease pathogen, now named *O. himal-ulmi*. *O. himal-ulmi* shares many other physiological similarities to *O. novo-ulmi* including being very aggressive to European elms, but is apparently in natural balance with the native Himalayan elms and elm bark beetles (Brasier and Mehrotra 1995).

Despite this development, the geographic origins of *O. ulmi* and *O. novo-ulmi*, and indeed the origins of the two subspecies of *O. novo-ulmi* (which may also have separate geographical origins; Brasier and Kirk 2001), still remain to be identified. Areas suggested for further surveys are the Eastern Himalayas, Burma, other parts of Southeast Asia bordering China; and the floristically unique Yunnan Province in China itself (Brasier and Mehrotra 1995).

Interactions between the Dutch elm disease pathogens

The migration of *O. novo-ulmi* has resulted in the sudden intermixing of the *O. ulmi* and *O. novo-ulmi* populations, and included, in Europe, the intermixing of the two *O. novo-ulmi* subspecies (Figure 1a). The taxa have, therefore, come into direct competition. The outcome of the resulting 'battle' (Figure 2) depends upon many factors, ranging from their different fitness attributes to the potential for gene flow between them (Brasier 1986).

Regarding the potential for gene flow, it is important to appreciate that *O. ulmi* and *O. novo-ulmi* are not totally reproductively isolated. Both species are obligatorily outcrossing, with two sexual incompatibility types. Crosses within each species are highly fertile and 'breed' true. In crosses between them, however, *O. novo-ulmi* strongly rejects *O. ulmi* as a fertilizing (male) sexual partner, whereas *O. ulmi* can be fertilized by *O. novo-ulmi*. The resulting progeny show a remarkable range of non-parental phenotypes, including female sterility. Many are of low vigour and fitness, and most are weaker pathogens even than the *O. ulmi* parent (Brasier 1977; Kile and Brasier 1990). *O. ulmi* and *O. novo-ulmi* are therefore strongly but not totally reproductively isolated at both pre-zygotic

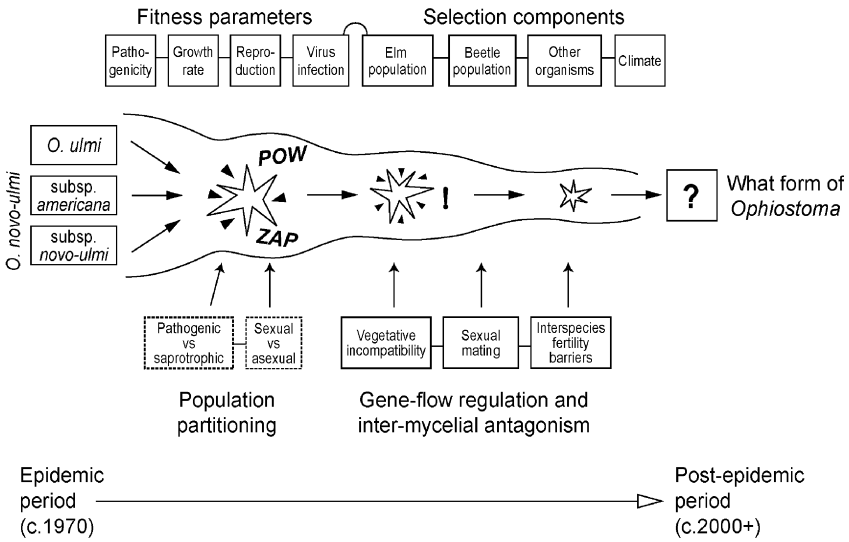

Figure 2. Concept of a battle between the Dutch elm disease Ophiostomas: the factors most likely to influence the outcome. Adapted from Brasier (1986).

and post-zygotic levels. This has been interpreted as evidence that they were once geographically separated species (Brasier 1977, 1986; Kile and Brasier 1990).

