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Virulence and transmission of infectious diseases in humans and insects: evolutionary and demographic patterns

Judith H. Myers and Lorne E. Rothman

The rate and degree of proliferation of disease organisms determine their pathogenicity and the efficiency of their transmission. These traits dictate the impact of a disease on individuals as well as populations. Virulence and transmission of diseases are molded by evolutionary forces – pathogens and hosts are each selected to reproduce and persist. New ideas about the evolution of human diseases also apply to the relationships between insects and their diseases. Evidence for close associations between insects and pathogens include the viral suppression of insect molting hormones and the occurrence of latent virus that can be activated by foreign viruses.

Judith Myers and Lorne Rothman are at the Depts of Zoology and Plant Science, Centre for Biodiversity Research, University of British Columbia, Vancouver, Canada V6T 1Z4.

In his recent book, *Evolution of Infectious Diseases*, Paul Ewald[1] explores the evolutionary relationships between virulence, transmission and persistence of disease. While Ewald primarily discusses the evolution of human disease, his interpretations also apply to the selective pressures molding the interactions between insects and their diseases. Insects provide useful models and experimental systems for evaluating the evolution of disease.

The interactions between insect diseases and population dynamics have been explored in theoretical and field studies[2,3]. Insect pathogens – bacteria, viruses, protozoans and fungi – can be categorized by their virulence and modes of transmission (Fig. 1). Highly pathogenic organisms, such as the nuclear polyhedral viruses of caterpillars and some bacterial and fungal diseases (Fig. 2), kill their hosts and are spread by the release of environmentally resistant, infectious particles. Epizootics can destabilize host populations. On the other hand, many of the protozoans and some viruses cause only benign disease, and these affect populations by reducing the vigor of infected hosts. Benign disease organisms can be transmitted efficiently between generations without having devastating influences on the populations. Recent studies shed light on how diseases are spread and maintained in wild populations of insects, and how they influence population dynamics. For both insect and human diseases, the transmission process is crucial to the dynamics and evolution of host–disease interactions.

Disease transmission

One way that disease is spread is through contact with bodily products, not entailing the death of the infected host. A human example is the common cold, which can be spread by a sneeze. An equivalent for insects is spread of disease through spores or polyhedra in fecal material or regurgitate. That 'spitting' can be detrimental to the health of caterpillars was first shown for pine loopers (*Bupalus piniarius*) by Gruys[4]. Interacting individuals regurgitate, and contact between individuals results in smaller adults with reduced fecundity and dispersal ability. This study did not determine whether the caterpillar 'spit' contained disease, but it is likely, since pine loopers are often infected by cytoplasmic polyhedral virus (CPV). These RNA viruses replicate in the cytoplasm of epithelial cells lining the midgut. Because new cells continue to differentiate, CPV reduces the efficiency of digestion, but infected caterpillars only die if infected very early or if they are cross infected with other pathogens, such as bacteria[5]. Polyhedra are released from the gut with feces, and other individuals can become infected by eating contaminated leaves. High-density and frequent interactions among individuals can thus spread sublethal disease organisms that reduce size, fecundity and possibly the dispersal of moths in the next generation.

Diseases are also transmitted by vectors or as persistent infective stages in the environment, and these diseases tend to be more pathogenic. Ewald[1] points out that humans are more likely to die of malaria transmitted by a mosquito bite

than of a cold transmitted by a sneeze. If a vector transmits the disease, movement of the initially infected host is not necessary – the mosquito does the work. Thus, the disease can 'afford' to be virulent, replicate rapidly and kill the host. Waterborne diseases of humans are also virulent, with the infective stage being long lived in the aquatic environment[1]. In this instance, water acts as the vector. Ewald predicts that the efficient transmission of disease by vectors or in the environment allows or depends on greater virulence.

Of the organisms that cause insect diseases – baculoviruses [nuclear polyhedral viruses (NPV) and granulosis virus], fungi and some bacteria – all have long-lived infective stages, for example, spores or polyhedra, which are released into the environment when the hosts die (Figs 1 and 2). The particles may be transported to new locations by benign vectors, such as flying wasp and fly parasitoids, walking beetles or in bird droppings, but transmission largely depends on the persistence and abundance of infective stages. With this 'sit-and-wait' strategy, the longer the infective stage lasts in the environment, the more likely it is to be ingested by a susceptible host. Since transmission requires the release of large numbers of particles into the environment, total conversion of host into pathogen improves the chances of transmission. This means death for the host.

