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Genetic Diversity in Host-Parasite Interactions

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1 Introduction

Probably a very small biochemical change will give a host species a substantial degree of resistance to a highly adapted microorganism. This has an important evolutionary effect. It means that it is an advantage to the individual to possess a rare biochemical phenotype. For just because of its rarity it will be resistant to diseases which attack the majority of its fellows. (Haldane 1949)

As is clear from this passage, Haldane recognized that the temporary resistance conferred by rarity could lead to the maintenance of alleles involved in antagonistic coevolution. He then set out to explain the 'surprising diversity revealed by serological tests,' and suggested that some of the proteins uncovered in these tests 'may play a part in disease resistance.'

Our purpose here is to review some of the more important theoretical and empirical advances since Haldane's time. We begin by considering simple genetic models of host-parasite interactions, with a particular interest in whether such interactions are expected to result in (1) stable equilibria for allele frequencies, (2) stable limit cycles of small amplitude, (3) stable limit cycles of large amplitude, or (4) unstable oscillations (see Fig. 1). We suggest that unstable oscillations, as well as stable oscillations having large amplitudes, can lead to the loss of genetic diversity, and discuss ways in which diversity could be protected, or at least re-supplied, if lost.

We then extend the discussion to consider the polygenic basis of resistance, and discuss the molecular evidence for the accrual of resistance genes in vertebrate genomes. It is clear that a profound revolution has taken place in our understanding of the genetic basis of disease resistance. The molecular mechanisms for accelerating the genetic evolution of parasites and for expressing genetic variation during the lifetime of an individual host could not have been anticipated in Haldane's time. We then consider the role that parasitism might play in the maintenance of sexual reproduction and the evolution of recombination rates in host populations. Finally, the evolution of inducible phenotypic responses to parasitism are considered. Because of the primacy of disease resistance in biomedical research, most of the discussion focuses on the vertebrate immune system. Nonetheless, advances in that arena



Figure 1. Phase planes showing the frequency of a host allele against the frequency of a matching parasite allele which result in infection: (A) stable equilibrium, as observed in the model by Mode (1958), and under very strict conditions in the model by Clarke (1976); (B) stable limit cycle having a small 'orbit', as observed under high mutation rates in the two-locus model by Seger (1988); (C) outward spiraling cycles, leading to fixation of alleles in either the host or the parasite, as observed in the one-locus model of Jayakar (1976); and (D) stable limit cycle having a large orbit as observed under low mutation rates in the model by Seger (1988), and under strong fitness effects and no migration in the model by Clarke (1976). The situations for either C or D lead to the loss of diversity either through deterministic (C) or stochastic processes (D).

have provided unexpected insights into the design and operating principles of resistance traits that can be applied more broadly.

2 Instability of host-parasite interactions

One of the first genetic models of antagonistic coevolution was contributed by Mode (1958). In an analysis of single 'gene-for-gene' interactions between flax and a rust described by Flor (1942), Mode found that a stable genetic polymorphism could readily exist.

Mode's model was followed by Jayakar's (1970) model, which made different assumptions concerning the genetic basis of the interaction and reached very different conclusions. Jayakar assumed first that both parasite and host Genetic diversity in host-parasite interactions

were haploid, and that parasites with allele **B** could infect hosts with allele **A**, but not allele **a**. However, parasites with allele **b** could infect both host types equally. Starting with different initial frequencies of **A** and **B**, he found that parasite allele **b** would usually fix before host allele **A** was lost from the population; and that host alleles **A** and **a** would persist in the frequencies they were at when allele **B** was lost from the parasite population.

Jayakar then introduced fitness differences. Hosts with allele a were less fit than hosts with allele A (as if there were a cost to a-bearing hosts for being resistant to B-bearing parasites), and parasites with allele b were less fit than parasites with allele B (as if there were a cost to b-bearing parasites for being infective to both host types). Under these biologically realistic conditions, there were oscillations around an internal equilibrium point. But in plots of successive recursions of host allele frequency against parasite allele frequency, the oscillations were seen to spiral out until one of the alleles fixed (as in Fig. 1C). Hence, in the first indication of genetic cycling, there was a loss of genetic diversity in either the host or the parasite (see Figs 10 and 11 in Jayakar 1970).

Following these models, Clarke (1976) examined two separate single-locus models: one of specific resistance, as in the flax-rust interaction examined by Mode (1958), and one of general resistance, analogous to Jayakar's model of general virulence just discussed. However, whereas Mode (1958) found a stable polymorphism in his model of specific resistance, Clarke reported a stable polymorphism for only a very restricted range of parameter values. Regarding these conditions he stated that (his italics):

These conditions can be specified, but they need not concern us because under all other conditions, ... the system moves into a stable limit cycle which will also maintain joint polymorphisms in hosts and parasites.

It is the cycles that interest us here. Such cycles had been recognized as important in ecological models (May 1972), but they had been under appreciated in theoretical population genetics (Clarke 1976). Clarke then added that when the conditions are set at their most extreme (meaning here that when parasites match their hosts, the host dies; and that when parasites do not match, the parasite dies):

... the system goes into a peculiar sort of cycle, in which the frequencies of the genotypes and phenotypes move round the outside edge of the diagram (plotting host gene frequency against parasite gene frequency). Thus hosts and parasites are alternatively polymorphic, presumably with some waiting at the corners for new mutations to occur.

