

## ***Intraclonal genetic variation: ecological and evolutionary aspects.***

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# **Microorganisms and parthenogenesis**

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Endosymbionts are being discovered in unprecedented numbers of invertebrate species. The bacterium *Wolbachia pipientis* alone is carried by some 20% of all insect species, in which it is responsible for such reproductive manipulations as cytoplasmic incompatibility, feminization of genetic males, male-killing and parthenogenesis. The discovery of *Wolbachia*-induced thelytoky in hymenopteran parasitoids has initiated the successful search in other groups of haplodiploid species. Haplodiploidy has arisen at least 17 times during animal evolution and intracellular bacteria that effect sexual development have been found in many of these haplodiploid groups, rekindling the question regarding the origin of haplodiploidy. The detection of *Wolbachia* in parthenogenetic thrips (Thysanoptera), booklice (Psocoptera), springtails (Collembola), mites (Acari) and *Xiphinematobacter* in dragger nematodes (Dorylaimida) raises the possibility of bacterial-induced parthenogenesis in diploid species as well. Recent studies indicate that *Wolbachia* might be just one of a rapidly increasing list of intracellular bacteria capable of inducing parthenogenesis in their host. The search for novel endosymbionts will rely heavily on previous taxonomic work, but the possibilities for discovery remain boundless. © 2003 The Linnean Society of London. *Biological Journal of the Linnean Society* 2003, **79**, 43–58.

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### INTRODUCTION

Parthenogenesis is surprisingly common among both plants and animals, perhaps accounting for up to 1% of the total number of species (Suomalainen, Saura & Lokki, 1987; Short & Balaban, 1994). With the exception of the most evolved taxa such as gymnosperms and mammals, in which it has not been reliably reported, parthenogenesis is practically universally distributed in the tree of life. This might reflect the ongoing battle between two basic forms of reproduction. Only bdelloid rotifers constitute an order that consists entirely of parthenogens. Most parthenogens have close relatives that are sexual, and often sexual and asexual populations of one species have been found geographically separated. Local adaptation easily explains these patterns. However, the sympatric occurrence of sexual and asexual populations defies mainstream evolutionary thinking. After all, cytoplasmic genes and not nuclear ones might be the decisive

evolutionary force behind some of the more interesting population structures of invertebrates.

### DISCOVERING ENDOSYMBIOTIC BACTERIA

Many invertebrates are known to have intimate intracellular relationships with a diverse array of microorganisms. The taxonomic range of the invertebrate hosts to the microorganisms is equally wide. Microorganisms associated with parthenogenesis can be placed into three general categories. The *Wolbachia pipientis* assemblage is a widespread group of closely related  $\alpha$ -Proteobacteria known to cause a variety of reproductive alterations, including cytoplasmic incompatibility, thelytokous parthenogenesis, feminization of genetic males, and male-killing (O'Neill, Hoffmann & Werren, 1997; Bourtzis & Braig, 1999; Stouthamer, Breeuwer & Hurst, 1999; Stevens, Giordano & Fialho, 2001). Bacteria belonging to the *Cytophaga–Flexibacter–Bacteroides* (CFB) group are associated with parthenogenetic wasps and feminized mites (Zchori-Fein *et al.*, 2001; Weeks, Marec & Breeuwer, 2001).

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*Xiphinematobacter* species belonging to the newly established Verrucomicrobia have been found in parthenogenetic nematodes attacking the rootlets of fruit crops (Vandekerckhove *et al.*, 2000). Table 1 reflects an ever increasing list of species subject to bacterial induced parthenogenesis.

Cytoplasmic microorganisms tend to be inherited exclusively from the mother, because males usually produce very small gametes where room for cytoplasmic bacteria is lacking. Hence cytoplasmic genes have no 'evolutionary interest' in being in a male, which for them is an evolutionary 'dead-end' (Hurst, 1993). Consequently, maternally inherited microorganisms are thought to evolve towards favouring the production of female offspring in order to maintain themselves and promote their own propagation. They are often referred to as 'sex ratio distorters' (Wrench & Ebbert, 1992; Hurst, 1993; O'Neill *et al.*, 1997). It should be noted that even though the existence of female-biased sex ratios may be an indication of extrachromosomal elements in an organism, a number of chromosomal mechanisms including meiotic drive, hybrid dysgenesis, sex linkage of a trait and maternal effects can also cause sex ratio distortion (Braig & Yan, 2002). Moreover, a wide variety of parasites are known to distort the sex ratios of their hosts without causing parthenogenesis (Braig *et al.*, 2002).

To verify the presence and character of endosymbionts within the female-biased species, a variety of investigations can be applied. The classic test for both maternal effect and uniparental inheritance is to perform reciprocal crosses involving back-crossing so as to replace the original maternal nuclear DNA with paternal DNA of an unaffected lineage. If the condition persists it must be cytoplasmic. However, in cases of obligate parthenogenesis, genetic analysis is not possible. Other methods of analysis include horizontal transfer of the trait and the removal of the trait by heat shock or antibiotics. Additionally, visual detection of the symbionts in every affected lineage, but never in unaffected lineages, is indirect but highly suggestive evidence (Hurst, 1993). This can be achieved, for example, by the bacterial stain lacmoid, followed by bleach treatment, or by using the double-stranded DNA stain, DAPI (4',6-diamidine-2-phenylindole-dihydrochloride) (Quicke, 1997). Sampling efforts for microorganism-induced thelytoky have clearly been taxonomically biased and relatively few non-hymenopteran organisms have been studied with respect to *Wolbachia* or to any other cytoplasmic endosymbiont. All these studies included only small samples of each host species and were therefore unlikely to detect the majority of sex ratio distorting strains, which commonly occur at low prevalence. It now looks possible that maternally inherited, thelytoky-associated microorganisms may also be wide-

spread in diploids and thus it can be expected that future research will involve a far wider range of taxa. The likelihood of detection of any endosymbiont depends on the detection method applied. Initial detection using *Wolbachia*-specific PCR (polymerase chain reaction) primers will discriminate against any unknown parthenogenesis inducing bacterium (Vandekerckhove *et al.*, 1999; Weeks *et al.*, 2001).

## BACTERIAL-INDUCED PARTHENOGENESIS IN HYMENOPTERA

In 1990, Stouthamer and colleagues reported that thelytokous strains of egg parasitoids in the genus *Trichogramma* could be induced to produce males if treated with specific antibiotics (tetracycline hydrochloride, sulfamethoxazole and rifampicin), or subjected to high temperatures (>30°C; Stouthamer, Luck & Hamilton, 1990a). They also conducted back-crossing experiments to determine if chromosomal factors had an effect on the inheritance of thelytoky. Wild-caught sexual (arrhenotokous) females were crossed with males derived from thelytokous strains through heat treatment. The back-crossed females remained arrhenotokous, showing that thelytoky was not inherited through the chromosomes of the wasps. It was concluded that microorganisms were the cause of thelytoky, and it was further suggested that their findings could explain the existence of many species of thelytokous wasps.