The two *O. novo-ulmi* subspecies, on the other hand, show only weak reproductive isolation. In sexual pairings, subsp. *novo-ulmi* exhibits a partial reproductive barrier against subsp. *americana*. When subsp. *novo-ulmi* is the recipient (♀) and subsp. *americana* the donor (♂), sexual reproduction is reduced by ca. 90% (Brasier 1979). However, the progeny show no unusual properties and no evident fitness reduction (Brasier 1986). These two subspecies, therefore, are expected to hybridize relatively freely in nature.

Replacement of *O. ulmi* by *O. novo-ulmi* and the potential for genetic exchange

During its current international migration, when *O. novo-ulmi* arrives at a 'new' location *O. ulmi* is already present. *O. novo-ulmi* then rapidly replaces *O. ulmi*, the latter declining at about 10% of the total pathogen population per annum (Figure 3) (Brasier 1986). This phenomenon represents a classic example of a fitter species replacing a less fit species. The fitness advantage exhibited by *O. novo-ulmi* probably has several components. First, when the two species come into close physical contact in the bark around the breeding galleries of the elm bark beetles, there appears to be direct competitive antagonism of *O. ulmi* by *O. novo-ulmi* (Mitchell 1988). Second, by virtue of its greater pathogenic ability, *O. novo-ulmi* captures more of the host resource than *O. ulmi* ('resource' meaning

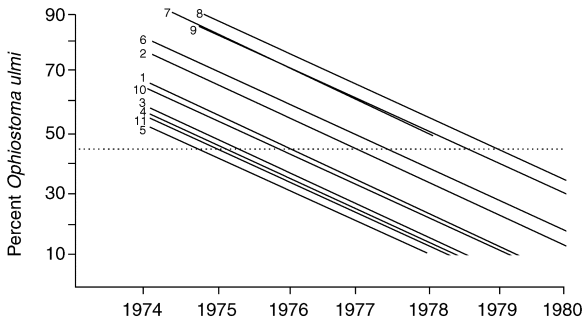

Figure 3. The replacement of *O. ulmi* by *O. novo-ulmi*. The figure shows the steady decline in the percentage of *O. ulmi*, following the arrival of *O. novo-ulmi*. The data are for 11 provinces of the Netherlands between 1974 and 1980. The dashed line is the 50% level (transformed). Redrawn from Brasier (1983).

here the internal sapstream or xylem of the tree and the highly nutritious inner bark around the beetle breeding galleries). Third, *O. novo-ulmi* may be better adapted to the temperate, elm-inhabiting regions of Europe and North America, and *O. ulmi* may be disadvantaged through being a tropically or sub-tropically adapted organism (Brasier and Mehrotra 1995).

During this replacement process, the close proximity of the mycelia of *O. ulmi* and *O. novo-ulmi* in the bark around the beetle galleries provides considerable physical opportunity for genetic exchange between them. Furthermore, as outlined above, *O. ulmi* and *O. novo-ulmi* are not fully reproductively isolated. Limited sexual hybridization between them is possible. Until recently, because of the low fitness of their progeny, it was considered that any hybrids produced would not survive in nature (Brasier 1977, 1986; Kyle and Brasier 1990). Now, however, it is known that rare *O. ulmi* × *O. novo-ulmi* hybrids do occur in nature, but are probably transient and unable to compete with the parent species (Brasier et al. 1998). Despite their transience, however, the hybrids could act as 'genetic bridges', allowing gene flow from one species to the other.

Evidence for gene transfer from *O. ulmi* to *O. novo-ulmi*

Several pieces of evidence indicate that introgressive gene flow from *O.ulmi* to *O. novo-ulmi* has occurred during the latter's migration.

