Host–symbiont conflict over the mixing of symbiotic lineages

STEVEN A. FRANK

Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92717, U.S.A.

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SUMMARY

Host and symbiont often conflict over patterns of symbiont transmission. Symbionts favour dispersal out of the host to avoid competition with close relatives. Migration leads to competition among different symbiotic lineages, with potentially virulent side-effects on the host. The hosts are favoured to restrict symbiont migration and reduce the virulent tendencies of the symbionts. Reduced mixing of symbionts would, in many cases, lower symbiont virulence and increase the mean fitness of the host population. But a host modifier allele that reduced symbiont mixing increases only when directly associated with reduced virulence. The association between modifiers and reduced virulence depends on the particular details of symbiont biology. The importance of this direct association between modifier and virulence was first noted by Hoekstra (1987) when studying the evolution of uniparental inheritance of cytoplasmic elements. I apply Hoekstra's insight to a wide range of host–symbiont life histories, expanding the scope beyond cytoplasmic inheritance and genomic conflict. My comparison of differing symbiont life histories leads to a careful analysis of the conditions under which hosts are favoured to control mixing of their symbionts.

1. INTRODUCTION

Many organisms harbour permanent symbiotic partners that contribute to metabolism or defence. These symbionts may be transmitted vertically from parent to offspring or horizontally from host to host. Host and symbiont conflict over patterns of symbiont transmission. The conflict arises from three inevitable consequences of natural selection.

First, selection favours some symbionts to disperse out of the vertical host lineage. This horizontal component of transmission arises from the Hamilton & May (1977) effect, which favours individuals to disperse away from close relatives and avoid competition with similar genotypes. Dispersal away from relatives can be favoured even when there is a low probability of successful colonization of new hosts.

Second, dispersal and mixing of symbiotic lineages reduces the relatedness among symbionts within hosts, favouring within-host competition and virulence. A symbiont's fitness can be partitioned into two components, competitive success and transmission relative to neighbours within the host, and overall success of the group of symbionts within the host. As relatedness declines within hosts, a genotype's success depends more on its ability to outcompete its neighbours and less on the overall success of the group (Hamilton 1972; Bremermann & Pickering 1983). Thus declining relatedness favours symbionts to compete more intensely. This competition may have virulent effects on the host and on the overall success of the group of symbionts.

Third, the hosts favour reduced mixing of symbionts, which leads to higher within-host relatedness among symbionts and lower virulence. This idea has been widely discussed in the context of genomic conflict and the evolution of uniparental inheritance of cytoplasmic elements (Eberhard 1980; Cosmides & Tooby 1981; Hurst 1994). When cytoplasmic lineages mix during syngamy, the relatedness among cytoplasmic elements is reduced. If the host can prevent mixing by imposing uniparental inheritance, then relatedness increases within hosts and lower virulence is favoured.

The hosts gain from low mixing and high relatedness of their symbionts. However, Hoekstra (1987) pointed out a complication with the evolution of host control over cytoplasmic mixing. Although reduced mixing would eventually cause symbionts to evolve lower virulence in response to higher relatedness, that evolutionary response would occur over time and would not provide an immediate benefit to an individual host that restricted mixing of its cytoplasmic elements. The benefit of restricted mixing is a delayed benefit to the mean fitness of the host population rather than to an individual host. Thus a host modifier allele that restricted mixing would not necessarily increase in frequency.

Individual hosts that restricted cytoplasmic mixing would gain an immediate advantage if they could avoid harmful parasites that invade during syngamy (Hoekstra 1990; Hastings 1992). Law & Hutson (1992) showed that a host may gain from sterilizing its own cytoplasm during transmission, so that all cytoplasmic elements in the progeny come from the other parent. Restricted cytoplasmic mixing is also advantageous when cytoplasmic elements increase expression of their competitive and virulent traits in direct response to local diversity (Hurst 1990).

I extend this work in two ways. First, I provide a careful analysis of the conditions under which hosts are favoured to control mixing of their symbionts. Second, I apply the theory to a wide range of host–symbiont life histories, expanding the scope beyond cytoplasmic inheritance and genomic conflict. The richly varied natural history of symbiosis provides opportunity to test comparative predictions over a broader range of phenomena.