Not surprisingly, ever since the discovery of Stouthamer and colleagues increasing numbers of researchers have been attracted to the phenomenon. Even prior to their findings, many authors had found that rearing the females of thelytokous parasitoid wasps – including members of the chalcidoid genera such as *Encarsia*, *Aphelinus*, *Muscidifurax*, *Ooencyrtus*, *Trichogramma*, and *Hexacola* members of the cynipoids – at elevated temperatures (>28–35°C) led to the production of males (Schlinger & Hall, 1959; Wilson & Woolcock, 1960a; Bowen & Stern, 1966; Orphanides & Gonzalez, 1970; Eskafi & Legner, 1974; Laraichi, 1978; Cabello-Garcia & Vargas-Piqueras, 1985; Legner, 1985). Interest in the parasitoid Hymenoptera was spurred by their potential as biological control agents and also as a means of clarifying the taxonomic status of uniparental species in relation to the biparental conspecifics. As summarized by Flanders (1965), most of the above authors concluded that sexuality and primary sex ratios in populations of many species of Hymenoptera were functions of the environment, which were acting indirectly on a pre-embryonic stage of the individual, changing it either from potentially female to male, or vice versa.

To confirm the presence of microorganisms in the thelytokous *Trichogramma* wasps, Stouthamer &

**Table 1.** Microorganisms in parthenogenetic species. (M = molecular identification; A = antibiotic treatment; H = heat treatment; O = optical detection)

Species	Bacterium	Mechanism	M	A	H	O	Reference
PHYLUM ARTHROPODA							
CLASS ELLIPLURA							
Order Collembola							
Superfamily Entomobryodea							
Family Isotomidae							
<i>Folsomia candida</i>	<i>Wolbachia</i>	Gamete duplication	✓	✓		✓	Vandekerckhove <i>et al.</i> (1999), Vandekerckhove (2001)
Superfamily Poduroidea							
Family Onychiuridae							
<i>Mesaphorura macrochaeta</i>	<i>Wolbachia</i>		✓				Czarnetzki & Tebbe (2002)
<i>Paratullbergia callipygos</i>	<i>Wolbachia</i>		✓				Czarnetzki & Tebbe (2002)
CLASS INSECTA							
Order Hymenoptera							
Superfamily Chalcidoidea							
Family Aphelinidae							
<i>Aphelinus semiflavus</i>	?				✓		Schlinger & Hall (1959)
<i>Aphytis chilensis</i>	<i>Wolbachia</i>		✓				Gottlieb <i>et al.</i> (1998)
<i>A. chrysomphae</i>	<i>Wolbachia</i>		✓				Gottlieb <i>et al.</i> (1998)
<i>A. diaspidis</i>	<i>Wolbachia</i>		✓	✓			Zchori-Fein <i>et al.</i> (1995); Gottlieb <i>et al.</i> (1998)
<i>A. lignanensis</i>	<i>Wolbachia</i>		✓	✓			Zchori-Fein <i>et al.</i> (1994, 995); Gottlieb <i>et al.</i> (1998)
<i>A. mytilaspidis</i>	?	Terminal fusion					Rössler & DeBach (1973)
<i>A. yanonensis</i>	<i>Wolbachia</i>		✓				Werren <i>et al.</i> (1995)
<i>Encarsia berlessei</i>	CFB group		✓				Zchori-Fein <i>et al.</i> (2001)
<i>E. citrina</i>	CFB group		✓				Zchori-Fein <i>et al.</i> (2001)
<i>E. formosa</i>	<i>Wolbachia</i>		✓	✓			Zchori-Fein <i>et al.</i> (1992); Werren <i>et al.</i> (1995)
<i>E. hispida</i>	CFB group		✓	✓			Hunter (1999), Zchori-Fein <i>et al.</i> (2001)
<i>E. meritoria</i>	?			✓			Giorgini (2001)
<i>E. pergandiella</i>	CFB group	Also in sexual lineage	✓	✓			Gottlieb <i>et al.</i> (2000), Zchori-Fein <i>et al.</i> (2001)
<i>E. perniciosi</i>	CFB group		✓				Zchori-Fein <i>et al.</i> (2001)
<i>E. protransvena</i>	CFB group		✓	✓			Giorgini (2001) Zchori-Fein <i>et al.</i> (2001)
<i>Eretmocerus mundus</i>	<i>Wolbachia</i>		✓	✓			De Barro & Hart (2001)
<i>E. stauferi</i>	<i>Wolbachia</i>		✓				van Meer <i>et al.</i> (1999)
Family Encyrtidae							
<i>Apoanagyrus diversicornis</i>	<i>Wolbachia</i>		✓	✓			Pijls <i>et al.</i> (1996); van Meer <i>et al.</i> (1999)
<i>Coccidoxenoides peregrinus</i>	<i>Wolbachia</i>		✓				van Meer <i>et al.</i> (1999)
<i>Habrolepis rouxi</i>	?				✓		Flanders (1965)
<i>Ooencyrtus submetallicus</i>	?				✓		Wilson & Woolcock (1960a,b)
<i>O. fecundus</i>	?				✓		Laraichi (1978)