Introgression of O. ulmi *DNA, including a pathogenicity gene*

An early clue that gene flow was occurring between *O. ulmi* and *O. novo-ulmi* came from RFLP-based DNA fingerprinting studies. In a search for cloned DNA fragments that unambiguously discriminated *O. ulmi* isolates from *O. novo-ulmi* isolates, some EAN *O. novo-ulmi* isolates were found to exhibit rare *O. ulmi*-like DNA polymorphisms, suggesting that they had acquired *O. ulmi* genes via introgression (Figure 4). The DNA of 50 EAN isolates from across Europe was therefore digested with two restriction enzymes and probed with a range of cloned DNA fragments that discriminated *O. ulmi* from *O. novo-ulmi*. Fifteen EAN isolates were found to carry

O. ulmi-like polymorphisms (Bates 1990). These isolates were concentrated around the Romania-Black Sea area (Figure 5), suggesting that the introgressed *O. ulmi* genes were being lost or 'discarded' as EAN *O. novo-ulmi* migrated away from its initial centre of appearance.

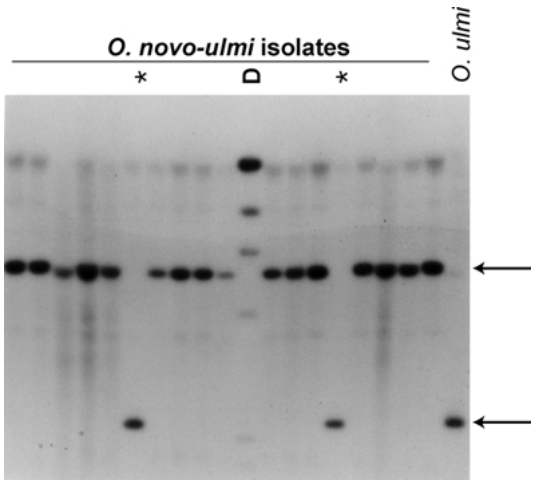

Figure 4. Presence of *O. ulmi* DNA in *O. novo-ulmi* subsp. *novo-ulmi* isolates. Nuclear DNA of 15 *O. novo-ulmi* and one *O. ulmi* isolate probed with a DNA clone from *O. ulmi*. Arrows show the presence of *O. ulmi*-like bands in two *O. novo-ulmi* isolates. D, DNA ladder. From Bates (1990).

Of the 15 EAN isolates with *O. ulmi*-like DNA, only one exhibited a non-*O. novo-ulmi*-like array of phenotypes. This isolate also had an unusually low level of pathogenic aggressiveness on elm. In a subsequent cross, the isolate's low aggressiveness was shown to be controlled by a single gene. An analysis based on cosegregation of AFLP markers has now shown that the gene involved is an *O. ulmi* pathogenicity gene that has been acquired by *O. novo-ulmi* via interspecific gene transfer (Abdelali et al. 1999).

Rapid changes in O. novo-ulmi *population structure*

Another clue to the occurrence of gene flow between *O. ulmi* and *O. novo-ulmi* has come from ultra-rapid changes in the genetic structure of local *O. novo-ulmi* populations. These changes involve, in particular, a sudden increase in the frequency of so-called 'vegetative incompatibility' (vc) types. Fungal vegetative incompatibility systems are analogous to tissue incompatibility systems in animals. They are controlled by multiple genes with multiple alleles so, potentially, many different 'vc types' can exist in a population. One function of vc systems is probably to restrict the spread of deleterious intra-hyphally transmitted viruses (Caten 1972) (Figure 6). If adjacent