2. BIOLOGICAL EXAMPLES

The examples in this section illustrate important concepts, set the assumptions and limits of my analysis, and define the wide diversity of problems that fall under the topic of host–symbiont conflict. I develop the theory more carefully in later sections, leading to an outline of assumptions and evolutionary consequences.

Before turning to the examples it is useful to define a few words more precisely. Virulence is reduction in host fitness caused by symbionts, associated with a reduction in the group fitness of the symbionts in a host. Competitiveness of symbionts influences relative success within the host. Increased competitiveness is often associated with increased virulence.

(*a*) *Figs and their pollinator wasps*

The life cycle of figs must be described first before turning to the host–symbiont conflict. A fig is an inflorescence that contains hundreds of tiny flowers within a sealed cavity. The female pollinator wasps arrive at a receptive fig and push their way in through a tiny opening. Once inside, the wasps lay eggs in the ovaries of some of the flowers, they pollinate other flowers with pollen carried from the fig in which they were born, and then they die. After several weeks the male offspring emerge and mate with the female offspring inside the sealed fig. The females then obtain pollen from the fig, fly off to find a new fig and continue the cycle.

The number of female wasps (foundresses) that lay eggs in each fig varies. In one sample 53, 27, 12 and 8 per cent of figs of *Ficus citrifolia* had one, two, three, and four or more foundresses, respectively, with a mean of 1.7 foundresses per fig (Frank 1985).

The sex ratio of the wasps is influenced by the relatedness among a group of foundresses (Hamilton 1967, 1979; Frank 1985; Herre 1985). A single foundress maximizes her number of grandchildren by producing mostly daughters and only enough sons to ensure fertilization. In this case each son competes with his brothers for access to mates. From the mother's point of view, additional sons are reproductively redundant.

The situation changes when there are two foundresses. A son is partly redundant because he competes against brothers one-half of the time. But a son also competes against non-related males one-half of the time, and so is valuable as a competitive agent of the mother. Consequently, the sex ratio, measured as portion of maternal resources allocated to sons, is favoured to increase as the number of foundresses increases and the relatedness among foundresses declines.

Investment of resources to sons is allocation to increased within-group competitiveness. The associated decline in allocation to daughters causes a decrease in group productivity because the total reproductive success of the group depends on the number of daughters that leave the fig to start a new generation. Group productivity, the number of daughters that carry pollen to new figs, is also associated with the dispersal of fig pollen. Reduced group productivity of the wasps therefore causes decreased fig (host) fitness. Male wasps are a competitive, virulent trait favoured to increase in response to declining relatedness.

The host–symbiont conflict over the wasps' sex ratio is clear (Frank 1985). The host trees favour few foundresses per fig, which increases relatedness and favours a rise in allocation to females: the agents that disperse pollen. From the wasps' point of view, there are typically more females searching for figs than there are figs available for colonization. Thus the wasps will tend to increase the number of foundresses per fig unless controlled by the trees.

We can now return to the problem outlined in § 1. If a modifier allele of the host tree restricted the number of foundresses, would that modifier increase in frequency? Suppose the wasp sex ratio were a genetically fixed trait that did not vary in response to local foundress number. The modifier would not increase because it is not directly associated with lower sex ratio and lower virulence. By contrast, suppose that sex ratio were a phenotypically plastic trait, with wasps increasing male production in response to a rise in local foundress number. In this case a host modifier that restricted foundress number would gain an immediate advantage through decreased allocation to male wasps and lower virulence.

Experimental studies on the pollinator wasp *Pegoscapus franki* show that wasps do increase their allocation to males in response to an increasing number of foundresses in their fig (Frank 1983*a*, 1985; for taxonomy, see Wiebes 1995). On the host side, the trees appear to seal off a fig soon after the first foundress wasp has entered (Frank 1983*b*). A virgin fig with no foundresses remains receptive to entering wasps for about ten days. After the first wasp has entered, the passage into the centre of the fig is sealed within 24–48 h. Additional foundresses must pass during this short period before the entry is closed.