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Hence, Clarke's analysis of specific resistance renders two key insights first, and most important, is the idea that genetic diversity can be maintained in the absence of stable equilibria; and second is the idea that diversity can be lost when the fitness effects are dire. Bell's (1982) two-locus model of time-lagged, frequency-dependent selection later makes clear a problem that is hinted at in Clarke's analysis of strong fitness effects. The problem in that host-parasite interactions may lead to (1) stable limit cycles of large amplitude, allowing the possibility of stochastic loss of alleles, or (2) large undamped oscillations that will lead to the loss of alleles (Fig. 1C). In general, oscillations of these potentially diversity-purging types are caused by both strong fitness effects and time lags (see Bell's Fig. 2.17), and it is exactly these kinds of effects that favor recombination (Hutson and Law 1981, Bell 1982). May and Anderson (1983) obtained similar results in their models of frequency-dependent selection, but added that making parasite transmission dependent on host density made the fluctuations even more extreme (see Fig. 5 in May and Anderson 1983). Since time lags, strong fitness effects, and density-dependent transmission are expected to be common features of hostparasite interactions, some mechanism is needed to prevent the extinction of rare alleles. In the absence of such a mechanism, it is hard to see how antagonistic coevolution alone could lead to the long-term maintenance of genetic variation. In what follows we discuss some ways that variation could

3 Diversity protection

3.1 Mutation

It is possible that variation could be continuously re-supplied by mutation. Using deterministic computer simulations, Seger (1988) found that high rates of mutation $(m = 10^{-3})$ resulted in oscillations of relatively low amplitude. Reducing the mutation rate, however, increased the amplitude of the oscillations until the orbits became dangerously close to the boundaries (Figs 3–5 in Seger 1988). For reasonable rates of mutation $(m = 10^{-7})$, alleles could be lost by drift, resulting in the kind of mutation-selection oscillation envisioned by Clarke (1976). Seger (1988) also found that intermediate rates of recombination tended to greatly reduce the amplitude of the oscillations, but made no claim that such rates of recombination should be expected to be evolutionarily stable.

Mutation-driven antigenic variation of influenza A virus promotes genetic diversity in the virus population within a cyclic epidemiological pattern. Point mutations alter the amino acids of the virus coat protein called hemagglutinin which allows variant viruses to escape immunological clearance (Wilson and Cox 1990). Mutation rates can be as high as 10^{-3} per nucleotide site per year TO DECLIC CREVED AREY THE DESCRIPTION OF THE OF ALL LINE

(Saitou and Nei 1986) due to the viral RNA polymerase being polymorphic with respect to fidelity of replication (Steinhauer and Holland 1987). Thus, the viral mutation rate can be selected to counteract the development of host resistance.

One mechanism used by long-lived vertebrate hosts to counter rapidly evolving parasites is the mutational fine tuning of antibody specificity. Point mutations are targeted to the nucleotide sequences that code the antigencombining site of antibodies (Wilson and Cox 1990). The process is termed nomatic hypermutation because it occurs throughout the life of an individual host at an accelerated rate, 10^{-3} to 10^{-4} per nucleotide site per 18–24 hr cell cycle (French *et al.* 1989). Empirical and theoretical evidence suggests that this particular mutation rate is matched to the kinetics of lymphocyte proliferation to permit rapid convergence to sequences that code for high affinity antigen-binding sites (Agur *et al.* 1991). These high affinity antibodies can persist for the lifetime of the individual and confer immunological memory.

Mutation of hemagglutinin proteins permits new variants to overcome immunity to prior infections and leads to recurring waves of influenza infection in one host generation. The host response tracks each wave of viral variants by an epigenetic immune response. This scenario resembles the single allele models whereby mutation is sufficient for producing entrained cyclical variation in virulence and resistance traits. In this case, resistance is an acquired trait that persists for the lifetime of an individual and leads to an additional level of cyclical behavior.

Figure 2 shows the prevalence of antibodies against a particular influenza variant that circulated widely between 1947 and 1957. Individuals exposed to this variant retained immunological memory as evidenced by the high prevalence of antibodies in surveys conducted in 1961, 1971 and 1977. This influenza variant was found circulating again in 1977 (Nakajima *et al.* 1978), presumably because individuals born after 1957 provided a suitably large cohort of susceptibles to support endemic transmission. Therefore mutation allows influenza A to evade immune recognition and persist in host populations. In addition, host demographic processes appear to permit persistence on a global scale. Where was the variant virus during this gap in transmission? Perhaps in another part of the world or in an alternate host species, such as aquatic birds (Gorman *et al.* 1992).

3.2 Movement

Another way to protect genetic variation may be through movement between structured populations (Hamilton 1986). Hamilton's idea is that if there are multiple subpopulations, they would be expected to oscillate out of phase with each other. If this is true, as seems reasonable, then migrants for one population are very likely to restore alleles in adjacent populations that have



Figure 2. The prevalence of antibodies directed against a particular influenza A variant (A/FM/1/47) as a function of age in the human population of Sheffield. The three lines represent the age-distributions at three successive sampling times. Notice that all individuals born before or during the period of endemic transmission of the variant have a high probability of retaining antibodies for a long period of time. Individuals born after 1957 failed to develop antibodies as the variant was no longer in circulation in that community (after Stuart-Harris 1981).

been lost, and vice versa. Such replacement, of course, depends on the rate of migration and the likelihood that two populations do not ever become in phase long enough for there to be a simultaneous loss of alleles in both populations. The latter problem is certainly diminished as the number of populations exchanging individuals increases.

The movement of hosts is certainly important in the epidemiology of influenza A virus, where broad host specificity allows long-distance transport by a variety of vertebrate hosts (Hinshaw and Webster 1982). Our current understanding of the epizootiology of wildlife rabies provides another example of how geographic subdivision and movement can maintain genetic diversity.

