1 The evolution of heritable symbionts

John H. Werren and Scott L. O'Neill

1.1 Introduction

Symbiotic microorganisms are extremely widespread in nature, having intimate and often obligatory associations with their `host' species. Despite the near ubiquity of arthropod symbionts, their study has been constrained by their fastidious nature and inability to be cultured out of the arthropod host. Recent advances in molecular biology have provided new tools for symbiosis research and stimulated new investigations of many symbiont systems that were described many years ago but were previously difficult to investigate in detail.

The term 'symbiosis' in its most general (and original) sense refers to the intimate 'living together' of dissimilar organisms (de Bary 1879). Symbiont interactions with hosts have been traditionally classified as mutualistic (beneficial), parasitic (harmful) or commensal (neutral). However, assigning a symbiotic association to these groupings is often problematic. The associations between symbiont and host.are complex and can shift between the different states both over time and depending upon the particular phenotype being considered (Clay 1988; Saffo 1991). Furthermore, it is not entirely clear to what extent mutualistic relationships are reciprocal. Although the benefits to the host are often clear and can be established by experimental elimination of the symbiont, it is much less clear whether mutualistic symbionts truly benefit from the association. It has been argued that such symbionts may better be considered slaves of the host than mutualists (Maynard Smith and Szatlunary 1995), although domesticated microorganisms may be a better analogy. Regardless of the relationship between symbiont and host (whether mutualistic, parasitic, or exploitative), there is considerable scope for reciprocal manipulation by both parties.

The term to the right of the '>' symbol is the average number of daughters produced per female in the population. For the symbiont to increase in frequency, the production of infected daughters by infected females must exceed the average production of daughters per female in the population. Note that if the symbiont controls the sex of the infected individual (rather than the sex ratio of its progeny), then the formula would be:

$$W_{xia} > (I - p)W_{„x„} + pW_{i}x_{i}.(a x_{i} + (I - a)x_{„}).$$

The differences in the two formulae occur simply because the probability of a revertant individual (an uninfected offspring of an uninfected mother) becoming female is different when the symbiont determines the sex ratio of the parent versus the sex of the progeny.

Consider for a moment the conditions for increase of a rare heritable symbiont in a population of uninfecteds, which is the same for both formulae, and is

$$aW_{i}x_{i} > W_{a}x_{„}.$$

Simply, infected females must produce more infected females than uninfected females produce uninfected females. This formulation also displays the alternative reproductive strategies available to strictly heritable symbionts. Below we will discuss briefly the alternative reproductive strategies. Heritable symbionts are already known to pursue most of the strategies described below. Other strategies discussed are predicted by this basic theory, but have not yet been documented in nature. Again, it is important to keep in mind that mixtures of these strategies can be expected to evolve in vertically transmitted symbionts.

1.2.1.1 Increase host fitness

Heritable symbionts can increase Wi, the fitness of hosts they are in (mutualism). This is the strategy generally thought of for heritable symbionts. Mutualistic symbionts (both heritable and infectious) are widespread among invertebrates (Buchner *1965).* Reproductive parasites are also subject to selection to increase host fitness, so long as increasing host fitness does not sufficiently reduce the advantageous fitness effects to the symbiont of manipulating host reproduction or its transmission rate (a). Note that the key parameter here is *fitness of infected females* (not males). Symbionts are not under direct selection to increase the fitness of infected males because males do not transmit the microorganisms.

1.2.1.2 Increase production of infected females

Heritable symbionts can increase the proportion of females produced (xi). Examples include parthenogenesis-inducing *Wolbachia* in parasitic Hymenoptera (Stouthamer *et al. 1993;* Chapter 4), feminizing *Wolbachia* found in isopods (Rousset *et al. 1992a;* Chapter 3) and feminizing microsporidia in shrimp (Dunn *et al. 1993a).* Note that the male-killing microbes do not cause an increase in the primary sex ratio (xi), but rather increase the fitness of infected females by inducing death of their sibling males. Fitness of the infected siblings is presumably enhanced by either freeing resources for them or by a reduction in the level of harmful inbreeding. In some systems, male-killing can provide an inoculum for horizontal transfer of the infection. These scenarios are reviewed in Chapter 5.

1.2.1.3 Decrease fitness of uninfecteds

Heritable symbionts that decrease the fitness of hosts in which they do not occur ($W_{„}$) can be selectively favoured. Here we consider three alternative strategies within the general category of reducing the fitness of hosts in which the symbiont does not occur:

(1) cytoplasmic incompatibility phenotype;

(2) killer and harmer phenotype; and

(3) Medea phenotype.

Cytoplasmic incompatibility phenotype

Reducing the fitness of uninfected hosts is precisely the strategy employed by cytoplasmic incompatibility (CI) microorganisms in diploid hosts. So far, all known cases of CI microbes occur in the rickettsial genus, *Wolbachia* (reviewed in Chapter 2). Although the biochemical mechanisms are not known, *CI Wolbachia* within the testes apparently modify developing sperm of infected males. When an egg is fertilized by sperm from an infected male, the same Cl-type bacterial strain must be present within the egg to rescue this modification. Otherwise, abnormal mitosis occurs which typically results in zygotic death (in diploid species). Thus, incompatibility can occur between the sperm of infected males and the eggs of uninfected females, or between the sperm of individuals infected with one strain and the eggs of individuals infected with a different strain. In the former case, incompatibility is unidirectional whereas in the latter case it can be either unidirectional or bidirectional. In effect, CI bacteria exploit infected males, which cannot normally transmit the bacterium, to reduce the fitness of uninfected individuals (or individuals infected with different strains).