Table 1. Continued

Species	Bacterium	Mechanism	M	A	H	O	Reference
<i>Pauridia peregrina</i>	?			✓			Flanders (1965)
<i>Plagiomerus diaspidis</i>	?			✓			Gordh & Lacey (1976)
<i>Trechnites psyllae</i>	<i>Wolbachia</i>		✓				Stouthamer, pers. comm.
Family Pteromalidae							
<i>Muscidifurax uniraptor</i>	<i>Wolbachia</i>	Gamete duplication	✓	✓	✓		Legner (1985); Stouthamer <i>et al.</i> (1993); Zehori-Fein <i>et al.</i> (2000)
<i>Spalangia fuscipes</i>	<i>Wolbachia</i>		✓				Werren <i>et al.</i> (1995)
Family Signiphoridae							
<i>Signiphora borinquensis</i>	?			✓			Quezada <i>et al.</i> (1973)
Family Torymidae							
<i>Torymus bedeguaris</i>	<i>Wolbachia</i>		✓				Schilthuizen & Stouthamer (1998)
Family Trichogrammatidae							
<i>Trichogramma brevicapillum</i>	<i>Wolbachia</i>		✓			✓	Stouthamer & Werren (1993); Schilthuizen & Stouthamer (1997)
<i>T. chilonis</i>	<i>Wolbachia</i>		✓	✓			Stouthamer <i>et al.</i> (1990ab); Chen <i>et al.</i> (1992); Schilthuizen & Stouthamer (1997)
<i>T. cordubensis</i>	<i>Wolbachia</i>		✓	✓	✓		Cabello & Vargas (1985); Stouthamer & Werren (1993); Schilthuizen & Stouthamer (1997); Pintureau <i>et al.</i> (1999a)
<i>T. deion</i>	<i>Wolbachia</i>	Gamete duplication	✓	✓		✓	Stouthamer <i>et al.</i> (1990a,b); Stouthamer & Werren (1993)
<i>T. nr deion</i>	<i>Wolbachia</i>	Gamete duplication	✓	✓			Stouthamer & Kazmer (1994); Schilthuizen & Stouthamer (1997)
<i>T. embryophagum</i>	<i>Wolbachia</i>		✓	✓	✓		Stouthamer & Werren (1993); Pintureau <i>et al.</i> (2000)
<i>T. evanescens</i>	<i>Wolbachia</i>		✓	✓	✓		Stouthamer <i>et al.</i> (1990b); Stouthamer (1997); Pintureau <i>et al.</i> (2000)
<i>T. kaykai</i>	<i>Wolbachia</i>		✓				Schilthuizen <i>et al.</i> (1998)
<i>T. nubilale</i>	<i>Wolbachia</i>		✓				Schilthuizen & Stouthamer (1997)
<i>T. oleae</i>	<i>Wolbachia</i>		✓				Pintureau <i>et al.</i> (2000)
<i>T. platneri</i>	<i>Wolbachia</i>		✓	✓	✓		Stouthamer <i>et al.</i> (1990a); Stouthamer & Werren (1993)
<i>T. pretiosum</i>	<i>Wolbachia</i>	Gamete duplication	✓	✓	✓	✓	Stouthamer <i>et al.</i> (1990a); Stouthamer & Werren (1993); Schilthuizen & Stouthamer (1997)
<i>T. nr pretiosum</i>	<i>Wolbachia</i>		✓				Schilthuizen & Stouthamer (1997)
<i>T. semblidis</i>	<i>Wolbachia</i>		✓				Pintureau <i>et al.</i> (2000)
<i>T. sibiricum</i>	<i>Wolbachia</i>		✓				Schilthuizen & Stouthamer (1997)
<i>T. nr sibiricum</i>	<i>Wolbachia</i>		✓				Schilthuizen & Stouthamer (1997)
<i>T. telengai</i>	?			✓			Stouthamer (1997)
Superfamily Cynipoidea							
Family Cynipidae							
<i>Diplolepis bicolor</i>	<i>Wolbachia</i>		✓				Plantard <i>et al.</i> (1999)
<i>D. californica</i>	<i>Wolbachia</i>		✓				Plantard <i>et al.</i> (1999)
<i>D. eglanteriae</i>	<i>Wolbachia</i>		✓				Plantard <i>et al.</i> (1999)
<i>D. fructuum</i>	<i>Wolbachia</i>		✓				Plantard <i>et al.</i> (1999)

<i>D. mayri</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>D. nodulosa</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>D. polita</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>D. radicum</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>D. rosae</i>	<i>Wolbachia</i>	✓	Stille & Däving (1980); van Meer <i>et al.</i> (1995)
<i>D. spinosa</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>D. spinosissimae</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1998)
<i>Liposthenes glechomae</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
<i>Phanacis lampsanae</i>	<i>Wolbachia</i>	✓	Plantard <i>et al.</i> (1999)
Family Eucolidae			
<i>Gronotoma micromorpha</i>	<i>Wolbachia</i>	✓	Arakaki <i>et al.</i> (2001b)
<i>Hexacola nr websteri</i>	?	✓	Eskafi & Legner (1974)
<i>Leptolina australis</i>	<i>Wolbachia</i>	✓	Werren <i>et al.</i> (1995)
<i>Leptolina clavipes</i>	<i>Wolbachia</i>	✓	Werren <i>et al.</i> (1995)
Superfamily Platygastridae			
Family Platygastridae			
<i>Amitus fuscipennis</i>	<i>Wolbachia</i>	✓	van Meer <i>et al.</i> (1999)
Family Scelionidae			
<i>Telenomus navai</i>	<i>Wolbachia</i>	✓	Arakaki <i>et al.</i> (2000)
Superfamily Tenthredinoidea			
Family Tenthredinoidea			
<i>Diprion hercyniae</i>	?	✓	Smith (1955)
<i>Pristiphora erichsonii</i>	?	✓	Smith (1955); Darwish <i>et al.</i> (1992)
Order Coleoptera			
Family Curculionidae			
<i>Aramigus tessellatus</i>	<i>Wolbachia</i>	✓	Werren <i>et al.</i> (1995)
<i>Cathormiocerus britannicus</i>	<i>Wolbachia</i>	✓	Piper <i>et al.</i> (2001)
<i>C. myrmecophilus</i>	<i>Wolbachia</i>	✓	Piper <i>et al.</i> (2001)
Family Scolytidae			
<i>Xyleborus ferrugineus</i>	<i>Staphylococcus</i>	✓	Peleg & Norris (1972)
Order Hemiptera			
Superfamily Coccidae			
Family Coccidae			
<i>Lecanium cerasifex</i>	?	✓	Nur (1972)
Order Psocoptera			
Family Liposcelididae			
<i>Liposcelis bostrychophila</i>	<i>Wolbachia</i>	✓	Yusuf <i>et al.</i> (2000)
Family Trogiidae			
<i>Cerobasis guestfalica</i>	<i>Wolbachia</i>	✓	Yusuf (1999)