On a continental scale, host-adapted variants of the rabies virus are geographically localized in North America (Fig. 3). The variants are characterized by a single antigenic profile which predominates in a single host species, with occasional spillover into alternate host species (Smith 1989). Movement of infected individuals between sub-populations permits the virus to invade new populations and leads to a disjunct distribution when observed at one point in time. A rabies variant associated with raccoons (*Procyon lotor*) spread across the state of Florida in a matter of decades then leaped to the middle Atlantic states in the mid 1970s. An antigenic profile typical of fox (*Vulpes* spp.) rabies is found in the northwest part of North America





and also in southern Ontario and Quebec, presumably as a result of an epidemic wave that spread southward across Canada between 1946 and 1962. In striped skunks (*Mephitis mephitis*), two rabies antigenic profiles are found in the center of the continent. One group appears to represent an endemic focus in the upper Mississippi valley. The same profile can be observed in skunk isolates from California. A different antigenic profile can be found in skunks in the lower Mississippi valley and westward through Texas. This distinct variant of skunk rabies is thought to represent the expansion of an epidemic focus centered in Texas. The occurrence of both skunk variants in Missouri and Arkansas in the central Mississippi valley is considered to be the result of the confluence of the two expanding rabies populations.

These disjunct and melding distributions are consistent with Hamilton's

hypothesis (1986) that movement between non-synchronized host-parasite cycles can maintain global genetic diversity. However, contrary to the expected oscillations, antigenic profiles appear to be relatively stable within geographic compartments (Smith and Baer 1988).

On a finer scale, cyclic behavior of rabies prevalence can be observed in an endemic focus of fox rabies in southern Ontario. In the central core, the number of rabies cases shows a periodic pattern (Figure 4a). In areas surrounding the core population, the epidemiological pattern appears irregular (Figure 4b). It is thought that the virus persists in the core population due to recurring invasion from marginal zones (Tinline 1988). At present, it is unclear whether this pattern is due to purely demographic processes (Coyne *et al.* 1989) or whether genetic turnover is occurring in host or parasite populations. The recent application of polymerase chain reaction methodology to genetically characterize rabies isolates has confirmed that syntopic host species share the same rabies variant and that geographic differentiation does occur within compartments (Sacramento *et al.* 1992, Nadin-Davis *et al.* 1993). If this mode of analysis is extended to a longer time frame, then it should be possible to directly test Hamilton's assertion of the importance of population subdivision and movement between compartments.

The interaction between freshwater snails and digenetic trematodes provides another setting where Hamilton's idea can be directly investigated. The larval stages of these helminths commonly sterilize their snail hosts, and therefore impose a strong selective effect. Where it has been investigated, freshwater snails also have highly structured populations (e.g. Mulvey and Vrijenhoek 1982, Phillips and Lambert 1987, review in Jarne and Delay 1991), and the associated trematodes have become adapted to infecting their local snail populations (Lively 1989). This pattern is consistent with movement between isolated host populations serving to foster genetic diversity. What is needed is an estimate of the rate of migration among locally adapted populations. Hamilton's idea would be strengthened by intermediate amounts of migration: enough to restore lost alleles, without homogenizing the locally adapted demes.

4 Complex genetic interactions

All of the models discussed above were concerned with very simple genetic interactions: one locus with two alleles, or two loci, each with two alleles. It seems possible that more complex genetic systems might show less extreme oscillations. For example, Hamilton *et al.* (1990) found that increasing the number of parasite species as well as the number of loci involved in combating these parasites had the effect of reducing the amplitudes for host gene frequencies. In fact, after 7,000 generations in a computer simulation of the model,





Figure 4. The number of cases of fox rabies cases in A) Renfrew County and B) Prescott County, Ontario, Canada. Renfrew County is located in the core of an endemic rabies focus, while Prescott is at the periphery (from Tinline 1988).

the original variation at host resistance loci had not been reduced. However, the parasites were asexual and their variation was continuously supplied by mutation (m = 0.005). Hence it is not clear that the antagonistic coevolution was sufficient to maintain genetic variation in both the host and its parasites.

Cohen and Newman (1989) used a game theoretic approach that disregarded mode of inheritance but only considered the evolutionarily stable strategy for antagonistic relationships. They suggested that continual diversification of tactics was the inevitable outcome of these interactions. This theoretical insight may help explain why gene-for-gene tactics might be overlaid with more complex virulence and resistance traits. Comparative immunological analysis reveals an evolutionary accrual of defense mechanisms. As innovative biochemical and cellular resistance traits evolve they are inte-

grated with rather than supplant ancestral resistance mechanisms (Marchalonis 1977, Cooper 1976, Herzenberg et al. 1992).

Mice selectively bred for resistance against a single nematode species (*Heligmosomoides polygyrus*) showed quantitative inheritance of that trait (Brindley *et al.* 1986). Although there are numerous examples of resistance associated with a single locus, resistance traits are typically polygenic (Wakelin and Blackwell 1988). Given that each individual host harbors a community of viral, prokaryotic, protozoan and metazoan parasites and that resistance to each parasite species may have a polygenic basis, then multilocus models should be more intuitively appealing. Furthermore Hamilton's approach of using multiple parasites portends greater realism of natural systems.

5 Multilocus genetics and meta-populations

Frank (1993) presented a model of antagonistic coevolution that evaluated population dynamics as well as gene-frequency dynamics. In addition (as in the model by Hamilton *et al.* 1990) he also allowed for multiple loci. Finally, Frank's model included the possibility of extinction and recolonization of demes.