Turelli *(1994)* has developed detailed population genetic models for CI bacteria, and Hoffmann and Turelli (Chapter 2) review the evolution and population biology of CI. CI microbes that cause a reduction in the fertility of infected females must exceed a threshold frequency before the advantages of CI induction (reduction in the frequency of uninfecteds) allows their spread

tract of the female (i.e. it need not be after egg-laying). We suspect that it is only a matter of time before symbionts that pursue this strategy are found. Note that a symbiont that also pursues a different strategy (e.g. mutualism, sex-ratio distortion) can be additionally selected to eliminate uninfecteds among the progeny of its host.

1.2.1.4 Decreasing sex ratio of uninfecteds

Heritable symbionts can be selected to reduce the primary sex ratio of hosts in which they do not occur (x,,). This, in fact, is what cytoplasmic incompatibility microbes *(Wolbachia)* do in haplodiploid organisms (Ryan and Saul 1968; Breeuwer and Werren 1990). It is accomplished by causing improper condensation and loss of paternal chromosomes in fertilized eggs, thus causing conversion of diploid (female) zygotes into haploid (male) zygotes (Ryan and Saul 1968; Reed and Werren 1995). Thus, haplodiploidy provides a ready mechanism for *Wolbachia* to reduce the proportion of females produced by uninfected individuals. In fact, it is possible that the same cytogenetic mechanism operates in *Wolbachia* causing CI in diploids and haplodiploids; in diploids destruction of the paternal chromatin results in zygotic lethality whereas in haplodiploids it results in male production.

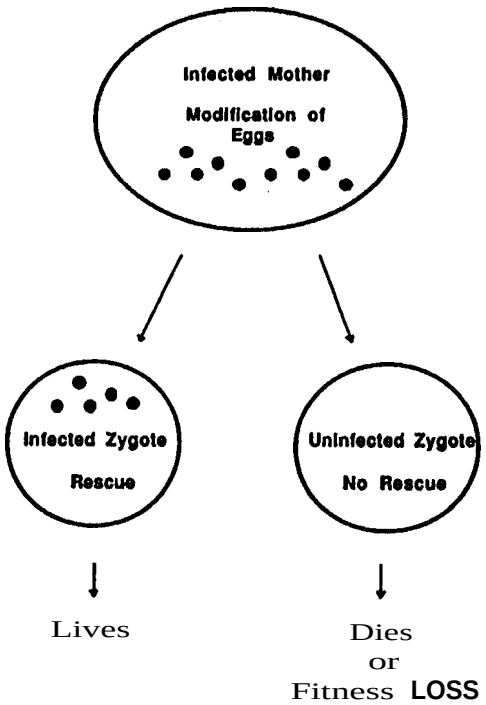

Figure 1.1 Action of Medea symbionts. The Medea effect could evolve in symbionts that have incomplete transmission to progeny. Symbionts present in the mother modify some egg component (e.g. cytoplasm or host genomic contribution) during its development. Symbionts must be present in the developing zygote to rescue this modification, otherwise the zygote dies (or suffers reduced fitness). Nuclear genetic regions are known to induce similar effects (e.g. Medea and meiotic drive chromosomes).

In theory, heritable symbionts will be selected to reduce the primary sex ratio of uninfecteds in diploid organisms. However, this has so far not been documented. How might it be accomplished? One scenario is as follows: symbionts present in testes produce DNA-binding proteins that associate with male- and female-determining genes in the developing sperm. Following fertilization by the sperm, the modifications (genomic imprinting) would then effect expression of the paternally derived sex determination genes during development of the zygote (i.e. sperm sex determination genes are imprinted). Specifically, the symbiont would be selected to increase expression of male-determining genes and decrease the expression of female-determining genes. To alter the sex ratio of uninfecteds *but not infecteds,* this modification would have to be rescued by the microorganisms in the egg (e.g. by increasing expression of female-determining genes). Symbiont-induced paternal sex-ratio modifications are shown in Fig. 1.2.

The scenario is not unreasonable, since microorganisms are already known to alter sex determination within infected hosts and to modify sperm function. A suggestive example occurs in *Clossina pallidipes.* Males infected with a vertically transmitted virus (when fertile) produce strong male-biased sex ratios when mated to uninfected females; however, uninfected females mated to uninfected males produce normal sex ratios (Jaenson 1986; Chapter 5). It remains to be seen whether symbionts pursuing the strategy of modifying sex determination through the male will be found. However, there is a simple and testable prediction for this strategy: uninfected females should produce more male-biased sex ratios when mated to infected males than when mated to uninfected males.