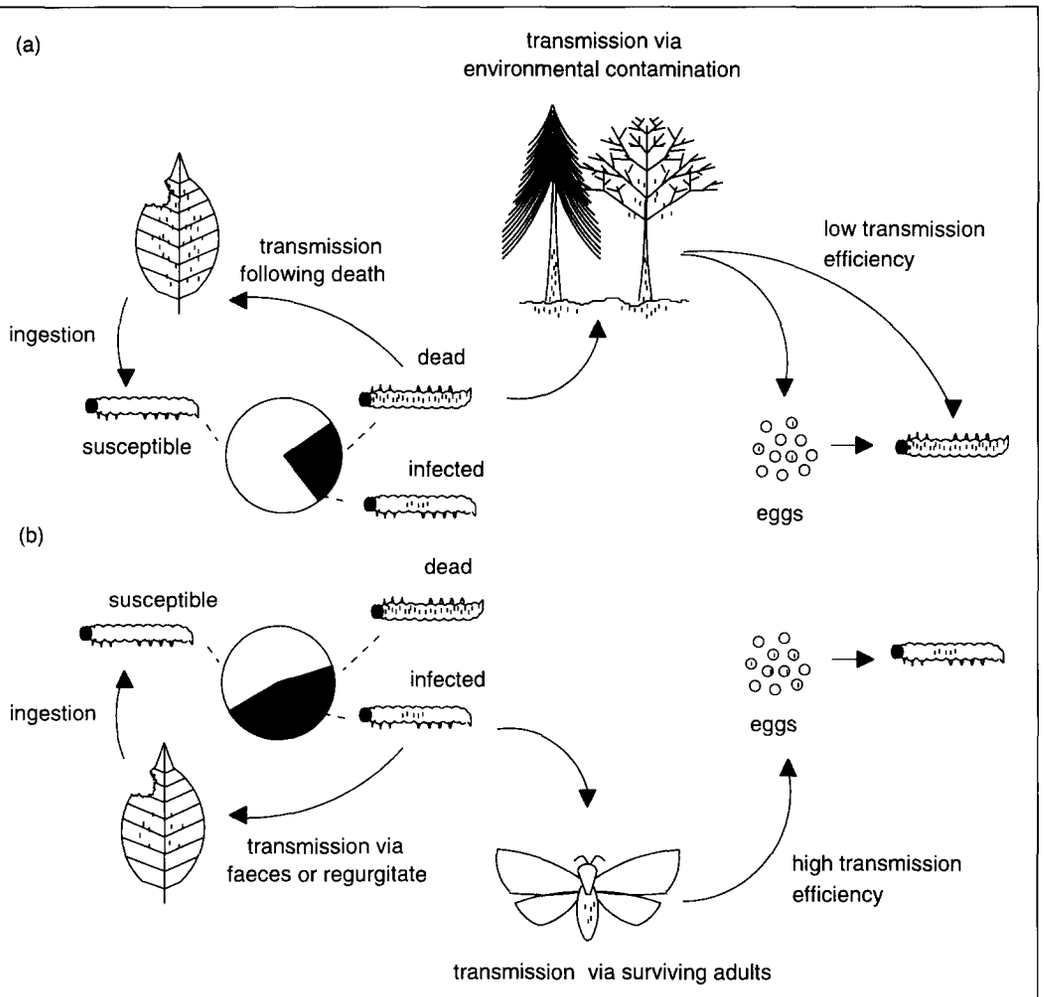

Fig. 1. Microorganisms that cause infectious disease of insects can be classified as being highly virulent with primarily horizontal transmission (NPV, fungi, bacteria, some protozoans) or less-virulent with both horizontal and vertical transmission (CPV, most protozoans, small RNA viruses). Sublethal effects are more likely with benign diseases, but can also occur under some conditions with virulent disease and potentially influence insect population dynamics. High virulence (a) is associated with low prevalence (except at high host density), high probability of death, and sublethal effects are not expected. Low virulence (b) is associated with high prevalence, low probability of death, and sublethal effects are expected.