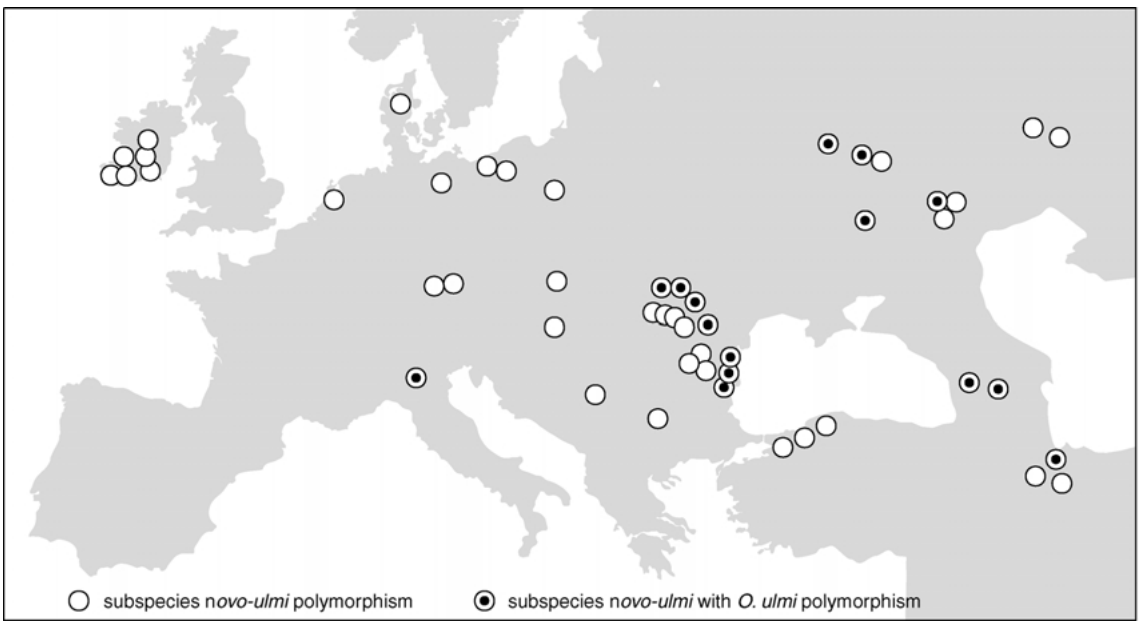

Figure 5. Sample positions of 50 *O. novo-ulmi* subsp. *novo-ulmi* isolates from across Europe and Central Asia, showing the distribution of isolates exhibiting *O. ulmi*-like DNA polymorphisms. M. Bates, K.W. Buck and C.M. Brasier, unpublished. Compiled from Bates (1990).

colonies of *O. novo-ulmi* are of a different vc type, i.e. vegetatively incompatible, viruses cannot pass readily from one colony to the other via hyphal fusions because the fusion cells die. However, when adjacent colonies are of the same vc type, i.e. vegetatively compatible, viruses can spread readily between them because the fusion cells are functional (Brasier 1986). The more vc types in a population, therefore, the more the spread of viruses should be restricted.

Each time *O. novo-ulmi* has arrived at a 'new' location in Europe it has usually spread as a clone of a single vc type. These clones are also of a uniform colony morphology, a uniform background genotype (Brasier and Kirk 2000) and of a single sexual mating type (Brasier 1988). Deleterious viruses (known as d-factors, Figure 6) tend to spread abundantly in the expanding vc clones. However, within only a few years, the clonal population diversifies into numerous new vc types (Figure 7a). This change is accompanied by a sudden increase in diversity in colony patterns, in the background genotype (M. Paoletti, personal communication), and by the appearance of the 'other' sexual mating type. As the new vc types appear, the frequency of deleterious viruses in the population falls rapidly.

Comparable studies on *O. novo-ulmi* populations migrating across North America show that a similar change from a clonal population to multiple vc types has occurred, but that the rate of change has been much slower and so far only partial (Brasier and Kirk 2000; Figure 7b). In addition, virus pressure on the clones in North America has remained low (Brasier 1996; Milgroom and Brasier 1997). In New Zealand (where *O. novo-ulmi* arrived in the late 1980s), an immigrant vc clone has continued to persist, apparently unchanged, for over a decade, and no viruses have been detected in the clone (Figure 7c) (Brasier and Gadgil 1992; and C.M. Brasier, unpublished). Moreover, in the New Zealand case, *O. ulmi* was not present prior to the arrival of *O. novo-ulmi*.