Table 1. Continued

Species	Bacterium	Mechanism	M	A	H	O	Reference
Order Thysanoptera							
Family Aeolothripidae							
<i>Franklinothrips vespiformis</i>	<i>Wolbachia</i>		✓	✓	✓		Arakaki (2001a)
Family Thripidae							
<i>Heliothrips haemorrhoidalis</i>	<i>Wolbachia</i>		✓				Pintureau <i>et al.</i> (1999b)
<i>Hercinothrips femoralis</i>	<i>Wolbachia</i>		✓				Pintureau <i>et al.</i> (1999b)
SUBPHYLUM CHELICERATA							
CLASS ARACHNIDA							
Subclass Arachnida							
Order Prostigmata							
Superfamily Tetranychoidae							
Family Tetranychidae							
<i>Bryobia graminum</i>	<i>Wolbachia</i>		✓				Weeks & Breeuwer (2001)
<i>B. kissophila</i>	<i>Wolbachia</i>		✓				Weeks & Breeuwer (2001)
<i>B. neopractiosa</i>	<i>Wolbachia</i>		✓				Weeks & Breeuwer (2001)
<i>B. praetiosa</i>	<i>Wolbachia</i>	Apomictic	✓	✓			Weeks & Breeuwer (2001)
<i>B. rubrioculus</i>	<i>Wolbachia</i>		✓				Weeks & Breeuwer (2001)
<i>B. sp. X</i>	<i>Wolbachia</i>	Apomictic	✓	✓			Weeks & Breeuwer (2001)
Family Tenuipalpidae							
<i>Brevipalpus phoenicis</i>	CFB group	Haplloid	✓	✓		✓	Weeks <i>et al.</i> (2001)
SUBPHYLUM CRUSTACEA							
CLASS OSTRACODA							
Subclass Podocopia							
Order Podocopida							
Superfamily Cypridoidea							
Family Cyprididae							
<i>Heterocypris incongruens</i>	?		✓			✓	Vandekerckhove (1998); Vandekerckhove (2001)
PHYLUM NEMATODA							
CLASS ENOPLA							
Subclass Enoplia							
Order Dorylaimida							
Family Longidoridae							
<i>Xiphinema americana</i>	<i>Xiphinematobacter americanus</i>		✓			✓	Vandekerckhove <i>et al.</i> (2000)
<i>X. brevicollum</i>	<i>Xi. brevicollum</i>		✓			✓	Vandekerckhove <i>et al.</i> (2000); Coomans <i>et al.</i> (2000)
<i>X. rivesi</i>	<i>Xi. rivesi</i>		✓			✓	Vandekerckhove <i>et al.</i> (2000)



Werren (1993) used 2% lacmoid stain to visualize them in wasp eggs. Eggs of field-collected thelytokous species examined in this way appeared spotted, whereas those from naturally sexual races lacked the dark-staining microorganisms in their cytoplasm. Similarly, staining of eggs from thelytokous and cured strains of *Trichogramma* showed that eggs of the former were infected with numerous bacterial cells, whilst these were absent from the latter.

The microorganisms in thelytokous *Trichogramma* species were subsequently identified as *Wolbachia* by sequence analysis. All parthenogenesis-inducing microorganisms were found to be closely related to cytoplasmic-incompatibility bacteria found in diverse insect taxa including beetles (Coleoptera), butterflies (Lepidoptera), flies (Diptera), spider mites (Prostigmata) and woodlice (Isopoda) (Rousset *et al.*, 1992; Stouthamer *et al.*, 1993; Braig *et al.*, 2002).

The induction of thelytoky by *Wolbachia* has now been found in a wide range of micro-Hymenoptera. It occurs in 12 parasitoid families and one species from the sawfly family Tenthredinidae (Stouthamer, 1997; Braig *et al.*, 2002).

Two forms of thelytoky are recognized in Hymenoptera – ‘revertible/reversible or microbe-associated thelytoky’ and ‘non-revertible/irreversible thelytoky’ (Stouthamer & Kazmer, 1994). In microorganism-associated thelytoky, *Wolbachia* cause parthenogenesis and the removal of the microbes by antibiotics or high-temperature treatment induces the production of males. In non-revertible parthenogenesis, microbes are not present and neither temperature nor antibiotic treatments cause a reversion to arrhenotoky (Stouthamer *et al.*, 1990b; Stouthamer & Werren, 1993). However over time, more and more cases have been detected where the outcome of antibiotic treatment covers the whole range from a clear-cut appearance of functional males to nothing more than a ‘hard to interpret’ increase in egg mortality. Since attempts to re-establish sexual lines have failed in all but one genus, most *Wolbachia*-induced cases of thelytoky are actually irreversible. Therefore, it might be clearer to only distinguish between microbial or cytoplasmic-induced and non-microbial or nuclear-induced parthenogenesis.

The sexual functioning of antibiotic-induced females and males in micro-Hymenoptera ranges widely. Australian parthenogenetic forms of *Eretmocerus mundus* Mercet (arrhenotokous in the rest of the world) are able to produce sperm and inseminate females, but fertilization leading to viable egg development does not appear to occur (De Barro & Hart, 2001). This is also reported in *Aphytis lingnanensis* Compere and *A. diaspidis* (Howard; Zchori-Fein *et al.*, 1995) and in the egg parasitoid *Telenomus nawai* Ashmead (Arakaki, Noda & Yamagishi, 2000). These are differ-

ent to the situation found in *Encarsia formosa* (Zchori-Fein, Roush & Hunter, 1992) where no insemination is observed despite sperm being produced by males. Further, in *Apoanagyrus diversicornus* (Howard) males from cured females can be successfully mated with sexual females, but not with cured thelytokous females (Pijls, van Steenberghe & van Alphen, 1996). It has been suggested that, after a considerable period of time, the accumulation of mutations in the genes governing sexuality as well as various behavioural and physical pre-zygotic barriers may arise to make thelytoky irreversible (Zchori-Fein *et al.*, 1992). In the case of *Muscidifurax uniraptor* Kogan & Legner, these reproductive barriers have now been more closely characterized. During the time of *Wolbachia*-induced thelytoky, genes for the maturation of sperm, for the mating behaviour of females and for a major muscle in the female sperm reservoir, the spermatheca, have been lost (Gottlieb & Zchori-Fein, 2001).

In all cases of *Wolbachia*-induced parthenogenesis, apart from *Trichogramma*, the infection has gone to fixation in the population. Although curing the parasitoid of *Wolbachia* restores male and female production, either the sons, or more usually the daughters, are unable or unwilling to mate, and so arrhenotokous lines are not established (Stouthamer, 1997; Gottlieb & Zchori-Fein, 2001). Interestingly, *Wolbachia*-induced thelytokous females of *Trichogramma pretiosum* Riley and *T. deion* Pinto & Oatman can and will mate with sexual males and the sperm is utilized to fertilize some of their eggs. These fertilized eggs give rise to females whose genome consists of one set of chromosomes from each parent (Stouthamer & Kazmer, 1994). In many populations of parasitoid Hymenoptera, thelytokous and arrhenotokous conspecifics occur sympatrically (Orphanides & Gonzalez, 1970; Rössler & DeBach, 1973; Stouthamer *et al.*, 1990a; Stouthamer *et al.*, 1990b; Chen, Kfir & Chen, 1992) and sometimes rare males are found in infected populations (Plantard *et al.*, 1998, 1999). In contrast to this, parthenogenesis-inducing *Wolbachia* infections in *Trichogramma kaykai* Pinto, Platner & Stouthamer, populations are at a low level and parthenogenetic individuals coexist and interbreed with uninfected conspecifics. Stouthamer *et al.* (2001) found that a male-biasing sex-ratio distorter, a parasitic B-chromosome, analogous to the ‘paternal sex ratio’ chromosome *psr* in the jewel wasp *Nasonia vitripennis* (Beukeboom & Werren, 2000), causes females to produce all-male offspring, preventing the fixation of parthenogenesis-inducing *Wolbachia*. It achieves this by converting the diploid fertilized egg into a male haploid egg by destroying the paternal set of chromosomes. This extremely selfish genetic element enhances its own transmission by preventing any outcrossing (Stouthamer *et al.*, 2001).