Host density and virulence of disease

Certain conditions may modify the virulence of disease or the susceptibility of hosts. For example, Ewald[1] discusses the possible impact of wars on the virulence of human disease. Soldiers living in close quarters provide excellent conditions for the spread of airborne diseases, such as influenza viruses, or waterborne diarrheal diseases. Efficient transmission among crowded hosts might explain what appeared to be increasingly pathogenic diarrheal disease during the Civil War in the USA, or the influenza pandemic that followed World War I. Diseases that formerly depended on the movement of infected hosts for transmission to new susceptible victims (nonvirulent diseases) now could be readily transmitted in crowded trenches and hospitals. Rapid transmission may have selected for more-virulent forms of the diseases.

Ewald's ideas on the evolution of virulence of human disease become more ominous when applied to the global increases in human populations and urbanization[6]. In 1900, <15% of the human population lived in cities, but by 2010, urban areas will house half of the world's population. It is predicted that by 2000, there will be 24 mega-cities with populations of >10 million. Most of these will be in the world's poorest countries where development of infrastructures for health care, sewage treatment and water systems are unlikely to keep pace with population increase. In this urbanized environment, diseases will be readily transmitted. Efficient transmission should favor the evolution of more-virulent forms of diseases that have plagued human populations in the past, as well as allowing the establishment of new virulent diseases, which previously could not persist in less-dense populations[6].

How might this model apply to insects during outbreaks, or to gregarious as opposed to solitary insects? For one thing, diseases that are too efficient in their spread among hosts or too virulent might eradicate local populations. In addition, organisms with short generation times such as insects can evolve resistance to disease more rapidly than human populations. Hochberg[7] hypothesized that gregarious caterpillars or those living in confined situations should be more resistant to disease, or their diseases less virulent than those of solitary caterpillars. To test this hypothesis he compared the LD_{50}s (number of polyhedra needed to kill half of the inoculated hosts) of baculoviruses of solitary caterpillar species to those of gregarious species. Gregarious caterpillars do appear to be more resistant to virus than solitary species.