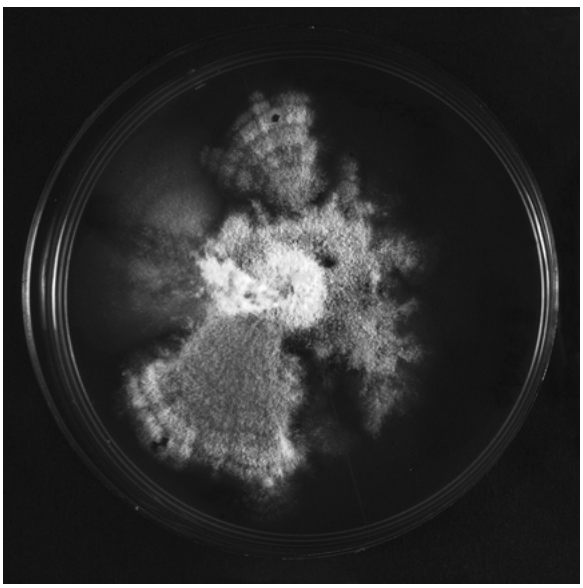

Figure 6. A virus (d-infected) culture of *O. novo-ulmi*, showing a typical irregular, unstable growth pattern associated with severe virus infection.

Two inferences may be drawn from the different outcomes in the European, American and New Zealand situations. First, *O. novo-ulmi* vc clones tend to diversify into new vc types only where *O. ulmi* was originally present, as in Europe and North America. Second, only where virus activity is widespread in the vc clones, as in Europe, do the clones diversify both rapidly and extensively. Those inferences suggest, in turn, that the novel *vc* genes are acquired by *O. novo-ulmi* from *O. ulmi*; and that the selection pressure exerted by the viruses favours the survival of novel vc types over the original vc clones. Results of a molecular study initiated to test the hypothesis that the novel *vc* genes come from *O. ulmi* are consistent with the hypothesis. Segments of *O. ulmi* DNA have been found flanking the novel *vc* genes in *O. novo-ulmi*. The results also indicate that the 'missing' mating type gene is acquired by the *O. novo-ulmi* vc clones from *O. ulmi* (M. Paoletti, K.W. Buck and C.M. Brasier, unpublished).

Transfer of viruses from O. ulmi *to* O. novo-ulmi*?*

There exists a further, equally biologically significant possibility: that the deleterious viruses that appear and spread in the *O. novo-ulmi* vc clones at epidemic fronts are also acquired from *O. ulmi*. A preliminary comparison of viruses in *O. ulmi* and *O. novo-ulmi* isolates obtained from the same epidemic front site in Europe indicates very close similarity in their RNA sequences (L. Crawford, K.W. Buck and C.M. Brasier, unpublished).

Together, these possibilities suggest that a remarkable series of events has occurred. *O. novo-ulmi* has competitively eliminated *O. ulmi* across much of the northern hemisphere, causing *O. ulmi* to become extinct. At the same time, *O. novo-ulmi* may have 'caught' debilitating virus infections from *O. ulmi*. These viruses could have brought about its demise, but for the fact that it simultaneously acquired 'useful'

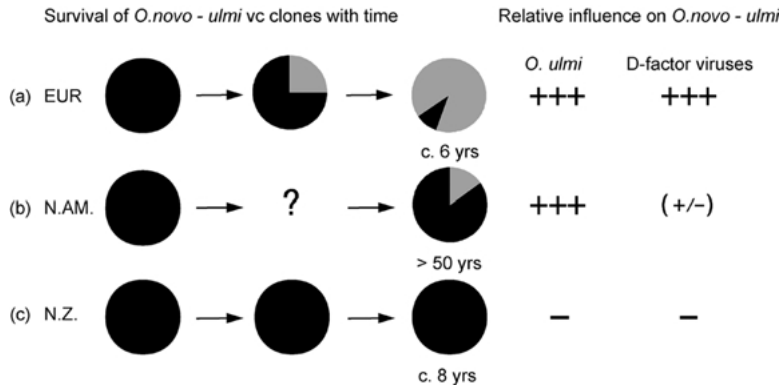

Figure 7. Changes in the structure of the *O. novo-ulmi* population from dominant single vc type clone(s) (black) to many new vc types (gray) since the beginning of the current DED epidemic. (a) Europe. (b) North America. (c) New Zealand. The level of involvement of two other variables in the survival of the clone is indicated: the presence (or absence) of *O. ulmi* before the arrival of *O. novo-ulmi*, and the level of virus infection in the dominant vc clone. +++, strong influence; +/−, weak or no influence; −, absent.