1.2.1.5' Increasing transmission rate

Heritable symbionts will almost always be selected to increase the vertical (maternal) transmission rate (a). The exception is when increasing transmission rate has negative pleiotropic effects on other adaptive phenotypes sufficient to offset the advantage of greater transmission. This point is made for cytoplasmic incompatibility microbes by Turelli (1994). For mutualistic symbionts, there will be strong selection on both the symbiont and the hosts to increase vertical transmission. Many of the transmission mechanisms for mutualistic symbionts described by Buchner (1965) reflect selection for stable transmission of mutualistic symbionts. Dunn *et al.* (1995) discuss some of the

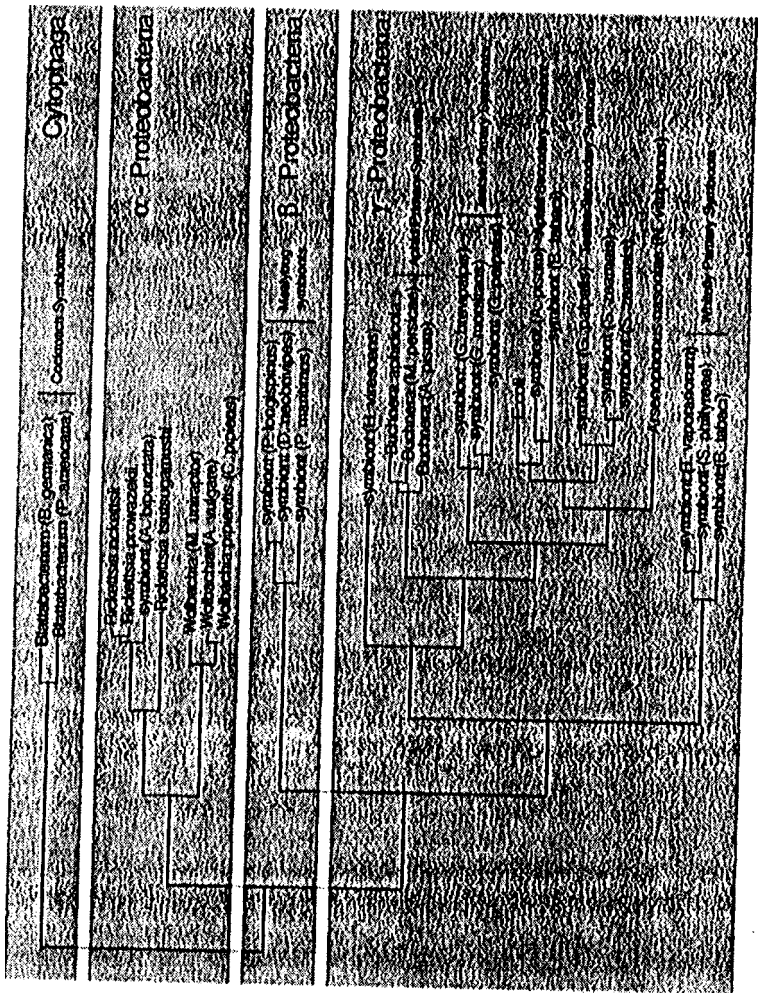

Figure 1.3 Phylogenetic tree based on 16S rRNA sequences. The arthropod

but not bund ill specialized tissues), The majority of symbionts (flat have been studied ill dclail are bacterial (and so they will receive greater attention ill this book than olllcr groups) but ill extremely wide diversity of organisms is known to associate with arthropods, including inherited viruses, extracellular arelluchac(criu, intracellular yeasts, and ex1racollular fungi and protims. Taken as a group, it is clear (flat the formation of both intracellular laid exlraccllular symbioses Mill arthropods has evolved many limes independendy (Fig. 1.:1), involving a diverse array of microorganisms and arthropods.

1.3.1 Taxonomy and phylogeny of microbial symbionts

Itticrohiolof;y has undergone a revolution ill recent years as a direct result of the application of I)NA and RNA sequencing to microbial syslcntatics. This rovolulicln has beep most keenly felt by scientists working will) fhslidious Illicroorgallisills. 'traditional microbial taxonomy was basal on nlorpltolof;ical and physiological el)aractcrs which were inadctluate to dclernline phylogenetic relationships between laxat with ally confidence, particularly of those organisms that were unable to be cultured on cell-five media (Worse 1987, 1994). The result was a very superficial tn)derstandilip, of (lie taxonomy and phylogenetic relationships of hlslidious nlicrool'gallisrlls, which included most arthropod symbionts.

The application of DNA-bused methodologies, ill particular polyn)crase chain rcaclion (PCIt) and sequencing of 16S rRNA genes, can provide an easily obtainable set of phylogenetic characters front ally microorganism, including crudely prepared samples of whole-insect DNA which contain intracellular bacteria. The IGS rRNA gene has now become a slanthlrd molecule ill microbial sys(cmatics. Sequences from this gene front a wide variety or cubacleria have been obt)lined and used for phylogenetic rccollslrtlclioll. These studies have olden provided results which have been III odds will) prior laxollollry based on more fradiliontd methods. These illcollgruellcics reflect the inadequacies of the characters used ill fllc past for classilicadon. This is particularly true will) regard to fnstidiotts lnicro-organisnls. For example, (lie currellily accepted nomenclature for (lie genus *Volhaahia* lists three species within the f;enus: *W*, pipirariis, lf', persicu, and lf'. urelophagi. 16S analysis shows that IV. pipirmris and f1'. persica tire quite unrelated bacteria-_- W. pipienlis belonging to file a-Protcobacteria, together with members of (lre germs Rickerisia (C)Ncill el al. 199?.), while IV. persica belongs to the y- Pro let) bacteria, most closely aligning with n)clllbers of the germs *hrancisella* (Weisburp el al. 1989), These discrepancies are proving it) be extrclllely common, and most likely reflect convergent evolution of dillercnl bacteria to the intracellular life style.