Does bacterial induced parthenogenesis come at a price? Since most infected thelytokous lines cannot revert back to sex this is not so easy to answer. When *Muscidifurax uniraptor* is treated with antibiotics at immature stages, no difference in adult fecundity or fitness are found between treated and non-treated lines (Horjus & Stouthamer, 1995; Zchori-Fein, Gottlieb & Coll, 2000). However, any effects on the egg or early larval stages cannot be assessed in this species, and that is where the cost of infection ultimately might lie. For *Trichogramma* species, *Wolbachia*-infected thelytokous and non-infected arrhenotokous lines can be compared directly. Only one third of the eggs of *Wolbachia*-infected strains develop compared to more than 78% of the non-infected strains. Half of the mortality stems from an arrest in the mitotic stage even 48 h after oviposition, whereas non-infected embryos develop a cellular blastoderm after six hours (Tagami, Miura & Stouthamer, 2001).

### TRANSMISSION

The final proof that microorganisms are indeed causal agents of thelytokous parthenogenesis is the transmission experiment. The first attempt had been reported by Grenier *et al.* (1998). These authors transferred purified *Wolbachia* from thelytokous *Trichogramma pretiosum* by microinjection into pupae of an uninfected species, *T. dendrolimi* Matsumura. The infection was still present 26 generations after the transfer, although only 4% of virgin females had between one and five females in their offspring at that time. Van Meer & Stouthamer (1999) encountered a similar fate when they attempted to transfer *Wolbachia* of parthenogenetic haplodiploid *Muscidifurax uniraptor* into diploid *Drosophila simulans* that are not adapted to thelytoky. No parthenogenesis was observed in the host and transmission rates of the bacterium decreased after each generation until it was 0% in the seventh generation. Interspecific transfers of *Wolbachia* resulted in some interesting phenotypes. *Wolbachia* from the mosquito *Aedes albopictus* (Skuse) caused a new crossing type and bidirectional incompatibility in *Drosophila simulans* Sturtevant (Braig *et al.*, 1994). Bouchon, Rigaud & Juchault (1998) reported that *Wolbachia* from *Porcellio pruinosus* Brandt (Crustacea: Isopoda) feminized *Porcellio dilatatus petiti* Vandel, the natural infection of which is associated with cytoplasmic incompatibility, demonstrating that a single host species can express different phenotypes with different *Wolbachia* strains. The most exciting transinfection of *Wolbachia* was accomplished by Fuji *et al.* (2001). They demonstrated that *Wolbachia* harboured by the adzuki bean borer, *Ostrina scapulalis* (Walker) (Lepidoptera) induced male-killing in the Mediterranean flour moth, *Ephes-*

*tia kuehniella* Keller, which is naturally infected with a strain expressing cytoplasmic incompatibility. In its natural host, the adzuki bean borer, *Wolbachia* causes the conversion of genetic males into functional females (feminization). Horizontal transfer of *Wolbachia* has been shown from *Wolbachia*-infected *Drosophila simulans* hosts to its parasitoid wasp, *Leptopilina bou-lardi* (Barbotin, Carton & Kelner-Pillault), but the infections were lost in subsequent generations (Heath *et al.*, 1999). In a like manner, a survey of 82 insect species from two temperate host-parasitoid communities did not show any evidence of horizontal transfer of *Wolbachia* between hosts and parasitoids (West *et al.*, 1998).

Lastly, Huigens *et al.* (2000) were able to show that *Wolbachia*-associated parthenogenesis is indeed infectious. Females of the parasitoid wasp *Trichogramma kaykai* in populations in Last Chance Canyon in the Mojave Desert of southern California are from 6 to 26% infected with *Wolbachia* (Stouthamer & Kazmer, 1994). The eggs of two *T. kaykai* females can share and mature in one and the same egg of a butterfly or moth host. Using a microsatellite marker, Huigens *et al.* (2000) were able to trace each offspring back to its mother. When a moth egg was parasitized by eggs from both a *Wolbachia*-infected and an uninfected female, 21 instances of horizontal transmission were recorded in 56 all-female broods. Intraspecifically, parthenogenesis-inducing *Wolbachia* are transferred at an unexpectedly high frequency.

Similar sequence comparisons between *Wolbachia* from different parthenogenetic host species and lineages suggest that the ability to induce parthenogenesis has either evolved on numerous occasions in *Wolbachia* or that the vast majority of *Wolbachia* strains have been lost (Gottlieb *et al.*, 1998; Plantard *et al.*, 1999; van Meer, Witteveldt & Stouthamer, 1999). These analyses are questioned with two recent reports about recombination between *Wolbachia* strains (Jiggins *et al.*, 2001; Werren & Bartos, 2001). The interspecies transmission of *Wolbachia*, which equals the *de novo* infection of a species, is very likely to be an extremely rare event, perhaps best measured on an evolutionary timescale.

### CYTOLOGY OF INFECTED PARTHENOGENS

Because parthenogenesis is a very widespread phenomenon within the animal kingdom, it is natural that cytological questions connected with it have been much studied, as demonstrated by the very extensive associated literature. However, only two publications describe in detail the cytogenetics of parthenogenetic wasps known to be infected with *Wolbachia* (Stille & Dävring, 1980; Stouthamer & Kazmer, 1994). The



cytological mechanism of parthenogenesis induced by *Wolbachia* has been determined in three species of *Trichogramma* parasitoids (*T. deion*, *T. nr deion* and *T. pretiosum*) but the molecular biology remains unknown. Normal meiosis in eggs was observed (with recombination) resulting in a haploid pronucleus consisting of five chromosomes and a total of 15 polar chromosomes. This stage was followed, after an interphase, by a mitotic metaphase in which five sets of metaphase chromosomes were visible. During the ensuing anaphase, chromosomes failed to segregate without a clear spindle formation, resulting in a nucleus containing ten chromosomes, five homozygous pairs. Throughout this process, the 15 polar chromosomes remained at the periphery of the egg. From then on, normal mitotic divisions continued (Stouthamer & Kazmer, 1994). In normal arrhenotokous gamete formation, the haploid cells formed after meiosis undergo two further mitotic divisions to form four haploid cells from each meiotic product, thereby producing twice as many haploid eggs as conspecific infected individuals do (Quicke, 1997).