(*b*) *General characteristics*

I began with the detailed fig example because it is a peculiar case among the range of intimate symbioses, which typically involve a small microorganism or cytoplasmic element living in a larger host (Buchner 1965; Grun 1976). The fig symbiosis helps to set the range of interactions under consideration and to tie together seemingly disparate fields of study.

The interactions that I focus on have four characteristics. First, the symbionts occur in all hosts because

they are obligate or have a net beneficial effect. Fig trees require wasps to disperse pollen. The relation between tree and wasp is an obligate symbiosis.

Second, the symbionts have competitive traits that can increase their success in reproduction and transmission relative to other symbionts within the host. Increase in allocation to competitive traits reduces the productivity of the group of symbionts and has a negative effect on host fitness (virulence). In fig wasps, males are a competitive, virulent trait.

Third, mixing of symbiotic lineages favours an increase in the competitive and virulent traits of symbionts. In fig wasps, higher foundress number favours increased production of males.

Fourth, hosts often have the potential to control symbiont movement and the mixing of symbiotic lineages. This establishes the potential for host–symbiont conflict.

In fig wasps, the number of foundresses determines the mixing of symbiotic lineages, and the hosts appear to limit the number of foundresses that enter each fig. Some of the luminescent symbioses of fishes and cephalopods have similar properties of symbiont transmission, mixing and host control. The host stories symbionts in special, luminescent organs. Young, uninfected hosts obtain symbionts from the water or substrate. In the squid *Euprymna scolopes* the special epithelial flaps that capture symbionts from the water regress after initial infection, reducing the likelihood of further infection (Douglas 1994). Thus transmission is purely by random association of host and symbiont, and the host appears to limit the number of symbiotic lineages that are allowed to colonize the luminescent organs.

(*c*) *Vertical transmission of symbionts*

Although figs and fishes obtain partners from the environment, many intimate symbioses have elaborate mechanisms of germline infection and vertical transmission (Buchner 1965). It will be useful to have a few examples in mind when I discuss models of host control over symbiont transmission.

Anoplura (sucking lice) have bacterial symbionts that provide essential vitamins not available in the normal diet of vertebrate blood. During larval development the symbionts are stored in an organ near the gut. A variety of mechanisms are used to move some of the symbionts to the ovaries for transmission to the offspring. These transmissible symbionts are stored in a second organ between the ovary and the oviduct. Each egg is infected before it matures.

Many groups lack direct ovariole transmission. In the drug-store beetle *Sitodrepa panicea*, symbiotic yeasts are smeared on the eggs during oviposition. The hatching larva devours the egg shell and ingests many yeast cells. The yeast reproduce rapidly in the gut, but only a few are admitted into the specialized gut organs that house the symbionts during the larval period. The few successful colonists are probably the only yeasts that will survive in the host. Thus only a limited number of yeasts are transmitted to the offspring.

Tsetse flies and other groups of insects bear live young. The larvae hatch within the mother and are fed by special 'milk glands.' These insects frequently transmit symbionts through the milk.

(*d*) *Symbiont dispersal*

Variation in symbiont dispersal and host control must be studied in light of the potential for host–symbiont conflict. From the symbionts' point of view, selection favours some dispersal out of the host and subsequent colonization of new hosts. This arises from the Hamilton & May (1977) effect, which favours individuals to disperse away from close relatives and avoid competition with similar genotypes. Thus some mixing of symbionts may occur even in hosts with elaborate mechanisms of vertical transmission.

For obligate, vertically transmitted symbionts there is little information about occasional transfer among hosts. But there are hints that dispersal sometimes occurs. For example, in the aphid *Schizoneura lanigera*, symbionts are housed in special organs. In older insects the symbionts escape their storage organs, permeate the body cavity, are reduced four-fold in size, and form particularly dense aggregations near the proboscis (Buchner 1965, p. 305). Many vertically transmitted symbionts also occupy the excretory organs (Buchner 1965, chapter 10).

(*e*) *Number of symbionts transferred to offspring*

The tendency for competitiveness and virulence increases as variation among genetic elements increases within a host. This may be the reason that metazoans pass through a single-celled stage in each generation with the formation of a fertilized egg (Maynard Smith 1988). The genetic bottleneck caused by single-celled reproduction means that differences among cells arise from *de novo* mutations during development. The high relatedness among cells promotes cooperation.