While molecular data have been to boon for many workers ill this field, as number of problems have not yet becn adequately resolved. For example, a

Table 1.1 16S rRNA-based classification of prokaryotes (archaebacteria and eubacteria) showing the major divisions and groupings (after Maidak et al. 1994). Shaded groups contain intracellular inherited bacterial symbionts from arthropods

Domainimajor divisions

Eucaryota

Archaea
- Euryarchaeota
  - Methanococcales
  - Methanobacteriales
  - Methanomicrobacteria and relatives
    - Methanomicrobiales
    - Methanosarcinales
    - Extreme halophiles
    - Thermoplasmales and relatives
    - Archaeoglobales
  - Thermococcales
- Crenarchaeota
  - Crenarchaeota group I
  - Crenarchaeota group II
  - Planktonic
  - Xenarchaea

Bacteria
- Thermophilic oxygen reducers
- Thermotogales
- Green non-sulphur bacteria and relatives
  - Chloroflexus subdivision
  - Deinococcus-Thermus subdivision
    - Thermus group
    - Deinococcus
- Flexibacter-Cytophaga-Bacteroides
  - Subdivision I
    - Bacteroides group
  - Subdivision II
    - Sphingobacterium group
    - Saprospira group
    - Flx. flexilis group
    - Flx. litoralis group
    - Cy. diffluens group
    - Thermonema
    - Rhodothermus
- Green sulphur bacteria
- Planctomyces and relatives
  - Planctomyces subdivision
  - Chlamydia subdivision
  - Verrucomicrobium subdivision
- Cyanobacteria and chloroplasts
  - Cyanobacteria
    - Oscillatoria group
    - Gloeothece gloeocapsa group
    - Anabaena group
    - Plectonema group
    - Synechococcus group
  - Chloroplasts and cyanelles
- Fibrobacter
  - *Fibrobacter* subdivision
    - *Fibrobacter* group .
  - Acidobacterium subdivision
- Spirochetes and relatives
  - Serpulina subdivision
  - Spirochetea-Treponema-Borrelia subdivision
    - Spi. halophila group
    - Treponema group
    - Spi. aurantia group
    - Borrelia group
  - Leptospira subdivision
    - Leptospira group
    - Leptonema group
- Proteobacteria
  - Alpha subdivision
    - R. rubrum assemblage
    - *Rhodobacter* group
    - *Hyphomonas* group
    - *Sphingomonas* group
    - Caulobacter group
    - *Rhizobium-Agrobacterium* group
  - Beta subdivision
    - Neissena group
    - *Spr.* volutans group
    - *Rhodocyclus* group
    - *Nitrosomonas* group
    - *Methylophilus* group
  - Gamma subdivision
    - *Ectothiorhodospira* assemblage
    - Chromatium group
    - Sulphur-oxidizing symbionts
    - *Xanthomonas* group
    - Cardiobacterium group
    - *Legionella* group
    - *Methylococcus* group
    - *Oceanospirillum* group
    - Pseudomonas and relatives
    - Colwellia assemblage
    - Alteromonas group
    - *Vibrio* group

Table 1.2 Known arthropod hosts of *Wolbachia*, phenotype associated with the infection where known and method by which the infection was detected

Species infected	Phenotype	Microscopy	Crossing/curing	PCR/sequencing
Phylum Nematoda				
Class Phasmidia				
Spirurida				
Filariidae				
Dirofilaria immitis				Sironi *et al.* (1995)
Phylum Arthropoda				
Class Crustacea				
Isopoda				
Armadillidiidae				
Armadillidium vulgare	Feminization	Rigaud *et al.* (1991a)	Martin *et al.* (1973)	Rousset *et al.* (1992a)
Armadillidium nasatum	Feminization	Juchault and Legrand (1979)	Juchault and Legrand (1979)	Rousset *et al.* (1992a)
Armadillidium album	Feminization	Juchault and Legrand (1979)		Bouchon (pers. comm.)
Oniscidae sp.				
Chaetophiloscia elongata	Feminization	Juchault *et al.* (1994)		Juchault *et al.* (1994)
Ligiidae				
Ligia oceanica	Feminization	Juchault *et al.* (1974)		Bouchon (pers. comm.)
Porcellionidae				
Porcellio dilatatus	CI		Legrand *et al.* 1986)	Rousset *et al.* (1992a)
Porcellionides pruinosus	Feminization	Juchault *et al.* (1994)	Juchault *et al.* (1994)	Juchault *et al.* (1994)
Sphaeromatidae				
Sphaeroma rugicauda	Feminization	Martin *et al.* (1994)	Martin *et al.* (1994)	Martin *et al.* (1994)
Class Arachnida				
Acari				
Phytoseiidae				
Metaseiulus occidentalis				Johanowicz and Hoy (1996)
Phytoseiulus persimilis				Breeuwer and Jacobs (1996)
Galendromus occidentalis				Breeuwer and Jacobs (1996)
Neoseiulus barkeri				Breeuwer and Jacobs (1996)
Neoseiulus bibens				Breeuwer and Jacobs (1996)
Tetranychidae				
Tetranychus kanzawai				Breeuwer and Jacobs (1996)
Tetranychus neocaledonicus				Breeuwer and Jacobs (1996)
Tetranychus urticae				Johanowicz and Hoy (1996)
Tetranychus turkestani				Johanowicz and Hoy (1996)
Oligonychus biharensis				Breeuwer and Jacobs (1996)
Eutetranychus orientalis				Breeuwer and Jacobs (1996)
Class Insecta				
Coleoptera				
Chrysomelidae				
Acromis sparsa				Werren *et al.* (1995b)
Chelymorpha alternans				Werren *et al.* (1995b)
Chersinetlina heteropunctata				Werren *et al.* (1995b)
Diabrotica v. virgifera				O'Neill *et al.* (1992)
Cerdae				
Phoceras sp.				Werren *et al.* (1995b)
Curculionidae				
Alaninus tesselatus				Werren *et al.* (1995b)
Bangasternus orientalis				Werren *et al.* (1995a)
Cossonus sp.				Werren *et al.* (1995b)
Hypera postica	CI	Hsiao and Hsiao (1985)	Blickenstaff (1965)	O'Neill *et al.* (1992)
Sitophilus oryzae				Werren *et al.* (1995a)

Table 1.2 (Cont.)