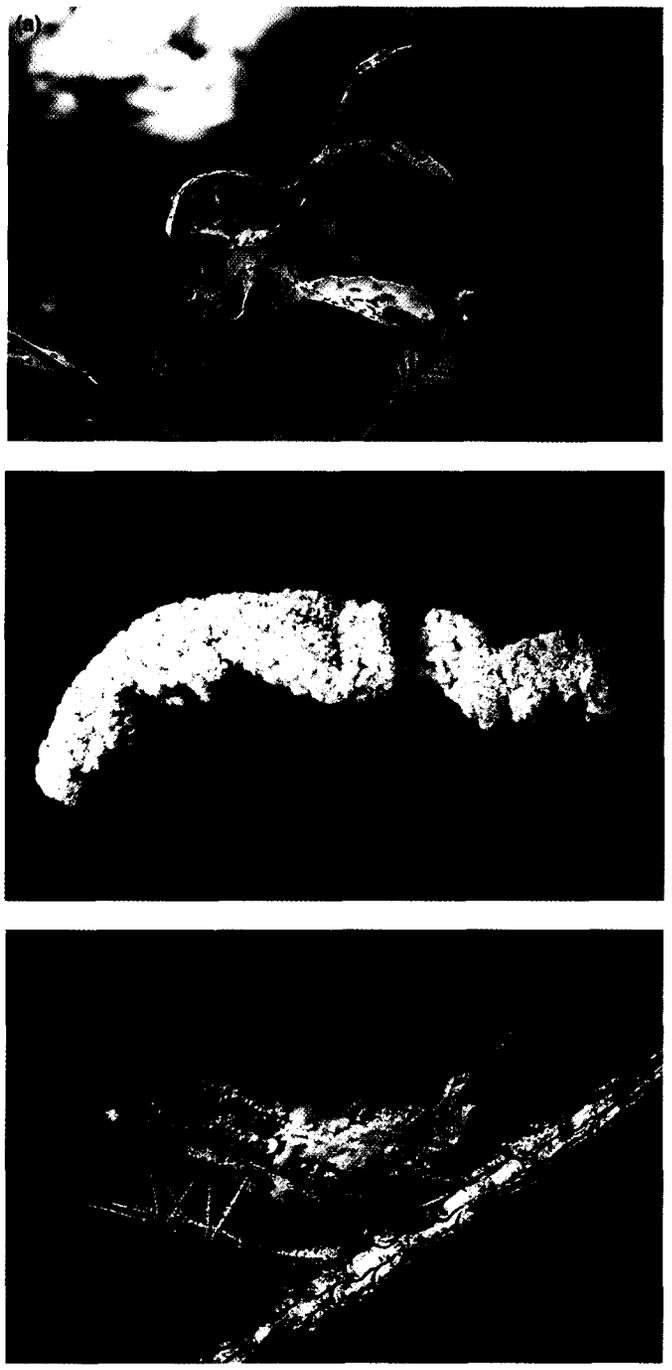

Fig. 2. (a) Deaths from NPV infection occur following outbreaks of tent caterpillars and other forest Lepidoptera. Dead caterpillars are shown here hanging from a tent in the decline phase of a tent caterpillar population. (b) Hemlock looper (*Lambdina fiscellaria fiscellaria*) larva in late stages of infection with the fungus *Entomophthora aulicae. Photograph provided by David Tyrrell, Forestry Canada.* (c) Spruce budworm (*Choristoneura fumiferana*) larva infected with the fungus *Zoophthora radicans*. Note the mycelial coating with conidia on the outside of the larva that will contaminate the environment and spread the disease. *Photograph provided by David Tyrrell, Forestry Canada.*

This result is the opposite of that predicted by Ewald for humans. Crowding was apparently associated with increased virulence of human disease. The discrepancy between these two situations is possibly explained by differences in the initial virulence of the diseases. Close association among hosts leads to increased virulence of more-benign human diseases. But the viral disease commonly associated with peak caterpillar populations is virulent, and depends on the death of the host for transmission. Also, resistance of hosts to viral infection may develop with strong selection at outbreak densities of insects. Changes in the virulence of human disease may be observed in war or with overpopulation, but experimental study of the evolution of disease transmission is more feasible with insects.

Vertical transmission of disease

Transmission of disease between generations – vertical transmission – is also expected to be associated with virulence. Highly pathogenic diseases are unlikely to be vertically transmitted because infected hosts will die before reproduction. An exception to this for humans is the transmission of HIV which causes AIDS. While infected humans eventually die, a period between infection and the development of disease symptoms allows HIV-positive mothers to reproduce. Infected offspring will eventually die of AIDS as well. However, because transmission of HIV to other individuals is through sexual contact or transfer of blood, HIV-positive children are unlikely to spread AIDS further. For insects, protozoan parasites and cytoplasmic polyhedral viruses often act sublethally, and can reduce offspring quality through vertical transmission within eggs or on the surface of eggs. Since they do not routinely kill their offspring, both the disease and hosts persist.

Although vertical transmission is not expected for virulent diseases, Fuxa and Richter[8] and Fuxa *et al.*[9] have documented vertical transmission of a normally virulent NPV in the army worm (*Spodoptera frugiperda*). The rate of vertical transmission in these caterpillars depended on the larval stage at which the parents were exposed to the virus; later instars were more likely to survive to adults and transmit the virus. Fuxa and Richter[8] were even able to select for increased vertical transmission. These exciting results show that vertical transmission with a genetic basis can occur even in a normally virulent insect pathogen.

Few data exist on the prevalence and virulence of diseases in field populations of Lepidoptera. Benign diseases that have vertical transmission might be expected to be more widespread than virulent diseases. But contrary to this prediction, in one study of European gypsy moth (*Lymantria dispar*), a microsporidian disease and NPV were very rare in low-density hosts[10]. The study of persistence and interactions between diseases in natural populations of forest Lepidoptera offers exciting possibilities for future research. These studies are being facilitated by dot-blot and PCR techniques for more-efficient identification of infected individuals.

Latent virus

How disease is maintained when hosts are rare will strongly influence the evolution of disease. Virulent diseases which are transmitted by the death of infected hosts and the release of spores or polyhedra into the environment can be easily spread when hosts are numerous. But when hosts are uncommon, virulent diseases are likely to be lost from the system. Thirty years ago, it was widely accepted that baculoviruses were maintained in a latent form in low-density host populations[11]. Recently, the existence of latent virus has been questioned, since environmental contamination can never be totally ruled out as an explanation for the appearance of sudden viral infection. For the first time, Hughes *et al.*[12] have conclusively demonstrated latent baculovirus by amplifying polyhedrin gene sequences in DNA from the moth (*Mamestra brassica*). Baculoviruses have evolved two forms: a virulent form that is transmitted horizontally with the death of infected caterpillars, and a benign, latent form that can be vertically transmitted. Hughes *et al.* confirmed that the latent form of the virus could be

activated by feeding caterpillars with viruses to which they were not otherwise susceptible.