Frank's results show that the intrinsic rate of increase for the parasite is the primary determinant of population dynamics in both the host and the parasite. Slow growing parasites lead to endemic disease and relatively stable population sizes. Fast growing parasites, by contrast, lead to epidemics, larger fluctuations in population sizes, and local extinctions. For rapidly growing parasite populations, the probability of extinction was also affected by the movement rate. Low rates of immigration and emigration increased the likelihood of local extinction, especially for the parasite (Frank's Fig. 4), by increasing the waiting times for the introduction of currently advantageous genotypes. Finally, and curiously, the effect of infection on individual hosts did not affect the dynamics.

Similar results were found for the gene-frequency dynamics. Relatively slow intrinsic growth by the parasite resulted in moderate fluctuations in gene frequency, whereas explosively growing parasites led to the loss of diversity during the crashes of local populations. Gene flow among demes tended to stabilize the meta-population. Hence, it would appear that greater complexity of genetic systems is not by itself enough to guarantee the maintenance of resistance and virulence alleles, at least where parasites are capable of rapid population growth.

6 Do parasites 'track' common genotypes?

The entrained cycling of host and parasite genotypes requires that parasites differentially respond to, or track, host genotypes as they become common. However, whereas population cycles are well known (e.g. Hudson and Dobson 1991; also this volume), reciprocal entrainment of genetic cycles is yet to be documented in the field. Nonetheless, recent studies have provided information consistent with the crucial idea that parasites do evolve to infect the most common local genotypes.

Ophiostoma ulmi is the fungal pathogen responsible for Dutch elm disease. Genetic analysis of a recent outbreak of a virulent subgroup indicated that fungal populations at the front of the epidemic were clonal and that genetic diversity increased toward the center of the outbreak. This parasite is itself infected by a virus-like cytoplasmic factor. The prevalence of this factor is greatest in genetically homogenous fungus populations and decreases in more diversified populations (Brasier 1988), which is a pattern consistent with parasite tracking.

Lively et al. (1990) compared the levels of infection by trematode larvae in natural populations of coexisting sexual and parthenogenetic fish (Poeciliopsis spp.). The parthenogens are triploid, and their eggs require activation by sperm to initiate development; hence, the parthenogens become sperm limited as they become common, and they cannot replace their sexual counterparts. The resulting coexistence of sexual and clonal fish means that a single clonal (but highly heterozygous) genotype can become and remain very common. Hence, one would expect to find that the parasites of these fish are better at infecting the most common clone. An analysis of encysted trematode larvae (Uvulifer sp.) was consistent with this basic prediction: clonal fish had more parasites per unit length (and a significantly lower residual variance) than did coexisting outcrossed fish. However, the result did not hold for a highly inbred founder population of sexual fish in another area. In fact, the inbred sexual fish had more parasites per unit length than the clones (perhaps due to the greater homozygosity of the inbred sexual subpopulation). But, two years after sexual fish were reintroduced by Vrijenhoek into the inbred founder population, the trend reversed, and clonal fish had more parasites and a lower variance than sexual fish (see also Vrijenhoek 1993). This rapid change by the parasite is evidence that they can rapidly track the changes in genotype frequency in natural host populations.

Jaenike (1993) recently showed similar tracking of a host by its parasite to an extreme degree. One strain of nematode, which was originally capable of infecting four different species of *Drosophila*, lost the ability to infect one of these species in less than three years. During this time the parasite was only exposed to one of the host species, which it could still infect. Hence, this study and the results of Boots and Begon (see Read *et al.* this volume,

Box 4) provides evidence for the evolution of host specificity, and the genetic trade-offs that are necessary to cause the cycling of genotypes.

Additional indirect evidence of genetic tracking by parasites comes from reciprocal cross-infection experiments. These experiments test the idea that parasites have become locally adapted to their host populations, which is suggestive of tracking. Recent studies of natural trypanosome-insect (Shykoff and Schmid-Hempel 1991), aphid-pea (Via 1991), fungi-plant (Parker 1985), thrips-composite (Karban 1989), trematode-fish (Ballabeni and Ward 1993), trematode-snail (Lively 1989), and nematode-mouse (Quinell *et al.* 1992) as sociations have been consistent with the expectation of local adaptation in structured coevolving populations, and add support for the assertion that negative-frequency dependent selection from parasites maintains genetic diversity. In addition, the concept of host-tracking is critical for the arguments pertaining to the advantages of sexual reproduction in the face of parasite selective pressure.

7 Parasitism and modes of host reproduction

If host-parasite interactions do in fact provide an explanation for the maintenance of genetic diversity, then they might also be a factor in the maintenance of diversity-generating phenomena such as sex and recombination (Hamilton 1975,1980,1982,1990, Levin 1975, Jaenike 1978, Glesener and Tilman 1978. Bremermann 1980, Lloyd 1980, Bell 1982). In its simplest form, the idea here is that if a sexual population is invaded by a rare mutant that reproduces solely by clonal means, it should be able to increase in frequency due to the advantage of not producing sons (Maynard Smith 1971). But as the mutant becomes common, it will also have the most common phenotype at resistance loci; parasites would rapidly counter the resistance trait and disproportionately exploit the common genotype. This kind of parasite-mediated selection against common clones could prevent them from fixing. One of three possibilities then exist: (1) the clone comes into a stable equilibrium with the sexual population (2) the clone is eliminated from the population, which would be expected if the parasite 'overshoots' (due to a time lag) and continues to infect the clone even after it is driven to extreme rarity, or (3) the clone is driven to rarity, but does not go extinct.