Species infected	Phenotype	Microscopy	Crossing/curing	PCR/sequencing
Delphacidae				
Laodelphax striatellus	CI	Noda (1984*b*)	Rousset *et al.* (1992*a*)	
Hymenoptera				
Agaonidae				
Tetrapus costaricensis				Werren *et al.* (1995*b*)
Aphelinidae				
Aphytis lingnanensis	Parthenogenesis		Zchori-Fein *et al.* (1995)	
Aphytis diaspidis	Parthenogenesis		Zchori-Fein *et al.* (1995)	
Aphytis yanonensis				Werren *et al.* (1995*a*)
Encarsia formosa	Parthenogenesis		Zchori-Fein *et al.* (1992)	Werren *et al.* (1995*a*)
Apidae				
Trigona sp.				Werren *et al.* (1995*b*)
Braconidae				
Asobara tabida			Werren *et al.* (1995*a*)	
Cynipidae				
Diplolepis rosae				van Meer *et al.* (1995)
Encyrtidae				
Apoanagyrus diversicornis			Pijls *et al.* (1996)	Werren (pers. comm.)
Eulophidae				
Mellitobia sp.				Werren *et al.* (1995*a*)
Eucoilidae				
Leptopilina australis	Parthenogenesis		van Alphen (pers. comm.)	Werren *et al.* (1995*a*)
Leptopilina clavipes	Parthenogenesis		van Alphen (pers. comm.)	Werren *et al.* (1995*a*)
Formicidae				
Ectatomma tuberculatum				Werren *et al.* (1995*b*)
Proctotrupoidae				
Trichopria drosophilae	CI		van Alphen (pers. comm.)	Werren *et al.* (1995*a*)
Pteromalidae				
Muscidifurax uniraptor	Parthenogenesis		Stouthamer et al. (1990)	
Nasonia vitripennis	CI		Saal (1951)	Breeuwer *et al.* (1992)
Nasonia giraulti	CI		Breeuwer et al. (1994)	Breeuwer *et al.* (1992)
Nasonia longicornis	CI			Breeuwer *et al.* (1992)
Spalangia fuscipes				Werren *et al.* (1995*a*)
Sphecidae				
Tropoxylon sp.				Werren *et al.* (1995*a*)
Trichogrammatidae				
Trichogramma brevicapillum	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer and Werren (1993)	Werren *et al.* (1995*a*)
Trichogramma chilonis	Parthenogenesis		Stouthamer *et al.* (1990*a*)	
Trichogramma cordubensis	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	Rousset *et al.* (1992*a*)
Trichogramma deion	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	Stouthamer *et al.* (1993)
Trichogramma embryophagum	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	
Trichogramma evanescens	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	
Trichogramma cf. deion	Parthenogenesis		Stouthamer and Kazmer (1994)	
Trichogramma oleae	Parthenogenesis	Louis *et al.* (1993)	Stouthamer *et al.* (1990*a*)	Rousset *et al.* (1992*a*)
Trichogramma platneri	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	
Trichogramma pretiosum	Parthenogenesis	Stouthamer and Werren (1993)	Stouthamer *et al.* (1990*a*)	Stouthamer *et al.* (1993)

Obligate relationships

Extracellular obligate symbiont associations have been studied in detail in a number of insect groups. For example, rich assemblages of various microorganisms are found in the hind-guts of termites and some cockroaches which have a predominantly cellulose diet. Indeed, termite guts have been described to contain representatives from the three domains of life, including bacteria, archaebacteria and eukaryotes (Ohkuma *et al. 1995;* Ohkuma and Kudo *1996).* Similarly, insects that feed solely on vertebrate blood or plant sap are often associated with obligate symbionts. A well-studied example is the extracellular, Gram-positive *Rhoclococcus* bacteria that infect triatomine bugs (Baines *1956).*

Many obligate associations also involve intracellular symbionts, predominantly bacterial or yeast-like organisms. Again, similar life histories of restricted diets are good indicators of insects that contain these symbionts. For example, aphids, mealybugs, and whiteflies are known to contain intracellular symbionts associated with bacteriocytes whose presence is required by the host. Often these insects contain a number of different symbiont associations, but the obligate bacteriocyte-associated symbionts are known as `primary' symbionts. Phylogenetic analysis of the 16S rRNA genes from these primary symbionts shows that they commonly have a concordant phylogeny with their host insects (Moran and Baumann *1994).* This indicates that these obligate nutritive associations have, for a long time, been solely dependent on strict vertical inheritance. This is in contrast to the facultative reproductive symbionts mentioned above which show no such concordance. In addition to the hemipteran examples, similar patterns of evolution have also been found for primary symbionts in cockroaches (Bandi *et al. 1994)* and in tsetse flies (Aksoy *et al. 1995).* Indeed, tsetse flies have a unique set of symbiont associations. Three categories of symbionts have been described from these blood-feeding flies to date, including *Wolbachia,* primary bacteriocyte-associated bacteria, and secondary facultative gut-associated intracellular bacteria. Of these three associations, only the primary symbionts are needed for the fly to survive and reproduce, and again these bacteria *(Wigglesivorthia glossinidia)* have a concordant evolution with their hosts, indicating strict vertical inheritance (Aksoy *et al. 1995).*