vc genes from *O. ulmi* that allowed it to 'escape' the consequences of the viruses. It also seems that 'unuseful' genes, such as *O. ulmi* pathogenicity genes, may have been acquired by *O. novo-ulmi*, but these genes appear to be eliminated by selection (Abdelali et al. 1999).

Yet to be tested is another fascinating possibility concerning *O. ulmi* and horizontal gene transfer. *O. quercus* (previously called the hardwood biological species group, or OPH group, of *O. piceae*) is a saprotrophic fungus believed to have been the resident associate of the elm bark beetles prior to the arrival of *O. ulmi* (Brasier 1990). When *O. ulmi* originally spread across Europe (Figure 4a) there is evidence that, like *O. novo-ulmi*, it probably did so as a single vc clone, which also subsequently became highly variable in vc types (Mitchell and Brasier 1994). *O. ulmi* may therefore have acquired its novel *vc* genes, and perhaps also its viruses (which were abundant in both European and North American *O. ulmi* populations) from *O. quercus*.

Unrestricted subsp. *novo-ulmi* × subsp. *americana* hybridization: emergence of a new form of *O. novo-ulmi*?

Unlike *O. ulmi* and *O. novo-ulmi*, the two *O. novo-ulmi* subspecies are hybridizing freely in the laboratory and in nature. Locations in Europe where the subspecies are known to overlap are shown in Figure 8. A preliminary study at two such locations, Limburg, in the Netherlands, and Orvieto, in Italy, has shown that hybrids with phenotypes intermediate between the two subspecies – and with recombinant vc and molecular (RAPD) genotypes – but no less aggressive to elm, are emerging and replacing the original 'pure' subspecies (C.M. Brasier and S.A. Kirk, unpublished). In the future, therefore, swarms of subsp. *novo-ulmi* × *americana* hybrids are likely to emerge at the overlap sites (Figure 8). Initially, the hybrids are likely to intercross with other hybrids and to backcross with the surviving 'pure' subspecies.

From such a swarm of recombinants, natural selection may in future favour a particular set of genotypes that will be of neither subspecies, but a new set of genotypes of the pathogen, effectively a new subspecies. *O. novo-ulmi* is therefore undergoing another phase of evolutionary development in Europe. Outside Europe – in North America and in Central and Southwest Asia, for example (Figure 1b) – the 'pure' subspecies may remain geographically isolated from these events for a while, and so survive into the future. Gradually, however, they are likely to be overtaken by hybrid genotypes as these migrate into the outlying areas or are introduced into them. The present situation in Europe therefore provides a unique opportunity to investigate the evolutionary development of a plant pathogen undergoing a hybridization process.

Potential for rapid evolution of introduced fungal pathogens

This glimpse of the migratory history of *O. novo-ulmi* from the 1940s to the 1990s demonstrates clearly how