Gamete duplication would normally result in purely homozygous offspring, but it is clear, at least in *Trichogramma*, that should a sperm from a male fuse with the egg before fusion of the first mitotic nuclei, it can prevent that fusion and a normal heteroparental zygote will develop. Thus, Stouthamer & Kazmer (1994) have shown that *Wolbachia*-induced thelytoky does not necessarily mean that populations will tend to homozygosity and that gene flow between thelytokous and arrhenotokous individuals can occur. The gene flow to thelytokous lines will also remove or reduce the presumed long-term costs of asexual reproduction, i.e. the accumulation of mutations via Muller's ratchet and the inability to adapt to changing environments (Muller, 1964; Stouthamer & Kazmer, 1994). Gamete duplication has also been confirmed as the cytological basis for parthenogenesis in the gall wasp, *Diplolepis rosae* (L.) (Stille & Dävring, 1980). Van Meer *et al.* (1995) later demonstrated, using 16S rDNA sequences, that the wasp was infected with *Wolbachia*. Examinations conducted by Legner (1985) showed that female *Muscidifurax uniraptor* achieves cytological diploidy also through endomitosis. It is also possible that *Wolbachia* can cause other cytological distortions. For example, in *Aphytis mytilaspidis* (Le Baron) where *Wolbachia* is thought to be responsible for thelytoky (Stouthamer, 1997), meiosis up to the beginning of anaphase was shown to be normal (Rössler & DeBach, 1973). From that stage on meiosis became unclear and two possible avenues were given for the peripheral nucleus: (1) completion of anaphase followed by endomitosis, or (2) the anaphase may or may not be completed, but the two anaphase groups of haploid products reunite to restore a diploid chromo-

some number. The female offspring of virgin heterozygous females consisted mainly of females homozygous for the markers, but a number of heterozygous offspring was also found, indicating fusion between the pronucleus and a polar body at some stage (Stouthamer, 1997). It was concluded that the cytogenetic mechanism is most likely terminal fusion (Rössler & DeBach, 1973).

A number of factors have been associated with microorganism-induced thelytoky in haplodiploids. Hurst (1993) proposed that typical species vulnerable to particular mechanisms of cytoplasmic distorters have an easily manipulated sex-determining/sex-ratio-determining system. That is because the sex ratio distorters presumably can only achieve simple manipulations. Looking at the intriguing physiology and pathogenesis of some microbes and the diversity of *Wolbachia*-induced phenotypes, this view is increasingly difficult to hold. Also, the greater the plasticity in sex ratio and sex determination, the greater will be a species' vulnerability to selfish sex ratio distorters. Werren (1997) states the suitability of haplodiploids for invasion by parthenogenesis-inducing *Wolbachia*, which restore diploidy by gamete duplication. Since gamete duplication results in complete homozygosity, it would expose recessive deleterious mutations normally present in out-breeding diploid species. In contrast, haplodiploids have considerably lower frequencies of deleterious recessives, due to their purging in the male (haploid) sex.

The distribution of thelytoky among different Hymenoptera taxa appears to be extensive. Luck, Stouthamer & Nunney (1993) report that at least 270 cases exist exclusive of the 2000 cases of Cynipoidea. Most of these have a haplodiploid sex determination mechanism. However, some species have single-locus complementary sex determination (CSD) in which fertilized (diploid) zygotes develop as females if the sex locus is heterozygous, but as sterile diploid males if it is homozygous (Cook, 1993; Cook & Crozier, 1995). Since the *Wolbachia* act by preventing the first cleavage division of a haploid egg, rendering the zygote diploid, this would lead species with CSD to sterile diploid males. The incompatibility of CSD with thelytoky via gamete duplication has been proposed as an explanation for the apparent absence of parthenogenesis-inducing *Wolbachia* in the Ichneumonidea (Cook & Butcher, 1999). Diploid males in Hymenoptera have been additionally reported in ants, several species of bees and sawflies (Bull, 1983; Luck *et al.*, 1993; Quicke, 1997). As discussed by Cook & Butcher (1999), gamete duplication could in theory induce thelytoky in all arthropod species except those that have sex determination by female heterogamety or analogous systems. The presumption that thelytoky induced by microorganisms is restricted to male haploid species

is based on the absence of a requirement for any fertilization or syngamy-associated checkpoints, or a requirement for paternally derived centrioles to derive astral microtubules (Werren, 1997; Cook & Butcher, 1999). It is generally thought that the male gamete is essential for the formation of the functional zygotic centrosome, which in turn allows duplication and organization of the first mitotic spindle. However, during parthenogenetic development the centrosome must be of maternal origin and egg cytoplasm must contain all the material necessary to form a zygotic centrosome. To clarify the reconstruction of a functional centrosome in *Wolbachia*-infected parthenogenetic eggs, Riparbelli *et al.* (1998) performed an ultrastructural study in which they confirmed/demonstrated that the cytoplasm of *Muscidifurax uniraptor* eggs does indeed contain a pool of inactive centrosomal precursor proteins that transform nucleate microtubules into well-defined asters containing centrioles after parthenogenetic activation. In the hymenopterous parasitoid, *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) unfertilized male eggs use maternal centrosomes whereas fertilized female eggs use the paternal centrioles brought in with the sperm and peri-centriole material to form centrosome. This is the first case in which egg cells switch between centrioles (Tram & Sullivan, 2000; Karr, 2000).

### INFECTED NON-HYMENOPTERAN PARTHENOGENS

Parthenogenetic thrips and haplodiploid mites were considered to be particularly important groups in searching for parthenogenesis-inducing *Wolbachia* (Werren, 1997; Cook & Butcher, 1999). Werren had already found *Wolbachia* in a parthenogenetic weevil, *Aramigus tessellatus* (Say) (Coleoptera: Curculionidae: Naupactini) in 1995. Curing with antibiotics or heat treatment was not possible because the population turned out to be triploid, but evidence of *Wolbachia* has not been found in related sexual lineages (Braig *et al.*, 2002).

During investigations on buprestid beetles and male-killing, Lawson *et al.* (2001) detected low levels of parthenogenetic production in 29% of field-collected females. A novel *Rickettsia* was found, called Btess (*Brachys tessellatus*), most closely related to a tick-associated bacterium and a pea-aphid *Rickettsia*, but also related to a *Rickettsia* that causes male-killing in ladybird beetle species. The field surveys revealed highly distorted sex ratios in some populations of *Brachys tessellatus*, ranging from 1.3 to 6 females per male. This was explained by possible selection for parthenogenetic development when males are scarce. Although the usual tetracycline treatment of the par-

thenogenetic females used in the experiment was conducted, males were introduced to the females, leaving unclear what the role of bacteria was in the parthenogenetic reproduction.