This same pattern is also seen with intracellular bacterial symbionts of cockroaches. These symbionts are localized to the fat body of the roaches and, although they are quite unrelated to the y-Proteobacteria which commonly form these mutualistic associations with insects, they still display a similar concordant phylogeny with their hosts. Indeed, even the primitive termite, *Mastotermes,* contains bacterial endosymbionts of the same group, which show a deep branching relationship to the cockroach endosymbionts. This parallels the commonly held insect phylogeny that relates termites and cockroaches (Bandi *et al. 1995).*

Similarly, attine ants (including leaf-cutter ants) have associated symbiotic fungi which they cultivate on plant material collected into subterranean gardens, upon which they are dependent for nutrition. Phylogenetic studies indicate that primitive attines have repeatedly acquired their symbionts from free-living fungi, whereas the fungi of derived attines have concordant phylogenies with the host, indicating heritable transmission (Chapela *et al. 1994).* This is consistent with the observation in some higher attine species, that the female collects a sample of fungus prior to dispersal and founding of a new colony (Chapela *et al. 1994).* It is likely that the specialized ant-fungus symbiosis has been a motor of evolutionary change in both the ants and associated fungi.

Many of these associations must he extremely ancient. To (late most of the studied primary symbionts have been bacterial, and predominantly members of the y-Proteobacteria (Table 1.1). However many known associations have yet to be studied in detail, including the yeast-like primary symbionts which are commonly found in hemipterans (Buchner *1965;* Noda *et al. 1995)* and some colcoplcrans (Nods and Kodama *1996).*

Facultative relationships

In contrast to the strictly inherited obligate nutritive symbionts of many insects, many associations are facultative. A spectrum of agents lulls into this grouping which shows the full range of abilities to be horizontally and vertically transmitted. Many of the studied examples are human pathogens, including the various *Rickettsia* species which are readily acquired orally by arthropods as well as being transmitted vertically (Azad *et al. 1992).* Here we will only consider the intracellular examples, although the majority of members of this class of association are probably extracellular. The best-studied examples are the intracellular secondary symbionts of aphids and tsetse flies (Beard *et al. 1993).* These bacteria are intracellular but not contained within bacteriocytes. In many cases the phenotypic consequence of these infections to the host is unknown and, owing to the difficulty in working with these systems, will likely remain difficult to determine. It is clear, however, that these agents are capable of horizontal transmission between hosts, as well as vertical transmission. The secondary symbionts of tsetse flies show no phylogenetic concordance with their hosts, unlike their primary counterparts (Aksoy *et al. 1995, 1997).* However, despite (his lack of linkage between the symbionts and hosts, it is still probable that transmission in these cases is commonly vertical.

1.4 Potential conflicts between symbionts and hosts

Symbionts, whether they he primarily mutualists, sex-ratio distorters or cytoplasmic incompatibility microbes, do not have completely convergent

(mitotic) divisions; however, in male eggs, the X chromosomes form a bivalent and one X segregates to the polar body. Thus, sex must be determined by factors placed into the egg during oogenesis that effect X chromoome behaviour. Buchner (1965) reports that symbionts are transmitted to both male and female eggs in most species. Aphid symbionts are expected to produce products that interfere with X chromosome loss and the host to counteract these effects. Similarly, aphid symbionts may produce products that sustain parthenogenetic reproduction, with host suppression of these effects during the sexual phase.

In some cases the possible footprint of male-killing symbionts is apparent. In some pcmphigine aphids, symbionts are not present in male embryos but arc in female embryos. In these species, the males are reduced in size and do not feed. It can be argued that symbionts are not needed in these species due to lack of male feeding (Buchner 1965); however, there is an alternative interpretation. In systems where male-killing mutualistic symbionts are selected for, suppression of the male-killing may be achieved by exclusion of the symbiont from male eggs. The cost of this exclusion will be frail males who are small and (to not feed. Thus, these situations are most likely to arise where there is parental brooding (to provide sustenance to the males), conditions that also favour male-killing (Werren 1987; L. D. Hurst 1993). In several coccid groups (Pseudococcus, Puto, Macrocerococcus) the mycetomes in embryonic males are very small or minute (Buchner 1965). Males in these groups also do not feed and females brood their young (Nur, personal communication). Buchner (1965) also reports that in coccid species with yeast symbionts, the symbionts are excluded from male eggs but not female eggs. One mechanism by which hosts could avoid the negative effects of male-killing nutritive symbionts is to conceal the sex of the progeny. Interestingly in this regard, coccids which undergo paternal genome loss in males actually retain the paternal genome within the symbiont-bearing mycetomes (Brown 1965). The patterns described above are merely suggestive. Clearly, much remains to be done to determine whether mutualistic symbionts are actually involved in host sex determination and sex-ratio distortion.

1.4.2 Regulation of symbiont numbers

The regulation of symbiont numbers in hosts is poorly understood. From the evolutionary perspective, the interests of host and mutualistic symbionts are generally concordant. Excessive replication of symbionts is likely to reduce the fitness of hosts, and therefore that of the symbionts, since the host is the vehicle for their propagation to future generations. Thus, in general we will expect the evolution of prudent symbionts that have reduced replication rates so as not to significantly harm the host. However, evolutionary interests of the symbiont and host are not completely concordant.