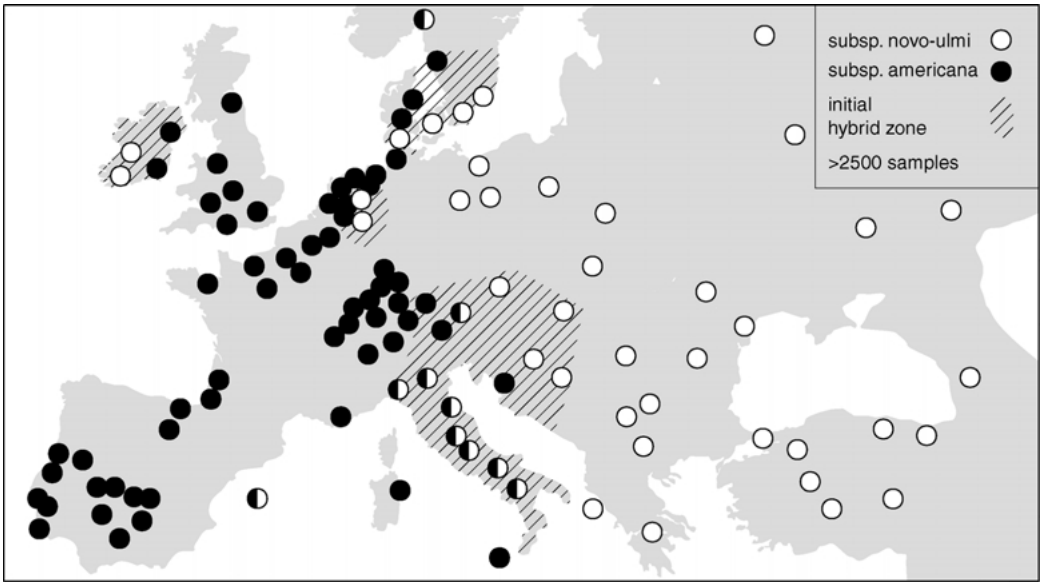

Figure 8. Distribution of the two of *O. novo-ulmi* subspecies across Europe in 1990 (representative sample points only). Locations where subsp. *novo-ulmi* and *americana* overlap occur in Ireland, Netherlands, Scandinavia, Germany and Italy. Hatched areas, known and probable zones of emergence of subsp. *novo-ulmi* × subsp. *americana* hybrids. Based on >2500 samples.

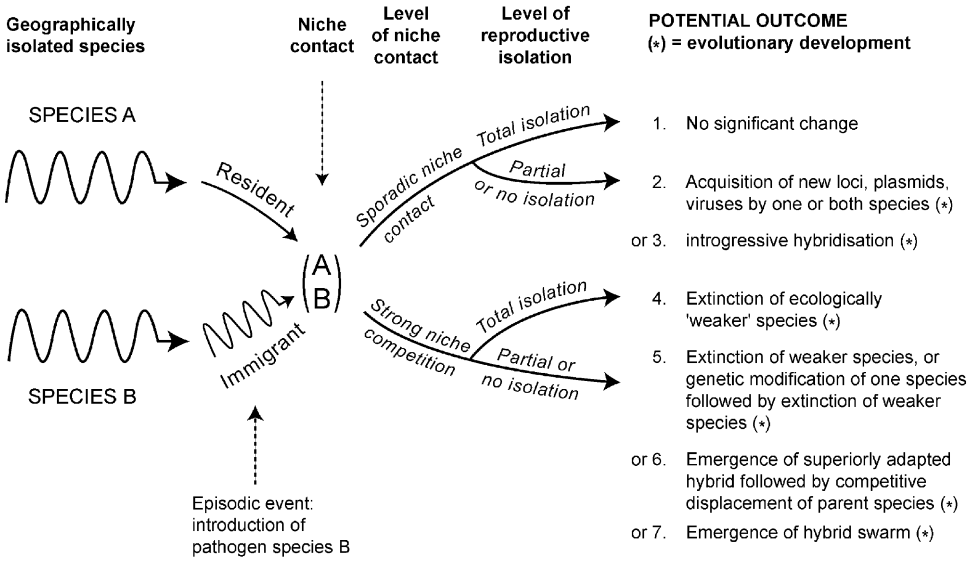

Figure 9. Potential evolutionary outcomes when closely related but previously geographically isolated pathogen species come into contact. The outcome will depend upon many factors, including the frequency of niche contact; the nature of any genetic barriers to hybridization; the degree to which the genomes of the two species can recombine and the ability of any resulting hybrids to compete with the 'parent' species. Redrawn from Brasier (1995).