Pintureau *et al.* (1999b) fulfilled the anticipation of *Wolbachia* in thelytokous Thysanoptera. They discovered the bacterium using PCR in two thrip species collected from Portugal and France. Once again, further substantiation of the role of *Wolbachia* in the species was left for the future, and therefore could be considered hypothetical. Nevertheless, in a recent publication by Arakaki, Miyoshi & Noda (2001a), the causative agent of the thelytoky in predatory thrips from Japan was indeed shown to be *Wolbachia*. Males of *Franklinothrips vespiformis* Crawford were produced after the thelytokous females were exposed to a high temperature (35°C) at the pupal stage and after treatment of newly emerged adults with tetracycline. Sequencing 16S rDNA revealed the identity of the bacteria. The males successfully mated and transmitted motile sperm to the spermathecae of females, but the females continued producing all-female progeny.

A speciose genus of haplodiploid phytophagous mites, the *Bryobia*, exists within the family of spider mites (Tetranychidae). Approximately 100 species have been described, most of them thought to reproduce by thelytokous parthenogenesis (Norton *et al.*, 1993). Weeks & Breeuwer (2001) screened six *Bryobia* species for *Wolbachia* using *ftsZ* (cell-cycle gene) primers and investigated the effect of the infection by curing two of the species with tetracycline. Most usefully, they used microsatellites for comparing genotypes of adults and their offspring to assess the mode of parthenogenesis. Predictably, *Wolbachia* infection was found in every individual tested and accordingly antibiotic-treated females produced significantly more male progeny than the untreated control. Infected individuals were observed to copulate with cured males but no genetic recombination was evident in the offspring, which all had identical genotypes to their mothers. The mode of parthenogenesis was found to be functionally apomictic since the maternal genotypes of three microsatellite loci tested passed unchanged to all progeny (mothers were heterozygous). This is significant, because it suggests that parthenogenesis-inducing *Wolbachia* might not be functionally restricted to haplodiploids, however, *Bryobia* species are haplodiploid.

Weeks *et al.* (2001) continued their studies with the phytophagous mite *Brevipalpus phoenicis* (Geijskes) and unexpectedly came across an extraordinary phenomenon. They discovered that the species exists only in a female haploid state. Large amounts of intracellular bacteria were noticed in the eggs, but in this case it was not identified as *Wolbachia*. Instead, after sequencing 16S rDNA, its closest relative was an

undescribed endosymbiotic bacterium belonging to the phylum the *Cytophaga–Flexibacter–Bacteroides* (CFB group) found in the tick, *Ixodes scapularis* Say. Tetracycline treatment led to the production of males. Weeks and colleagues suggested that infection of a sexual haplodiploid ancestor with feminizing intracellular bacteria caused haploid eggs that normally developed as males to develop into females. The authors deliberated that similar mechanisms that cause feminization in the isopod, *Armadillidium vulgare* Latreille, could be involved in *B. phoenicis*. In the former, *Wolbachia* induces feminization by blocking the formation of the androgenic gland responsible for male differentiation (Rousset *et al.*, 1992; Martin *et al.*, 1999). Whether or not diploid sexual females can be generated in *B. phoenicis* has yet to be demonstrated. It would also be worthwhile seeing if this bacterium is involved in thelytoky induction in two closely related species, *B. obovatus* Donnadieu and *B. californicus* (Banks). By successfully infecting the closest sexual relative, the haplodiploid *B. russulus* (Boisduval) with bacteria from *B. phoenicis*, it might be possible to gain the first empirical data on the relative importance of somatic and germline deleterious mutations for the maintenance of diploidy in animals (Weeks, Marec & Breeuwer, 2001; Otto & Jarne, 2001).

Despite the fact that research had long ago been recommended by Nur (1972) into the mode of reproduction in haplodiploid soft scale insects (Coccoidea: Homoptera), on a suggestion that it would prove rewarding, no work has been conducted/published to date. In a detailed study, he observed that some of the uninseminated females of *Parthenolecanium (Lecanium) cerasifex* Fitch (Homoptera: Coccidae) produced only female offspring in the presence of needle-like bacterial symbionts, whereas other females lacking this bacterium produced male offspring only. He cleared the puzzle by suggesting that *L. cerasifex* females belonged to at least two races: a diploid arrhenotokous race and an obligate automictic thelytokous one. Diploidy is restored by a fusion of the first haploid cleavage nuclei.

It is now clear that the ability to induce thelytokous parthenogenesis is neither a unique property of *Wolbachia* nor even of the Proteobacteria. The finding of an undescribed vertically transmitted bacterium in association with thelytokous parthenogenetic reproduction in *Encarsia*, a genus of parasitoid wasps (Hymenoptera: Aphelinidae), further substantiates the above statement (Zchori-Fein *et al.*, 2001). Six species of this genus are infected with an 'Encarsia bacterium (EB)'. The 16S rDNA sequence closely matches with the CFB group and the bacterium in the haploid female mite. Furthermore, analysis of the different EB sequences from four not-closely related infected *Encarsia* populations and one infected sexual popula-

tion showed a very high degree of sequence identity (99.7%), indicating horizontal transmission. Rifampicin treatment affected the oviposition behaviour of the *Encarsia pergandiella* Howard females, which laid significantly fewer eggs than the untreated wasps. The authors concluded that EB was the most likely cause of the parthenogenesis and that the bacterium might have multiple reproductive effects like *Wolbachia*, since one of the populations was bisexual. This conclusion is corroborated by previous study of Hunter (1999) who demonstrated that thelytokous *E. hispida* produced male progeny after been fed with antibiotics.

The springtail, *Folsomia candida* (Willem) (Collembola) represents the first significant case of a diploid species found to reproduce by *Wolbachia*-induced thelytoky. In their search for novel endocytobioses, Vandekerckhove *et al.* (1999), using transmission electron microscopy, PCR and fluorescence microscopy with DAPI staining, detected *Wolbachia* in this species. DAPI fluorescence microscopy revealed, as expected for diploid parthenogenetic *Wolbachia* hosts, that uninfected eggs from cured virgins aborted because of haploidy. However, complete elimination of parthenogenesis by antibiotics was not achieved. Using electron microscopy, it was observed that during *Wolbachia*-induced parthenogenesis, at the end of the anaphase of the first cleavage, diploidy was restored through non-disjunction, or fusion of the two haploid division products. Further mitoses continued normally, giving rise to 100% homozygous females (Vandekerckhove, 2001). This is concordant with Stouthamer & Kazmer's (1994) findings that gamete duplication is the mechanism for diploidy restoration in *Wolbachia*-induced parthenogenesis.