Although NPV are usually quite host specific, activation of latent virus by foreign virus can potentially cause interspecific interactions among Lepidoptera living in the same area, and this could be important in insect control projects using viral insecticides. Understanding how latent virus is activated could provide key information on the process of viral replication, and might explain viral persistence in low-density caterpillar populations.

Sublethal effects of diseases

Although sublethal effects of disease are expected to be associated with less-pathogenic infections, heterogeneity in time of infection, resistance of hosts or disease virulence might lead to sublethal influences of normally lethal disease. For example, baculoviruses, which usually kill infected hosts, may slow development and/or reduce the size and reproduction of lightly infected individuals or individuals infected shortly before pupation. Sublethal effects have been observed in some, but not all, studies of caterpillars surviving exposure to virus[13], and they might be attributable to: (1) the diversion of host energy reserves to support or combat the pathogen[14]; (2) direct infection of host reproductive organs[15]; or (3) hormonal changes caused by the disease organism. An example of the latter occurs with NPV that produce an enzyme that disrupts the activity of the molting hormone of the host. The expression of this viral gene retards the growth and molting of the host caterpillar[16].

Symptoms of sublethal infections are more likely to be observed in insects with higher resistance to normally lethal diseases. The high efficiency of disease transmission in crowded situations may select for resistance[7], and caterpillars living in confined conditions (e.g. stored grain insects) and gregarious caterpillars, such as forest caterpillars, with periodic population outbreaks[17] are likely candidates for sublethal disease.

Indian meal moth (*Plodia interpunctella*) live in stored grain and have a granulosis virus. A laboratory study found that larval development rate, egg production and egg viability were all reduced for offspring of sublethally infected parents[18]. The greatest sublethal influences occurred following infection of late-instar caterpillars. Perhaps most surprising was the observation that control females mated to sublethally infected males still laid fewer and less-viable eggs. How sublethal infection of males reduces the reproductive output of their mates is not understood, but the impact on the next generation could be great. In laboratory cultures of Indian meal moth, dynamics of virus-infected populations differed from virus-free populations in both the period and amplitude of cyclic fluctuations[19].

Sublethal disease is largely unstudied in field populations of insects. However, infection by a neogregarine protozoan (*Mattesia* sp.) reduces the adult lifespan and egg development of spruce needle miners (*Epinotia tedella*) in a delayed density-dependent manner[20]. These shifts in fecundity accompanied by variation in parasitism determine the period of cyclic host dynamics, and are thought by Munster-Swendsen[20] to be particularly important in determining the amplitude of population fluctuations.

Models of sublethal effects of insect disease

Theoretical models can explore the roles of sublethal disease in population dynamics. The joint influences of reduced fecundity from sublethal infection and vertical transmission of NPV were investigated with a simulation model based on the western tent caterpillar (*Malacosoma pluviale californicum*), a species that fluctuates in density with a periodicity of approximately eight to ten years[21]. Cyclic dynamics could be generated with reduced fecundity as a sublethal effect and mortality from virus with additional density-dependent mortality. In fact, in these simulations, reduced fecundity was necessary for population cycles. The simulated change in mortality and fecundity over the cycle was realistic in magnitude, and provided the lag in recovery lasting several generations, which is necessary for cycles with a periodicity of approximately ten generations. The sit-and-wait strategy of diseases and the resulting environmental contamination could contribute to a delay in the recovery of populations after the decline[20]. Such lags are likely to cause 'cycles' in populations of forest Lepidoptera.

A more general model by Ginzberg and Taneyhill[22] demonstrates the potential role of maternal effects on offspring quality and population dynamics of forest Lepidoptera. Their two-dimensional difference-equation model, which includes maternal effects on offspring as a delayed density-dependent process, predicts the periods of oscillation of six species of forest moths better than a delayed logistic model. Sublethal disease could influence insect quality, and with vertical transmission would act as a maternal effect. These models support the suggested association between sublethal infection and population dynamics of forest Lepidoptera through a lag in the recovery of populations after decline.