In many respects the population genetics of heritable symbionts is analogous to that of mitochondria, a topic extensively investigated by Birky and colleagues (Birky 1978; Backer and Birky 1985; Birky et al. 1989). As with mitochondria, symbionts are inherited uniparentally through the maternal line. As a result, there is little or no mixing between symbiont lineages. A second feature in common with mitochondria is the hierarchical structure of symbiont populations. There is the individual symbiont, population of symbionts within individual host cells (nutritive symbionts are often localized in specialized cells, mycetocytes or bacteriocytes, until the time of host reproduction and transmission), population of infected host cells within an individual host organism, and populations of infected hosts. The population dynamics of symbionts will be dependent upon stochastic processes of transmission and selection at the different levels.

Selection on'symbiont-induced phenotypes will occur at each of the organizational levels, and can sometimes be antagonistic at different levels. For example, both within-host selection and between-host selection act upon symbionts. Phcnolypes that favour individual symbionts in competition within a host can be detrimental to the fitness of the host and associated symbiont lineages (Maynard Smith and Szathmary 1995). Consider a mutation that arises within an individual symbiont that confers a replication advantage (e.g. higher replication rate) relative to the other symbionts within a host cell. The frequency of the mutant will increase during successive replication cycles within the host cell or within the host cell lineage. Eventually the mutant can go to fixation within the host lineage, whether or not the symbiont variant is harmful to the host. Variants that have a replication advantage but are harmful to host fitness will be selected for by within-host selection but selected against by between-host selection.

Clearly, symbiont mutants can also have a replication advantage without being detrimental to the host, and such symbionts should become common in host populations. The rate at which this occurs will depend upon how readily mutations for higher replication rates occur and to what extent there is mixing of symbionts within and between hosts. Symbiont mutations that increase relative replication rates but are not harmful to the host can occur, for instance, when symbionts compete for a limiting resource provided by the host. Competition among symbionts within a host will be most intense at the time of transmission to progeny. Competition will occur for transmission to the eggs and for representation within the egg, thus favouring higher replication rates at this time. Interestingly in this regard, Buchner (1965) observed that, in general, a conspicuous spontaneous increase of the symbionts goes hand in hand with egg infection. This is particularly dramatic in Camponotus ants, where a stormy increase of symbionts temporarily results in a large proportion of the egg biomass being made up by symbionts. However, in general, symbiont mutations that are detrimental to the host cannot persist

mutations that impart an advantage during transmission will be selectively favoured by within-host selection. Eventually, we expect the refinement of symbiont phenotypes that restrict competitive behaviour to these particular times, thus minimizing negative effects on host (and therefore symbiont lineage) fitness.

As previously described, bacterial symbionts in *Camponotus* and *Formica* ants are transmitted to the eggs and undergo high rates of replication that are far in excess of what would be necessary to merely ensure transmission (Buchner 1965; Koch 1967). The pattern is best explained in terms of intrahost competition among symbionts. In cicadas, at the time of ovarian development, the symbionts swarm out of the mycetomes and arrive at the place of infection by way of the lymph where they enter specialized wedge cells near the follicles prior to entering the egg (Koch 1967, p. 45). Again, although anecdotal, these accounts imply .t much greater movement of symbionts than would be required merely to ensure transmission. In *Macrocerococcus superbus,* intact mycetocytes actually enter the developing egg and fuse with yolk cells in a kind of somatic fertilization (Koch 1967). ']'his incclaanisan would reduce (lie potential for symbiont competition, depending upon how many different mycetocytes contribute to each egg.

For reproductive parasites, the evolutionary interests of host and symbiont are less likely to be concordant. The host is not necessarily selected to ensure symbiont transmission, and in some cases may be favoured to suppress it. For example, hosts infected with male-killing or other sex-ratio distorting microbes will generally be selected to reduce or eliminate transmission of the reproductive parasites (Uyenoyama and Feldman 1978; Werren 1987). For cytoplasmic incompatibility (CI) bacteria, the situation is more complex (Turelli 1994). During initial invasion of a population by CI bacteria, selection can favour host genotypes that reduce CI bacterial transmission. However, if the infection is near fixation within the host population, the host can actually be selected to enhance transmission of the bacteria to progeny (at least to daughters) because the daughters will then be reproductively compatible with infected males in the population. Reproductive symbionts themselves are under strong selection to enhance their transmission rates, so long as the cost to (female) host fertility is not too great. In this regard, the CI *Wolbachia of Nasonia* wasps localize to the pole *of* the egg where germ cells develop, thus presumably enhancing their transmission through the germline (Breeuwer and Werren.1990). This contrasts with CI *Wolbachia* in *Drosophila simulans* and *D. melanogasler,* where bacteria are distributed throughout the developing syncytial blastoderm (ONeill and Karr 1990), although they are found predominantly within the gonadal tissue of adults. This may reflect a more ancient association of *Wolbachia* in *Nasonia,* resulting in specific adaptations for germline transmission within that host.

1.4.3.2 Germline determination

Could symbionts be selectively favoured to influence germline determination in the developing embryo'? Under some circumstances, the answer may be yes. First, it should be clear that symbiont mutants that induce gross abnormalities in germ-cell formation will generally not be selected for. Although such mutants may gain a short-term transmission advantage (i.e. greater representation among the gametes) in the first host generation, they are likely to cause sufficient detrimental effects upon host fitness to be selectively eliminated by interhost selection. However, more subtle influences on germline development may evolve.