7.1 Difficulties for theory

Regarding the last possibility, a clone could be driven to a single individual and still recover (because of its ability to reproduce without a mate). The clone would then be expected to engage in the same initial spread as that initiated by the original ameiotic mutant, resulting in a parasite-mediated oscillation in the frequency of the clone. This kind of result might cause a problem for the parasite theory of sex, because selection would favor the accumulation of clones. The advantage of sex would be eroded in direct proportion to the number of clones with different resistance phenotypes. This is true unless there is some additional mechanism that functions to eliminate clones as fast or faster than they are generated. Mutation accumulation by Muller's ratchet (Muller 1964, Bell 1988, Lynch and Gabriel 1990, Chao 1990) may provide such a mechanism. The idea here is that each time a host clone is driven to rarity (but not extinction) by the parasite, the mutational load in the least-loaded clone should increase. Hence, parasites could prevent the fixation of clones, and Muller's ratchet could function to eliminate them (Lively 1992, Howard and Lively 1994).

Along the same lines, there may be another problem for parasites, at least as they apply to the maintenance of biparental sex in hosts. In contrast to the situation of invasion by a invariant clonal lineage, consider the effect of an allele for selfing. In the absence of fairly severe inbreeding depression, the selfing allele should spread (Lloyd 1979). The problem here is that there is no single genotype associated with uniparental reproduction (as there is for the situation involving obligately apomictic reproduction). Nonetheless, parasite pressure could prevent the allele from fixing, leading to a stable level of mixed reproduction within individuals. Aggregation of sibs (as seems reasonable in plants) combined with density-dependent transmission of disease might be expected to push the equilibrium (or point of attraction) towards more outcrossing. Conversely, the intermixing of families might be expected to push the equilibrium more towards complete selfing.

One final concern for the idea that parasites maintain sex is that, for sex to be favored, the effect of parasites have to be severe (May and Anderson 1983). One possible solution to this problem is truncation selection (Hamilton et al. 1990). The idea is that the most intensely infected individuals will be less able to compete for some limiting and discrete resource, and as a consequence they will be unable to survive. Hence, parasites need not kill or sterilize their host to be effective in maintaining sex. In addition, as they point out, the elimination of individuals under this kind of truncation selection is independent of the intensity of parasitism. Thus some intensely infected individuals could still reproduce, which might act to protect genetic variation. Recent experimental evidence for possible truncation selection in the wild comes from the study of red grouse (Lagopus lagopus) infected by a nematode (Trichostrongylus tenuis). These parasites made the grouse more susceptible to predation; worm burdens for depredated animals were intermediate compared to randomly collected (shot) birds and those killed by the parasite (Hudson et al. 1992, Hudson and Dobson this volume).

7.2 Empirical support

These difficulties aside, the parasite theory is the best supported of the ecological theories for the maintenance of outcrossing. In an elegant experimental study, Antonovics and Ellstrand (1984) tested for frequency-dependent selection, and whether it was generated by density-dependent or densityindependent factors. A density-dependent advantage to being rare would be evidence for the competition-based 'tangled bank' hypothesis (Bell 1982), provided the competitive advantage was not associated with a light parasite load. A density-independent advantage to being rare would be evidence for the parasite hypothesis. By manipulating densities of plant tillers (*Anthoxanthum odoratum*), they found that rare genotypes had an advantage that was independent of density, but the exact mechanism underlying the advantage was not determined. In a later study, an unexpected invasion by aphids suggested that attack by this insect may either directly or indirectly favor rare genotypes, due to the transmission of viruses (Schmitt and Antonovics 1986).

The biogeographic distribution of parthenogenesis has also been found to be consistent with the parasite hypothesis for sex. For example, the dioecious freshwater snail, *Potamopyrgus antipodarum*, contains both biparental and uniparental populations, as well as populations that are mixtures of both sexual and parthenogenetic individuals. Hence, the opportunity exists for rapid replacement of sexual females by parthenogenetic females. The frequency of sexual females in this species was found to be correlated with the prevalence of infection by digenetic trematodes, which suggests that males and crossfertilization have been lost from populations where the parasite is rare or absent, and have persisted where the parasite is common (Lively 1987, 1992). It is not known whether infected snails are more likely to be removed by truncation selection, but because the parasites consume the reproductive organs of infected individuals, the strong fitness effects required by theory (May and Anderson 1983) would seem to be met.

Schrag et al. (1994a) found strikingly similar results in their study of a simultaneously hermaphroditic snail from Nigeria. Two morphs of this snail exist in natural populations: an aphallic morph, which can either self fertilize or cross fertilize its eggs, but cannot donate sperm; and a euphallic morph, which can donate sperm to either aphallic or euphallic individuals. As they point out, the frequency of cross fertilization in the population must be positively related to the proportion of euphallic individuals it contains, which is highly variable (Brown and Wright 1972). In a thorough examination of the biotic and abiotic correlates of euphally in this snail, Schrag et al. found that parasites were the best predictor of the frequency of the euphallic type. Specifically, the most common trematode taxon explained most of the variance in the frequency of euphallics, even after the effects of season and snail

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age were statistically removed. Moreover, euphally is environmentally determined (Schrag and Read 1992), and in those populations that develop the euphallic morph, the induction of this condition seems closely timed so that broods exposed to the highly seasonal parasites are cross fertilized (Schrag *et al.* 1994b).

These correlational studies on snails tend to suggest that parasites influence the level of uniparental reproduction such that, across populations, biparental reproduction is favored where there is a high risk of parasitism, and uniparental reproduction is favored where there is a low risk of parasitism. Within populations, however, the parasite theory predicts that uniparental reproduction should be associated with greater parasite loads. Moritz *et al.* (1991) in a study of parasite (mite) loads of sexual and parthenogenetic geckos found that, where both reproductive modes were represented, parthenogenetic geckos were significantly more likely to be infected, and they had significantly higher mite burdens. Moreover, in the 6 locations they sampled where mites were found on at least 25% of the geckos, parthenogens were 150 times more likely to be infected than sexuals. This result held for multiple clonal types. Hence, as in the study of parasite loads in coexisting sexual and clonal fish (Lively *et al.* 1990) described above, parasites do seem to disproportionately attack clones where both clonal and sexual types coexist.