Applications

Virulence of human and of insect diseases is related to transmission; diseases transmitted by vectors or by environmental contamination of resistant particles appear to be more virulent than those transmitted by bodily products, such as feces or regurgitate for insects, or coughing and sneezing for humans. Pathogenic diseases have received the most attention in studies of population dynamics of insects, but benign or sublethal diseases can also influence the amplitude and periodicity of fluctuations. For insects, benign diseases can be transmitted vertically within or on eggs. Vertical transmission also can occur with the HIV in humans, and although this disease is ultimately lethal, it also has a prolonged benign period. In humans, efficient disease transmission in crowded conditions may select for increased virulence, but in insects, gregarious species may be more resistant to disease or their diseases less virulent.

Practical applications of these relationships might be used in very different ways for the benefit of humans or to combat insects. For humans, it could be beneficial to reduce the virulence of disease by manipulating selection pressures. Ewald suggests that reducing the efficiency of transmission of disease – by mosquito-proofing houses for malaria control, purifying water for control of diarrheal disease or introducing vigorous needle exchange programs for AIDS – could select for reduced virulence of the pathogens. For insect control, greater virulence might be the goal. Microbial insecticides are being modified through genetic engineering, with the goal of killing the insect hosts before they damage valuable plant parts. Genes coding for toxins have been incorporated into viruses to achieve rapid kill[23]. However, to initiate a natural epizootic, diseases that kill the hosts more slowly might be beneficial since they allow the production and release of more-infectious particles into the environment[24]. The cost-effectiveness of microbial insecticides will be maximized by reducing the concentrations required for control. Increasing the persistence of infectious polyhedra or spores in the environment is one way to achieve this goal. Another is to initiate an epizootic that allows replication of the microbe in the field.

For control of human disease through public health programs, and of insect pests with microbial insecticides, the evolutionary interactions between diseases and hosts should be considered. It is more efficient to work with Mother Nature than against her.

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The use of chloroplast DNA polymorphism in studies of gene flow in plants

David E. McCauley

In many species of plants, the dispersal of genes is mediated by the movement of both seeds and pollen. The relative contributions of seed and pollen movement to total gene flow can be difficult to estimate. Chloroplast DNA (cpDNA) may prove useful for resolving this problem. Over the past several years, studies of numerous species of plants have shown that intraspecific variation in cpDNA is often sufficiently abundant to serve as a marker for studies of gene flow. Recent theoretical models have shown that estimates of population structure based on cpDNA polymorphism should be especially sensitive to the impact of seed movement on gene flow, because cpDNA is often maternally inherited.

David McCauley is at the Dept of Biology, Vanderbilt University, Nashville, TN 37235, USA.

Over the past 15 years, information from organelle DNA has proven quite useful in a variety of evolutionary studies. Organelle DNA often displays uniparental inheritance, little or no crossing over, and a rate of evolution different from that seen in nuclear DNA. In animals, mitochondrial DNA (mtDNA) has been used to resolve phylogenetic relationships at a variety of taxonomic levels, analyze patterns of introgression associated with hybrid zones, and has served as a genetic marker in the analysis of intraspecific population structure and gene flow[1]. In plants, information from chloroplast DNA (cpDNA) (and to a lesser extent mtDNA) has become a standard tool in phylogenetic analysis[2] and has also provided considerable insight into the dynamics of hybrid zones[3].

At first glance, cpDNA would seem very useful in population level studies as well. For example, when cpDNA is maternally inherited, it can disperse in seeds but not pollen. In that case, contrasting the spatial distribution of cpDNA polymorphism with that of biparentally inherited mendelian variation might help to evaluate the relative influences of seed and pollen dispersal on total gene flow. In contrast to animal mtDNA, however, cpDNA has been used relatively little by plant population biologists. In general, it is quite highly conserved[4] and the conventional wisdom has been that only rarely does it display the type of intraspecific variation that is useful to population level studies. Recently, however, several authors[5–7] have suggested that intraspecific cpDNA polymorphism is more widespread than was once believed and could, in fact, be useful in the analysis of population level processes in plants. Furthermore, several recent papers[8–10] now provide a theoretical context for such an analysis by considering explicitly how the mode of inheritance and dispersal can result in very different genetic structures for nuclear and chloroplast genes.

Population genetics of organelle DNA

Indirect studies of gene flow focus on how genetic variation is distributed within and among natural populations, as indicated by the distribution of variation in a variety of genetic markers[11]. This partitioning of genetic variance is usually quantified by Wright's[12] F_{st} or Nei's[13] G_{st}; each is a measure of the probability that two genes taken from within a population are identical, relative to the same