Germ cells are determined early in development in most animals. Imagine the following situation. Suppose that germ cells are determined by a gradient of germline determinant products within the egg cytoplasm. Cells that form within (lie region containing germline determinants will develop into primordial germ cells. This is the pattern observed in a number of species. For example, in 1)rosophilu, germline determinants called polar granules localize to the gernapole of the egg and are composed of the products (both protein and mRNA) of several different genes (Lehmann and Ephrussi 1994). Germ-specific granules arc also found in such diverse organisms as nematodes and frogs (Lehmann and Ephrussi 1994). Intracellular symbionts that are free within the host cytoplasm will be selected to localize within the general region containing germline determinants (as described above). However, there is likely to be a gradient in such determinants within the cytoplasm. Those symbionts in the periphery of the germ determinant region will be less likely to be incorporated within germ cells. A symbiont mutant that increases its probability of incorporation, either by interacting with or producing germ-cell determinants, will increase in frequency so long as such interactions do not dramatically disrupt host fatness.

1.5 Other evolutionary consequences of heritable symbiosis

1.5.1 Evolution of novel phenotypes

It is now widely recognized that the union of symbiont and host provides opportunities for the evolution of novel phenotypes, by combining genomes with different biochemical capabilities (Margulis and Fester 1991). Examples of this phenomenon abound, including the symbiotic origin of mitochondria and chloroplasts (Margulis 1981), sulphide-oxidizing bacteria found in various invertebrates in hydrothermal vents and other sulphide-rich environments (Vetter 1991), and a variety of nutritional endosymbionts in arthropods (Buchner 1965).

with aphids is ancient, going back at least 200 million years (Moran and Baumann 1994). Thus, reduction in genome size is apparently not inevitable, and the factors that may cause symbionts to evolve in different directions are, at this point, unclear.

What factors may lead to the reduction in genome size and movement of vital genes to the nuclear genome? We can imagine several processes that could be involved, including:

(1) loss of unnecessary genes;

(2) intrahost competition among symbionts for increased replication rate;

(3) mutational degeneration (Muller's ratchet); and

(4) male-function degeneration.

As with other parasites, symbionts will lose unnecessary biochemical functions when the products are already provided by the host environment. This process probably explains the reduced genome size in rickettsia and chlamydia, obligatory intracellular parasites. However, the process does not predict transmission of vital symbiont genes to the nuclear genome, because the products are already provided by the nuclear genome.

Intrahost competition is one possible explanation for genome streamlining in incipient organelles. According to this model, if symbionts with smaller genome sizes have higher replication rates, then symbionts with deletions can increase by intrahost selection. As the defective symbiont became more abundant within hosts, this would select for compensatory mutations on the part of the host. One such mutation would be translocation of the functional gene to the nucleus, assuming the gene was appropriately expressed to rescue the defective variants. This model assumes that the product was not vital for individual symbiont survival and replication, or that the product was diffusible so that functional symbionts rescue defective symbionts in heteroplasmic hosts.

A second mechanism for genome streamlining is mutation accumulation. Because most heritable symbionts are effectively asexual, occur in small diSCI'ete 1)uptila0otts within hosts, and undergo bottlenecks each generation, deleterious mutations are expected to accumulate via genetic drift , a process known as Muller's ratchet. During mutational degradation of particular genes, rare translocations of the functional allele (presumably present in some symbionts) to the nucleus would be selectively favoured if they enhanced symbiont performance (and therefore host survival). Similarly, increase of symbiont genes that disrupted male function (either by active selection or drift) would select for nuclear translocations of the symbiont allele that functions within males. The extent to which streamlining occurs within different symbionts, and the extent to which these different processes may be involved, is currently unknown.

1.6 Conclusion

Evolutionary interactions between inherited symbionts and hosts can be complex. In some cases, evolutionary interests of the symbiont and host are concordant, but in many cases they are not. In addition to mutualism, a variety of alternative adaptive strategies are available to heritable symbionts, including induction of cytoplasmic incompatibility, killer and Medea phenotypes, and manipulation of host sex determination. The tools provided by recent advances in molecular biology have reinvigorated studies of microbial symbionts. These fastidious microorganisms can now be identified based on gene sequence information, their genomes can be characterized, and interactions with hosts can be studied at a level not previously possible. An increasing number of studies over the past decade have demonstrated that not only are classical nutritional mutualistic symbionts very common, as has been suspected for many years, but that intracellular reproductive symbionts are also pervasive among invertebrates. It is quite possible that many more examples of inherited symbionts employing strategies of reproductive manipulation are yet to be discovered and characterized. Much work remains to be done in order to address the mechanisms by which these microorganisms are able to subvert the machinery of host reproduction to their own advantage.

Acknowledgements

The authors would like to thank Rolf Weinzierl, Ary Hoffmann, Steve Perlman, Cesar Perez-Gonzalez and Rebecca Weston for comments on the manuscript. JHW thanks Bryant McAllister and Rence Goodwin for long-distance assistance, Uzi Nur for discussions, Nico Michaels and Leo Beukeboom for an excellent sabbatical environment, and the NSF and Alexander von Humboldt Foundation for financial support. SLO thanks Thierry Rigaud, Richard Stouthamer and Frangois Rousset for help in compiling Table 1.2, and the NIH and WHO/TDR for financial support.