Similarly, Burt and Bell (1991) compared parasite-induced leaf damage for 106 pairs of vegetatively produced suckers and sexually produced seedlings of American Beech (*Fagus grandifolia*). For trees 0.5 m tall, suckers had significantly (1.7 times) more damage than seedlings. This asymmetry decreased with increasing tree height, until at 2 m there was no difference in the degree of damage between the two types. Hence there was a transient advantage to the sexually produced seedlings.

7.3 Parasitism and recombination

There are viable alternatives to the parasite theory of biparental sex, particularly those that deal with the irreversible accumulation of mutations in obligately uniparental populations (see Kondrashov 1988, Lynch and Gabriel 1990). But, however sex is maintained, parasites could still be the primary force selecting for rates of recombination (Levin 1975). The most striking evidence of this comes from a study of excess chiasma frequency by Burt and Bell (1987a,b). They reasoned that longer lived species should be selected to have higher rates of recombination to partially compensate for the disadvantage of long life in the face of rapidly evolving pathogens. For non-domesticated mammals, they found that a highly significant fraction (75%) of the variance in excess chiasma frequency was explained by age at maturity.

As recombination shuffles genes within the genome between generations, gene re-arrangements perform the same function during the lifetime of an

individual. This process is best understood in the ontogeny of the vertebrate immune system. In fact, the permutational assortment of immunoglobulin genes can be observed twice in the lifetime of a metamorphosing amphibian as it switches from aquatic to terrestrial habitats (Flajnik *et al.* 1987). By analogy to somatic hypermutation, it may be convenient to consider this pro cess as somatic hyperrecombination because it occurs at an accelerated rate during an individual's lifetime. Like somatic hypermutation, it is targeted to specific parts of the genome, namely those that code for the antigen receptors of lymphocytes.

Gene re-arrangements generate approximately 10^8 to 10^{10} specific antigen receptors from a limited number, on the order of 10^3 , germline genes. This array of antigen receptors, one specific type per lymphocyte clone, constitute an individual's primary immune repertoire, which becomes available for clonal expansion and somatic hypermutation following exposure to parasite antigens (Rajewsky *et al.* 1987). It is believed that this primary immune receptor repertoire is sufficient for initially recognizing any biochemical identity posed by parasites (Klein 1990).

Mutation-driven variation of parasite antigens provides only a temporary respite from negative selection by the vertebrate immune system. Typically this window of vulnerability lasts for only several days as accelerated lympho cyte proliferation, mutational refinement and competition for antigen between cellular clones leads to a population of lymphocytes bearing high affinity re ceptors that efficiently seek and destroy invading parasites (Klein 1990). Al though parasites are considered to have rapid generation times permitting rapid genetic evolution, this appears to be countered by somatic recombination and mutation in long-lived vertebrate hosts.

Parasites have also capitalized on gene re-arrangements for the somatic evolution of virulence. Major epidemics of influenza A are associated with reassortment of the 8 RNA molecules that constitutes its genome (Webster *et al.* 1982). In addition to recombination through sexual reproduction, some trypanosomes (e.g. *Trypanosoma brucei*) have an extensive library of genes that code for surface coat proteins that are sequentially expressed via a gene rearrangement mechanism (Barbet and Kamper 1993). Although textbooks depict trypanosome population dynamics within the host as negative-frequency dependent cycles produced by the lag in immune clearance, the true picture is more complex (Barry and Turner 1991). The initial progeny from a clonal infection are less virulent and show pronounced cycles. Successive waves of progeny become more virulent and coexist with other previous progeny leading to non-cyclic chronic infections illustrated in Figure 5. Nonetheless, the behavior of these interactions based on gene re-arrangements superficially resembles the cyclical genetic changes predicted by simple gene-for-gene models.

In vertebrates, the set of germline genes coding for antigen-binding recep-



Figure 5. (A) The number of *Trypanosoma rhodesiense* trypomastigotes in the circulation of infected mice after infection by a single parasite clone (Lou-TAT 1). (B) Designation of daughter clones collected at different time points during the infection. (C) Virulence of the daughter clones as indicated by the survival time of mice of the same strain as the original infection (from Mansfield 1990).

tors genes appears to have diversified over geologic time through gene duplication via unequal crossing-over events (Warr and Dover 1991). In the murine and human genomes, approximately 10³ variable (V) region immunoglobulin genes are available for re-arrangement and incorporation into the primary immune repertoire. These V-region genes can be grouped into a limited number of families based on nucleotide sequence similarity. In a comparative analysis, V-region gene complexity increases with the divergence of each chordate lineage. Elasmobranchs have a limited diversity of V-region genes and are also characterized as having a more primitive genomic architecture (Hinds and Litman 1986). Higher vertebrates, except birds, possess a greater

number of V-region families and a greater number of members per family. The pattern suggests that immunoglobulin gene diversity increases with each derived vertebrate class. Curiously, birds use 25 pseudo-genes as donors of sequence motifs which are expressed in the single functional reading frame via a gene conversion mechanism to generate antibody diversity (McCormack *et al.* 1991).