A similar problem arises when we consider the number of vertically transmitted symbionts that colonize an offspring. If only one symbiont is transmitted, then variation among symbionts will be low, with all differences arising from *de novo* mutations within the host. In general, the variation among vertically transmitted symbionts depends on the number of colonists in each generation and the mutation rate of the relevant life history characters (Szathmáry & Demeter 1987; Frank 1994*a*).

It is difficult to find data for the number of symbionts transferred. A few anecdotes from Buchner (1965) will at least suggest the kinds of transmission patterns that can occur. In *Pediculus*, a type of sucking louse discussed above, the ovarial storage organs contain 3000–6000 symbionts. Approximately five eggs are produced daily, with 150–250 symbionts transferred to each egg. In the drug-store beetle mentioned above, it appears that relatively small numbers of yeasts colonize each offspring. In general, hosts commonly limit symbiont transmission to a subset of symbiotic lineages. I recently reviewed the observations on this topic and suggested that hosts divide their symbionts into transmissible, germline lineages and non-transmissible, somatic lineages (Frank 1996*a*).

3. HOST CONTROL OF SYMBIONT MIGRATION

The spread of host traits that restrict symbiont mixing depends on the details of symbiont biology. In this section I summarize seven factors that may influence the evolution of host traits. Each factor is expressed in terms of the spread or decline of a rare host modifier allele that influences symbiont mixing. I assume that restriction of symbiont migration requires investment by the host because of the conflicting tendency of the symbionts to favour some dispersal out of a host. Thus the host modifier typically has a cost; a neutral modifier is treated as a limiting case with zero cost. I also assume that mutations of small effect occur in host modifiers and symbiont traits. Potential variation is not a limiting factor in my analysis (see below, Alternative Models).

1. Modifiers fail to control immigration when virulence depends on genetically fixed traits. Hosts gain from limited mixing and low variation among their symbionts. However, a host modifier that restricts symbiont mixing does not necessarily increase in frequency (Hoekstra 1987). Symbionts may have a genetically fixed division of resources between beneficial, metabolic traits and virulent, competitive traits. Restricted immigration would eventually cause the symbiont population to evolve lower average virulence and therefore increase the mean fitness of the host population. A costly modifier that reduces symbiont immigration can only increase by association with relatively less virulent symbionts. Although such associations may occur transiently, there is nothing in this system to maintain associations between hosts that restrict immigration and relatively low virulence of their symbionts.

2. Modifiers fail to control immigration when virulence depends on local variance of symbiont characters. The previous case described host evolution when symbiont virulence depends on genetically fixed traits. Hurst (1990) emphasized that host modifiers could be favoured when the symbionts increase their competitiveness and virulence in response to locally high levels of mixing and genetic variation. My purpose here is to develop this idea in a more precise way, showing the particular properties of symbiont competitiveness that support Hurst's conclusions.

To separate the role of competitiveness from virulence, let us begin with a quantitative trait that influences virulence but not competitiveness. Assume virulence increaes with the standard deviation among the trait values of the symbionts within the host.

Clearly a neutral (cost-free) host modifier can spread because reduction of within-host variation provides immediate benefits to the modifier. The spread of a costly modifier depends on the relative strength of opposing forces. The modifier is favoured when reduced immigration into the host lineage provides a sufficient loss of symbiont variation to offset the cost of the modifier. When total variation in the population is low, then reduced immigration rarely provides sufficient reduction of virulence to outweigh the cost of the modifier. Total variation tends to be low in this model because a uniform set of symbionts within a host yields the highest symbiont fitness. Symbionts that deviate from the average have lower fitness.

A model that invokes conditional virulence in response to local symbiont variation must specify a mechanism that maintains variation in the symbiont population. Because the virulence (group-level) component of symbiont life history imposes stabilizing selection, within-host competition must provide the disruptive or frequency dependent component of symbiont fitness that maintains variation.

3. Modifiers may be favoured to control immigration when symbiont competition increases with local variation. Many microorganisms produce allelopathic compounds that kill competitors (Rice 1984). For example, several medicinal antibiotics are derived from the allelopathic chemicals of fungi. Bacteria often carry plasmids that produce a toxin (bacteriocin) that kills competitors.