sudden 'removal' of a fungal pathogen from the routine selection constraints of its endemic environment, i.e. its exposure to episodic selection, can present it with new evolutionary opportunities. It also shows that the traditional focus of concern about the risk posed by introduced exotic plant pathogens – namely, disease impact – must be extended to include the risk of accelerated pathogen evolution and the emergence of new or altered pathogens. Gene flow between fungal species is, however, a neglected area of mycology and plant

pathology. Reports of species hybrids in fungi are surprisingly rare, with only about six clear examples prior to 1990 (Burnett 1983; Brasier 1995).

The potential for hybridization is probably greatest when related but previously geographically isolated fungal species, lacking strong barriers to hybridization, came into contact in the same niche either through migration or through geographic transposition by man (Brasier 1995, 2000). Among the main variables involved are the level of niche contact and the degree of reproductive isolation between the immigrant and resident species (Figure 9); and the potential for survival of any resulting hybrids or introgressants e.g. whether or not any hybrids exhibit greater fitness than the 'parent' species; and whether new substrates or hosts are available for their exploitation. The possible evolutionary outcomes range from relatively small (if highly significant) genetic modifications, such as the acquisition by one 'parent' species of a virus or of a single gene for a new host specificity; to the emergence of an entirely new fungal taxon combining the genomes of both parents (Figure 9). Recent events with the Dutch elm disease pathogens encompass several of these outcomes.

Further new examples of hybridization between pathogens

Recently, several additional new examples of pathogen hybrids have come to light, all involving important plant pathogens. In the Netherlands, a new *Phytophthora* pathogen on *Primula* and *Spathyphllum* has been shown to be a hybrid between *P. cactorum*, which is probably an endemic resident in the Netherlands, and *P. nicotianae*, an introduced species (Man in 't Veldt et al. 1998). Also in Europe, a new, aggressive *Phytophthora* pathogen of alder has been found spreading along river systems and in crop shelterbelts in several countries. This appears to be a swarm of recent allopolyploid interspecific hybrids between the introduced *P. cambivora*, which is a well-known pathogen of hardwood trees but not a pathogen of *Alnus*; and another *Phytophthora* species close to *P. fragariae*, is a pathogen of raspberry and strawberry (Brasier et al. 1999). In this case, therefore, the hybrids appear to have acquired the ability to attack a new host – a possibility already demonstrated with laboratory-generated *Phytophthora* hybrids (Ersek et al. 1995). In nature, hybridization between *Phytophthora* species may have a higher potential frequency because the asexual zoospores can fuse like protoplasts. It may also tend to be promoted in hydroponics facilities or in intensive horticultural nurseries (Brasier 2001).

In North America and New Zealand, swarms of newly evolved interspecific hybrids have been found between different introduced and resident *Melampsora* rust species on poplar trees (Spiers and Hopcroft 1994; Newcombe et al. 2000; Frey et al. 1999). In America, the hybrids are attacking previously rust-resistant commercial poplar clones. There is now a risk that the hybrids may allow transfer of novel pathogenicity genes into the resident *M. occidentalis* population (Newcombe et al. 2000). In the forests of Northeastern California, hybrids have been identified between the host-specialized 'S' and 'P' species of the conifer root pathogen *Heterobasidion* (Garbelotto et al. 1996). In this case the emergence of the hybrids may be associated with human disturbance events such as fire suppression (M. Garbelotto, personal communication).

These latest reports, and the continuing evolution of *O. novo-ulmi*, indicate that many cases of emerging hybrid pathogens remain to be discovered. They raise a range of additional evolutionary and plant health issues, such as the role of man's activities in promoting hybridization, the risk of evolution of 'super pathogens', and the problem of detecting and defining hybrids effectively for quarantine legislation. Many of these issues are discussed elsewhere (Brasier 1995, 2000, 2001). Most of them are illustrated by the history of the Dutch elm disease pathogens.

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