Psocoptera is yet another non-hymenopteran order where a *Wolbachia*-like rickettsial bacterium is associated with parthenogenesis. *Liposcelis bostrychophila* Bondonnel (Psocoptera: Liposcelididae), a stored-product pest, is parthenogenetic. Intracellular bacteria have been detected in its oocytes with electron microscopy (Yusuf *et al.*, 2000). Thelytoky was reported in 13 of the 32 families in Mockford's (1971) review of parthenogenesis in psocids. Among the small subset of psocids that are pests, *L. bostrychophila* is the only species that is parthenogenetic (Mockford, 1971). Cytological studies conducted by Goss (1954) suggest that there is only a single maturation division in the oocyte (ordinarily mitosis). This division is an equatorial one, so that there is no chromosome synapses and no chromosome reduction, thus preventing new gene combinations. Nevertheless, in their study of allozyme patterns among 111 British populations of *L. bostrychophila*, Ali & Turner (2001) found 47 distinct genotypes.

An unexpected discovery was made when endosymbiotic bacteria associated with parthenogenesis were



detected in plant nematodes (Vandekerckhove *et al.*, 2000). Species of the *Xiphinema americanum* group (Longidoridae), *X. americanum* Cobb, *X. rivesi* Dalmasso and *X. brevicollum* Lordello & da Costa, all reproduce by thelytokous parthenogenesis (Coomans, Vandekerckhove & Claey's, 2000), each harbouring their own specific, maternally inherited verrucomicrobial endosymbionts. The first cytoplasmic species identified among the Verrucomicrobia, these were described on the basis of transmission electron microscopy (TEM) and 16S rDNA sequence analysis and named *Xiphinematobacter americani*, *X. rivesi* and *X. brevicolli*. The microorganisms were only found in the gut epithelium and in the ovaries of *X. americanum* group specimens. In females they prevail in the ovarian wall cells, while oögonia and primary oocytes are scarcely populated. The endosymbionts in the egg move to the pole where the gut primordial cell will arise, so that all bacteria populate the intestinal epithelium of juvenile worms. During the moult from last juvenile stage to adult female, the bacteria concentrate around the developing ovaries. The endosymbionts become enclosed in the ripening oocytes when they start to grow (Vandekerckhove, 2001). This is different from *Wolbachia*, which invade the gonadal primordium directly (Hadfield & Axton, 1999).

Sequence divergence between the three bacteria suggest that the infection in the common ancestor of the *Xiphinema* species would have originated some 100–140 million years ago (Vandekerckhove *et al.*, 2000). It is still unclear whether the bacteria have a role in the metabolic activity of their hosts or whether they might be the driving force behind the thelytokous parthenogenesis. Curiously, the verrucomicrobial endosymbionts are only found in parthenogenetic *Xiphinema* species belonging to the *X. americanum* group, all the other *Xiphinema* species, sexual or parthenogenetic, are free of endosymbionts (Vandekerckhove *et al.*, 2000). Lastly, ostracod crustaceans represent the latest addition to the taxa where parthenogenesis and intracellular microbes are intertwined. Using transmission electron microscopy, unidentified intracellular bacteria have been seen in great numbers in the gonadal cells of the Ostracod, *Heterocypris incongruens* (Ramdohr) (Vandekerckhove, 1998). DAPI staining showed the preference of the bacterium for superficial tissues associated with the mantle (Vandekerckhove *et al.*, 2000).

#### SYMBIONTS AND CYCLICAL PARTHENOGENESIS

Soon after the discovery of microbial-induced parthenogenesis, Stouthamer, Luck and Hamilton speculated that a moderate heatwave might uncouple the bacterial-induced parthenogenesis without actually

killing the endosymbiont. They foresaw a scenario where a temporary controlled incapacitation of the endosymbiont without eliminating it could evolve into a potentially powerful adaptation for the host, affording entry to facultative or cyclic parthenogenesis (Stouthamer *et al.*, 1990a). If sex ratio distorting symbionts had been instrumental in the evolution of cyclic parthenogenesis, one would expect that the two major clades expressing cyclic parthenogenesis would also harbour reminiscent lineages that are still all sexual. This is not the case (Bell, 1982).

The only study so far addressing this question was conducted on oak gallwasps (Hymenoptera; Cynipidae, tribe Cynipini), aiming to establish the prevalence of *Wolbachia* infection and its phenotypic consequences within the cyclically parthenogenetic Cynipini. Here, it was shown that there was no correlation between life-cycle structure and the infection status (Rokas *et al.*, 2002). Also, preliminary surveys of aphid species that exist as both cyclic and obligate parthenogenetic populations have not revealed any involvement of *Wolbachia*. The broader association between endosymbionts and cyclic parthenogenesis needs further investigation.

#### SYMBIONTS AND HAPLODIPLOIDY

The production of all-male progenies has been a puzzle for some time. Virgin females of the hymenopteran spider egg parasitoid, *Baeus achaeareaneus* (Loiacono) (Hymenoptera: Scelionidae) produce all-male progenies in a close inbreeding system. It has been suggested that these males serve as prey for emerging spiderlings to secure access to future spider eggs (Valerio, 1976). *Wolbachia* is not only known to induce parthenogenesis in haplodiploid species. Several strains of *Wolbachia* can also cause cytoplasmic incompatibility. Whereas cytoplasmic incompatibility will lead to embryonic mortality in diploid species, it will generate all-male progenies in haplodiploid species. Considering the implications of close inbreeding populations, W.D. Hamilton speculated that maternally inherited endosymbionts might be involved in the origins of haplodiploidy (Hamilton, 1993). He suggested that the development of haploids as males is essentially a counter-adaptation by the host to the elimination by endosymbionts of all-male-determining elements from the genome. Evidence for this hypothesis is still very scarce; however, the idea is getting increasing attention (Normark, Jordell & Farrell, 1999; Braig *et al.*, 2002). Peleg & Norris (1972) describe a beetle, *Xyleborus ferrugineus* (F.) (Coleoptera: Scolytidae) in which an endosymbiotic bacterium has taken over the role of the sperm in activating the egg cell. The direct induction of arrhenotokous male-production by endosymbionts seems

counter-intuitive, and may have the simple explanation that the bacteria provide needed nutrients for oviposition. The beetles in this clade are haplodiploid and exhibit regular sib mating with highly female-biased sex ratios (Normark *et al.*, 1999). Normark and colleagues argue that microbial control of arrhenotoky may explain why none of the over 1300 species in this arrhenotokous clade have ever evolved anything close to 50:50 sex ratios.

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