There is no direct evidence that symbiont populations within hosts compete by allelopathy. But allelopathy is so widespread that it is useful to consider the consequences of allelopathic competition on the course of symbiotic evolution.

Bacteriocin systems have three important components: toxin, anti-toxin and immunity (Reeves 1972; Hardy 1975). Different plasmids produce different toxins, each with a specific, matching anti-toxin. The anti-toxins work intracellularly, perhaps by cleaving the toxin molecule. In addition, there is often specific immunity to a toxin. This appears to work on the cell surface. To destroy a cell, the toxin enters through a surface receptor, for example, a receptor that transports nutrients. Immunity is obtained by modification of the receptor to prevent entry by the toxin. This sort of attack–defence recognition system often maintains variation by frequency dependent selection (Frank 1994*b*).

A host modifier that restricted immigration would significantly reduce polymorphism in allelopathy among its symbionts. However, the modifier spreads only when a reduction in local variation leads to an immediate decrease in symbiont competition and virulence. Lower virulence of unmixed relative to mixed symbionts could arise in two ways. First, mixtures of incompatible strains may lead to significant mortality among symbionts, lowering the effectiveness of the symbionts in contributing to host success. Second, the symbionts may respond to increased local variation by allocating more resources to competition and fewer resources to beneficial metabolic traits.

4. Modifiers are favoured to control immigration when symbiont competition increases conditionally with a decrease in relatedness. In the previous examples virulence increased with the absolute level of symbiont variation within hosts. Significant amounts of competition and virulence occur only when frequency dependent selection maintains high levels of symbiont polymorphism.

Fig wasps provide an interesting contrast. Wasp competition and virulence increase when genetic relatedness within figs declines. In this case sons are a competitive trait of female wasps; the wasps increase

their proportion of sons in response to the number of competing foundresses within a fig. Relatedness is approximately $1-V_w/V_t$, where V_w and V_t are the genetic variance within hosts and the total variance in the population. Because relatedness depends on the ratio V_w/V_t, the relative level of within-host variance determines virulence rather than the absolute amount of polymorphism. Thus the evolution of host control does not require widespread frequency dependent polymorphism of symbionts when the symbionts adjust their competitiveness and virulence in direct response to local levels of relatedness. Conditional response to relatedness seems more likely in wasps than in symbiotic bacteria, but no study of microorganisms has focused on this problem.

5. Modifiers are favoured to restrict symbiont dispersal associated with harmful movement and reproduction. Selection on the host to restrict harmful movements and growth by symbionts may conflict with selective pressures on the symbionts that favour dispersal. The hosts, although not directly favoured to restrict dispersal, may restrict traits correlated with dispersal. Thus host control of symbiont migration may frequently evolve for reasons unrelated to symbiont competition within hosts. The ultimate reduction in symbiont mixing and the associated decline in virulence is simply a side-effect of other selective pressures.

6. Modifiers may control patterns of offspring infection in vertically transmitted symbionts. Variation among symbionts and within-host competition can occur in vertically transmitted symbionts. The variaiton among symbionts depends on the number of individuals that colonize each egg and on the mutation rate of the relevant life history characters (Szathmáry & Demeter 1987; Frank 1994*a*). Occasional horizontal transmission also increases within-host variation.

Hosts may reduce variation among symbionts by decreasing the number of symbionts that are transferred to each offspring. Host modifiers that influence vertical transmission are affected by the same complications as modifiers of symbiont migration. Put another way, immigration control does not depend on the source of the symbionts. This is a bit surprising at first glance because it would seem that, with purely vertical transmission, host and symbiont interests would coincide. However, key components of virulence do not depend on the mode of symbiont transmission–vertical infection from the parent or horizontal infection from another host – but on the diversity of colonists, the types of within-host competition, and the correlation between competitive traits and virulence (Frank 1996*b*).