Within a vertebrate clade, the complexity of V-region families can be dynamic over evolutionary time. In rodents, family complexity can increase in one family while decrease in another family over an interval of 1 to 30 million years (Tutter and Riblet 1988). It remains to be seen whether these changes in genetic diversity correspond to shifts in the immune repertoire and if these shifts are a counterploy to parasite tracking. It would be especially illuminating to evaluate immunoglobulin gene diversity in sexually and parthenogenetically reproducing sister taxa.

These molecular observations provide generality for the role of recombinational processes in mediating antagonistic interactions. As with mutation, current theoretical models use a single recombination rate to express the lability of genetic variation in parasite and host interactions. Our present understanding of the genetic basis of virulence and resistance, especially in vertebrates, shows that these genes are subject to rates and processes that differ from other regions of the genome. Accounting for this intragenomic heterogeneity of rates and incorporating the dynamics of somatic responses provide daunting challenges for future theoretical analysis of genetic coevolution.

8 Evolution of phenotypically plastic responses

The central tenet of Haldane's principle of parasites driving host genetic diversity is the importance of individuality. Modern agriculture and some natural systems discussed above (see Parasite Tracking and Modes of Reproduction) provide examples of how parasites successfully exploit genetically uniform host populations. We have discussed how, in genetic terms, individuality is generated by mutation and sexual reproduction and why parasites may be important in these processes. The interaction between the genome and environment further differentiates individuals within a population. Genotype by environment interactions figure prominently in the development of the resistant phenotype because of the predominance of inducible defenses.

As exposure to natural enemies, or intensity of exposure, becomes unpredictable and the fitness consequences severe, then the benefit of inducible defenses increases (Lloyd 1984, Lively 1986, Harvell 1990, Clark and Harvell 1992). Host-parasite interactions are characterized by features that promote inducible defenses: (1) mutations which produce novel virulence traits appear sporadically; (2) movement of parasite infectious stages or infected individuals may be haphazard; (3) climatic or local environment conditions may favor transmission on an irregular basis; and (4) reservoir or intermediate host population dynamics may alter transmission independent of the focal host. Most importantly, the suite of potential parasite species that can infect a host species is greater than the number of species an individual host encounters during its lifespan. All of these aspects favor the evolution of inducible resistance traits.

Germ-free animals have a paucity of immunocytes compared to healthy animals reared in even the relatively sanitary conditions of the laboratory (Gordon and Pesti 1971). This indicates a significant inductive effect of 'benign' microbial flora on the development of the lymphoid compartment. In chronic parasitic infections, host responses are closely modulated to track the intensity of infection. Demographic models of unicellular parasites and lymphocytes within a single host show that the number of lymphocytes at the onset of infection is an important determinant of the outcome of infection (Schweitzer and Anderson 1992). Yet the vertebrate immune system generates immune effector cells on demand rather than maintaining a large reserve. Taken together, these divergent sources of evidence suggest that inducible resistance traits are costly and are carefully regulated to minimize the physiological cost to the host. Direct evidence for the cost of immune function is scarce but can be seen in the retardation of growth rates of chickens undergoing induced immune and inflammatory responses (Klasing et al. 1987).

The resource-dependent expression of the resistance traits is considered more fully in the chapter by Lloyd (this volume). Here, we wish to point out that the interrelationship between parasitism, resistance and nutritional resources poses serious challenges for future genetic research. The covariances between resource availability and effectiveness of host resistance will confound the relationship between the intensity of parasitism and host fitness. Thus, genetic variation in foraging performance or nutrient acquisition could be mistakenly attributed to the genetic component of parasite resistance. This underscores the difficulty of assessing the role of parasitism in natural wildlife populations as discussed by Gulland (this volume). Furthermore, the nutritional-dependence of resistance will render some questions unanswerable, such as genetic predisposition to geohelminth infections (Woolhouse 1992).

In addition to inducibility, the host immune system has two other critical design features: specificity and memory. As discussed previously (see Recombination), an elaborate genetic mechanism generates a practically limitless set of exquisitely specific antigen-binding sites. The antigen-driven expansion and focusing of this antigen-receptor repertoire is not only a form of

genotype by environment interaction but also has the hallmarks of cognitive process (Cohen 1992). There is positive and negative selection of the receptor repertoire as the lymphocytes 'learn' to discriminate between self tissues and foreign antigens (Boehmer and Kisielow 1990, Goodnow *et al.* 1990). Quantitative information about exposure to parasite antigens forms the basis for short-term immunoregulatory feedback loops (Schweitzer and Anderson 1992). In this way, proximate immune responses are scaled to the dose of the parasite antigen. Long-term retention of antigenic exposure is preserved in the memory cell compartment (Gray 1993). The fact that immune responses display dose-dependency and memory can impart complex dynamics in hostparasite relationships when these factors are taken into account (see chapters by Grenfell *et al.* (this volume)).

A model that incorporates immunological memory as a driving force in parasite evolution has been developed and analyzed by Pease (1978). It is based on the immunoselection of influenza A variants in a host population capable of resisting the previous epidemic variants. It assumes that the parasite's ability to invade the host population is linearly related to the time- (not replication) dependent mutation rate of the parasite genome. A provocative outcome of the analysis is the suggestion that a transmission threshold for the host population does not exist. This is because the virus can persist in small host populations because susceptibles are not introduced by a host birth process but rather by the ability to evade previous immunity by a mutation/selection process of the parasite that can match the host demographic characteristics. The model treats immunological memory and parasite mutation rate as converse yet identical processes, i.e. a higher mutation rate of the parasite implies that immunity is transient rather than phenotypically stable. This appears to be a tenuous assumption as it would allow cycling of parasite genotypes within one host generation. At least for the case of influenza A, immunological memory appears to be long-term (Figure 2).

The benefit of immunological discrimination between self and nonself comes at the cost of molecular mimicry by parasites. By resembling host self antigens, either through conservation or convergence, the parasite can evade immunological detection and destruction (Damian 1987). In a study where 14 human organs were screened with 635 monoclonal antibodies against 11 viruses, cross-reactivity between human host and viral antigens was observed in 3.5% of the combinations (Srinivasappa *et al.* 1986). Thus, all but the most critical functional sites of enzymes and structural proteins would be expected to be polymorphic in host populations in order to prevent parasites from fixing on common self-antigens. The fact that immunological non-responsiveness to self or tolerance is a phenotypically acquired trait assures biochemical individuality. The joint processes of molecular mimicry by parasites, genetic drift and the host's ability to discriminate between self and nonself on an individual basis may be epistemologically sufficient to account for the perological polymorphism that Haldane sought to explain.

The major histocompatibility complex (MHC) is a genetic locus of vertebrates that is remarkable for its extreme polymorphism and its retention of alleles across speciation events (reviewed in Nei and Hughes 1991) Coded within this locus are cell-surface receptors that present non-self antigens to reactive lymphocytes, thereby initiating specific immune responses (Klein 1986). A number of hypotheses have been advanced to explain the extreme degree of polymorphism and all rely, to some extent, on diversification from parasite selection pressure (Potts and Wakeland 1990, Hughes and Nei 1988,1989, Jones *et al.* 1990, Ohta 1991).

Recent evidence suggests that specific alleles are associated with resistance to specific parasites, cerebral malaria from *Plamodium falciparum* for example (Hill *et al.* 1991). The non-equilibrial frequencies of MHC haplotypes in Africans with severe clinical signs of parasite-induced disease and the geographic concordance between haplotypes and endemic malaria serve as evidence. Yet additional processes such as negative frequency-dependent selection cycling or population subdivision and movement need to be invoked to explain the persistence of alleles over time.

It is possible that the MHC is so polymorphic precisely because it is constrained by its functional role. Outside the immune system, biochemical individuality can increase through mutation-selection balance. The process of tolerance induction assures that each individual can recognize self from nonself tissue so that parasites can be recognized and controlled by highly specific immune responses. This discrimination is acquired through a single gateway-the MHC molecule. MHC molecules are constrained in one significant feature. There needs to be a conserved functional site for cell-cell contact and communication during antigen presentation. This crucial role for initiating specific immune responses will constrain variation and make this site predictable and exploitable to parasites. The recent discovery that Theileria parva sporozoites use MHC molecules to gain entry into host cells (Shaw et al. 1991) and the manipulation of immunity via binding of bacterial enterotoxins to a conserved site of the MHC protein (Johnson et al. 1991) are just two examples that illustrate this threat . Thus, the extreme degree of MHC polymorphism may arise from the mutation-selection processes typical of other loci, except that selection is exceptionally strong because of its central role in generating phenotypically plastic immune responses that are otherwise unpredictable and, hence, non-exploitable by parasites.

9 Conclusion

Models of host-parasite coevolution have confirmed Haldane's (1949) insight that the advantage enjoyed by rare alleles can lead to the maintenance of genetic variation. However, at least in models of very simple genetic systems (one or two loci with two alleles), there are several factors that could lead to the fixation of alleles, rather than their protection. These include long time lags, strong fitness effects, and parasite transmission that is determined by host density. More complicated genetic systems (several loci with two or more alleles) may help to reduce the gene frequency 'orbits', but even these could be insufficient for protecting variation when the population dynamics are strongly oscillatory (Frank 1993). This potential for loss of diversity provides a subtle challenge for Haldane's idea. Empirical validation of these theoretical studies is limited. There is evidence of cyclical genetic variation in influenza A epidemiology, but host genetic changes are unknown since immunity depends on epigenetic mechanisms. Trypanosomes and the vertebrate response is also posited to show cyclical genetic interaction, but again within the lifetime of an individual host. Studies based on simple allelic determinants of parasite virulence and host resistance have not examined cyclical dynamics of these genes. Studies that combine a molecular genetic understanding of virulence and resistance with a population genetic analysis are critically needed.

Current theoretical models and existing empirical information challenges (perhaps more stridently) the parasite theory for sexual reproduction. This is because strong negative effects of parasites on their hosts are required if outcrossing is to overcome its reproductive and genetic disadvantages. Yet, these conditions lead to large orbits in gene frequency phase planes. Clearly if diversity is lost, there is no advantage to outcrossing, and uniparental forms of reproduction should replace cross fertilization. Movement between subdivided populations could be a viable mechanism for restoring lost alleles to local populations, and maintaining diversity (Hamilton 1986, Seger 1988, Frank 1991). Studies of local genetic differentiation in natural populations in concert with estimates of gene flow are needed to resolve the question.

Truncation selection as envisioned by Hamilton *et al.* (1990) could be a potent force maintaining variation in the face of large selective differentials and time lagged frequency-dependent selection. It also obviates the need for the parasites themselves to cause host mortality or infertility in order for antagonistic interactions to favor sex. The available evidence for the parasite theory of sex, recombination, and genetic diversity is supportive, but not definitive.

Our current understanding of the genetic processes determining virulence and resistance traits imposes conceptual difficulties for unifying the genetic effects of parasitism into a single framework. Recently documented processes such as gene capture, gene re-arrangements, gene conversion and somatic

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hypermutation confer resistance for hosts and some cases, provide for accelerated evolution of virulence genes of parasites. These discoveries blur the conventional distinction between genotype and phenotype and new models incorporating these biological innovations are needed.

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