7. Sexual and asexual hosts may differ in their tendency to mix their symbiotic lineages. If the host is asexual and the symbiont is purely vertically transmitted, then host and symbiont alleles are locked together. Processes that favour recombination, such as changing environments or escape from mutation accumulation in a partner gene, may favour asexual hosts to 'recombine' their symbiont partners by mixing symbiotic lineages. By contrast, sexual hosts recombine with vertical symbiotic lineages even though the symbionts do not mix. Thus, if there are proceses that favour host–symbiont recombination, those processes will favour asexual hosts to mix their symbiotic lineages but will not favour sexual hosts to mix symbionts. A specific theory is difficult to develop because there is no general understanding of the processes that influence the evolution of recombination. But it may be worthwhile to compare observations between closely related sexual and asexual host species for the rates at which their symbiotic lineages mix.

4. ALTERNATIVE MODELS

I have assumed that mutations of small effect recur, causing traits to vary continuously. Recurrent mutations also cause decay of transient associations (linkage disequilibrium) between host modifiers and symbiont traits. The importance of this assumption can be seen by a contrasting model that emphasizes unique mutations and the long-term importance of linkage disequilibrium.

Suppose, for example, that an asexual population of hosts has symbionts that are transmitted by a mixture of vertical and horizontal transmission. Symbiont traits are genetically determined as in case 1 above. Consider a host mutation that restricts immigration of symbionts and has a cost c. This mutation increases only if it occurs in a host with symbionts less virulent than the population average by an amount sufficient to offset c (Hoekstra 1987, 1990).

Such chance associations could explain how hosts evolve to control immigration when virulence depends on genetically fixed traits, contrary to my case 1 above. But this explanation is fragile because recurrent mutations of varying effect destroy the directional tendency for host traits to evolve toward restricted immigration. For example, suppose the initial host mutation, with cost c, restricts immigration and causes purely vertical transmission. This mutation spreads to fixation if associated with symbionts of sufficiently reduced virulence. With fixation of the host mutant, there is also fixation of reduced symbiont virulence.

This joint state of pure vertical transmission and lowered virulence is not stable. A new host mutation that allowed horizontal transmission, with a cost less than c, spreads because all symbionts are monomorphic for lowered virulence and there is no gain in restricted immigration. If the original cost, c, is zero, then other mutations with $c=0$ but differing levels of mixed horizontal and vertical transmissions are selectively neutral. Thus the level of symbiont mixing drifts. As the symbiont population also accumulates genetic variation for virulence, another round of restricted immigration and linkage disequilibrium can fix vertical transmission. But, again, this state is unstable with respect to new mutations. Thus a complete transition to vertical transmission can only be explained by transient associations if subsequent mutations are not possible.

Models that depend on the uniqueness of mutations are fragile. Obtaining data on rare events is necessarily difficult or impossible, so one cannot rule out the unique-mutations models. But they do not provide

compelling explanations. The conclusions I provided above are based on previous analytical models (e.g. Frank 1994*a*) and new computer simulations. These analyses assume rare but recurrent mutations of varying effect in both host and symbiont.

5. CONCLUSION

Symbiont dispersal and immigration tend to reduce host fitness in several ways but, surprisingly, only special circumstances favour hosts to control symbiont migration. For example, the recognition properties and consequent frequency dependence of allelopathy maintain a special kind of symbiont polymorphism that favours host control over symbiont immigration. In this case a host that restricted the number of colonists would gain direct advantage by reducing the damage that different symbiont strains may cause each other and by reducing any conditional tendency of symbionts to increase their allocation to internal combat. Hosts are also favoured to restrict symbiont dispersal when dispersal has correlated traits that directly harm the host.

If the net effect of the symbionts is harmful, then selection on clearing the symbionts or preventing infection dominates the evolution of host traits. My emphasis here is on symbionts that have net beneficial effects, but also have the potential for variation in their overall contributions to the host. For example, leeches cannot survive on their blood diet without symbionts; clearly the net effect of the symbionts is beneficial. But all of the points above about conflict of host and symbiont over dispersal and immigration apply to the symbionts of leeches. Thus duality of benefit and harm to the host occurs because selection acts independently on uncorrelated traits. For an obligate symbiont, selection can favour the increase of beneficial traits associated with metabolism and defence while simultaneously favouring harmful traits associated with competition and dispersal.

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